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Research Paper

Effect of spraying with colchicine alkaloid on seed viability of bread wheat varieties

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ABSTRACT

This study was conducted in the laboratories of the Seed Testing and Certification Department of Baqubah City in Diyala Governorate during the years 2022 and 2023, with the aim of studying the effect of the difference in the genetic composition of bread wheat and its response to spraying with colchicine alkaloid and its reflection on the vitality of its resulting seeds, as the experiment included two factors, the first is the varieties (Jad, Thariyah, Adna and Ibaa 99), and the second is spraying the vegetative group with colchicine alkaloid (at a concentration of 100 mg L-1 in one spray and at a concentration of 200 mg L-1 in two batches, each spraying half the amount) in addition to the comparison treatment (spraying with distilled water only). The experiment was applied using a completely randomized design (CRD) with three replicates. The results of the statistical analysis showed that wheat varieties had a significant effect on all studied traits, and the variety Ebaa 99 recorded the highest average for field emergence percentage (88.20%), seedling length (7.767 cm), dry radicle weight (47.34 mg) and dry seedling weight (50.92 mg). The results showed that spraying with colchicine alkaloid with an average of two sprays was significantly superior to the traits of field germination percentage (88.30%), seedling length (7.625 cm), radicle length (6.250 cm), dry radicle weight (47.90 mg) and dry seedling weight (50.03 mg). We conclude from this study that bread wheat varieties vary in seed vitality indicators, and the use of colchicine significantly improved these indicators, especially when using more than one spray.

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1. INTRODUCTION

Wheat is one of the oldest crops that humans have cultivated and improved for thousands of years until today [1], and wheat is considered the first cereal crop in Iraq and the world [2]. Due to its nutritional importance, wheat is at the forefront of strategic crops, and the most important crop for food security at the global level, as it constitutes a food source for more than 35% of the world's population and provides about 20% of the protein and calories consumed by humans [3], [4]. Given the large increase in population, there is a need to increase global production by 1.6% annually to cover the increasing needs for this crop, which necessitated the search for new ways to increase production and productivity by using new genetic sources in wheat genetic improvement programs on the one hand and applying scientific research methods in its cultivation and production programs [5], [6]. Crop production in general and wheat in particular is affected by the planted genetic composition, as varieties, soil management factors and crop play a decisive role in determining yield and increasing production [7], which is represented by the environmental genetic interaction (E × G), which is very important in developing or evaluating crop varieties [8], and storage conditions and seed treatment before planting [6], [9], [10]. One of the most important factors in increasing wheat production is the seeds used in agriculture, which must be of high quality and are essential for successful and commercial agriculture [11], [12], [8].

It is necessary to increase wheat productivity by making effective use of available resources, which is crucial to meet the growing demand for food [13]. Therefore, plant breeders focus on enhancing grain yield by improving traits associated with them directly, indirectly, or both, as several goals can be achieved by using methods that can improve growth and yield traits when applied directly to the plant during different growth stages or by producing strong seeds that can germinate and grow actively and withstand extreme conditions more [14]. Among these, the most important methods used by plant breeders is the use of mutation technology, as an important source of genetic diversity [15]. Among the most important mutagenic means used is the alkaloid colchicine ($C_{22}H_{25}O_6N$), which works by inhibiting the formation of spindle threads during the regular reproduction of somatic cells, and as a result, the number of chromosomes in the cell doubles, which leads to improving plant productivity by improving seed germination, seedling activity, accelerating germination, and the growth of many crops [16], [17]. confirmed in their study of the effect of colchicine at concentrations (0.05, 0.10, 0.15, and 0.20 g dL^{-1}) on the germination of bean seeds that the germination percentage was high with a concentration of (0.05 g dL^{-1}) and found significant differences for most quantitative traits such as the number of leaves, branches, and seeds per plant. In general, choosing the concentration of colchicine or the date of its addition is one of the most important things that give the best benefit from the study factors. Accordingly, this study aimed to evaluate the performance of seeds of several genetic combinations of bread wheat and to determine the most appropriate number of sprays of colchicine mutagen on the vegetative group plant.

2. RELATED WORK

Previous studies have explored the role of colchicine in plant breeding, particularly its ability to induce polyploidy by interfering with spindle fiber formation during mitosis. This mechanism has been used to enhance various genetic and agronomic traits in wheat and other crops. Research has shown that colchicine treatment can lead to chromosome doubling, which is often associated with improved morphological characteristics and genetic stability in hybrid lines.

Various application methods have been assessed, with spraying emerging as a more practical and less damaging alternative to traditional methods like seed soaking or direct tissue exposure. Spraying allows for more localized application and can be adjusted to suit different developmental stages of the plant. However, the effectiveness of this method can be influenced by factors such as colchicine concentration, frequency of application, and the developmental stage of the plant at the time of treatment. Comparative assessments among wheat varieties have revealed that genetic background plays a significant role in determining the outcome of colchicine treatment. Some varieties show greater resilience to its

cytotoxic effects, while others may suffer reduced germination and lower seedling vigor. Consequently, studies have emphasized the importance of varietal screening to identify genotypes that respond favorably to colchicine treatment.

Additionally, attention has been given to the balance between inducing polyploidy and maintaining seed viability. An optimal concentration of colchicine is crucial; excessive exposure may inhibit cell division to the point of lethality, whereas insufficient dosage may result in incomplete chromosome doubling. Thus, ongoing research continues to focus on identifying treatment protocols that achieve desired genetic outcomes without compromising seed health and viability.

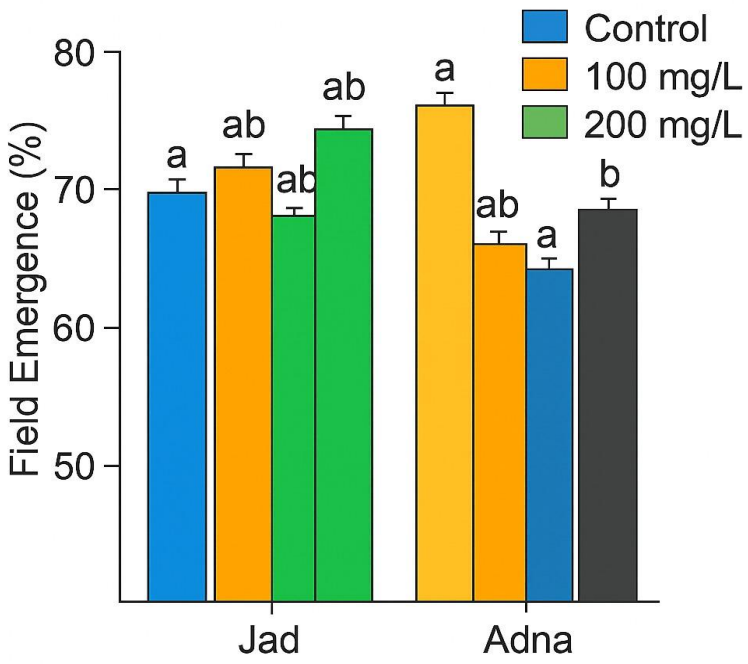


Figure 1. Effect of Colchicine Alkaloid Spraying on Field Emergence Percentage of Bread Wheat Varieties

As shown in Figure 1, the diagram illustrates the effect of colchicine alkaloid spraying on field emergence percentage in two bread wheat varieties, Jad and Adna, under three treatment conditions: Control (no colchicine), 100 mg/L, and 200 mg/L concentrations. The bar chart clearly shows an increasing trend in field emergence percentage with higher colchicine concentration, particularly under the 200 mg/L treatment. In the Jad variety, field emergence increased noticeably from the control to 200 mg/L, indicating a positive response to colchicine application. Similarly, in the Adna variety, the highest emergence was also observed under the 200 mg/L treatment. The 100 mg/L treatment showed moderate improvement in both varieties compared to the control. Error bars indicate standard deviation across replicates, and statistical groupings are labeled above each bar using letters. Bars labeled with different letters (e.g., "a", "b") signify significant differences at $p < 0.005$, confirming the statistical reliability of the results. The use of color-coded bars improves visual clarity in distinguishing treatment effects. These results demonstrate that colchicine can enhance seed viability by improving field emergence.

3. METHODOLOGY

A laboratory experiment was conducted in the Seed Technology Laboratory of the Seed Testing and Certification Authority located in Baqubah city / Diyala Governorate during the seasons 2022-2023,

with the aim of studying the effect of spraying with colchicine alkaloid on the vegetative group of bread wheat varieties on the vital characteristics of the resulting seeds.

The experiment was carried out in a completely randomized design (CRD) with factorial experiments arranged with three replicates. The first factor included four bread wheat varieties (Jad, Thariyah, Adna, and Ibaa99), and the second was spraying the vegetative group with colchicine alkaloid (at a concentration of 100 mg L^{-1} in one spray and at a concentration of 200 mg L^{-1} in two batches, each spraying half the amount). The number of experimental units = 36. The planting was done on 11/1/2022, and the colchicine mutagen was sprayed during the seed formation stages on the plant, as the first spray was done when the plant reached the stage of 50% emergence of spikes (ZGS: 55), and the second spray was done when flowering was complete (ZGS: 69) [18]. After harvesting, seed samples were taken from each experimental unit and after mixing them manually, a laboratory sample was taken using a mechanical divider device and the following laboratory tests were performed on the seeds:

1. Standard Germination Percentage in the First and Final Counts(%)

The total number of natural seedlings germinated after 4 and 10 days of growth chamber the seeds in the incubator for the first and second counts, respectively [19], and the germination percentage was calculated as in the following equation:

Germination percentage in the first count = (Number of natural seedlings after 4 days / Total number of seeds) × 100.

Germination percentage in the final count = (Number of natural seedlings after 10 days / Total number of seeds) × 100.

2. Length of the Radicle and Plumule in the Standard Germination Test (Cm)

After the end of the test period in the second count, 10 natural seedlings were taken and then the radicle and plumule were separated from their point of contact with the seed coat [20], and they were measured each on Sharpness using a ruler and the average of these two characteristics was extracted.

3. Dry Weight of Seedling and Radicle (Mg)

The seedlings whose radicle and plumule lengths were measured were taken and placed in a perforated paper bag and dried at 80°C for 24 hours, and the average dry weight of the seedling was calculated by dividing the weight of the total dry seedlings by their number [20], [19].

4. Seedling Vigor Index

It was Calculated According to the Following Equation

Seedling vigor index = % of germination ×(radicle length (cm) + plumule length (cm)) [21].

4. RESULTS AND DISCUSSION

4.1 Standard Germination Percentage in the First Count (%)

It appears from the results of Table 1 that there are significant differences between the genetic compositions and colchicine treatments and the interaction between them in the standard germination percentage in the first count. The cultivar Ibaa99 gave the highest average of 73.68%. While the cultivar Thuraya gave the lowest average of the trait of 55.11% without significantly differing from the cultivar Jad. The reason for the difference between the seeds of the cultivars in the germination percentages is due to the difference in the vital and physiological processes and the activity of growth regulators and enzymes that are under the control of the genetic mechanism of each cultivar [22]. This result is consistent with what was reached by [23], which obtained significant differences between the Iraqi wheat cultivars in the standard germination percentage in the first count. The treatment of spraying with the mutagen showed significant differences in the germination percentage trait in the first count, as the treatment sprayed twice with the mutagen gave the highest germination percentage of 67.84%, compared to spraying once and the comparison treatment, which gave the lowest germination percentage of 60.29%. The reason is that

colchicine improves plant productivity by improving seed germination, seedling activity, accelerating germination and growth of many crops [16].

As for the effect of the interaction between varieties and spraying with colchicine on the germination percentage in the first count of wheat seeds, the results showed significant differences between the different interactions, as the variety Ibaa99, whose plants were sprayed with two batches of colchicine (concentration 200 mg L^{-1}), recorded the highest germination percentage of 76.13%. While the variety Thraya with the comparison treatment recorded the lowest value of interaction of 50.75%.

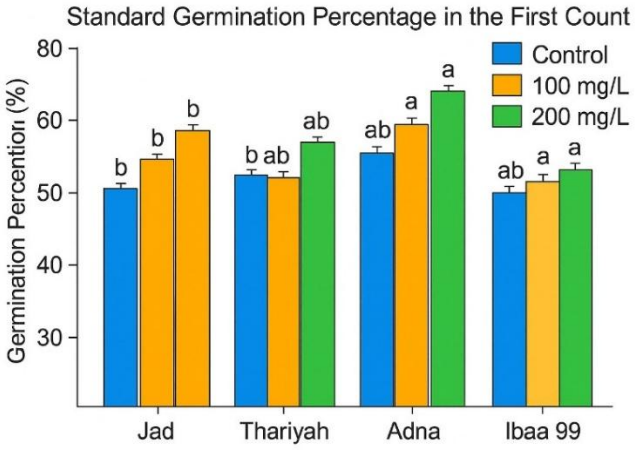


Figure 2. Effect of Colchicine Alkaloid Spraying on Standard Germination Percentage in the First Count of Bread Wheat Varieties

As shown in Figure 2 Effect of Colchicine Alkaloid Spraying on Standard Germination Percentage in the First Count of Bread Wheat Varieties, the diagram illustrates the standard germination percentage in the first count for four bread wheat varieties under three treatments: control, 100 mg/L, and 200 mg/L colchicine sprays. The variety Ibaa 99 treated with 200 mg/L colchicine recorded the highest germination percentage, demonstrating colchicine's positive influence. In contrast, Thariyah under control treatment exhibited the lowest germination rate. The figure clearly shows a trend of increasing germination with higher colchicine concentration across most varieties. These results support the hypothesis that colchicine enhances early seed vigor and germination potential.

Table 1. The Effect of Genetic Compositions and Spraying with Colchicine on the Standard Germination Percentage in the First Count (%)

Cultivars (Cv.)	Colchicine Treatment (Col.)			Mean (Cv.)
	Control	One Spray	Tow Spray	
Jad	52.50	55.63	62.13	56.75
Thuraya	50.75	54.25	60.33	55.11
Adna	66.78	70.25	72.78	69.94
Iba 99	71.13	73.78	76.13	73.68
Mean (Col.)	60.29	63.48	67.84	
LSD %5	cv. = 1.84	Col.= 1.68	cv. × Col.= 2.02	

4.2 Standard Germination Percentage in the Final Count(%)

The results of Table 2 showed that the variety Ibaa99 gave the highest germination percentage in the final count, reaching 88.20%. With a significant difference from the rest of the varieties, the variety Jad gave the lowest average for this trait, reaching 85.07%, which did not differ significantly from the germination of the seeds of the variety Thraya. The difference in varieties in this trait gives a clear

indication of the variation of these varieties in the genetic structure, which is reflected in the structural structure of the seeds and the activity of their seedlings and germination [9].

The results also showed that the effect of spraying with the mutagen on the germination percentage trait was significant, as the treatment sprayed twice with the mutagen outperformed (88.30%), compared to the control treatment, which gave the lowest average for this trait (84.26%). The reason is that the use of colchicine led to an improvement in seed vitality and seedling activity compared to the control treatment [16].

As for the effect of the interaction between varieties and spraying with colchicine, the results show significant differences, as the variety Ibaa99, which was sprayed in two batches at a concentration of 200 mg L^{-1}, recorded the highest germination percentage of 90.46%. While the variety Thraya with the comparison treatment recorded the lowest average for the trait.(%82.66)

Table 2. The Effect of Genetic Compositions and Spraying with Colchicine on the Standard Germination Percentage Trait in the Final Count (%)

Cultivars (Cv.)	Colchicine Treatment (Col.)			Mean (Cv.)
	Control	One Spray	Tow Spray	
Jad	83.23	85.80	86.20	85.07
Thuraya	82.66	85.20	87.63	85.16
Adna	85.60	87.56	88.90	87.35
Iba 99	85.56	88.56	90.46	88.20
Mean (Col.)	84.26	86.78	88.30	
LSD %5	cv. =0.48	Col.= 0.42	cv. × Col.= 0.84	

4.3 Radicle Length (Cm)

The results showed that there were significant differences between the genetic compositions in the radicle length. The Aba99 variety gave the highest average radicle length (6.233 cm), while the Jad and Thuraya varieties gave the lowest average for this trait, reaching 5.533 and 5.556 cm, respectively Table 3. The reason is that the varieties are a quantitative trait governed by genes that are affected by the environment with the effect of the host gene on the main genes [24].

The treatment sprayed with the mutagen twice (6.250 cm) was significantly superior to the treatment sprayed once and the control treatment which gave the lowest average for this trait, reaching 5.208 cm. The reason is that the use of colchicine solution had a positive effect on accelerating cell division, accelerating vital activities and growth of the basic embryo parts (radicle and plumule) which appeared in the traits of germination speed and percentage Table 1 and Table 2 [25].

The cultivar Ibaa99 which was sprayed twice with colchicine recorded the highest average of radical length (6.833 cm), while the seedlings of the cultivars Jad and Thuraya with the control treatment recorded the lowest average (5.200 and 5.300 cm).

Table 3. Effect of Genetic Compositions and Spraying with Colchicine on Radical Length (Cm)

Cultivars (Cv.)	Colchicine Treatment (Col.)			Mean (Cv.)
	Control	One Spray	Tow Spray	
Jad	5.200	5.600	5.800	5.533
Thuraya	5.300	5.500	5.867	5.556
Adna	5.133	6.376	6.500	6.000
Iba 99	5.200	6.667	6.833	6.233
Mean (Col.)	5.208	6.033	6.250	
LSD %5	cv. = 0.082	Col.= 0.071	cv. × Col.= 0.143	

4.4 Plumule Length (Cm)

The results of Table 4 showed that there are significant differences between the genetic compositions in the average length of the plumule. The variety Ibaa99 gave the highest average (7.767 cm), while the variety Jad gave the lowest average for this trait, which amounted to 6.767 cm. The reason is that the seedlings of the variety Ibaa are more active and growing compared to the rest of the varieties Table 1, Table 2 and

Table 3, which was also reflected in the growth of the escarpment of this variety. This confirms what was indicated by [26], [9], who indicated that the trait is under the control of genetic factors, which led to differences in genetic compositions in this trait.

The treatment sprayed twice with the mutagen was significantly superior in the trait of plumule length, with an average of 7.625 cm, compared to the unsprayed treatment, which gave the lowest average for this trait, which amounted to 6.626 cm. The improvement and increase in these traits when using colchicine alkaloid may be due to the doubling of chromosomes, which led to an increase in vegetative growth indicators, germination strength, and increased cell size [25].

As for the effect of interaction between varieties and spraying with colchicine on the length of plumule, the variety Ibaa99, whose mother plants were sprayed twice with the mutagen, recorded the highest average length of the plumule, reaching 8.400 cm. While the varieties Jad and Thraya with the comparison treatment recorded the lowest average for both of them, reaching 6.500 cm.

Table 4. Effect of Genetic Compositions and Spraying with Colchicine on the Length of the Plumule

Cultivars (Cv.)	Colchicine Treatment (Col.)			Mean (Cv.)
	Control	One Spray	Tow Spray	
Jad	6.500	6.800	7.000	6.767
Thuraya	6.500	7.133	7.300	6.978
Adna	6.800	7.500	7.800	7.367
Iba 99	6.700	8.200	8.400	7.767
Mean (Col.)	6.625	7.408	7.625	
LSD %5	cv. = 0.094	Col.= 0.081	cv. × Col.= 0.163	

4.5 Dry Weight of the Radical (Mg)

The seeds of the cultivar Ibaa99 gave the highest average dry weight of the radical, which reached (47.34 mg), with a significant difference from the rest of the cultivars, while the cultivar Thuraya gave the lowest average for this trait, which reached (42.73 mg) Table 5. The results also showed that the effect of spraying with the mutagen on the dry weight of the radical was significant, as the treatment sprayed twice with the mutagen (47.90 mg) outperformed the unsprayed treatment, which gave the lowest average for this trait, which reached (41.47 mg).

The interaction of the cultivar Ibaa99, whose mother plants were sprayed twice with the mutagen, recorded the highest average dry weight of the radical, which reached (50.26 mg), while the cultivar Thuraya and Jad, with the comparison treatment, recorded the lowest average, which reached (39.43 and 40.86 mg). The reason for the superiority of the seedlings of the cultivar Ibaa99 or the treatment of spraying twice with the mutagen is due to their superiority in the radical length trait Table 3, which was reflected in the dry weight of the radical.

Table 5. Effect of Genetic Compositions and Spraying with Colchicine on the Radical Dry Weight (Mg)

Cultivars (Cv.)	Colchicine Treatment (Col.)			Mean (Cv.)
	Control	One Spray	Tow Spray	
Jad	40.86	43.70	45.36	43.31
Thuraya	39.43	42.66	46.10	42.73
Adna	42.96	48.00	50.26	47.08
Iba 99	42.63	49.53	49.86	47.34
Mean (Col.)	41.47	45.97	47.90	

LSD %5	cv. = 0.52	Col.= 0.45	cv. × Col.= 0.91

4.6 Dry Weight of the Seedling (Mg)

The results of Table 6 showed that there were significant differences between the genetic compositions in the average dry weight of the seedling. The cultivar Ibaa99 gave the highest average of (50.92 mg), while the cultivar Thuraya gave the lowest average for this trait of (44.76 mg). The superiority of the Aba99 variety in this trait may be attributed to the superiority of this variety in all indicators of germination and growth of seedlings Table 1, Table 2, Table 3, Table 4, and Table 5, which led to its superiority in the dry weight of seedlings. These results are consistent with what reached regarding the existence of a difference between wheat varieties in this trait, in which the Aba99 variety showed the highest average for this trait. The treatment of spraying twice with the mutagen was significantly superior in this trait, which amounted to 50.03 mg, compared to the control treatment, which gave the lowest average for this trait (44.75 mg). This is due to the fact that the appropriate concentration of the mutagen led to an increase in the efficiency of the photosynthesis process, which led to the accumulation of nutrients in the seeds [27], and that large seeds ultimately produce large embryos and seedlings [7]. The cultivar Ibaa99, with two sprays, recorded the highest average plant length of 53.16 mg, while the cultivar Thuraya, with the unsprayed treatment, recorded the lowest average of (41.30 mg).

Table 6. Effect of Genetic Compositions and Spraying with Colchicine on the Seedling Dry Weight Seedling (Mg)

Cultivars (Cv.)	**Colchicine Treatment (Col.)**			**Mean (Cv.)**
	Control	**One Spray**	**Tow Spray**	
Jad	43.26	46.40	48.33	46.00
Thuraya	41.76	45.06	47.46	44.76
Adna	46.30	48.90	51.16	48.78
Iba 99	47.70	51.90	53.16	50.92
Mean (Col.)	44.75	48.06	50.03	
LSD %5	cv. = 0.49	Col.= 0.43	cv. × Col.= 0.86	

4.7 Germination Strength Index

The seeds of the cultivar Ibaa99 gave the highest average for the germination strength index, reaching 1234.80, while the cultivar Jad gave the lowest average for this trait (1046.36) Table 7. The results also showed that the treatment of spraying twice with the mutagen was superior in this trait, reaching 1225.16, compared to the control treatment, which gave the lowest average for the trait (997.05). The reason for the superiority of the cultivar Ibaa over spraying with the mutagen twice may be attributed to their superiority in the germination percentage in the final count, plumule length and plumule length Table 2, Table 3 and Table 4, and these traits are directly related to seed strength. The interaction of spraying the cultivar Ibaa99 twice with the mutagen recorded the highest average for the germination strength index (1377.98), while the two cultivars Jad and Thuraya with the control treatment recorded the lowest value for the interaction, reaching 973.79 and 975.39, respectively.

Table 7. Effect of Genetic Compositions and Spraying with Colchicine on the Dry Weight of the Seedling (Mg)

Cultivars (Cv.)	**Colchicine Treatment (Col.)**			**Mean (Cv.)**
	Control	**One Spray**	**Tow Spray**	
Jad	973.79	1063.92	1103.36	1046.36
Thuraya	975.39	1076.33	1153.82	1067.39
Adna	1021.46	1214.98	1271.27	1167.61
Iba 99	1018.16	1316.62	1377.98	1234.80

Mean (Col.)	997.05	1166.41	1225.16	
LSD %5	cv. = 17.11	Col.= 15.09	cv. × Col.= 20.32	

5. CONCLUSION

The germination and seed vitality indicators are evidence of the genetic variation of the different genetic compositions of bread wheat, in which the variety Aba99 emerged, which is one of the most important reasons that led to the superiority of this variety in grain yield. Also, spraying the mother plants of the different genetic compositions of wheat with colchicine twice was reflected in the improvement of the vitality and activity of the resulting seeds, whether due to the effect of colchicine alone or by its interaction with wheat varieties.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Hassan Ali Majeed	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The author declares no conflict of interest.

Informed Consent

Not Applicable.

Ethical Approval

Not Applicable.

Data Availability

All data supporting the findings of this study are available within the article and/or its supplementary materials.

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