Unveiling the Induced Drought Stress by Polyethylene Glycol (PEG-6000); Effects on Seed Germination, Morphological and Gaseous Exchange Parameters of Grass Species from Cholistan Rangeland, Pakistan

Muhammad Abid[1], Muhammad Rafay[1], Tanveer Hussain Khan[1], Muneeb Khalid[1], Muhammad Azeem Sabir[1], Muhammad Madnee[1], Hussain Ahmad Makki[1], Ahmer Saleem[1], Hamza Basit[1], Sana Ghafar[1] and Waqas Iqbal[2]

[1]*Institute of Forest Sciences, The Islamia University of Bahawalpur, 63100, Pakistan.*

[2]*Department of Forestry and Range Management, University of Agriculture Faisalabad, Pakistan.*

Abstract

Severe drought stress is the prolonged low rainfall that limits rangeland biomass production. Due to drought severity in Cholistan rangeland, an experiment on seed germination, biomass production and gaseous exchange activity of *Aeluropus lagopoides* and *Ochthochloa compressa* against induced drought stress by Polyethylene Glycol (PEG-6000) was conducted. Seeds germinated using a completely randomized design (CRD) at T1=0, T2=10% and T3=20% PEG-6000 concentrations. Data was analyzed by simple linear correlation and CR design. Results showed that PEG-6000 significantly affects germination, biomass production and plant physiology. Seed germination at treatments T1 was 78% with 0.873 coefficient of correlation (R-squared) and minimum was in T3 (20% PEG-6000) which was germination 44% with R-squared 0.78 in *O. compressa*. The highest seed germination in *A. lagopoides* was at T1 72% with 0.826 coefficient of determination (R-squared) and the minimum was at T3 40% with R-squared 0.74. The results also showed that PEG-6000-induced drought stress negatively correlates with physiological and morphological parameters. Conclusively PEG-6000 induced drought stress significantly lower the seed germination and biomass production. Various plants adapt different strategies for coping with drought stress. Exploration of such strategies helps us better understand sustainable range management.

Keywords: *Aeluropus lagopoides*, Biomass, *Ochthochloa compressa*, Gaseous exchange Photosynthesis, Sustainable range management

Introduction

Drought is a significant environmental stress that greatly impacts the productivity and geographic ranges of plants (Brendan et al., 2012). Drought stress arises from a water shortage, causing plants to lose their turgidity and lack the required amount of water for vital functions (Blackman et al., 2018; Ali et al., 2022). Drought stress is a common phenomenon in semi and semi-dry regions, which make up over half of the world's cultivated land areas (Huang et al., 2016). In arid regions, the water cycle, which refers to the transport of water through the ecosystem, is typically so fragile due to the limited availability of water, even in normal circumstances. Consequently, anthropogenic activities, such as diverting water for agricultural purposes or facilitating urban growth, have disrupted the equilibrium of water budgets in dry regions, leading to a rising imbalance in modern era (Brum *et al*., 2021; Lian *et al*., 2021; Iqbal et al., 2022). Plants are significantly harmed by environmental stresses such as drought, salinity, excessive temperature, heavy metals, and pollution, which result in biochemical, physiological, and molecular alterations (Ali et al., 2023; Akbar et al., 2022; Asif et al., 2023). Drought is a significant constraint that restricts plant growth and development and distributed worldwide, especially in arid and semiarid regions. Even minor drought stress primarily lowers the water absorption, cell turgor, expansion, elongation, and overall plant growth (Taiz and Zeiger 2010; Goswami et al., 2020; Zafar et al., 2023). Seed germination and seedling growth are critical developmental stages that determine the growth, establishment, and survival of plants. The limited availability of water is a significant environmental factor that plays a crucial role in determining the success or failure of plant establishment (Li et al., 2013; Afzal et al., 2023). Drought stress causes significant harm to pigments, the photosynthetic apparatus, and thylakoid membranes, ultimately leading to a decrease in carbon absorption and biomass production (Ashraf and Harris 2013; Goswami *et al*., 2020; Ullah et al., 2023).

Seed germination is generally the crucial phase in the establishment of seedlings, as it determines future growth (Almansouri *et al*., 2001; Ismail et al., 2023). Plant growth and production is influenced by the seedbed environment as well as seed condition (Brown *et al*., 1989; Khajeh-Hosseini *et al*., 2003; Zafa et al., 2023). Water is required for the preservation of several plant activities, such as the emergence of seedlings till ther maturity of plants (Shao et al., 2009; Ullah et al., 2023). Different plant species respond differently to water-stressed conditions, which are related to their evolutionary and physiologic modification to the particular climate

(Ennajeh *et al*., 2010). Drought-stressed plants are thought to benefit from structural modifications that assist minimize water loss. The capability of a plant to germinate in the face of adversity is aided by structural alterations (Wolters and Jurgens, 2009; Iqbal et al., 2023).

In the present study, Polyethylene glycol (PEG-6000) was used to induce water stress. PEG simulates and causes drought stress in plants (Jiang *et al* 1995; Aminullah et al., 2023). Water stress in the plant's leaf hurts biomass production in general (Rawson and Turner, 1982., Saxena., 1993). The primary plants' ability to photosynthesize is directly correlated with the relative amount of chlorophyll (Fotovat *et al.,* 2007). In addition to chlorophyll levels, drought stress has a significant impact on the Calvin cycle enzymes (Monakhova and Chernyadev, 2002). PEG-6000 produces osmotic stress, which lowers photosynthetic rate and in turn, affects chlorophyll-a as well as chlorophyll-b contents. Any stress experienced by a plant has an impact on the cellular mechanisms involved in photosynthesis, including pigments, photosystems, the electron transport system, and pathways for reducing carbon dioxide (Shivakrishna *et al.,* 2018). According to reports, PEG significantly increased water stress in plants while having no toxic effects (Emmerich and Hardegree 1990). However, PEG or contaminants have been blamed for toxic side effects that have been documented by (Lesham., 1966) and PEG entered the plants studied by (Lagerwerff *et al*., 1961; Macklon and wetherley 1965). By lowering water potential, the PEG inhibits seed germination and growth, with the effect being more pronounced on shoots than primary roots (Khajeh-Hosseini, 2003; Yavari and Sadeghian, 2003).

Plants have the capacity to react to drought stress through several biological mechanisms, including strategies like drought avoidance, drought tolerance, drought resistance, and improvements to water use efficiency (WUE) (Yang et al., 2012; Hughes et al., 2017). Plants possess various adaptations, including morphological, physiological, and genetic mechanisms, to carry out strategies such as coping with drought stress. These adaptations involve regulating stomatal opening, adjusting osmotic potential, activating hormonal pathways, and potentially shortening their lifecycles (Yang et al., 2020).

Methodology

Study area

The Cholistan Desert is situated in southern Bahawalpur Punjab, Pakistan, covering a 26000 km^2 region between 27 42^0 and 29 45^0 N latitude and 69 52^0 and 75 24^0 E longitude, at an altitude of almost 112 m (Akhter and Arshad, 2006). Desert of Cholistan is two-thirds of Bahawalpur Division which makes about 8% of Punjab's total land area. Earlier governed as a portion of the districts of Bahawalpur (50% of the territory), Rahimyar Khan (40% of the area), and Bahawalnagar (10% of the land), Cholistan is now a portion of the formerly princely state of Bahawalpur (FAO, 1993; Rasheed et al., 2022). The climatic data of study duration is shown in figure 1.

The desert is too hot and can attain the temperature of 51.6 ^{0}C and extend from March to mid-September. Tremendously high temperatures divergence with restrained cold but mild winter. According to Arshad and Akbar (2002), summer temperature attains a height of 34 -48 ^{0}C, while the mean winter temperature is 15^0C. Underground water is brackish; so main source of water for agriculture, livestock or domestic purposes is rainfall water (Rasheed et al., 2022).

Seed physiology

Seed germination of selected grass species *A. lagopoides* and *O. compressa* under different drought conditions were studied. The research was conducted in the experimental area of Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan.

Polyethylene glycol (PEG-6000)

Seeds were collected from the Cholistan Desert of Bahawalpur from the healthy plants and were Surface sterilized using 0.1 percent sodium hypochlorite solution for 5 minutes, rinsed completely with distilled water. Then the seeds were grown in the pots having 5kg loamy soil for five months during summer season and treated with 10 ml deionized water (control), 10% and 20% PEG-6000. These germinating seeds were kept under environmental conditions. Pots were placed under green shed of the nursery of Institute of Forest Sciences to avoid dew and rain water availability to plants.

Experimental design

Each treatment given to plants was in solution form as water and was labeled with its name; T1 (which was control and 100% water), T2 (10% PEG-6000) and T3 (which was 20% PEG-6000). The experiment for this research work was completed in randomized design (CRD). In this design, there were three factors of drought stress levels and varieties with each treatment.

Morphological Attributes

Germination percentage

Emergence of seedlings was observed on daily basis till it achieved maximum germination.

Shoot length (cm),

Height of all replicas of tagged plants was measured by the scale (cm) from the end of last spikelet to the starting point of shoot.

Root length (cm)

All pots were irrigated well before removing the entire plant from the soil.

Leaf length (cm^2)

Leaf length was measured by the scale and then mean was calculated and recorded.

Number of leaves and number of nodes per plant

The number of leaves and nodes of all replicas of each treatment was noted by counting method.

Inter-nodal distance

The distance between two nodes is called inter-nodal distance or length. Inter-nodal length was calculated using a scale.

Relative Leaf Water Contents

Young leaves were used to measure the leaf relative water contents. For these young leaves from each treatment of every species were taken. Leaves were separated from the base of lamina and sealed in polythene bags. These were shifted to laboratory just after harvesting. Fresh weight (FW) was measured within 2 hours after harvesting. Then leaves were soaked in distilled water for 16-18 hour at 25^0 C and then carefully dry by using blotting tissue paper. After drying turgid weight (TW) was measured by using electrical weighing balance. Dry weight (DW) was measured after drying the samples in oven at 70^0 C for 72 hours. LRWC was calculated by using the formula as giving by (Lazcano-Ferrat and Lovatt 1999).

LRWC % = (FW-DW) / (TW-DW) x 100

Membrane Stability Index (MSI)

Membrane Stability Index (MSI) was measured by using the method of (Sairan and Saxena 2000). Leaf disc of (100 mg) were made and carefully cleaned by using tape water followed by wash with distilled water. Washed discs were heated in 10 ml distilled water at 40^0 C for 30 minutes. After this, Electrical Conductivity (C_1) was measured by using EC meter. The samples were kept in boiling water at (100^0 C) for 10 minutes. EC (C_2) was also noted at that time. MSI was calculated as MSI= [1-(C_1/C_2)] x 100

Gaseous exchange parameters

Gaseous exchange attributes such as Carbon dioxide absorbed, carbon assimilation, stomatal conductance (gs mmol H2O m^{-2} s^{-1}), photosynthesis rate (A µmol CO_2 $m^{-2}s^{-1}$, transpiration rate (E mmol H_2O $m^{-2}s^{-1}$) and water use efficiency (WUE µmolCO_2−2 s−/mmol$m^{-2}s^{-1}$) were recorded by using IRGA (infrared gas analyzer). Leaves of each germinated seedling from every treatment were kept under 10 cm^3 chamber of IRGA. Temperature of Leaf chamber was set at 37° C and reading was taken after 20 seconds placement. Data was collected during day time from 10 AM to 2 PM under full sunlight. Leaf temperature during gas exchange measurements was set 20 °C and constant gas flow was 500 µ$mols^{-1}$ (Farooq et al., 2022).

Statistical analysis

Analysis of variance for CR design, Mean values ± standard deviation (SD) were determined by using SPSS software. Linear correlation of seed germination was measured by using Microsoft excel. Pearson's correlation coefficient of morphological and physiological character against PEG-6000 was determined by using SPSS at a significance level of 0.01.

Results

Seed physiology of *Ochthochloa compressa* and *Aeluropus lagopoides*

Seed germination of *O. compressa* and *A. lagopoides* were observed under induced drought stress by using PEG-6000. At 3rd day germination was observed. Maximum seed germination was found at treatment T1 (control) in both species that was 78% in *O. compressa* and 72% in *A. lagopoides* as shown in figure 1 & 2. Linear correlation of seed germination of *O. compressa* and *A. lagopoides* was measured as shown in figure 1 & 2. The highest seed germination within treatments level was maximum in T1 which 78% germination with 0.873 coefficient of correlation (R-squared) in *O. compressa* species as shown in figure 1. The highest in *A. lagopoides* within treatments during T1 was maximum with 72% seed germination and 0.826 coefficient of determination (R-squared) and minimum was T3 which were 40 with R-squared 0.74 as shown in figure 2. Table 1 explains the significant effect of PEG-6000 on seed germination of both species.

Table 1: ANOVA of CR design for seed germination against induced drought stress PEG6000

Ochthochloa compressa					
Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	2	2.92	1.46	6.62	0.0018
Error	147	32.4	0.22		
Total	149	35.34			
Aeluropus lagopoides					
Treatment	2	2.77	1.38	6.07	0.0029
Error	147	33.6	0.228		
Total	149	33.37			

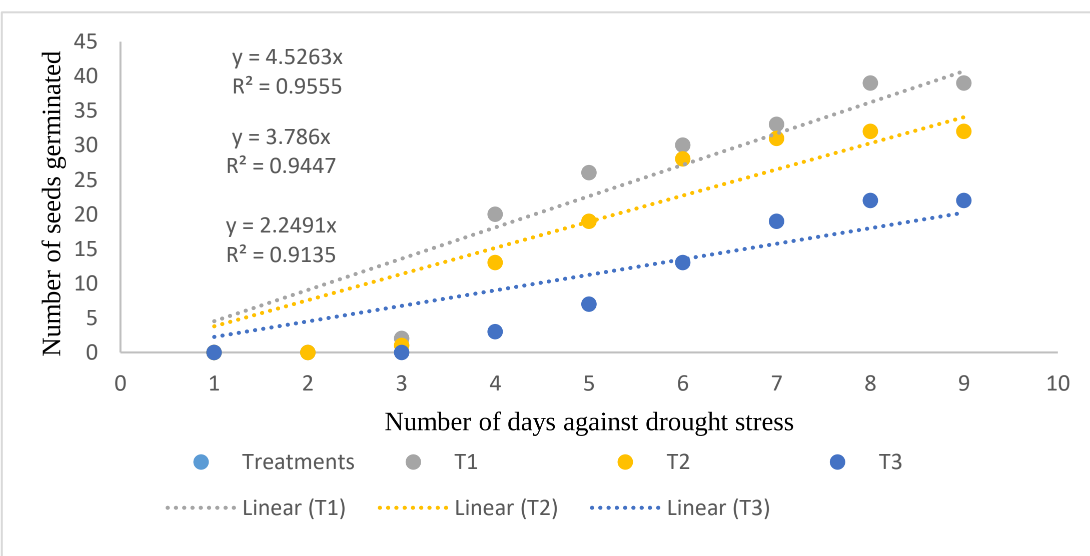

Figure. 1: Correlation and linear regression in *O. compressa* under induced drought stress PEG-6000

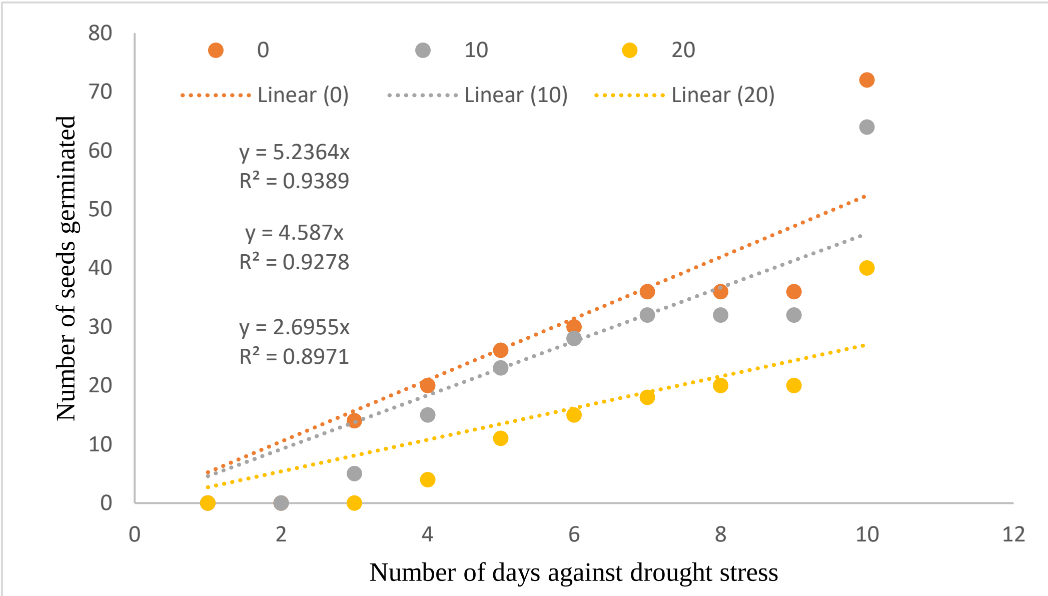

Figure. 2: Correlation and linear regression in *A. lagopoides* under induced drought stress PEG6000

Correlation among plants productivity and physiological parameters of *Ochthochloa compressa* and *Aeluropus lagopoides* against induced drought stress by PEG-6000

Pearson correlation was developed to study the effects of different induced drought stress PEG 6000 levels on plants production. The results showed that PEG-6000 induced drought stress has negative correlation on physiological and morphological parameters. Notably the highest negative correlation was found in MSI that was 0.958 followed by chlorophyll contents that was 0.954 as shown in table 2. While number of nodes has the lowest negative correlation that is 0.623. Table 3 describes the correlation of PEG-6000 in *A. lagopoides* against induced drought PEG-6000. Results showed that there strong negative correlation among all the parameters. Strongest negative correlation was found in MSI and gs 0.939 and 0.936 respectively (table3).

Table 2: Correlation among plants productivity and physiological parameters of *Ochthochloa compressa* against induced drought stress PEG6000

Pearson Correlation	Treatm-ents	Number of Leaves	Number of nodes	Fresh weight	MSI	LRWC	Chloroph-yll	Shoot length	Root length	A	E	gs	WUE
Treatments	1	-.876**	-.623**	-0.774	-.958[a]	-.953**	-.954**	-0.916	-.919[a]	-.932**	-.912**	-.914**	-0.833
Number of Leaves	-.876**	1	.614**	.725**	.874[a]	0.862	.815**	.850**	.813[a]	0.821	.845**	.845**	.766**
Number of nodes	-.623**	.614**	1	.661**	.601[a]	.600**	0.577	.547**	.619[a]	.606**	0.557	.589**	.406**
Fresh weight	-.774**	.725**	.661**	1**	.746[a]	.753**	.754**	.723**	.781[a]	.715**	.696**	0.749	.628**
MSI	-.958**	.874**	.601**	.746**	1[a]	.946**	.910**	.886**	.897[a]	.895**	.897**	.871**	.824**
LRWC	-.953**	.862**	.600**	.753**	.946[a]	1**	.910**	.895**	.903[a]	.898**	.902**	.907**	.801**
Chlorophyll	-.954**	.815**	.577**	.754**	.910[a]	.910**	1**	.851**	.864[a]	.879**	.865**	.864**	.772**
Shoot length	-.916**	.850**	.547**	.723**	.886[a]	.895**	.851**	1**	.895[a]	.876**	.857**	.878**	.845**
Root length	-.919**	.813**	.619**	.781**	.897[a]	.903**	.864**	.895**	1[a]	.867**	.839**	.865**	.799**
A	-.932**	.821**	.606**	.715**	.895[a]	.898**	.879**	.876**	.867[a]	1**	.895**	.887**	.783**
E	-0.912	0.845	0.557	0.696	0.897	0.902	0.865	0.857	0.839	0.895	1	0.831	0.789
Gs	-0.914	0.845	0.589	0.749	0.871	0.907	0.864	0.878	0.865	0.887	0.831	1	0.786
WUE	-0.833	0.766	0.406	0.628	0.824	0.801	0.772	0.845	0.799	0.783	0.789	0.786	1

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

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

Table 3: Correlation among plants productivity and physiological parameters of *Aeluropus*

Pearson Correlation	Treatments	Number of nodes	Number of Leaves	fresh weight	MSI	LRWC	Chlorophyll	shoot length	root length	A	E	gs	WUE
Treatments	1	-.877**	-.884**	-.878**	-0.939	-.868**	-.233**	-.924**	-0.691	-.811**	-.813**	-.936**	-.876**
Number of nodes	-.877**	1	.779**	.759**	.788**	0.747	.179**	.822**	.566**	0.754	.739**	.826**	.796**
Number of Leaves	-.884**	.779**	1	.779**	.823**	.761**	0.209	.829**	.666**	.700**	0.739	.863**	.775**
fresh weight	-.878**	.759**	.779**	1	.818**	.762**	.221**	0.803	.585**	.761**	.743**	0.795	.755**
MSI	-.939**	.788**	.823**	.818**	1**	.834**	.153**	.887**	.670**	.776**	.722**	.898**	0.836
LRWC	-.868**	.747**	.761**	.762**	.834**	1**	.171**	.855**	.529**	.666**	.669**	.812**	.743**
Chlorophyll	-.233*	0.179	0.209	.221*	.153*	0.171	1	.196*	.117*	0.284	0.162	.252*	0.235
shoot length	-.924**	.822**	.829**	.803**	.887**	.855**	.196**	1**	.609**	.767**	.778**	.855**	.820**
root length	-.691**	.566**	.666**	.585**	.670**	.529**	.117**	.609**	1**	.626**	.520**	.640**	.567**
A	-.811**	.754**	.700**	.761**	.776**	.666**	.284**	.767**	.626**	1**	.704**	.737**	.774**
E	-.813**	.739**	.739**	.743**	.722**	.669**	.162**	.778**	.520**	.704**	1**	.735**	.650**
gs	-0.936	0.826	0.863	0.795	0.898	0.812	0.252	0.855	0.64	0.737	0.735	1	0.844
WUE	-0.876	0.796	0.775	0.755	0.836	0.743	0.235	0.82	0.567	0.774	0.65	0.844	1

***lagopoides* against induced drought stress PEG-6000**

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

*. Correlation is significant at the 0.05 level (2-taile

Biomass production

Drought stress always has profound negative impact on biomass production in rangelands. Availability of judicious water supply increases biomass production. During the study maximum biomass was calculated at control T1 and its productivity decrease with the higher induced stress. Number of leaves and number of nodes are shown in figure 3 that describe the maximum number of leaves and nodes. At higher drought stress at T3=20% PEG6000 both the number of leaves and nodes were reduced to 2.54 and 2.3 accordingly. Similar kind of results were also observed in *A. lagopoides* as shown in figure 3.

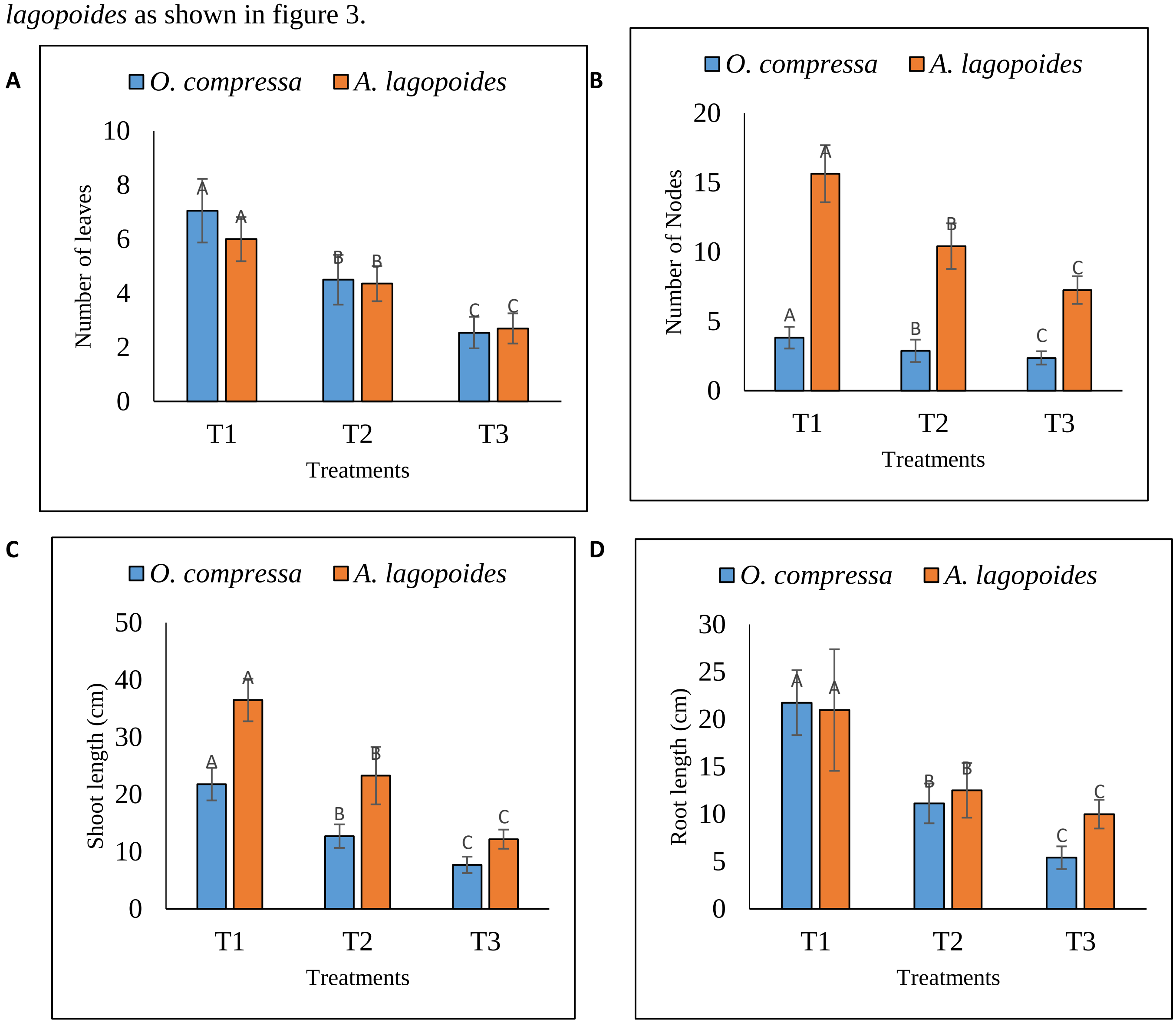

Figure 3: Effects of induced drought stress by PEG6000 on morphological attributes of *O. compressa* and *A. lagopoides*; **A:** Number of leaves; **B:** Number of nodes; **C:** Shoot length; **D:** Root length; Lettering A, B and C shows the highly significant results against all the treatments;

Alpha 0.05; Critical T Value 1.98

Biomass and chlorophyll

Maximum fresh weight and chlorophyll were observed at T1 8.1 and 0.8 at T2 5.4 and 0.6 while at T3 these values reduced to 3.2 and 0.4 as shown in figure 3. Chlorophyll in *A. lagopoides* observed the similar results and found maximum fresh weight and chlorophyll at T1 25.8 and 0.49 and reduced to 10.2 and 0.36 at T3 respectively as shown in figure 3.

Root/shoot ratio

Root and shoot always vary in xerophytes. In normal conditions; roots length in xerophytes are 3 to 4 time to the length of shoot. Shoot length in *ochthochloa compressa* at T1, T2 and T3 were 21.7, 12.7 and 7.6 respectively as shown in figure 3. In *Aeluropus lagopoides* at T1, T2 and T3 root length was 20.96, 12.4 and 9.9 respectively while shoot length was 36.4, 23.2 and 12.18 at each treatment accordingly.

Plant Physiology

Relative Leaf Water Contents (RLWC) and Membrane Stability Index (MSI)

RLWC and MSI decrease significantly with the elevated induced stress. At T1, T2 and T3 RLWC and MSI are shown in figure 4 for *A. lagopoides & O. compressa.* Maximum RLWC were 73.5 at T1, T2 52.7 and T3 39.1 while MSI at T1 44.8, T2 32.2 and T3 20.3 in *O. compressa.* RLWC and MSI in *A. lagopoides* at three treatments T1, T2 and T3 are 76.3, 60.6 and 41.5 respectively.

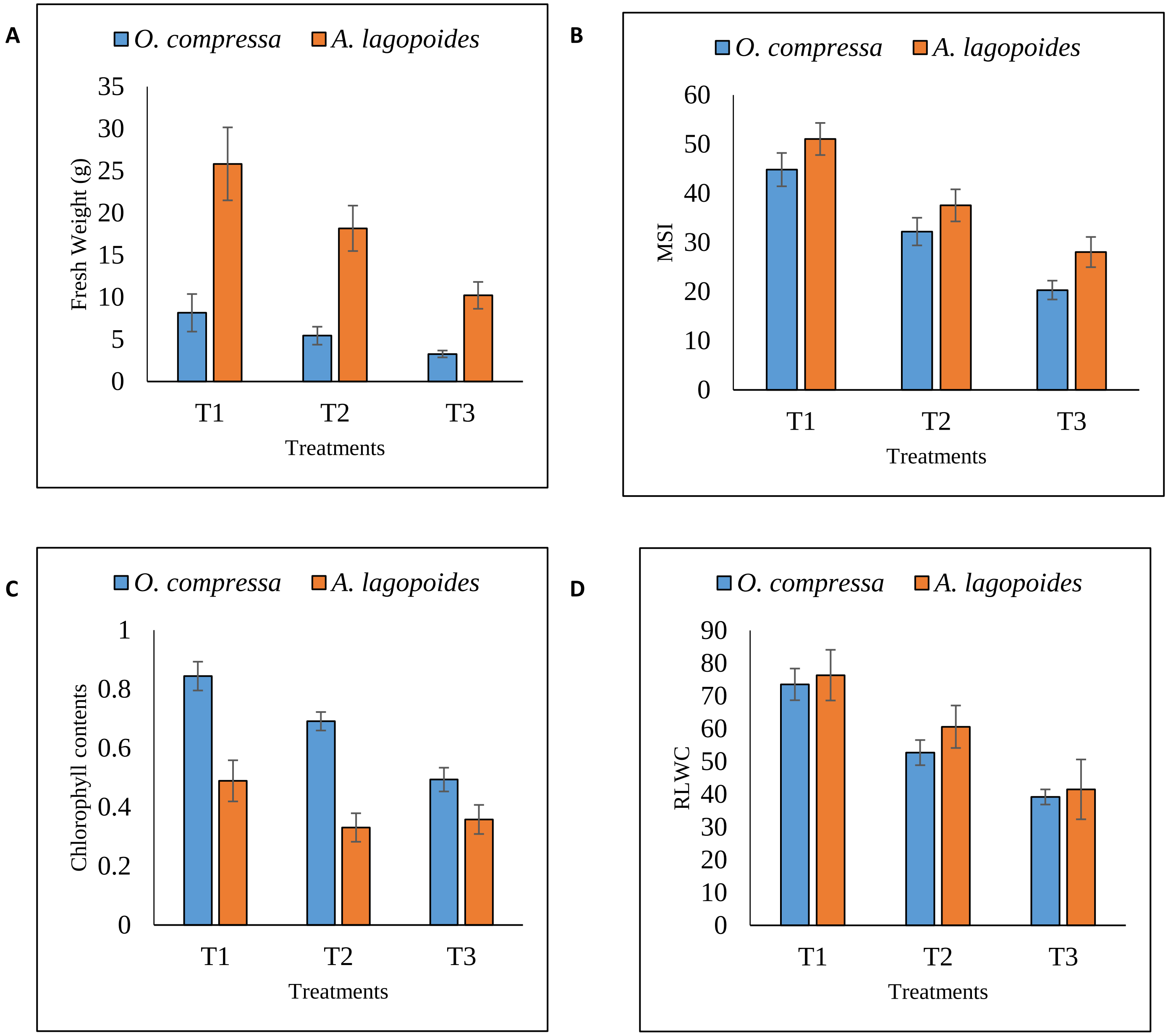

Figure 4: Effects of induced drought stress by PEG6000 on biomass and physiological characteristics of *O. compressa* and *A. lagopoides*; **A:** Fresh weight; **B:** Membrane Stability Index (MSI); **C:** Chlorophyll contents; **D:** Relative Leaf Water Contents (RLWC); Lettering A, B and C shows the highly significant results against all the treatments; Alpha 0.05; Critical T Value 1.98

Gaseous exchange

Gaseous exchange photosynthesis, stomatal conductance and transpiration is very important because all of the metabolic process run through these gaseous exchange in plants. Photosynthesis rate (A) and transpiration (E) in *O. compressa* against induced drought PEG6000 at T1 maximum transpiration and photosynthesis was noticed maximum that is 1.21 and 3.5 respectively. Photosynthesis and transpiration rate decrease significantly with increasing drought at T2 and T3 as shown in figure 5. Similar pattern was behaved by *A. lagopoides* against the stress and show maximum results at T1 for photosynthesis rate (A), stomatal conductance (gs), transpiration (E) and water use efficiency (WUE) as shown in figure 5.

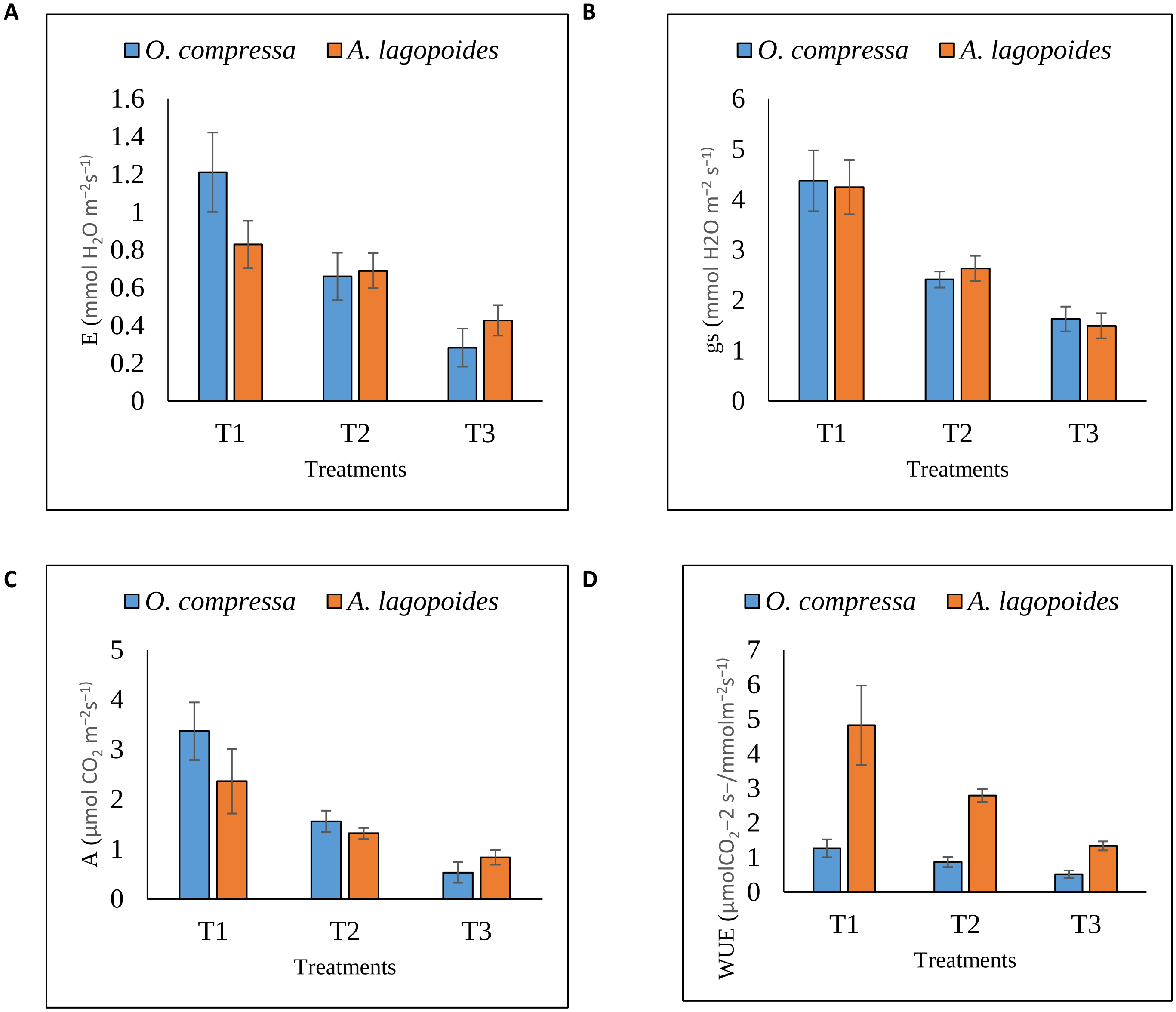

Figure 5: Effects of induced drought stress by PEG6000 on gaseous exchange attributes of *O.*

compressa and *A. lagopoides*; **A:** Transpiration rate (E mmol H_2O $m^{-2}s^{-1}$); **B:** stomatal conductance (gs mmol H2O m^{-2} s^{-1}); **C:** photosynthesis rate (A μmol CO_2 $m^{-2}s^{-1}$; **D:** water use efficiency (WUE μmolCO_2−2 s−/mmol$m^{-2}s^{-1}$); Lettering A, B and C shows the highly significant results against all the treatments; Alpha 0.05; Critical T Value 1.98

Discussion

There are number of environmental factors that affect the plant germination, production and geographical representation while drought stress is the most hazardous factor that limit the seed viability and germination (Choat et al., 2012). Our results are correlated with the Choat et al., (2012), in this study lowers the seed germination, its productivity and physiological functions with the elevated induced drought stress through PEG-6000 as shown in figure 1 and 2. Blackman et al., (2018) studied that drought stress is the one when there is water deficiency in the root zone and plants loose its turgid pressure, however they fail to perform its physiological functions. Liu et al., (2022) also studied the drought tolerance in *Psammochloa villosa* species belonging to family Poaceae. The species is forage species in arid region of Inner Mongolian Plateau and the Qinghai- Tibet Plateau of China desert (Chen and Phillips 2006). Liu et al., (2022) found that seeds of *P. villosa* germination under induced drought through PEG-6000 reduced under 10% PEG-6000 and further decreased in seed germination at 20% PEG-6000 while control level shows the maximum seed germination. Similar findings were noticed in our results that both species showed reduction in seed germination at 10% and 20% PEG-6000 induced drought stress. The species are indigenous to desert region and are very important from forage point of view.

Plants production were also lower at elevated drought stress figure 3 and 4. Wang et al., (2021) found reduction in buds growth under drought stress which is an indication of lower plant production and are in line with our findings as we have shown in our results. Goswami, and Rankawat (2020) studied the fresh & dry weight, Relative Leaf Water Content RLWC, chlorophyll, root/shoot length, and physiological traits. They found the variation in these parameters and their values reduced against higher induced stress by using PEG-6000. Further study in our research includes the physiological parameters. Liu et al., (2022) also found that chlorophyll contents reduced with the increasing drought stress and we have found the same results and chlorophyll contents reduce at 10% and 20% PEG-6000 treatments as shown in figure 4. They also discussed the similar results of (Hortensteiner, and Krautler 2011) which are related to chlorophyll contents. Kizilgeci et al., (2017) studied the impact of five distinct drought stress (using PEG solution at 0, -0.3, -0.6, -0.9 and -0.12 MPa) on the germination of four different bread wheat cultivars (Tekin, Pehlivan, DZT13-2, and DZT13-1). They found that seedling growth dramatically impacted with the increasing concentration of PEG solution. Germination percentage, seedling vigor, coleoptile length, root length, and shoot length were decreased with the increasing concentration of induced drought by PEG.

Drought stress reduced the plant's production as water deficiency makes them looseness of turgid pressure and unable to perform various physiological functions such as photosynthesis, transpiration, water use efficiency (Arora et al., 2002; Nadeem et al., 2021). Nadeem et al., (2021) studied the drought effect on physiology and growth of maize and found that drought stress showed negative correlation on A, E, gs and WUE ads same results were witnessed in our research that induced drought by using PEG6000 destructively affected the physiological factors photosynthesis rate (A), transpiration rate (E), water-use efficiency (WUE) and stomatal conductance (gs). Drought effect also witnessed the severe damage to plant physiology (Arora et al., 2002) and this is because of reduction on leaf area , WUE gs that ultimately reduce the A and E rate (Osmolovskaya et al., 2018). Nadeem et al (2021) also discussed the results of Ahmad et al., (2013) and they said induced drought stress are negatively correlated with the physiology of plants. Our results are similar with the Zhu et al., (2021) who investigate the PEG-DS induced drought against rice and find the reduction in A, gs, Ci, with the increasing drought stress. Screening of drought tolerant species would provide us a useful insight in successful re-vegetation of rangelands based on their adaptability to various drought levels and climatic conditions.

Acknowledgement

Muhammad Rafay, Tanveer Hussain, Muneeb Khalid, Muhammad Madnee, Hussain Ahmad Makki are highly acknowledged for their contribution during the research work.

References

Afzal, S., Gul, S., & Mehmood, H. M. Z. (2023). Conversion of land utilization, Chickpea to Eucalyptus on marginal lands for the betterment of countryside. *Global Forests*, *2*(01), 26-32.

Ahmad, M., Zahir, Z. A., Khalid, M., Nazli, F., & Arshad, M. (2013). Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiology and Biochemistry, 63, 170-176.

Akbar, G. and Arshad, M., 2000. Developing Sustainable Strategies for Cholistan Desert: Opportunities and perspectives. Science Jision, 5 (2): 77-85.

Akbar, M., Qadir, I., & Arif, A. (2022). Assessment of carbon sequestration rate in eucalyptus-based irrigated forest plantation Bahawalpur. *Global Forests*, *1*(01), 33-53.

Akhter, R. And M. Arshad. 2006. Arid rangelands in the Cholistan desert (Pakistan). Science et changements planétaires/Sécheresse, 17: 210-217

Ali, M. A., Shah, Y. R., Din, S., & Saqib, M. (2023). Climatic Variability's Influence on Cereal Harvests in Punjab, Pakistan: A Comprehensive Analysis and Mitigation Strategies. Journal of Food and Agricultural Technology Research, 2(01), 92-104.

Ali, M. A., Shah, Y. R., Din, S., & Saqib, M. (2023). Climatic Variability's Influence on Cereal Harvests in Punjab, Pakistan: A Comprehensive Analysis and Mitigation Strategies. *Journal of Food and Agricultural Technology Research*, *2*(01), 92-104.

Ali, R., Turabi, T. H., & Naseer, J. (2022). Community participation and support for urban forestry in Bahawalpur: Barriers and opportunities. *Global Forests*, *1*(01), 54-65.

Almansouri, M., Kinet, J. M., & Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.). *Plant and soil*, *231*, 243-254.

Aminullah, A., Ashfaq, M., Khan, M. A., Khan, S. Z., Ashraf, M. R., Tariq, N., ... & Khan, A. (2023). Integrated Pest Management (IPM) of Fruit Fly (Diptera: Tephritidea) in Citrus at District Faisalabad. *Journal of Food and Agricultural Technology Research*, *2*(01), 69-91.

Arora, A., Sairam, R. K., & Srivastava, G. C. (2002). Oxidative stress and antioxidative system in plants. *Current science*, 1227-1238.

Arshad, M. and Akbar, G., 2002. Benchmark of plant communities of Cholistan desert [Pakistan]. Pakistan Journal of Biological Sciences (Pakistan).

Ashraf, M. H. P. J. C., & Harris, P. J. (2013). Photosynthesis under stressful environments: An overview. Photosynthetica, 51, 163-190.

Asif, M., Naseer, J., Turabi, T. H., Khan, R. I., & Maryam, A. (2023). Drought induced molecular and physiological adaptations in Ziziphus mauritiana and Salvadora oleoides under climate change scenario. *Global Forests*, *2*(01), 1-17.

Baghyalakshmi, K., Ramchander, S., Raveendran, M., & Jeyaprakash, P. (2020). Unravelling of Osmotic genes involved in Drought tolerance in Backcross inbred lines of rice (Oryzasativa L.) cultivars. Research Journal of Biotechnology Vol, 15, 7.

Blackman, C. J. (2018). Leaf turgor loss as a predictor of plant drought response strategies. Tree physiology, 38(5), 655-657.

Brown, S. C., Gregory, P. J., Cooper, P. J. M., & Keatinge, J. D. H. (1989). Root and shoot growth and water use of chickpea (Cicer arietinum) grown in dryland conditions: effects of sowing date and genotype. The Journal of Agricultural Science, 113(1): 41-49.

Brum, M., Oliveira, R. S., López, J. G., Licata, J., Pypker, T., Chia, G. S., ... & Asbjornsen, H. (2021). Effects of irrigation on oil palm transpiration during ENSO-induced drought in the Brazilian Eastern Amazon. Agricultural Water Management, 245, 106569.

Chen, S.L., Phillips, S.M., 2006. Orinus. In: Wu, C.Y., Raven, P.H., Hong, D.Y. (Eds.), Flora of China 22. Science Press and St. Louis: Missouri Botanical Garden Press, Beijing, pp. 464-465.

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., ... & Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. Nature, 491(7426), 752-755.

Emmerich, W. E., & Hardegree, S. P. (1990). Polyethylene glycol solution contact effects on seed germination. Agronomy Journal, 82(6), 1103-1107.

Ennajeh, M., Vadel, A. M., Cochard, H., & Khemira, H. (2010). Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. The Journal of Horticultural Science and Biotechnology, 85(4), 289-294.

Farooq, T. H., Rafay, M., Basit, H., Shakoor, A., Shabbir, R., Riaz, M. U., ... & Jaremko, M. (2022). Morpho-physiological growth performance and phytoremediation capabilities of selected xerophyte grass species toward Cr and Pb stress. Frontiers in Plant Science, 13, 997120.

Fotovat, R., Valizadeh, M., & Toorchi, M. (2007). Association between water-use efficiency components and total chlorophyll content (SPAD) in wheat (Triticum aestivum L.) under well-watered and drought stress conditions. Journal of Food Agriculture and Environment, 5(3/4), 225.

Goswami, B., Rankawat, R., & Gadi, B. R. (2020). Physiological and antioxidative responses associated with drought tolerance of Lasiurus sindicus Henr. endemic to Thar desert, India. Brazilian Journal of Botany, 43, 761-773.

Hortensteiner, S., & Krautler, B. (2011). Chlorophyll breakdown in higher plants. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1807(8), 977-988.

Huang, Y. C., Niu, C. Y., Yang, C. R., & Jinn, T. L. (2016). The heat stress factor HSFA6b connects ABA signaling and ABA-mediated heat responses. Plant physiology, 172(2), 1182-1199.

Hughes, J., Hepworth, C., Dutton, C., Dunn, J. A., Hunt, L., Stephens, J., ... & Gray, J. E. (2017). Reducing stomatal density in barley improves drought tolerance without impacting on yield. Plant physiology, 174(2), 776-787.

Iqbal, A., Zulfiqar, U., Ehsan, G. M., & Khan, R. (2022). Application of urea significantly enhanced the growth and biomass production in Terminalia arjuna seedlings. *Global Forests*, *1*(01), 25-32.

Iqbal, M., Hussain, D., Javed, M. Y., Ellahi, F., Asim, M., Adeel, M., ... & Baqir, M. (2023). In Vitro Efficacy Evaluation of Agrochemical Fungicides in the Mitigation of Colletotrichum Capsici Infection. *Journal of Food and Agricultural Technology Research*, *2*(02), 128-135.

Ismail, M., Mujtaba, A., Ahmed, E., Saleem, M. A., & Jakhro, M. I. (2023). Adoption of Drip Irrigation System in Southern Punjab-Pakistan: Challenges and Options. *Journal of Food and Agricultural Technology Research*, *2*(01), 35-45.

Jiang, Y., MacDonald, S. E., & Zwiazek, J. J. (1995). Effects of cold storage and water stress on water relations and gas exchange of white spruce (Picea glauca) seedlings. Tree physiology, 15(4), 267-273.

Khajeh-Hosseini, M., Powell, A. A., & Bingham, I. J. (2003). The interaction between salinity stress and seed vigour during germination of soyabean seeds. Seed Science and technology, 31(3), 715-725.

Kizilgeci, F., Tazebay, N., Namli, M., Albayrak, O., & Yildirim, M. (2017). The drought effect on seed germination and seedling growth in bread wheat (Triticum aestivum L.). *International Journal of Agriculture Environment and Food Sciences*, *1*(1), 33-37.

Lagerwerff, J. V., Ogata, G., & Eagle, H. E. (1961). Control of osmotic pressure of culture solutions with polyethylene glycol. Science, 133(3463), 1486-1487.

Lazcano-Ferrat, I. and C.J. Lovatt. (1999). Relationship between relative water content, nitrogen pools, and growth of *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray during water deficit. *Crop Science*, 39(2), 467-475.

Li, H., Li, X., Zhang, D., Liu, H., & Guan, K. (2013). Effects of drought stress on the seed germination and early seedling growth of the endemic desert plant Eremosparton songoricum (Fabaceae). Excli Journal, 12, 89.

Lian, X., Piao, S., Chen, A., Huntingford, C., Fu, B., Li, L. Z., ... & Roderick, M. L. (2021). Multifaceted characteristics of dryland aridity changes in a warming world. Nature Reviews Earth & Environment, 2(4), 232-250.

Liu, T., Liu, Y., Fu, G., Chen, J., Lv, T., Su, D., ... & Harris, A. J. (2022). Identification of genes involved in drought tolerance in seedlings of the desert grass, Psammochloa villosa (Poaceae), based on full-length isoform sequencing and de novo assembly from short reads. Journal of Plant Physiology, 271, 153630.

Macklon, A. E. S., & Weatherley, P. E. (1965). Controlled environment studies of the nature and origins of water deficits in plants. New Phytologist, 64(3), 414-414.

Monakhova, O. F., & Chernyad'ev, I. I. (2002). Protective role of kartolin-4 in wheat plants exposed to soil draught. Applied Biochemistry and Microbiology, 38, 373-380.

Nadeem, S. M., Ahmad, M., Tufail, M. A., Asghar, H. N., Nazli, F., & Zahir, Z. A. (2021). Appraising the potential of EPS-producing rhizobacteria with ACC-deaminase activity to improve growth and physiology of maize under drought stress. Physiologia plantarum, 172(2), 463-476.

Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., ... & Wessjohann, L. A. (2018). Methodology of drought stress research: Experimental setup and physiological characterization. International journal of molecular sciences, 19(12), 4089.

Rasheed, S. et al., 2022. Ecological assessment and indicator species analyses of the Cholistan desert using multivariate statistical tools. Pak. J. Bot, 54(2): 683-694.

Rawson, H. M., & Turner, N. C. (1982). Recovery from water stress in five sunflower (Helianthus annuus L.) cultivars. II. The development of leaf area. Functional Plant Biology, 9(4), 449-460.

Sairam, R. K. and D.C. Saxena. (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *Journal of Agronomy and Crop Science*, 184(1), 55-61.

Saxena, M. C. & Singh, K. B., (Eds.). (1993). Breeding for stress tolerance in cool-season food legumes (No. 635.65 S52). Chichester, UK: Wiley.

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., ... & Battaglia, M. L. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants, 10(2), 259.

Shao, H. B., Chu, L. Y., Jaleel, C. A., Manivannan, P., Panneerselvam, R., & Shao, M. A. (2009). Understanding water deficit stress-induced changes in the basic metabolism of higher plants–biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Critical reviews in biotechnology, 29(2), 131-151.

Shivakrishna, P., Reddy, K. A., & Rao, D. M. (2018). Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi journal of biological sciences, 25(2), 285-289.

Siddiqui, M. N., Léon, J., Naz, A. A., & Ballvora, A. (2021). Genetics and genomics of root system variation in adaptation to drought stress in cereal crops. Journal of Experimental Botany, 72(4), 1007-1019.

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates Inc., Publishers

Ullah, H., Ahmed, N., Khan, N. A., Senkus, P., Borda, R. C., & Huamán, J. M. C. (2023). Data-driven Approach to Assessing the Sustainability of Agricultural Supply Chains: A Review of Frameworks and Methodologies. *Journal of Food and Agricultural Technology Research*, *2*(01), 01-34.

Ullah, M. I., Ahmad, I., Yasin, G., Saeed, A., Asif, M., Asghar, M., & Tariq, M. U. (2023). Economic Share of Agroforestry in Boosting Living Status of Farmers Community: A Case Study of District Faisalabad. *Journal of Food and Agricultural Technology Research*, *2*(01), 54-68.

Wang, Y.G., Zhang, S.J., Liu, Y.L., Fan, Y.X., Guo, C.Q., Ma, Y.M., 2021. Identification and screening of drought tolerance in winter wheat cultivars in Xinjiang during germination period. Xinjiang Agric. Sci. 58, 2024–2034.

Wolters, H., & Jürgens, G. (2009). Survival of the flexible: hormonal growth control and adaptation in plant development. Nature Reviews Genetics, 10(5), 305-317.

Xiao, S., Liu, L., Zhang, Y., Sun, H., Zhang, K., Bai, Z., ... & Li, C. (2020). Fine root and root hair morphology of cotton under drought stress revealed with RhizoPot. Journal of Agronomy and Crop Science, 206(6), 679-693.

Xing, X., Jiang, H., Zhou, Q., Xing, H., Jiang, H., & Wang, S. (2016). Improved drought tolerance by early IAA-and ABA-dependent H 2 O 2 accumulation induced by α-naphthaleneacetic acid in soybean plants. Plant Growth Regulation, 80, 303-314.

Yang, J., & Wang, Y. (2012). Effects of drought stress simulated by PEG on seed germination of four desert plant species. Acta Prataculturae Sinica, 21(6), 23-29.

Yang, L., Feng, Y. X., & Yu, X. Z. (2020). Comparative response of SOD in different plants against cadmium and drought stress at the molecular. Appl. Environ. Biotechnol., 5(1), 15-28.

Yavari, N., & Sadeghian, Y. (2003). Use of mannitol as a stress factor in the germination stage and early seedling growth of sugar beet cultivation in vitro. J Sugar Beet, 17, 37-43.

Zafa, M. S., Mehmood, M., Iqbal, S., & Qureshi, M. A. (2023). Varying Sowing Time Influences Morphological Attributes of Zinnia Cultivars. *Journal of Food and Agricultural Technology Research*, *2*(01), 46-53.

Zafar, F., ul Haq, H., & Shafae, G. (2023). Phytoremediation potential of Vachellia nilotica under cadmium-contaminated soil by using organic amendments. *Global Forests*, *2*(01), 18-25.

Zhu, G., Gu, L., Shi, Y., Chen, H., Liu, Y., Lu, F., ... & Zhou, G. (2021). Plant hydraulic conductivity determines photosynthesis in rice under PEG-induced drought stress. *Pak. J. Bot*, *53*(2), 409-417.