Effect of barassinolide on growth characteristics of wheat (Triticum aestivum L.) under water stress

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Abstract. This experiment was conducted in the laboratories of the Seed Testing and Certification Division- Babylon province in 2018 in a completely randomized design (CRD) with four replicates and two factors. The first factor included the addition of polyethylene glycol 6000 (PEG 6000) with three levels of water stress of 0 bar, -6 bar, and -9 bar which are herein denoted by S1, S2, and S3, respectively. The second factor included three stimulation treatments of wheat seeds by soaking the seeds in the plant hormone, Brassinolide for 24 hours with concentrations of 0 mg liter⁻¹, 2 mg liter⁻¹, and 4 mg liter⁻¹ which are denoted by B0, B1, and B2, respectively. The results showed that water stress adversely affected wheat seedling characteristics. With the water stress level S2, the lowest average period of germination initiation was 3.33 days, germination period was 3.38 days, drought resistance index at germination reached 1.01, total chlorophyll content was 27.91 mg/100 g fresh weight, and gibberellin content was 29.8 micromolar. The treatments with Brassinolide achieved a significant improvement in plant characteristics where B2 treatment realized the lowest average period of germination initiation of 2.58 days and drought resistance index during germination of 1.11. B2 treatment also achieved 22.13% increase in total chlorophyll content, 66.53% increase in gibberellin content, and 43.81% increase in Indole-3-acetic acid (IAA) compared to the control treatment B0 without Brassinolide.

1. Introduction

Seed germination and seedlings are critical to plant growth, especially in drought-prone areas. In facts, water stress inhibits wheat germination characteristics including a decrease in germination rate[10]. Landscape heterogeneity caused by unfavorable environmental factors results in wheat germination failure which adversely affects all stages of the plant growth [12]. Besides, seed germination also requires an effective enzymatic system to carry out the metabolic processes during germination [5]. The effect of water stress on this critical stage of germination and wheat seedlings, including radicle length, can be attributed to decreased water absorption by seeds, and inhibition of cell division and elongation [7]. In a study on barley seedlings, [17] found that at water stress of -3 bar the average plumule length decreased by 25.5% while the radicle length decreased by 10.5%. In other words, the average plumule length was more affected by water stress than the radicle length. The use of plant growth hormones to increase plant tolerance and resistance to stresses through seed treatment is an
important technology characterized by ease of application and low cost, and low risk, as well as its
great importance in plant life through its mechanisms in regulating growth such as accelerating and
delaying germination, flowering, and maturation. Plant hormones also play a role in the response of
plants to environmental stresses [19]. Technically, the aim of seed stimulation is to increase the
germination ratio, reduce the time required from planting to seedling emergence, and achieve
homogeneity under a wide range of unfavorable conditions [26],[27] stated that seed stimulation by
soaking contributes to germination improvement and increase of germination rate compared to
unstimulated seeds. Brassinolide growth regulator is a natural plant steroid hormone which contains a
wide range of compounds that have a role in the biological processes within the plant by regulating
many aspects of plant growth and development, the most important of which is seeds germination
[22]. It has been found that the addition of Brassinosteroids can stimulate seed germination by
significantly increasing the activity of Amylase enzyme [16].

2. Materials and Methods
The experiment was applied in the laboratories of the Seed Testing and Certification Division in
Babylon province in 2018 as a factorial experiment in a completely randomized design (CRD) with
four replications and two factors. The first factor included exposing wheat seeds from Ebaa-99 variety to
polyethylene glycol 6000 (PEG 6000) solution under three levels of water stress of 0 bar, -6 bar, and -
9 bar which are herein denoted by S0, S1, and S2, respectively while the second factor involved seed
stimulation treatment by soaking the wheat seeds for 24 hours in Brassinolide plant growth regulator
with three different concentrations of 0 mg liter-1 , 2 mg liter-1 , and 4 mg liter-1 which are denoted
by B0, B1, and B2, respectively. To simulate water stress conditions in the laboratory, polyethylene
glycol was used and the solutions were prepared according to [21]. Solutions of Brassinolide growth
regulator (American made from Sigma Co.) prepared according to the required concentrations.
Healthy undamaged wheat seeds were selected and washed with water to remove dust. Then, the seeds
were sterilized with hypochlorite 0.01% solution for 3 minutes. The seeds were then washed several
times with distilled water. After that, fifty seeds were placed on sterile filter paper in Petri dishes and
PEG 6000 solutions were added so all seeds were completely immersed. The experiment was
implemented under controlled germinating conditions according to the International Seed Testing
Association [18] to study the following attributes:

- First day of germination (day after planting (DAP)) [20].
- Last day of germination (day after planting (DAP)) [20].
- Germination period (day) [20].
- Drought resistance at seed germination [9].
- Drought tolerance index in terms of seedling dry weight: the ratio of the seedling dry weight under
  stress to the seedling dry weight without stress (control) [4].
- Total chlorophyll content in leaves [14].
- Plant hormones contents of Indole-3-acetic acid and gibberellin [28].

3. Results and Discussions

3.1. First and Last Days of Germination
The results in Figure 1 and Figure 2 show significant differences between water stress coefficients and
Brassinolide concentrations for the first and last days of germination under different treatments. It can
be noticed that S0 treatment (control treatment) gave the fastest germination initiation as the first and
last days of germination were 2.25 and 6.83 DAP, respectively. Longest germination initiation was
recorded under S2 treatment with the first day of germination of 3.33 DAP which was slightly
different than S1 treatment that gave first day of germination of 3.08 days. Regarding the last day of
germination, S1 treatment resulted in the highest average which was 7.17 DAP with a significant
difference from S2 treatment. Treatments with Brassinolide achieved a substantial response. For
instance, B2 treatment resulted in the shortest germination initiation with first day and last day of germination were 2.58 and 6.33 DAP, respectively while the germination initiation under B0 treatment required a longer time with first and last day of germination of 3.08 DAP and 8 DAP, respectively.

![First day of germination (DAP)](image1)

L.S.D0.05 = S= 0.61, B =0.61  S*B=n.s

**Figure 1.** Effect of Brassinolide and water stress and their combinations on first day of germination

![Last day of germination (DAP)](image2)

L.S.D0.05 = S= 0.66, B =0.66  S*B=n.s

**Figure 2.** Effect of Brassinolide and water stress and their combinations on last day of germination

3.2. Germination Period

Figure 3 shows differences in the germination period under the different water stress treatments. It is obvious that S0 treatment gave the longest germination period which reached 4.75 days with an increase of 26.31% compared to S2 treatment which reduced germination period to 3.83 days. Concerning the effect of treatment with Brassinolide on the germination period, B2 treatment has significantly improved this attribute by reducing germination period to 3.83 days compared to 4.67 days under the control treatment B0.

![Germination period (day)](image3)

L.S.D0.05 = S= 0.76  B =0.76  S*B=n.s

**Figure 3.** Effect of Brassinolide and water stress and their interaction on germination period
3.3. Drought Resistance Index at Germination and Drought Tolerance Index in Terms of Seedling Dry Weight

The results of these attributes are presented in Figure 4 and Figure 5 which show considerable differences between the studied factors. Water stress treatments significantly affected the drought resistance index for germination and the drought tolerance index where S2 treatment resulted in the lowest readings of these two attributes which were 1.01 and 95.52%, respectively. Highest attributes were observed under the control treatment S0 which were 1.07 and 112.40%, respectively. As with the previous attributes, treatment with Brassinolide has also affected these two attributes. B2 treatment achieved the highest drought resistance and tolerance indexes that were 1.11 and 118.98%, respectively compared to 0.99 and 87.3, respectively under the control treatment B0. Overlap between the two factors has been noticed as the change in the levels of one factor affected the other. That was evident in the comparison of the treatment combination S0B2 which gave the highest attributes of 1.21 and 131.81% respectively with the treatment combination S2B0 that resulted in the lowest attributes of 0.97 and 80.61%, respectively.

![Figure 4](image1.png)

L.S.D$_{0.05}$ = S = 0.03 B = 0.03   S*B = 0.06

**Figure 4.** Effect of Brassinolide and water stress and their interaction on drought resistance index at germination

![Figure 5](image2.png)

L.S.D$_{0.05}$ = S = 2.86 B = 2.86   S*B = 4.95

**Figure 5.** Effect of Brassinolide and water stress and their interaction on drought tolerance index

3.4. Physiological Attributes of Seedlings

Figures 6, 7 and 8 indicate noticeable differences in the water stress treatments. S2 treatment resulted in a reduction in chlorophyll, gibberellin, and Indole-3-acetic acid contents which reached the lowest as 27.91 mg/100 g fresh weight, 29.8 micromolar, 42.23 micromolar, respectively compared to the control treatment S0 which gave the highest attributes as 30.95 mg/100 g fresh weight, 40.36 micromolar, 58.26 micromolar, respectively. In the case of treatment with Brassinolide, B2 treatment
gave the highest attributes as 31.95 mg/100 g fresh weight, 45.93 micromolar, 64.76 micromolar, respectively which are corresponding to an increase of 22.13%, 66.53%, and 43.81%, respectively compared to B0 treatment.

Figure 6. Effect of Brassinolide and water stress and their interaction on chlorophyll content.

Figure 7. Effect of Brassinolide and water stress and their interaction on gibberellin content.

Figure 8. Effect of Brassinolide and water stress and their interaction on IAA content.

4. Discussion
Effects of water stress on wheat germination and seedling characteristics were studied in the lab by adding polyethylene glycol to simulate field conditions. The water stress treatment S2 led to the
acceleration of the first and last days of germination (Figures 1 and 2). This response is attributed to the fact that water stress creates osmotic potential and that seeds are negatively affected by water absorption [1] or it could be due to inhibition of the enzymes responsible for germination such as α-amylase and β-amylase and their role in germination improvement [3]. The prolonged germination time, which is the time between the first and last germination incidents, under water stress (Figure 3) may be due to the fact that water stress has affected the metabolic processes within the seed. Water stress hinders water absorption by seeds and as a result, metabolic processes within the seeds will occur slowly. Thus, more time will be needed for the radicle to emerge from the seed resulting in longer germination time and lower germination rate [8].

Drought resistance index is considered one of the important indicators under water stress because it gives high accuracy in drought tolerance evaluation tests. The effect of water stress on drought tolerance during germination can be attributed to the delay in germination (Figure 3). It is believed that decreased drought tolerance index in terms of seedling dry weight is a result of reducing cell division rate and radicle and plumule elongation [11]. These results and observations correspond to [3].

The low leaf content of chlorophyll under water stress (Figure 6) is due to the lack of water which is necessary to produce energy used in photosynthesis [23]. It is known that gibberellin helps in increasing the germination rate. Therefore, the decrease and reduction of growth regulator content (Figures 7 and 8) under water stress may be attributed to the lack of molecules of enzyme cofactor, Acetyl-CoA needed by Mevalonic acid to produce gibberellin [22]. Free radicals from the active oxygen group, such as hydrogen peroxide, are thought to inhibit hormone synthesis under water stress [2].

As it was mentioned in the previous section, treatment with brassinolide resulted in significant improvement in germination characteristics (Figures 1, 2 and 3). This improvement is attributed to biological effects that contribute to the regulation of seed germination [29]. Besides, treatment with brassinolide stimulates seed germination by activation of regulatory substances including plant hormones (Figures 7 and 8). Furthermore, brassinolide increases the effectiveness of the DNA and RNA as well as enhancement of protein substances. Brassinolide also improved drought resistance index during germination and drought tolerance index in terms of seedling dry weight. This response can be due to the role of brassinolide as a catalyst for proteins and enzymes. It increases the level and activity of the amylase which is positively reflected on the germination process under stress as well as the role of brassinolide in increasing the radicle and plumule elongation and cells expansion [6].

The increased concentration of chlorophyll in brassinolide-treated seedlings is related to brassinolide role in activating the production of gibberellin (Figure 7). Brassinolide is also responsible for activating the RuBisCO enzyme in the chloroplasts and the thiamine concentration in the synthesis of Acetyl-CoA enzymatic compounds. On the other hand, it has an important role in stimulating the production of enzymatic antioxidants [25]. Regarding the increase in the growth hormones the gibberellin and IAA (Figures 7 and 8) under treatment with brassinolide, it can be attributed to the gene expression in the metabolic acid of Mevalonic Acid which is considered required for hormones synthesis [15].

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