

# Response of early seedling inbred lines of wheat against 20% PEG<sub>6000</sub> induced condition

## Abstract

At the germination and seedling stages, the drought resistance of six genotypes of winter wheat was investigated under lab condition. PEG<sub>6000</sub> was employed in research under controlled circumstances to simulate drought stress at 20% concentration. Our finding showed that the inbred lines (Sakha-94 x Misr-1) and (Weiber x Sids-12) demonstrated highest drought tolerant. In order to achieve high yield under water stress, these lines have a great deal of potential to be incorporated with the current commercial wheat types.

**Keywords:** wheat lines, germination, PEG<sub>6000</sub>

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## Introduction

Seed germination, the first stage of growth, is susceptible to water scarcity because soil moisture at the time of planting may hinder seedling emergence, which in turn affects yield output.<sup>1,2</sup> Due to a water deficit, plant physiological and biochemical processes as well as growth and development may all be significantly impacted.<sup>3</sup> The plant integrates a variety of responses and adaptive mechanisms at the morphological, physiological, and molecular levels to respond to drought stress.<sup>4</sup> Yet, the use of these systems varies significantly between different plant species or genotypes within a species. When there is a water shortage early on plants, typically have the capacity to efficiently absorb water from the soil through their root systems, partly close their stomata to reduce water loss through transpiration, and change their metabolism to accommodate the available carbon resource.<sup>5,6</sup> Prolines, soluble sugars, spermines, and betaine are examples of osmolytic substances that build in plant cells as stress levels rise to maintain the cell turgor pressure.<sup>7</sup> Therefore, the physical and molecular reactions that occur when plants respond to drought involve a variety of biological macro and micro molecules, including nucleic acids (DNA, RNA, and microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, and mineral elements.<sup>8</sup>

Due to PEG<sub>6000</sub> high molecular weight it is used for seedling stage selection for drought tolerance genotypes.<sup>9</sup> PEG<sub>6000</sub> molecules are too large to be absorbed by plants, too small to affect osmotic potential, and even too massive to be anticipated to quickly penetrate intact plant tissues.<sup>10</sup> Because PEG does not penetrate the apoplast, water is withdrawn from the cell, making PEG<sub>6000</sub> solution more like dry soil than other solutions.<sup>11</sup>

The goal of this study was to determine whether there were differences in the responses of six winter wheat inbred lines to water stress in order to be grown in marginal and dry land by estimating several morphological drought resistance parameters under PEG<sub>6000</sub> as a water deficiency at early seedling stage.

## Materials and methods

Six wheat genotypes made up the experimental materials for this investigation (Table 1). The seeds were collected from Genetics Dept.,

Faculty of Agric., Sohag Univ., Sohag, Egypt. These genotypes were directly seeded in moisten petri dishes, and their drought tolerance and various root and shoot characteristics were assessed over the interval of three replications.

**Table 1** List of wheat inbred lines used for present investigation

Lines No.	Lines Source
1	Misr-1 X Sids-12
2	Weiber X Sids-12
3	Katela X Sakha-94
4	Weiber X Misr-1
5	Katela X Sids-12
6	Sakha-94 X Misr-1

Mean performance was recorded for seedling length, root numbers, seedling fresh weight, seedling dry weight, germination percentage, vigor index and tissue water content.

## Preparation of priming solution

Drought-stressed plants were watered daily with a 20% PEG<sub>6000</sub> solution (dissolving 20 gm of PEG<sub>6000</sub> in 100 ml of purified water), which was applied for 15 days.

## Seed placement for germination

The surface of each seed was sterilised with 5% sodium hypochloride solution for 20 mins, followed by a sterile water rinse and room temperature air drying. The priming media was made in 20% PEG<sub>6000</sub> solution and distilled water, and it was primed for nine hours. Seeds that had been soaked were then air dried and put in petridish. For each replicate, 15 seeds were put in a petri dish with a diameter of 90 cm, layered with filter paper moistened with 10 ml of 20% PEG<sub>6000</sub> and exposed to conditions that included a photoperiod of 114 hours, 250 μmole<sup>-2</sup>s<sup>-1</sup> day/night temperature of 25°C, and a relative humidity of 60/75%.

## Experimental design

Three replications were used in the experiment's Complete Randomized Design (CRD). The eximend wheat lines presented in Table 1 under primed seed with 20% PEG<sub>6000</sub> solution.

## Recording data

Considering the data collection for different parameters, the mean data of seedling length, root numbers, Seedling fresh weight, seedling dry weight, germination percentage, vigor index<sup>12</sup> and tissue water content (Muscolo et al., 2013) were recorded after 15 days of planting seeds. In each petri dishes ten seedlings were evaluated. Shoot and root length of single seedling were measured using a ruler and metre scale two weeks after seeding. After that, each seedling's roots were numbered. The seedling's shoot and root were then dried for 48 hours, and their dry weights were registered using an electric scale.

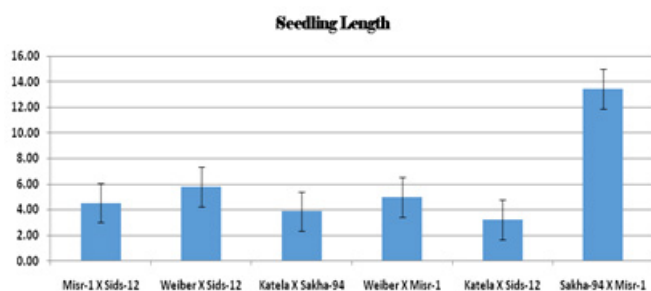
## Statistical analysis

The collected CRD design data was used to statistically analysis by Excel software.

## Results and discussion

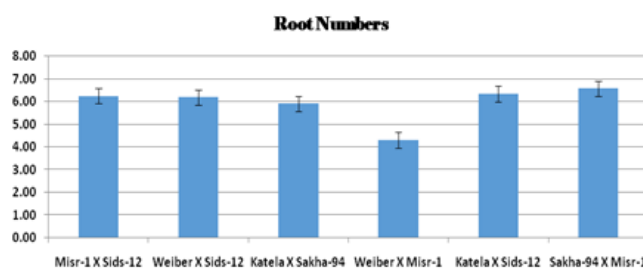
The process and trait of plant drought resistance is quite complicated. Drought resistance in plants is correlated with a number of activities at specific developmental stages, including stomatal movement, photosynthesis, cell osmotic regulation, synthesis of protective macromolecules, and antioxidant synthesis. Plant growth and development are typically quite vulnerable to water sacrificial mechanisms<sup>13</sup> it might cause significant increase in electrolyte leakage from leaves. Keeping a high water content gave researchers the chance to study physiological processes both during droughts and after rehydrating plants. When there is a water shortage in early plants stage, typically have the capacity to efficiently absorb water from the soil through their root systems, partially close their stomata to limit water loss through transpiration, and change their metabolism to adapt the available carbon resource.<sup>5,6</sup> Consequently, determining drought resistance is significantly harder and more complicated than determining other stress resistances.<sup>14</sup>

Mean values regarding seedling length under 20% PEG<sub>6000</sub> are presented in Figure 1. The highest seedling length was recorded in (Sakha-94 X Misr-1). Ahmad et al.,<sup>15</sup> and Baque et al.,<sup>16</sup> reported that when the seeds were induced with a 10% PEG<sub>6000</sub> solution, the highest seedling length of wheat was secured.



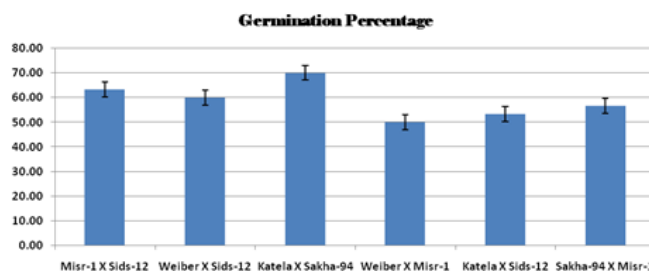
**Figure 1** Effect of 20% PEG<sub>6000</sub> on Seedling Length of tested wheat inbred lines (all means [ $\pm$  S.E.] are significantly different at  $P<0.05$ ).

When plants are subjected to drought stress in the soil, the roots become the first organ to experience. According to the presented investigation, the majority of the examined inbred lines were affected by drought stress as exhibited in Figure 2. Fenta et al.,<sup>17</sup> stated that an important factor in determining a plant's capacity to extract essential resources from the soil, such as water and mobile nutrients, is the distribution of roots, particularly those that can reach deeper into the soil. Hence, root architecture has a significant impact on the development and productivity of crop plants.



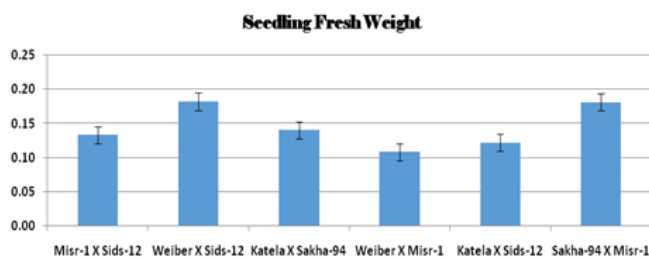
**Figure 2** Effect of 20% PEG<sub>6000</sub> on Root Numbers of tested wheat inbred lines (all means [ $\pm$  S.E.] are significantly different at  $P<0.05$ ).

Germination percentage significantly affected by 20% level PEG<sub>6000</sub> resulted declined progressively under 20% PEG<sub>6000</sub> in all six inbred lines. The inbred line (Katela X Sakha-94) demonstrated the highest level of tolerance, and the rate of decline was low relative to the other lines (Figure 3). Similar to this, Jatoi et al.,<sup>9</sup> found that 25% PEG induced a distract reduction, while 19% PEG caused a reduction in germination percentage, which refer to early seedling germination stage water deficit stress causes delayed and reduced germination. This is consistent with Ashraf & Abu-Shakra's<sup>18</sup> research, which found that at low moisture levels, germination percentage and seedling growth both decreased.

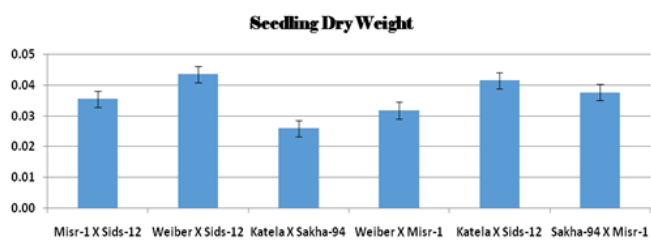


**Figure 3** Effect of 20% PEG<sub>6000</sub> on Germination Percentage of tested wheat inbred lines (all means [ $\pm$  S.E.] are significantly different at  $P<0.05$ ).

Seedling fresh and dry weights were significantly decreased under 20% PEG<sub>6000</sub> (Figures 4 and 5). The seedling fresh weight was highest in (Weiber X Sids-12) and (Sakha-94 X Misr-1). While, the variation in seedling dry weight was observed, and the results showed that (Katela X Sakha-94) was constantly decreased value, that is caused by the treatment. And So far, other genotypes reacted to the observations in various ways. Sayar et al.,<sup>19</sup> reported that when osmotic potential increased, the fresh weight of seedlings of both drought-tolerant and sensitive durum wheat genotypes decreased. Numerous other scientists have noted the declining trend in seedling fresh and dry weight, Chachar et al.<sup>20</sup>; Kamran et al.<sup>21</sup>; Moucheshi et al.<sup>22</sup> and Ahmad et al.<sup>23</sup> who showed that the fresh and dried weight of seedlings was significantly impacted by water stress.

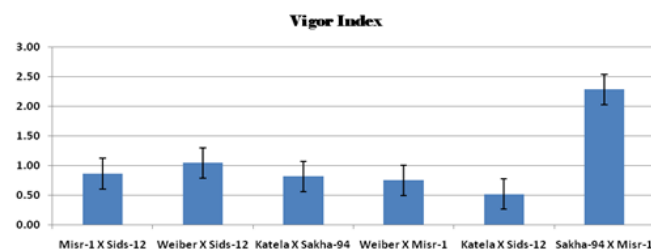


**Figure 4** Effect of 20% PEG<sub>6000</sub> on Seedling Fresh Weight of tested wheat inbred lines (all means [ $\pm$  S.E.] are significantly different at  $P<0.05$ ).



**Figure 5** Effect of 20% PEG<sub>6000</sub> on Seedling Dry Weight of tested wheat inbred lines (all means [± S.E.] are significantly different at  $P<0.05$ ).

The vigor index and tissue water content values were affected by the level of 20% PEG<sub>6000</sub>. The highest vigor index were presented in (Sakha-94 x Misr-1) (Figure 6). Meanwhile, the values regarding tissue water content responded differently to the observations (Figure 7). Hassan et al.<sup>24</sup>; El-Hamamsy and Behairy,<sup>25</sup> and Alamri et al.<sup>26</sup> illustrated that seedling vigor traits and tissue water content decreased with increasing of PEG and NaCl concentrations. Biesaga-Kościelniak et al.,<sup>27</sup> reported that wheat seedlings under drought stress had considerably decreased growth vigour.



**Figure 6** Effect of 20% PEG<sub>6000</sub> on Vigor Index of tested wheat inbred lines (all means [± S.E.] are significantly different at  $P<0.05$ ).

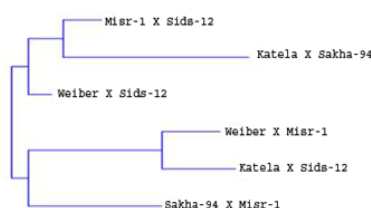
**Table 2** Pearson correlation matrix among studied parameters

	Seedling Length	Root Numbers	Seedling Fresh Weight	Seedling Dry Weight	Germination Percentage	Vigor Index
Root Numbers	0.312					
Seedling Fresh Weight	0.689	0.594				
Seedling Dry Weight	0.317	<b>0.771*</b>	0.472			
Germination Percentage	-0.165	0.412	0.267	-0.12		
Vigor Index	<b>0.990**</b>	0.39	<b>0.744*</b>	0.321	-0.024	
Tissue Water Content	0.464	0.251	0.645	-0.211	0.709	0.567

\*\*Correlation is significant at the 0.01 level (2-tailed)

\*Correlation is significant at the 0.05 level (2-tailed)

To evaluate the degree of similarity and dissimilarity among examined genotypes against induced 20% PEG<sub>6000</sub>, the Euclidean Similarity Index was used (Figure 8). The results indicated two clusters. The first cluster illustrates (Sakha-94 x Misr-1), (Katela x Sids-12) and (Weiber x Misr-1). Meanwhile, the second cluster viewed (Weiber x Sids-12), (Katela x Sakha-94) and (Misr-1 x Sids-12). These clusters had a stable performance in 20% PEG<sub>6000</sub> stress, which provides visibility performance response during the experiment. Therefore, similarity index (Euclidean) was used to identify superior tolerant genotypes under salinity stress conditions for accuracy and reliability index.<sup>33</sup>



**Figure 8** Paired group (UPGMA), similarity index (Euclidean) of six wheat inbred lines based on Seedling Length, Root Numbers, Germination Percentage, Seedling Fresh and Dry Weight, Vigor Index and Tissue Water Content under 20% PEG<sub>6000</sub> condition.

Overall, our finding showed that the inbred lines (Sakha-94 x Misr-1) and (Weiber x Sids-12) demonstrated highest drought tolerant because they did not statistically significantly reduced under 20% PEG<sub>6000</sub>.

## Conclusion

In regard to the previously mentioned findings from the current study, it's possible to conclude that 20% PEG<sub>6000</sub> osmotic stress has a positive significant effect at early seedling growth, which helps to promote wheat seed germination and seedling growth in drought-stressed conditions.

## Acknowledgments

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## Conflicts of interest

Authors declare that there is no conflict of interest.

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