IDENTIFICATION OF DROUGHT-TOLERANT WHEAT (*TRITICUM AESTIVUM L*) CULTIVARS BASED ON THE ASSOCIATIONS OF *IN-VITRO & IN-VIVO* PREDICTORS THROUGH POLYETHYLENE GLYCOL (PEG 6000) MEDIATED OSMOTIC STRESS

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ABSTRACT

Drought tolerance has been the criterion under water stress conditions. The present study was therefore evaluated in six wheat genotypes viz. (IBWSN-1010, IBWSN-1025, TD-1, ESW-9525,Khirman and Chakwal-86). Experiment first occupied with different polyethylene glycol (PEG 6000) concentration 0.0, -0.5, -0.75, -1.0 MPa, where is another experiment was laid out complete randomized design (CRD) along with two treatments (T1 control normal four irrigations) and (T2 terminal drought T. drought) conditions with replication thrice, has been used to induce drought tolerance. Different seedling parameters as agronomical traits, named, Plant height (cm), Spike length (cm), Number of Tiller Plant⁻¹, Straw weight/plant (g), 100 Grain weight (g), Seed index (g), Days to 75% heading; Days to 75% maturity. The results depicted that ESW-9525 and IBWSN-1010 wheat cultivars performed better as compared to both check varieties (Khirman and Chakwal-86). The mean squares of variance showed that different osmotic potential had meaningful impact on most of the characters excluding root dry weight. Moreover, using all seedling growth parameters, under consideration can be utilized as selective characters to evaluate among resistant and susceptible cultivars in water stress under laboratory experiment and breeding programs seems to be additional desired for evaluation in water stress.

Keywords: In-Vitro & In-Vivo screening, wheat cultivars, early seedling stage, ionic content, agronomical traits, PEG (6000), osmotic stress.

INTRODUCTION

Wheat (Triticumaestivum L.) is a major cereal above than 35% percent of all around the world residents and wheat is also the first grain crop in most emerging countries.(Metwaliet al., 2011). Bread wheat is the major food cereal for many people's living in different countries and about 80 % of protein and 70 % of calories of human is supplied from its consumption (Tareghet al., 2011).Environmental stress, particularly water stress, is considered a major problem and severely restricts global plant production (Pan et al., 2002). It is one of the main reasons for yield loss worldwide, which translates into more than 50% lower average yields for most crops (Wang et al., 2003; Bayoumi et al., 2008). Maximum plant yield under normal irrigation conditions is not necessarily associated with high yield under drought stress (Vahidi et al., 2009). Experience has shown that a plant reacts differently to stress under water stress at each stage of growth (Galies et al., 1983).Drought can affect at any time of the plant life, but there are some specific stages that are more serious such as germination and seedling

growth (Pessarakliet al., 1999).Some of the most important factors in determining yield are seed germination and seedling growth (Raufet al., 2007; Dhandas et al., 2004 and Heikalet al., 1981) showed vigour index of grains and length of shoot are considered as the greatest complex for drought stress, similarly by length of root and length of coleoptiles as well. Several researches like choosing plant of genus or else treatment of seeds which are supportive for improving the harmful effect of water stress on plant (Iqbalet al.,2007). Estimation of drought resistance during the initial stage of a seedling is often accomplished by imitation efficiency tempted by substances like PEG-6000. PEGs are mainly used to modify the osmotic potential of cultures in a nutrient solution, that is, the water shortage of artificial plants, in a relatively controlled way (Carpita et al., 1979; Money et al., 1989; Zhu et al., 1997; Luand Neuman 1998; Kulkarni and Deshpande, 2007) found that PEG molecules are immobile, non-ionic, almost resistant to water for cell membranes and can stimulate uniform drought stress that does not cause deterioration

direct physical. Poly ethylene glycol PEG as an aspect water stress caused by tumbling water potential outcome in decreasing of development in seed germinate. (Khaheh et al., 2000; Zhu et al., 2006; Dodd and Donovan 1999). It examine that Poly ethylene glycol stops water assimilation by seeds, penetrable ions by falling potential inside cell results in water assimilation and initial to germinated. Therefore the experiments were conducted to isolate 5 genotypes of wheat that possess drought tolerance at germination, seedling period. This study is about to see the use of Poly ethylene glycol and osmoticum, to induce water stress conditions.

MATERIALS AND METHODS

The research trail was conducted at Nuclear institute of Agriculture Tandojam. Both experiments were performed to evaluate the effect of drought stress conditions with PEG6000 on the growth of seedling parameters in wheat. The experiment was configured in factorial and completely randomized CRD design with three replications. Six varieties of wheat were used (IBWSN1010, IBWSN1025, TD1, ESW9525, Khirman and Chakwal86). The grains of the six varieties were obtained from the Department of Genetics and Plant Improvement of the Tandojam Nuclear Institute of Agriculture. The PEG6000 was adjusted by dissolving the desired amount of PEG6000 in distilled water at 30 ° C. Subsequently, the grains of six wheat genotypes were sterilized with a 10% sodium hypochlorite solution for approximately 30 seconds. The beans were washed twice separately after treatment with distilled water. 20 seeds of each variety were sown in glass dishes (intensive 15 and 10 cm) treated with PEG6000. The glass plates are placed in an incubator under a photoperiod (4.9 ¹/₄ mol m2 s1) for 20 days. Grains were measured as sprouts if they had an additional root space of> 3 mm. After soaking every 24 hours, the germinated grains were produced daily during the experiment to determine the following parameters of the seedling. seed germination, radicle length, coleoptile length, shoot length cm, root length cm, seedling weight. fresh shoot g, fresh root weight g, dry shoot weight g, dry root weight g and the content of ions (Ca and K) as the total content of chlorophyll (mg g1 Fresh weight), shoot potassium K + (%), Root potassium content (%), draft calcium (%), root calcium (%) were also observed in the shoots and roots of wheat varieties using the flame photometer method. While the other experiment was carried out in the pot house, the seeds were sown in cemented tanks under

control (size 3x3x1 cubic meters), filled with sandy loam soil, with three repetitions in the CRD Complete Random Design, agronomic characteristics were observed, named, plant height (cm), ear length (cm), number of plow plants1, straw/plant weight (g), 100-grain weight (g), days up to 75% of the ear, days to 75% maturity. The objective of this study was the screening of wheat varieties (Triticum aestivum L.) under conditions of water stress in the seedling stage using PEG 6000.

Statistical analysis: The data recorded were subjected to analysis of variance to discriminate the superiority of treatment means and LSD tests were applied by the following method of (Gomez and Gomez 1984) to compare the means.

RESULTS AND DISCUSSIONS

Seed germination (%) under PEG water stress: In the present study, the capacity of the six genotypes was evaluated with two control varieties Khirman & Chakwal86 of wheat under chemical drying induced by PEG (6000) during the early seedling stage under in vitro conditions. The relevant data for the effect of PEG-induced osmotic stress on seed germination are shown in Figure 1.





Seeds of six wheat genotypes were germinated after 120 hours at various PEG values (0, 0.5, 0.75, 1.0 MPa). In all wheat genotypes, seed germination decreased with increasing water stress. It was found that the reduction was greater than 1.0 MPa, followed by 0.75, 0.5 MPa and the control, since the corresponding mean values for seed germination were 67.77, 88.33, 92.22 and 99.44%, respectively. Under control conditions, varieties IBWS1010, IBWS1025, TD1, ESW9525 and Chakwal86 showed maximum seed

germination (100%), while in Khirman under control conditions germination of 96.66% was observed. (Chachar et al., 2016) reported that the germination percentage of various wheat genotypes decreased with increasing water stress. When the maximum PEG concentration (6000) (1.0 MPa) was used, the Chakwal86 and Khirman genotypes also exceeded the maximum seed germination percentage (98.33 and 95%. respectively). On the other hand, in ESW9525 (40%), minimal germination was recorded under high water stress (1.0 MPa). The results found in our study regarding the germination of wheat genotypes with the conditions induced by PEG with a reduction of germination of wheat genotypes fully agree with the result of Mirbahar et al., 2013; Chachar et al., 2016; Surbhaiyya et al., 2018).

Coleoptile length (cm): The coleoptile length results of various wheat genotypes that are influenced by PEG stress (6000) are shown in Fig. 2.



Figure-2: Effects of polyethylene glycol PEG (6000) on coleoptiles length of different wheat cultivars

A minimum coleoptile length was observed compared to the control condition in all treatments at a concentration of 1.0 Mega Pascal (MPa). Seedling development under laboratory conditions has been accepted and it could be speculated that an appropriate growth stage to test drought tolerance in wheat is that the presence of high levels of PEG during seedling growth inhibits traits of development and survival of wheat seedlings. In our study, the length of the coleoptile decreased with increasing PEG concentration. While working under the controlled conditions induced by PEG (Surbhaiyya et al., 2018), it was observed that with the increase in the

concentration of PEG 6000, the length of the coleoptile of various wheat genotypes decreased significantly. The coleoptile length of different cultivars differed due to the different osmotic potentials of PEG. Under normal conditions (control) the maximum length value of coleoptile was recorded for IBWSN1010 (10.68 cm), while the TD1 variety recorded the lowest value (10.88) cm). followed by IBWSN1025 (9.93cm), ESW9525 (15.86 cm) compared to local controls Khirman & Chakwa 186 (11.9, 12.22 cm). Under treatment with high PEG (1.0 MPa), the maximum coleoptile length was recorded in ESW9525 (4.09 cm), followed by the genotypes IBWSN1010 (5.29 cm) and IBWSN1025 (5.06 cm), while the minimum coleoptile length in TD1 (9.68 cm) was recorded. Chachar et al., (2014a, 2014b) and (Mirbahar et al., 2013) reported the tendency to decrease the length of coleoptiles under increasing osmotic stress was reported by Chachar et al., (2014a, 2014b) & (Mirbahar et al., 2013).

Radicle length (cm): In our experiment, the radicle length parameter fig3, the wheat variety ESW9525 showed the maximum root length (10.04 cm), while the wheat variety IBWSN1010 (8.62 cm), IBWSN1025, (6.98 cm) and TD1 (5.34 cm) maximum observed radical length compared to local test truths Khirman and chakwal86 (8.92, 9.92) under control treatment. While the wheat variety IBWSN1010 (4.83 cm) exhibited the minimum radicle length associated with 1.0 MPa PEG6000, the wheat variety TD1 (5.67 cm) observed the maximum root length treatment, as shown in FIG. Many other scientists (Chachar et al., 2016; Chachar et al., 2014a, 2014b) have also reported the decreasing trend in radical length, and found that water stress had a significant impact on radical length.



Figure-3: Effects of polyethylene glycol PEG (6000) on radicle length of different wheat cultivars.

Shoot length cm: Under the selection technique, the survival capability of six wheat genotypes for resistant chemical dehydration by PEG-6000 during the seedling stage showed fig-4.



Figure 4: Effects of polyethylene glycol PEG (6000) on shoot Length of different wheat cultivars

Among most the treatments, -1.0 MPa concentrations revealed minimum shoot length as compared to other concentrations. At the -0.5 MPa PEG6000 concentration, the IBWSN-1025 produced a minimum shoot length 12.56 cm, whereas IBWS-1010 revealed a maximum length 16.43cm as compared to the check varieties Khirman 17.60cm Chakwal-86 15.69cm. The relative decrease over control exhibited more in IBWS-1025 -7.13. Moreover, the osmotic stress at -0.75MPa PEG, TD-1 showed a minimum shoot length 12.33cm and IBWSN-1010 showed a maximum shoot of 15.72 cm. At the highest PEG stress -1.0MPa the cultivars ESW-9525 produced a minimum shoot length 4.09cm and TD-1 revealed a maximum length 9.68cm. This value of PEG stress was more decreased over the control recorded as 24.85cm as compared with checks. Accordingly, TD-1 exposed an overall relative decrease for 0.75 MPa 4.25 cm and 1.0MPa 24.85 cm as compared to the checks. It is also reported by (Almaghrabiet al., 2012) that PEG effect on shoot length of different wheat cultivars.

Root length (cm): Effects of poly ethylene glycol PEG (6000) stress on root length the results of root length of different wheat cultivars, the results for the effect of PEG stress on root length are shown in fig-5.



Figure-5: Effects of polyethylene glycol PEG (6000) on root Length of different wheat cultivars

Among all the treatments -1.0MPa concentration revealed minimum root length as compared to other concentrations. At the -0.5 MPa PEG6000 concentration, the IBWSN-1025 produced a maximum root length 14.11cm, whereasTD-1 revealed a minimum length 10.30cm as compared to the check varieties Khirman 12.92cm Chakwal-86 11.81cm. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 showed a minimum root length 8.17cm and IBWSN-1025 showed maximum root as 12.07cm. At the highest PEG stress -1.0MPa the cultivarsTD-1 produced maximum root length 8.67cm and IBSWN-1010 and ESW9-525 revealed minimum root length 4.83, 4.82cm (Chachar et al., 2014) stated that restriction for the length of roots in drought stress because of an obstruction of cell division and elongation leading to kind tuberization.

Shoot fresh weight (g/10 shoots): The results for the effect of PEG6000 stress on Plant biomass are presented in fig-6.



Figure-6: Effects of polyethylene glycol PEG (6000) on shoot fresh weight of different wheat cultivars.

Among all the treatments -1.0MPa concentration revealed minimum shoot fresh weight SFW as compared to other concentrations. At the -0.5 MPa PEG (6000) concentration, the ESW-9525 produced a minimum shoot fresh weight 49.48g. IBWSN-1010 whereas revealed maximum SFW86.64g as compared to the check varieties Khirman 85.77g Chakwal-86 91.86g. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 showed minimum SFW 31.75g and IBWSN-1010 showed maximum SFW as 79.35g. At the highest PEG stress -1.0MPa the cultivarsTD1 produced a maximum SFW 54.63g and ESW-9525 revealed a minimum SFW 10.82g. Reduced in fresh weight of shoot was recognized to minimum number and growth of slighter leaves with augmented PEG-6000 meditation of the growth. It is significant that drought tolerance is considered by little reduction of growth of shoot in water-stressed condition (Mingn et al., 2012; Moucheshi et al., 2012; Saghafikhadem 2012).

Root fresh weight (g/10 roots): The impact of PEG poly ethylene glycol on plant biomass were recorded in terms of root fresh weight (R.F. Wt) results for the root fresh weight of different wheat cultivars are presented in fig-7.



Figure-7: Effects of polyethylene glycol PEG (6000) on root fresh weight of different wheat cultivars

Among all the treatments -1.0MPa concentration revealed minimum Root fresh weight RFW as compared to other concentrations at the -0.5 MPa PEG-6000 concentration, the ESW-9525 produced a minimum root fresh weight16.18g, whereas IBWSN-1010 revealed maximum RFW 82.36g as compared to the check varieties Khirman 44.69g Chakwal-86 41.01g. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 showed minimum SFW19.76g and IBWSN-1010 showed maximum RFW as 45.43g. At the highest PEG stress -1.0MPa the cultivarsIBWSN-1010 produced maximum RFW 27.22g and ESW-9525 revealed minimum RFW 8.07g. The results showed that with the increase in water stress and high water stress (1.0 MPa) there was a significant decrease in plant biomass with the increase in water stress in the growing media, which led to a comparatively greater reduction.

Shoot dry weight (g/10 shoots): The PEG (6000) effects on dry matter yield were noted in terms of shoot dry weight (SDW) and the data is presented in fig-8.



Figure-8: Effects of polyethylene glycol PEG (6000) on shoot dry weight of different wheat cultivars

Among all the treatments -1.0MPa concentration revealed minimum shoot fresh weight SDW as compared to other concentrations. At the -0.5 MPa PEG-6000 concentration, the TD-1 produced a minimum shoot dry weight 8.52g, whereas IBWSN-1010 revealed a maximum SDW13.36g compared the check varieties as to (Khirman10.95g Chakwal-86 12.98g. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 showed minimum SDW5.62g and IBWSN-1010 showed maximum SDW as 12.37g. At the highest PEG stress -1.0MPa the cultivars ESW-9525 produced a minimum SDW 1.75g and TD-1 revealed a maximum SDW 10.32 g. The decreasing trend in root and shoot dry weight was also reported by other researchers (Kamran et al.,2009) who found that water stress had a significant effect on root and shoot dry matter production.

Root dry weight (g/10 roots): The PEG (6000) effect on root dry matter yield was recorded in terms of root dry weight (RDW) and the data is presented in fig-9.



Figure-9: Effects of polyethylene glycol PEG (6000) on root dry weight of different wheat cultivars

Among all the treatments -1.0MPa concentration revealed minimum root dry weight RDW as compared to other concentrations. At the -0.5 MPa PEG6000 concentration, the TD-1 produced a minimum shoot dry weight 5.95g, whereas IBWSN-1010 revealed a maximum SDW12.42g as compared to the check varieties Khirman 6.61g Chakwal-86 9.52g. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 showed minimum SDW4.29g and IBWSN-1010 showed maximum SDW as 8.76g. At the highest PEG stress -1.0MPa the cultivars ESW9-525 produced minimum SDW 2.87g and IBWSN-1010revealed maximum SDW 5.82g. It was reported by (Jiguang et al., 2013) in his research that decreasing trend in shoot and root dry weight was also reported by many other scientists they mentioned that it was significantly increased shoot and root masses in both cultivars under wellwatered conditions. PEG6000 alone obviously decreased shoot and root dry masses in both cultivars.

Total Chlorophyll content (mg g^{-1} fresh weight): The data regarding total chlorophyll content mg g^{-1} fresh weight is presented in fig-10.



Figure10: Effects of polyethylene glycol PEG (6000) on total chlorophyll content of different wheat cultivars

Decrease in total chlorophyll content was found with the increase of PEG stresses. Under -0.5 MPa PEG-6000 stresses, the cultivarsIBWSN-1025 produced minimum total chlorophyll content 0.19mg g⁻¹ fresh weight, whereas IBWSN-1010 revealed maximum total chlorophyll content0.40mg g⁻¹ fresh weight as compared to the check varieties Khirman 0.72 and chakwall-86 0.54mg g⁻¹ fresh weight. Moreover, at -0.75MPa PEG stress, IBWSN-1025 produced minimum total chlorophyll content 0.19mg g⁻¹ fresh weight. However, the cultivars ESW-9525 produced more chlorophyll content as 0.37mg g⁻¹ fresh weight at

the highest PEG stress -1.0MPa, the cultivars IBWSN-1010 revealed the lowest chlorophyll among the cultivars 0.12mg g^{-1} fresh weight and TD-1 showed to have more chlorophyll content 0.26mg g⁻¹ fresh weight. It was also reported by (Paknejad et al., 2007 that the improvement of cultivar yield under drought stress has resulted from a more extended grain filling duration, a higher chlorophyll content, a more sustained turgor, or a combination of them. On the other hand, (Rong-hua et al., 2006) reported that the values of chlorophyll content in drought tolerance genotypes of barley were significantly higher than those in drought-sensitive genotypes under drought stress.

Shoot potassium K⁺: The data of shoot potassium K^+ is presented in fig-11.



Figure-11: Effects of polyethylene glycol PEG (6000) on shoot potassium content (%) of different wheat cultivar

The potassium content increased in almost all the wheat cultivars with different PEG stresses. Under -0.5 MPa PEG-6000 concentration the IBWSN-1010 produced minimum shoot potassium content 1.41%, whereas ESW-9525 revealed the highest shoot potassium content 1.82% as compared to the check varieties Khirman 1.63 and Chakwal-86 11.27%. Moreover, the osmotic stress at -0.75 MPa PEG, the cultivarsIBWSN-1025 produced minimum shoot potassium content 0.73%. Whereas, the cultivars ESW-9525 revealed more potassium content 1.86% content at this stress. At the highest PEG stress -1.0MPa the cultivarsIBWSN-1010 and IBWSN-1025 possessed more potassium content 0.21% and 0.61%, respectively. It was mentioned by Jiguang et al., (2013) in his study, adequate external K meaningfully increased K+ substances in both shoot and root of PEG6000 stressed plants. This might be explained that higher K+ concentration in plant growing medium offered more opportunities for roots absorbing K+ cellular membrane recovery enhanced K+ conservation in plant tissues.

Root potassium content (%): The data regarding root potassium content (%) is presented in fig-12.



Figure-12: Effects of polyethylene glycol PEG (6000) on root potassium content (%) of different wheat cultivars.

For this trait, almost all the PEG treatments affectted the wheat cultivars as compared to the control. The PEG treatment -0.75 was observed to have more root potassium compared to other treatments. Under -0.5MPa PEG6000 concentrations, the cultivarsIBWSN-1010 produced mini-mum root potassium content0.41% and TD-1 revealed maximum root potassium content 0.70% as compared to the check varieties Khirman 0.48 and Chakwal-86 0.53%. Moreover, the osmotic stress at -0.75 MPa PEG, IBWSN-1010 produced reduced root potassium content 0.40%. However, the cultivars TD-1 produced maximum potassium content 0.90 % in roots. At the highest PEG stress -1.0MPa, the cultivarsIBWSN-1010 produced minimum potassium in roots 0.44% and TD-1 was observed to contain maximum potassium content 0.54%. In the year 2013 a scientist (Jiguang) mentioned hi study that adequate external K significantly increased K+ contents in together shoot and root of PEG6000-stressed plants.

Shoot root calcium Ca (%): The data regarding shoot calcium (%) is presented in fig-13.



Figure-13: Effects of polyethylene glycol PEG (6000) on shoot calcium (%) of different wheat cultivars

Among overall treatments, -1.0MPa PEG osmotic stress produced reduced calcium (%) in shoots as compared to other concentrations. Under -0.5 MPa PEG6000 concentrations, the cultivars I BWSN-1025 produced maximum shoot calcium 1.53%, whereas ESW-9525 revealed minimum shoot calcium 0.72% as compared to the check varieties Khirman 0.89 and chakwal-86 0.78%. Moreover, the osmotic stress at -0.75MPa PEG, IBWSN-1025 produced more calcium 1.31% in shoots and the cultivars ESW-9525produced minimum shoot calcium 0.91%. At the highest PEG stress -1.0MPa, the cultivarsTD-1produced maximum Shoot calcium 1.13% and ESW-9525 produced reduced shoot calcium 0.33%. The data regarding root calcium (%) is presented in fig-14.



Figure-14: Effects of polyethylene glycol PEG (6000) on root calcium (%) of different wheat cultivars

Among all the treatments, root calcium (%) was found to be more in -0.75 MPa and -1.0MPa PEG6000 concentrations. Accordingly, with the PEG concentration -0.5 MPa, the cultivarsTD-1 produced maximum root calcium 0.65%, whereas IBWSN-1010 revealed minimum root calcium 0.52% as compared to the check varieties Khirman 0.52% and Chakwal-86 0.55%. Moreover, the osmotic stress at -0.75MPa PEG, ESW-9525 produced more calcium 0.74% in roots and the cultivars IBWSN-1010 produced a minimum calcium 0.50%. At the highest PEG stress -1.0MPa, the cultivars I BWSN-1025 produced maximum root calcium 0.62% and ESW-9525 revealed minimum root calcium 0.40%. Mujtaba observed the same result in his 2016 study. He mentioned that the PEG 6000 ratio in Root K + / Ca2 + wasnot significantly affected by osmotic stress.

Plant height (cm): For the trait plant height (cm) fig 15, most of the wheat cultivars reduced their height at terminal drought as compared to control treatment.



Figure-15: Plant height affected by water stress in different wheat cultivars.

The cultivar TD-1 produced a shorter height and was recorded as 52.7cm at control condition, whereas the cultivar ESW-9525 revealed a taller plant having a height of 79.7cm, respectively as compared with the check varieties, Chakwal-86 and Khirman with height recorded as 81.0 and 91.3cm, under control condition. The cultivar TD-1 produced minimum plant height at the highest water stress terminal drought and was recorded as 46.00 cm. Moreover, the cultivar ESW-9525 produced a taller plant height and was observed as 66.3cm, respectively. Comparison with the check varieties Chakwal-86 and Khirman produced plant height of 63.0 and 69.7 cm, respectively under water stress condition terminal drought. Plants were found to have the capability to adjust to environmental conditions, which is usually unstable due to the various environmental factors. In all wheat varieties used in the experiment, water stress has a drastic effect on plant height (Mirbahar et al., 2009). The height of the plant plays an important role in photosynthesis (Malik and Hassan 2002; khanzada et al., 2001). The shoot length of guar genotypes has also been previously reported to be similarly significantly reduced under water stress (Inamullah et al., 1999).

Spike length (cm): Spike length was reduced at water stress level fig-16.



Figure-16: Spike length affected by water stress in different wheat cultivars.

The cultivar TD-1 produced the shortest spike length and was recorded as 10.8 cm at the control condition, whereas the cultivar ESW-9525 produced longest spike length as 14.7 cm. In comparison with the check varieties, Chakwal-86 and Khirman produced spike lengths of 13.8 and 14.7 cm, respectively under the controlled condition. Moreover, the cultivar TD-1produced a minimum spike length at the highest water stress terminal drought and recorded as 7.6 cm, whereas the cultivar ESW-9525 produced a maximum spike length 13.2cmas compared with the check varieties Chakwal-86 and Khirman having spike lengths of 11.3 and 12.1, respectively under water stress condition terminal drought. It was reported that the significant suppressive effect of water stress on a number of spike lengths. It has previously been reported that water stress during vegetative and reproductive development caused a signifycant reduction in the length of the wheat spike (Tompkins et al., 1991; Qadir et al., 1999).

Number of tillers plant⁻¹: The number of tillers plant⁻¹ also reduced at water stress level terminal drought as compared to control treatment fig-17.



Figure-17: Number of tillers affected by water stress in different wheat cultivars.

The cultivars IBWSN-1010 produced a minimum number of tillers plant⁻¹ and recorded as 8.0 tillers plant⁻¹ under control conditions, whereas the cultivar ESW-9525 produced a maximum number of tillers plant⁻¹(12.0), respectively. In comparison with the check varieties, Chakwal-86 and Khirman produced a number of tillers as 12.0 and 12.0 respectively under control conditions. However, the cultivar IBWSN-1010 produced a minimum number of tillers plant⁻¹ at the highest water stress (5.0). The cultivar ESW-9525 produced a maximum number of tillers plant⁻¹ and recorded as 10.0, compared with the check varieties Chakwal-86 and Khirman having a number of tillers 10.0 and 10.0, respectively under water stress condition terminal drought. It was also observed that tillers plant⁻¹ in wheat cultivars reduced significantly under water stress when it was compared with irrigated (Mirbahar et al., 2009). In 1999 it was also reported that water stress compact the tillers plant⁻¹ in wheat cultivars (Qadir et al., 1999).

Straw weight (g): Straw weight was also reduced during water stress level fig-18. The reduction was more at the terminal drought stress as compared to control treatments.



Figure-18: Straw weight affected by water stress in different wheat cultivars

The cultivars IBWSN-1010 produced minimum straw weight gas 20.1 g under control condition, whereas the cultivar ESW-9525 produced maximum straw weight g as 39.1 g, respectively, compared to the check varieties, Chakwal-86 and Khirman influenced moderate straw weight as 30.3and 36.2 g, respectively under the controlled condition, respectively. The cultivar IBWSN-1010 produced minimum straw weight under water stress condition terminal drought and recorded as 8.4 g. However, the cultivar ESW-9525 revealed a maximum straw weight 14.3 gcomparison with

the check varieties, Chakwal-86 and Khirman produced straw weight as11.2 and 13.7 g, respectively under the highest water stress condition terminal drought. it was also reported by (Katerji et al.,2009) that imposition of water stress during ear formation and flowering stages had caused 37% and 18% decline in grain and straw yields in wheat crops.

100 Grain weight (g): Overall wheat cultivars showed that the grain weight also reduced at water stress level terminal drought as compared to control treatment fig-19.



Figure-19: Grain weight affected by water stress in different wheat cultivars.

TD-1 produced reduced grain weight and recorded as 19.7 g under control conditions, whereas the cultivar ESW-9525 produced more grain weight as 23.3 gm, respectively. In comparison with the check varieties, Chakwal-86 and Khirman produced grain weight of 21.3 and 23.4 gm, respecttively under the controlled condition. Whereas the cultivar IBWSN-1010 produced minimum grain weight under the highest water stress condition terminal drought and recorded as 14.8gm. However, the cultivar ESW-9525 produced a maximum grain weight 19.7 gmas compared to the check varieties Chakwal-86, and Khirman, having grain weight 17.2 and 19.2 gm, respectively under the highest water stress condition terminal drought. Drought escape is highly heritable but it is associated with lower 100 grain weight (Wortmann, 1998) as shown by these results.

Days to 75% heading: For the trait days to heading, most of the cultivars heading reduced at terminal drought as compared to control treatment fig-20.



Figure-20: Days to heading affected by water stress in different Cultivars.

The cultivars ESW-952 head earlier 78.2 under control condition, whereas the cultivar IBWS-1010 mature lately 92.7, as compared with the check varieties, Chakwal-86 and Khirman. The cultivar ESW-9525 also produced earlier 84.83 under highest water stress condition terminal drought. Compare to the check varieties Chakwal-86 and Khirman also produced earlier heading as 73.5 and 78.5 under highest water stress condition terminal drought. It was reported that drought stress reduced the number of days to heading (Hasan Kiliç and Tacettin Yağbasanlar, 2010).

Days to 75% maturity: Most of the cultivars reduced maturity at terminal drought as compared to control treatment fig-21.



Figure-21: Days to maturity affected by water stress in different Cultivars.

The cultivars IBWS-1010 matured earlier 118.7 under the control condition, whereas cultivar ESW-952 matured late 122.1, as compared with the check varieties, Chakwal-86 and Khirman.

The cultivar ESW-952 matured earlier 113.7 under the highest water stress condition terminal drought as compared with both the check varieties Chakwal-86 and Khirman (113.8 and 115.8). It was also observed by (Hasan Kiliç and Tacettin Yağbasanlar, 2010) that drought stress reduced the number of days to maturity.

Conclusion: Plants have developed biochemical and physiological approaches to tolerate in dehydrated environments. From the present study, it is concluded that osmotic stress under laboratory conditions significantly reduced the length of the sprout/incubation root of the seed and the dry weight of the six varieties of wheat. The maximum reduction was found to be significant with higher PEG6000 osmotic stress (T4, 1.0 MPa). The effect of osmotic stress was found to be stronger at a PEG level of 1.0 MPa compared to other treatments at the seedling stage. The growth in this stage of the treatment was in seed germination, coleoptile length, radicle length, shoot length cm, root length cm, the weight of fresh shoots g, weight of fresh root g, weight of dry shoots g, the weight of dry roots g, total chlorophyll content (mg g1 fresh weight), Ca and ion content. In this case, K reduces osmotic stress. IBWS1010 varieties performed better for various growth traits and can be considered tolerant varieties. ESW9525 also had the maximum content of total chlorophyll, potassium k + calcium Ca in the roots, which were considered drought-tolerant, also because the accumulation of ionic contents was more.

REFERENCES

- Almaghrabi, O.A., Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. *Life Science* Journal 9(1): 590-598 (2012).
- Almansouri M., Kinet, J. M., Lutts, S., Effect of salt and osmotic stresses on germination in durum wheat (*Triticum aestivum* L). *Plant. Soil*. 231: 243-254 (2001).
- Ashraf, M., Bokhari, H., Cristiti, S.N., Variation in osmotic adjustment of lentil (*Lensculimaris* Medic) in response to drought. Acta Bot. Neerlandica 41: 51-62 (1992).
- Bayoumi, T.Y., Manal, H. Eid., Metwali, E.M., Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *African Journal of Biotechnol*. 7(14): 2341-2352 (2008).
- Carpita, N., Sabularse, D., Monfezinos, D., delmer D.P., Determination of the pore size of

cell walls of living plant cells. Sci. 205:1144-1147. (1979).

- Chachar, M.H., Chachar N.A, Chachar S.D., Chachar, Q.I., Mujtaba, S.M., Yousafzai, A., *In-vitro* screening technique for drought tolerance of wheat (*Triticum aestivium* L.) genotypes at early seedling stage. J. Agri. Technol. 10(6): 1439-1450 (2014).
- Chachar, M., Hanif, C.,Ahmed, N.,Chachar, Q.,Mujtaba, S.M.,Chachar, S., Chachar, Z., Physiological characterization of six wheat genotypes for drought tolerance. International Journal of Research Granth Pp.2394-3629 (2016).
- Chachar, N.A., Chachar, M.H., Chachar, Q.I., Chachar, Z., Chachar, G., Nadeem, A.F., Exploration of genetic diversity between six wheat genotypes for drought tolerance. Climate Change outlook and adaptation 2(1): 27-33 (2014).
- Dhanda, S. S., Sethi, G. S., Behl, R., KIndices of drought tolerance in wheat genotypes at early stages of plant growth. *Journal of Agronomy Crop Sciences* 190: 6-12 (2004).
- Dodd, G, L., Donovan, L. A., Water potential and ionic effects on germination andseedling growth of two cold desert shrubs. *American Journal of Botany* 86; 1146-1153 (1999).
- Galies, J. Ho., T, D., Multiple molecular forms of the gibberellin-induced 8-amaylase from the aleurone layer of barley seeds. *Arch Biochem Biophys* 224 (1983).
- Gomez, A.K., Gomez, A.A., Statistical procedures for agricultural research. (2nd edition). John, Wiley and Sons. New York. (1984).
- Heikal, M.M., Shaddad, M.A., Ahmed, A.M., Effect of water stress and gibberellic acidon germination of flax, sesame and onion seed. *Biology Plantarum* 24(2): 124-129 (1981).
- Hasan, K., Tacettin, Y., The effect of drought stress on grain yield, yield components and some quality traits of durum wheat (*Triticum turgidum* ssp. durum) Cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38(1): 164—170 (2010).
- Iqbal, M., Ashraf, M., Seed treatment with auxins modulates growth and ion partitioning in salt stressed wheat plants. *Journal of Integrative Plant Biology* 49: 1003-1015 (2007).
- Inamullah, Z., Swati, A., Latif, A. and Siraju-Din., Evaluation of lines for drought tolerance in wheat (*Triticum aestivum* L.). *Science Khyber* 12(2): 39--48 (1999).
- Jiguang, W., Caihong, L., Yong, L., Gaoming, J., Guanglei, C., Yanhai, Z., Effects of External Potassium (K) Supply on Drought Tolera-

- nal pone 0069737 (2013). Kamran, M.M., Shahbaz, M., Ashraf and Akram N.A., Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as pre-sowing seed treatment. *Pakistan journal of Botany* 41(2): 621-632 (2009).
- Kaya, M.D., Okcu, G., Atak, M., Cikili, Y., Kolsarici, O., Seed treatments to overcome salt and drought stress during germination in sunflower (Helianthus annusL.). *European Journal of Agronomy* 24: 291-295 (2006).
- Khaheh, H., Bingham, M., Powel, A., The effects of reduced water availability and salinity on the early seedling growth of soybean. Proceeding of the Third International Crop Science Congress, Humbarg Germany (2000).
- Kulkarni, M., Deshpande, U., *In-vitro* screening of tomato genotypes for drought resistance using polyethylene glycol. *African Journal of Biotechnology* 6: 691-696 (2007).
- Khanzada, B., Yasin, M., Ashraf, S., Ahmed, A., Alam, S. M., Shirazi, M.U.and Ansari, R., Water relations in different Guar (Cyamopsis tetragonoloba (L.) Taub) genotypes under water stress. *Pakistan Journal of Botany* 33 (3): 279–287 (2001).
- Lichtenthaler, H. K., Chlorophyll and carotenoids pigments of photosynthetic biomembranes Methods Enzymol. 148: 350-382 (1987).
- Lu, Z., Neumann, P M., Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. *International Journal of Experimental*, *Botany* 49: 5-1952 (1998)
- Metwali, M.R., Ehab, Manal., Eid, H., Tarek, Y. Bayoumi., Agronomical traits and biochemical genetic markers associated with salt tolerance in wheat cultivars (*Triticum aestivum* L.). Australian Journal of Basic and Applied Sciences 5(5): 174-183 (2011).
- Micheal, B.E., Kaufman, M.R., The osmotic potential of polyethylene glycol 6000. *Plant Physiology* 51: 914-916 (1973).
- Ming, D. F., Pei, Z. F., Naeem, M. S., Gong, H. J., Zhan, W. J., Silicon alleviates PEG- induced water-deficit stress in upland rice seedling by enhancing osmotic adjustment, *Journal of Agronomy and Crop Sciences* 198: 14-26 (2012).
- Mirbahar, A, A., Saeed, R., Markhand, G, S., Effect of polyethylene glycol-6000 on wheat (*Triticum aestivum* L.) seed germination. J. Biol & Biot. 10(3): 401-405 (2013).

- Money, N.P., Osmotic pressure of aqueous polyethylene glycols. Relation between molecular weight and vapor pressure deficit. *Plant Physiology* 91: 497-500 (1989).
- Moucheshi, A., Heidari, B., Assad, M.T., Alleviation of drought stress effects on wheat using arbuscular mycorrhizal symbiosis. *International Journal of Agriculture Science* 291: 35-47 (2012).
- Mujtaba, S.M., Faisal, S., Khan, M.A., Mumtaz, S., Barkat,K., Physiological studies on six wheat (*Triticum aestivum* 1.) genotypes for drought stress tolerance at seedling stage. *Pakistan Journal of Botany* 49(2): 1-5 (2016).
- Mirbahar, A.A., Markhand, G.S., Mahar, A.R., Abro, S.A., Kanhar, N.A., Effect of water stress on yield and yield components of wheat (*Triticum aestivum* L.) varieties. *Pakistan journal of Botany*, 41(3): 1303 -1310 (2009).
- Malik, M.A. and Hassan,F., Response of wheat genotypes on suppression of weeds under rainfed conditions. *Pakistan Journal of Agricultural Engineering Veterinary Sciences* 18(1-2): 18-22 (2002).
- Okcu, G., Kaya, M. D., Atak, M., Effect of sat and drought stresses on germination and seedling growth of pea (Pisum sativum L.).*Turkish Journal of Agriculture Forestry* 29: 237-242. (2005).
- Paknejad, F., Nasri, M., Moghadam, H.R.T., Zahedi, H.,&Alahmadi, M.J., Effects of drought stress on chlorophyll fluorescence parameters, chlorophyll content and grain yield of wheat cultivars. *Journal of Biology Science* 7(6): 841-847 (2007).
- Pan,X. Y., Wang, Y. F., Wang, G. X., Cao, Q. D., Wang, J., Relationship between growth redundancy and size inequality in spring wheat population mulched with clear plastic film. *Acta Phytoecological Sinica* 26: 177-184 (2002).
- Pessarakli, M., Handbook of plant and crop stress, 2nd Ed., New York: Marcel Dekker Inc, Pp 247-259 (1999).
- Qadir, G., Mohammad, S. and Cheema, M.A., Effect of water stress on growth and yield performance of four wheat cultivars. *Journal of Biological Sciences* 2(1): 236–239 (1999).
- Rauf, M., Munir, M., UI-Hassan, M., Ahmed, M., Afzai, M., Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. *African Journal of Biotechnology* 8: 971-975 (2007).

- Ronghual, L., Guo, P., Michael, B., Stefania, G., and Salvatore, C., Evaluation of Chlorophyll Content and Fluorescence Parameters as Indicators of Drought Tolerance in Barley. *Agriculture Science in China* 5(10): 751-757 (2006).
- Saghafikhadem, A., The effect of drought on growth and yield of wheat. *American Journal Science Research* 44: 110-115 (2012).
- Sayar, R., Khemira, H., Kameli, A., Mosbahi, M., Physiological tests as predictive appreciation for drought tolerance in durum wheat (Triticum durum Desf). Agronomy Research 6: 79-90 (2008).
- Surbhaiyya, S.D., Gahukar, S.J., Jadhav, P.V., Bhagatm, S.Y., Moharil, M.P., Potdukhe, N.R. and Singh, P.K., *In-vitro* based screening of promising wheat (*Triticum aestivum L.*) genotypes for osmotic stress imposed at seedling stage.*International Journal Current Microbiology Applied Science* (6): 2500-2508 (2018).
- Taregh, G., Mostafa, V. S., Hossein, S., Effect of drought on germination indices and seedling growth of 12 bread wheat genotypes. *Advance in Environmental Biology* 5(6): 1034-1039 (2011).
- Tompkins, D.K., Fowler, D. B. and Wright, A. T., Water use by no till winter wheat influence of seed rate and row spacing. *Agronomy Journal* 83(4): 766–769 (1991).
- Vahidi, J., Effect of water stress on germination indices in seven wheat cultivar. 597 World Academy of Science. Engineering and Technology Pp 49 (2009).
- Wang, X.Y., Vinocur, P., Altman, A., Plant responses to drought, salinity and extreme temperatures towards genetics engineering for stress tolerance. *Planta* 218: 1-14 (2003).
- Wortmann, C. S., An adaptation breeding strategy for water deficit in bean developed with application of the DSSAT 3 dry Bean Model. *African Crop Science Journal* 6: 215–225 (1998).
- Zhu, J. K., Hasegawa, P. M., Bressan, R. A., Molecular aspect of osmotic stress in plants. Critical Rev. *Plant Sciences* 16: 253-277 (1997).
- Zhu, J., Effects of drought stresses induced by polyethylene glycol on germination of Pinus sylvestris var mongolica seeds from pollination forests on sandy land. *Natural and Pollination Forests on sandy Land Journal of Forest Research* 11(5): 319-328 (2006).