Screening of Wheat (Triticum Aestivum L.) Genotypes for Drought Tolerance through Estimation of Chlorophyll and Proline Contents

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Abstract

Wheat (Triticum aestivum L) is a staple food cereal crop for the majority of the global population. Water scarcity is a major challenge to wheat productivity under changing climate conditions, especially in arid and semi-arid regions. During recent years, different agronomic, physiological, and molecular approaches have been used to overcome the problems related to drought stress. The adverse effects of single or multiple environmental stresses, such as drought, high and low temperatures, and nutrient deficiencies on plant growth and yield have become severe in recent years due to increased global climate change and the occurrence of extreme weather events. This study was designed to investigate the screening of wheat genotypes under drought and irrigated conditions and to identify the best-performing genotypes under both conditions. Five different genotypes were used in two factors and three replications at Gomal University, Dera Ismail Khan, and investigated for yield and drought tolerance under irrigated and drought conditions by the estimation of chlorophyll and proline contents. Data collected for yield/pot (g) and drought tolerance by estimating chlorophyll and proline contents was subjected to Statistix 8.1 software at the 5% probability level. Results revealed significant differences ($P \leq 0.01$) among genotypes for chlorophyll and proline contents, and yield/pot. Under both irrigated and drought treatments, AZRC Dera exhibited the highest chlorophyll content value (46.33 and 45.11, respectively), followed by RK-2022 (43.32 and 41.89, respectively). Similarly, under drought stress conditions, RK-2022 (20.21) and AZRC Dera (18.86) recorded the accumulation of the highest proline content, followed by Wadan (18.21). Moreover, the irrigation treatments and stressed plants varied the grain yield from 0.72 to 0.49 and 0.52 to 0.28 grams/plot, respectively. AZRC Dera and RK-2022 exhibited a higher yield/plot (g) under both irrigated (0.72g and 0.65g, respectively) and drought (0.52g and 0.48g, respectively) conditions. Considering yield and drought-responsive high chlorophyll and proline contents, AZRC Dera and RK-2022 showed greater drought tolerance and revealed potential to grow under water deficit conditions in comparison to other cultivars.

Keywords: Drought Stress; Irrigation; Genotypes; Chlorophyll, Proline

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Introduction

Wheat (*Triticum aestivum* L.) is our staple food cereal crop; wheat belongs to the family Gramineae, and it is a very important cereal crop in Pakistan. It is cultivated on one-sixth of the total arable land in the world. Pakistan ranks 8th in the top ten countries. Besides many factors, low and imbalanced fertilizer as well as drought stress are the main factors limiting wheat productivity in Pakistan. The wheat crop is a source of starch, protein, vitamins, and fiber, which are important for human health. Environmental stresses due to a changing climate have emerged as big threats to staple food production (Ahad et al.,2023).

In all, drought-induced osmotic stress limits plants' uptake of water and nutrients, resulting in reduced initial growth. The reduced area for photosynthesis in plants minimizes the accumulation of photosynthetic products, ultimately leading to lower yields. Therefore, restricted water and nutrient uptake in crops is one of the main reasons for the decreased yield under the environmental stresses of drought (Niinemets, 2007). Among these solutes, proline is the most widely studied. The beneficial roles of proline in conferring osmotic tolerance have been widely reported (Ashraf and Foolad, 2007; Hayat et al., 2012; Liang et al., 2013; Hosseinifard et al., 2022). It has been widely reported that plant cells achieve their osmotic adjustment by the accumulation of some kind of compatible solutes such as proline, betaine, and polyols to protect membranes and proteins. Compatible solutes are overproduced under osmotic stress, aiming to facilitate osmotic adjustment. These compounds accumulated in high amounts mainly in the cytoplasm of stressed cells without interfering with macromolecules and behaved as osmoprotectants. It has been shown that proline also has a key role in stabilizing cellular proteins and membranes in the presence of high concentrations of osmoticum (Altendorf et al., 2009; Slama et al., 2015).

Drought significantly limits crop production, and the duration of drought periods is increasing due to climate change, declining ground and surface water resources, and warming air temperature in most of the cereal cropping regions around the world (Dhakal, 2021). Drought is a serious problem in semi-arid and arid areas worldwide (Hamarash et al., 2022). It can cause losses in wheat grain yields ranging from 10% and 100%. Furthermore, climate change is likely to increase drought risk in the 21st century worldwide (Mesterházy et al., 2020). Hence, it is important to develop wheat cultivars with high drought tolerance for improving food security. An understanding of the morphological, physiological, and genetic mechanisms allowing plants to cope with environmental challenges is of vital importance for breeding crops with improved stress tolerance and performance under stress. Screening wheat germplasm for drought tolerance is a key strategy to select and develop cultivars that perform better in terms of yield under drought stress conditions (Ahmad et al., 2022). This study was designed to evaluate six Pakistani wheat genotypes under irrigated and drought stress conditions to select the best-performing wheat genotype for the agro-climatic conditions of Dera Ismail Khan.

Material & Method

Current research was conducted at the Department of Plant Breeding and Genetics, Gomal University Dera Ismail Khan, in the wheat growing season of 2023-2024.

Research Design

The experiment was performed using a two-factorial split-plot design having a main plot and subplot using three replications. The experiment's total plot area was kept at 192 m^2 with 5 wheat germplasm per replication. Plant-to-plant distance was kept at 30cm, and row-to-row distance was standardized at 30cm.

Treatments and agronomic Practices

Drought treatment was applied by growing the genotypes under irrigated (supplemental irrigation; four times irrigation from heading to mid-grain filling stages) and rainfed (as drought stress) conditions during one cropping season (2023–2024). The soil texture at the station was clay loam. Land was prepared with 2-3 deep ploughing followed by rotavator operation. There was the application of necessary agronomic practices. The recommended dose of fertilizer was applied at the rate of 120:90:60 kg/ha^{-1}.

Germplasm

Five types of Pakistani wheat varieties (Table 1) were tested to see how they respond to drought by measuring chlorophyll and proline levels, as well as the amount of yield per plot (g) in both watered and dry conditions.

Table 1 Germplasm under study

S. No	Genotype name
1	Hashim-08
2	Wadan
3	Gulzar-19
4	RK-2022
5	AZRC Dera

Data collection

Data were recorded by keeping selection intensity at 5% for each line. The protocol for data collection of parameters is under.

1. Chlorophyll Contents

Chlorophyll contents were recorded with the help of a SPAD meter as shown in Fig. 1.

Fig. 1 Estimation of chlorophyll contents in wheat using SPAD Meter.

2. Estimation of proline contents

Estimation of proline contents from wheat leaves was conducted using the method of Sarver et al. (1999). Fresh leaves (0.5 g) were taken to homogenized with 10 mL of 3% sulpho-salicylic acid solution and then filtered. Out of these plant extracts, 2 mL of each plant sample was heated with ninhydrin and glacial acetic acid solution at 98 °C in a water bath for an hour and cooled instantly. After that, 4 mL of toluene was poured into the above mixture and shaken vigorously for 20-30 seconds. This resulted in the formation of two layers. The upper pink colored layer was separated for measuring OD at 520 nm against the blank.

Calculated formula of proline content

GRI = (OD or (Rep value) + 0.024)/ 0.055

Proline = ((GRI /2) *)/ (115.5)/(0.5 plant wt.)/(10)

3. Grain yield plant/plot (g)

Data for grain yield/plot (g) was recorded per/m2 of plants after hand-threshing all the spikes, cleaning the whole grains, and recording their weight with the help of an electric balance.

3. Statistical Analysis

Software Statistix 8.1 was used for data analysis; the calculation of LSD values was at the 5% probability level.

Results & Discussion

1. Response to drought stress via chlorophyll content accumulation

Significant differences (P ≤ 0.01) were noted among genotypes for chlorophyll contents (Table 2). Under irrigated treatments, AZRC Dera exhibited the highest chlorophyll content value (46.33) for drought treatment (45.11). AZRC Dera was followed by RK-2022 (43.32 under irrigated conditions and 41.89 under drought stress). The lowest chlorophyll contents were recorded for Wadan under both irrigated (42.23) and drought stress (40.21) conditions. Drought treatment significantly decreased chlorophyll contents among all genotypes as compared to irrigated conditions (Table 2).

Table 2. Chlorophyll content of wheat genotypes under irrigated and drought conditions.

Treatment	Irrigated	Drought	Mean
Hashim-08	42.23	40.21	41.22 BC
Wadan	37.96	37.61	37.78 C
Gulzar-19	40.12	38.56	39.34 ABC
RK-2022	43.32	41.89	42.60 AB
AZRC Dera	46.33	45.11	45.72 A
Treat Mean	41.99 A	40.67 B	

Different letters followed by values in a column indicate significant differences at a 5% level of probability, tested by Turkey's test.

Plants under drought stress, the green tissues of chlorophyll in leaves of resistant wheat cultivars, play a vital role in mitigating drought stress tolerance. Our results are in line with the findings of Khayatnezhad *et al.* (2011) who reported that drought stress conditions drastically decreased the leaf chlorophyll content in wheat genotypes non-tolerant to drought stress, while drought-tolerant wheat genotypes experienced a mild decrease in leaf chlorophyll content. Wheat genotypes with higher leaf chlorophyll content are tolerant to drought stress. In our study, the higher chlorophyll contents of AZRC Dera and RK-2022 relative to other genotypes make them ideal candidates to be grown under drought-prone wheat growing zones.

2. Response to drought stress via proline content accumulation

The data regarding proline content showed that under irrigated conditions, RK-2022 and AZRC Dera showed high proline contents (17.12 and 16.88, respectively), followed by Gulzar-19 (15.22). Under drought stress conditions, RK-2022 and AZRC-DK accumulated the highest proline contents (20.21 and 18.86, respectively), followed by Gulzar-19 (17.89). Wadan showed the lowest proline contents under both irrigated and drought stress conditions (13.22 and 15.88, respectively). In general, drought stress enhanced the accumulation of proline contents in all wheat genotypes under study (Fig. 2).

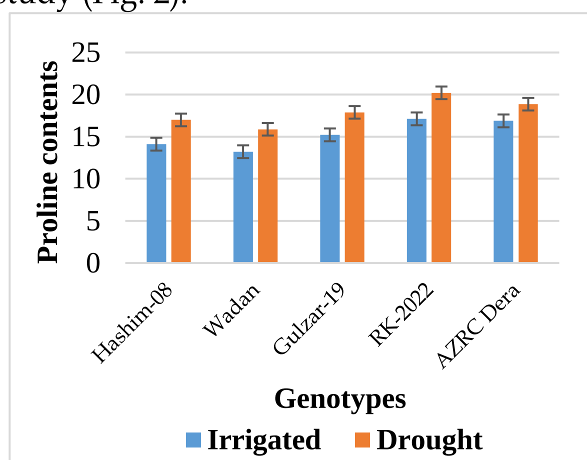

Fig. 2 Accumulation of proline contents in wheat genotypes under irrigated and drought stress.

Proline plays a critical role in enhancing drought tolerance in wheat by acting as a multifunctional osmoprotectant and stress mitigator. In dry conditions, wheat plants gather proline, which helps control the water balance in their cells, keeping them firm and functioning properly even when there isn't enough water. This osmotic adjustment helps preserve cellular integrity and physiological processes despite water scarcity (Ahmed and Hassan, 2011). Certain wheat genotypes accumulate high proline contents to minimize the adverse effect of drought stress and show better yield potential. Mwadzingeni et al. (2016) screened 98 wheat genotypes from the CIMMYT collection under both irrigated and drought conditions for proline contents and yield. Their findings revealed that genotypes with high yield under both drought and optimal conditions maintained robust yield-related traits. Proline levels increased significantly under drought but showed weak correlations with agronomic traits across conditions. Importantly, the positive link between grain yield and proline during drought indicates that higher proline levels might help in choosing plants that can handle dry conditions better.-. The study identified 12 genotypes with high drought-stress grain yield and adaptive traits suitable for breeding programs. The findings of our study also revealed that both RK-2022 and AZRC Dera maintained the highest proline contents and could be used as excellent breeding materials for drought-stress breeding.

3. Grain yield/plot

Grain yield/ per plot showed that the irrigation treatments and stressed plants varied the grain yield from 0.72 to 0.49 and 0.52 to 0.28 grams/plot, respectively. AZRC Dera and RK-2022 exhibited a higher yield/plot (g) under both irrigated (0.72g and 0.65g, respectively) and drought (0.52g and 0.48g, respectively) stress conditions. The lowest grain yield/plot was exhibited by Hashim-08 under both irrigated (0.49g) and drought stress (0.28 g). In general, drought stress decreased grain yield/plot among all genotypes (Table 3). Our results are in line with Pradhan et al., (2012), who showed that the development of high-yielding cultivars with acceptable stability and adaptability across different environments is one promising strategy for improving wheat yield under drought-prone environments. A significant reduction in grain yields due to water stress at the post-anthesis stage may result in a severe reduction in the production of photo-assimilates, depending on the severity of the stress and the crop growth stage during which drought was imposed. Both AZRC Dera and RK-2022 with higher grain yield/plot under both irrigated and drought stress conditions imply that these two genotypes should be prioritized by growers for higher yield potential in drought-prone wheat growth zones.

Table 3. Grain yield/plot of wheat genotypes under irrigated and drought conditions.

Treatment	Irrigated	Drought	V- Mean
Hashim-08	0.49	0.28	0.38 C
Wadan	0.50	0.32	0.41 C
Gulzar-19	0.56	0.35	0.45 ABC
RK-2022	**0.65**	**0.48**	0.56 A
AZRC Dera	**0.72**	**0.52**	0.62 A
Treat Mean	**0.58 A**	0.39 B	

Different letters followed by values in a column indicate significant differences at a 5% level of probability, tested by Turkey's test.

Conclusion & Recommendations

Based on chlorophyll contents, proline accumulation, and grain yield/plot, wheat varieties AZRC Dera and RK-2022 were found highly tolerant to drought stress and are hereby recommended for farmers to grow them in semi-arid drought-prone areas of Dera Ismail Khan and elsewhere in Pakistan.

Authors Contribution

Nasr Ullah Khan and Adnan Shehzad conceived the idea, designed the study, and drafted the manuscript. Abdul Majid, Ubairah, and Hafiz Ullah helped in data collection and data analysis. Adnan

Shehzad and Abdul Majid helped in drafting and proofreading the manuscript. All authors read the drafted manuscript.

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