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EFFECT OF POLYETHYLENE GLYCOL-6000 ON WHEAT (*TRITICUM AESTIVUM* L.) SEED GERMINATION

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ABSTRACT

The response of twenty-five wheat (*Triticum aestivum* L.) varieties to different water stress levels were studied in a series of experiments conducted in the laboratory on Polyethylene glycol-6000. The experiments on germination with twenty-five wheat varieties were conducted in a growth cabinet maintained at 30/25^{0}C day/night temperature with 16 hours photoperiod. Seeds were germinated in glass tray lined with filter paper moistened with polyethylene glycol (PEG-6000) solutions of 0.0, -0.25, –0.50, -0.75 and –1.0 MPa osmotic potential. Germination percentage decreased with decrease in water potential of the medium. The water uptake reached at a peak value within 48 hours in all the genotypes under all the water stress conditions. The varieties Sarsabz and Kiran-95 showed comparatively better performance of germination under water stress than other wheat varieties.

Key-words: Wheat varieties, Polyethylene glycol-6000, seed germination.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major cereal crop in Pakistan (Arain *et al.*, 2011 and Anwar *et al*., 2011). Wheat is a major staple food crop of Pakistan grown over 9.046 million hectares with annual production of 24.032 million tones (Sial *et al.,* 2012), wheat occupies central position in the agricultural policies of the country (Mirbahar *et al.,* 2009). Water deficiency is generally considered as one of the limiting factors for crop productivity which affects physiological as well as biochemical processes in plants. Wheat is a major crop of rainfed agriculture in Pakistan (Mujtaba *et al.,* 2007). Wheat is the largest grain crop in the world. It provides food to 36% of the global population and contributes 20% of food calories. With progressive global climatic change and increasing shortage of water resources and worsening eco-environment, wheat production is influenced greatly (Khan and Naqvi, 2011).

Water deficit is one of major abiotic stresses to reduce crop production worldwide, limiting the productivity of crop species, especially in arid and semiarid zone (Chutipaijit *et al*., 2012). Plants are subjected to various abiotic stresses due to unfavorable environmental conditions that affect their growth, metabolism and yield and drought is one of the major abiotic stresses which limit the crop production in arid and semi-arid tropics (Kumar *et al.*, 2011). Osmotic adjustment is a part of drought avoidance mechanisms to counteract the loss of turgor by increasing and maintaining higher amount of intracellular compatible solutes in cytosol and vacuole has been proved to be particularly significant among all the stress adaptation mechanisms. Proline (Pro) and quaternary ammonium compounds (QAC's) e.g. glycinebetaine, choline, prolinebetaine are key osmolytes contributing towards Osmotic Adjustment (Nayyar, 2003).

Germination is the first critical phase most affected by drought (Ashraf and Mehmood, 1990). For achieving a successful crop production, it determines the crop density and consequently yields. Three different stages can be distinguished during seed germination: (i) Imbibition, during which water absorption by the seed takes place (ii) initiation of biochemical and meristematic activities takes place (iii) differentiation of axis into root and shoot. The sequence of seedling growth governed by water uptake from the external substrate i.e. the soil or solution (Khanzada, 1996). The seed weight influenced germination to a greater extent more or less linearly. The genotypes with higher seed weight in general had higher germination percentage (Bashir and Khanzada, 1997). The best option for crop production, yield improvement and yield stability under soil moisture deficient conditions is to develop drought tolerant crop varieties. A physiological approach would be the most attractive way to develop the new varieties rapidly, but breeding for specific, sub-optimal environments involves deeper understanding of the yield determining process (Siddique *et al.,* 2000).

Conditions of water stress can be created in laboratory using an osmotic medium Polyethylene glycol-6000. PEG-6000 is chemically an inert and non-toxic chemical substance with high molecular weight. Conditions of varying degrees of dryness/water stress can be achieved by preparing PEG-6000 solutions of different concentrations. High molecular weight of PEG-6000 induce the conditions of water stress similar to that caused by dry soil. The objective of this research was to screen out the water stress tolerant and water stress sensitive wheat varieties.

MATERIALS AND METHODS

Seeds of twenty five wheat varieties were surface sterilized with 10% Sodium hypochlorite (NaOCl) solution for five minutes and washed three times with distilled water. After washing, the seeds of each variety were placed on the moistened filter papers in glass trays. Seeds of twenty five wheat (*Triticum aestivum* L.) varieties were grown hydroponcially by incorporating Polyethylene glycol-6000 (PEG-6000) in solution to evaluate the comparative sensitivity of these varieties towards different stress levels. Different PEG-6000 concentrations were prepared i.e., control 0.0, -0.25, -0.50, -0.75 and -1.0 Megapascal (MPa) water potential. The experiment was carried out in growth room, photoperiod of 16/8hr and temperature was maintained at 22^0C. Filter papers were moistened at regular intervals with different concentrations of polyethylene glycol (PEG-6000) solution and each treatment was replicated three times.

RESULT AND DISCUSSION

Significant differences in germination percentage between the wheat varieties under the different water stress levels were observed. Emergence of 5 mm radical was considered as the criterion for seed germination. Percentage germination was calculated at the end of the experiment. The results indicated that germination percentage reduced with increasing water stress level (Table. 1). However, germination percentage increased with the passage of time and it was maximum on the 8th day under all the treatments. Under treatment 1 (control) the maximum germination was achieved within two days after that non-significant increase was observed in all the varieties. Germination, seedling establishment and vigor play very important roles in the subsequent capability of crop to withstand water shortage and indirectly they determine crop stand, density and consequently the yield of the resultant crop (Gelmond, 1978). Singh and Singh 1982 and 1983 observed that the reduction in germination took place at –1.0 MPa in wheat and rice. Khan and Naqvi 1984 testing mungbean found varietial differences in germination and seedling growth under water stress applied through NaC1 and PEG-6000 at (i.e. –1.0 MPa). On 6th day the observations were similar except in case of Sarsabz and Kiran-95, where differences between both the treatments were non-significant.

But on 8th day, the differences between control and T2 were non-significant in case of Sarsabz and Kiran-95. However in case of other varieties, differences were significant. The differences between T2 and T3 (-0.5 MPa) were significant throughout the experiment in all the varieties. The maximum germination under T3 was observed in Sarsabz and Kiran-95 under all time period. Similarly our results are in agreement with Ashraf and Mehmood (1990) working with sorghum found reduction in percentage germination even at –0.1 MPa in the case of sensitive genotypes. However, in case of tolerant genotype a similar reduction occurred at –0.5 MPa. On the other hand, germination percentage under treatment 2 (T2) increased with the passage of time and it was maximum upto 6th day. The differences between control and T2 (-0.25 MPa) were significant upto 4th day in all the varieties. The differences were clear between T4 (-0.75 MPa) and T5 (-1.0 MPa) at all the intervals of time in all the genotypes for 8 days. T5 is the highest stress level, on the 8th day maximum germination percentage was recorded for the varieties Sarsabz and Kiran-95. The differences between Sarsabz and Kiran-95 were non-significant under T5 but the differences were significant with other varieties at all the interval of time in all varieties. On the basis of these results, the wheat varieties under study can be classified into two groups i.e. Sarsabz and Kiran-95 (tolerant), showing non-significant differences between them. While Abadgar-93, Bakhtawar, Inqlab-91, Iqbal, Johar-78, Mangla, Mehran-89, Pavon, Soghat-90, Sonalika, WL-711, Yecora, Zardana, ZA-77, RWM-9313, SI-9590, and ESW-9613 non-resistant (sensitive) to drought. Significant differences were observed among them at all intervals of time. These varieties were selected on the basis of highest germination percentage on highest stress levels in the laboratory experiments.

It is obvious from the foregoing discussion that the effect of external water potential depends not only on the performance of the varieties, but also on the crops tested. Twenty-five wheat varieties were used in the present study, 23 varieties could be classified as sensitive, where reduction in germination occurred at –0.1 MPa. In case of Sarsabz and Kiran-95, the higher germination recorded at the same treatment (Table 1). The germination generally depends upon the absorption of water by the germinating seeds. Many workers found a progressive fall in water uptake during germination with lowering external water potential. The peak of water uptake and germinating seeds was between 48 hours in different genotypes of rice (Singh and Singh, 1982) and between 24-30 hrs. in different wheat genotypes (Singh and Singh, 1983), 24-30 hours in sorghum (Ashraf and Mehmood, 1990) and 24 hours in mungbean (Khan and Naqvi, 1984). The peak of water uptake in the present study was also 27-48 hours. An analysis of data presented here makes one thing clear that the peak of water absorption occurs at the same time irrespective of external water potentials and the cultivars used. It is perhaps more coincidental than a strategy for survival.

Table 1. Germination (%) of Wheat varieties after 192 hours in PEG-6000 stress.

Varieties	Control (T1)	-0.25Mpa (T2)	-0.5Mpa (T3)	-0.75Mpa (T4)	-1.0Mpa (T5)	Mean
Abadgar-93	100	100	86.56	63.45	40.03	78.08
Anmol-91	100	100	90.65	83.65	58.60 *	93.57
Bakhtawar	100	100	85.01	61.65	50.24	79.38
Inqilab-91.	100	100	90.35	75.65	50.65	83.33
Iqbal	100	100	80.35	56.65	40.24	75.44
Johar78	100	100	89.56	68.35	51.05	81.79
Khirman	100	100	91.23	83.35	60.35*	93.64
Kiran-95	100	100	92.35	84.15	65.45**	94.12
Mangla	100	100	83.35	66.65	50.21	80.04
Marvi	100	100	90.65	78.09	60.65*	92.18
Mehran-89	100	100	88.9	70.35	46.35	81.12
Pavon	100	100	84.56	59.45	45.24	77.85
Sarsabz	100	100	90.64	85.71	67.87 **	94.08
Sindh-81	100	100	97.25	80.42	60.45 *	94.41
Soghat-90	100	100	89.14	70.54	45.71	81.07
Sonalika	100	100	85.68	71.34	52.16	81.83
T-J 83	100	100	89.35	71.24	60.35**	90.14
WL-711	100	100	82.59	70.35	50.15	80.61
Yacora	100	100	87.56	71.35	45.31	80.84
Zardana	100	100	83.35	73.35	53.35	82.01
Z-A 77	100	100	89.56	78.07	51.35	83.79
RWM 9313	100	100	80.35	63.35	38.25	76.39
ESW 9525	100	100	88.35	74.35	60.34 *	90.67
SI 9590	100	100	79.87	68.35	48.65	79.37
ESW 9613	100	100	90.43	76.35	58.35	85.02
Mean	100	100	87.05	72.25	48.07	81.47

The decrease in total water uptake may be more significant. The role of the rate and percentage of germination in the subsequent performance of the plant has generated quite a bit of controversy. Theoretically, it should have a positive effect. A seed germinating in an early time would have an advantage in establishing itself before the surface moisture depletes. Williams *et al.* (1967) and Richards (1978) worked on Brassica and Corn, respectively were of the views that germination test could be useful in screening for drought tolerance. On the other hand Blum *et al.*, (1980), Boustama and Schapaugh (1984) and Ashraf *et al.*, (1992) reported that germination test did not reflect stress tolerance responses but rather it reflects seed quality differences in wheat and sorghum. This test also did not reflect the yield stability of the genotypes.

It has been suggested that several factors involved in seed quality such as the age of seed, environmental conditions during development, harvest and storage conditions may also affect the germination. As a result, the varieties may respond differently resulting in erroneous conclusion. In this study, every effort was made to minimize the effect of above factors by selecting the seeds of same age, collected from similar plants and stored under similar conditions. It seems that such precautions eliminated the factors mentioned above and the differences observed were real rather than apparent. Although, differences between control and treatment (-0.25 MPa) external pressure were not clear, but a visual pattern between the tolerant and sensitive varieties could be discerned. At –1.0 MPa, these differences were very clear and lucid. The pattern that emerges from the present study supports the view that germination test could be used as an indicator of a drought tolerance. A word of caution in all these studies is appropriate, no matter, how vigorous the seed selection is, there would always be a possibility of gene/genes for germination interacting differently with the genetic background of the seed. As appropriately pointed out by Richards (1978), the only way the value of a particular trait can be assessed with some certainty is by using isogenic or near-isogenic lines. Creation of an isogenic line poses its own problem, especially in case of polygenic characters. The isogenic lines may provide an appropriate answer to the value of the trait, but its real value will have to be determined under different genetic backgrounds to be a significance or potential value in the application of practical agriculture.

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