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Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes

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ABSTRACT

The objective of the present study was to find out a straightforward technique for screening the tolerance of ten wheat genotypes to two levels of osmotic stress at early seedling stage. Data revealed that polyethylene glycol-induced drought had general negative effect on seedling morphological characters indicated by plumule and radicle length, number of adventitious roots as well as seedling biomass and water content. Water deficit could also suppress membrane integrity by stimulating lipid peroxidation with marked increase in membrane leakage and subsequent decrease in its stability index. For all the addressed germination parameters and seedling membrane features, the impact of severe drought was more pronounced than that of moderate drought. Simultaneously, moderate stress could activate peroxidase, polyphenol oxidase and ascorbic peroxidase of the studied genotypes; but these enzymes were inhibited by severe stress. The activity of catalase, superoxide dismutase and glutathione reductase was conversely retarded by drought whether at moderate or severe level. More interestingly, a novel function "Stress Impact Index; SII" was introduced to rank the estimated morpho-physiological traits (SII_{trait}) as well as the considered genotypes ($SII_{genotype}$) according to their sensitivity to stress. Values of SII_{trait} implied that germination parameters were generally affected by drought more intensively than membrane characteristics and finally came the antioxidant enzymes with the least degree of suppression when applying stress. Based on the magnitudes of $SII_{genotype}$, Sids 13 seemed to be the most drought-tolerant wheat cultivar while Shandawel 1 could be the most sensitive one at their juvenile growth stage.

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1. Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop globally cultivated for human consumption as a prime source of carbohydrates, proteins, fats, vitamins, minerals and other nutritional constituents. World production of wheat could be rated in the third level after that of maize and rice [1]. However, great attention is paid to bridge the gap between wheat production and consumption especially with various environmental stresses multiplying readily. Among these stresses, drought is a deleterious factor that can reduce wheat yield by 50–90% [2]. In this context, the final yield of any crop is well known to depend on plant performance during the successive stages of its life cycle; the most critical of which are seed germination and seedling growth [3].

At the same time, seed germination and seedling establishment in the majority of crop species are the most sensitive phases to abiotic stress particularly water deficit [4]. Drought is documented to delay seed germination and suppress its rate. Furthermore, water shortage can induce significant alterations in seedling physiology and biochemistry [5]. Nevertheless, certain plants exhibit a set of physiological adaptations that enable them to withstand water stress. Among these adaptive strategies, enhanced activity of antioxidant enzymes may induce plant tolerance by scavenging reactive oxygen species (ROS) [6]. Overproduction of ROS causes the damage of essential biomolecules present in cell compartments and/ or membranes [7]. Therefore, the status of cellular membranes also indicates the degree of plant acclimation to stress. The impact of water stress on antioxidant defense system and membrane features in wheat and other plants was intensively studied [8,9].

Screening drought-resistant plant genotypes is thus a fundamental goal obviously targeted in arid and semi-arid regions. Nonetheless, drought cannot be easily controlled in the field because of rainfall that can impede water deficit [10]. Therefore, assessing plant response to drought at early seedling stage was commonly achieved using chemical desiccators such as polyethylene glycol (PEG). It was inferred that PEG can be employed to shift the water potential of nutrient media inducing plant-water deficit in a relatively programmed manner compatible with experimental protocols [11]. In this regard, Gou et al. [12] and Homayoun et al. [13] evaluated different wheat genotypes under gradual doses of PEG and recorded considerable deterioration in germination indices and seedling traits in susceptible varieties rather than their tolerant synonyms.

Accordingly, the present study aims at exploring differential method to verify the response of different wheat genotypes to two levels of PEG-induced drought during germination and seedling growth stage. In addition, associations between drought-related seedling traits would be investigated *via* in-depth statistical analysis of the addressed traits as well as the checked genotypes.

2. Materials and methods

2.1. Plant material and germination conditions

A collection of different wheat genotypes was kindly supplied by Sakha Agricultural Research Center, Kafr El-Shakh Governorate, Egypt. The obtained wheat cultivars included Masr 1, Masr 2, Gimmaza 9, Gimmaza 11, Sids 12, Sids 13, Sakha 93, Sakha 94, Shandawel 1 and Giza 186. In a germination trial, grains of the studied genotypes were surface sterilized with sodium hypochlorite then soaked for 8 hours in water. The grains of each cultivar were then allowed to germinate in dark at 25 ± 2 °C for 6 days in 3 sets; the first was supplied with water when required to serve as control, while the second and the third ones were treated with PEG 6000 at 15% (–2.95 bar osmotic pressure) and 25% (–7.35 bar); respectively.

2.2. Determination of germination parameters

Plumule and radicle length, the number of adventitious roots as well as the fresh and dry mass of 6-day old wheat seedlings were recorded. Seedling water content, the amount of water per unit seedling fresh mass, was additionally calculated as cited from Mickky [14] where;

Water content = (fresh mass – dry mass)/fresh mass

2.3. Determination of membrane status

Membrane stability index (MSI) and membrane leakage (ML) were determined following Sairam et al. [15] and Vahala et al. [16], respectively. Furthermore, lipid peroxidation indicated by malondialdehyde (MDA) content was determined according to Doria et al. [17].

2.4. Assay of antioxidant enzymes

Enzyme extracts were prepared as recommended by Agrawal and Shaheen [18] in phosphate buffer at pH 6.8 for peroxidase (POX; EC 1.11.1.7.), polyphenol oxidase (PPO; EC 1.14.18.1.), catalase (CAT; EC 1.11.1.6.) and glutathione reductase (GR; EC 1.8.1.7.); and pH 7.8 for superoxide dismutase (SOD; EC 1.15.1.1.) and ascorbic peroxidase (APX; EC 1.11.1.11.).

The activity of POX and that of PPO were estimated as cited from Devi [19]. CAT and APX were assayed according to Devi [20] and Barka [21], respectively. Meanwhile, the protocol designed by Nishikimi et al. [22] was followed for SOD assay and that of Goldberg and Spooner [23] for GR.

2.5. Data processing

Means of ten determinations for germination parameters and three for the other biochemical investigations were computed along with standard deviation. Obtained data were subjected to one way completely randomized ANOVA (analysis of variance) test at 5% probability level using CoHort/ CoStat software. According to the values of LSD (least significance difference), small letters were denoted with different letters referring to significant variation. For each criterion, the mean value was calculated for all genotypes under control as well as moderate and severe drought conditions. Thereafter, the individual % of difference between the values at each drought level and that at control was calculated so that the total % of difference between drought in general and control

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