



### Full Length Article

## Effects of Drought Stress on Growth and Development of Wheat Seedlings

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### Abstract

In this study, two wheat cultivars XM13 and AK58 under different drought tolerance were compared by evaluating seed germination, seedling growth and microstructures in order to identify the drought resistance of two wheat cultivars. Results showed that the germination of wheat seeds could be inhibited by drought stress, the germination energy significantly decreased with the increase of drought degree, and the germination energy of AK58 was significantly stronger compared with XM13. Although the root length of wheat XM13 were little influenced by drought stress, AK58 can be promoted and appeared significant differences with the control except the stress of 25% PEG<sub>6000</sub>. Along with the increase of drought degree, the volume of cortex cell in root had a trend of decline, even shrank at the stress of 15–25% PEG<sub>6000</sub>, but effect of drought stress on root growth of wheat XM13 was especially significant compared with wheat AK58. In addition, leaf area of wheat seedling decreased under drought stress, but the decreasing amplitude of wheat AK58 leaf area was greater than XM13. Further study identified that palisade tissue cell in leaf gradually became shorter and disorder, and the thickness of spongy tissue decreased, which was especially significant under the stress of 20–25% PEG<sub>6000</sub>. Compared with wheat XM13, the atrophy of endodermis cell in leaf of wheat AK58 should be later. Therefore, the drought-resistant ability of two cultivars wheat was different, and wheat AK58 might be stronger and could better adapt to drought stress compared with wheat XM13. © 2017 Friends Science Publishers

**Keywords:** *Triticum aestivum*; Drought stress; Leaf; Root; Microstructure

### Introduction

In recent years, drought is one of the most common abiotic factors which harmed growth and development of plant, and is becoming an increasingly severe problem in many regions of the world (Zalibekov, 2011; Hossain *et al.*, 2013; Lipiec *et al.*, 2013). Extreme water shortage causes considerable physiological, metabolic and morphological changes in plant, and ultimately reduces crop yield and quality (Maqsood *et al.*, 2012). However, there are some protection mechanisms in plant which could be beneficial to plant in response to drought stress. Under drought stress, the morphology, photosynthesis, antioxidant enzymes or osmotic adjustment substances in a plant would be adversely influenced and changed. The severity of drought depends on the growth stage of plant (Maqsood *et al.*, 2013). In addition, root is the key organ of plant against drought, because it could absorb water and nutrients from soil (Cseuz *et al.*, 2009; Shen *et al.*, 2013). The response of root growth to water deficits is important in drought tolerance (Carvalho *et al.*, 2014; Wang *et al.*, 2014a). Under drought stress, leaf of plant could also protect water and improve utilization rate of water. Therefore, the morphology

of plant could show a variety of strategies to adapt to drought stress, which is important for plant to survive and grow under drought stress (Bhargava and Sawant, 2013; Grzesiak *et al.*, 2013).

Wheat (*Triticum aestivum* L.) is one of the important crops in the world, contains starch, protein, sugar and provides food for human population (Peleg *et al.*, 2011; Liu *et al.*, 2016; Yu *et al.*, 2016). At present, drought stress has become the main one in abiotic stresses, and could restrain growth and yield of wheat (Lopes and Reynolds, 2010; Boscaiu *et al.*, 2011; Gong, 2014). According to the Food and Agriculture Organization of the United Nations, Human farming, grazing took possession of 25–30%. Humans cannot effectively use the remaining dry land for plant production (Hu and Xiong, 2014; Zhang *et al.*, 2016). Thus, it is urgent to clarify effect of drought stress on growth and development of wheat. Polyethylene glycol (PEG) is non-ionic, inert, non-penetrating and osmotically active polymer, could induce dehydration by decreasing water potential of soil, and so is generally used to simulate soil water environment of plants such as wheat (Ramya *et al.*, 2016), rice (Ma *et al.*, 2015) and maize (Khodarahmpour and Motamedi, 2001). In this research, seed germination,

seedling growth and microstructure of two wheat cultivars under drought stress were studied to explore effect mechanism of drought stress on wheat seedlings, which would provide theoretical basis for breeding and evaluation of drought-resistant wheat.

## Materials and Methods

### Plant Materials

Wheat seeds (*Triticum aestivum*) cultivar, Xinmai13 (XM13) and Aikang58 (AK58) were respectively provided by Xinxiang Academy of Agricultural Sciences and Henan Institute of Science and Technology, P. R. China.

### Germination of Wheat Seeds

Wheat seeds were disinfected for 10 min in beaker with 0.1%  $\text{HgCl}_2$ , running water for 50 min and then washed 3–5 times with distilled water. 100 wheat seeds of each group were grown in 12 cm sterile petri dishes containing two filter papers, and moistened with 30 ml 0%, 5%, 10%, 15%, 20% and 25%  $\text{PEG}_{6000}$  (Yan, 2015), respectively. Subsequently, the petri dish was placed in the incubator at  $24 \pm 1^\circ\text{C}$  with 45% relative humidity, and 14/10 h light/dark photoperiod (4500 Lux) except for the first three days. The germination energy (GE) and germination rate (GR) of wheat seeds were respectively calculated after sown in hydroponics culture for 4 d and 8 d (Jung *et al.*, 2006; El Harfi *et al.*, 2016),  $\text{GE} = (\text{N}_4/100) \times 100$ ,  $\text{GR} = (\text{N}_8/100) \times 100$ ,  $\text{N}_4$ =number of seed germinated on the 4 day,  $\text{N}_8$ =number of seed germinated on the 8 day. In addition, there were three replications for each group.

### Cultivation of Wheat Seedlings

Wheat seeds were sown in 15 cm pots with the equal mixture of soil, peat and vermiculite, and irrigated with distilled water once every 2 days, then were kept at  $24 \pm 1^\circ\text{C}$  in the light (14/10 h light/dark photoperiod, 4500LUX). Seven days later, irrigated with 100 mL 0%, 5%, 10%, 15%, 20% or 25%  $\text{PEG}_{6000}$ , respectively. At the three-leaf stage, growth of wheat seedling was observed, root length was measured from the collar to the root tip, and the leaf area was measured by length-width method. In addition, there were 300 wheat seeds in each group with three replications.

### Preparation of Paraffin Section

Small pieces of  $0.2 \text{ cm} \times 0.5 \text{ cm}$  were cut off from root and leaf of wheat seedling in three-leaf stage, and were quickly fixed with 50% FAA solution for more than 48 h. After, these pieces were processed as the followings: dehydration by a series of gradient-concentration ethanol, transparency with gradient-concentration xylene, immersion and embedment with paraffin. Subsequently, the tissue sample

was sliced by microtome, the thickness of transection was  $10 \mu\text{m}$ , and then paraffin section was observed by Olympus photo microscope (Duan *et al.*, 2009; Wang *et al.*, 2014b). In addition, there was repeated three times for each tissue sample in this research.

### Statistics Analysis of Data

The results were analyzed by analysis of variance (ANOVA) and multiple comparisons of least significant difference (LSD) using SPSS software.

## Results

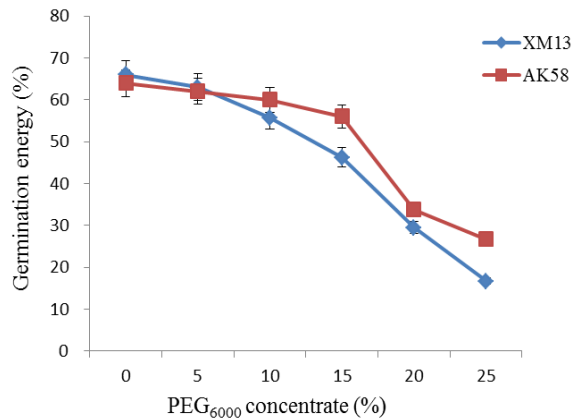
### Effect of Drought Stress on Germination of Wheat Seeds

Compared with the control, the germination energy and germination rate of two wheat cultivars were low, and reduced significantly with the increased concentration of  $\text{PEG}_{6000}$  (Fig. 1; Fig. 2). Under the normal condition, the germination energy of wheat XM13 was greater than wheat AK58, however was significantly lower than wheat AK58 under the stress of  $\text{PEG}_{6000}$ . The germinate rate of seed treated with 25%  $\text{PEG}_{6000}$  was higher in wheat AK58 than wheat XM13, which was 26.7% and 16.7%, respectively, and significantly decreased compared with the control. The result indicated that seed germination was inhibited by  $\text{PEG}_{6000}$ , and the drought resistant ability of wheat AK58 was stronger than wheat XM13.

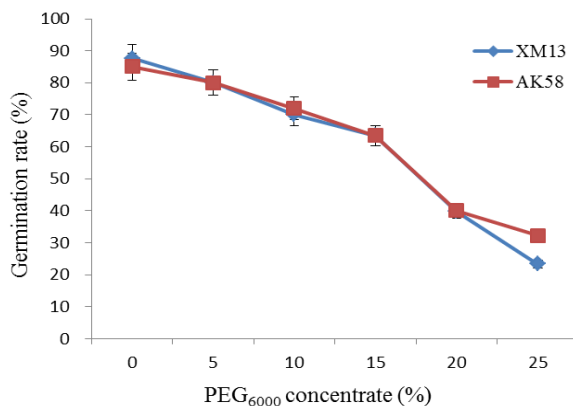
### Effect of Drought Stress on Growth of Wheat Seedlings

Under the normal condition, root length of wheat XM13 was longer than wheat AK58, however shorter than wheat AK58 under drought stress (Fig. 3). Compared to the control, root length of wheat AK58 increased and showed a trend of inverted bell along with the increase of  $\text{PEG}_{6000}$  concentration, but root length of wheat XM13 had no significant change. As stressed with 10%  $\text{PEG}_{6000}$ , the root of wheat AK58 was the longest and about 16.21 cm, and appeared extremely significant compared with the control. In addition, under the stress of 25%  $\text{PEG}_{6000}$ , root length of wheat AK58 was only 11.51 cm and also longer compared with the control.

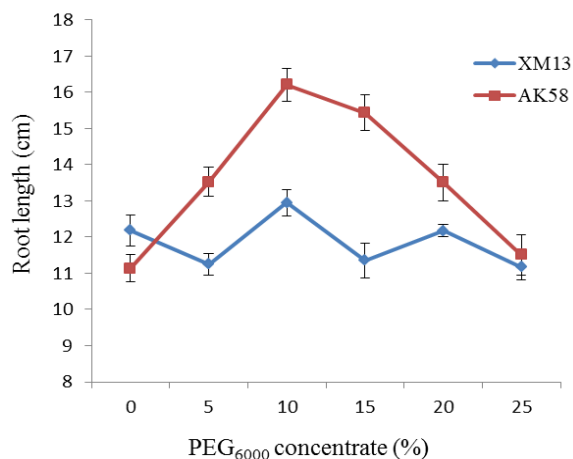
Under the normal condition, leaf area of wheat AK58 was significantly greater than wheat XM13 (Fig. 4). Compared with control, leaf area of wheat AK58 decreased and became smaller with the increasing concentration of  $\text{PEG}_{6000}$ . As treated with 15%, 20% and 25%  $\text{PEG}_{6000}$ , leaf area of wheat AK58 was respectively  $3.5 \text{ cm}^2$ ,  $2.05 \text{ cm}^2$  or  $1.68 \text{ cm}^2$ , and reached a significant level compared with the control. Although leaf area of wheat XM13 was about  $5.975 \text{ cm}^2$  at 5%  $\text{PEG}_{6000}$  and larger than the control, decreased gradually along with the increasing concentration of  $\text{PEG}_{6000}$ , even was smaller under the stress of 15–25%  $\text{PEG}_{6000}$  and reached significant difference with the control.



**Fig. 1:** The germination energy of wheat seeds under drought stress. The error bars represented standard errors



**Fig. 2:** The germination rate of wheat seeds under drought stress. The error bars represented standard errors



**Fig. 3:** The root length of wheat seedling under drought stress. The error bars represented standard errors

Thus, the leaf area of wheat seedling reduced under drought stress, and the decreasing amplitude of wheat AK58 was greater than wheat XM13.

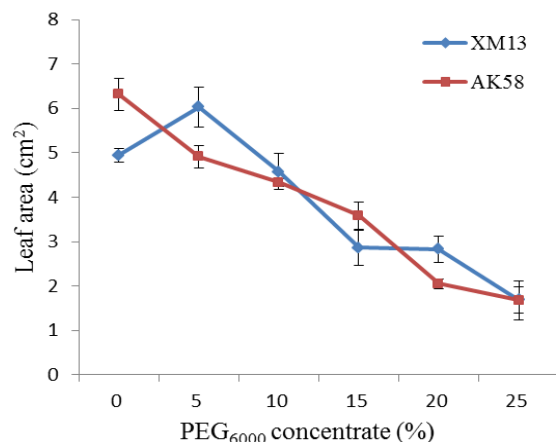
### Effect of Drought Stress on Microstructure of Wheat Seedlings

Under the normal condition, the volume of exodermal cell in seedling roots of wheat XM13 and AK58 was large, cortical parenchyma cell and endodermal cell were full, and the main xylem vessels were circular (Fig. 5a; 6a). Furthermore the difference was not significant between two wheat cultivars. Compared with the control, the volume of endodermal cell in root of wheat XM13 reduced as treated with 10% PEG<sub>6000</sub> (Fig. 5c), but changes of endodermal cell in root of wheat AK58 were not obvious (Fig. 6c). Under the stress of 15% PEG<sub>6000</sub>, the diameter of main xylem vessels in wheat root decreased significantly, exodermal cell began to shrink and endodermal cell shrink slightly (Fig. 5d; 6d), even the endodermis in root of wheat AK58 appeared slightly thickened (Fig. 6d). In addition, cell in exodermis and endodermis of root also shrank when wheat seedlings of XM13 and AK58 were treated with 20% PEG<sub>6000</sub> (Fig. 5e; 6e), and the endodermis in root of wheat AK58 showed distinct thick (Fig. 6e), but the necrosis of exodermises was found in seedling root of wheat XM13 (Fig. 5e).

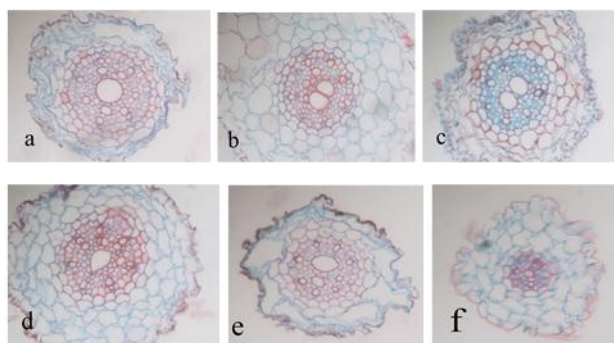
As shown in Fig. 7a and Fig. 8a, in seedling leaf of wheat XM13 and AK58 under the normal condition, the upper epidermis and lower epidermis kept complete, palisade tissue was thicker and the arrangement of its cells were close, and there were a large number of spongy tissue cell arranged in an orderly manner. Under the stress of 5–15% PEG<sub>6000</sub>, compared with the control, palisade tissue cells in leaf of wheat XM13 became shorter, the arrangement of spongy tissue was disorder (Fig. 7b, 7c and 7d), while there were no significant changes in leaf of wheat AK58. When the concentration of PEG<sub>6000</sub> increased to 20%, palisade tissue cell in seedling leaf arranged loosely, the thickness of spongy tissue decreased, and the arrangement of spongy tissue cell was irregular (Fig. 7e; 8e). Under the stress of 25% PEG<sub>6000</sub>, palisade tissue in seedling leaf of two wheat cultivars reduced significantly, spongy tissue cell appeared no regular arrangement (Fig. 7f; 8f), indicating that tissue structure in leaf of wheat seedling were destroyed.

### Discussion

It is well known, drought is one of serious environment problems that could influence growth and development of plant. When water is shortage, plant could appear the relative adaptability from morphological structure, physiological and biochemical in order to ensure the relatively stable growth of plant, for example, leaf area reduces and root activity increases (Ashraf, 2010; Mwadzingeni *et al.*, 2016). In this study, the germination rate and germination potential of two wheat cultivars significantly decreased along with the increase of PEG<sub>6000</sub> concentration, similar result was also found in citrus (Zaher-Ara *et al.*, 2016). Compared with wheat



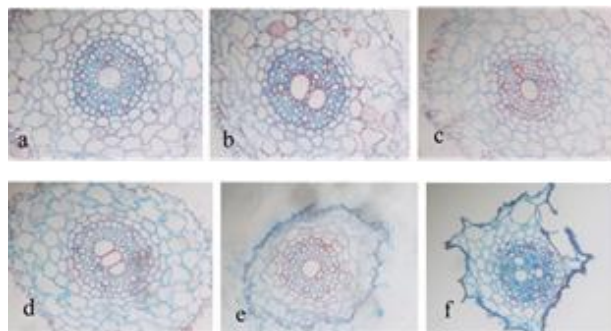
**Fig. 4:** The leaf area of wheat seedling under drought stress. The error bars represented standard errors



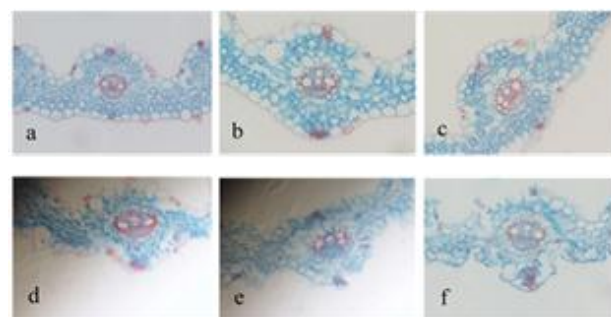
**Fig. 5:** Paraffin transection of root from wheat XM13 under different drought stress ( $\times 200$ ). a, b, c, d, e and f represented root from wheat seedlings treated with 0% PEG<sub>6000</sub>, 5% PEG<sub>6000</sub>, 10% PEG<sub>6000</sub>, 15% PEG<sub>6000</sub>, 20% PEG<sub>6000</sub> or 25% PEG<sub>6000</sub>, respectively

AK58, the germination energy of wheat XM13 was greater under the normal condition, yet was significant lower than wheat AK58 under drought stress, and the germination rate of wheat AK58 was higher than wheat XM13 at 25% PEG<sub>6000</sub>. Germination energy and germination rate are important indicators to detect the quality of seeds, and strong germination energy and high germination rate indicate the emergence of neat and fast (Farooq *et al.*, 2013; Allahverdiyev, 2015). Thus, the germination of wheat seeds could be inhibited by drought stress, and the drought-resistant ability of wheat AK58 might be stronger than XM13.

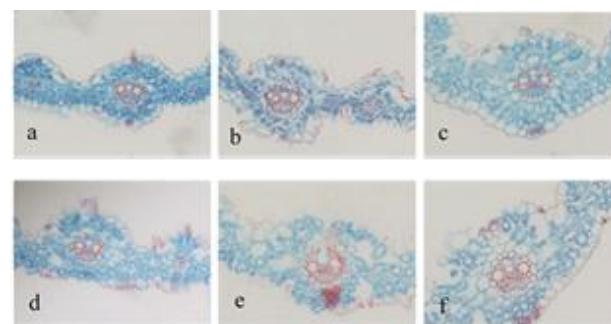
Root is the main organ of plant to absorb water and nutrient, could firstly feel effects of water deficits (Comas *et al.*, 2013; Janiak *et al.*, 2015), and plays a key role in response to drought stress. Effects of drought stress on plant root are reflected in many aspects, such as root length and root number. In this study, the formation of wheat root and the length of wheat XM13 root were little



**Fig. 6:** Paraffin transection of root from wheat AK58 under different drought stress ( $\times 200$ ). a, b, c, d, e and f represented root from wheat seedlings treated with 0% PEG<sub>6000</sub>, 5% PEG<sub>6000</sub>, 10% PEG<sub>6000</sub>, 15% PEG<sub>6000</sub>, 20% PEG<sub>6000</sub> or 25% PEG<sub>6000</sub>, respectively



**Fig. 7:** Paraffin transection of leaf from wheat XM13 under different drought stress ( $\times 200$ ). a, b, c, d, e and f represented leaf from wheat seedlings treated with 0% PEG<sub>6000</sub>, 5% PEG<sub>6000</sub>, 10% PEG<sub>6000</sub>, 15% PEG<sub>6000</sub>, 20% PEG<sub>6000</sub> or 25% PEG<sub>6000</sub>, respectively



**Fig. 8:** Paraffin transection of leaf from wheat AK58 under different drought stress ( $\times 200$ ). a, b, c, d, e and f represented leaf from wheat seedlings treated with 0% PEG<sub>6000</sub>, 5% PEG<sub>6000</sub>, 10% PEG<sub>6000</sub>, 15% PEG<sub>6000</sub>, 20% PEG<sub>6000</sub> or 25% PEG<sub>6000</sub>, respectively

influenced under drought stress, however root length of wheat AK58 can be promoted, its root was the longest as stressed with 10% PEG<sub>6000</sub> and appeared extremely significant compared with the control. It was also found that

root growth of *baicalensis* could be promoted by low concentration of PEG<sub>6000</sub> and inhibited at high concentration (Hua and Li, 2011). Further study showed that the cell volume of endodermis and exodermis in wheat root had a trend of decline along with the increase of PEG<sub>6000</sub> concentration, and then shrink at the stress of 15–25% PEG<sub>6000</sub>, but effect of drought stress on root of wheat XM13 was especially significant compared with wheat AK58 (Tang *et al.*, 2014). Moreover, the diameter of main xylem vessels in wheat seedling root also decreased under the stress of 15% PEG<sub>6000</sub>, which was especially significant under the high concentration of PEG<sub>6000</sub>, and the endodermis in root of wheat AK58 showed distinct thick. These results indicated that growth of wheat seedling root was influenced by drought stress, and this effect would be obviously stronger along with the increase of drought degree, especially was significant on root of wheat XM13.

Leaf is the main part of plant photosynthesis which can directly affect the strength of photosynthesis. Under drought stress, leaf would protect water and improve the utilization rate of water in plant by reducing growth rate of leaf, which can effectively reduce the transpiration and loss of water. In this study, leaf area of wheat seedling decreased under drought stress, even was smaller under the stress of 15–25% PEG<sub>6000</sub>, which reached significant difference with the control. Under the normal condition, leaf area of wheat AK58 was significantly greater than wheat XM13, but the decreasing amplitude of wheat AK58 leaf area was greater than wheat XM13 under drought stress. It is found in some research that once plant is short of water, cell volume of leaf could be inhibited first, and then leaf area would be smaller (Chen *et al.*, 2012). Further, study identified palisade tissue cell in wheat leaf gradually became shorter and disorder, and the thickness of spongy tissue decreased under drought stress, (Zhang *et al.*, 2015; Han *et al.*, 2016). However, under the stress of 20–25% PEG<sub>6000</sub>, the arrangement of palisade tissue cell or spongy tissue cell in wheat leaf was significantly loose and irregular, inferring that this degree of drought stress might be serious to wheat seedling. Furthermore, the atrophy of endodermis cell in leaf of wheat AK58 should be later compared with wheat XM13, which is consistent with structure changes of wheat root under drought stress, thus wheat AK58 seedling might have strong adaptability to drought stress compared with wheat XM13.

## Conclusion

In this study, it was found that germination, root and leaf growth of wheat cultivars were considerably influenced by drought stress, increasing drought stress level would obviously affect stronger cv. XM13 than cv. AK58. However, the defense response of plant under drought stress is very complicated, further research is needed to explore the mechanism of wheat seedling in response to drought stress.

## Acknowledgments

This work was supported by the National Science-technology Support Plan Projects (2013BAD07B14) and Project for Innovative Talent of Science and Technology in University of Henan Province (17HASTIT034) in P. R. China.

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(Received 08 March 2017; Accepted 22 May 2017)