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Barley Seed Germination and Seedling Growth Responses to Polyethylene Glycol (PEG)-Induced Drought Stress

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Abstract: Drought is becoming more prevalent and negatively affects the growth and development of barley. To explore the genetic variation in barley under drought stress, ten breeding genotypes were tested using polyethylene glycol-6000 to simulate drought conditions. We observed that drought stress significantly affected germination-related traits, depending on the specific genotypes. Some parameters, such as root length, reduced by up to 85% under drought conditions compared to the control. Overall, considering the barley growth performance, the drought tolerance index was an ideal criterion for selecting drought-tolerant genotypes, as it well characterized the gradient responses of barley genotypes to drought stress. Based on this indicator, genotype OB1878-ON-50 is recommended as a significant germplasm resource for low-precipitation regions.

Keywords: barley; drought stress; seed germination; roots; polyethylene glycol

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1. Introduction

Canada is the fourth-largest producer of barley (*Hordeum vulgaris* L.) worldwide [1]. It ranks third in harvested area, following wheat and canola, producing over 8.9 million tons of barley grain in 2023 [2]. This extensive cultivation is driven by barley's important roles in animal feed, alcoholic beverage production, and human table consumption [3]. In addition, barley is often cultivated in crop rotation and intercropping systems attributed to its strong adaptability to diverse climates and soil conditions [4]. This makes it a vital component in efforts to develop sustainable agroecosystems in the face of unpredictable climate changes.

Climate change may cause severe weather events, such as extreme precipitation, heat waves, and drought. Drought is one of the major environmental factors that affects the growth and development of crop plants, and drought stress is expected to become more frequent and intensive in the coming future. Therefore, it is critical to fill our knowledge gap on how drought stress impacts different stages of barley growth and development and identify genotypes with better drought tolerance for future cultivar development. Drought stress can be induced artificially using chemicals such as polyethylene glycol 6000 (PEG) due to its ability to restrict the water uptake and decrease the overall osmotic potential [5]. Thus, PEG can be used in vitro screening against drought conditions because it reduces water potential and simulates drought stress under in vitro conditions [6–9]. Specifically, PEG hinders water transport through the xylem by effectively increasing the size of water molecules when added to water [10]. The current study aimed to investigate the responses of ten barley genotypes to PEG-induced drought stress and identify drought-tolerant barley genotypes for potential use in breeding programs to develop drought-resistant barley varieties. We hypothesized that PEG-induced drought stress would reduce seed germination and alter root number and length of both root and shoot in barley genotypes.

2. Materials and Methods

2.1. Plant Materials

Ten barley genotypes, including CH1808-ON-28, CH1808-ON-69, CH1818-PE-34, CH1819-PE-05, OB1864-ON-16, OB1870-ON-27, OB1877-ON-41, OB1878-ON-50, OB1879-ON-13, and OB1882-ON-75, were used in this study. These genotypes were advanced barley breeding lines with good yield performance from the Ottawa barley breeding program, Ottawa, ON. The seeds were produced in the field in 2019 at the Charlottetown Research and Development Centre, Charlottetown, PEI.

2.2. Experimental Design

Before the experiment, 10 mL of distilled water was added to each Petri dish, followed by the addition of either 5 mL of distilled water (control) or 5 mL of 25% PEG solution (drought) after 24 h [11]. Uniformly sized seeds were selected, surface-sterilized in 1% (v/v) sodium hypochlorite solution for 5 min, rinsed three times with distilled water, and air-dried. Fifteen seeds of each genotype were placed ventral side down on double layers of filter papers in Petri dishes. Temperatures were maintained at 23 °C during the daytime (16 h) and 18 °C at nighttime (8 h), and relative humidity was maintained at approximately 50% [7]. The experiment was carried out in a randomized complete block design with three replications.

2.3. Trait Measurements

In line with our objectives, we concentrated on key parameters that effectively capture barley's responses to drought conditions. The number of germinated seeds, defined as those with both the plumule and radicle emerged to at least 2 mm, was recorded daily for seven days. On the 7th day, seed germination percent (GP), root number (RN), root length (RL), and shoot length (SL) were measured for each Petri dish. In addition, germination drought tolerance index (GDTI) of each genotype was calculated as follows [12]:

$$\text{GDTI} = \text{GR under PEG stress}/\text{GR under control}$$

2.4. Statistical Analysis

The analysis of variance (ANOVA) was performed with SAS (SAS Institute, Cary Inc., Cary, NC, USA) using the mixed procedure. Drought treatment and genotype were treated as fixed effects while the replication was treated as the random effect. The differences between treatments and genotypes were investigated using Duncan's multiple comparison at the level of $p < 0.05$. Shapiro–Wilk's statistic was used to test the normality assumption on the residuals of each model, while residual plots were used to verify the homogeneity of variances.

3. Results

3.1. Seed Germination

Among all genotypes, CH1819-PE-05 had the highest germination percent of 85% under sufficient water conditions. This germination percent gradually declined to 24%, with an average of 60% under the same growing environments. However, the germination percent rate reduced to 27%, on average, under drought stress, ranging from 16% to 49%. All genotypes, except for OB1882-ON-75, exhibited a negative response to drought stress in terms of germination percent (Figure 1). The genotype OB1878-ON-50 had the highest germination percent in PEG-induced drought stress conditions.

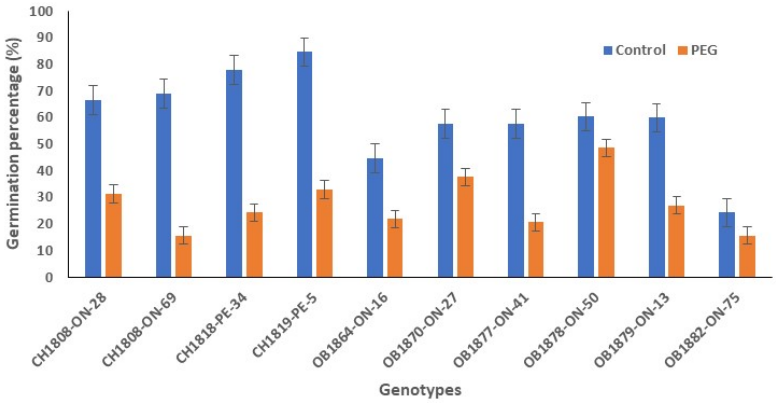

Figure 1. Mean and standard error of germination percent (%) in no stress and PEG-induced drought stress condition.

3.2. Root Length

Root length was significantly affected by drought treatment. The longest root, 111 mm, was observed for CH1808-ON-69 and gradually reduced to 62 mm for OB1882-ON-75 under control conditions. However, root length of drought-stressed seedlings decreased to 13–45 mm, with an average of 28 mm (Figure 2). Of the 10 genotypes, OB1878-ON-50 had the longest root of 45 mm under PEG-induced drought stress.

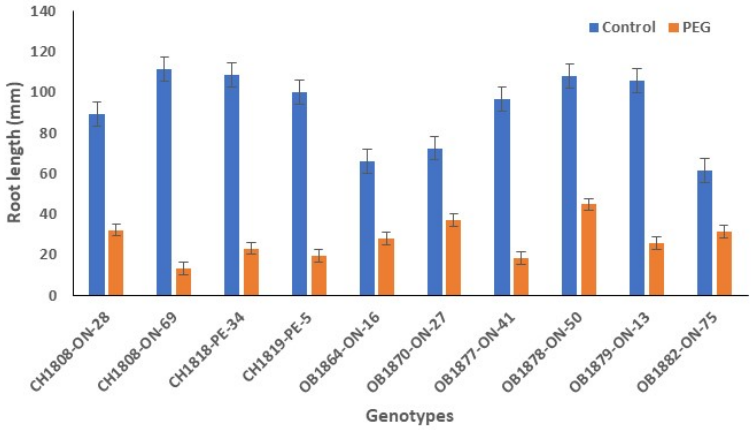

Figure 2. Mean and standard error of root length (mm) in no stress and PEG-induced drought stress condition.

3.3. Shoot Length

There was a significant interaction effect between genotype and drought treatment on shoot length. PEG-induced conditions significantly shortened the shoot length in all genotypes (Figure 3). The shoot length of genotypes under no-stress conditions varied between 65 mm and 143 mm, averaging 98 mm, which was over 12-fold greater than that under PEG-induced drought stress conditions. Genotype OB1878-ON-50 had the longest shoot of 25 mm under PEG-induced drought stress.

3.4. Number of Roots

The difference in root number between PEG-induced drought stress and no-stress conditions was more evenly distributed compared to other traits (Figure 4). The number of roots under no-stress conditions varied between six and seven, with an average of six roots per genotype. Under PEG-induced drought stress, root numbers ranged from two to six, with an average of four. Genotype CH1818-PE-34 had the highest number of roots, averaging seven roots under the no-stress condition. In contrast, OB1878-ON-50 had the highest number of roots under drought conditions with an average number of

six. Notably, OB1878-ON-50 had the most consistent root number across both stress and no-stress conditions.

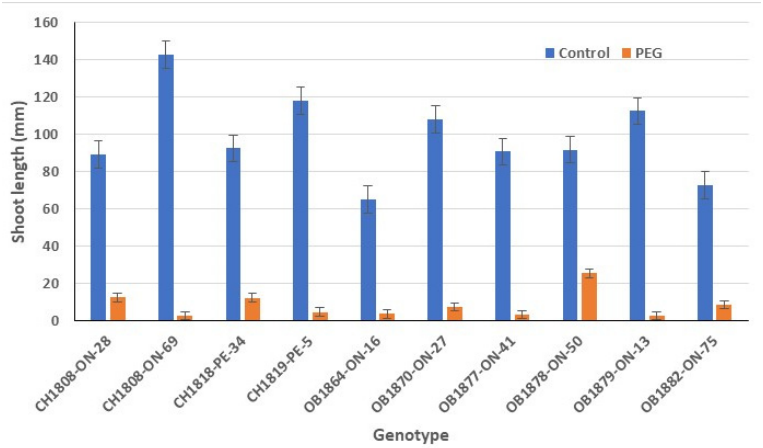

Figure 3. Mean and standard error of shoot length (mm) in no stress and PEG-induced drought stress condition.

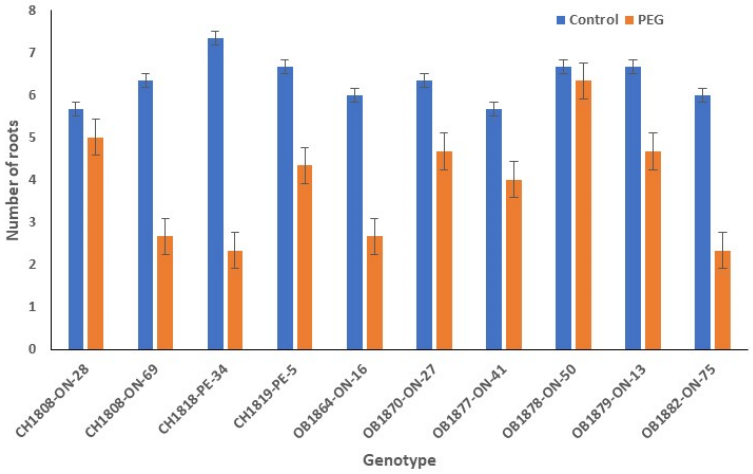

Figure 4. Mean and standard error of number of roots in no stress and PEG-induced drought stress condition.

3.5. Germination Drought Tolerance Index

To evaluate the drought response of the tested genotypes, GDTI was calculated by dividing its germination percent under stress conditions by that under the control condition. The index categorized the 10 genotypes into four groups of tolerant (GDTI > 0.80), moderately tolerant (0.60–0.79), moderately susceptible (0.40–0.59), and susceptible (GDTI < 0.40). Among the tested genotypes, OB1878-ON-50 showed the highest GDTI while CH1808-ON-69 had the lowest value (Table 1).

Table 1. Performance of germination percent (%), root length (mm), shoot length (mm), number of roots, and corresponding germination drought tolerance index (GDTI) for each barley genotype under both control and drought conditions.

Genotype	Germination Percent		Root Length		Shoot Length		Number of Roots		GDTI
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	
CH1808-ON-28	67	31	89	32	89	12	6	5	0.47
CH1808-ON-69	69	16	111	13	143	3	6	3	0.23
CH1818-PE-34	78	22	109	23	92	12	7	2	0.29

Table 1. *Cont.*

Genotype	Germination Percent		Root Length		Shoot Length		Number of Roots		GDTI
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	
CH1819-PE-05	85	33	100	20	118	5	7	4	0.39
OB1864-ON-16	45	22	66	28	65	4	6	3	0.49
OB1870-ON-27	58	38	73	37	108	7	6	5	0.65
OB1877-ON-41	58	21	97	18	91	3	6	4	0.36
OB1878-ON-50	60	49	108	45	92	25	7	6	0.81
OB1879-ON-13	60	27	106	26	112	3	7	5	0.45
OB1882-ON-75	24	16	62	32	73	8	6	2	0.65
Range	24–85	16–49	62–111	13–45	65–143	3–25	6–7	2–6	
Mean	60	27	92	28	98	8	6	4	

4. Discussion

Drought has become one of the most important abiotic stresses to plants. With global warming, climate change-induced drought intensity and frequency are expected to increase in the coming years. Studies have shown that it significantly inhibits crop growth and development, leading to yield losses up to 80% [13]. This is due to its negative effects on plant morphological, physiological, and biochemical responses [14]. This study aimed to examine the effects of PEG-induced drought stress and assess if different barley genotypes exhibited specific resistance towards drought stress.

Most of the recent studies have focused on the trait variation of different crops in response to drought stress [6,8,9,15,16]. For example, Hellal et al. [7] reported that the increase in PEG concentrations decreased the germination percentage of barley cultivars relative to the untreated barley cultivars. Similarly, Lateef et al. [8] reported PEG-induced drought stress significantly minimized germination of 59 barley genotypes. Our results aligned with previous studies and provided further evidence that germination percent reduced with PEG-induced drought stress. Tarnawa et al. [17] reported that water is crucial for seed germination, and without sufficient water, seeds cannot properly imbibe due to the osmotic imbalance [18] and oxidative stress [19], which is the first step in the germination process. In addition, under drought conditions, the metabolic activities within the seed are reduced. This includes lower enzyme activities that are essential for breaking down stored food reserves in the seed, which are necessary for seedling growth [20].

In addition to the seed germination, PEG-simulated drought also reduced root length, shoot length, and root number compared to those under favorable growing environments. Similar results were reported on oat crops [21]. Drought stress decreases the biosynthesis of key plant hormones, such as abscisic acid and gibberellins, which play crucial roles in promoting seed germination [22,23]. Water is essential for cell turgor, which drives cell expansion and elongation. That is, without adequate water, cells cannot expand properly, leading to shorter roots and shoots [24]. However, genotypes CH1818-PE-34 and OB1878-ON-50 maintained root lengths exceeding 15 mm. Sayed [25] highlighted the roles of exotic alleles in enhancing root length under drought conditions, suggesting that these two genotypes may carry favorable alleles that contribute to increased root length under stress. In this study, barley genotypes showed slow shoot growth under PEG-induced drought, whereas fast shoot growth occurred under control conditions. This trend is likely due to the PEG limiting the seeds' ability to absorb sufficient water for shoot growth and development. The number of roots did not change significantly between the control and PEG-induced drought stress conditions. This could be attributed to the fact that both treatments received 10 mL of water 24 h prior to the first PEG application, providing equal opportunities for germination to start.

This study identified OB1878-ON-50 as a promising drought-tolerant barley genotype as it excelled in three out of the four traits studied under drought conditions. This suggests that it could be used as a breeding material in future strategies to develop superior genotypes for regions with low precipitation. Future research can focus on in-depth genetic

analysis to pinpoint specific loci or genetic regions responsible for drought resistance using methods such as QTL mapping and genome-wide association studies. Additionally, developing molecular markers can support marker-assisted breeding for enhanced drought tolerance in barley.

5. Conclusions

Drought has become a major constraint to crop production, particularly in low precipitation, rainfed regions. Early selection of optimal barley genotypes is crucial for improving drought tolerance in these areas. In this study, we used PEG to simulate drought stress and investigated the responses of 10 barley genotypes. As expected, seed germination and growth traits in both roots and shoots were significantly inhibited by drought, with notable genotype-specific differences. Based on these responses, we developed a drought index to identify drought-tolerant genotypes and found that OB1878-ON-50 shows strong potential for low-precipitation regions. Further field trials are necessary for more detailed evaluation.

Author Contributions: R.K. conceptualized the experiment; M.D.-W. performed the experiment, collected data, and interpreted the results; B.S. and R.K. supervised the experiment; R.K. wrote the manuscript with the assistance of G.W. and R.K.; G.W. and B.S. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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