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Screening Wheat Parents of Mapping Population for Heat and Drought Tolerance, Detection of Wheat Genetic Variation

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Abstract**—**To evaluate genetic variation of wheat (*Triticum aestivum*) affected by heat and drought stress on eight Australian wheat genotypes that are parents of Doubled Haploid (HD) mapping populations at the vegetative stage, the water stress experiment was conducted at 65% field capacity in growth room. Heat stress experiment was conducted in the research field under irrigation over summer. Result show that water stress decreased dry shoot weight and RWC but increased osmolarity and means of Fv/Fm values in all varieties except for Krichauff. Krichauff and Kukri had the maximum RWC under drought stress. Trident variety was shown maximum WUE, osmolarity (610 mM/Kg), dry mater, quantum yield and Fv/Fm 0.815 under water stress condition. However, the recovery of quantum yield was apparent between 4 to 7 days after stress in all varieties. Nevertheless, increase in water stress after that lead to strong decrease in quantum yield. There was a genetic variation for leaf pigments content among varieties under heat stress. Heat stress decreased significantly the total chlorophyll content that measured by SPAD. Krichauff had maximum value of Anthocyanin content (2.978 A/g FW), chlorophyll a+b (2.001 mg/g FW) and chlorophyll a (1.502 mg/g FW). Maximum value of chlorophyll b (0.515 mg/g FW) and Carotenoids (0.234 mg/g FW) content belonged to Kukri. The quantum yield of all varieties decreased significantly, when the weather temperature increased from 28 ◦C to 36 ◦C during the 6 days. However, the recovery of quantum yield was apparent after 8th day in all varieties. The maximum decrease and recovery in quantum yield was observed in Krichauff. Drought and heat tolerant and moderately tolerant wheat genotypes were included Trident, Krichauff, Kukri and RAC875. Molineux, Berkut and Excalibur were clustered into most sensitive and moderately sensitive genotypes. Finally, the results show that there was a significantly genetic variation among the eight varieties that were studied under heat and water stress.

Keywords**—**Abiotic stress, Genetic variation, Fluorescence, Wheat genotypes.

I. INTRODUCTION

WHEAT is the most important cereal grown in the world, and it is the most important cereal crop in Australia. Two thirds of the Australian wheat crop is exported. In Australia, most wheat in grown in rain field systems in which drought and heat stress occur frequently and with greatest severity during the post anthesis.

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Growth and primary production of plants are severely reduced by water deficit. Even in habitats with high average rainfall, plants may experience water stress in certain periods of the year, or at times during the day [12]. Drought is an important limitation to grain yield in many crops [10].

Two stresses, high temperature and drought stress are common environmental phenomena encounter by wheat throughout the world. High temperature or heat stress is often accompanied by drought stress under field conditions. In Australia, wheat is grown mainly as a rain fed crop and it is exposed to drought and heat stresses at various times in its life cycle but most commonly during grain filling.

Drought is a major factor limiting the productivity of wheat throughout the world and particularly in arid, semi arid and Mediterranean climates due to the unpredictable and erratic rainfall in these regions [27]. Breeding and selection for high yield under drought has been an important objective of crop breeders working in these environments.

Drought is an important environmental constraint that limits the productivity of many crops and affects both the quality and quantity of yield. Drought stress brings about a reduction in growth rate, stem elongation, leaf expansion and stomatal movements, and causes changes in a number of physiological and biochemical processes governing plant growth and productivity. The stresses may cause a variety of plant responses, which can be additive, synergistic or antagonistic. The response of genotypes to water stress is determined by measurement of a combination of traits.

Moderate to severe water stress drastically affects various morpho-physiological traits in wheat such as chlorophyll fluorescence, water use efficiency and dry matter yield [13]. Since genotypic differences for these traits have been reported for various crop species including wheat, these traits have been used to identify drought tolerant genotypes in various crops.

Various physiological traits related to drought tolerance in wheat have been used to select tolerant genotypes.

Jia [26] after the results of eight years of pot and field experiments found that, winter wheat genotypes with high water use efficiency had greater yield potential. Similarly, Gan [17] reported 60% increase in grain yield of winter wheat, spring and durum wheat due to the improved water use efficiency under semi arid climates. Also, reported that

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drought tolerant genotypes of wheat had higher water use efficiency, chlorophyll a/b contents and dry weight than drought susceptible genotypes [52]-[39].

Photosynthesis is sensitive to heat and drought stresses and it is often the first process that is affected by stress. Various traits are linked strongly to photosynthesis like chlorophyll content and components, chlorophyll fluorescence, relative leaf water content and some leaf pigments.

Photosynthetic activity is reduced by water stress. One of the earliest responses to drought is stomatal closure, which limits CO_2 diffusion to chloroplasts [35]. With short periods of drought, or with relatively mild water stress, stomatal limitations account for most of the decrease in photosynthesis [12]. However, when drought is prolonged and/or more severe, the breakdown of the photosynthetic apparatus may contribute to the inhibition of CO_2 gain in water-stressed plants. In leaves experiencing a 30% or higher leaf water deficit, there was a significant decrease in photosynthetic O_2 evolution at high CO_2 concentration, i.e. where stomatal limitations to CO_2 have a minimal impact [12]. Photosystem activity and photophosphorylation have been shown to decrease in droughted plants [20]-[23]. Non-stomatal limitations to photosynthesis in droughted plants might be related to oxidative damage to chloroplast lipids, pigments and proteins. In droughted wheat, sunflower and pea leaves [33, 34]-[43, 44, 45] there was an increase in the production of reactive oxygen species (ROS) and in lipid peroxidation, probably initiated through photo-reduction of O_2 by the photosynthetic electron transport system [33]-[45]. ROS in plants are removed by a variety of anti-oxidant enzymes (e.g. superoxide dismutase, ascorbate peroxidase) and by small ROS scavenging molecules [15].

Chlorophyll fluorescence has been used in several studies to detect the genotypic differences in response to heat stress in many plant species, including wheat [49]. Araus [4] have examined durum wheat cultivars at the flag leaf stage under the hot field conditions and measuring chlorophyll fluorescence parameters.

Various studies reported that Fv/Fm ratios indicate the maximum efficiency of photosystem II [49] and in healthy plants, the value of Fv/Fm is 0.83. A similar effect of water stress on the PS II efficiency and a significant decline in Fv/Fm values were reported in intact wheat leaves [53]. Use of a chlorophyll fluorescence technique as a tool to investigate drought tolerance in different wheat genotypes has been reported.

Many studies have been conducted to observe the seedling response to heat stress in various crops. For example; rice [51], maize and wheat [11]. Babani and Mathis [7] exposed five wheat cultivars to 40^{0C} for 4h and reported a large decrease in variable fluorescence parameters and PS II efficiency.

Likewise, Lu and Zhang [32] reported that moderately high temperatures ($25\text{-}37.5^{0C}$) did not show significant changes in Fv/Fm ratio. However, a significant decrease in the Fv/Fm ratio was observed when plants were exposed to high temperatures ($40\text{-}45.5^{0C}$). However, Balota and Lichenthaler [9] are reported the effect of moderate heat stress (35^{0C}) in wheat seedlings under field conditions and have shown that such temperatures affected the chlorophyll fluorescence measurements and net photosynthesis in wheat seedlings.

One of the major goals for plant breeders is to develop genotypes with a high yield potential and the ability to maintain yield across environments. This is particularly true in the Mediterranean basin where harsh and fluctuating climatic conditions lead to high Genotype × Environment (G×E) interactions. With the development of molecular markers, breeders have a complementary tool to traditional selection, and markers linked to the variation in a trait of interest could be used to assist breeding programs. However, the identification of relevant markers linked to the variation of yield components and stability is difficult and time-consuming. Nevertheless, quantitative trait loci (QTLs) for such traits were described. As the Mediterranean area is often drought-prone (low rainfall, high temperature and light intensity leading to high evaporative demands), the identification of QTLs for agronomic traits in wheat in some Mediterranean environments will be of interest for yield improvement in this area. Screening wheat parents of mapping population for heat and drought tolerance and detection of wheat genetic variation is the first step to identify suitable parents and aide to plant breeding programs by QTL mapping methods.

In this study, the effects of water and heat stress on wheat seedlings were carried out experimentally to investigate the genetic variation by treatments. Also, mention it was done using parents of mapping populations. The Anthocyanin levels, chlorophyll content, osmotic potential and Chlorophyll fluorescence of treated seedlings were analyzed and compare with those of the untreated seedlings.

II. Materials and Methods

A. Experimental design and treatments

To evaluate genetic variation of wheat (*Triticum aestivum*) affected by heat and drought stress on eight wheat genotypes that are parents of Doubled Haploid (HD) mapping populations (Table 1) at the vegetative stage, two experiments were conducted at Crop Physiology Laboratory, School of Agriculture Food and Wine, Waite Campus, Adelaide University, in Australia.

The water stress experiment was conducted in growth room whit $15/20^{0C}$ day/night temperature and 12 hours of photoperiod. The light intensity was 800µmole quantum.m-2.sec^{-1} throughout the experimental period. Sixteen small pots, eight each for the control and drought stress treatments, were prepared and filled with 700 g of soil for each replicate. In each pot, four seeds were sown and thinned after one week to give final population of two uniform seedlings per pot. Moisture level was maintained by adding the nano-pure water daily in the morning at field capacity after weighting the pots (Table 2). After two weeks of sowing, pots allowed to dry and

TABLE I
GENOTYPES INFORMATION THAT USED IN THIS EXPERIMENT. THERE ARE PARENTS OF DOUBLED HAPLOID (HD) MAPPING POPULATIONS.

Serial no	Genotype	Year of Release	Origin	Pedigree
1	Cascades	1994	WA	Aroona*3/c Ausenvii-95 Tadorna. India66
2	Excalibur	1991	SA	RAC177(Sr26)/Uniculm492//RAC311s
3	Molineux	1988	SA	Pitic62/Festigur/12*Warigal
4	Krichauff	1996	SA	Wariquam//Kloka/Pitic2/3/Warimek/Halberd/4/3 ag3 Aroona
5	Kukri	1999	SA	Srl3*3//76 ECN 44/76 ECN 36=RAC 820
6	Trident	1993	SA	VPM11.5 *Cook//4*Spear
7	RAC875-2	-	SA	Rac655/sr214*Lance//4*Bayonet
8	Berkut	2002	CIMMYT	Irene/Babax//Pastor

Source For Pedigree: International Crop Information System (ICIS, 2000 CD V1.0); CIMMYT & IRRI; Australian Winter Cereal Collection (AWCC) and Howard Eagles (press.com.2002).

water were added to bring it up to 65% field capacity.

Another experiment was conducted in the research field at the Waite Campus of Adelaide University for study the effect of heat stress. Then, we sowed the seeds of eight varieties in three rows (1m) for each variety and two replications in the field. Plants were grown under irrigation over summer (25$^{\circ C}$ to 39$^{\circ C}$).

TABLE II
DATA OF SOIL ANALYSIS IN DROUGHT STRESS

	10 KPa 'field capacity'	1500 KPa permanent welting point	
Name of soil	Mean water content	Mean water content	FC-PWP (g/g)
Coco Peat Potting Mix	1.447	0.685	0.761

B. Measurements

Biochemical analyses were carried out on fresh plant material that were immediately extracted and assayed according to the appropriate methods listed here.

C. Anthocyanin

To determine the concentration of Anthocyanin, 0.5 g leaves was extracted in 10 mL of acidified methanol (HCl: methanol, 1:99, v/v) and tubes of samples were centrifuged in 10 min and 3000 rpm and kept overnight in the dark. Absorption spectra of the extracts were determined using UV-VIS spectrophotometer (model 2100 Shimadzu, Columbia, MD, USA). The spectra were scanned from 350-750 nm on a split beam UV-VIS spectrophotometer (model 2100 Shimadzu, Columbia, MD, USA) and absorbance at 560 nm determined. Anthocyanin concentrations were shown by Absorbance per fresh weight.

D. Chlorophyll fluorescence

In vivo chlorophyll fluorescence was measured with a pulse amplitude modulation fluorometer (model PAM 2000, Walz, Effelrich, Germany). Fluorescence signals were analyzed as described by Andrews [3] to provide estimates of the Fv/Fm (primary maximal PSII efficiency), measured after 15 min of dark adaptation. ΦPSII (actual quantum yield of PSII electron transport) was calculated from chlorophyll fluorescence data obtained from leaves exposed to light [19], measured at a PPFD of 800 μmol m^{-2} s^{-1}, which was similar to the minimum mean growth PPFD. F_o was measured at very low irradiance (0.6 $mmol.m^{-2}.s^{-1}$; modulated light). Fm was determined by application of a saturating pulse of white light (3000 $mM.m^{-2}.s^{-1}$). Variable fluorescence (Fv) was determined as Fm-Fo. The used fluorescence parameter was ΔF/F′m (effective quantum yield of photosynthesis), which is calculated as (F′m-Ft)/F′m. The chlorophyll a fluorescence was measured before and after the heat stress, at the same position of the leaf in the same time per measurement (10-12 am).

E. Chlorophyll a and b and Carotenoids Content

The chlorophyll content was measured 4 times from the stunt of the water and heat stress paned. Measurements were made on the second youngest emerged blade on two seedlings per pot, with a chlorophyll meter, (SPAD-502, Soil Plant Analysis Development (SPAD) Section, Minolta Camera Co, Osaka, Japan). Three readings were taken along the middle section of the leaf, and mean used for analysis and values were expressed as SPAD units.

Total chlorophyll pigments were extracted by 85% acetone and estimated according to Arnon [5]. Leaf samples (0.5 g f. wt) were ground in 10 ml of 85% acetone. The homogenate was centrifuged at 3000 rpm for 10 min. The concentrations of chlorophyll a and b and Carotenoids (xanthophyll and β-carotene) were determined by colorimetry measuring the absorbance of chlorophyll at 646, 663 and 470 nm and then using equations described by Lichtenthaler and Wellburn [31].

F. Relative leaf water content (RWC)

Plant water status was estimated by measuring the relative leaf water content (RWC) as previously described [48]. The RWC measured on the youngest emerging leaf to ensure uniformity across all the plants. Leaves were harvested directly in to 15 mL eppendorf tubes and place on ice to prevent any further water loss, and then weight to determine fresh weight (FW). Two mL of nano-pure water was added to the tubes and the leaves place in a cold room overnight (for 24 h at 4$^{\circ C}$) to allow for rehydration. Following rehydration, the leaves were re-weighted for turgid weight (TW). The leaves were dried at 65$^{\circ C}$ for 24 hours and weighted for dry weight (DW).

RWC was determined by: RWC% = ((FW-DW)/(TW-DW)) x 100

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G.Dry Shoot Weight (DM)

Shoots (leaf and stem) from two seedlings were oven dried at 65^{oC} for 48 h to constant weight for the determination of dry weight.

H.Total Water Use (TWU) and Water Use Efficiency (WUE)

Total water use (g) of each genotype was calculated by adding the daily water used by two seedlings. Water-use efficiency was calculated by integrating over the vegetation period dividing accumulated dry matter by cumulative water use (WUE).

I. Osmolarity

Osmolarity measurements were made using a model 5520 Vapor Pressure Osmometer for samples in drought stress

J. Data and Statistical analyses

Statistical analyses were performed using the Genstat 6 for Windows [18]. Data were analyzed by ANOVA using models appropriate to the experimental design. These were complete randomize design for drought stress and randomize complete block design for heat stress by three and two replication respectively. Differences between means were assessed using the least significant differences (LSD) at the 5% and 1% probability levels. Relationships between variables were examined by simple linear correlation (r) and by regression analysis. The measurements of all traits were made on the same leaves at the same time, which allowed the data to be analyzed using a repeated measurement ANOVA. The data were then analyzed using agglomerative hierarchical cluster analysis in order to explore the grouping within the genotypes. The further neighbor method was used to cluster the genotypes.

III. Result and Discussion

A. Effects of Drought Stress

There were highly significant differences among the variances for all the traits measured. The result of data variance analysis show the mean square values of total chlorophyll (SPAD), WUE, growth stage (GS), stem number, dry shoot weight (DM), quantum yield, Osmolarity, RWC in 5 days, primary maximal PSII efficiency (Fv/Fm) 11 days and Anthocyanin content 5 days after stress for the variety were highly significant. In addition, there was a highly significant for mean square values of relative water content (RWC) in 5 days, stem number, Osmolarity, primary maximal PSII efficiency (Fv/Fm) 7, 11 days, and quantum yield 11 days after stress between treatments. Varieties x Treatment interactions were significant for Osmolarity and quantum yield 1 and 11 days after stress. There was not any significant difference for Anthocyanin content after 12 days of water stress among varieties and treatments.

B. Plant water

Water stress decreased the relative water content (RWC) up to 6 % in all varieties except for Krichauff. The minimum RWC was observed in RAC 875 and Berkut under water stress. Krichauff and Kukri had the maximum RWC under drought stress (Figure 1).

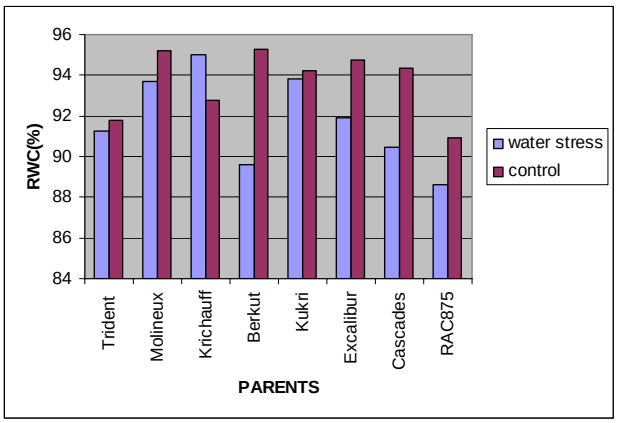


Fig. 1 Effect of water stress on RWC percentage in eight Australian wheat parents

Sairam and Saxena, [40] reported that the relative water content (RWC) in leaves of wheat cultivars under irrigated and stress conditions showed a decreasing trend with age in all the genotypes. There was significant reduction in RWC under moisture stress in all the cultivars. The results are in agreement with the findings of Al-Hakimi and Mannoveux [1] who reported high leaf water potential and RWC under drought in tetraploids. Significant differences in RWC/water potential in tolerant and susceptible genotypes of barley and wheat [30] have also been reported. Tambussi, [49] reported that water stressed plants showed a significant decrease in RWC during the experiment, reaching values of 85 and 55% after 6 and 8 day of withholding water, respectively.

Water stress did not have any significant effect on water use efficiency of eight varieties in this study but there was significant variation among the wheat varieties for WUE. Therefore, minimum and maximum WUE were shown in Excalibur and Trident respectively (Figure 2). Selection for high water use efficiency in wheat might improve the yield potential under drought conditions [38].

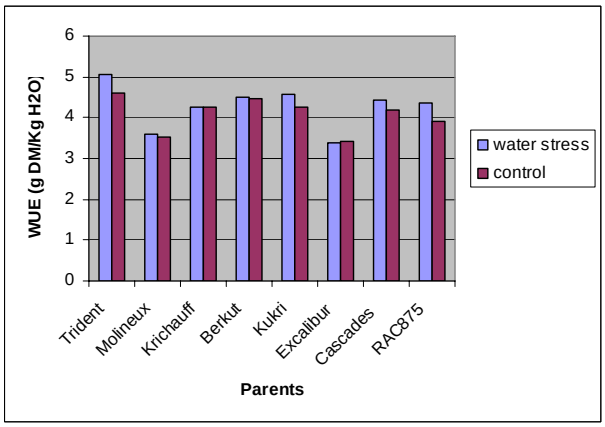


Fig. 2 WUE differences of eight Australian wheat parents under water stress condition

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Result show that water stress increased osmolarity in all varieties. Maximum osmolarity was observed in RAC 875-2 (740 mM/Kg) and after that in Trident (610 mM/Kg) under water stress condition. Berkut had minimum osmolarity (420 mM/Kg) after water stress condition (Fig. 3).

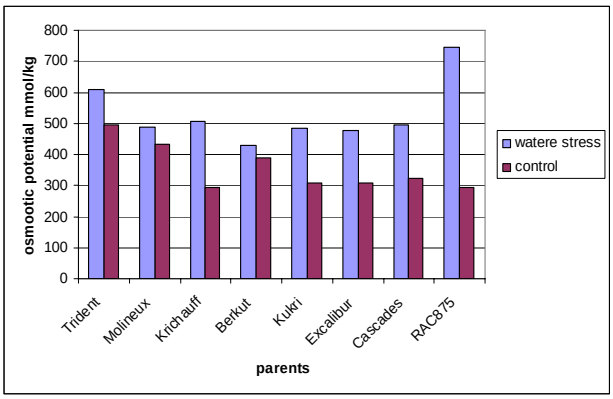


Fig. 3 Osmotic potential differences of eight Australian wheat parents under water stress

Flagella [14] also reported that drought tolerant cultivars showed a smaller decrease in photosynthetic efficiency (Fv/Fm ratios) and higher osmotic adjustment and leaf water potential under both water regimes, however, drought susceptible cultivars despite good osmotic adjustment and leaf water potential showed drastic decreases in photo efficiency under severe drought stress.

C. Growth and development

Results show that water stress decreased the stem number significantly in all varieties (Figure 4). The maximum decrease in stem number was observed in Berkut under drought stress and control condition. The minimum decrease in stem number under water stress was observed in Molineux. The varieties Molineux, Krichauff and Kukri had maximum stem number in control condition (Fig. 4).

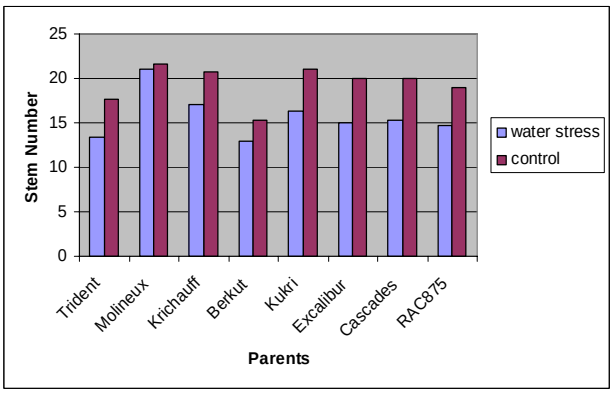


Fig. 4 Effect of water stress on Stem number in eight Australian wheat parents at 13 DAS

Result show that water stress decreased the dry shoot weight in six of eight varieties. Minimum and maximum dry mater was observed in Excalibur and Trident under both drought and control conditions respectively (Figure 5). James [25] reported that dry matter yield increased by on average of 53% in 18 different species including cereals such as wheat, rice, barely and sorghum, due to the increase in WUE variations unconcern of photosynthesis [13].

D. Fluorescence

The means of Fv/Fm values were increased in all varieties after water stress. Maximum Fv/Fm was showed in Trident 0.815 and 0.820 for both non-stress and stress conditions respectively. Minimum Fv/Fm value was observed in Excalibur 0.795 and 0.802 for both control and water stress conditions respectively (Fig. 6).

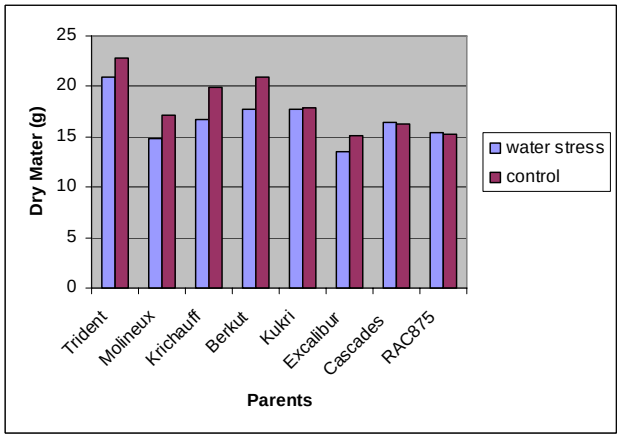


Fig. 5 Effect of water stress on Shoot dry weight in eight Australian wheat parents

Quenching modulated Chl fluorescence was used for screening wheat (*Triticum aestivum* L.) cultivars for water stress tolerance. The same was also true for screening wheat (*Triticum durum* Desf.) for drought tolerance [49]. In addition, in wheat, Fv/Fm was used to distinguish stomatal and non-stomatal limitations to photosynthesis under water stress [29], and to separate effects of water stress from photo inhibition [32]. The ratio Fv/Fm was used to study effects of water stress on PSII in wheat and components of the xanthophyll cycle [46]-[36]-[54]-[56].

Result show that water stress decreased quantum yield of all varieties significantly in this study. Maximum decrease in quantum yield was observed in Berkut and Excalibur at the same time as Berkut had a maximum quantum yield 11 days after stress in control condition among the varieties. Nevertheless, the quantum yield values were increased after drought stress in Trident and RAC857-2 against the other varieties in the same condition (Figure 7).

However, the recovery of quantum yield was apparent between 4 to 7 days after stress in all varieties. Nevertheless, increase in water stress after that lead to strong decrease in quantum yield. The recovery of photosynthetic efficiency after various abiotic stresses has been examined in a number of crops including rice, maize and soybean [24].

Similarly, water stress imposed at different stages after anthesis resulted in an increase in lipid peroxidation and a

decrease in membrane stability and chlorophyll and carotenoid contents [40]. Sairam and Saxena, [40] found that total chlorophyll (Chl) and carotenoid (Car) contents showed a decreasing trend with age, under both control and stress conditions.

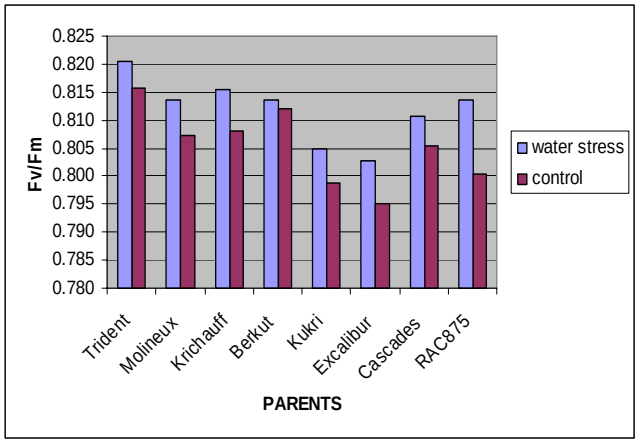


Fig. 6 Fv/Fm differences of eight Australian wheat parents under water stress condition.

There was marked reduction in Chl and Car contents under water stress in all cultivars. Oxidative injury at the cellular level because of water or other stresses (temperature, pollution, etc.) is a major cause of crop damage [2]. Genotypes respond differentially to such stresses because of variations in their antioxidant systems [30]. Carotenoids are responsible for scavenging of singlet oxygen, and hence their comparative levels in a genotype will determine its relative tolerance. Higher Chl and Car contents in tolerant genotypes have also been reported earlier [30]. Chlorophyll maintenance Helpful Hints is essential for photosynthesis under drought stress. Higher Chl content and lower per cent decrease under stress in tolerant genotype of wheat [30]-[41, 42] has also been reported.

Limitation of photosynthesis by water stress, especially when it is combined with conditions of high temperature and light, may cause photo-oxidative damage to the photosynthetic apparatus if the plant does not avoid or dissipate the excess excitation energy [6]. In this case, water stress can damage several components of cells, including proteins and lipids [23]-[49].

However, if the photoprotective mechanisms are insufficient, the leaves are protected from stress-induced oxidative damage by several antioxidant systems [15]. Carotenoids, like β-carotene, are key scavengers of reactive oxygen species such as singlet oxygen, and so protect thylakoidal membranes from oxidative damage [57].

E. Pigments

Water stress did not have a significant effect on leaf Anthocyanin content in our study but the means of leaf Anthocyanin content show different result among the varieties.

Therefore, water stress increased Anthocyanin content in Krichauff and Kukri whereas it decreased in Berkut, Molineux and Trident (Figure 8). This result may be due to low UV light levels in the growth room.

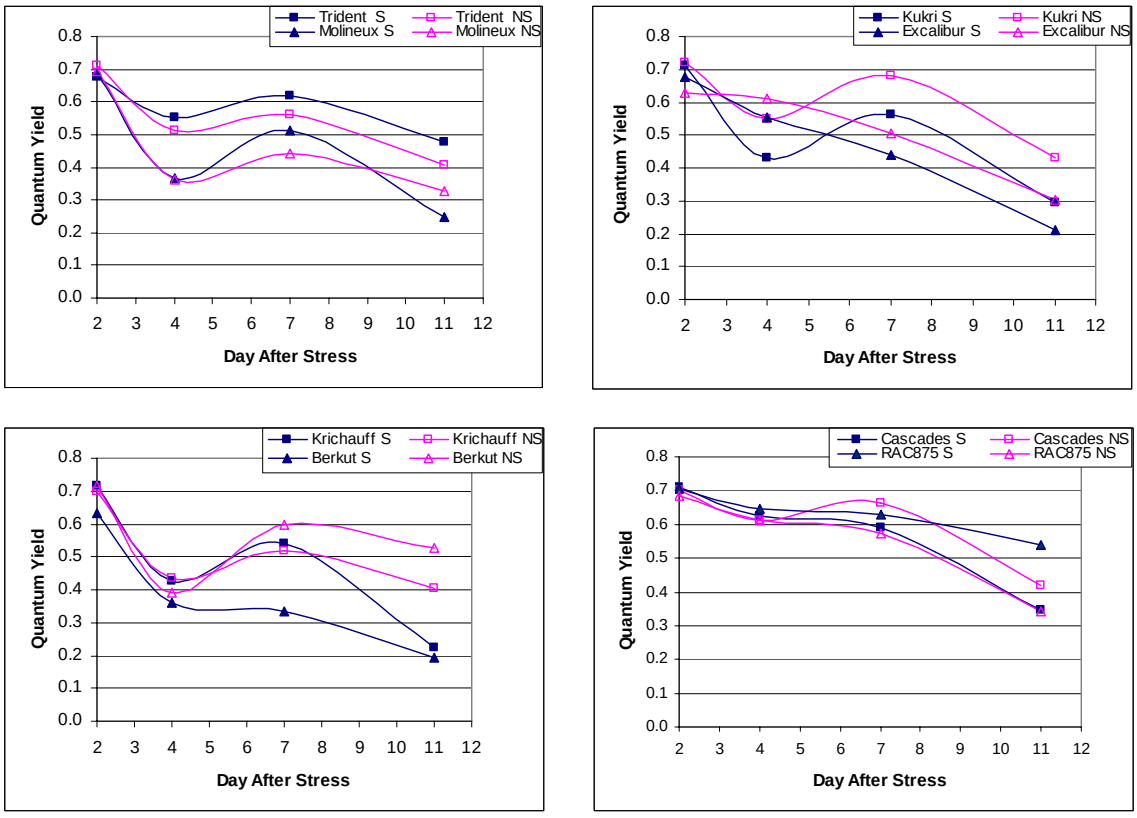


Fig. 7 Quantum yield differences of eight Australian wheat parents under water stress

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Carotenoids are responsible for the scavenging of singlet oxygen. Higher levels of Car in tolerant genotypes have also been reported by, Kraus [30] and Sairam [42].

Carotenoids protect against photo-oxidation because they actively quench singlet oxygen and minimize its formation by absorbing excess energy from excited triplet states of Chi. Zhang and Kirkham [59] reported that Drought did not generally affect carotenoid content in either crop. Their results suggest that the physiological levels of Carotenoids in sorghum and sunflower might be high enough to protect against photo-oxidation. Price and Hendry [37] also reported unchanged concentration of Carotenoids in wheat leaves under drought.

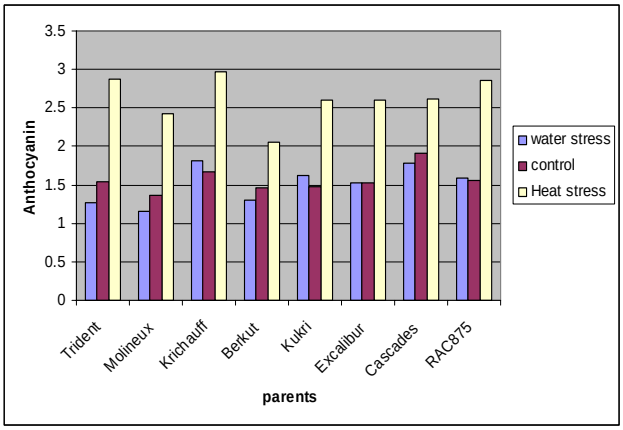


Fig. 8 Effect of water stress on Anthocyanin content in eight Australian wheat parents

Although drought is said to increase pigmentation [55], no evidence of drought-induced anthocyanin synthesis could be found. Combinations of high UV-B radiation and water stress increased pigmentation in cowpea [8] and cucumber [55] seedlings, but not relative to UV alone. On its own, water stress had no significant effect on pigmentation.

Finally, the results show that there was a significantly genetic variation among the eight varieties that were studied under water stress.

The hierarchical cluster analysis grouped based on all traits the cultivars into 5 groups at the 60 % level. Group 1: Trident (1); group 2: Krichauff (3) and Kukri (5); group 3: Cascades (7) and RAC875 (8); group 4: Molineux (2); group 5: Berkut (4) and Excalibur (6). (Figure 9)

The dendrogram revealed that drought tolerant and moderately drought tolerant wheat genotypes were clustered into group 1, 2 and 3. A number of most sensitive and as well as moderately sensitive genotypes clustered together in-group 4 and 5.

F. Effects of Heat Stress

There was a genetic variation for SPAD values among eight Australian wheat varieties that were studied under heat stress in the field. The result show that heat stress decreased significantly the total chlorophyll content that measured by SPAD. Maximum SPAD value was observed in RAC 875-2 (55%) and the minimum SPAD value was showed in Berkut 40% (Figure 10).

Also, differences in leaf pigments content values of Australian wheat varieties under heat stress show that there was genetic variation among these varieties. The results of means leaf pigments content show that the Berkut had minimum value of Anthocyanin content (2.053 A/g FW), chlorophyll a+b (1.171 mg/g FW), chlorophyll a (0.894 mg/g FW) and chlorophyll b content (0.276 mg/g FW). Whereas, Krichauff had maximum value of anthocyanin content (2.978 A/g FW), chlorophyll a+b (2.001 mg/g FW) and chlorophyll a (1.502 mg/g FW). Maximum value of chlorophyll b content belonged to Kukri (0.515 mg/g FW) (Table 3)

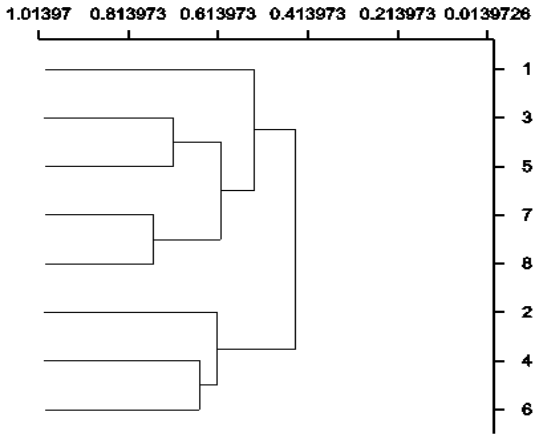


Fig. 9 The hierarchical cluster analysis grouped the cultivars into 5 groups of 8 Australian

Zaharieva [58] reported that leaf color and chlorophyll content were correlated, as expected, since chlorophyll loss is the main factor responsible for change in leaf color. Chlorophyll content was positively correlated with biomass and grain weight per plant. Plant temperature depression (the difference between air and plant temperature) was positively correlated with chlorophyll content when all accessions were considered and when some origin, such as Lebanon, were considered separately. They found the negative correlation for the Jordanian accessions with pale green leaves.

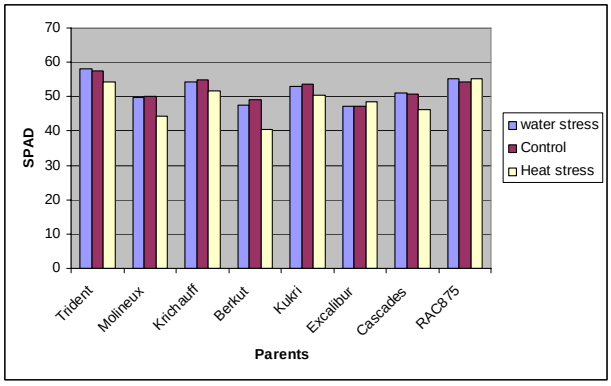


Fig. 10 SPAD differences of eight Australian wheat parents under heat and water stress.

In addition, pale green color and low chlorophyll content

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could play an important role in leaf temperature regulation, as suggested for barley [22], whereas earliness of varieties could permit them to complete their growth cycle before the hottest and driest period.

Tardy [50] showed that the light green color leaves of Tadmor was due to a non-specific decrease in both chlorophyll and Carotenoids, without repartitioning of the pigments between or within the pigmented thylakoid complexes. This pigment alteration appears to be an adaptation to high temperature rather than to strong light.

Another important pigment in the wheat leaves is Carotenoids that was measured in this study. Maximum Carotenoids content was observed in Kukri (0.234 mg/g FW) and RAC 875-2 had minimum value of Carotenoids (0.138 mg/g FW). In addition, Krichauff had high Carotenoids content after Kukri by 0.197 mg/g FW (Table 3).

The Carotenoids serve at least two important functions in photosynthesis, namely light harvesting and photo-protection [16]. Carotenoids are vital as photo protective agents that prevent the chlorophyll-photosensitized formation of highly destructive singlet oxygen by intercepting the chlorophyll triplet states and by scavenging any additional singlet oxygen present.

Results from the field experiment show that the quantum yield of all varieties decreased significantly, when the weather temperature increased from 28$^{\circ C}$ to 36$^{\circ C}$ during the 6 days. However, after 6th day temperature dropped off to 28$^{\circ C}$ for 2 days whereas the quantum yield was obtained dropped to minimum value on 8th day. After 8th day, the quantum yield of all varieties increased until 16th day, except for Kukri that did not have any change in quantum yield in these days. The weather temperature was increased from 28$^{\circ C}$ to 35$^{\circ C}$ after 8th day. We think that low temperature for 2 days between 6th and 8th day lead to acclimate the varieties to heat stress. This acclimation caused to an increase in the quantum yield even in high temperature after 8th day. However, the recovery of quantum yield was apparent after 8th day in all varieties. The maximum decrease in quantum yield was observed in

TABLE III
LEAF PIGMENTS CONTENT DIFFERENCES OF EIGHT AUSTRALIAN WHEAT PARENTS UNDER HEAT STRESS CONDITION.

Parents	Carotenoids (mg/g Fw)	Anthocyanin (A/g Fw)	Chlorophyll a+b (mg/g Fw)	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)
Trident	0.141	2.868	1.641	1.239	0.401
Molineux	0.189	2.424	1.457	1.100	0.357
Berkut	0.161	2.053	1.171	0.894	0.276
Krichauff	0.197	2.978	2.001	1.502	0.498
Cascades	0.152	2.617	1.471	1.113	0.357
RAC875	0.138	2.850	1.603	1.213	0.389
Excalibur	0.164	2.595	1.279	0.968	0.311
Kukri	0.234	2.608	1.996	1.480	0.515
CV%	27.9	16.5	18.9	18.4	21.0
e.s.e	0.01955	0.17635	0.1216	0.0891	0.0332

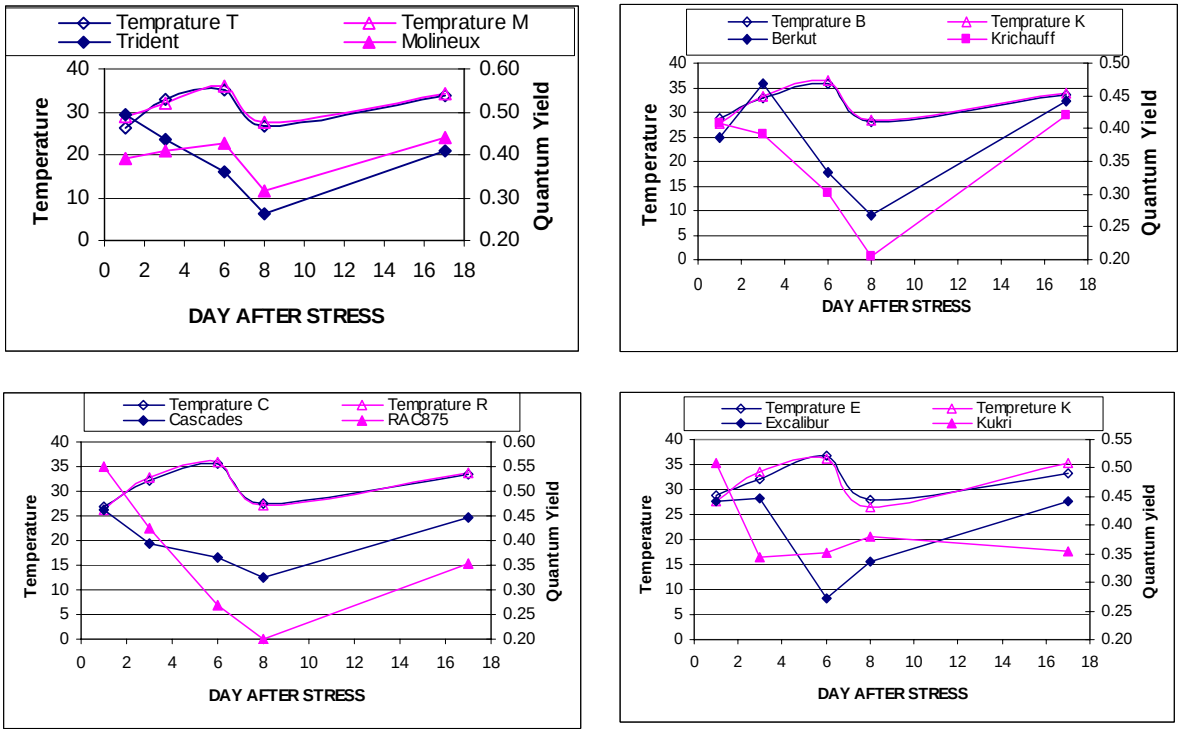


Fig. 11 Effect of heat stress on quantum yield in 8 Australian wheat parents.

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Krichauff and RAC 875-2 and maximum recovery was in Krichauff, Molineux, Cascades and Excalibur. Kukri did not show any acclimation or recovery in our study (Figure 11).

Karim [28] reported that both Fv/Fm and ΦII PSII were very stable in heat stressed developing leaves, whereas they decreased strongly in the developed leaves and did not recover, even after 72 h. This indicates that irreversible damage occurred to the PSII functions of the developed leaves because of the heat stress [21].

Sharkey [47] reported that the quantum yield estimated by chlorophyll fluorescence declined during heat stress of the tobacco (w38) leaf but recovered after the stress. Chlorophyll fluorescence was measured as leaves were heated from 30 to 42°C then cooled back to 30°C. Regardless of the conditions, photosynthesis could be inhibited by up to 50% by high temperature and still rapidly recover when the temperature was lowered.

Results show that highly significant negative correlation between weather temperature and quantum yield (r = -0.741) and also, positive association with Carotenoids content (r = 0.752). So, increasing the temperature lead to increasing the Carotenoids content and decreasing the quantum yield as well (Figure 11 and Table 3) so as to this result was a strong evidence for negative correlation between quantum yield and Carotenoids content in heat stress condition.

The hierarchical cluster analysis grouped the cultivars into four groups at the 65% level (figure 12). The dendrogram revealed that heat tolerant and moderately heat tolerant wheat genotypes were clustered into group 1 and 2. A number of most sensitive and as well as moderately sensitive genotypes clustered together in-group 3 and 4.

IV. Conclusion

Result show that water stress decreased dry shoot weight and RWC but increased osmolarity and means of Fv/Fm values in all varieties except for Krichauff. Krichauff and Kukri had the maximum RWC under drought stress. Trident variety was shown maximum WUE, osmolarity (610 mM/Kg), dry mater, quantum yield and Fv/Fm 0.815 under water stress condition. However, the recovery of quantum yield was apparent between 4 to 7 days after stress in all varieties. Nevertheless, increase in water stress after that lead to strong decrease in quantum yield. There was a genetic variation for leaf pigments content among varieties under heat stress. Heat stress decreased significantly the total chlorophyll content that measured by SPAD. Krichauff had maximum value of Anthocyanin content (2.978 A/g FW), chlorophyll a+b (2.001 mg/g FW) and chlorophyll a (1.502 mg/g FW). Maximum value of chlorophyll b (0.515 mg/g FW) and Carotenoids (0.234 mg/g FW) content belonged to Kukri. The quantum yield of all varieties decreased significantly, when the weather temperature increased from 28°C to 36°C during the 6 days. However, the recovery of quantum yield was apparent after 8th day in all varieties. The maximum decrease and recovery in quantum yield was observed in Krichauff. Drought and heat tolerant and moderately tolerant wheat genotypes were included Trident, Krichauff, Kukri and RAC875. Molineux, Berkut and Excalibur were clustered into most sensitive and moderately sensitive genotypes. Finally, the results show that there was a significantly genetic variation among the eight varieties that were studied under heat and water stress.

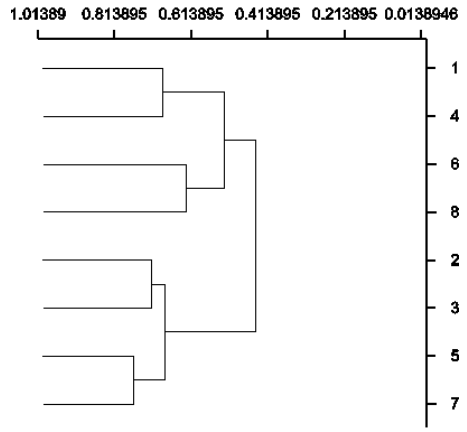


Fig. 12 The hierarchical cluster analysis grouped the cultivars into 4 groups of 8 Australian wheat parents under heat stress. Group 1: Trident (1) and Krichauff (4); group 2: RAC875 (6) and Kukri (8); group 3: Molineux (2) and Berkut (3); group 4: Cascades (5) and Excalibur (7).

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