Early Growth Response of Wheat Genotypes to Varying Concentrations of Hydrogen Peroxide (H2O2)

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Abstract

Wheat is an important cereal crop and is grown globally as a staple food and an energy source. The germination and early growth of wheat seedlings are adversely affected by various biotic and abiotic factors, more importantly, insect pests, soil texture, temperature, moisture and soil nutrient availability. The exogenous application of hydrogen peroxide (H2O2) is thought to promote both germination and early growth response of wheat. However, its application is limited by optimizing the proper concentration and time duration of H2O2 for different wheat genotypes. This study was designed to determine the optimum H2O2 concentration and time required for H2O2 application to promote germination and early growth response of three wheat genotypes. The seeds of wheat genotypes namely AZRC Dera variety, landrace 49 and black wheat were subjected to varying concentrations (0.5%, 1% and 3%) of H2O2 for different time intervals (30 minutes, 2 hours and 4 hours) before the seeds soaking and growing in Petri-dishes using Completely Randomized Design (CRD) with three biological replicates. Data collected for germination percentage, root length, fresh root weight and fresh biomass weight was subjected to an R-package for conducting ANOVA and calculating standard means (p = 0.05). Results showed that all four early growth parameters among three genotypes were significantly and adversely affected by higher H2O2 concentrations (1% and 3%) for longer periods (2 hours and 4 hours). On the contrary, the application of low H2O2 concentration (0.5%) for a short period (30 minutes) significantly enhanced germination percentage, root length, and fresh root and fresh biomass weight among all genotypes with landrace 49 being the most responsive (P = 0.05). At a concentration of 0.5% H2O2 and treatment for 30 minutes, all three genotypes exhibited the best germination enhancement and achieved a 100% germination percentage. In conclusion, pre-treatment of wheat seeds with 0.5% H2O2 for 30 minutes is recommended before sowing the seeds in the field.

Keywords: Landrace, Black wheat, AZRC Dera, H2O2, Germination percentage.

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Introduction

Wheat is one of the 'big three' cereal crops and ranks third after ric and maize. It is the dominant crop in temperate countries being used for human food and livestock feed. Its success depends partly on its adaptability and high yield potential but also on the gluten protein fraction which confers the viscoelastic properties that allow the dough to be processed into bread, pasta, noodles, and other food products. Wheat is also a repertoire of essential components, commonly known as primary and secondary metabolites, such as beneficial phytochemicals, dietary fibres, vitamins, essential amino acids, proteins, starch and glutens. All these components are particularly enriched in whole-grain products (Tadesse *et al*., 2017; Igrejas and Branlard, 2020). The average annual rate of wheat consumption in the developed countries of the world has levelled off at about 175 kg per capita. In developing countries, however, wheat is now an important source of calories and, to a lesser extent, protein in the diet of millions of people (Shewry and Hey, 2015).

The first cultivation of wheat occurred about 10,000 years ago, as part of the 'Neolithic Revolution', which saw a transition from hunting and gathering of food to settled agriculture. These earliest cultivated forms were diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheat and their genetic relationships indicate that they originated from the south-eastern part of Turkey (Heun *et al*., 1997; Nesbitt, 1998; Dubcovsky and Dvorak, 2007). Cultivation spread to the Near East about 9000 years ago when hexaploid bread wheat made its first appearance. The earliest cultivated forms of wheat were essentially landraces selected by farmers from wild populations, presumably because of their superior yield and other characteristics, an early and non-scientific form of plant breeding. Later on, two domestication events produced high-yielding and free-thrashing bread wheat. The first is the loss of shattering of the spike at maturity, which results in seed loss at harvesting. This is an important trait for ensuring seed dispersal in natural populations and the non-shattering trait is determined by mutations at the *Br* (*brittle rachis*) locus (Nalam *et al*., 2006). The second important trait is the change from hulled forms, in which the glumes adhere tightly to the grain, to free-threshing naked forms. The free forms arose from a dominant mutant at the *Q* locus which modified the effects of recessive mutations at the *Tg* (*tenacious glume*) locus (Jantasuriyarat *et al*., 2004; Dubkovsky and Dvorak, 2007). Cultivated forms of diploid, tetraploid, and hexaploid wheat all have tough rachis apart from the spelt form of bread wheat. Similarly, the early domesticated forms of einkorn, emmer, and spelt are all hulled, whereas modern forms of tetraploid and hexaploid wheat are free-threshing.

Germination and early growth response of wheat is a problem in Pakistan, especially in the calcareous soil of Dera Ismail Khan. Hydrogen peroxide (H_2O_2) has been found to break the dormancy of seeds by pre-treatment of seeds with specific concentrations of H_2O_2 for different time intervals (Hameed *et al*., 2004; He and Gao, 2009; Li *et al*., 2011; Lu *et al*., 2013; Szopińska, 2014). However, the optimum concentration of H_2O_2 and specific time duration of treatment yet need to be investigated. Black wheat, having winter growth habit, used in this study has a naturally low germination rate and low early growth response in the semi-arid spring wheat growth region of Dera Ismail Khan. The current study was designed to evaluate the early growth response of wheat genotypes including

black wheat to varying concentrations of H_2O_2 and at different time intervals. The key objective of the study was to optimize the best concentration and duration of treatment of H_2O_2 for potential early growth enhancement of wheat genotypes.

Material and method

Current research was conducted at the graduate research lab of the Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University Dera Ismail Khan in the wheat growing season of 2023-2024.

Material

In this study, Chinese black wheat, landrace 49 and wheat variety AZRC Dera were acquired from the Department of Plant Breeding & Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan.

Experimental design

The current study was conducted to investigate the response of three wheat genotypes to different H_2O_2 treatments. The experiment was conducted using Completely Randomized Design (CRD) with three biological replicates for each genotype and each treatment. The seeds were grown in Petri plates for examining days to germination and germination percentage. All the sowing of wheat seeds was done after three days' interval. Experiments were performed in Petri dishes while sand and mud were used for sowing in pots. The very 1st data for seed germination was noted on 20-11-2024. Transplantation of seeds was done on 28-11-2024 to sand and mud-filled pots. About 30ml of water was given to each pot for irrigation.

Chemicals

Chemicals used in this study include distilled water and H_2O_2 at varying concentrations (0, 0.5%, 1% and 3%) at different time points (30 minutes, 2 hours and 4 hours).

Parameters studied

1. Germination Percentage

Sprouted seeds in each Petri dish were counted and accepted as germinated. Data regarding germination was recorded on 3rd day after soaking and completed on the 10th day after soaking. A total of 15 seeds/genotypes were soaked and grown in each Petri plate to determine germination percentage. A total of three biological replicates were applied for each treatment and each genotype. The germination percentage was computed as follows;

Germination percentage = (No of germinated seeds /total no. of seeds) x 100

2. Root length (cm)

Randomly single seedling was taken out of the root seedling on the 21st and 24th day after sowing from each pot and the lengthy root was measured in centimetres [cm].

3. Fresh shoot weight (g)

Shoot from the plant were excised and their fresh weight was measured in grams using digital weight balance.

4. Fresh root weight (g)

Roots weight was measured by taking out the entire plant from the pot and was calculated on the digital weight machine in grams.

5. Fresh biomass weight (g)

The fresh weight of the plant was calculated by adding the fresh shoot and root weight taken in grams.

Statistical Analysis

After the collection of data, mean values and variance analysis were performed using R-package (https://www.r-project.org/).

Results

Germination percentage

Germination percentage of all three genotypes decreased by increasing the concentration of H_2O_2 and time duration of treatment, especially at 3% H_2O_2 concentration for 4 hours. Among three genotypes, germination of landrace 49 was most severely affected while black wheat

was less affected by varying concentration and time intervals of H_2O_2 treatments. At a concentration of 0.5% of H_2O_2 and treatment for 30 minutes, all three genotypes exhibited best germination enhancement and achieved 100% germination percentage (Fig. 1).

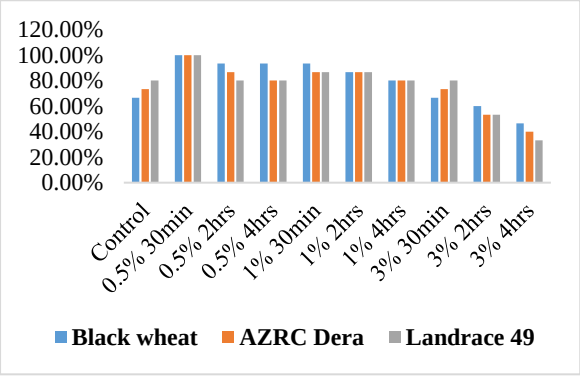

Fig. 1 Germination percentage of wheat genotypes exposed to varying concentrations of H_2O_2 at different time intervals.

Mean performance for Root length

These mean root length values indicate that H_2O_2 treatment and its concentration have varying effects on the growth of wheat roots, with Landrace-49 showing the most substantial increase in root length under certain H_2O_2 concentrations and durations, especially at 0.5% concentration for 30 minutes (Fig. 2). Black Wheat and AZRC Dera also responded differently to H_2O_2 treatment (Table 1).

Fig. 2 Variation in root length and density of wheat genotypes in response to H_2O_2 treatment (0.5% for 30 minutes). **WLR**: wheat landrace 49, **BW**: Black wheat, **AZD**: AZRC Dera.

Table 1 Response of root length of wheat genotypes to varying concentrations and time intervals of H_2O_2 treatments.

	Control	0.5% (30 min)	0.5% (2 hrs)	0.5% (4 hrs)	1% (30 min)	1% (2 hrs)	1% (4 hrs)	3% (30 min)	3% (2 hrs)	3% (4 hrs)
Black wheat	4.2000 B	3.3000 D	3.2500 D	4.2667 B	4.3000 B	4.3367 B	3.2667 D	3.7500 C	4.0667 D	4.8333 A
AZRC Dera	4.1333 BC	5.9167 C	3.5000 D	4.3000 AB	4.5000 A	3.4000 D	4.3000 AB	4.1000 BC	4.0167 BC	3.4000 D
Landrace 49	4.0167 F	9.5000 E	4.2000 EF	4.3000 EF	5.1667 C	4.2000 D	4.5333 B	4.3333 EF	4.2000 A	4.3000 EF

Mean performance for Root weight

The results revealed that the H_2O_2 treatments had diverse effects on root weight in different wheat varieties. For instance, in BW, the root weight increased significantly (category "A") when exposed to 1% H_2O_2 for 30 minutes or 2 hours, but not at other concentrations or durations. In contrast, AZRC showed significant root weight enhancement (category "A") with 1% H_2O_2 for 30 minutes or 4 hours. Landrace 49 exhibited the most prominent response to H_2O_2 treatment, with significant increases in root weight (category "A") observed for multiple combinations, such as 1% H_2O_2 for 30 minutes, 1% H_2O_2 for 2 hours, and 3% H_2O_2ethanol for 4 hours (Table 2).

Table 2 Response of root weight of wheat genotypes to varying concentrations and time intervals of H_2O_2 treatments.

	Control	0.5% (30 min)	0.5% (2 hrs)	0.5% (4 hrs)	1% (30 min)	1% (2 hrs)	1% (4 hrs)	3% (30 min)	3% (2 hrs)	3% (4 hrs)
Black wheat		3.3000	3.2500 D	4.2667 B	4.3000 B	4.3367 B	3.2667 D	3.7500 C	4.0667 D	4.8333 A
AZRC Dera	4.1333 Bc	3.9167 C	3.5000 D	4.3000 AB	4.5000 A	3.4000 D	4.3000 AB	4.1000 BC	4.0167 BC	3.4000 D
Landrace 49	4.0167 F	4.5000 E	4.2000 EF	4.3000EF	7.1667 C	5.2000 D	8.5333 B	4.3333 EF	9.2000 A	4.3000 EF

Mean performance for Fresh biomass

Results showed that the H_2O_2 treatments had diverse effects on fresh biomass in the different wheat genotypes. For instance, significant increases in fresh biomass (category "A") were recorded with 0.5% ethanol for 30 minutes for black wheat, 0.5% H_2O_2 for 2 hours, and 1% H_2O_2 for 4 hours. On the other hand, AZRC Dera showed significant fresh biomass enhancement (category "A") with 1% H_2O_2 for 30 minutes and 1% H2O2 for 4 hours. Landrace 49 exhibited substantial responses to H_2O_2 treatment, with significant increases in fresh biomass (category "A") observed for various combinations, such as 0.5% $H_2O_2$for 30 minutes, 0.5% H_2O_2 for 2 hours, 1% H_2O_2 for 2 hours, 1% H_2O_2 for 4 hours, and 3% H_2O_2 for 4 hours (Table 3).

Table 3 Response of fresh biomass of wheat genotypes to varying concentrations and time intervals of H_2O_2 treatments.

	Control	**0.5% (30 min)**	**0.5% (2 hrs)**	**0.5% (4 hrs)**	**1% (30 min)**	**1% (2 hrs)**	**1% (4 hrs)**	**3% (30 min)**	**3% (2 hrs)**	**3% (4 hrs)**
Black wheat	0.2722 A	0.5877 A	0.5447 D	0.4333 H	0.4333 H	0.5483 C	0.5357 E	0.5630 B	0.5630 B	0.4763 F
AZRC Dera	0.5757 D	0.4750 H	0.5193 E	0.4460 J	0.7320 B	0.4653 I	0.4770 G	0.7760 A	0.6207 C	0.4860 F
Landrace 49	0.8770 C	0.7593 D	0.7130 F	0.4630 J	1.0570 B	0.7353 E	0.5243 I	0.6860 G	1.2040 A	0.5950 H

Cushionion

The germination and early growth response of wheat is affected by various types of abiotic stresses such as soil texture, soil microbiota profile, availability of moisture and nutrients crucial for germination and growth of seedlings, and insect pests that damage the germination seeds and young seedlings. Besides, the genetic background of wheat genotype and the age of wheat kernels also affect both germination and early growth response (Lamichhane *et al.*, 2018). Several strategies have been devised by breeders to enhance wheat germination and early growth response. For instance, H_2O_2 has been previously found to enhance wheat's early growth. Our findings show that H_2O_2 treatment had varying effects on the growth parameters of the three wheat genotypes used in this study. The impact was most noticeable in the Landrace-49, where the application of H_2O_2 at higher concentrations and longer durations resulted in significantly higher values for germination percentage, root length, root weight and fresh biomass weight compared to the control group. On the other hand, Black Wheat showed a mixed response to H_2O_2 treatment, with some concentrations and durations resulting in enhanced growth, while others showed no significant change or even reduced growth (Table 1-3).

Previous studies have shown that due to genetic background effects, different wheat genotypes respond differently to H_2O_2 treatments (He *et al.*, 2011; Chakraborty *et al.*, 2012; Kamruzzaman *et al.*, 2022). Our results also indicated that different wheat genotypes exhibited varying responses to H_2O_2 treatment. While Landrace 49 exhibited a more pronounced positive response, AZRC Dera and black wheat showed less consistent results, with some parameters responding positively and others not showing significant improvement. This suggests that the effect of H_2O_2 treatment may be genotype-dependent. Based on the mean performance data, certain H_2O_2 concentrations and durations seem to be more beneficial for promoting germination and early growth in wheat genotypes. For example, a 0.5% ethanol treatment for 30 minutes appeared to be effective in enhancing growth in all three wheat varieties, as seen in the increased values for germination percentage, root length, root fresh weight and fresh biomass weight. Our results are by

He *et al*., (2011), Chakraborty *et al*., (2012) and Kamruzzaman *et al*., (2022).

Conclusion and recommendations

The H_2O_2 concentration of 0.5% for 30 minutes has a significant effect on all parameters studied in this experiment. Pre-treatment of wheat seeds with 0.5% concentration of H_2O_2 for 30 minutes is recommended before sowing the seeds in the field.

Conflicts of interest

The authors declare no conflicts of interest.

Authors contribution

Nasr Ullah Khan conceived the idea, designed the study and drafted the manuscript. Iqra Arooj, Iqra Gohar and Rifat Mustansir conducted the experiments and collected the data. Rima Bibi and Muhammad Raza helped in data collection and data analysis.

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