Physiological and Molecular Characterization of Bread Wheat (Triticum aestivum L.) For Drought Resistance

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Abstract

The present study evaluated three wheat genotypes (SD-28, SD-32 and Chirya-1) were evaluated for physiological attributes like Relative Water Content, Proline Content, Membrane Stability Index and Chlorophyll Content where Opata was used as a control check under three different levels of drought stress (100% FC, 80% FC and 60% FC). Results revealed that chlorophyll content was significantly affected under stressed conditions in all the studied genotypes and genotypes. Molecular diagnosis of the selected wheat genotypes and genotypes was carried out with RT-PCR using expression profile of 06 genes (TaLhca1, TaLhca2, TaLhca3, TaLhcb1, TaLhcb4 and TaLhcb6) that encodes for LHCI and LHCII proteins. RT–PCR indicated variable expression of the selected genes in response to different level of drought stress. The results obtained clearly showed the relation between genotypes and severity of drought stress condition. Among the studied genotypes Chirya-1 and SD-28 performed well with higher level of gene expression under drought stress condition; and may be considered drought tolerant genotypes with potential to enrich the genetic background of locally adapted wheat lines against drought stress.

ABSTRACT

The present study evaluated three wheat genotypes (SD-28, SD-32 and Chirya-1) were evaluated for physiological attributes like Relative Water Content, Proline Content, Membrane Stability Index and Chlorophyll Content where Opata was used as a control check under three different levels of drought stress (100% FC, 80% FC and 60% FC). Results revealed that chlorophyll content was significantly affected under stressed conditions in all the studied genotypes and genotypes. Molecular diagnosis of the selected wheat genotypes and genotypes was carried out with RT-PCR using expression profile of 06 genes (*TaLhca1, TaLhca2, TaLhca3, TaLhcb1, TaLhcb4* and *TaLhcb6*) that encodes for LHCI and LHCII proteins. RT–PCR indicated variable expression of the selected genes in response to different level of drought stress. The results obtained clearly showed the relation between genotypes and severity of drought stress condition. Among the studied genotypes Chirya-1 and SD-28 performed well with higher level of gene expression under drought stress condition; and may be considered drought tolerant genotypes with potential to enrich the genetic background of locally adapted wheat lines against drought stress.

Key Words: Wheat; Drought Stress; Stay-Green; Gene Expression; Physiological attributes.

INTRODUCTION

Importance of wheat as staple food is well known as being life line of 35% of the world population. But in past two-three decades, unpredictable climatic conditions have resulted in stagnation in wheat production. Drought is a major environmental stress, which can effect growth of cereals crops and has decreased the production and performance of the plant (Shao *et al.* , 2009), (Rad *et al.* , 2012). The significant rise in drought stress condition has enforced us to develop climate resilient high yielding genotypes; hence, there is a need to develop better understanding of the traits which respond to drought by exploiting key traits (Halford and Hey, 2009) It has been suggested that due to climatic changes, the shortage of water may be increased which will affect the cereals crops in many areas of the world.

Leaf senescence is an intricate process where various cellular processes occurs consistently, parallel or sequentially (Lim *et al.* , 2007). It normally starts with change in genes expression and genes are expressed by environmental stimuli during various developmental stages. If senescence starts at mature stage then the changes will not appear at earlier stages of senescence until the stress is applied at initial stages. These changes include the chlorophyll pigment breakdown normally followed by break down of mitochondria, plastids, nuclei and vacuoles, which leading to death of cell (Buchanan-Wollaston and Ainsworth, 1997). To know about the whole mechanisms of leaf senescence it is important to dissect the leaf senescence process one such approach is to evaluate transgenic plants which show different leaf senescence phenotype which we called stay green (Kusaba *et al.* , 2013).

Two main types of chlorophyll are present in higher plants, Chlorophyll$a$ and Chlorophyll b . Chlorophyll a is a part of all chlorophyll–protein complexes, whereas Chlorophyll b is confined only in PSI-associated light-harvesting complex I (LHCI) and PSII-associated LHCII. Light-harvesting complex I and light-harvesting complex II are present in thylakoid membranes and its function is energy production and transfer. LHCII is mostly present in grana, and due to intermolecular forces its main function is formation and maintenance of grana stacks (Allen and Forsberg, 2001). The *Lhca* and *Lhcb*gene families encoded the apoproteins of light-harvesting complex I and light-harvesting complex II respectively. *Lhca1–Lhca4* genes formed the protein of LHCI which are associated with PSI. *Lhcb1* ,*Lhcb2* , and *Lhcb3* genes code the polypeptides of trimeric LHCII. Lhcb4, Lhcb5, and Lhcb6 proteins (also known as CP29, CP26, and CP24,) are proposed to be monomeric proteins which are found one set per PSII unit. The Lhca and Lhcb genes expression and LHCI and LHCII stability are very important to keep the photosynthetic process at high level (Standfuss *et al.* , 2005).

One of the most important goals of researcher to increase grain yield and it can be achieved by improving the rate of photosynthesis or by carbon assimilation (Zhu *et al.* , 2010) the approach to achieve this goal would be to delay senescence alongside the photosynthetic activity of plant for lengthier period of time (Dohleman and Long, 2009). To achieve these objectives in wheat plant, it is necessary to understand the leaf senescence mechanisms at molecular level.

In the present study, the selection and photosynthetic characteristics of 'stay green' mutants of wheat are described and to characterize wheat genotypes in their response to drought stress. The characterization was focused on both physiological and molecular aspects of wheat genotypes to select the wheat genotype with desirable traits

2. Materials and Methods

2.1 Laboratory Experiment

A pot experiment were conducted under laboratory condition with temperature 10 to 15 C and 13 to 14 hours light duration, to assess the effect of three drought level Control 100% Field Capacity, Stress 80% Field Capacity, stress 60% Field Capacity. Field soil, sand and peat compost were mixed in equal proportion and filled in 36 pots at the rate of 1835g in each pot. Two conventional wheat genotypes Opata and chirya-1, and two Synthetic genotypes i.e. SD-28 and SD-32 were studied. Nine pots were used for each wheat genotype to test each genotype at three drought levels with three replications. The pots were tapped on the floor enough to bring the soil mixture to a uniform height to get soil bulk density (SBD) and corresponding total soil porosity (TSP)

The height of soil mixture in each pot required to get the desired SBD were calculated by following formula.

VSM (cm3)

Height (cm) = ---

π × (*Diameter of pot (cm) 2*

2

Where VSM (volume of soil mixture) were calculated by formula

Mass of soil mixture taken in each pot (g)

VSM (cm3) = ---

Desired SBD (g cm -3)

The pots were irrigated with a uniform volume of tap water to saturate all the pore space of soil mixture and kept aside for drying. The volume of water (VW) required saturating the soil mixture in the pots were calculated by the formula.

VW (cm3) = TSP (cm3 cm-3) × VSM (cm3)

Where TSP were calculated assuming soil particles density (SPD) of 2.65 (g cm3) using the formula

SBD (g cm3)

TSP (cm3 cm-3) = 1- ---

SPD (g cm3)

Volumetric soil water content (VSWC) were monitored daily from each pot with Domain Reflectometer (TDR) model TRIME –FM (IMKO Micromodultechnik GmbH, Germany) the seed were sown in pots when VSWC was at FC (assuming FC as 50% of the saturation and taking saturation =TSP). VSWC will be calculated by formula.

TSP (cm3ncm-3 ×50

VSWC at FC (cm3 cm-3) = ---

100

Similarly, VSWC at other drought levels was calculate with their respective formulae.

TSP (cm3 cm3) ×40

VSWC at 80% FC (cm3 cm-3) = ---

100

TSP (cm3 cm3) ×30

VSWC at 80% FC (cm3 cm-3) = ---

100

After germination VSWC were maintained in each pot at FC for three weeks, Monitoring VSWC and fulfilling the irrigation requirements daily. The VW require maintaining each pot at FC was calculated by the formula:

VW (cm3) = (VSWC at FC (cm3 cm-3) – VSWC from TDR (cm3 cm-3)) × VSM (cm3)

After three weeks the irrigation supply was cut off from the pots to which drought treatment were applied and VSWC was monitored daily until the respective drought level will achieved. Afterwards, VW required

3maintaining different drought levels were applied daily to respective pots calculating by their respective formulas.

VW (cm3) = (VSWC at 80% FC (cm3 cm-3) – VSWC from TDR (cm3 cm-3)) × VSM (cm3)

VW (cm3) = (VSWC at 60% FC (cm3 cm-3) – VSWC from TDR (cm3 cm-3)) × VSM (cm3)

The drought stress will be maintained for 30 days and after that samples were collected for physiological and molecular study.

2.2. Physiological evaluation of wheat genotypes:

After applying drought stress the data for various physiological characters as relative water content (RWC), proline, chlorophyll content and membrane stability index (MSI) were recorded.

2.3. Molecular Characterization of Selected Wheat Genotypes

The genes related to pigment-binding proteins were selected to examine the different expression in four genotypes of wheat which was grown in normal condition 100% field capacity and drought stress condition 80% field capacity and 60% field capacity. Total 6 genes were studied and specific primers for genes encoding LHCI and LHCII were designed based on published expressed sequence tags. The primers were: *TaLhca1* - F 5' CAACCTGCCGACCATCCTG-3' and *TaLhca1*- R 5'CAGCCGCCCGTTCTTGAT-3' and *TaLhca2* - F 5'CCCCAACCGCAAGAACC-3' and *TaLhca2*- R 5'CCGACGAAGGCGAGCAT-3', and *TaLhca3* - F 5'CCTCACCAGCCTCAAGTTCC-3', and *TaLhca3*- R 5'CGCACGCTCACGTTTCC-3', and *TaLhcb1* - F 5'GGAGAACACACAATACACC-3' and *TaLhcb1*- R 5'CCCATTATGTGTGCAGTTC-3' and *TaLhcb4* - F 5'AAAGGCCGAGGAGGACAA-3' and *TaLhcb4*- R 5' CCACCGACCACTTAAGAGG-3' and *TaLhcb6* - F TCAGCGACCTCACCGTCA-3' and *TaLhcb6*- R 5'CCCCAAAGAAGTCACGGACA-3', and amplification of the *T. aestivum tubulin* gene using primer *Tubulin- F* 5'ACCGCCAGCTCTTCCACCCT- 3' and *Tubulin-R* 5 TCACTGGGGCATAGGAGGAA- 3' exhibiting constitutive expression was used as a positive control, from which a linear relationship between the amount of RNA used for amplification and the amount of cDNA fragment amplified, as well as the quality of both extracted RNA and RT–PCRs were determined.

2.4. RNA Extraction by using Gene JET Plant RNA Purification Minikit

Leaf samples were collected from each replication and immediately transferred to liquid nitrogen. Put 100 mg of plant tissue into liquid nitrogen and grind thoroughly with a mortar and pestle. after grinding the tissue powder were quickly transferred into 1.5 ml micro centrifuge tube which Containing 500 µL of Plant RNA Lysis Solution and Vortex all the mixture for 15-20 seconds to mix thoroughly and after that Incubate the samples for 3 min at 56°C and then Centrifuge for 5 min at [?]20,000 x g ([?]14,000 rpm). Collect the supernatant 550 µL and transfer to the clean micro centrifuge tube and then Add 250 µL of 96% ethanol and Mix by pipetting and then transferred the mixture into purification column inserted in a collection tube. Centrifuge the column for 1 min at 12,000 × g (˜11,000 rpm). Discard the flow through solution and reassemble column and collection tube. Then we Add 700 µL of Wash Buffer WB 1 to the purification column (ethanol has been added to Wash Buffer WB 1). Centrifuge for 1 min at 12,000 × g (˜11,000 rpm). Discard the flow-through and collection tube. Place the purification column into a clean 2 mL collection tube. Then Add 500 µL of Wash Buffer 2 to the purification column (ethanol has been added to Wash Buffer 2). Centrifuge for 1 min at 12,000 × g (˜11,000 rpm). Discard the flow-through solution and reassemble column and collection tube after that we repeat this step again. To elute the RNA, add 50 µL of nuclease-free water to the centre of the purification column membrane and centrifuge for 1 min at 12,000 × g (˜11,000 rpm). Discard the purification column and extracted RNA immediately stored at -20°C for further analysis.

2.5. Synthesis of cDNA

The complementary DNA was synthesized from RNA by using a kit (Revert Aid Reverse Transcriptase, Thermo Scientific, Catalog # EP0442). Briefly 11 uL template RNA were mixed with 1.0 µl of Oligo (dt) 18 primers (#S0132) and incubate for 5 mints at 65°C. Then 4.0 µl of RT buffer, also add 1 uLRibolock Rnase inhibitor and 1 µl of reverse transcriptase (Revert Aid) and 2 µl of dNTPs mix (#R0181) were added

to making a total volume of 20 µl. now the mixture was centrifuged briefly and incubated at 42°C for 80 minutes. Then the reaction was Terminate the by heating at 70 °C for 5 minutes cDNA were stored at minus 20 for RT-PCR analysis.

2.6. Expression analysis of LHCI and LHCII genes through RT-PCR

2.0 µl of cDNA from reverse transcription was used as template and LHCI and LHCII genes specific primers were employed to amplify the desired fragment through RT- PCR with optimized condition as initial denaturation at 95 °C for 10 minutes, denaturation at 95 °C for 20 seconds, annealing at 58 °C for 40 seconds extension at 72 °C for 30 seconds by 34 cycles. The final product of RT-PCR was separated running on 1% agrose gel by the process of electrophoresis and visualized on alpha in notech gel documentation system.

2.7. Statistical analysis:

Data obtained were subjected to descriptive statistics, analysis of variance and Correlation test. The analysis over treatments will also performed by using STATISTIX 10 and means will be compared by using Fisher's least significant difference ($P<0.01$ and 0.05)

3. RESULTS

3.1. Physiological analyses

3.1.1. Membrane Stability Index (MSI)

The characterization of wheat genotypes contrasting in their response to drought stress was performed by checking membrane stability index. The results showed that the mean value for membrane stability 55.227 was recorded in control 100% field capacity. Similarly in drought condition 80% field capacity the mean value for membrane stability was 60.69 was recorded. Likewise in severe drought condition 60% field capacity condition the mean value 54.106 was recorded. (Table. 1, Fig. 1) Analysis of variance (ANOVA) regarding MSI revealed that the effect of drought stress is highly significant while genotypes and their interaction with different level of drought stress are non-significant as shown in (Table 2). The least significant difference (LSD) showed that in normal condition 100% field capacity the membrane stability not different significantly and stay-green chirya-1 genotype showed maximum 58.415 membrane stability index followed by SD-32. In drought condition 80% field capacity and 60% field capacity the means are not significantly different from each other and highest value for membrane stability 62.685 and 55.966 were recorded for SD-28 wheat genotype respectively (Table 3).

3.1.2. Relative Water Content (RWC)

The relative water content in all the studied genotypes showed significant difference in both control and drought stress condition. The mean value under normal condition 100% field capacity 94.580% was recorded while in drought stress condition 80% field capacity the mean value for relative water content 81.887% was recorded for all the studied genotypes. In drought stress 60% field capacity the mean value for relative water content 73.659% was recorded (Table 1, Fig. 2). In general, RWCs declined during water stress in all the studied wheat genotypes. The Analysis of variance (ANOVA) result showed that in drought condition the interaction between genotypes and different levels of drought stress are not positively significant (Table 2). The least significant difference (LSD) indicated that in 100% field capacity the relative water content were not affected and maximum relative water content 96.477% were recorded for SD-32 genotype followed by chirya-1 genotype. Similarly in drought stress 80% and 60% field capacity the maximum relative water content was recorded for chirya-1 genotype which was 82.8% and 76% respectively (Table 3)

3.1.3. Proline Contents

The results regarding proline content revealed that mean proline content under 100% FC 0.828 were observed. Similarly under drought stress condition 80% FC the mean value for proline content 1.433 was recorded. Likewise in drought stress 60% the mean value for proline content 2.509 was recorded shown in (Table1 and Fig 3.). The results indicate that proline content was increased under drought stress condition as compared

to control condition. As drought condition were increased the proline content were also increased. Analysis of variance (ANOVA) regarding proline content revealed that the effect of drought stress and response the genotypes were highly significant and the interaction of genotypes and different level of drought stress were also highly significant as shown in (Table 2). The least significant difference (LSD) regarding proline content showed that in 100% FC the proline content are not significantly different and maximum proline content 0.872 were recorded for genotype SD-28 followed by SD-32 wheat genotype. Similarly under drought stress 80% FC the maximum proline content 1.667 was recorded for stay green chirya-1 genotype followed by SD-32. Similarly in drought stress 60% FC the maximum proline content 3.091 was recorded for Opata genotype followed by stay green chirya-1 genotype as shown in (Table 3). The results clearly demonstrated that drought stress could improve proline content and different genotypes have different response to various drought levels.

3.1.4 Chlorophyll Contents

Data concerning Chlorophyll "a" presented in Table 1 demonstrated that there were significant differences in chlorophyll "a" after different level of drought stress. The mean value for chlorophyll "a" 1.186 was recorded and under drought stress 80% FC the mean value for chlorophyll "a" 1.041was recorded. Likewise under drought stress 60% FC the mean value for chlorophyll "a" 0.542 was recorded as shown in Fig 1. The results clearly indicate that different drought stress level effect the chlorophyll content in all studied wheat genotypes. The least significant difference (LSD) regarding chlorophyll "a" presented in Table 3 showed that in 100% FC the chlorophyll "a' content are not significantly different and maximum chlorophyll "a" content 1.230 were recorded for stay green chirya-1 genotype followed by SD-28 wheat genotype. Similarly under drought stress 80% FC the mean maximum chlorophyll a content 1.099 was recorded for SD-32 followed by Opata wheat genotype. Similarly in drought stress 60% FC the maximum chlorophyll "a' content 0.576 was recorded SD 28 followed by stay green chirya-1 genotype.

Data regarding chlorophyll "b" presented in Table 1 demonstrated that drought stress can affect the chlorophyll "b" content. In control condition 100% FC the mean value 1.108 was recorded and under drought stress 80% Fc the mean value 0.926 was recorded. Likewise in drought stress 60% FC the mean value 0.559 was recorded as shown in (Table 1 and Fig 4). The least significant difference (LSD) regarding chlorophyll "b" presented in Table 3 revealed that in control condition 100% FC the chlorophyll "b" content are not significantly different and maximum chlorophyll "b" content 1.247 were recorded for stay green chirya-1 genotype followed by Opata wheat genotype. Similarly under drought stress 80% FC the maximum chlorophyll "b" content 1.159 was recorded for stay green chirya-1 genotype followed by Opata wheat genotype. Similarly in drought stress 60% FC the maximum chlorophyll "b" content 0.576 was recorded SD 28 followed by stay green chirya-1 genotype.

The data regarding total chlorophyll presented in (Table 1) revealed that drought stress affect the total chlorophyll content. The mean total chlorophyll value under control condition 100% FC 2.295 and drought stress 80% FC 1.968 mean value were recorded for total chlorophyll. In drought stress 60% FC the mean value 1.101with CV 4.846 was recorded shown in Fig 1. Analysis of variance (ANOVA) regarding Chlorophyll "a" chlorophyll "b" and total chlorophyll presented in (Table 3.) showed that drought stresses are highly significant and the response of genotypes was also highly significant. The interaction of drought stress and wheat genotypes was observed highly significant. The least significant difference (LSD) regarding total chlorophyll presented in Table 6 revealed that in control condition 100% FC the total chlorophyll content are not significantly different and maximum total content 2.477 were recorded for stay green chirya-1 genotype followed by Opata wheat genotype. Similarly under drought stress 80% FC the maximum total chlorophyll content 2.477 was recorded for Opata wheat genotype followed by SD-28. Similarly in drought stress 60% FC the maximum total chlorophyll content 1.143 was recorded for stay green chirya-1 genotype followed by SD 28 wheat genotype. The Overall results regarding chlorophyll contend suggested that different level of drought stress highly affect the chlorophyll "a" "b" and total chlorophyll contents. The results clearly indicate that stay-green chirya-1 genotype perform well under drought stress condition regarding chlorophyll content.

3.2. Expression levels of genes involved in LHCI and LHCII in response to drought stress.

Six genes which are responsible for coding pigment binding proteins were studied to find out different transcriptional responses to different level of drought Stress in two synthetic genotypes (SD-28 and SD-32) and two conventional wheat genotypes (Chirya-1 and Opata). As shown by semi-quantitative RT–PCR, that the expression pattern was different among the studied genes in response to different level of drought stress, and different level of genes expression was observed. However it is observed that expression of each genes were related to drought stress condition and the genotypes.

The expression level of genes which are encoding proteins for LHCI, namely *TaLhca1, TaLhca2* and *TaLhca3, showed* different level of expression in SD-28 genotype as shown in (Fig 5). The *TaLhca1* gene show down regulation in drought stress condition while *TaLhca2* gene show up regulation, similarly *TaLhca3* are down regulated in 80% FC but in severe drought stress condition show high level of expression. Similarly the genes which are involved in LHCII namely, *TaLhcb1, TaLhcb4* and *TaLhcb6* also show distinct level of expression as shown in (Fig 6). The *TaLhcb1* show down regulation under drought stress condition and expression level was decreased as the drought level was increased. *The TaLhcb4* show up regulation in both drought stress level 80% FC and 60% capacity while the expression of *TaLhcb6* show down regulation under 80 FC but the expression was induced in severe drought stress condition 60% FC.

The expression level of genes involved in LHCI, in genotype SD-32 were not affected by drought stress condition as shown in (Fig 7). The *TaLhca1* genes slightly affected by drought stress and show low level of expression as compare to control condition, while the Expression level of *TaLhca2* showed up regulation under 80% and 60% FC. Similarly *TaLhca3* gene was also not affected by drought stress and show up regulation. The expression level of genes which are responsible for LHCII show similar expression pattern with those involved in LHCI as shown in (Fig 8). The expression level of *TaLhcb1, TaLhcb4,* and *TaLhcb6* was not affected by drought stress and showed up expression.

The expression level of genes which are responsible encoding proteins for LHCI in drought sensitive wheat genotype Opata showed distinct level of expression as shown in (Fig 9). The *TaLhca1* genes were down regulated and expressions level decreased slowly as drought condition were increased. Similarly TaLhca2 gene expression was not significantly affected by drought stress condition, while *TaLhca3* did not decrease significantly and still remained high at severe drought stress condition under 60% FC. The expression level of genes which are responsible for LHCII also showed different level of expression as shown in (Fig 10). *TaLhcb1* genes show down regulation under drought stress condition while *Talhcb4* genes were highly sensitive to severe drought stress condition but not affected under 80% FC. The *TaLhcb6* gene also shows up regulation and not affected by drought stress condition. The results indicate that Opata genotype is drought sensitive as compare to synthetic derivatives SD-28.

The expression level of genes which encode proteins for pigment binding molecule LHCI showed highly significant results in Stay-Green Chirya-1 genotype as shown in (Fig 11). All the genes which involved in LHCI, namely The TaLhca1, TaLhca2 and TaLhca3 show higher level of expression and their expression is not effected by drought stress condition. Similarly the genes which involve in LHCII, namely TaLhcb1, TaLhcb4 and TaLhcb6 also show similar results like those which involved in LHCI as shown in (Fig 12). The expression levels of these genes were not significantly affected by drought stress condition which is and evident that Stay-Green Chirya-1 genotype is drought resistant as compared to Opata and Synthetic derivatives genotypes. The studied morphological parameters are also evident that Chirya-1 Varity performs well under drought stress condition.

3. DISCUSSION

The four selected wheat genotypes under different drought level showed significant difference regarding physiological parameters. Chlorophyll “a” and chlorophyll “b” is one of the events which are used for showing of water stress. The genotypes and stress level show different results regarding chlorophyll content. Data concerning Chlorophyll “a” and “b” presented in Table 1 demonstrated that there were significant differences in chlorophyll “a” and “b” after different level of drought stress. The data regarding total chlorophyll presented

in (Table 1) revealed that drought stress affect the total chlorophyll content. Similar results under different drought level were observed by (Kumar*et al.* , 2013) that chlorophyll content of leaf was demolished under drought stress treatment and also stops it from making. A number of investigators have observed that harm to chlorophyll content of leave as a result of drought stress (Arjenaki *et al.* , 2012; Nilsen and Orcutt, 1996). The cause for decrease in chlorophyll content of leaves as affected by water scarcity is that due to drought treatment reactive oxygen species (ROS) such as O2- and H2O2, was produced in which an escort to lipid peroxidation and as a result, chlorophyll demolition (Saeidi *et al.* , 2015; Foyer *et al.* , 1994; Schlemmer*et al.* , 2005) also reported that drought stress highly affect chlorophyll content in which changing the green color of leaf into yellow color, the reflectance of the event radiation is enlarged.

Relative water content is the relation between fully turgid water content and actual water content of plant tissues when they are subjected to drought stress condition. Therefore leaf relative water content indicates the ability of plants to keep their water status adequate enough to sustain water stress. In the current research the RWCs declined during water stress in all the studied wheat genotypes. Similar results were reported by (Siddique *et al.* , 2001) that drought stress condition considerably reduced the leaf potential and relative water content and transpiration rate with an associated raised in leaf temperature. This current result was also supported by the statement of (Almeselmani *et al.* , 2011) that drought regime lead to decrease status of water during the growth of crop, soil moisture potential and plant osmotic potential for water and nutrient uptake which finally moderate leaf turgor pressure as results metabolic activities of crop was disturbed.

Membrane stability and integrity is one of the significant selection measurements of non-irrigation stress charitable genotypes (Tripathy*et al.* , 2000). Because under water deficit environment membrane stability and integrity sure water scarcity resistance (Bewley, 1979). Analysis of variance (ANOVA) Water stress caused water loss from plant tissues which seriously impair both membrane structure and function (Cave *et al.* , 1981). Our results in agreement with (Vasquez-Tello*et al.* , 1990) that electrolyte leakage was correlated with drought tolerance. Our results also support the finding of (Sayar*et al.* , 2008) that they reported drought stress highly effect the membrane stability of the plant.

The results indicate that proline content was increased under drought stress condition as compared to control condition. As drought condition were increased the proline content were also increased. Our current experimental work was also supported the finding of (Parida *et al.* , 2007) that they reported that proline are highly accumulate in drought stress condition.

Relative expression of six genes which encode proteins for LHCI and LHCII were studied to check the effect of drought stress at molecular level. As shown by semi-quantitative RT–PCR, that the expression pattern was different among the studied genes in response to different level of drought stress, and different level of genes expression was observed. However it is observed that expression of each genes were related to drought stress condition and the genotypes. The results revealed that the Chirya-1 genotype perform well under water stress condition followed by Synthetic derivatives genotypes. The results regarding level of gene expression were related with the observation of (Zhao *et al.* , 2007) who reported that in drought stress condition the photosynthesis in the chloroplast is the most sensitive region. In the previous work it was observed that expression level of genes in Stay-Green was found to be greater than that of the wild type under drought condition (Tian *et al.* , 2012). The genes involved in LHCII namely *TaLhcb1, TaLhcb4* and *TaLhcb6* show higher level of gene expression in Stay-Green genotype and similar results was also observed by Tian *et al* . (2013) they observed higher level of gene expression in Stay-Green Genotype as compare to wild type wheat genotype. Our results revealed that LHCI genes *TaLhca2, TaLhca3* and LHCII genes *TaLhcb4* and *TaLhcb6* show up regulation in SD-28, Opata and Chirya-1 and their expression level is not affected by drought stress condition but *TaLhca1* and*TaLhcb1* are the most sensitive genes to drought stress condition. In comparison the expression level of genes is more affected in SD-32 followed by Opata wheat genotype. The overall results regarding level of genes expression is in general agreements with (Oksman-Caldentey and Saito, 2005; Reinders and Sickmann, 2007) who reported that the levels of regulation based on post-transcriptional and post-translational mechanisms are involved in the abiotic stress response.

Conclusion

It is concluded from current research work that Different drought levels had substantial effects on physiological traits of wheat. Photosynthetic pigments and Relative Water Contents were decrease when increase in drought stress. It is observed that over expression of genes (*TaLhca1, TaLhca2, TaLhca3, TaLhcb1, TaLhcb4* and *TaLhcb6*) that encodes for LHCI and LHCII proteins is directly proportional to drought stress. Among the studied genotypes wheat variety Chirya-1 and SD-28 performed well with higher level of gene expression under drought stress condition; considered drought tolerant genotypes with potential to enrich the genetic background of locally adapted wheat lines against drought stress.

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Authors Contribution

Ahmad Ali: Conceptualization. **Shahid Maqsood Gill:**Methodology, Software. **Ahmad Zada** : Data curation, Writing- Original draft preparation. **Irtiza Hussain** : Visualization, Investigation. **Azhar Hussain Shah** *:* Supervision:**Zahid Ullah** : Software, Validation. **Hassan Sher:** Writing- Reviewing and Editing,

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Table 1: Descriptive Statistics of study wheat genotypes under different field capacity

Variables		Mean	SD	Variance	SE
Membrane Stability Index	**Membrane Stability Index**	**Membrane Stability Index**			
100% FC		55.227	3.552	12.619	1.02
80% FC		60.691	1.978	3.912	0.57
60% FC		54.106	5.012	25.124	1.44
Proline Content					
100% FC		0.828	0.089	7.951	0.02
80% FC		1.433	0.242	0.059	0.07
60% FC		2.509	0.472	0.223	0.13
Relative Water Content	**Relative Water Content**	**Relative Water Content**			
100% FC		94.580	2.506	6.282	0.72
80% FC		81.887	2.386	5.694	0.68
60% FC		73.659	2.870	8.238	0.82
Chlorophyll a					
100% FC		1.186	0.039	1.515	0.01
80% FC		1.041	0.044	1.909	0.01
60% FC		0.542	0.037	1.378	0.01
Chlorophyll b					
100% FC		1.108	0.174	0.030	0.05
80% FC		0.926	0.156	0.024	0.04
60% FC		0.559	0.035	1.239	0.01
Total Chlorophyll					
100% FC		2.295	0.180	0.032	0.05
80% FC		1.968	0.141	0.020	0.04
60% FC		1.101	0.053	2.848	0.01

Table 2Mean Square of studied wheat lines

sov	DF	MSI	Proline	RWC	Chla	Chlb	Total Chl
Stress	2	148.9***	8.692***	1333***	1.371***	0.938***	4.563***
Genotypes	3	21.82NS	0.244***	7.35NS	0.004**	0.120***	0.106***
stress* Genotypes	6	20.07NS	0.323 ***	7.38NS	0.004***	0.033***	0.031***
Error	22	11.980	0.022	7.380	0.001	0.002	0.031

MSI Membrane Stability Index, RWC Relative Water Content, Chla Chlorophyll a, Chlb Chlorophyll b, Total Chl Total Chlorophyll

Table 3 LSD for membrane stability, proline, relative water content, chlorophyll a and b and total chlorophyll under control and drought stress condition

Treatment	Genotypes	MSI	Proline	RWC	Chla	Chlb	Total chl
100% FC	**SD-28**	58.176AB	0.872 H	93.701 A	1.200 AB	0.914 CD	2.115 B
100% FC	**SD-32**	50.679 C	0.864 H	96.477 A	1.168 BC	0.985 C	2.153 B
100% FC	**Opata**	53.637BC	0.857 H	93.010 A	1.144 C	1.285 A	2.431 A
100% FC	**Chirya-1**	58.415AB	0.719 H	95.131A	1.230 A	1.247 A	2.477 A
80% FC	**SD-28**	62.685 A	1.148 G	82.303 B	1.017 E	0.850 DE	1.869 C
80% FC	**SD-32**	62.047 A	1.556 EF	80.193 BC	1.099 D	0.777 E	1.876 C
80% FC	**Opata**	59.268AB	1.358 FG	82.175 B	1.034 E	0.917 CD	1.952 C
80% FC	**Chirya-1**	58.764AB	1.667 E	82.876 B	1.014 E	1.159 B	2.173 B

Treatment	Genotypes	MSI	Proline	RWC	Chla	Chlb	Total chl
60% FC	**SD-28**	55.966BC	2.272 C	73.396 DE	0.576 F	0.562 F	1.115 D
60% FC	**SD-32**	53.740BC	1.971 D	71.46 E	0.547 F	0.542 F	1.090 D
60% FC	**Opata**	54.707BC	3.091 A	73.659 DE	0.492 G	0.562 F	1.055 D
60% FC	**Chirya-1**	52.009 C	2.698 B	76.111 CD	0.550 F	0.592 F	1.143 D

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