SCREENING WHEAT SEEDLINGS FOR HEAT AND DROUGHT TOLERANCE BY *IN VIVO* CHLOROPHYLL FLUORESCENCE

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Thesis submitted to the University of Adelaide for the degree of
Master of Agriculture Science

Discipline of Plant and Pest Science
School of Agriculture and Wine
The University of Adelaide

January 2005

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SUMMARY

Heat and drought stresses affect the yield and total production of wheat in Australia and Pakistan. In Australia, these stresses occur most frequently and with greatest severity during the post anthesis period, but in Pakistan drought and high temperature can occur during seedling emergence of wheat as well as grain filling stage. These stresses adversely affect the seedling emergence, coleoptile length, seedling growth and photosynthesis in wheat (Alkhatib and Paulsen, 1984; Shipler and Blum, 1986; Radford, 1987; Krause, 1988; Wardlaw *et al.*, 1989). This study examined the effects of high temperature and drought on seedlings of wheat. A diverse range of genotypes were used, a number of which have been previously characterised for their tolerance to heat stress during grain filling. A series of experiments was conducted to examine the effect of heat-stress and drought-stress on the *in vivo* chlorophyll fluorescence, seedling emergence, coleoptile length and different morpho-physiological traits in wheat seedlings. The main objective was to examine tolerance to these stresses during seedling growth using the chlorophyll fluorescence technique.

To examine the genotypic variability in wheat seedlings for heat-tolerance, *in vivo* chlorophyll fluorescence technique was used to screen 100 wheat genotype seedlings after standardizing a screening method in a preliminary experiment. Wheat genotypes of diverse genetic background representing different agro-climatic zones of Australia and some of the other world regions were selected. Many of these genotypes have been tested previously for heat-tolerance at the anthesis or grain filling stage in Australia (Wardlaw *et al.*, 1989; Stone and Nicolas, 1994 & 1995, Blumenthal *et al.*, 1994). Seedlings of these genotypes were grown in small pots (300 mL) for 14 days in a

control growth room with 25/20°C day/night temperature with 10 hours photoperiod. Heat-stress (40°C) was imposed for 6 hours on 14 days old seedlings in a hot air incubator under the controlled growth room conditions. *In vivo* chlorophyll fluorescence measurements were recorded in two leaves of seedlings using Plant Efficiency Analyser (Hansatech, Ltd), before and after heat-stress. Recovery of the chlorophyll fluorescence was also recorded usually after 24 hours of heat-stress.

Considerable genotypic variation was observed. Normally, 6 hours of heat-stress (40°C) reduced the chlorophyll fluorescence (Fv/Fm ratio) in seedlings of all wheat genotype. Percent reduction in Fv/Fm ratios ranged from minimum 2% to 4.5% which included the heat-tolerant and moderately heat-tolerant genotypes such as Kulin, Buckley, Anlace, Krichauff, Kukri, Janz, Excalibur, Molineux, Aroona and Bindawara, while maximum percent reduction was from 6% to 11% and heat-sensitive genotypes included were such as Vulcan, Batavia, Cascade, Machete, Kalyansona, Cook, Lyllapur-73, WW15, Millewa and ME-71. The data of Fv/Fm ratios at control (0 time), after 6h of heat-stress and recovery after 24h was used to group genotypes using the hierarchical cluster analysis. Genotypes were clustered as expected into 6 distinct groups. Heat-tolerant and moderately heat-tolerant genotypes clustered together into three groups. The most heat-tolerant genotypes clustered together included, Amery, Fane, Krichauff, Anlace, Buckley, Kulin, Meering and Halberd. Whereas, most heat-sensitive genotypes clustered in group 4, these genotypes were such as Lyallpur, Cook, WW15, Millewa, Schomburgk, Bodallin and Kalyansona, while group 6 included only one heat-sensitive genotype ME-71. The reason for these distinct groups could be due to their recovery in Fv/Fm ratios, as differences in the recovery of Fv/Fm ratios were observed in some genotype seedlings after removing the heat-stress.

Following this initial screening, subsets of genotypes that represented a range in thermo-tolerance and thermo-sensitivity were selected for more detailed examinations of their response to high temperature and drought stresses. Further experiments have shown considerable reductions in Fv/Fm ratios after 6 hours of heat-stress (40°C) particularly in some of the heat-sensitive genotypes such as Millewa, Cook, Lyallpur and ME-71, while there was very minimal reductions observed in Fv/Fm ratios in heat-tolerant genotypes including Buckley, Kukri, Anlace, Kingswhite and Kulin. Recovery of the Fv/Fm ratios after removing the heat-stress was an important factor associated with heat-tolerance and heat-susceptibility. A relatively slow recovery of PSII efficiency was recorded in most of the heat-susceptible genotype seedlings while reasonably fast recovery of Fv/Fm ratios was observed in heat-tolerant genotype seedlings.

Heat-stress affected all chlorophyll fluorescence parameters including initial fluorescence (Fo), variable fluorescence (Fv), maximum fluorescence (Fm), ratio of variable to maximum fluorescence (Fv/Fm) and time to reach maximum fluorescence (Tm). Initial fluorescence (Fo) increased drastically even after one hour of heat-stress in all genotypes and particularly in heat-sensitive genotype seedlings such a Lyallpur, Millewa and Oxley. The drastic changes in all *in vivo* chlorophyll fluorescence measurements most probably indicates the physical dissociation of PSII reaction centres from light harvesting complexes, a substantial accumulation of inactivated PSII centres as well as photoinhibition. However, changes in fluorescence measurements during and after removing heat-stress indicated the reversibility of damage to photosystems. These changes were more likely involved in the relatively fast recovery of PSII efficiency in

heat-tolerant genotype seedlings and slow recovery in heat-sensitive genotype seedlings.

Drought-stress also affected *in vivo* chlorophyll fluorescence measurements in wheat seedlings. However, the main objective of the study was to detect whether heat-tolerant genotype seedlings, which were selected after screening 100 genotypes are also water-deficit tolerant on the basis of *invivo* chlorophyll fluorescence. Many heat-tolerant genotypes were also drought-tolerant. Other morph-physiological traits were also examined in wheat seedlings under gradual water-stress. Two traits, total chlorophyll contents (SPAD values) and photoefficiency (Fv/Fm ratios) have shown a strong positive correlation. Although, we did not measure the SPAD values in all experiments, the strong correlation between these two parameters suggested the possible use of both measurements in selecting drought-tolerant and perhaps heat-tolerant genotypes at the seedling stage. On the whole, results have suggested the possible use of *in vivo* Fv/Fm ratios to screen large number of wheat genotype seedlings for heat-tolerance and drought tolerance.

Seedling emergence and coleoptile length in wheat genotypes were also affected by the heat-stress. A strong association was found between percent reduction in coleoptile length and reduction in seedling emergence. The reduction in coleoptile length was not associated with the sensitivity of seedlings to heat-stress, assessed using chlorophyll fluorescence, however, heat-tolerant genotypes tended to emerge slightly more rapidly than the heat-sensitive genotypes.

DECLARATION

I hereby declare that this thesis contains no material that has been accepted for the award of any degree or diploma in any other university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

SIGNED **DATE:** 4/4/2002

ACKNOWLEDGEMENTS

I wish to extend my sincere gratitude to my supervisors Dr. Glenn K. McDonald and Gilbert Hollamby for their support, assistance, advice and guidance throughout the project. I would also like to thank Dr. Arun P. Aryan for his advice, guidance and support during the first half of this study. I am also grateful to the Plant Breeding units at Waite and Roseworthy for providing me with seeds of wheat germplasm for this study. I would also like to thanks to all the people of Waite Campus who provided their invaluable assistance throughout my study period.

The financial support to complete this study through the Australian Agency for International Development (AusAID) and Government of Australia is gratefully acknowledged. I would also like to thank the people of AusAID who gave me valuable assistance and support. I am also grateful to the Pakistan Agricultural Research Council for giving me permission to complete this study.

Last but not least, I fully appreciate the continued encouragement of my late father, mother, brothers and sisters for their support during studies. Particularly, I fully adore my wife for her patience and encouragement and love to our pretty baby Ahd.

CHAPTER 1

GENERAL INTRODUCTION

Wheat is the most important cereal grown in the world (FAO, 2000). It is the most important cereal crop in Australia and Pakistan. While two thirds of the Australian wheat crop is exported, South Asian countries still need to import substantial quantities of wheat to meet the food demand of their growing populations. The average yield of wheat in Pakistan is about 2.0 t/ha (FAO, 2001), and it can vary considerably from year to year. The low average yield of wheat is due to many factors, but drought and heat stresses are important limitations to yield. Therefore, improving production in these countries is a major priority.

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 rainfed crop and it is exposed to drought and heat-stresses at various times in its life cycle but most commonly during grain filling. In Pakistan, high temperature stress can occur during crop establishment and seedling growth as well as during grain filling. Much of the wheat in Pakistan is grown on residual soil moisture. Therefore, both high temperature and drought stress can occur soon after sowing and potentially reduce the wheat establishment and early growth. While there has been considerable work done on the effects of high temperature on wheat during grain growth and development, less attention has been paid to thermo-tolerance during germination, establishment and seedling growth. The genetic variations in tolerance to drought during these stages also need to be considered.

Photosynthesis is sensitive to heat and drought stresses and it is often the first process that is affected by stress. Various techniques have been used to measure the

photosynthetic response of plants to these stresses. One technique is chlorophyll fluorescence, which has been used successfully to measure responses to high temperature and water stresses in higher plants (Syed *et al.*, 1989; Xu *et al.*, 1995). Heat-tolerance during grain filling has been correlated to differences in chlorophyll fluorescence (Moffat *et al.*, 1990). The advantages of the technique are that it is quick and non-destructive.

Responses and sensitivities to heat-stress and to water-stress may be linked both ecologically and physiologically. In the rainfed system, periods of water-deficits and high temperature stress can occur co-incidentally. Stomatal closure, which occurs with water deficits, reduces the ability of the plant to dissipate energy by evaporative cooling. The altered energy balance affects leaf temperature and chlorophyll fluorescence. Thus, the aims of the present study are:

1. To examine the heat-tolerance of diverse wheat genotypes by *in vivo* chlorophyll fluorescence technique at the seedling stage
2. To assess the physiological response of wheat genotype seedlings to heat and drought at early growth stages (3-4 leaf stage)
3. To investigate the effect of moisture-stress on different morpho-physiological traits in wheat seedlings
4. To investigate the effect of high temperature stress on seedling emergence and coleoptile length in different wheat genotypes

Furthermore, this study might contribute in the development of a simple, reliable and cost effective technique to select heat-tolerant and drought-tolerant wheat genotypes at their early stages of development for use in breeding programs.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The following review discusses the effect of high temperature and water stress on wheat. These abiotic stresses affect crop productivity in the wheat growing regions of Australia and South Asian countries, including Pakistan. The first part of the review addresses the issues of heat-stress. Firstly, the importance of wheat in Australia and Pakistan is described briefly, and then the effect of high temperature or heat-stress on the important physiological processes in higher plants is discussed. Emphasis is given to seedling emergence, coleoptile length, grain yield and quality as well as effects of high temperature on photosynthesis. The second part of the review examines the responses of important physiological attributes of higher plants to water deficit. These include photosynthesis, early vigour, leaf area and water use efficiency and transpiration efficiency. The last part of the review deals with techniques to measure the responses of higher plants to these stresses, which can be used to screen varieties for heat or drought tolerance. Particular attention is paid to the chlorophyll fluorescence technique. This technique has been recognized as a powerful tool to study the photosynthetic response in higher plants particularly in stress physiology.

2.2. Importance of wheat in Australia and Pakistan

2.2.1. Australia

Wheat is the major cereal grain produced in Australia and is grown in a specific area called the "wheat belt" (Figure 2.1), that is mainly determined by available water and suitable topography. Table 2.1 shows that during 2000/01 total production of wheat was 21.1 Mt with a national average yield of 1.75 t/ha. Barley was the next

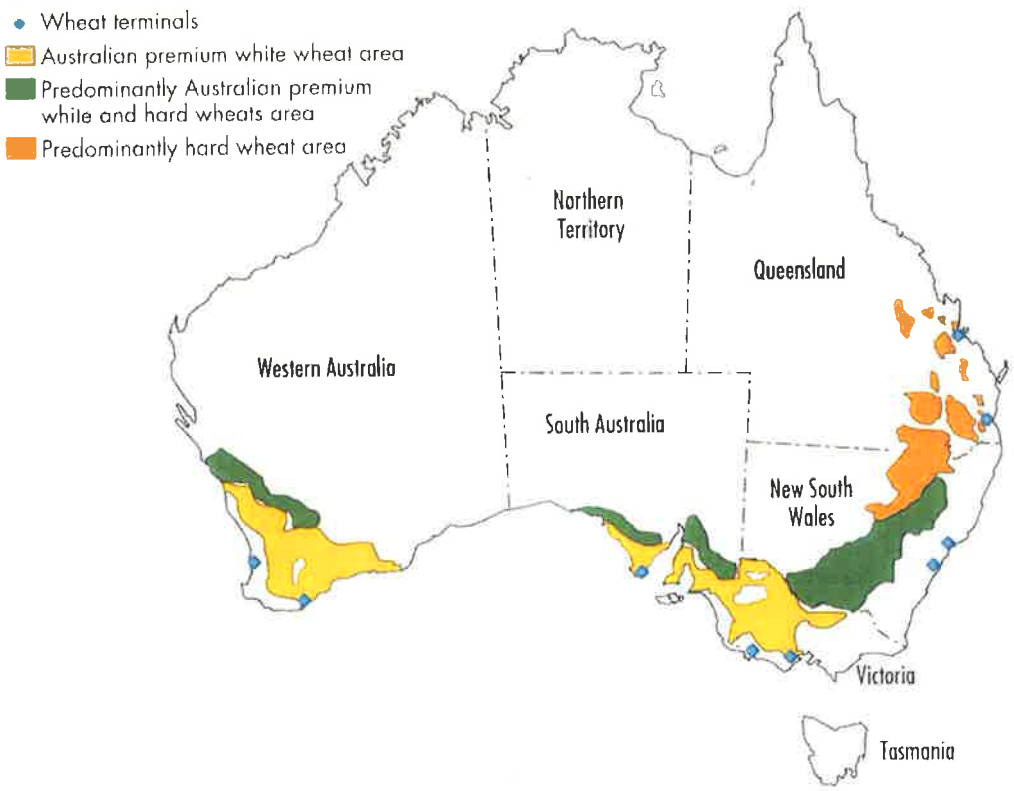


Figure 2.1: Distribution of wheat production in Australia according to quality grade (Australian Bureau of Agricultural and Resource Economics, Crop Report, June 2005)

largest cereal crop with grain production of 5.6 Mt and a national average yield of 1.85 t/ha.

Wheat is predominantly grown under rainfed conditions in Australia with no supplementary irrigation. In general, crops rely on utilization of growing season rainfall, although this is sometimes supplemented by fallow-stored water, especially in the cereal belt of northern New South Wales and Queensland. Wheat production is based on spring wheat cultivars. Sowing generally occurs from late April to mid June and harvest occurs from October to December. In the southern and western regions, sowing starts predominantly from May to mid June and harvest in November and December.

Table 2.1. Area (Mha), production (Mt) and grain yield (t/ha) of wheat in Australia in 2000 (ABARE, 2001)

State	Area (Mha)	Production (Mt)	Yield (t/ha)
Queensland	1.027	1.350	1.310
New South Wales	3.548	6.700	1.880
Victoria	1.200	3.200	2.670
South Australia	1.971	4.200	2.130
Western Australia	4.327	5.700	1.320
Australia	12.079	21.168	1.750

2.2.2. Pakistan

Pakistan is the seventh most populous country in the world with an estimated population of more than 120 million people. About 65-70% of population is directly involved in agricultural production and its related activities. Wheat is the most important staple food in the country. It is produced under both irrigated and dry land conditions. Under dry land conditions, wheat is mainly grown on the reserve soil water of post monsoon rainfall. It is sown during September and October and harvested in May and June.

Table 2.2 shows that there was a slight increase in area sown to wheat between 1995/96 and 2000/2001, while grain production also increased from 16.9 Mt in 1995/96 to 21.1 Mt in 1999/00 associated with a steady increase in yields. However, there was an 11% decrease in wheat grain production during 2000/01, which was associated with severe drought.

Table 2.2. Changes in area, production and yield of wheat in Pakistan between 1995/96 and 2000/01.

Year	Area (Mt)	Production (Mt)	Yield (t/ha)
1995/96	8.379	16.901	2.01
1996/97	8.409	16.630	2.05
1997/98	8.355	18.694	2.24
1998/99	8.230	17.857	2.17
1999/2000	7.468	21.079	2.49
2000/01	8.430	18.735	2.22

2.3. Important climatic features affecting wheat production in Australia and Pakistan.

2.3.1. Abiotic stresses

Both heat and drought stresses are important abiotic stresses that limit wheat yield. throughout the world. High temperature stress and drought stress are important climatic features in Australia and Pakistan, which adversely affects wheat growth and grain production in both countries.

2.3.2. Australia

Mean daily temperatures and monthly average rainfall during wheat growing season at four sites in southern Australia are shown in Figure 2.2. Throughout the Australian wheat belt, mean daily maximum and daily minimum temperatures can fluctuate considerably during the growing season. Wheat is sown mainly from autumn to early winter (May to June). In southern Australia the climate is described as

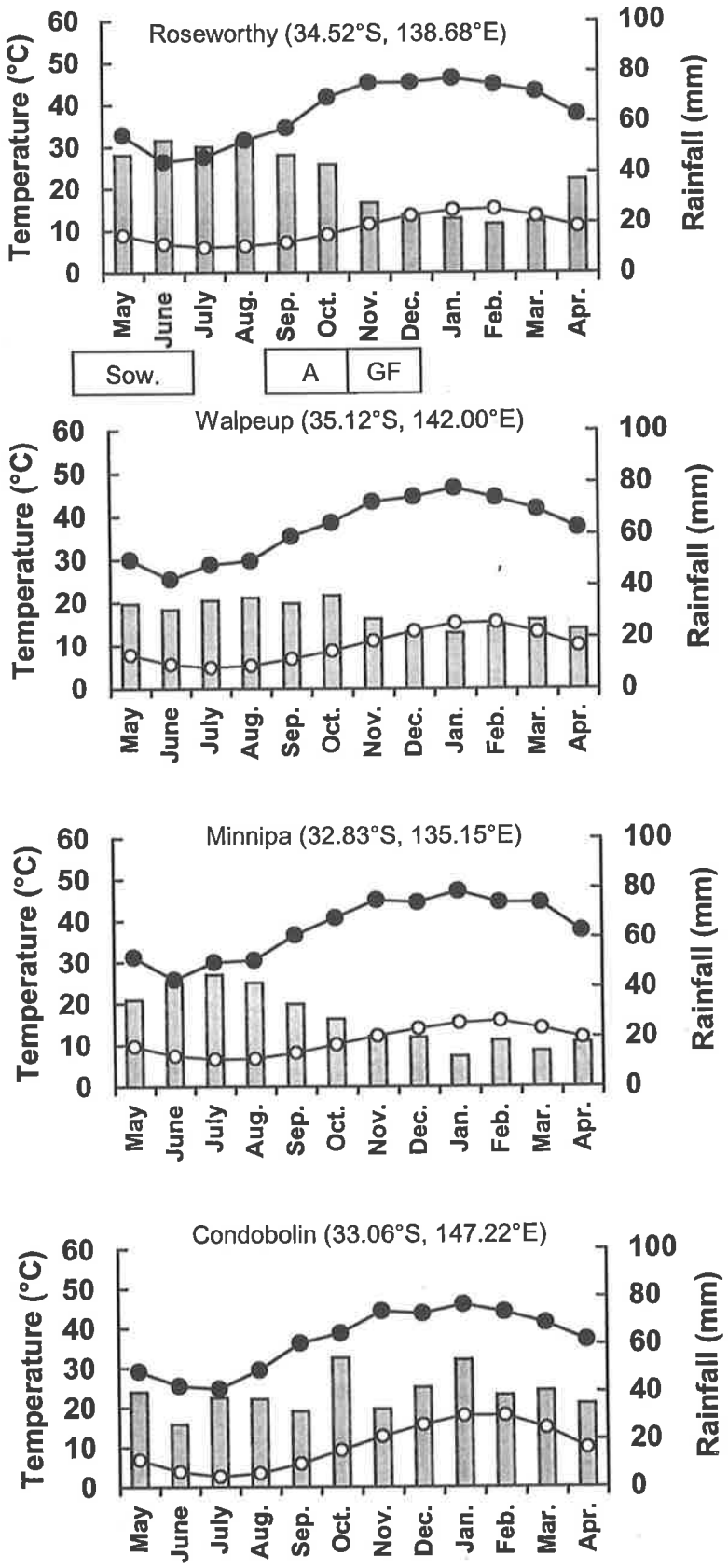


Figure 2.2. Mean maximum (•) and minimum (○) temperatures and monthly rainfall (histogram) during the wheat growing season at four sites in southern Australia (Source: www. Bom.gov.au). Sowing –Sow; anthesis-A; grain filling-GF.

Mediterranean-like, with a winter-dominant rainfall pattern and hot, dry summers. Generally, sowing is done after sufficient rain has fallen in autumn or winter to allow germination of seed (the 'break' of the season). Seedling emergence and tillering occur under short days, frequent rain showers and cool temperatures. Usually heading, anthesis and grain filling occur under increasing temperature and decreasing soil water during spring (October to November) and harvesting is done in December. The increasing temperature at the time of grain filling and grain growth can adversely affect grain yield and grain quality. Consequently, much work has been done on the effect of high temperature stress on grain growth and grain quality in Australia (Wardlaw *et al.*, 1989; Blumenthal *et al.*, 1991, 1994, 1995; Stone and Nicolas, 1994, 1995) and relatively less attention has been paid to heat and drought stress during the early stages of crop growth.

2.3.3. Pakistan

Figure 2.3, shows the maximum and mean temperatures in some important regions of Pakistan where wheat is grown. Wheat is sown during autumn (September – October) when mean daily temperatures can rise above 20°C in Peshawar (34.01 ° N, 71.35 ° E), Karachi (24.54° N, 67.09° E), and Lahore (24.54° N, 67.09° E). However, perhaps more importantly, maximum daily temperatures during the sowing period can rise to more than 35°C in Peshawar and Lahore and more than 30°C in Karachi and Quetta. During January to March, when wheat is booting and flowering, the mean daily temperature remains in the range of 22° C to 31°C in these regions. However, terminal heat-stress (more than 35°C) during grain filling is also important, especially when wheat is planted late.

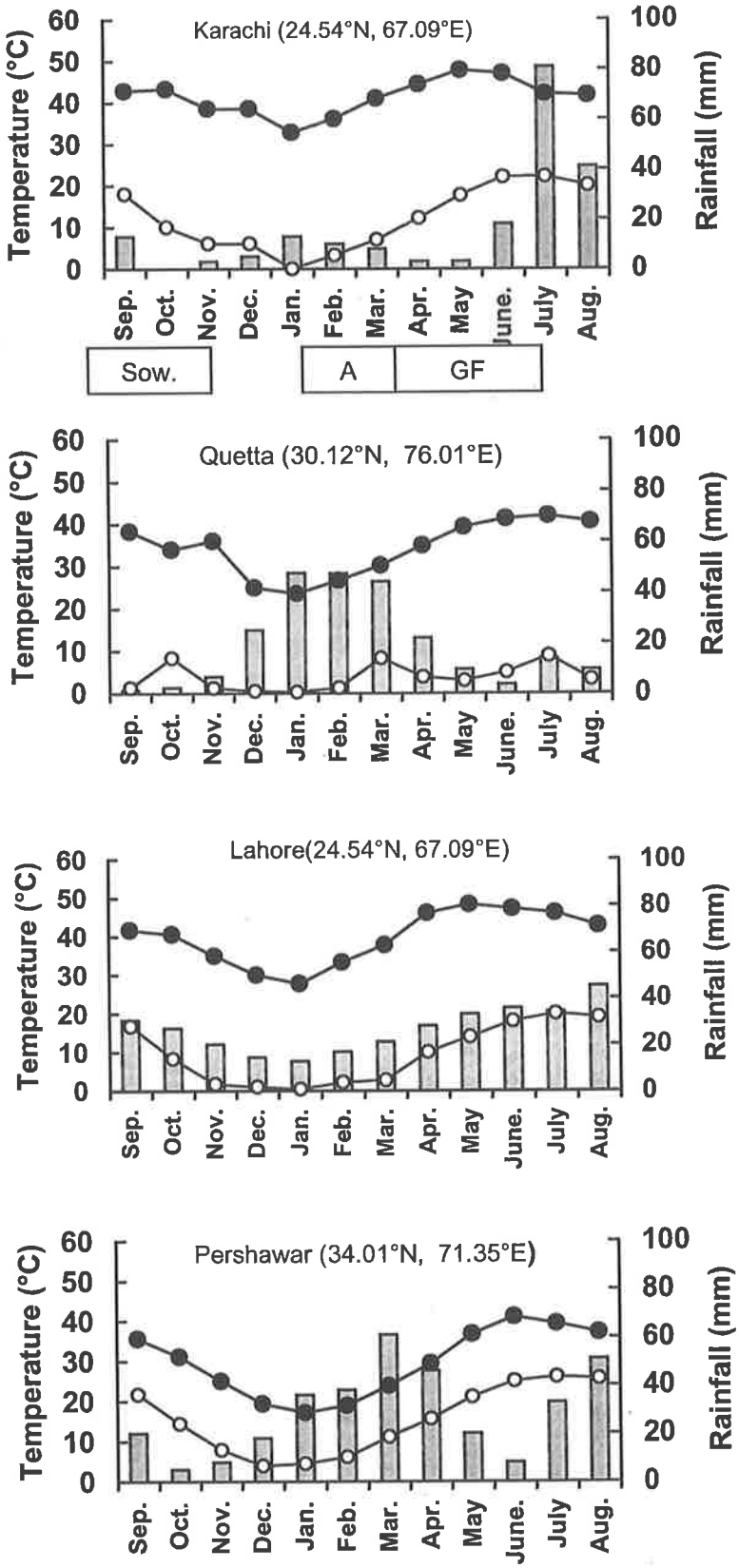


Figure 2.3. Mean maximum (•) and minimum (○) temperatures and monthly rainfall (histogram) during the wheat growing season at four sites in Pakistan (Source: www. Met.gov.pk). Sowing –Sow; antthesis-A; grain filling-GF.

2.3.4. Similarities in climatic features of Australia and Pakistan

There are some climatic and environmental features, which are similar in Australia and Pakistan. For example, the variation in the timing of the opening rains and the recent trend to earlier sowing means that wheat crops in southern Australia may occasionally be exposed to periods of water stress during emergence. In Pakistan, the reliance on stored water means that water stress may also occur during germination and emergence. A major difference, however, is the markedly higher temperatures in Pakistan during crop establishment.

Temperatures rise during the late spring and summer with diminishing soil water in Australia, whereas temperature doesn't rise during spring in Pakistan. However, spring and late drought can occur in Pakistan when monsoon rainfall is low and sometimes temperatures can rise along with moderate to severe drought during the grain growth period in Pakistan, which affects the total production of wheat.

2.4. Heat-stress in cereals

Important growth stages in cereals, including wheat, described by Large (1954), are shown in Figure 2.4. In Pakistan, high temperature stress can occur at two main stages of wheat development, during seedling establishment and tillering (Stages 1-5) and during grain filling (after Stage 10.5). In southern Australia, heat-stress is most likely to occur during flowering and grain filling. Hence, the effect of high temperature at these stages will be considered.

Figure 2.2 presents the maximum daily and mean daily temperatures at different sites of wheat belt in Australia and shows that maximum daily temperatures at these sites don't exceed 20°-25° C at the time of sowing and during much of the period of anthesis.

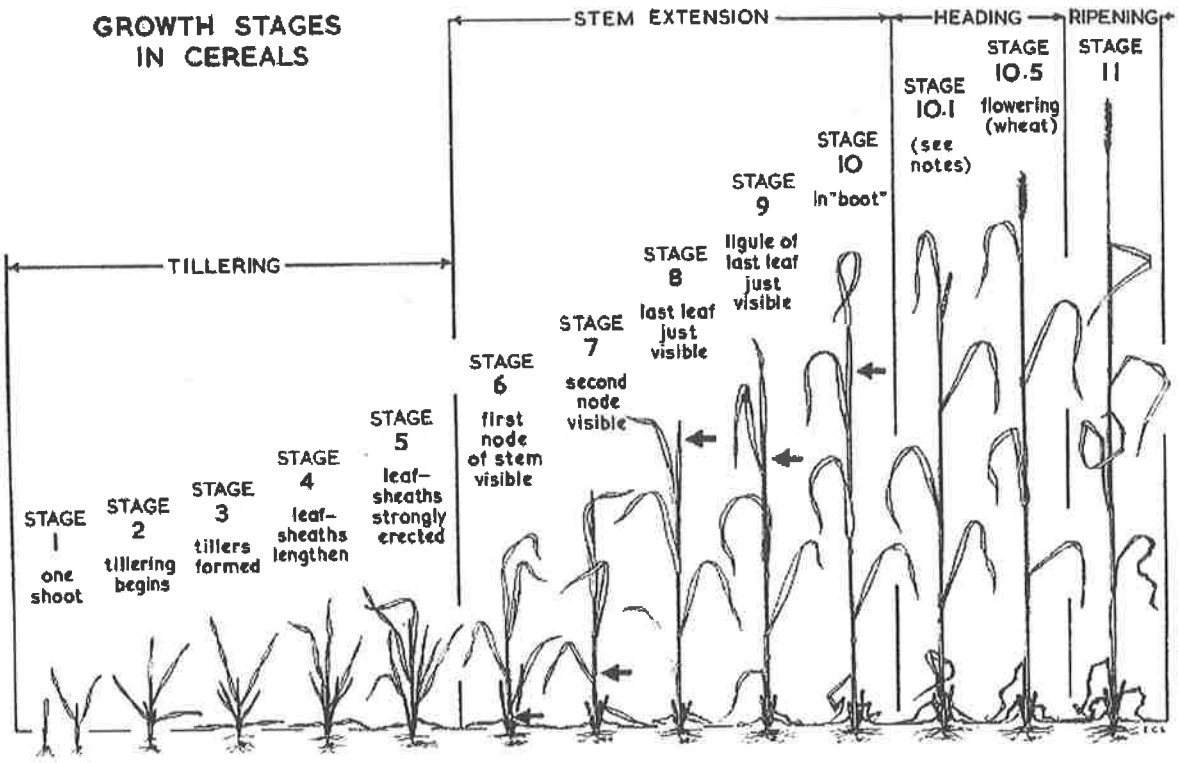


Figure 2.4. Growth stages in cereals (Large, 1954)

However some studies have suggested that in parts of the Australian wheat belt maximum daily temperatures can rise ranging from moderately high (25°-32°C) to very high (33°-40°C) temperatures at the time of wheat sowing (Powel, 1985). Similarly, in Pakistan mean daily and maximum daily temperatures can rise to more than 30°-32°C at the time of sowing and seedling emergence. Figure 2.3 shows that maximum daily and mean daily temperatures ranging between 25°-30°C can occur at some sites in Pakistan by the time wheat is sown and also at later growth stages.

2.4.1. Effect of high temperatures on wheat sowing in Pakistan and Australia.

High temperatures at the time of wheat sowing can occur during wheat emergence in Pakistan, which may affect stand density, early plant growth and grain yield. However, very few studies have been reported in Pakistan for seedling emergence under high temperature stress. Ali-Dib *et al.* (1994) reported the effect of different

temperatures regimes on seed germination in wheat ranging from 5°C to 40°C. They observed no germination at 40°C and only 35% germination at 35°C temperature. Similarly, coleoptile length, which is related to the seedling emergence in cereals and wheat, could be affected under the high temperature stress (Radford, 1987). Therefore, in next section, a review of the effect of high temperature stress on germination, emergence and coleoptile length in different cereal crops and wheat is presented.

2.4.2 Effect of heat stress on germination and seedling emergence in different cereal crops

Different seeds have different temperature ranges within which they germinate. At very low temperatures or very high temperatures, depending on the species, the germination of seeds of all cereals is reduced (Lafond and Baker, 1986). Similarly, soil temperatures at the time of sowing affect seed germination in different cereal crops. Significant variation in seed germination and in seedling emergence ranging from 25% to 100% under different temperature regimes have been reported in different crop species (Radford, 1987; Radford and Henzell, 1990; Peacock *et al.*, 1990). Radford and Henzell, (1990) reported poor emergence in sorghum genotypes due to inadequate coleoptile length after high temperature stress >35°C. Kasalu and Mason., (1993) also reported a significant reduction in seedling emergence in sorghum as the temperature increased from 30° to 45°C. However, many studies were conducted to observe the effect of very high temperatures ranging from 40°C to 50°C in various other crop species such as grain legumes (Ellis *et al.*, 1986) and annual legumes *Trifolium balansae* and *T. resupinatum* (Jansen and Ison, 1994). All these studies have shown that very high temperatures (more than 40°C) affected the seed germination and field emergence in these crops. While these temperatures are high, they are not atypical of temperatures that are possible in seed beds when air temperatures are high.

2.4.3. Effect of heat stress on coleoptile length in different cereal crops

The elongation of the mesocotyl and the coleoptile is related to the seedling emergence in cereals. Seedlings emerge by elongation of both mesocotyl and coleoptile in maize, sorghum, oats and rice, whereas in wheat only coleoptile length is related to seedling emergence (Redford and Henzell, 1990). Several researchers have found a direct response of coleoptile length to temperature fluctuations in wheat, sorghum, pearl millet and other crops (Bhatt and Qualset, 1976; Garcia-Huidobro *et al.*, 1982b; Radford and Henzell, 1990).

Maximum and minimum temperatures affect the mesocotyl and coleoptile length (Agnew, 1988). Turner *et al.* (1982) suggested that in rice, temperature and sowing depth of seed were directly related to the total length of the mesocotyl and coleoptile. Radford and Key (1993) germinated the seeds of sixteen oat (*Avena sativa*) cultivars under different constant temperatures and reported the optimum temperature to be 15°C for maximum length of coleoptile and mesocotyl. They also observed that temperatures above 20°C reduced the coleoptile length but not the mesocotyl length in oats. However, a wide range of genetic variability for seedling emergence and coleoptile length was reported in many cereal crops such as barley, oats and wheat (Allan *et al.*, 1965; Kaufmann, 1968; Radford and Key, 1993).which suggests that it may be possible to select for improved emergence at high temperatures.

2.4.4. Effect of high temperature on seedling emergence and coleoptile length in wheat

Temperature affects both germination and coleoptile elongation and consequently emergence and establishment of the crop. Seedling emergence and

coleoptile length in wheat can be affected severely by very low (< 0°C) and very high (> 35°C) temperatures (DeJong and Best, 1979; Radford, 1987). Peterson (1965) reported the minimum, optimum and maximum temperatures for germination and seedling emergence in wheat to be 3.5°C to 5.5°C, 20°-25°C and 35°C, respectively. In wheat, a positive correlation between coleoptile length and field emergence has been demonstrated (Whan, 1976). Liang and Richards (1994) reported that faster coleoptile growth is associated with early emergence and leaf area development in wheat. Radford (1987) reported that wheat cultivars differ in their range of optimum temperature for coleoptile length. He tested eight cultivars and found coleoptile length was longest at 10°C and 15°C and became shorter as temperature increased from 15° to 35°C.

Genetic variability in coleoptile length and seedling emergence occurs in wheat (Allan, 1962, 1965). Seedling emergence of semi-dwarf wheat cultivars, which contain the Rht1 or Rht2 genes, has been poor, particularly when field temperature is high (Radford, 1987). Radford also investigated the effect of different temperatures on coleoptile length in wheat cultivars and found genetic variability in mean coleoptile length. Richards *et al.* (1998) demonstrated that selecting for long coleoptiles could improve seedling emergence and establishment under high temperatures and in dry soils. They have also shown that long coleoptile can be combined with semi-dwarf wheat by using genes such as Rht8 and Rht9.

Many studies have shown that coleoptile length is associated with emergence capability in tall and semi-dwarf wheat cultivars due to the presence and absence of semi-dwarf genes Rht1 or Rht2 (Allan *et al.*, 1965, Allan 1980; DeJong and Best, 1979; Schillinger *et al.*, 1998). All these studies confirmed that wheat cultivars differ in seedling

emergence rate and coleoptile length. Therefore, in the present study, the effect of high temperature stress on seedling emergence and coleoptile length will be examined using a range of wheat genotypes. However, high temperature stress also affects many other physiological processes including photosynthesis in higher plants.

2.5. Heat stress and photosynthesis

Photosynthesis is a fundamental physiological process in higher plants, which is affected by high temperatures. Heat stress of seedlings and young crops can reduce photosynthesis, crop growth rate and ultimately may limit yield. Various studies have demonstrated that thermal inactivation of photosynthesis occurs at temperatures higher than 37°C, but the effect also depends on plant species and their environmental habitat (Kobaza and Edwards 1987; Sayed *et al.* 1989; Al-Khatib and Paulsen, 1990). It has also been demonstrated that at temperatures greater than 35°C most crop species have a significantly reduced carbon assimilation rate and utilization of photosynthate. Metabolic processes, including net photosynthesis and activities of photosynthetic enzymes, are also affected (Berry and Bjorkman, 1980). The extractable activities of photosynthetic enzymes such as ribulose-1,5-biphosphate carboxylase (Rubisco) and fructose-1,6-biphosphate are observed to decline with increasing leaf temperature (Krause and Weis, 1984).

The integrity of the photosynthetic apparatus, that includes protoplast, chloroplast and thylakoid membrane, is affected by high temperature stress (Kobza and Edwards, 1987). The inhibition of whole leaf photosynthesis by high temperature has been found to be due to the disruption of the photosynthetic apparatus at the chloroplast level (Weis, 1982; Weis and Berry, 1987). Thyalkoid membranes in the chloroplast are particularly

heat sensitive and are considered to be the most thermolabile component. Moderately-high to high temperatures, depending on the plant species, can modify structure of the chloroplast. This includes damage to thylakoid membranes and redistribution of chlorophyll-protein complexes. However, Al-Khatib and Paulsen, (1990) reported that moderately high temperatures (25° to 32°C) result in the reversible inhibition of photosynthesis in plants. After returning plants to their original growth conditions, the chloroplast ultra-structure recovers gradually followed by resumption of photosynthetic activity.

Higher plants from different environmental habitat such as tropical, sub-tropical, temperate, arid, semi-arid and Mediterranean climates show differences in their photosynthetic responses to high temperatures, and these differences are considered to be genotypic (Sayed *et al.* 1989; Barro *et al.* 1996). The potential for photosynthetic response to various environmental factors including high or low temperatures is quite variable between and among different species. This important photosynthetic response of higher plants can be measured *in vitro* and *in vivo* by the chlorophyll fluorescence technique. The use of chlorophyll fluorescence technique to measure these responses under various abiotic stresses will be discussed in a later section of this review.

2.6. Drought-stress

Water is an essential component of plants, being about 85-90% of total fresh weight of higher plants and involved in all physiological and metabolic processes. Water deficit or drought affects plants drastically. It causes reduction in cell turgor and cell growth, closure of stomata, decline in leaf area, and ultimately reduction in plant growth and grain yield (Bunce, 1988).

Drought has been defined in meteorological, hydrological and agricultural terms. Meteorological drought is defined as an extended period of time during which rainfall is scarce and the amount of water is less than required for normal plant growth. Hydrologists consider drought a situation where the underground water level is very low, whereas agricultural drought is considered to be when availability of soil water becomes too low to sustain the normal plant growth (Levitt, 1980). Long drought periods have been reported in the history of human civilization since 3000 B.C. All drought periods resulted in severe food scarcity, losses in agricultural productivity, spread of diseases and loss of human life due to famine (Waterlow *et al.* 1998).

2.6.1 In Australia

Australia has one of the most variable rainfall patterns in the world. Due to diverse and variable rainfall climate, Gibbs and Maher (1967) suggested that "rainfall" is the best single indicator of drought in Australia. Water supply, through the amount and distribution of rainfall, therefore plays a major role in cereal growth and production. Srivastava (1987) reported that 70-80% of the variation in wheat yield in South and Western Australia was due to the variation in annual rainfall. Lovett (1973) gave a detailed survey of statistical records on wheat production and yield losses due to drought in the country from 1894 to 1969 which highlights the impact of drought on cereal production in Australia. The lowest national average wheat grain yield in Australia was 0.16 t/ha, which was reported in 1902 when all states in the country were severely affected by drought (Lovett, 1973). He also reported the wheat production losses from drought ranged from 1.63 to 2.72 Mt during 1940 to 1967. Drought periods during 1895-1903, 1958-68, 1982-83 and 1991-95 were most devastating in terms of their effect on agriculture production and economy of Australia. The effect of

widespread drought on the economy is illustrated by the effects of the drought period of 1991-95, which is estimated to have resulted in loss of about $A5.0 billion to the economy.

2.6.2 In Pakistan

Drought is a prominent feature of the climate of the South Asian countries including Pakistan. However, mention here will only be made of the recent drought in 2000/01 in Pakistan and its impact on the economy of the country, to illustrate the effect.

The Food and Agricultural Organization of the United Nations and World Food Programme reported in July 2001 the effect of three consecutive years of drought period in Pakistan that prevailed during 1999-2001. According to the report, the rainfall during the cereal-growing season, and especially during anthesis and grain growth (January-March), was 50 to 80% below the average in most parts of the country. Moreover, the monsoon rainfall was also less than 40% of the average. This had a major impact on wheat yields because about 60% of wheat in the country is sown on the soil water reserves from the previous monsoon rainfall. The most effected areas were the province of Baluchistan and parts of the Sind and Punjab provinces. Table 2.3 shows the reduction in major cereal crop production, including wheat, due to the recent drought in Pakistan. An 11% reduction in the wheat grain production was observed while a 24% reduction was observed in rice (milled), which is one of the main exporting commodities.

Table 2.3. Pakistan: Cereal Production in 2000/2001 compared to 1999/00 ('000 tonnes) (Ref. FAO, 2001)

Cereals	Grain production (Mt)			Change
	Average[A]	1999/00	2000/01	(%)
Wheat	18.238	21.079	18.735	-11
Rice (milled)	4.487	5.155	3.900	-24
Maize	1.565	1.652	1.489	-10
Others	0.559	0.494	0.457	-8
Total	24.849	28.380	27.581	-13

[A]**Average production 1995/96 to 1999/00**

2.7 Improving drought resistance in cereals

Passioura (1977) proposed that grain yield of crops in water-limited environments depends on three major components, which are water transpired, transpiration efficiency and harvest index. Consequently, selection for improved drought tolerance aims to improve one or a combination of these traits, which are considered by crop breeders or physiologists as drought resistant or drought tolerant traits. Thus, in water-limited regions, crop breeders particularly try to improve these traits, either directly or indirectly.

While drought stress is widely acknowledged to be a major limitation to rainfed wheat production, there has been considerable debate about the effectiveness of previous efforts to breed for drought resistance using physiological criteria. In the past, crop breeders have often considered that the high yield to be the best indicator of drought tolerance in cereals (Passioura, 1997; Ludlow and Muchow, 1990) and have relied on yield potential in the field as a major selection criterion. However, some researchers have considered the strategy of breeding wheat simply for high yield potential might not

be the best approach to improve drought tolerance in cereals because the selection is not specifically targeted to drought-tolerant traits. While improvements in yield under rainfed conditions have occurred, this may have resulted from selection for a range of traits, such as disease resistance or nutritional characteristics, that indirectly are associated with drought tolerance. An alternative approach to improve performance under drought is to screen genotypes at an early stage of crop development (seedling stage) using different morpho-physiological traits that are linked to drought resistance. This may be particularly important for environments where drought stress occurs early in the growing season.

2.7.1 Drought resistant traits

Plants cope with seasonal water stress by two main strategies – drought escape drought resistance. Plants escape drought by having a developmental pattern that avoids sensitive stage of growth being exposed to drought, while drought resistance involves mechanisms allows plants to yield well under soil or atmospheric drought (Hall 2001). There are many traits that can contribute to improved growth and yield of wheat under drought stress. Many of these are beyond the scope of the present study, which focuses on stand establishment and seedling growth. Therefore, only the role of crop vigour will be considered.

2.7.2 Early vigour

Seedling establishment and early vigour are considered important traits for improved yield in the southern and western Australia cereal belt. In a Mediterranean-type climate where rainfall is usually frequent in winter and gradually declines in late spring, genotypes with high levels of seedling vigour tend to use water efficiently (Richards, 1984). The reason for this is that greater early vigour reduces the soil

evaporative losses early in the growing season and increases the amount of water available for transpiration. Therefore, vigorous crops increase the transpiration component of the yield model proposed by Passioura (1997). In the southern and western regions evaporation during emergence and tillering (May-July) is low, while it increases during spring season (September-October) at the time of flowering and anthesis. Therefore, during spring, as the evaporation increases and rainfall declines, water deficits increase, which may limit growth and yield. Richards (1984) suggested that for the Australian wheat areas, wheat genotypes should be developed with good seedling establishment and early vigour, which will enhance transpiration at the expense of soil evaporation. Turner and Nicolas (1987) found that vigorous early growth in wheat resulted in higher dry matter yield as well as higher grain yield. They have also suggested that vigorous seedling growth has helped with greater root development, which enabled the genotypes to survive under water stress situation in sandy soils. Richards, (1991) also indicated that early vigour is correlated with deeper root system in wheat. Some studies have shown that early vigour has a positive influence on yield potential due to the increased radiation interception (Liang and Richards, 1994). Lopez-Castenada *et al.* (1995) reported that barley grows faster than wheat at the seedling stage and is usually more drought tolerant.

Seedling vigour is a genetically controlled trait and has the potential to increase biomass and grain yield in cereals, especially in arid, semi-arid and Mediterranean environments. Whan *et al.* (1991) suggested increasing grain yield by selecting a wide range of genotypes with early vigorous growth at different locations. Van Ooesterom and Acevedo (1992) established a strong correlation between early vigour and grain yield in barley. However, this trait may have a positive or negative effect on yield stability under

different climatic conditions, particularly in Mediterranean environment. It is recommended in some studies that early vigour and faster leaf area development may result in exhaustion of soil water where crops depends on water stored in the soil. Therefore, in these environments early vigour must be combining with early flowering in cereals (Lopez-Castenada *et al.*, 1995).

2.7.3 Transpiration efficiency and water use efficiency

Transpiration efficiency is the amount of dry matter or grain yield produced per unit of water use (transpired), whereas water use efficiency is the amount of dry matter or grain yield produced per unit of total water use (i.e. soil evaporation plus transpiration). Passioura (1977) suggested that under the arid and semi-arid Mediterranean environments, dry matter and yield is a function of the water use by the crop and the efficiency with which it is converted into the biomass.

Any method that reduces losses from evaporation and increases transpiration ultimately increases the biomass and will increase water use efficiency for biomass production. Richards *et al.*, (1998) suggested that grain yield and biomass could be improved by improving the water use efficiency, leaf area growth and early establishment in cereal crops. They also reported that early establishment and vigorous growth might be possible by selecting for long coleoptiles in wheat with short stature or semi-dwarf wheat. Jia *et al.*, (1999) after the results of 8 years of pot and field experiments, found that winter wheat genotypes with high water use efficiency had greater yield potential. Similarly, Gan *et al.*, (2000) reported 60% increase in grain yield of winter wheat, spring and durum wheat, rye and barley due to the improved water use efficiency and evapo-transpiration under the semi-arid climates.

2.7.4 Water use efficiency and drought tolerance

Efficient water use is an important trait for adaptation to drought-prone environments. Gummuluru *et al.*, (1989) measured different drought tolerance traits in durum wheat genotypes and reported that drought-tolerant genotypes had higher water use efficiency, chlorophyll a/b contents, leaf area and shoot dry weight than the drought susceptible genotypes. Waines (1994) compared wheat, rye and triticale and found that rye was more tolerant of water stress than wheat due to the efficient water use and better rate of photosynthesis. Saadalla (2001) found significant variations for water use efficiency (on a grain yield basis) among spring wheat cultivars. This shows that there is a substantial potential to improve wheat yield and water use efficiency in the drought-prone areas of semi-arid Australia and sub-tropical areas of South Asia. However, cost-effective selection techniques are difficult to develop. In the past much research has been based on the later stages of cereal development, such as the grain filling stage to maturity. Where early season drought is a problem, selection based on early stages of crop development, such as seedling emergence and establishment, leaf area growth and better water use efficiency at seedling stage, may improve the biomass and grain yield in cereals.

2.8. Screening for response to heat and drought stresses

2.8.1 The use of seedlings in screening

Many studies have been conducted to observe the seedling response to heat stress in various crops. For example, rice (Vani *et al.*, 2001), *Phaseolus vulagaris* L. (El-Tohamy *et al.*, 1999), maize (Karim, *et al.*, 2000), and wheat (Foker *et al.*, 1998; Blum *et al.*, 2001). Seedling responses to drought stress have been investigated in different crop species, *Vicia faba* (El-Tayeb and Hassanein, 2000), barley (Ramanjulu

et al., 1999), maize (Li and Van, 1998), cotton (Xue *et al.*, 1997), wheat (Malik and Aeright, 1998; Aliyev *et al.*, 2000; Aydin *et al.*, 2000). Most of these studies were conducted under laboratory and green house conditions except for a few studies that were conducted both in laboratory and under field conditions to compare the seedling response under both conditions. For example, Aydin *et al.*, (2000) tested 20 wheat genotype seedlings for drought resistance under greenhouse, laboratory and field conditions. They observed a direct correlation between different drought susceptibility index and stability parameters when the data obtained from field trails was compared to seedling stage through correlation analysis. Malik and Aeright (1998) conducted a series of field and pot experiment to evaluate leaf water potential, osmotic adjustment, relative water contents, stomatal resistance and seedling survival in drought tolerant and susceptible seedlings of spring wheat cultivars. They suggested that the responses of all genotypes were generally consistent for drought resistant traits between 3rd leaf stage (seedling stage) and flag leaf stage. By using these traits at the seedling stage researchers could screen wheat genotypes for the improved drought tolerance. Various other techniques are also available for screening the heat and drought resistance, which will be discussed in the next section.

2.8.2. Screening techniques for heat and drought tolerance in seedlings

Various techniques are available to screen crops for tolerance to heat and water stress. These techniques have been used in various studies to evaluate thermo-tolerant and drought tolerant genotypes in crops (Ismail and Hall 1998). One of the approaches has been the selection of high-yielding genotypes that were grown directly under the very hot and water stress conditions in the field. However while improved growth and yield is the ultimate goal in selecting for improved stress tolerance, other abiotic and

biotic stresses can affect the results of field screening for specific traits related to stress tolerance. The other approach has been the selection of genotypes grown in pots under controlled-stress conditions and which has focused on specific traits linked to improved tolerance.

Many techniques for screening for heat and drought tolerant genotypes have been used by various researchers. For example, membrane thermostability (MT) has been successfully used to screen various crop species for their heat tolerance (Blum, 1988. Saadala *et al.* 1990a). In wheat, MT was reported to be associated with heat tolerance during grain filling (Shanahan *et al.* 1990). It may be possible to screen a large number of seedlings effectively using this technique and it could be used for breeding purpose (Saadala *et al.* 1990b). A positive association between MT and grain yield under heat stress has been reported in spring wheat (Blum *et al.* 2000). However, researchers have suggested that MT should not be used as an exclusive selection criteria for heat tolerance.

In wheat, cell viability test under the high temperature stress could be performed by measuring the reduction of the chemical compound TTC (2,3,5 triphenyl tertrazolium chloride) (Sun and Xu. 1998). Wang and Clarke. (1993) studied the cellular response in wheat after *in vitro* culturing the cells under high temperatures stress (48°C). They observed that 45% of the cells of a tolerant cell line (M-48) survived when returned to normal temperatures (22°C). These lines also maintained the production of normal proteins under heat stress (40°C) along with the synthesis of several low molecular weight heat shock proteins. This study also demonstrated that heat tolerant lines could be obtained from *in vitro* selection.

Likewise, various techniques are available to evaluate drought resistant or tolerance in several crop species. Simple traits in cereal crops such as yield and its components which include kernels per ears, kernels per spike, kernel per spikelet and grain weight have been used to select high yielding varieties under drought conditions (Richards, 1989; Ludlow and Muchow, 1990; Calhoun *et al.* 1994; van Ginkel *et al.* 1998). As well, various physiological traits related to drought tolerance in wheat and other cereal crops have been used to select tolerant genotypes. These physiological characteristics are leaf water potential, leaf water contents, stomatal conductance, maintenance of turgor pressure, osmotic adjustment, osmoregulation, accumulation of proline and abscisic acid in cells (Angus and Moncur, 1977; Gupta and Berkowitz, 1987; Blum, 1988; Blum and Pnuel, 1990; Lu and Zeiger1994).

Chlorophyll fluorescence has also been used in several studies to evaluate heat and drought tolerance, however, most studies have been conducted during grain filling. Therefore, in the present study, chlorophyll fluorescence measurements will be used to evaluate large numbers of wheat genotypes at the seedling stage along with some other simple techniques such as seed germination and coleoptile elongation under high temperatures and transpiration efficiency under water stress. In the last section of this review the chlorophyll fluorescence technique and its use in stress physiology will be discussed.

2.8.3 Role of chlorophyll fluorescence technique in stress physiology

Both heat and water stresses affect the chlorophyll fluorescence measurements in higher plants. Chlorophyll fluorescence was first used in 1977 to measure the

fluorescence emission in plants (Butler, 1978). Several studies have shown that plant species show differences in their fluorescence characteristics under stress (Smillie, 1979; Smillie and Hertherington, 1983; Sayed *et al.* 1989). The chlorophyll fluorescence measurements have also been used to screen for thermo-tolerance in various crop plants including wheat (Smillie and Gibbsons, 1981; Sayed *et al.* 1989). In the next section information on the chlorophyll fluorescence and its use in plant physiology especially under heat and drought stress is discussed.

2.8.4. Chlorophyll fluorescence

Plants contain chlorophyll, which captures light energy from the sun and passes it to photosynthetic reaction centres in the chloroplast. Two types of chlorophyll - chlorophyll a and chlorophyll b - are present in the thylakoid membranes of the chloroplast. There are two different reaction centres, photosystem-I (PSI) and photosystem-II (PSII). The light harvesting protein complexes (LHCI and LHCII) mainly associated with PSII, absorbs the energy, which drives the photosynthetic electron transport through PSII and PSI in thylakoid membranes. The capture of energy and the movement of electrons through the photosytems provide the chemical energy, in the form of ATP and NADPH, used for CO_2 fixation in the Calvin cycle for production of carbohydrates. PS-II also extracts electrons from water-releasing oxygen in the process as part of the electron transport chain. A small portion of this energy is re-emitted as fluorescence, whereas some portion is dissipated by a variety of non-photochemical processes such as fluorescence quenching, which was reported by many researchers (Kruase and Weis, 1984; Kobza *et al.*, 1984; Yucel *et al.,* 1992; Takeuchi and Thornber, 1994; Xu *et al.*, 1995).

Fluorescent light has a longer wavelength (red light) and can be detected by placing an appropriate filter in front of a camera. This is also known as fluorescence quenching. When a plant is placed in darkness for 30 minutes or more, the photochemical and non-photochemical processes become inactive and when a dim light (< 1 μmol m^{-2} s^{-1}) is turned on then initial chlorophyll fluorescence (Fo) can be measured. When very high light (> 4000 μmol m^{-2} s^{-1}) is turned on, fluorescence rises very quickly to a maximum value, Fm. Over the next minute, the photochemical and non-photochemical processes become active and fluorescence falls to Fo or initial level. From these two values, the fluorescence can be measured as variable fluorescence (Fv)

$$Fv = Fm-Fo$$

The ratio of variable fluorescence to maximum fluorescence (Fv/Fm) can also be calculated:

$$Fv/Fm = (Fm-Fo)/Fm$$

Initial fluorescence (Fo), Fm and Fv change in response to high light, temperature and water stress. The damage due to these stresses is reflected in changes of fluorescence characteristics including Fo, Fm and Fv/Fm. Various studies reported that Fv/Fm ratios indicate the maximum efficiency of photosystem-II (Balota and Lichetenthaler, 1999; Tambussi *et al.*, 2000) and in healthy plants the value of Fv/Fm is 0.83. Therefore, chlorophyll fluorescence measurements, especially Fv/Fm, is a sensitive indicator for these damages in PSII and electron transport chains in thylakoid membrane. Moreover, 90% of chlorophyll fluorescence originates from PSII; therefore, changes in the fluorescence measurements reflect changes in PSII and electron transport chain. However, various studies have reported that PSI is very heat stable and significant changes or inactivation of this system is detected above the temperatures that cause

complete inactivation of PSII, depending on plant species and environment (Kruase and Weis, 1984). Several studies indicate that PSII driven electron transport activity that is damaged due to high temperature can be restored in the isolated chloroplast after returning to original growth temperature (Mishra and Singhal, 1992; Feller *et al.* 1998). The response of initial chlorophyll fluorescence (Fo) to high light intensity was examined in various plant species. It was observed that photochemical efficiency measured as Fv/Fm of PSII declined as Fo level increased in leaves of soybean and cotton plants, whereas, photochemical efficiency increased as Fo level decreased in leaves of wheat and barley leaves (Hong and Xu 1999). Furthermore, this technique has been used to study the effects of heat stress and drought stress on photosynthetic efficiency in higher plants.

2.8.5 Heat-stress and chlorophyll fluorescence

Differential effects of moderate and high temperatures on the chlorophyll fluorescence parameters have been reported in various studies. Mishra and Singhai (1992) reported that high temperature treatment of intact leaves of wheat resulted in the reduction in Fv/Fm. This reduction in the variable fluorescence ratio indicates that photochemical efficiency of the PSII is reduced. This shows inefficient energy transfer from the light harvesting complex (Chl a/b) to the reaction centre. Furthermore, changes in the fluorescence induction and its derived parameters (Fo, Fm, Fv) can also be used to evaluate the thermo-tolerance of wheat genotypes (Babani and Mathis, 1995). They exposed five wheat cultivars to 40°C for 4 h. and reported a large decrease in variable fluorescence parameters and PSII efficiency. It was also concluded that changes in fluorescence induction patterns and its parameters could be used to estimate damage caused by high temperature stress in wheat.

Feller *et al.* (1998), suggested that initial and maximum chlorophyll fluorescence were not significantly altered until the temperature exceeded 40°C, which showed that electron transport was more stable at moderately high temperatures. Likewise, Lu and Zhang (2000) investigated the *in vivo* effects of heat-stress on the various functional aspects of PSII in leaves of wheat. The leaves were exposed to temperatures that ranged from moderately high (25°C) to very high (45°C) in the dark for 10 minutes. The results revealed that moderately high temperatures (25°-37.5° C) 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°-45.5°C), which showed that photo-efficiency of PSII had been severely effected as well as a significant decrease in electron transport activity. However, Balota and Lichenthaler (1999) reported the effect of moderate heat stress (35°C) in wheat seedlings under field conditions and have shown that such temperatures affected the chlorophyll fluorescence measurements and net photosynthesis in wheat seedlings.

2.8.6 Drought stress and chlorophyll fluorescence

Drought-stress also affects the net photosynthetic rate as well as fluorescence quenching in higher plants (Kaiser, 1987; Critchley, 1981). Significant decreases in maximal photochemical efficiency of PSII (Fv/Fm) were reported in bean (*Phaseolus vulgaris L.*) under drought stress (Cornic and Briantais, 1991), which indicates a decrease in electron transport activity and PSII activity. McKersie and Leshem (1994) have reported that under mild drought stress, photosynthesis and chlorophyll fluorescence decrease as a result of stomatal closure and a reduction in CO_2 availability in plants. Other studies have indicated similar results such as Balota and Lichenthaler

(1999) assessed different wheat cultivars under water stress conditions in the field and reported a significant reduction in the net photosynthesis and chlorophyll fluorescence ratios in all wheat genotypes. A similar effect of water stress on the PSII efficiency and a significant decline in Fv/Fm values were reported in intact wheat leaves (Xu *et al.* 1999).

Use of the chlorophyll fluorescence technique as a tool to investigate drought tolerance in different wheat genotypes has been reported. Ali-Dib *et al.* (1994) investigated the possibility of using the chlorophyll fluorescence measurements and proline accumulation as a screening test for drought tolerance in durum wheat cultivars. They evaluated 25 genotypes under irrigated and non-irrigated conditions and reported that this measurement can be used for screening drought tolerant genotypes. Similarly, Al-Hakami *et al.* (1995) reported the use of chlorophyll fluorescence technique for drought tolerance test in wheat genotypes. Aruas *et al.* (1998) suggested a relationship between different chlorophyll fluorescence parameters under three different water regimes in wheat. They utilized two rain-fed and one irrigated environment and fluorescence measurements were recorded on a flag leaf after anthesis. The photochemical capacity of PSII was measured by means of the ratio of variable to maximum fluorescence (Fv/Fm) in addition to absolute values of Fo, Fm and half time of the increase from Fo to Fm (t ½) values. The parameters, which showed the best genetic correlation with grain yield, were half time (t ½, r = 0.92), followed by Fo (r = 0.88), Fm (r = 0.74) and Fv (r = 0.71). In view of these coordinated parameters, they suggested that more productive genotypes are those that can avoid severe water stress during anthesis.

2.9 General conclusions

- Heat and drought stresses are the important abiotic stresses in the wheat belt areas in semi-arid and Mediterranean climates of Australia and tropical, sub-tropical, arid and semi-arid areas in South Asian countries.
- Heat and drought stress can occur during germination and stand establishment in Pakistan, which can reduce growth and grain yield.
- Heat stress is more likely to occur during grain filling in southern and western Australia, but emergence and seedling growth may be affected by drought stress.
- Various techniques are available to screen for heat and drought tolerance in wheat but only few studies have suggested screening of large number of seedlings using different techniques.
- Chlorophyll fluorescence has been used to screen heat and drought tolerance in cereal crop seedlings, however most studies were conducted at grain filling and maturity and some involved very few numbers of seedlings.
- Therefore the present study will be conducted involving a diverse range of wheat genotype seedlings to see the possible use of chlorophyll fluorescence as a screening tool for heat and drought tolerance at 3 to 4 leaf stage of plant growth.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Introduction

During February 2000 to October 2001, a series of experiments was performed to evaluate some physiological parameters to screen for heat tolerance and drought tolerance among wheat genotypes at the seedling stage. These experiments were conducted in growth rooms at the Waite Institute, Adelaide. This chapter presents the general materials and methodology used in this thesis.

3.2 Cultivar selection

Up to 100 cultivars were used in the present study. Some were selected from previous studies that have been conducted to evaluate heat-tolerance in wheat genotypes at the grain filling stage (Wardlaw *et al.*, 1991; Wrigley *et al.*, 1994; Stone and Nicolas, 1995, Blumenthal *et al.* 1995). The cultivars represented a range of both current and early Australian cultivars as well as some from other countries. The list includes tall and semi-dwarf cultivars, mostly spring type and few winter types. Most of cultivars were bread wheat (*Triticum aestivum*) and some durum wheats (*T. durum*) were selected. A complete list of these genotypes with their heat-tolerance and heat-susceptibility with reference for that particular genotype is presented in Table 3.1.

Genotypes were selected to represent different breeding programmes and breeding Institutes working in Australia at different agro-climatic zones. The seeds of these genotypes were provided by the Plant Breeding Units at Roseworthy and at the Waite Institute..

Table 3.1. The 100 genotypes used in the study, with details of their year of release, origin, relative heat tolerance/sensitivity (Heat T/S) at grain filling stage with reference and pedigree, when known.

Serial no.	Genotype[A]	Year of release	Origin[B]	Thermo-tolerance at grain stage[C]	Reference for thermotolerance	Pedigree[D]
1	Amery					Lr21-Srx/2*SHORTIM//3*BODALLIN
2	Anlace	1999	SA			Amigo/4*Tatiara
3	Aroona	1981	SA			Ww-15/Raven
4	Arrino	1997	WA			77w:660/Eradu
5	Barunga	1993	SA			Bt-Schomburggk#4/2*Molineux
6	Bass	1983	QLD			Flinders`S'/2*Cook
7	Batavia	1991	QLD	S	Blumenthal *et al.* (1995).	Brochis`S'/Banks;Ciano67/Bluedird//Caerdinal/4/SietteCerros/3/LlermaRojo64/Inia 66//Inia66/Blubird/5/Ban
8	Bayonet	1984	SA			Ptic 'S'/Glaive
9	Bindawara	1980	SA			Mexico 120/Koda/2/Raven
10	Bodallin	1981	WA	S	Stone & Nicolas, (1995).	Bokal/Siete Cerros
11	Brookton	1997	WA			Torres/Cranbrook//Emblem.P1640..Nuri70/Cranbrook
12	Buckley	1997	SA			Pitic62/Festiguay//Warimba/3/Mexico20/Quadrat/2/Kloka/Pitic/2//Bayonet/4/3*Tatiara
13	Cadoux	1992	WA			Centrifen/2*Gamenya//Jacup
14	Camm	1998	WA			Vpm1.5*Cook/4*Spear
15	Canna	1982	WA			Gamenya/Siete Cerros

16	Cascade	1994	WA			Aroona*3/(Ausenvii-95) Tadorna.Inia66
17	Chara	1999	VIC			Beulah Sib/Pavon`S`//Condor
18	Chough	1997	NSW			M2293/Qurrion//Rosella (M2293= Ww15/M1238-2//Kite/Ww15)
19	Chris	1965	USA			Frontana/3*Thatcher/3/Kenya 58/Newthath/2/2* Thather
20	Condor	1973	NSW	S	Wardlaw *et al.* (1989)	Penjamo62/4*Gabo56/2/Tezanos Pintos Precoznainari60/4/2*Lerma Rojo/2/Norin 10/3/3*Andes
21	Cook	1977	Qld		Randall & Moss, (1990.)	Timgalen/Condor 'S'/2/Condor
22	Cranbrook	1984	CIMMY T (WA)			Wren/2/Ciano'S'/Noroeste 66/3/Zambezi
23	Dackicyn*					
24	Dagger	1984	SA.			Sabre/Mec 3/2/Insignia
25	Darkan	1968	WA			Kenya C6041/Eureka2//Unknown
26	Dollabird	1987	CIMMY T/ NSW			Wren/Gaboto//Kalyansona/Bluedird
27	Dundee	1927	NSW			Hard Federation/Cleveland/2/Sands
28	Excalibur	1991	SA			Rac177(Dr26)Uniculm492/Rac311s
29	Fane	1916	Aust.			Marshall'S'no 3a/Queen Fan (Wa) Or Earl/Queen Fan(Sa)
30	Flinders	1982	QLD	S	Stone & Nicolas, (1995)	Pwth/Condor 'S'/2/2*Condor
31	Frame	1994	SA			Molineu/Dagger#3
32	Gabo	1945	NSW	S	Wardlaw et al. (1989).	Bobin*2/Gaza
33	Gamut	1965	Aust.			Gamenya/Gabo54
34	Gatcher	1969	SYD			Chater/3*Gabo/3/Santa Atalina/Thather/2/Mayo
35	Goldmark	1996	VIC			Pavon " S" /Tm56

36	Grebe	1977		T	Blumenthal et al. (1995)	Skorospeklka/3* Egret
37	Gutha	1983	WA			Gamenya/2/Gabo*3/Khapstein/3/Falcon*3/Chili
38	H-45	1998	NSW			Ciano/2*01olympic//Ww15/Qt 605
39	Halberd	1969	SA	S	Blumenthal *et al.* (1990)	Scmitar/Kenyac6042/Bobin/2/Insignia49
40	Hartog	1982	(QLD)			Vicam71/2/Ciano'S'/Sietecerros/3/Kalyansona/Blubird
41	Hyden	1982	WA	S	Stone & Nicolas, 1995.	Gamenya/Inia
42	Jacup	1982	WA			Bencubbin/3/Charter/2/Sword/Kenya C 6041/4/ Pwth//2*7165
43	Janz	1989	QLD			3ag/4*Condor/Cook
44	Kalgrin	1999	WA			Spear//Bodallin/Eradu
45	Kalyansona	1967	India	S	Wardlaw *et al.* (1989)	
46	King	1983	QLD	S	Stone & Nicolas, (1995)	Pwth//2*7165
47	Kingswhite	1907	SA			
48	Kite	1973	SA			Norin10/Brevor(Seln.14)/2/4*Eureka2/3t-A/3*Falcon/4/T-A/4*Falcon/5/T-A/5*Falcon
49	Krichauff	1996	SA			Wariquam//Kloka/Pitic2/3/Warimek/Halberd /4/3ag3aroona
50	Kronos*	1993	USA			
51	Kukri	1999	SA			Sr13*3//76 ECN 44/76 ECN 36 =RAC 820
52	Kulin	1985	WA			Bodallin/2/Gamenya/Inia 66
53	Lance	1978	SA			Collafen/Raven
54	Lark	1989	NSW	S	Blumenthal *et al.* (1995)	Canrock1/2* Csp44/Banks
55	Lyallpur-73	1973	Pakistan	S	Wardlaw *et al.*	Blubird/Nortino 69

					(1989.)	
56	Machete	1985	SA	S	Blumenthal *et al.* (1995).	Sonora64/2/Tezanospintosrecoz/Yaqui 54/3/ *Gabo/4madden
57	Matong	1982	VIC	S	Blumenthal *et al.* (1995).	Kalyansona/Olympic
58	ME 71		Mexico	S	Blumenthal *et al.* (1995).	
59	Meering	1985	VIC	S	Stone & Nicolas, (1994)	Condor Seln
60	Mendos	1986	Aust.			Spica/Koda/2/Gabo/3/Mengavi 'S'
61	Miling	1978	WA			Bencubbin/3/Charter/2/Sword/Kenya C6041/4/ Mexico/5/Gamenya
62	Millewa	1978	VIC	S	Stone & Nicolas, (1994)	Sonora64/Yaqui 50e/2/Gaboto/Mexico 8156
63	Mira	1999	VIC			Cw-Pc C162/Matong//Xd85
64	Mokoan	1985	VIC			Ww15/Olympic/2/Kalyansona/Olympic
65	Molineux	1988	SA			Pitic62/Festiguy//2*Warigal
66	Mustang	1984	Mexico			
67	Olympic	1956	VIC			Baldmin/Quadrat
68	Osprey	1984	NSW	S	Stone & Nicolas, (1994)	Condor*2/Ww33b
69	Oxley	1974	QLD	S	Stone & Nicolas, (1998a)	Oenjamo 62/4* Gabo 56/2/Tezanos Pintos Precoznainari 60/4/2*Lerma Rojo/2/Norin10 /Brevor//3/3*Andes
70	P1226573*					
71	Pavon`S					
72	Psathia*					
73	Quarrion	1983	NSW			Condor/Ta3 Pnb3p/Ww33g/3/Condor/Ww33b
74	RH911996*		Aust.			

75	Schomburgk	1986	SA			W3589/Oxley/2/2*Warigal/3/2* Aroona
76	Silverstar	1996	VIC			Pavan "S"/Tam56
77	Spada*					Trebbo/Kansas/T. Trugidum Var Pseud0-Cervinum
78	Spear	1983	SA			Sabre/Mec 3/2/Insignia
79	SR839426*					
80	Sunbird	1987	SYD			Condor Selection*4/3ag14
81	Sunco	1986	Aust.	S	Blumenthal *et al.* (1995).	Sun9e-27*4/3ag 14/2/Ww15/3/3*Cook
82	Sundor	1985	Aust.			Condor Seln.4*/3 Ag14
83	Suneca	1982	SYD	T	Blumenthal *et al.* (1995)	Ciano/2/Spica/Amber Mutant Sonora 64
84	Tamaroi*	1998	SA			Altar84/4/Tam1b-17/Kamilaroi/3/ Mengavi/ Siete Cerros
85	Tatiara	1987	SA			Mexico20/Koda/2/Raven/3/Menguaui/ Siete Cerros
86	Tincurrin	1977	WA			Gluclub/3/Chile 1b/2/Insignia/Falcon
87	Torres	1983	QLD			3 Agent 3/3*Condor
89	Trident	1993	SA			Vpm1/5*Cook//4*Spear
89	Ulla			T	Blumenthal *et al.* (1995)	
90	Veery	1977	Mexico	S	Stone & Nicolas, (1994)	
91	Vulcan	1985	NSW			Condor/Pitic 62/2/Condor Sib
92	Westonia	1997	WA			Spica.Timgalen.Tosca/Cranbrook..2*Jacup.Bobwhite
93	Wilgoyne	1977	Mexico (WA)			Ciano/Gallo
94	Wlly9-256					
95	Wlly9-263*					

96	Wlly9xTam					
97	Worrakatta	1998	SA			Wariquam//Klotic 62/3/Warimek/Halberd/4/ 3ag3 Aroona
98	WW-15		Mexico (Aust.)			Penjamo 62/4*Gabo 56/2/Tezanos Pintos Precoz /Nainairo 60/4/2* Lerma Rojo/Norin
99	Wyuna	1984	VIC			Dx 3-134/Olympic
100	Yitpi	1999	SA			Chamlein*8156/Mengavi2siete Cerros/Chamlein*8156*Heron/Mengavi*Siete Cerros/Frame

[A] Durum wheat indicated by *; all other genoypes are bread wheat
[B] Origin: Aust. = Australia; WA = Western Australia; SA = South Australia; Qld = Queensland; NSW = New South Wales; SYD = University of Sydney; VIC = Victoria
[C] Thermotolerance at anthesis/grain filling stage: T= heat-tolerant; S= heat sensitive), References are included for those genotypes that have reported in a particular study.
[D] Source for pedigree: International Crop Information System (ICIS, 2000 CD V 1.0 CIMMYT & IRRI; Australian Winter Cereal Collection (AWCC) and Howard Eagles (Pres. Com. 2002)

3.3 Plant growth

3.3.1 Surface sterilisation of seeds

Usually, the seeds were surface sterilised by soaking in 90% Ethanol for 15 to 20 seconds and rinsing 3 times with nano-pure water under a laminar flow hood. If the seeds found to be contaminated with fungus, then they were soaked in 25% Milton bleach for 30 seconds and were rinsed 3 to 4 times with nano-pure water before sowing.

3.3.2. Soil preparation and sowing of seeds

The soil mixture used was a prepared potting mix which contained adequate amounts of nutrients for normal growth. The mix contains soil, peat and sand in the ratio of 2:1:1 and this mixture was autoclaved and oven dried usually 24 hours prior seed sowing. Small plastic pots of 300 mL were prepared with 3 to 4 holes at the bottom and filled with 300g autoclaved soil mixture. Pots were placed in small plastic trays. After surface sterilization, 12 to 14 seeds of each genotype were sown in these pots. These pots were transferred to a control growth room with the conditions mentioned below.

3.3.3. Growth room conditions

Pots were placed in a growth room at the Waite Institute. The day/night temperature (25/20°C) was maintained with 10 hours of photoperiod unless otherwise stated in the individual experiments. The light intensity in the growth room was 600µE m^{-2} sec^{-1} through out the experimental period.

3.3.4. Thinning of pots and watering

After 4 to 5 days of germination, pots were thinned to give final population of 6 uniform seedlings per pot. Moisture level was maintained at 12.5% field capacity after weighing the pots. The moisture level was maintained by adding the nano-pure water daily in the morning. To maintain the nutrients in the pot, 25mL of Hoagland's (10%) solution at day 7 and day 14 was added to each pot.

3.4. Induction of heat stress

Usually, heat-stress of 40°C (unless otherwise stated) was imposed on 14 day old wheat seedlings using a hot air incubator (Orbital Mixer Incubator, Ratek Instruments, Australia; Plate 1). Every time the incubator was placed in the same growth room and the growth room conditions were maintained throughout the experimental period as mentioned in section 3.3.3. The temperature (40°C) of the hot air incubator was set 1.5 to 2 hours prior to the experiment. To monitor the temperature of the incubator, two thermometers were used, one thermometer was inserted from the top through a hole and another was placed inside the incubator. Usually, 10 pots were placed inside the incubator because only 10-15 pots at a time can fit easily in the hot air incubator.

3.5. Chlorophyll fluorescence measurements

In vivo chlorophyll fluorescence measurements were recorded using a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., England). Measurements were done on two fully-expanded leaves of two seedlings, which were dark-adapted for 15 minutes by clamping with leaf clips. The measurements were generally taken on 2

Plate 1. Imposing heat stress (40°C) in the hot air incubator.

Plate 2. Clamping of 2 leaves of wheat seedlings under control conditions (25°C) with leaf clips of a Plant Efficiency Analyser (PEA).

Plate-3. Recording *in-vivo* chlorophyll fluorescence data on a leaf of wheat seedling under control conditions (25°C) using a Plant Efficiency Analyser (PEA).

week-old seedlings and were made on the abaxial surface of middle portion of the leaves. Usually, the repeated measurements were done on the same leaves by marking them. Generally light of approximately 3000 micromoles/m^2/sec was used to illuminate the leaf surface. The peak wavelength was 650nm.

The potential quantum yield of the photosystem-II (PS-II) was determined by the means of the ratios of variable to maximum fluorescence (Fv/Fm), which is the efficiency of excitation capture by the open PS-II centres. In addition the changes in the variable fluorescence (Fv = Fm – Fo), the initial fluorescence (Fo), maximum fluorescence (Fm) and the time at which maximum fluorescence occurs (Tm), were recorded. The control measurements were done under the growth room conditions with 25/20°C day/night temperature mentioned in section 3.3.3. While heat-stress measurements were recorded by placing the pots inside the incubator and the leaves were dark adapted for 15 minutes after clamping the leaves. Recovery of the PSII efficiency after cessation of the heat stress treatment was also determined by removing the seedlings from the incubator and growing the plants under the control growth conditions.

3.6. Statistical analysis

Statistical analyses were performed using the GENSTAT-5 Statistical package (GENSTAT-5 Committee, 1987). Data were analysed by ANOVA using models appropriate to the experimental design. These are described in each experimental chapter. Differences between means were assessed using the least significant difference (LSD) at the 5% and 1% probability levels. Relationships between variables were

examined by simple linear correlation (r) and by regression analysis. Other statistical tests were used in the studies and these are described in the relevant chapters.

CHAPTER 4

SCREENING OF WHEAT SEEDLINGS FOR HEAT TOLERANCE USING CHLOROPHYLL FLUORESCENCE

4.1 Introduction

Moderately high temperatures (25-35°C) and very high temperatures (>37.5°C) adversely affect vegetative growth (Shipler and Blum, 1986) and reproductive growth (Wardlaw *et al.*, 1990; Stone and Nicolas *et al.*, 1994) in wheat. Heat stress can occur during any growth stage in wheat but seedling emergence and grain filling are especially sensitive periods (Raynolds *et al.*, 1994). Temperatures can rise from 30°C and above at seedling emergence, tillering (early growth) and grain filling stages in many arid, semi-arid, and tropical regions of the world where wheat is grown (Woodruff, 1984).

Chlorophyll fluorescence has been used in several studies to detect the genotypic differences in response to heat stress in many plant species, including wheat (Smillie and Nott.,1979; Smillie and Gibbens, 1981; Syed *et al.*, 1989; Moffatt *et al.*, 1990; Galiba *et al.*, 1997; Tambussi *et al.*, 2000). These studies have been performed at different growth stages in wheat; for example, Moffatt *et al.*, (1990) examined the effect of high temperatures at grain growth and maturity stages, while Araus *et al.* (1998) have examined durum wheat cultivars at the flag leaf stage under the hot field conditions using chlorophyll fluorescence parameters. A few studies have been done at the early growth stages in wheat, such as 3-4 leaf stage, to study the responses of wheat genotypes to heat stress and moisture stress using chlorophyll fluorescence technique (Alkhatib and Paulsen, 1990; Balota and Lichtenthaler, 2000). However, these studies

have used a small number of genotypes to observe responses to heat or drought stresses. Therefore, the present studies have been conducted to examine the effect of heat stress on a diverse range of wheat genotypes at the seedling stage. The responses to heat stress and the recovery of photosynthetic efficiency after removing the heat stress were examined.

The objectives of this study were:

(i) To screen one hundred wheat genotypes for thermo-tolerance at the seedling stage using the chlorophyll fluorescence technique,

(ii) To investigate the effect of heat damage to the maximal efficiency of photochemistry (Fv/Fm) including other chlorophyll fluorescence parameters Fo, Fm, and Fv in thermo-tolerant and thermo-sensitive genotypes at the seedling stage,

(iii) To investigate which fluorescence parameter is the most suitable to screen large number of genotypes at the seedling stage.

4.2 PRELIMINARY EXPERIMENT

To determine the time course of heat-stress on the chlorophyll fluorescence Fv/Fm ratio and its recovery after heat stress.

Two preliminary experiments were conducted to establish the experimental protocol for screening a large number of genotypes at the seedling stage using two temperature regimes and different time intervals.

4.2.1 Materials and Methods

The experiment was conducted to optimise the conditions for screening 100 genotypes and other related heat stress experiments. In this experiment two cultivars

were selected, cv. Banks, relatively heat sensitive (Wardlaw *et al.*, 1989) and cv. Frame, relatively heat tolerant (Dr Arun. P. Aryan personnel communication). Two sets (4 pots) of these genotypes were grown for 14 days under growth room conditions (Materials and Methods section 3.4). At day 15, heat stress (40°C) from 0h to 10 hour was imposed on one set and other set was kept under the control growth room conditions (section 3.4) with the day/night temperature of 25/20°C.

Another experiment was conducted using 15 different genotypes. Two sets of pots were prepared for control conditions and heat stress treatments (Materials and Methods, sections 3.3 to 3.4). Heat stress was imposed using a hot air incubator (section 3.5), for 6 h. and allowed to recover for 24 h.while the chlorophyll fluorescence parameters were recorded as described in (section 3.6).

4.2.2 Results

The results show that heat-stress (40°C) decreased the chlorophyll fluorescence Fv/Fm values significantly in both genotypes after 10 hours of heat-stress (Fig-A and B). However, the recovery of Fv/Fm ratio was not apparent after 24 hours in either genotype. In the additional experiment with 15 different genotypes, the Fv/Fm values decreased in all genotypes after 6 hours of heat stress and recovered up to the initial level (0h) in all genotypes after 24 hours, when returned to the control growth room conditions (Fig-C). Therefore, the above mentioned method was used for screening 100 wheat genotypes as well as in the subsequent experiments.

Plate 4. Seedlings of a heat-tolerant wheat cultivar Anlace under control conditions (25°C, left) and after 6h of heat stress (40°C, right).

Plate-5. Seedlings of a heat-sensitive wheat cultivar Lyallpur-73 after 6h of heat stress (40°C, left) and under control conditions (25°C, right).

Plate-6. Seedlings of a moderately heat-tolerant wheat cultivar Kukri after 6h of heat-stress (40°C, right) and under control condition (25°C, left).

Plate-7. Seedlings of a heat-sensitive wheat cultivar WW15 after 6h of heat-stress (40°C, right) and under control condition (25°C, left).

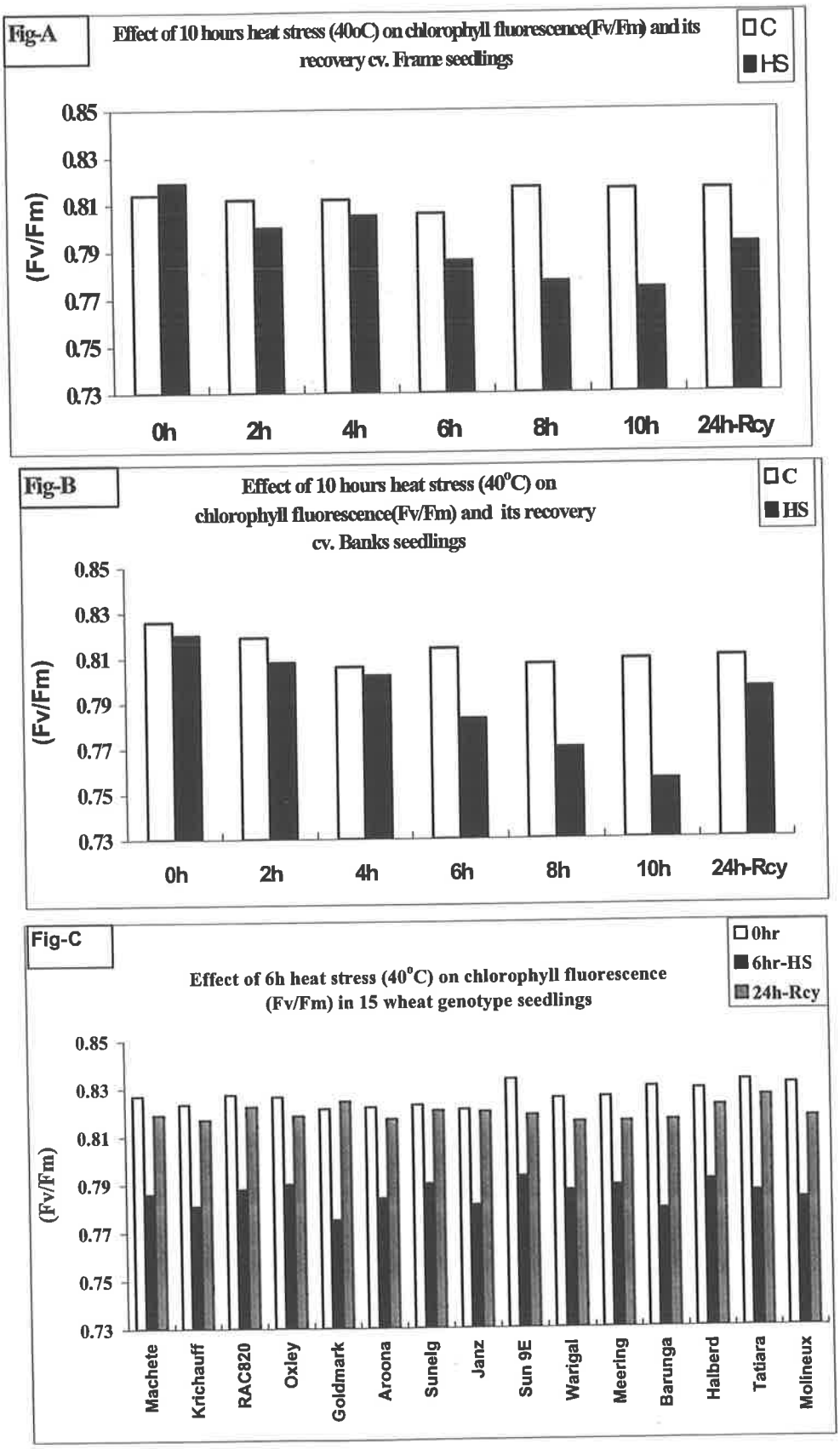


Figure 4.1 The effects of various heat treatments on Fv/Fm of different varieties of wheat during and after heat stress treatments.

4.3 EXPERIMENT-1.

Screening of 100 wheat genotype seedlings for heat tolerance.

4.3.1 Introduction

In the past many studies have been conducted to evaluate or even screen various crops species based on several techniques for heat and drought tolerance (Havaux *et al.*, 1988; Moffat *et al.*, 1990; Wardlaw *et al.*, 1989a&b; Stone and Nicolas, 1994 & 1995). However, no study has been reported involving large number of genotypes to evaluate heat or drought tolerance at the seedling stage. In the present study, *in vivo* chlorophyll fluorescence technique was used to evaluate 100 genotypes for heat tolerance.

Several studies have shown that diverse genotypes depict similar response to heat and drought stresses based on the morpho-physiological traits such as leaf photosynthesis, membrane thermostability and chlorophyll contents (Lynch *et al.*, 1992; Gonzalez *et al.*, 1995; Saadalla, 1997; Zhang *et al.*, 1997). Similarly, the coefficient of parentage (COP) has been used to estimate the expected percentage of alleles common by descent at loci polymorphic within a population. In general, a COP is a measure of overall common ancestry of two genotypes. Crop species such as oats and wheat are completely in-bred species. It is assumed that two cultivars when crossed, contribute equally to the offspring despite inbreeding and selection (Martin, 1982; Cox *et al.*, 1985).

4.3.2 Materials and Methods

One hundred diverse wheat genotypes of *Triticum aestivum* and *T. durum* were selected representing the different ago-ecological zones of wheat belt in Australia, including some genotypes from other regions of the world. The pedigree, year of release, origin and thermo-tolerance, when known, are presented in Table 4.1. Usually, 6 seedlings were grown in small pots (300mL) under the control growth room

conditions for 14 days as described in Chapter 3 (section 3.3.1 to 3.3.4). Seedlings of these genotypes were screened for thermo-tolerance and sensitivity using the chlorophyll fluorescence technique Chapter 3 (section 3.4 and 3.5).

It was not possible to screen all 100 genotypes at one time due to the limited space in the hot air incubator and limited numbers of clamps used to dark adapt the leaves. Therefore, ten non-replicated experiments were conducted under the control growth room conditions (section 3.4, Chapter 3), and 6 hours of heat stress (40°C) was imposed using the hot air incubator placed in the same growth room. Genotypes were compared under the control temperature (25/20°C, day/night) and during 6h exposure to the heat stress (40°C) as well as recovery from the heat stress under controlled growth room conditions (section 3.3.3, Chapter 3). A number of check varieties such as Goldmark, Oxley, Lyallpur-73, Halberd, Anlace and Buckley were included in each run to use as a check for variability and reproducibility of data. These genotypes were selected on their previous performance under heat stress and recovery periods. A Plant Efficiency Analyzer (Hanstech, Ltd) was used to record the chlorophyll fluorescence Fv/Fm values on the two fully expanded leaves of 14 days old seedlings after 15 minutes of dark adaptation. These values were recorded at day 14, immediately before imposing the heat stress (0h) and after 6 hours of heat stress. The recovery of chlorophyll fluorescence Fv/Fm values in the control and heat stress plants was recorded after 24 hours and one week after the heat stress.

4.3.3 Data analysis

The measurements of chlorophyll fluorescence were made on the same leaves at each time, which allowed the data to be analyzed using a repeated measurements

ANOVA. Data of Fv/Fm from 0h. 6h., 24h., and 21d., were used in the analysis (Table-4.1). The mean square values for the Variety, Time and Time x Variety were highly significant (Table-4.1). The data were then analyzed using agglomerative hierarchical cluster analysis in order to explore the groupings within the genotypes (Manley, 1998). The values of Fv/Fm at 0h, 6h, 24h and 21d were expressed as a percentage of the values measured at 14d. The analysis was based on the relative Fv/Fm measurements at the 4 times of measurement during heat stress and recovery period. The dissimilarity was measured using the squared Euclidean distance and the further neighbour method was used to cluster the genotypes. The analysis was truncated at the 90% level of similarity (two cluster groups are identical when they have a similarity of 100%).

Table 4.1. The ANOVA for Fv/Fm based on repeated measures

SOV	d.f.	SS	MS
Variety	98	363.64	3.71**
Residual	101	9.94	0.098
Time	3	3211.9	1070.6**
Time x Variety	290	555.3	1.91**
Residual	299	25.35	0.0847
Total	791		

The 100 genotypes were arranged according to the frequency distribution, as from Tolerant-T, Moderately tolerant-MT, Moderately sensitive-MS and Sensitive-S genotypes that corresponds to the Figure 4.1 (Table 4.2). Heat tolerant genotypes were grouped together whose chlorophyll fluorescence reduction was less then 3.5% after 6h

of heat stress, moderately tolerant genotypes were grouped together with more than 3.5% and less than 5.5% reduction in chlorophyll fluorescence, moderately sensitive genotypes were those with more than 5.5% but less than 7% reduction while most heat sensitive genotypes were considered with more than 7% reduction in chlorophyll fluorescence after 6h of heat stress (Table 4.2). The International Crop Information System (ICIS 2000) software package developed by CIMMYT was used to determine the coefficient of parentage (COP) in some of the extreme tolerant or sensitive genotypes in this study to examine if there were genotypes in common among the tolerant and sensitive genotypes.

4.3.4 Results

Screening of 100 genotypes in un-replicated experiments showed a range of heat tolerance. The 100 genotypes were arranged according to the percent decrease in the ratio of Fv/Fm from 0h to 6 hours of heat-stress (Figure 4.1). Genotypes were classified as tolerant, if their Fv/Fm ratio fell by less then 3.5% after 6h of heat stress. Some of the most thermo-tolerant genotypes were Kulin, Buckley, Mira, Meering, Psathia, Brookton, Anlace, Kingswhite, Tamoroie, Kronos and RH-911996 and Arrino, where the Fv/Fm ratios decreased by less then 3.5%. Some of the moderately heat tolerant genotypes such as Veery-S, WLY9-256, Spear, Sundor, Krichauff, Halberd, Molineux, Aroona, Janz, Milling, Kukri, Wyuna and Sunberd, grouped together whose Fv/Fm ratios fell between 3.5% to 5.0%. While moderately sensitive and most sensitive genotypes were those that grouped together in the last 30 genotypes (Figure 4.1), the

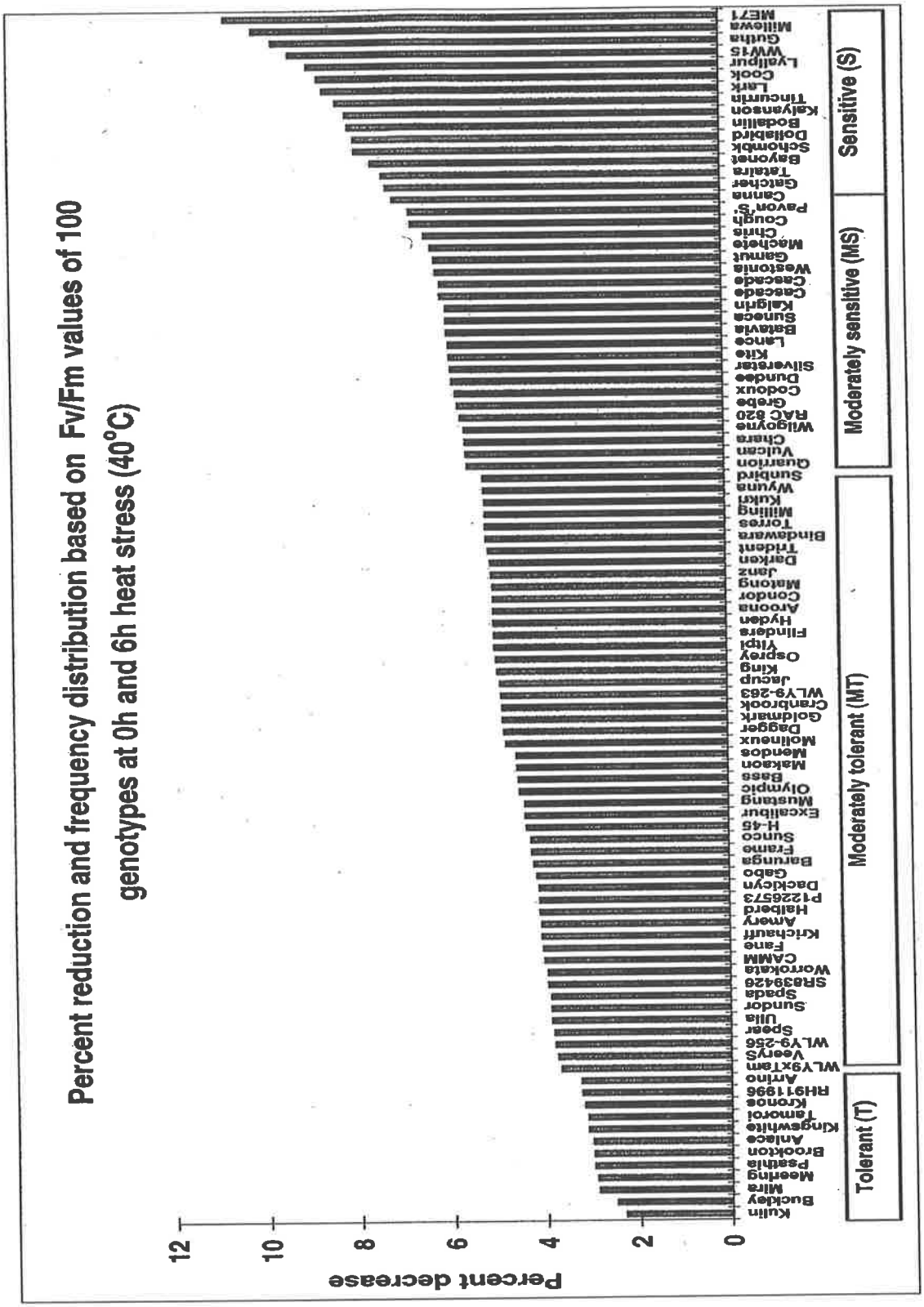


Figure 4.2. Relative reduction in Fv/Fm after 6h. of heat stress among 100 genotypes of wheat.

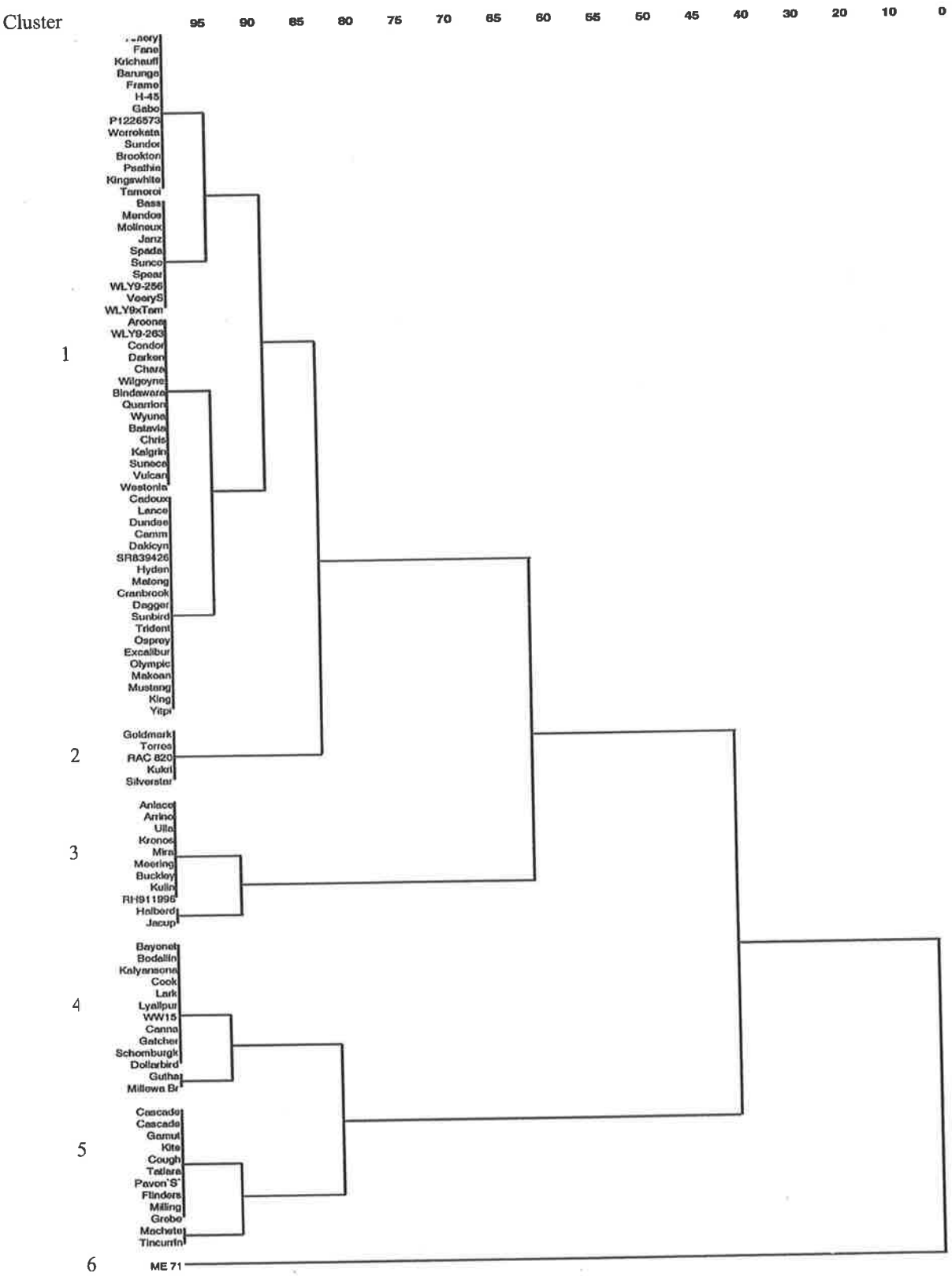


Figure 4.3. Dendrogram of 100 genotypes based on hierarchical cluster analysis of Fv/Fm values at 0h, 6h heat stress (40°C) and recovery of Fv/Fm. Cluster numbers are shown on the left hand side.

Table 4.2. Range of percent reduction in chlorophyll fluorescence in wheat seedlings after 6h of heat stress (40°C).

Heat tolerance	Range of percent reduction (%) of chlorophyll fluorescence after 6h of heat stress
Heat Tolerant (T)	<3.5%
Moderately heat tolerant (MT)	3.5% to 5.5%
Moderately heat sensitive (MS)	5.5% to 7.0%
Heat Sensitive (S)	> 7.0%

ratio of Fv/Fm in these moderately and most sensitive genotypes decreased from 5.5% to 10.8%. Thermotolerance rating at the seedling stage did not correspond with the thermotolerance rating at anthesis/grain filling (Table 4.3) for a number of genotypes within the limited set of data available.

The hierarchical cluster analysis grouped the cultivars into 6 groups at the 90% level (Figure 4.3). The mean values for each cluster are shown in Table 4.4. The dendrogram revealed that heat tolerant and moderately heat tolerant wheat genotypes of *T. aestivum* and *T. durum* were clustered into group-1, group-2 and group- 3 (Figure 4.2), while group-1 is the largest group containing 58 genotypes and group 2 and 3 consisting 16 genotypes.

A number of most thermo-sensitive and as well as moderately sensitive genotypes clustered together in group 4. Some of moderately tolerant, sensitive and moderately sensitive cultivars clustered to gather in group 5. One of a highly thermo-sensitive genotype ME-71 was clustered separately in group 6. A significant decrease in Fv/Fm

Table 4.3. Classification of thermotolerance at the seedling stage of 100 genotypes and their thermotolerance at grain filling. Genotypes are ordered as in Figure 4.1.

Genotype[A]	Origin[B]	Thermo-tolerance at seedling stage[C]	Thermo-tolerance at anthesis or grainfilling stage[C]	Reference for (anthesis/grain stage)
Kulin	WA	T		
Buckley	SA	T		
Mira	VIC	T		
Meering	VIC	T	S	Stone & Nicolas, 1994
Psathia*		T		
Brookton	WA	T		
Anlace	SA	T		
Kingswhite	SA	T		
Tamaroi*	SA	T		
Kronos*	USA	T		
RH911996*	Aust.	T		
Arrino	WA	T		
Wlly9xTam		MT		
Veery	Mexico	MT	S	Stone & Nicolas, 1995
Wlly9-256		MT		
Spear	SA	MT		
Ulla		MT	T	Blumenthal et al. 1995
Sundor	Aust.	MT		
Spada*		MT		
SR839426*		MT		
Worrakatta	SA	MT		
Camm	WA	MT		
Fane	Aust.	MT		
Krichauff	SA	MT		
Amery		MT		
Halberd	SA	MT	S	Blumenthal et al. 1990
P1226573*		MT		
Dackicyn*		MT		
Gabo	NSW	MT	S	Wardlaw et al. 1989.
Barunga	SA	MT		
Frame	SA	MT		
Sunco	Aust.	MT	S	Blumenthal et al. 1990.
H-45	NSW	MT		
Excalibur	SA	MT		
Mustang	Mexico	MT		
Olympic	VIC	MT		
Bass	QLD	MT		
Mokoan	VIC	MT		
Mendos	Aust.	MT		
Molineux	SA	MT		
Dagger	SA.	MT		
Goldmark	VIC	MT		
Cranbrook	CIMMYT(WA)	MT		
Wlly9-263*		MT		
Jacup	WA	MT		
King	QLD	MT	S	Stone & Nicolas, 1995.
Osprey	NSW	MT	S	Stone & Nicolas, 1995.
Yitpi	SA	MT		
Flinders	QLD	MT	S	Stone & Nicolas, 1995.
Hyden	WA	MT	S	Stone & Nicolas, 1995.

Aroona	SA	MT		
Condor	NSW	MT	S	Wardlaw et al. 1989
Matong	VIC	MT	S	Blumenthal et al. 1995.
Janz	QLD	MT		
Darkan	WA	MT		
Trident	SA	MT		
Bindawara	SA	MT		
Torres	QLD	MT		
Miling	WA	MT		
Kukri	SA	MT		
Wyuna	VIC	MT		
Sunbird	SYD	MS		
Quarrion	NSW	MS		
Vulcan	NSW	MS		
Chara	VIC	MS		
Wilgoyne	CIMMYT WA	MS		
Hartog	CIMMYT Qld	MS		
Grebe		MS	T	Blumenthal et al. 1995.
Cadoux	WA	MS		
Dundee	NSW	MS		
Silverstar	VIC	MS		
Kite	SA	MS		
Lance	SA	MS		
Suneca	SYD	MS	T	Blumenthal et al. 1995
Batavia	QLD	MS	S	Blumenthal et al. 1995.
Kalgrin	WA	MS		
Oxley	QLD	MS	S	Stone& Nicolas, 1998.
Cascade	WA	MS		
Westonia	WA	MS		
Gamut	Aust.	MS		
Machete	SA	MS	S	Blumenthal et al. 1995.
Chris	USA	MS		
Chough	NSW	MS		
Pavon`S	CIMMYT	MS		
Canna	WA	S		
Gatcher	SYD	S		
Tatiara	SA	S		
Bayonet	SA	S		
Schomburgk	SA	S		
Dollabird	NSW	S		
Bodallin	WA	S	S	Stone & Nicolas, 1995.
Kalyansona	India	S	S	Wardlaw et al. 1989
Tincurrin	WA	S		
Lark	NSW	S	S	Blumenthal et al. 1995.
Cook	CIMMYT Qld	S		Randall & Moss, 1990.
Lyallpur-73	Pakistan	S	S	Wardlaw et al. 1989.
WW-15	Mexico (Aust.)	S		
Gutha	WA	S		
Millewa	VIC	S	S	Stone & Nicolas, 1995.
ME 71	Iraq	S	S	Blumenthal et al. 1994.

[A] Durum wheat shown as *; all others are bread wheat

[B] Aust. = Australia,; WA = Western Australia, SA = South Australia; NSW=New South Wales,; Qld = Queensland; VIC = Victoria; SYD = Sydney

[C] T= tolerant; MT = moderately tolerant; MS = moderately sensitive; S = sensitive

Table 4.4. Mean Fv/Fm values (± SEM) at the start of heat stress (0h), after 6h of heat stress and two recovery periods expressed as percentage of 14d for each cluster group. The number of genotypes (n) in each group is indicated.

		Period of heat stress (h.)		Period of recovery	
Cluster	Number of genotypes	0	6	24 h.	21 d.
1	58	100 (±0.044)	95.3 (± 0.11)	98.6 (± 0.098)	99.9 (±0.44)
2	5	99.9 (± 0.09)	94.6 (±0.18)	97.9 (±0.26)	98.7 (±0.20)
3	11	99.6 (± 0.17)	96.7 (±0.23)	99.5 (±0.16)	100.7 (±0.14)
4	13	100 (± 0.059)	91.6 (± 0.257)	98.4 (± 0.295)	99.8 (±0.065)
5	12	99.8 (±0.14)	93.7 (±0.26)	98.6 (±0.14)	100.7 (±0.12)
6	1	99.9	89.3	89.1	98.5

values was observed in genotype ME-71, as it decreased by 11.7% after 6 hours of heat stress.

The mean values of Fv/Fm after 6 hours of heat stress decreased by 4.7% (95.3 ± 0.11), 5.4% (94.6 ± 0.18) and 4.3% (96.7 ± 0.23) in groups 1, 2 and 3, respectively. While, in two sensitive groups 4 and 5, the Fv/Fm values decreased to 9.4% (91.6 ± 0.25) and 7.3% (93.7 ± 0.26). The ratio of Fv/Fm dropped to 11.7% (89.3) in group-6 that clustered only one genotype ME-71. Coefficient of parentage (COP) was calculated based on the pedigree information for all the pair wise combinations of some selected genotypes from the tolerant, moderately tolerant and sensitive grouping's (Table 3.1, Chapter 3). COP was used to estimate the common ancestry of some selected heat-

Table 4.5: Coefficient of parentage of lines used in all experiments. Meering, Condor and Oxley were grouped together because they were siblings derived from the same cross. Empty cells are because of insufficient expansion of pedigree

Genotype	Buckley	Kukri	Anlace	Kulin	Kingswhite	Cook	WW15	ME71	Millewa	Lyallpur 73S	Machete	Halberd	Krichauff	Meering/ Condor/ Oxley
Thermotolerance	T	T	T	T	T	S	S	S	S	S	MS	MT	MT	T/MT/S
Buckley	1.000													
Kukri	0.165	1.000												
Anlace	0.832*	0.154	1.000											
Kulin	0.192	0.230	0.195	1.000										
Kingwhite					1.000									
Cook	0.065	0.083	0.068	0.079		1.000								
WW15	0.059	0.073	0.052	0.065		0.526*	1.000							
ME71								1.000						
Millewa	0.091	0.128	0.089	0.134		0.093	0.111		1.000					
Lyallpur		0.105		0.191		0.105	0.128		0.213	1.000				
Machete	0.204	0.332*	0.207	0.313*		0.061	0.026		0.112	0.141	1.000			
Halberd	0.115	0.159	0.115	0.169		0.033	0.011		0.048	0.061	0.251	1.000		
Krichauff	0.140	0.069	0.137	0.073			0.270		0.062		0.069	0.158	1.000	
Meering Condor Oxley	0.074	0.096	0.069	0.088		0.591*	0.691*		0.109	0.127	0.065	0.031	0.243	1.000

tolerant and heat-sensitive genotypes. The results showed that most COP values ranged from as low as 0.011 to the highest 0.832 (excluding similarity values of the genotype with themselves, which is =1). The higher COP values of some of the genotypes also showed a close relationship between two heat-tolerant genotypes such as Buckley and Anlace (0.832), while their pedigree information (Table 3.1) showed that Tatiara was common in both of the genotypes. However, Tatiara was classified as heat sensitive (Table 4.4).

Similarly, three of the genotypes that were grouped together including Meering, Condor and Oxley were siblings of the same cross (Penjamo 62/4* Gabo65/2/Tezanos Pintos Precoznian Nainari 60/4/2* Lerma Rojo/2/Norin10/Brevor//3/3*Andes), however our results showed that Meering was a heat-tolerant and Condor was moderately heat-tolerant cultivars, while Oxley was heat-sensitive genotype (Figure 4.2), however, genotype Meering was heat-sensitive at the grain filling stage (Stone and Nicolas, 1994). These differences could be due to different physiological mechanisms involved in heat-tolerance and sensitivity at two different stages of crop development i.e., seedling and grain filling stages. Higher COP values were found among some genotypes such as the sensitive genotypes Cook and WW15 (0.526), and the moderately tolerant genotype Condor and the sensitive genotype Cook (0.591), and between Condor and WW15 (0.691). However, the COP data showed that many of the heat tolerant genotypes and as well as many of the heat sensitive genotypes were unrelated.

4.4 EXPERIMENT 2.
Effect of heat stress (40°c) on Fv/Fm and recovery after heat-stress in thermo-tolerant and thermo-sensitive wheat seedlings.

4.4.1 Introduction

Screening of 100 genotypes in Experiment 1, showed a range of heat tolerance and sensitivity in wheat genotype seedlings. The survey of 100 genotypes was done in un-replicated experiments and the results need to be confirmed. Therefore, a replicated experiment was conducted using fewer genotypes that represented tolerant, moderately tolerant and sensitive responses to heat stress. The selection was made based on percent decrease in Fv/Fm values at 0 hour and 6 hours heat stress of 40°C (Figure 4.2). The recovery of photosynthetic efficiency (Fv/Fm) after various abiotic stresses has been examined in a number of crops including rice (Lal *et al.,* 1996), maize (Aguilera *et al.*, 1999) and soybean (Hong and Xu, 1999). Experiment 1 indicated that there are differences in the ability of plants to recover from heat stress. Therefore, effect of heat stress on the quantum yield of PS-II and its recovery after heat stress was examined in selected tolerant and sensitive wheat seedlings in greater detail then Experiment 1.

4.4.2 Materials and Methods

Two weeks old seedlings were exposed to 40°C heat stress for 6 hours and then allowed to recover. Measurements of chlorophyll fluorescence were made using the same method and schedule described in Experiment 1. Five heat-tolerant genotypes including one moderately-tolerant genotype and five heat-sensitive genotypes were selected after screening 100 genotypes according to their tolerance and sensitivity to heat stress. The tolerant genotypes were Kulin, Buckley, Anlace, and Kingswhite, the moderately tolerant genotype was Kukri, and five sensitive genotypes were Cook, Lyallpur-73, WW15, Millewa, and ME-71, (Table 4.3). The experiment was repeated

four times, which were considered as replicates, and the experimental design was a factorial, randomized block.

4.4.3 Results

Genotypes differed significantly for Fv/Fm ratios (Table 4.6). They also differed significantly in their response to temperature as evident by highly significant Variety x Treatment interaction (Table 4.6). The Fv/Fm values decreased both in tolerant and sensitive genotypes after 6 hours of heat stress (40°C). The Fv/Fm ratio decreased by 4.3%, 4.6%, 4.9%, 5.2% and 5.3% in Kulin, Buckley, Kingswhite, Anlace and Kukri, respectively, which were thermo-tolerant genotypes (Figure 4.4). By comparison, the Fv/Fm values dropped by 6.9%, 7.6%, 9.2%, 9.3% and 9.6% in thermo-sensitive genotypes, WW15, Cook, ME-71, Lyallpur-73 and Millewa (Figure 4.4).

Table 4.6. Analysis of variance for Fv/Fm values under control and 6 hours heat stress (40°C)

SOV	d.f.	S.S.	M.S.	V.R	F pr.
Replication	3	0.0012	0.00041	3.52	
Variety (Var.)	9	0.0055	0.00062**	5.26	<0.001
Treatment (Trt.)	1	0.0162	00.0617**	526.26	<0.001
Var. x Trt.	9	0.0052	0.00058**	4.90	<0.001
Residual	47	0.0055	0.00012		
Total	69	0.0696			

Genotypes differed significantly in their recovery after removal of heat-stress treatment (Figure 4.4). The Fm/Fv values in thermo-tolerant genotypes increased approximately to the same level of un-stressed control values (0h) within 24 hours after the removal of heat-stress treatment. However, the recovery for Fv/Fm in sensitive genotypes was much poorer then tolerant and moderately tolerant genotypes (Figure 4.4).

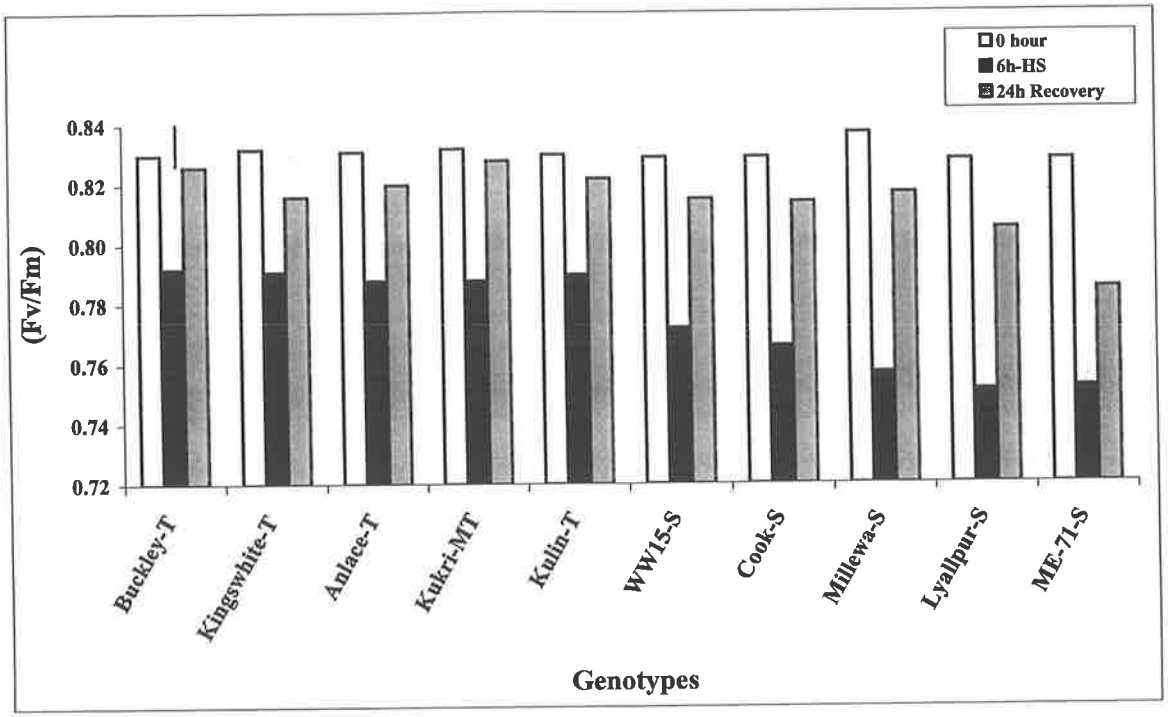


Figure 4.4. Effect of 6 hour heat stress (40°C) on Fv/Fm and recovery after heat-stress in wheat seedlings. Vertical bar is the LSD at 5% level for 6h heat stress. (T= heat tolerant, MT= Moderately tolerant, S = sensitive)

4.5 EXPERIMENT 3.
Effect of time course heat-stress (40°C) on Fv/Fm and recovery during night and daytime in heat-tolerant and heat-sensitive wheat genotype seedlings.

4.5.1 Introduction

This experiment was conducted to observe the time course of Fv/Fm during heat stress (40°C) and subsequent recovery period during night and day cycles. As in Experiment 2, it was observed that after 24 hours (during day time) the recovery of photosynthetic efficiency of PSII was different in some tolerant and sensitive genotypes. Therefore, this study will be conducted to observe that how quickly the PSII efficiency recovers after removing the heat stress in wheat seedlings growing in night and day cycles.

4.5.2 Materials and Methods

The experiments was conducted under the same conditions as in Experiments 1 and 2, except that chlorophyll fluorescence was recorded at 0h, 1h, 2,h, 3h, 4h, 5h, and 6h under the light. Three recovery phases after the end of the heat stress, which include 1h (dark), 2h (dark) and 18h (18h included the 4h light period). The dark period begins when the lights go off after 10 hours of photoperiod (from 9:00h to 19:00h) and temperature decreases from 25°C day to 20°C night temperature. Therefore, in proceeding section, 18h recovery also includes the 4h light period. Genotypes were the same ones used in Experiment 2. The chlorophyll fluorescence measurements (Fv/Fm) were recorded as mentioned in earlier section (section 3.6). The experiment was repeated twice, providing two replicates.

4.5.3 Results

Table 4.7 shows the mean Fv/Fm values and the percent decrease in Fv/Fm ratios from 0h during heat stress period and subsequent recovery periods. After 1h of heat stress, the Fv/Fm ratios decreased least in cultivars Buckley (3.8%) and Anlace (4.2%), while the greatest reductions was observed in genotypes ME-71 (6.5%) Lyallpur-73 (6.1%). After 2 hours the greatest reduction was observed again in Lyallpur-73 (7.5%) followed by WW-15 (6.4%). This decrease in Fv/Fm ratios was consistent throughout the heat stress period in Lyallpur73. At the end of 6 hours heat stress, the maximum decrease in Fv/Fm ratio was observed in Lyallpur-73 followed by Kingswhite (9.4%) and Millewa (9.0%). The minimum decrease in Fv/Fm value was observed in Buckley (6.7%), however, other genotypes showed almost the same ratio of decrease in Fv/Fm ratios after 6 hours of heat stress. During the first phase of the recovery period of 1h (dark) all other genotypes except Lyallpur-73, recovered quickly to values similar to those measured at 0hrs level (Table 4.7). In the second phase of

recovery i.e., 2h (dark), the minimum recovery of Fv/Fm ratio was observed in Lyallpur-73 (0.802), while all other genotypes had almost fully recovered. A similar trend was observed after 18h recovery. Figures (4.4-A, 4.4-B, & 4.4-C) illustrate the responses of individual genotypes and their post stress recovery. Differential responses in genotypes were observed during time course of heat stress from 1h, 2h, 3h, 4h, 5h and 6h including three recovery phases, 1h and 2h (dark) and 4h (light). The most rapid decline in Fv/Fm ratios occurred in the genotypes ME-71 and Lyallpur-73 (Figure 4.4-C), while a major decline in Fv/Fm ratios was also observed after 6 hours of heat stress in Kingswhite (0.755), Millewa (0.760), ME-71 (0.761) and Lyallpur-73 (0.745) (Figures 4.4, A, B and C). Similarly, during three recovery periods from 1h and 2h (dark) and 4h (light), the response among genotypes seedlings were different. Full recovery in Fv/Fm ratios occurred in Buckley, Kukri, Anlace, Kingswhite, Cook, WW-15 and Millewa, while there was incomplete recovery in Kulin, ME-71 and Lyallpur-73 (Figures 4.4-A, B, C). The irreversible decreases in Fv/Fm ratios during recovery periods in ME-71 and Lyallpur-73 are in agreement with the results of Experiments, 1 and 2. The irreversible decreases in Fv/Fm ratios suggest that the PSII efficiency in these genotypes might have been damaged considerably than the other genotypes (Kim *et al.*, 1997).

4.6 EXPERIMENT 4.
Effect of heat-stress on chlorophyll fluorescence and its components in wheat seedlings.

4.6.1 Introduction

This study was conducted to compare two treatments, control (20/15°C day/night) and heat stress of 38.5°C. However, instead of using a hot air incubator, which was used in all previous experiments, two growth rooms were used as an

Table 4.7. Reduction in Fv/Fm ratio during 6h of heat stress and during an 18h recovery period. Values are expressed as percentage change relative to initial value. The measurement at 2h recovery coincided with a dark period during the 24-h. lighting cycle

	Reduction in Fv/Fm values relative to 0 h. (%)									
Genotypes	Initial Fv/Fm	Period of heat stress (h.)						Period of recovery (h.)		
		1	2	3	4	5	6h	1	2	18
Buckley	0.835	3.8	4.5	5.3	5.6	5.6	6.7	0.5	0.5	1.8
Kingswhite	0.833	5.0	5.2	5.2	7.2	7.8	9.4	2.2	1.4	1.8
Anlace	0.834	4.2	4.2	5.5	4.7	7.3	7.9	3.0	0.8	1.7
Kukri	0.832	4.9	4.9	4.8	5.8	7.5	7.9	1.3	0.5	1.3
Kulin	0.832	5.3	5.6	6.1	5.6	6.6	8.4	2.3	0.4	0.4
WW15	0.833	5.5	6.4	4.9	5.5	7.1	8.5	1.3	-0.4	2.3
Cook	0.833	4.4	4.7	5.3	6.6	7.6	8.3	2.4	0.4	1.8
Millewa	0.835	5.4	5.3	5.1	6.3	8.4	9.0	0.7	1.0	1.8
Lyallpur-73	0.831	6.1	7.5	7.5	9.1	10.2	10.7	5.3	3.5	4.6
ME-71	0.832	6.5	5.6	6.0	6.7	7.8	8.5	1.2	-1.1	1.4
Mean	0.833	5.2	5.6	5.6	6.4	7.7	8.5	2.0	0.7	

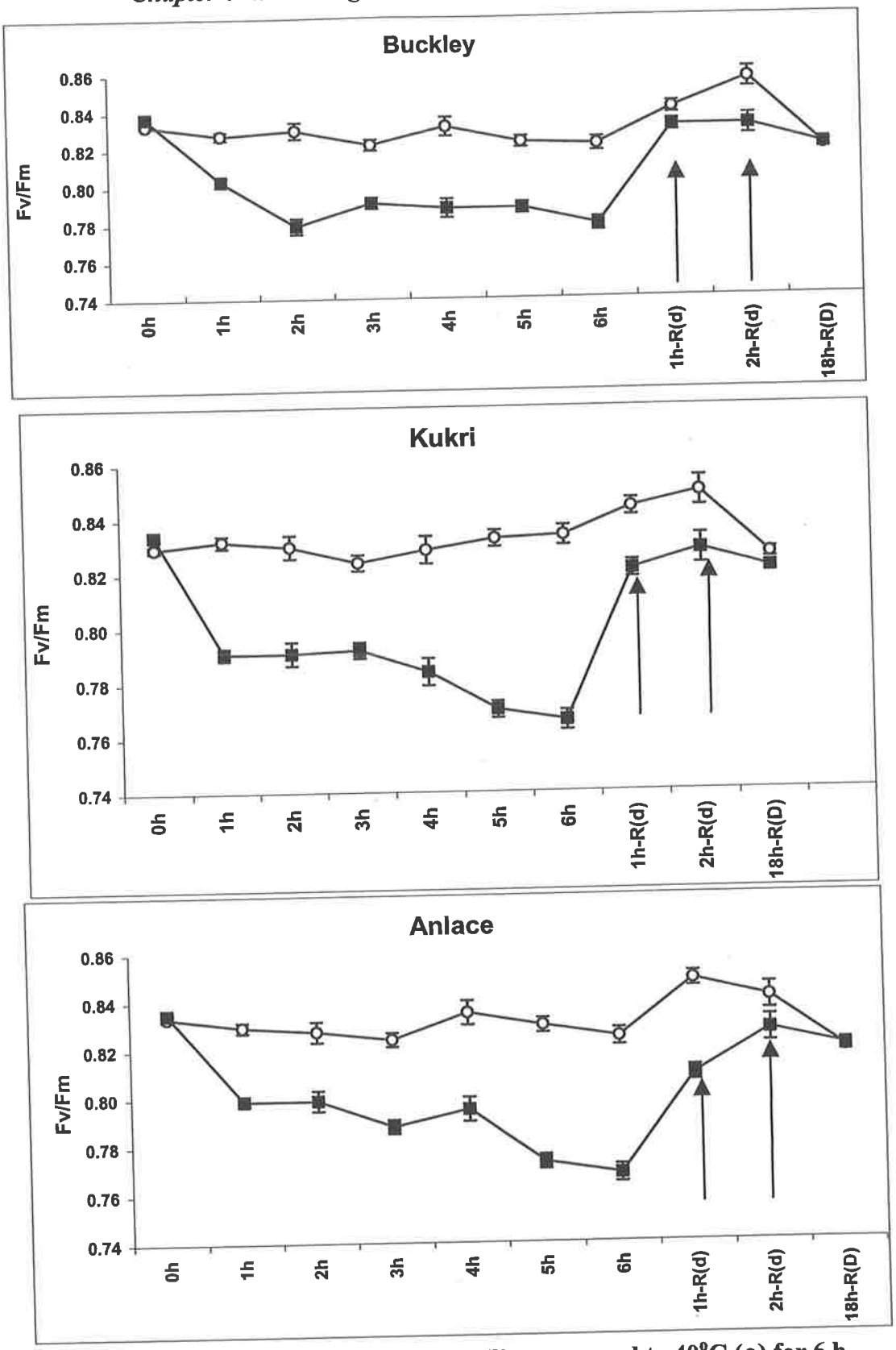


Figure 4.5-A. Time course of Fv/Fm in seedlings exposed to 40°C (•) for 6 h. followed by removal of heat stress or grown at 25°C/20°C day/night (○). Arrows indicate the dark period. (Vertical bars represent s.e.m.).

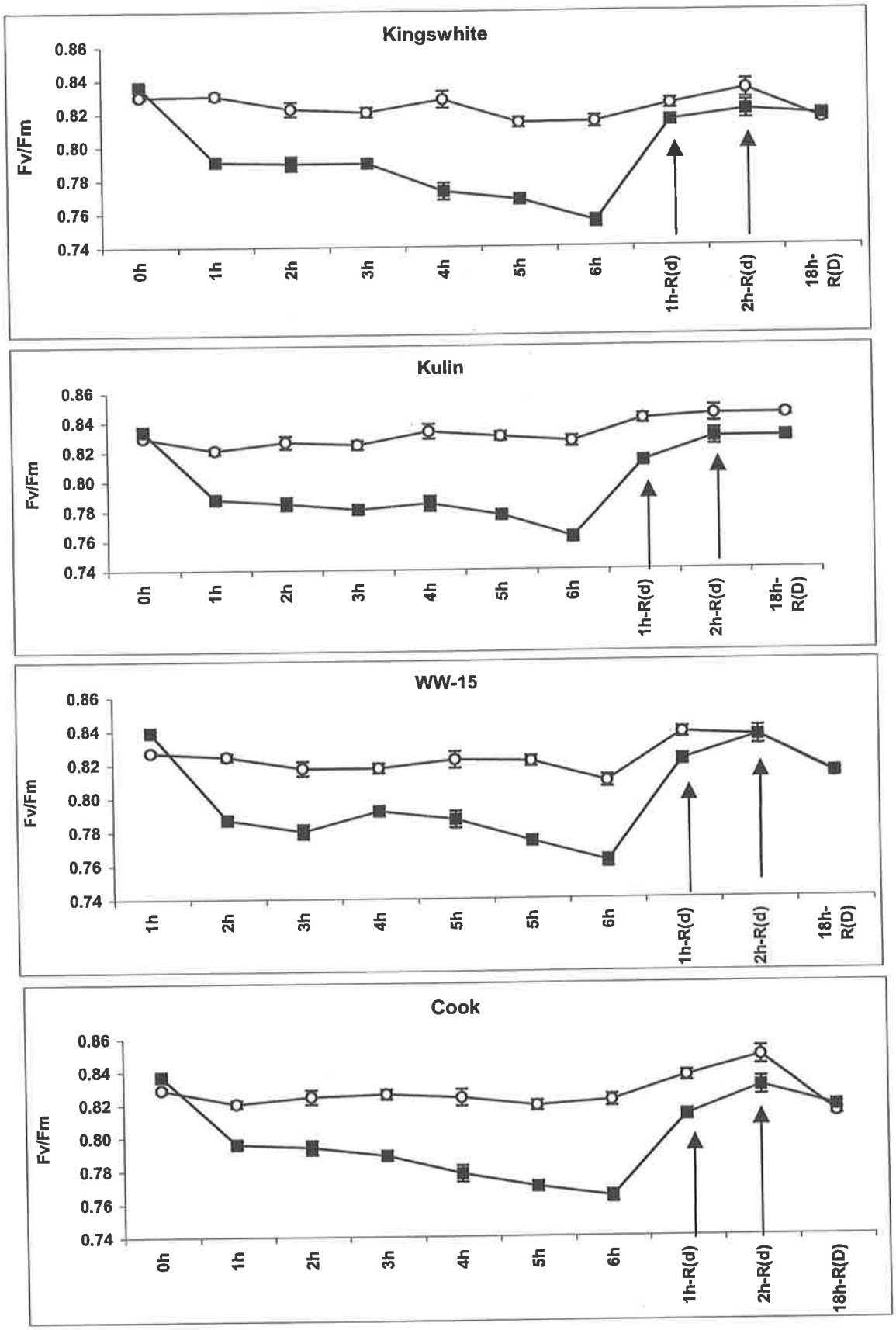


Figure 4.5B. Time course of Fv/Fm on wheat seedlings grown at 40°C for 6 hours followed by removal of heat stress (●) or grown at 25°/20° (○) day/night. Arrows indicate the dark period. Vertical bars represent the s.e.m.

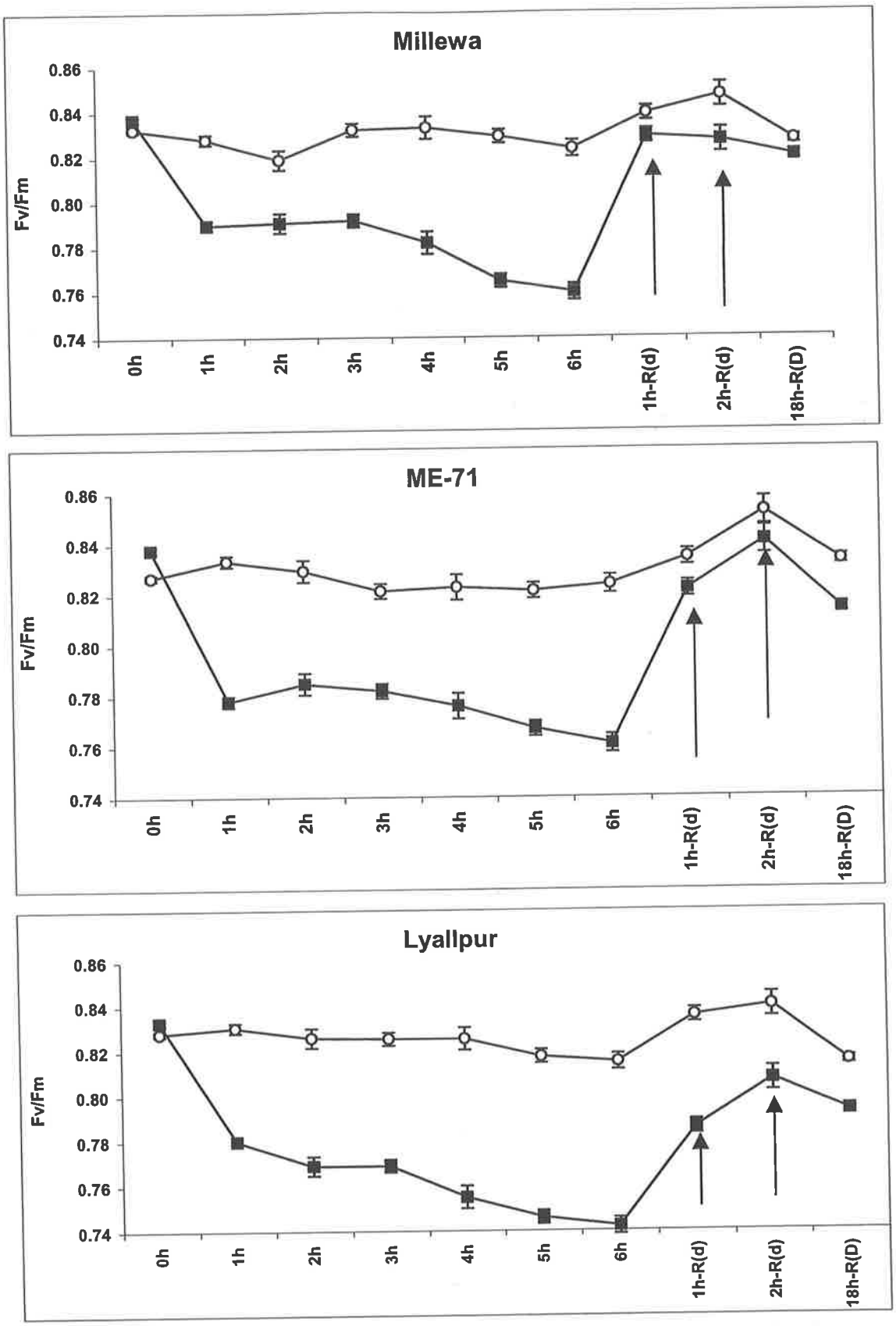


Figure 4.5C. Time course of Fv/Fm on wheat seedlings grown at 40°C for 6 hours followed by removal of heat stress (•) or grown at 25°/20° (○) day/night. Arrows indicate the dark period. Vertical bars represent the s.e.m.

to the hot air incubator. In previous experiments seedlings were grown to the 2-3 leaves stage before exposing them to heat stress, but in this study, seedlings were grown for 28 days till 4-5 leaves stage under the control conditions and then the heat stress (38.5°C) was imposed. In addition, the effect of heat stress was observed on all chlorophyll fluorescence parameters including Fo, Fv, Fm, Fv/Fm and Tm values in detail. .

4.6.2 Materials and Methods

A split plot design with three replications, two treatments and ten genotypes, was used to conduct the experiment under the growth room conditions. In this experiment heat-stress 38.5°C was applied in a growth room, described below. The control growth room conditions were 20/15°C day/night temperature with 8 hours of photoperiod. Ten genotypes including some of which the same were used in previous experiments and some new genotypes were also selected from the 100 genotypes (Table-4.3). These genotypes were selected as heat tolerant (Anlace, Meering), moderately tolerant (Kukri, Halberd, Krichauff, Condor), moderately heat sensitive (Machete) and sensitive (Oxley, Lyallpur-73, Millewa). The sowing method and soil material were the same as mentioned in section 3.3 of Materials and Methods. Pots were thinned to 3 plants/pot after 5 days of germination. Heat-stress (38.5°C) was imposed on 28 days old seedlings.

Two growth rooms were used for control and heat-stress treatments. Control experiments were performed under 20/15°C day/night while the heat stress treatment was 38.5/15°C day/night temperature. An 8-hour photoperiod was used in both growth rooms. Initially all the plants were grown in the same growth room and half the pots were transferred to the high temperature growth room for the heat stress treatment. The

growth room conditions were set 24 hours prior the heat-stress commenced. The pots were placed in a plastic tray (39 x 28 x 11 cm^3) filled with 500mL of reverse osmosis (RO) water to avoid any possible moisture-stress during the heat-stress period. Additional RO water was added whenever considered necessary during the entire stress period.

Measurements were made before placing the pots in the pre-heated (38.5°C) growth room (0 h.). Subsequent measurements were made after 1h, 6h, 24h and 48h in the light, while three recovery periods include 12h (dark)-R, and two light periods 72h-R and 96h-R. Chlorophyll fluorescence parameters were recorded using Plant Efficiency Analyser (PEA) described in section-3.6. Data on all fluorescence parameters were recorded to compare different fluorescence parameters in order to assess which can be used to obtain the greatest differences between tolerant and sensitive genotypes during heat stress and recovery periods.

4.6.3 Results

Significant Variety x Treatment effects were observed for Fv/Fm ratios during 1h, 6h, and 12h heat stress periods (Table 4.8), while all other parameters related to chlorophyll fluorescence parameters including Fo, Fm, and Fv did not show the variety x treatment interactions. However, a significant (P=0.05) effect of heat stress was observed for Fo at (48h-heat stress & 72h-recovery), Fv (1h, 24h, 48h), Fm (1h, 6h, & 24h), Tm (1h & 24h).

Results in Table 4.9 show the percent change in chlorophyll fluorescence parameters during the heat stress treatment and the recovery. After 1h of heat stress, Fv/Fm ratios

decreased by 10.3% in Anlace and by 21% in Lyallpur 73, whereas, after 6h of heat stress the greatest decline in Fv/Fm ratio was observed in Krichauff (19.3%) followed by Millewa (17.3%), Halberd (16.8%) and Lyallpur 73 (15.8%). However, different responses in genotypes were observed during the 12h (night) recovery period. The Fv/Fm ratios recovered fully in Anlace, Kukri, Condor, Meering, Machete and Millewa while genotypes that did not recover completely were Krichauff, Halberd, Oxley and Lyallpur-73. After 48h of heat stress, Fv/Fm ratios decreased further in all genotypes and by between 10.4% (Anlace) to 21.6% (Millewa). However, the Fv/Fm ratio in Lyallpur-73, after decreasing to 29% at 24h, showed a small increase atr 48h but it did not change further in the subsequent recovery periods of 72h-R and 96h-R. Anlace showed the greatest recovery after 48 h. recovery. Both components of Fv /Fm also decreased (Table 4.9), however Fv declined considerably more than Fm. For example, after 1h of heat stress, Fv decreased by between 27.8% (Anlace) and 46.8% (Lyallpur 73), while Fm decrease by 19.6% (Anlace-) to 33.2% (Lyallpur-73). Likewise, both Fv and Fm decreased correspondingly to almost the same level through out the heat stress periods (1h, 6h, 24h, 48h) and during the second phase of recovery 72h-R. However, initial fluorescence (Fo) increased during heat stress periods and 72h-R recovery period (Table 4.9). Tm values, which are related to the number of antenna complexes in PSII, also declined with heat stress and showed differential responses in all genotypes. The decline ranged from 12.5% in Meering to 43.2% in Oxley-S after 1h of heat stress. The greatest decreases in Tm values were observed after 24h and 48h of heat stress, and ranged from 25.6% in Halberd to 49.1% in Lyallpur-73 at 24h. and from 28.3% in Meering to 47.2% in Lyallpur-73 after 48h heat stress (Table 4.9). In general, the declines in Fv/Fm ratios were predominantly caused by the maximum reduction in Fv.

Table 4.8. Mean squares for chlorophyll fluorescence parameters Fo, Fm, Fv, Fv/Fm, and Tm values under control and heat stress in 28 day-old wheat seedlings. Significance: * - P<0.05; ** - P<0.01

0 hour						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	342.7	334	1333	0.0005	2884
Treatment	1	1006	4709	7732	0.00091	1944
Residual (a)	2	1416	2567	1201	0.00011	14.6
Variety	9	845.6	1631	1502	0.00010	1758
Var. x Trt.	9	439.3	2878	2607	0.00013	1499
Residual (b)	36	821.8	2799	2432	0.00012	815.4
Total	59					
1 hour						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	3446	5050	6441	0.00186	6100
Treatment	1	2021	9121*	1254*	0.2236**	2324*
Residual (a)	2	8286	1386	1366	0.000282	194.1
Variety	9	2258	7923*	8693*	0.00139*	1114
Var. x Trt.	9	1040	3563	3554	0.000958*	531.6
Residual (b)	36	1521	2434	2386	0.000592	770
Total	59					
6 hour						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	155	1508	16349	0.00018	1485
Treatment	1	1333	1247*	1542	0.2087**	9779
Residual (a)	2	2297	3626	4957	0.00059	1044
Variety	9	2405	4560*	4019*	0.0007**	1791
Var. x Trt.	9	2359	1725	2904	0.0001**	1824
Residual (b)	36	1346	1714	1866	0.00003	1589
Total	59					
12 hour (Dark recovery)						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Trt.	1	7504	6994	3272	0.00038**	1788
Residual (a)	2	650	5501	8496	0.000064	4083
Variety	9	3536	3011	1393	0.00107*	1563
Var. x Trt.	9	5903	5632	1773	0.00014**	1773
Residual (b)	36		4466	6774	0.000041	1869
Total	59					

Continued

Table 4.8 (continued)

24 hour						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	6060	2730	8813	0.00028	925.4
Treatment	1	5436	7620*	1279*	0.3010**	1288*
Residual (a)	2	687	2489	1866	0.00034	1556
Variety	9	1338	6636*	8957	0.00357*	1364
Var. x Trt.	9	15106	3127	5052	0.003067*	569.1
Residual (b)	36	8054	2896	35257	0.00183	880.9
Total	59					
48 hour						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	2187	8238	877	0.001040	2053
Treatment	1	1336*	8237	111*	0.16089**	4521
Residual (a)	2	274	5083	589	0.000859	3681
Variety	9	2490	6584	672	0.001733	954.6
Var. x Trt.	9	3923	3055	366	0.001814	443
Residual (b)	36			371	0.01232	711.5
Total	59					
Recovery 72 hour (Day time)						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	2778	55374	34469	0.000552	108.3
Treatment	1	1286*	1712	2781	0.0494**	256.3
Residual (a)	2	345.1	1713	1771	0.000414	766.2
Variety	9	639.7	5632	6099	0.001025	446.2
Var. x Trt.	9	2463	4521	5315	0.000841	1103
Residual (b)	36	955.8	3931	4094	0.000483	786.7
Total	59					
Recovery 96 hour (Day time)						
SOV	df	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	870.4	19562	54569	0.00020	859.7
Treatment	1	673.3	1254	6895	0.00117**	24.1
Residual (a)	2	505.4	3141	2090	0.000173	2034
Variety	9	1470	1056	5796	0.000336	554.2
Var. x Trt.	9	1901	5354	1848	0.000158	1389
Residual (b)	36	842.6	3693	3227	0.000123	930.5
Total	59					

The most responsive of the fluorescence parameter was Fv in almost all genotypes ranging from 27.8% (Anlace) to 46.8% (Lyallpur) after 1h, 24h and 48h heat stress periods as well as 72h-recovery period. This shows that reductions in Fv/Fm ratios were mainly caused by the large reduction in the variable fluorescence (Fv) than maximum fluorescence (Fm) (Alkhatib and Steven, 1990). Figures 4.6A and 4.6B show the time course in Fv/Fm ratios of individual genotypes during the period of heat stress and three recovery phases 12h (dark), 72h (light) and 96h (light) periods. These results are in agreement for Fv/Fm ratios as we have described earlier results in Table 4.7.

4.7 Discussion

4.7.1. Effect of heat stress on photosynthetic efficiency in wheat seedlings

Photosynthetic efficiency in 100 wheat genotype seedlings was measured by the chlorophyll fluorescence parameter Fv/Fm. The Fv/Fm ratios, which reveal the photochemical capacity of PS-II (Bjorkman, 1980), showed a range of responses in wheat seedlings. After screening 100 genotype seedlings for heat tolerance on the basis of Fv/Fm ratios, a wide range of heat tolerance and sensitivity was observed. The genotypes were arranged into tolerant (T), moderately tolerant (MT), moderately sensitive (MS) and sensitive (S) genotypes according to the response of chlorophyll fluorescence parameter Fv/Fm to heat-stress and after cluster analysis (Figure 4.1 & 4.2). The Fv/Fm values decreased considerably in most sensitive genotypes Cook, Lyallpur-73, WW-15, Gutha, Millewa, and ME-71, when compared to tolerant and moderately tolerant genotypes such as Kulin, Buckley, Mira, Meering, Psathia, Brookton and Anlace (Table 4.2). These results indicated that Fv/Fm might provide a

Table 4.9. The changes in chlorophyll fluorescence parameters between 0 h and 48 h heat stress (38.5°C) and during recovery periods of 72h & 96h.

Fv/Fm

Genotype	0h	1h	%	6h	%	12h-dark	%	24h	%	48h	%	72h-Recy	%	96h-Recy	%
Anlace	0.826	0.741	10.3	0.73	11.7	0.848	-2.7	0.725	12.2	0.740	10.4	0.810	1.9	0.831	-0.6
Kukri	0.824	0.692	16.0	0.71	14.0	0.840	-1.9	0.721	12.5	0.728	11.7	0.768	6.8	0.831	-0.8
Meering	0.827	0.677	18.1	0.71	13.6	0.848	-2.6	0.699	15.4	0.733	11.3	0.770	6.8	0.800	3.2
Krichauff	0.824	0.719	12.7	0.67	19.3	0.797	3.3	0.666	19.2	0.690	16.3	0.765	7.2	0.824	0.0
Halberd	0.823	0.711	13.6	0.69	16.8	0.787	4.4	0.695	15.6	0.757	8.02	0.780	5.2	0.819	0.5
Machete	0.818	0.686	16.1	0.73	10.6	0.814	0.5	0.675	17.5	0.732	10.5	0.785	4.0	0.816	0.2
Condor	0.821	0.712	13.2	0.72	12.7	0.834	-1.6	0.707	13.8	0.720	12.2	0.757	7.7	0.814	0.8
Oxley	0.827	0.699	15.4	0.73	12.2	0.795	3.8	0.709	14.2	0.740	10.5	0.761	7.9	0.810	2.1
Millewa	0.818	0.724	11.4	0.68	17.3	0.817	0.1	0.624	23.7	0.641	21.6	0.772	5.6	0.800	1.8
Lyallpur	0.818	0.646	21.0	0.69	15.8	0.764	6.6	0.579	29.2	0.714	12.7	0.714	12.7	0.800	2.4

Fv

Genotype	0h	1h	%	6h	%	12h-dark	%	24h	%	48h	%	72h-Recy	%	96h-Recy	%
Anlace	2587	1867	27.8	1769	31.6	3122	-20.7	1763	31.9	1735	32.9	2458	5.0	2541	1.8
Kukri	2520	1526	39.4	1547	38.6	2741	-8.8	1701	32.5	1702	32.5	2117	16.0	2607	-3.5
Meering	2575	1448	43.8	1583	38.5	2982	-15.8	1647	36.0	1754	31.9	2063	19.9	2364	8.2
Krichauff	2518	1601	36.4	1427	43.3	2466	2.1	1563	37.9	1554	38.3	2169	13.9	2392	5.0
Halberd	2598	1585	39.0	1546	40.5	2289	11.9	1706	34.3	1842	29.1	2183	16.0	2347	9.7
Machete	2505	1487	40.6	1664	33.6	2578	-2.9	1616	35.5	1746	30.3	2151	14.1	2398	4.3
Condor	2472	1583	36.0	1604	35.1	2830	-14.5	1645	33.5	1586	35.8	1964	20.6	2424	1.9
Oxley	2535	1549	38.9	1671	34.1	2339	7.7	1675	33.9	1716	32.3	2063	18.6	2261	10.8
Millewa	2474	1649	33.3	1378	44.3	2542	-2.7	1323	46.5	1322	46.6	2096	15.3	2342	5.3
Lyallpur	2455	1306	46.8	1484	39.6	2196	10.5	1340	45.4	1541	37.2	1726	29.7	2260	7.9

Fm

Genotype	0h	1h	%	6h	%	12h-dark	%	24h	%	48h	%	72h-Recy	%	96h-Recy	%
Anlace	3127	2514	19.6	2423	22.5	3677	-17.6	2428	22.4	2342	25.1	3031	3.1	3056	2.3
Kukri	3053	2196	28.1	2181	28.6	3262	-6.8	2353	22.9	2250	26.3	2744	10.1	3134	-2.7
Meering	3110	2130	31.5	2215	28.8	3514	-13.0	2348	24.5	2383	23.4	2676	14.0	2951	5.1
Krichauff	3052	2224	27.1	2142	29.8	3090	-1.2	2342	23.3	2243	26.5	2820	7.6	2889	5.3
Halberd	3140	2229	29.0	2253	28.2	2908	7.4	2459	21.7	2433	22.5	2795	11.0	2866	8.7
Machete	3065	2166	29.3	2270	25.9	3159	-3.1	2382	22.3	2378	22.4	2738	10.7	2934	4.3
Condor	3011	2222	26.2	2236	25.7	3386	-12.5	2315	23.1	2196	27.1	2594	13.8	2607	13.4
Oxley	3049	2211	27.5	2300	24.6	2938	3.6	2347	23.0	2315	24.1	2708	11.2	2789	8.5
Millewa	2991	2276	23.9	2032	32.1	3100	-3.6	2186	26.9	2021	32.4	2703	9.6	2912	2.6
Lyallpur	2999	2004	33.2	2148	28.4	2868	4.4	2311	22.9	2158	28.0	2396	20.1	2824	5.8

Continued

Table 4.9 (continued)

Fo															
Genotype	0h	1h	%	6h	%	12h-dark	%	24h	%	48h	%	72h-Recy	%	96h-Recy	%
Anlace	553	647	-17.0	653	-18.1	555	-0.4	665	-20.3	607	-9.8	572	-3.4	516	6.7
Kukri	537	670	-24.8	634	-18.1	521	3.0	652	-21.4	608	-13.2	627	-16.8	527	1.9
Meering	529	683	-29.1	632	-19.5	499	5.7	701	-32.5	629	-18.9	613	-15.9	588	-11.2
Krichauff	530	623	-17.5	715	-34.9	623	-17.5	779	-47.0	689	-30.0	651	-22.8	497	6.2
Halberd	529	643	-21.6	700	-32.3	638	-20.6	745	-40.8	590	-11.5	612	-15.7	519	1.9
Machete	561	680	-21.2	604	-7.7	580	-3.4	766	-36.5	632	-12.7	587	-4.6	536	4.5
Condor	544	639	-17.5	632	-16.2	556	-2.2	670	-23.2	611	-12.3	630	-15.8	550	-1.1
Oxley	530	663	-25.1	629	-18.7	598	-12.8	672	-26.8	598	-12.8	645	-21.7	570	-7.5
Millewa	528	627	-18.8	654	-23.9	558	-5.7	803	-52.1	700	-32.6	607	-15.0	529	-0.2
Lyallpur	547	698	-27.6	664	-21.4	672	-22.9	963	-76.1	617	-12.8	669	-22.3	564	-3.1
Tm															
Anlace	219	171	21.9	149	32.0	303	-38.4	120	45.2	144	34.2	171	21.9	165	24.7
Kukri	202	137	32.2	132	34.7	262	-29.7	118	41.6	137	32.2	168	16.8	166	17.8
Meering	184	161	12.5	135	26.6	273	-48.4	126	31.5	132	28.3	175	4.9	215	-16.8
Krichauff	206	153	25.7	134	35.0	231	-12.1	130	36.9	124	39.8	167	18.9	152	26.2
Halberd	195	161	17.4	168	13.8	243	-24.6	145	25.6	145	25.6	161	17.4	168	13.8
Machete	218	132	39.4	145	33.5	275	-26.1	145	33.5	125	42.7	178	18.3	165	24.3
Condor	211	137	35.1	139	34.1	272	-28.9	132	37.4	129	38.9	115	45.5	186	11.8
Oxley	243	138	43.2	182	25.1	263	-8.2	148	39.1	146	39.9	175	28.0	171	29.6
Millewa	189	132	30.2	126	33.3	248	-31.2	131	30.7	129	31.7	165	12.7	179	5.3
Lyallpur	218	152	30.3	144	33.9	258	-18.3	111	49.1	115	47.2	148	32.1	221	-1.4

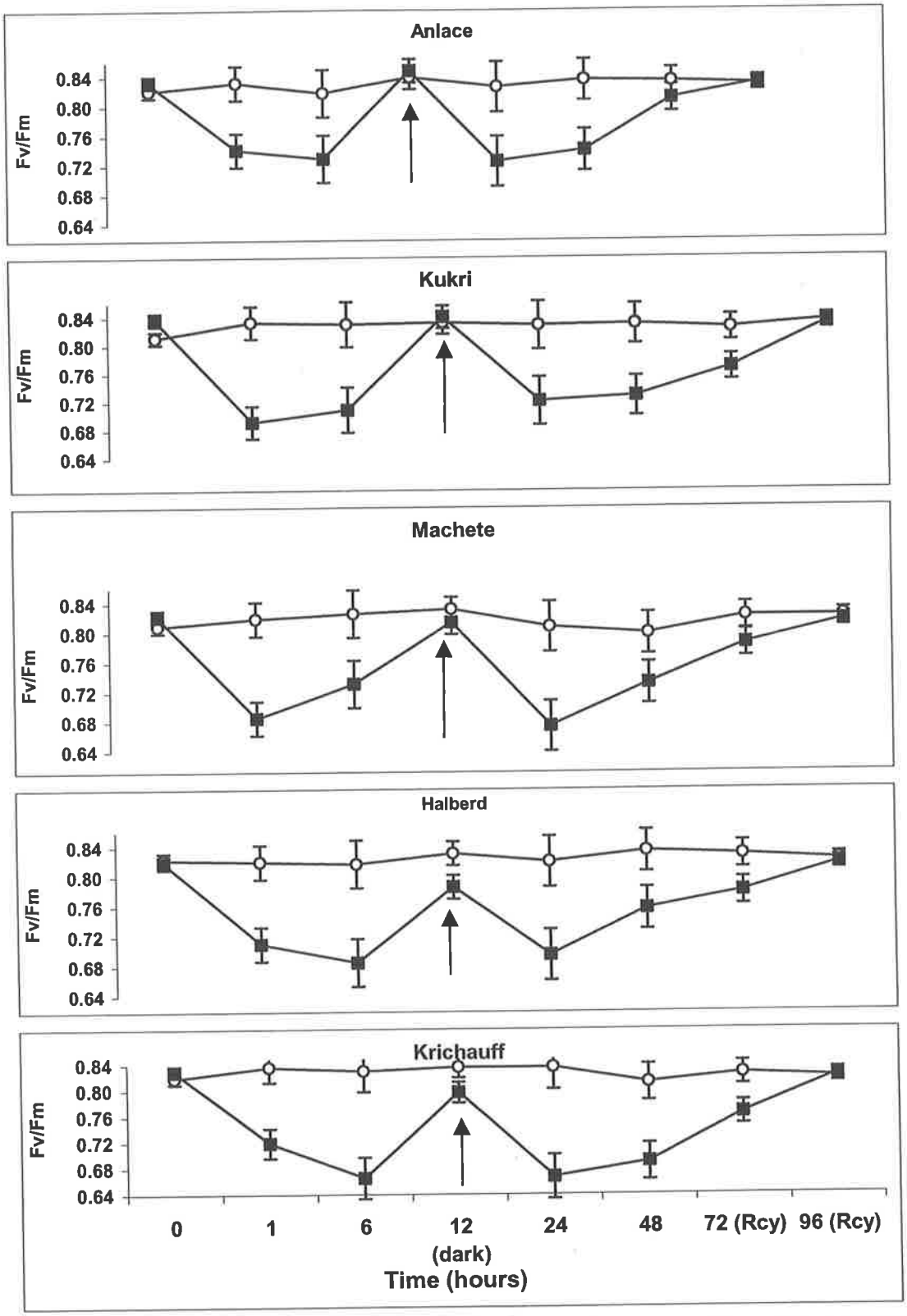


Figure 4.6A. Effect of heat stress on Fv/Fm ratios over 48h with subsequent recovery over 96 h. Plants were grown at 38.5°/15°C (day/night) with an 8 h. photoperiod before being transferred to 20°C/15°C during the recovery phase. The 12 h. measurement was made at 15°C in the dark period of the heat stress treatment. (Vertical bars presenting s.e.d.).

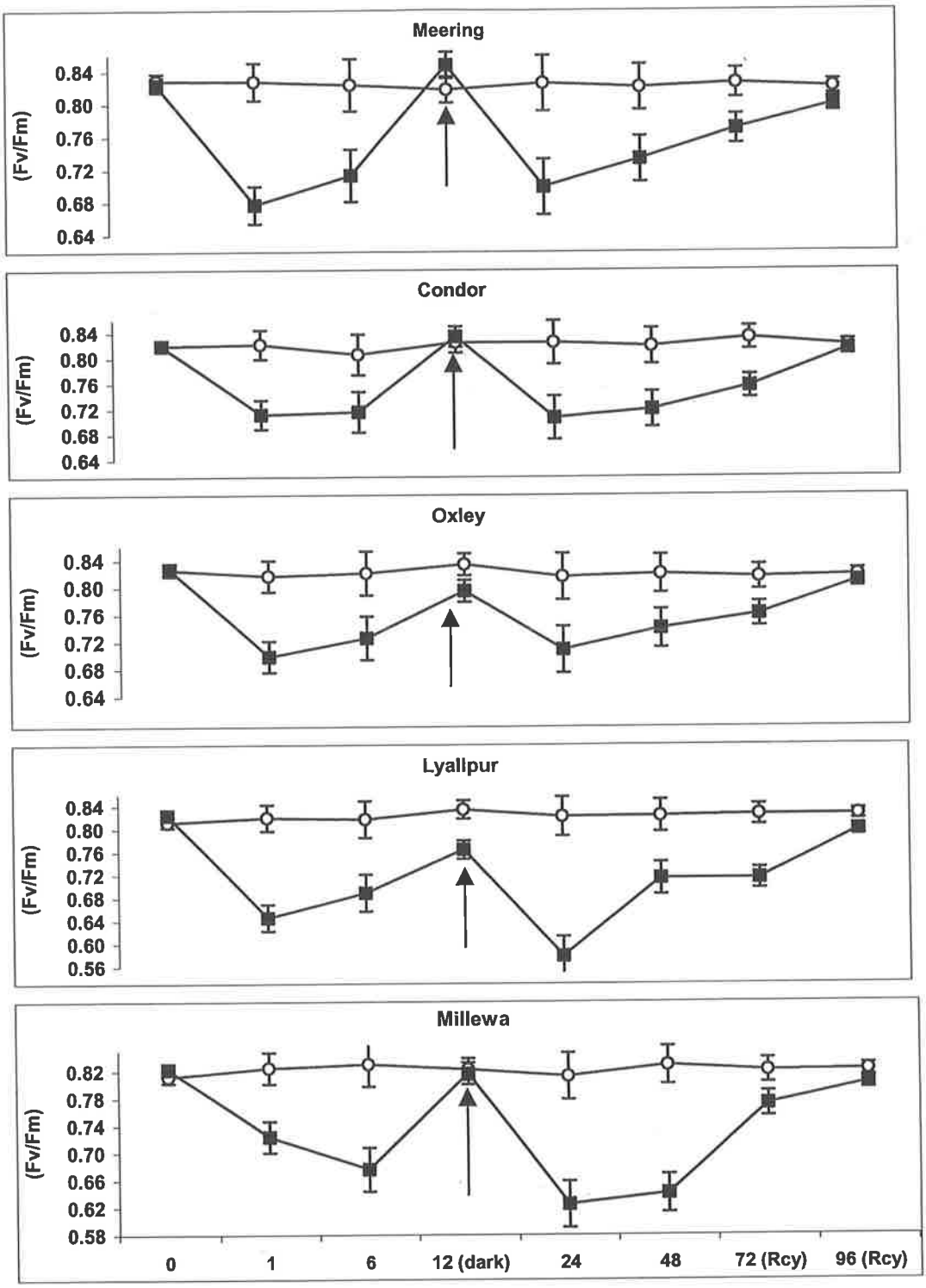


Figure 4.6B. Effect of heat stress on Fv/Fm ratios over 48h with subsequent recovery over 96 h. Plants were grown at 38.5°/15°C (day/night) with an 8 h. photoperiod before being transferred to 20°C/15°C during the recovery phase. The 12 h. measurement was made at 15°C in the dark period of the heat stress treatment. (Vertical bars presenting s.e.d.).

sensitive indicator of differential responses of wheat seedlings to high temperature stress (Bjorkman and Demming, 1987).

4.7.2. Effect of heat stress on the recovery of photoefficiency

Various studies have suggested the effect of various abiotic stresses on the photoefficiency is reversible. For example, chilling stress and recovery of PSII efficiency in tomato and rice seedlings was reported by Bruggemann *et al.*, 1992 and Kim *et al.*, 1997. However, no such studies have been reported so far in wheat seedlings and especially after heat-stress.

Results of the present studies also showed a range in the ability to recovery of photochemical efficiency of PSII from heat-stress in wheat seedlings. The initial screening of 100 genotypes allowed them to be classified as tolerant, moderately tolerant, moderately sensitive and sensitive based on the response in Fv/Fm during a period of heat stress and recovery. More detailed assessment of the responses of a subset of genotypes confirmed these responses. After removing the heat-stress, the Fv/Fm ratio of the genotypes identified as thermo-tolerant recovered more quickly than that of the sensitive genotypes. Photoefficiency recovered to a greater degree in the tolerant and moderately tolerant genotypes Buckley, Anlace and Kukri when compared to the sensitive genotypes, Lyallpur-73, ME-71, and Millewa (Experiments 2 and 3).

The substantial decrease and increase in the magnitude of Fv/Fm showed a time dependent increase or decrease when stress was imposed from 1 hour to 48 hours and during the un-stressed period in the dark (1h and 2h), including the recovery periods under control conditions (Experiment 2 and 3). The recovery of PSII activity was rapid

within 1 and 2 hours at the lower temperature in the dark in all the tolerant and moderately tolerant genotypes except Kulin (Fig. 4.6). Within 2 h. the Fv/Fm in these genotypes returned to the level measured prior to the imposition of the heat stress (0h). In contrast the recovery in Fv/Fm in the sensitive genotypes (Lyallpur-73 and ME-71) was incomplete (Fig 4.6). The pattern of changes in Fv/Fm during and after removing the heat-stress might suggest that the greater heat tolerance of the tolerant and moderately tolerant genotypes is caused by their ability recover quickly after removing the heat stress as compared to sensitive genotypes.

4.7.3 Effect of heat stress on chlorophyll fluorescence parameters

Generally, heat stress had a substantial affect on all chlorophyll fluorescence parameters, Fo, Fm, Fv, Fv/Fm and Tm (Table 4.8). Interactions between genotypes and treatments were significant only for Fv/Fm values after 1h, 6h, 12h, and 24h (Table 4.5), while all other parameters did not show interaction.

Heat-stress affected all chlorophyll fluorescence parameters considerably (Table 4.9). In particular, substantial decreases were observed in the ratios of variable to maximum fluorescence (Fv/Fm) and its two components, Fv and Fm. For example, the Fv/Fm ratios decreased in Lyallpur-73 and Oxley by 21.0% and 15.4% after 1h of heat stress, because of the drastic reductions in its two components, Fv and Fm: Fv decreased by 46.8% in Lyallpur-73 and 38.9% in Oxley, whereas Fm decreased by 33.2% and 27.5% in same genotypes (Table 4.7). Thus variations in Fv/Fm values were more associated with drastic declines in Fv values than Fm (Alkhatib and Steven, 1990). A decline in Fv, Fm and their ratios (Fv/Fm) in response to heat stress may indicate the photoinhibitory impairment of the photosynthetic apparatus and especially damage to

thyalkoid membranes in the chloroplast (Demmig and Bjorkman, 1987). Heat induced structural disorganizations have been reported, which cause the blockage of PS-II and disassociation of light harvesting complexes LHC-II (Krause, 1988). Although this study did not cover this aspect, the results show that heat stress (40°C) for 6 hours (Experiments 2 and 3), have drastic effect on Fv/Fm ratios and its components, especially in genotypes such as Lyallpur-73, ME-71, Cook, Millewa. However, severe declines in Fv/Fm ratios in heat-sensitive genotypes and their very slow recovery after 24 to 48 hours, indicates severe damage to the thyalkoid membranes and photoinhibitory impairment due to heat stress (Yang *et al.*, 1996). Similarly, comparatively low reductions of Fv/Fm ratios in seedlings of heat-tolerant genotypes such as Buckley, Anlace, Kukri and higher reductions in heat-sensitive genotype seedlings (Lyallpur-73, ME71, Millewa) represented either a reversible damage to PSII in heat-tolerant genotypes or irreversible damage in heat-sensitive genotypes (Baker and Bowyer, 1994). Therefore, our results are in agreement of various studies involving reversible or irreversible damage to photochemical efficiency in higher plants.

Other fluorescence parameters such as initial fluorescence (Fo) increased in response to heat stress (Table 4.9). Initial fluorescence (Fo) at control level or 0 hour, indicates that all reaction centers are open, while, an increase in Fo during heat-stress period specify that proportion of inactive centers might have increased (Bolhar *et al.*, 1989). Similarly, increase or decrease in Fo values reflects the redox state of the primary electron acceptor of PSII. Hence, increase in the level of Fo might indicate that heat-stress inhibited the photo-reduction process in PSII, because the photo-oxidizing side of PSII will quench the yield of fluorescence, whereas, an inhibition on photo-reducing side of PSII will enhance the quenching (Baker and Horton, 1987, Bolhar *et al.*, 1989). Thus

the efficient quenching of excitons/electrons is necessary for the protection of photosynthetic apparatus; therefore any drastic increase in initial fluorescence indicates that the photosynthetic apparatus might have been damaged (Long *et al.*, 1994).

Tm values, which indicate the number of antennae in PSII and LCH-II, have shown differential responses in all genotypes. As in the light harvesting complexes (LHC-I & II), the antenna size and numbers are involved in the heat dissipation and subsequent migration of electrons to thyalkoid membranes (Bilger *et al.*, 1995), Table 4.9 shows that there was a substantial decreases in the antennae numbers (Tm values) in most of the genotypes after 24h and 48h of heat-stresses even including recovery phases. e.g., after 24h of heat-stress, Tm values decreased 42% and 45% in heat-tolerant genotypes Kukri and Anlace, while maximum decrease was observed in a heat-sensitive genotype Lyallpur-73 (49%), whereas, after 48h of heat-stress Tm values decreased only to 32%, 34% and 47% in Kukri, Anlace and Lyallpur-73. Results also showed that the Tm values tend to decrease in recovery phases. These results suggested that there might be imbalance in light harvesting complexes and as well as between light absorption and utilization in PSII (Powles, 1984).

4.7.4 Possible use of fluorescence parameters for evaluation of heat tolerance at the seedling stage

Various fluorescence parameters have been used to evaluate tolerance of different crop species under various abiotic stresses such as chilling, drought, and heat stresses. For example, initial fluorescence (Fo) and variable fluorescence (Fv) have been used to measure chilling-tolerance and heat-tolerance in barley, pea, pearl millet and wheat ((Hetherington and Smillie, 1982; Smillie and Heterington, 1983), but they have used leaf discs from 14 days old seedlings. Moffat *et al.*, (1990) tested six wheat

cultivars at anthesis for Fo, Fm and Fv measurements on flag leaves along with other grain yield parameters under control and field conditions. They observed substantial decline in Fv values under high temperature stress but also found differences in genotypes for chlorophyll fluorescence parameters due to accelerated development and senescence under the field conditions. Similarly, Alkhatib and Paulsen (1990) tested ten winter wheat genotypes for photosynthetic rate and variable fluorescence (Fv) at the seedling stage and maturity. They found an association between photosynthesis and productivity of both seedlings and maturing plants under high temperature stress. They also observed considerable declines in Fv values from moderately (22/17°C) to high (32/27°C) day/night temperatures in wheat seedlings. The present results showed that the temperature regime of (25/20°C, day/night) in Experiments 1 to 3 did not affect any fluorescence parameters considerably in seedlings and remained almost unchanged during the experimental periods in all genotypes. This difference could be that Alkhatib and Paulsen (1990) had used the seedlings of winter wheat, whereas in present studies we used spring wheat genotypes that might have adapted to the moderate temperature regimes in Australia. Differences in the fluorescence responses between winter and spring types have been observed in other cereals. Rizza *et al.* (2001) used about one month old winter and spring oat cultivars seedlings to determine the freezing tolerance by the reduction of Fv/Fm ratios in winter and spring oat genotypes, under control conditions and in the field. They observed minimum reductions in Fv/Fm ratios in winter oats (freezing tolerant) compared to spring (freezing sensitive) genotypes and as well as recovery of Fv/Fm ratios to almost to the control level in tolerant genotypes and vice versa. Although the chilling-stress or cold-stress is different from the heat-stress, their mode of PSII damage in seedlings seems to be the same. Cold and freezing stresses also affect the photosynthetic efficiency drastically in cold-sensitive seedlings

than cold-tolerant seedlings. Hence, we can compare these results with the present study.

The results of the present study (Experiments1, 3 & 4) also suggested the possible use of Fv/Fm ratios to determine heat-tolerance during heat-stress and recovery after removing the heat-stress in wheat seedlings. Small reductions in Fv/Fm ratios were observed in a number of wheat genotypes which indicates that PSII was less affected by heat stress than other genotypes and so the photosynthetic efficiency is maintained under stress. This indicates that these genotypes have a high degree of heat tolerance. In a similar vein, there were a number of sensitive genotypes identified and therefore there is evidence of significant genetic variation in heat tolerance. For example, Table 4.9 shows that mean values of Fv/Fm ratios under the control conditions were 0.818 to 0.824, while after 1 h. of heat-stress the range was 0.646 (Lyallpur-73) to 0.741 (Anlace). Similarly, during the recovery phase at 72h and 96 h, the Fv/Fm values in tolerant genotypes like Anlace recovered quickly, while Fv/Fm did not recover in sensitive genotypes such as Lyallpur-73, Millewa and Oxley. This suggests that heat tolerance is related to two responses: the ability to maintain PSII effcieicny during heat stress as well as the ability to recover after the removal of the heat stress. These results are in agreement with work on cold tolerance by Rizza *et al.*, (2001), who reported fast recovery of Fv/Fm values in cold-tolerant and slow recovery in cold-sensitive oats seedlings after removing the cold-stress. The results from the present experiments showed that the Fv/Fm ratios and Fo could be used to screen heat tolerant and heat sensitive seedlings. In particular, the recovery of Fv/Fm ratios after the heat-stress is important in screening the seedlings.

4.7.5. Comparison between wheat genotypes at seedling and anthesis/grain filling stages for heat-tolerance and heat-sensitivity

Various researchers in Australia have reported heat-tolerance and sensitivity of many wheat genotypes at the grain filling stage. Wardlaw *et al.*, (1989) reported the cultivar Lyallpur-73 as being heat-sensitive genotype at the grain filling stage. Similarly, many other genotypes have been identified as heat-sensitive genotypes at anthesis or grain filling stage, including Cook (Randall and Moss, 1990), Millewa (Stone and Nicolas, 1995), Lark and Machete (Blumenthal *et al.*, 1995), Oxley (Stone and Nicolas, 1995a, 1998a). Our results also showed Oxley, Cook and Lyallpur-73 to be heat sensitive at the seedling stage on the basis of Fv/Fm ratios (Figure 4.1 and Table 4.1). The Fv/Fm ratio differed notably between heat tolerant and heat-sensitive genotype seedlings under the heat stress. Small to modest decreases in Fv/Fm ratios were observed in heat-tolerant and moderately heat-tolerant genotypes after 6 hours of heat-stress (40°C). Reductions in Fv/Fm ranged from 2.2% to 4.0% heat-tolerant genotypes including Kulin, Buckley, Mira, Halberd, Gabo, Krichauff, while reductions of more than 7% in Fv/Fm ratios were observed in genotypes Lark, Cook, Lyallpur-73, WW15, Gutha, Millewa and ME71 (Figure 4.1). Most of these results are consistent with many previous studies showing a range of genotypic differences in heat-tolerance of wheat genotypes for their responses to heat-stress at anthesis or grain filling stages in Australia (Stone and Nicolas, 1995; Wardlaw and Moncur, 1995). However, some genotypes which have shown heat-tolerant at the seedling stage in the present study, like Meering, Veery, Halberd, Sunco (Table 4.3), have been reported to be heat-sensitive at the grain filling stage (Stone and Nicolas, 1995a,b; Blumenthal *et al*, 1990). These differences could be due to many physiological and biochemical differences involved in thermotolerance at the seedling and grain filling stages. For example, thermotolerance during grain filling is associated with the thermostability of key

enzymes involved in starch synthesis, whereas the thermotolerance of wheat seedlings was assessed by the effect of high temperatures on photosynthesis. Thermotolerance based on the decline of Fv/Fm of the flag leaf may be a more valid basis of comparing genotypes as seedlings and during grain filling.

The consistent response of selected heat-tolerant and heat-sensitive genotypes, identified in an initial screen of 100 genotypes, in consectutive experiments suggested there are consistent responses to heat stress in Fv/Fm during the period of heat stress recovery, which might be the best criteria to screen the seedlings quickly and efficiently. However, more experiments could be suggested to confirm these results before any definite conclusion.

4.8. Conclusions:

i) A broad range of genetic variability was observed in 100 wheat genotype seedlings for heat-tolerance and heat-susceptibility on the basis of *invivo* chlorophyll fluorescence.

ii) Generally, the results showed that heat-stress reduced the maximum quantum yield of photoefficiency of PSII, as it was indicated by severe decreases in fluorescence parameters including variable fluorescence (Fv), maximum fluorescence (Fm) and particularly the ratio of variable to maximum fluorescence Fv/Fm.

iii) Efficiency of PS-II was reversible more or less effectively in heat-tolerant and moderately heat-tolerant genotype seedlings after removing the heat-stress when compared to heat-sensitive genotypes.

iv) Fluorescence parameters changed in a coordinated manner under heat-stress. Values decreasing quickly after one hour of heat-stress in all genotypes, however, the recovery of these parameters were observed to be different in heat-tolerant than heat-sensitive genotype seedlings when the heat-stress was removed.

v) Photoefficiency of PSII in heat-tolerant genotype seedlings recovered more quickly after removing the heat-stress than heat-sensitive genotypes.

vi) Drastic increases in the level of initial fluorescence (Fo) showed that heat-stress inhibited the photo-reduction process in PSII in wheat seedlings.

vii) Results of present study suggested the possible use of the *in vivo* chlorophyll fluorescence parameter the photosynthetic efficiency (ratio of Fv/Fm). Particularly, decrease in the ratio of variable to maximum fluorescence (Fv/Fm) and its recovery after heat-stress, for the purpose of screening wheat seedlings for heat-tolerance.

CHAPTER 5

EFFECT OF DROUGHT STRESS ON PHOTOSYNTHETIC EFFICIENCY AND WATER USE EFFICIENCY IN SEEDLINGS OF WHEAT GENOTYPES DIFFERING IN HEAT TOLERANCE

5.1 Introduction

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 (Jones and Bradley 1992). Breeding and selection for high yield under drought has been an important objective of crop breeders working in these environments. However, selection and screening of genotypes is a long and tedious task, particularly when based on later stages of crop development, for example, when booting, heading, anthesis and total yield are considered (Edmeades *et al.*, 1989; Annichiarico and Pecetti, 1990; Clarke *et al.*, 1992). Thus, simple traits linked to drought tolerance that could be used for screening and selection would undoubtedly be important to the development of drought resistant or tolerant cultivars.

In many parts of the world, particularly in tropical and subtropical areas in South Asia and South East Asia, drought stress is often confounded with heat stress, because drought is usually accompanied by high temperatures (>30°C) at sowing, seedling emergence and establishment (Pfeiffer, 1987). Due to the high temperatures at this time, the rate of transpiration can be high, which causes water deficits to develop in plants. Initially, stomata close and transpiration rate falls, which in turn can cause leaf

temperature to increase. If the plant water deficit becomes more severe, damage the photosynthetic processes can occur.

Moderate to severe water-stress drastically affects various morpho-physiological traits in wheat such as chlorophyll fluorescence (Havaux and Lannoye, 1985), water use efficiency (Farquhar, 1983; Condon and Richards, 1992) leaf area (James *et al.*, 1984), specific leaf weight (Morgan and LeCain, 1991; Cedola *et al.*, 1994) and dry matter yield (Ehdaie *et al.*, 1991). 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. Havaux and Lannoye (1985), for example, reported genetic variation in wheat cultivars on the basis of *in vivo* chlorophyll fluorescence measurements in drought-tolerant and sensitive durum wheat cultivars. In another study, a positive relationship between quantum yield of photosynthetic electron transport of photosystem-II (PSII) and osmotic adjustment was found in drought-tolerant wheat cultivars (Flagella *et al.*, 1996).

Selection for high water use efficiency in wheat might improve the yield potential under drought conditions (Farquhar and Richards, 1984; Richards *et al.*, 1998; Condon *et al.*, 1990). Indirect selection for efficient water use and greater earlier leaf area at seedling stage in wheat improved the early vigour and plant establishments in dry land areas (Lopez-Castaneda *et al.*, 1996). James *et al.*, (1984) reported that dry matter yield increased by an average of 53% in 18 different species, including cereals such as wheat, barley, rice and sorghum, due to the increase in water use efficiency. Variations in water use efficiency between cultivars and among genotypes could result from the differences in photosynthesis (Ehdaie *et al.*, 1991).

Previously, 100 genotypes of wheat were screened for heat tolerance (Chapter 4). As drought and heat stresses often occur simultaneously under field conditions, and the response to water stress is manifested in higher leaf temperatures, it would be of interest to examine whether heat tolerance provides some protection against plant water deficits. Therefore the objectives of the current study were to:

i) Examine the physiological responses of heat-tolerant and heat-sensitive genotype seedlings under different levels of drought-stresses.

ii) Investigate whether heat-tolerant genotype seedlings are also drought-tolerant

iii) Study if photosynthetic efficiency, transpiration efficiency and related traits are associated with drought tolerance in wheat seedlings?

5.2. PRELIMINARY EXPERIMENT 1

The effect of moderate to severe drought stress on *in vivo* chlorophyll fluorescence (Fv/Fm) in wheat seedlings.

5.2.1 Introduction

This preliminary experiment was conducted using relatively heat tolerant and sensitive genotypes selected after screening the 100 genotypes for tolerance to heat stress (Chapter 4). The aim was to develop a protocol for exposing seedlings to moderate to severe drought stress level in small pots and the time interval required to see the effect of these drought stresses on the chlorophyll fluorescence parameter (Fv/Fm) in wheat seedlings.

5.2.2 Materials and Methods

Four genotypes differing in heat tolerance were selected after screening 100 genotypes. Meering was heat tolerant, Frame and Goldmark were relatively heat

tolerant, and Oxley was heat sensitive genotypes (Table 4.3). Eight small pots, four each for the control and drought stress treatments, were prepared and filled with the 300g of soil, peat moss and sand medium in the ratio of (2:1:1) after sterilization and oven drying (~60°C) for 24 hours. In each pot 10-12 seeds were sown which were later thinned to 7-8 healthy seedlings 5 days after germination. The soil moisture content was maintained at 12.5% (w/w) by weighing the pots daily and adding nano-pure water to replace the water used by seedlings. The pots were weighed in the morning. At day 7 and 14, 25 mL Hoagland`s solution was added in each pot. The seedlings were grown in a growth room, which was set at 25/20 °C day/night temperatures, and under day/night length of 10 h/14 h. The experiment was unreplicated

Drought stress was imposed on 14-day old seedlings by withholding the water from one set of pots for 36 hours, while the other set was watered daily to the control 12.5% moisture level. Chlorophyll fluorescence (Fv/Fm) was recorded on control and drought stressed seedlings, as described in Section 3.5, starting at 0 h. and then every 4 hours until 36h. Pots were re-watered to the control 12.5% (w/w) moisture content after 36 h. and the recovery of Fv/Fm was recorded at 48h., 52 h., 56h. and 60h. During the entire period of drought stress and recovery from 0 h. to 60 h., the loss of water from pots was recorded.

5.2.3 Results and Discussion

At the end of 36 h. of water stress the seedlings were wilted and showing signs of severe water stress (Plates 8 and 9). As drought stress developed the average chlorophyll fluorescence (Fv/Fm) progressively declined, although it recovered during the dark period (Figure 5.1). The Fv/Fm ratios decreased by 2.0% at 28 h., 2.4% (30

h.), 2.9% (32 h.) and 3.1% (36 h.) (Figure 5.1). Apart from the recovery during the dark period, the Fv/Fm values for Goldmark, Oxley and Meering showed little change and only showed a small decline after 36h of moisture-stress. In contrast, Frame was more sensitive (Figure 5.2). This would suggest that the level of thermotolerance was unrelated to the response to a rapidly-developing water stress. While the plants had wilted, the minimum levels reached (ca. 0.80) would indicate that the photosystem was operating normally. Therefore the level of dehydration was insufficient or the length of time over which the seedling were dehydrated was too short to damage the photosystem.

5.3 EXPERIMENT 2

Evaluation of chlorophyll fluorescence (Fv/Fm) to screen for drought tolerance in seedlings of wheat genotypes differing in heat tolerance.

5.3.1 Introduction

To investigate an association between heat tolerance and seedling drought tolerance, genotypes were selected from Experiments 2 and 3 of Chapter 4. Two non-replicated experiments were conducted to evaluate, whether the heat tolerant genotype seedlings were also drought-tolerant on the basis of Fv/Fm. The results of the preliminary experiment showed that a drought stress treatment of 36 h. during which time soil moisture content in the pots reached about 3% (w/w), reduced the Fv/Fm ratios slightly but, except for cv. Frame, no other genotypes showed a marked decline after 36 h. (Figure 5.2). Therefore, length of drought stress was increased from 36 hours to 48 hours.

Plate-8. Seedlings of different wheat cultivars prior to the imposition of drought stress. Varieties are (left to right) Frame, Oxley, Goldmark and Meering.

Plate-9. Seedlings of different wheat cultivars after 36h of drought stress. Varieties are (left to right) Frame, Oxley, Goldmark and Meering.

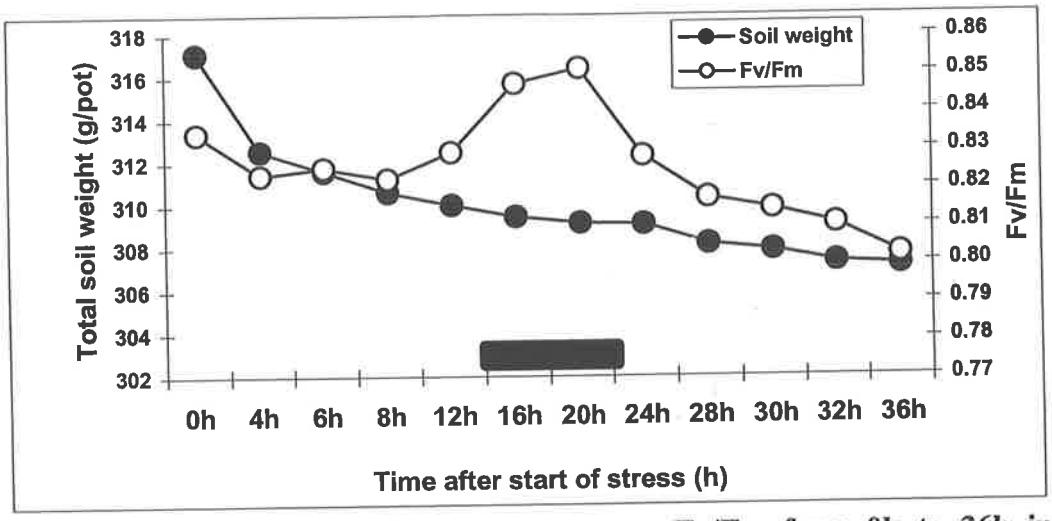


Figure 5.1: Effect of drought stress on mean Fv/Fm from 0h to 36h in seedlings of four heat tolerant and sensitive wheat genotypes. Soil weight is the weight of moist soil within the pot during the drying cycle. Each pot contains 300g. of dry soil and was initially watered to a moisture content of 12.5% (w/w). Each point is the average of four genotypes. The dark period is indicated by ■.

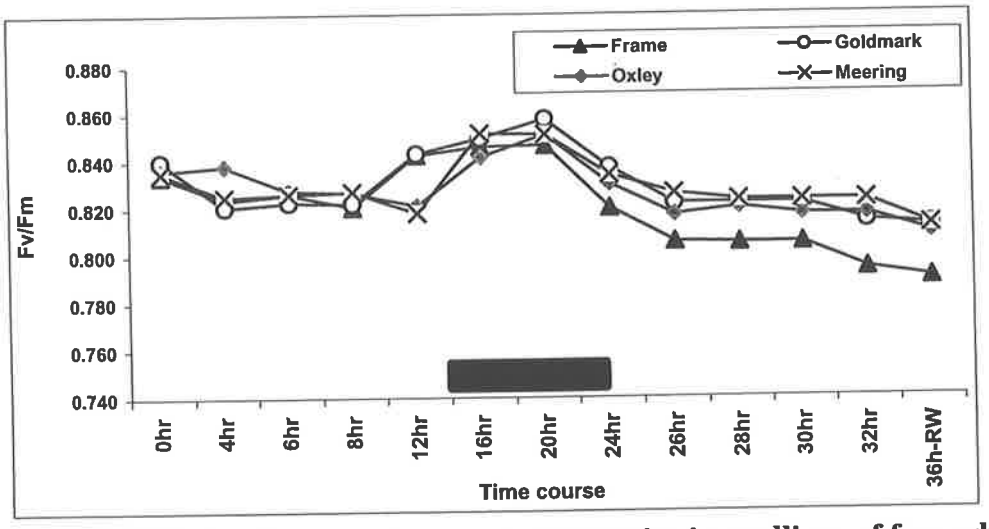


Figure 5.2: Effect of drought stress on Fv/Fm ratios in seedlings of four wheat genotype from 0 h. to 36 h. The dark period is indicated by ■.

5.3.2 Materials and Methods

The results of preliminary Experiment 1 showed that more than 36 hours might be required to cause marked reductions on Fv/Fm and allow differences between genotypes to the distinguished in wheat seedlings. Therefore, length of drought stress was increased from 36h to 48h. The general materials and methods were the same as described in section 5.1.3. Due to the limited number of leaf clamps that were used for dark adaptation of the leaves to record Fv/Fm, two un-replicated experiments were conducted under the growth room conditions described in section (5.1.3). The two sets of results were considered as replicates and subsequently combined in the data analysis. Growing medium included soil, peat moss and sand medium in the ratio of (2:1:1) was used after sterilizing and hot oven drying for 24 hours.

Ten genotypes were examined, including those that were used in Experiments 2 and 3 of Chapter 4. Drought stress was imposed on 14-day old seedlings by withholding the water for 48 hours and then rewatering the pots to control level (12.5% (w/w)) to examine the recovery of PSII efficiency, while control pots were watered regularly. Chlorophyll fluorescence was recorded every 4 hours. Measurements at 12 h. and 36 h. corresponded to the dark periods of the lighting cycle. After the stressed pots were rewatered at 48 h., measurements were made during the recovery periods at 52 h., 56 h., 80 h. and finally after 5days.

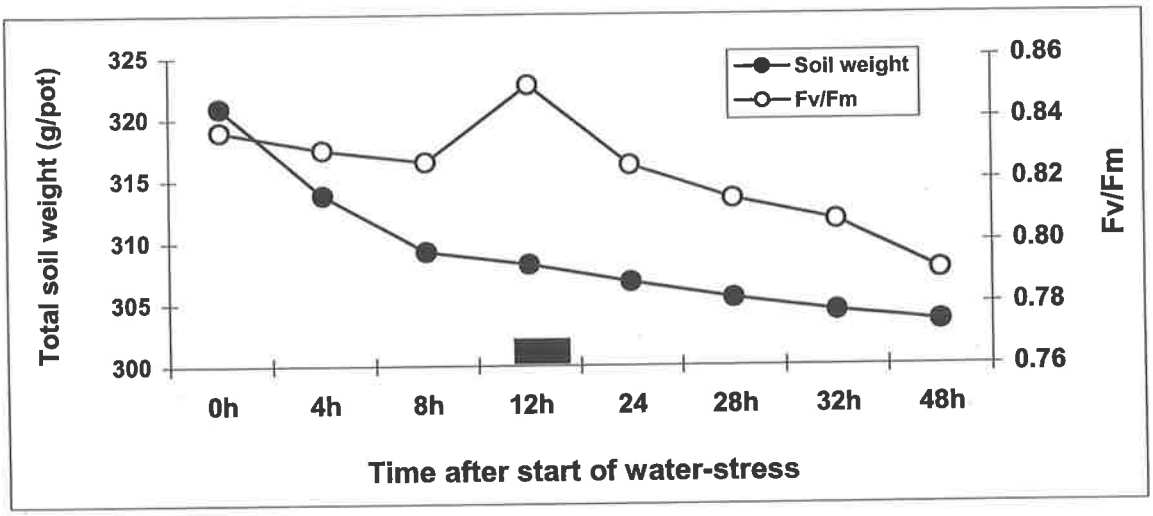


Figure 5.3: Average effect of drought stress on average chlorophyll fluorescence (Fv/Fm) ratios in 10 genotypes of wheat seedlings. Soil weight is the weight of moist soil within the pot during the drying cycle. Each pot contains 300g. of dry soil and was initially watered to a moisture content of 12.5% (w/w). The time when the measurement was taken in the dark period is indicated by ■.

5.3.3 Results

Figure 5.3 shows the average responses of the 10 genotypes to 48h of drought stress. At the beginning the available water in drought stressed pots was about 21g (7% w/w). As moisture level decreased gradually, the Fv/Fm ratios also decreased except at 12 h. (dark) period in which fluorescence increased and then again gradually decreased with increasing drought stress. Minimum average Fv/Fm (0.790) occurred when the moisture level was 3 g/pot (1% (w/w)). This indicated that the fluorescence Fv/Fm ratios decreased on an average in all genotypes with increasing drought stress. However the minimum Fv/Fm value is still high, indicating the PSII was not damaged even at this level of stress.

Results in Table 5.1 also show that drought stress significantly affected the Fv/Fm not only during the period of drought stress, from 4h to 48h, but also during the recovery

Table 5.1: Mean square values for chlorophyll fluorescence parameter Fv/Fm from 0h to 48h drought stress and 3 recovery phases in wheat seedlings.

SOV	df	0h	4h	8h	12h (dark)	24h	28h
Rep.	1	0.0000	0.00009	0.00029	0.00087	0.0012	0.0002116
Var.	9	0.00002	0.00015**	0.00013**	0.00043**	0.00075**	0.000168
Trt.	1	0.0000	0.0042**	0.00024**	0.00044**	0.00028**	0.008123**
Var. x Trt.	9	0.00002	0.000001	0.00019**	0.000013**	0.000020	0.0001523
Res.	19	0.000016	0.000001	0.00002	0.00004	0.00002	0.0001576
Total	39						

SOV	df	32h	36h (dark)	48h	52h Recovery	56h Recovery	80h Recovery
Rep.	1	0.00000	0.00000	0.00095	0.00169	0.0146	0.0000
Var.	9	0.0001770	0.0007**	0.00016	0.00012	0.00042	0.0010**
Trt.	1	0.01173**	0.0030**	0.0199**	0.0062**	0.003**	0.0189**
Var. x Trt.	9	0.0001993	0.0059**	0.0011**	0.00014	0.0019*	0.00009**
Res.	19	0.0002276	0.000001	0.00013	0.0003	0.0004	0.000001
Total	39						

***, ** Significant at P > 0.05 & 0.001**

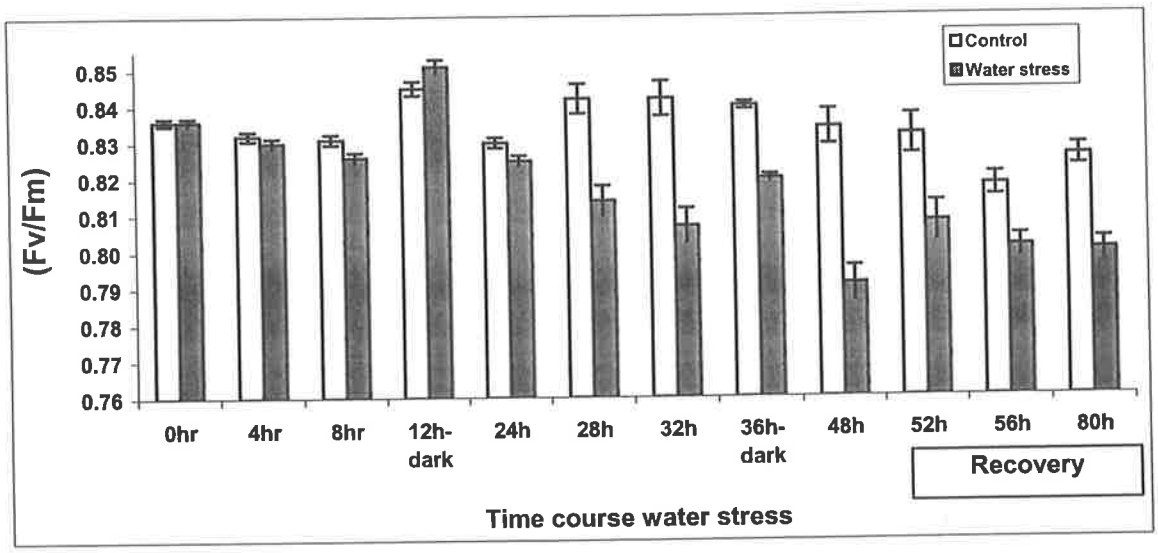


Figure. 5.4 Variable to maximum fluorescence (Fv/Fm) values, average of 10 genotypes under time course of drought-stress. Vertical bars represent the standard error of difference of means of 2 replicates.

phase, from 52h to 80h. The Genotype x Treatment interaction was significant for Fv/Fm at 8 h, 12 h (dark), 36 h (dark), 48 h, and two recovery periods at 56 h and 80 h. Significant genotypic differences for treatment affects were evident during the whole drought stress period and during the recovery phase.

The average response in Fv/Fm for the 10 genotypes is shown in Figure-5.4. The maximum reduction of 5.2% was observed after 48 hours of drought stress. However, during the recovery period from 52h to 80h, the Fv/Fm ratios did not recover completely and further decreased by 2.9%, 2.1% and 3.1% at 52h, 56h and 80h respectively. This shows that drought stress adversely affected the efficiency of photosystem-II in wheat seedlings beyond the period of water deficit.

Figure 5.5 shows the effect of drought stress from 0 h. to 48 h. and the recovery up to 5 days after rewatering in the different genotypes. The first significant effect of drought stress was observed at 28 h. when Fv/Fm ratios were decreased in all genotypes except Kukri. In this variety the first significant decrease in Fv/Fm ratio was evident after 48h of drought stress.

Gradual decreases in Fv/Fm were observed from 48 h. in almost all heat-tolerant genotypes including Buckley, Kukri, Anlace, Kingswhite and Kulin. However, significant decreases in Fv/Fm ratios were observed in heat-sensitive genotypes including WW15, Cook, Millewa, ME-71 and Lyallpur from 48h of drought stress and during the recovery phases. Recovery of the photoefficiency at 48 h was observed after rewatering the stressed seedlings to the control moisture content. Heat-tolerant genotypes, Buckley, Kukri, Anlace, Kingswhite and Kulin showed a rapid recovery after rewatering at 48 h. and during 52 h. to 80 h. as well as full recovery after 5 days.

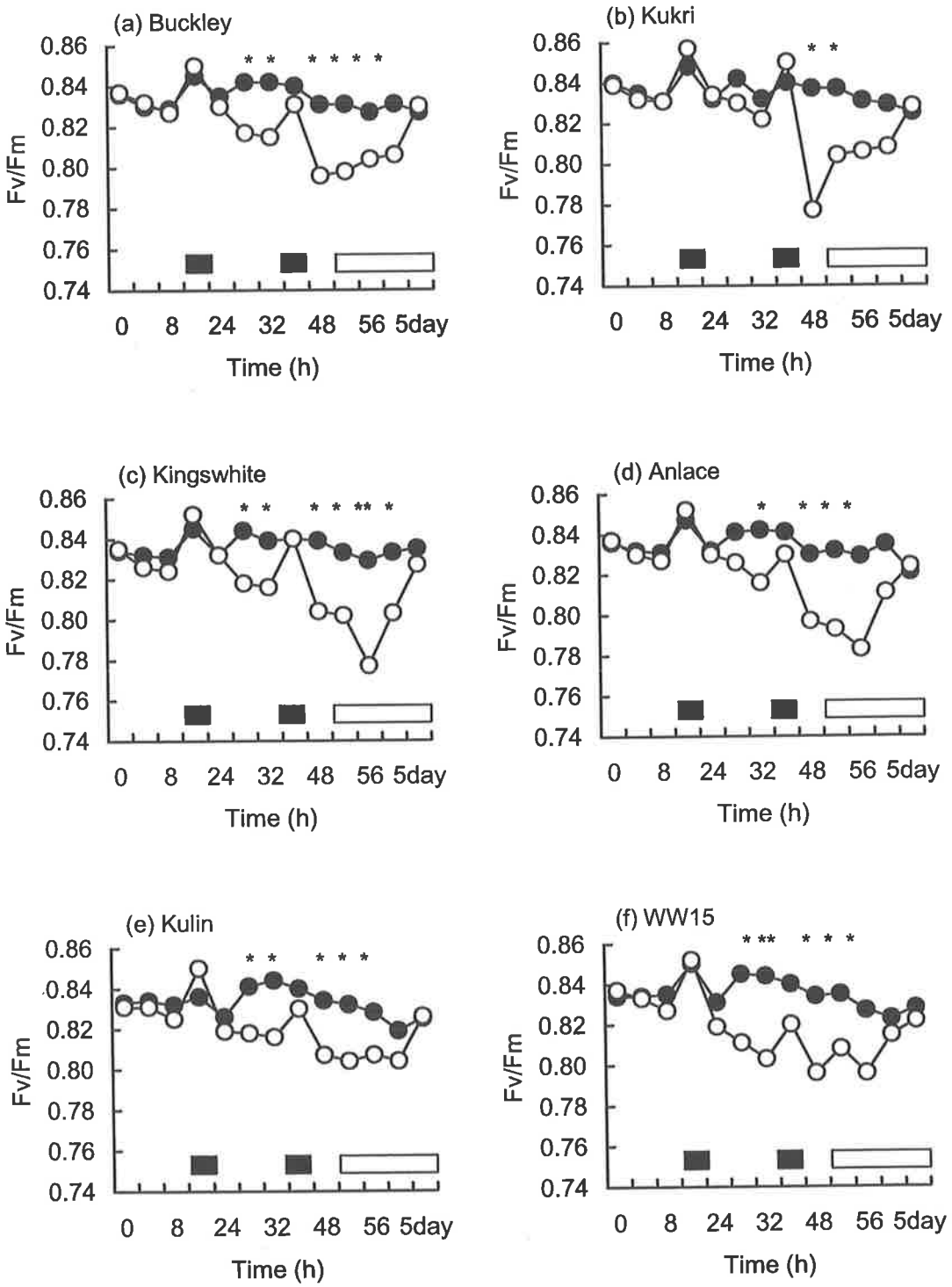


Figure 5.5. The changes in Fv/Fm in 10 varieties of wheat when seedlings were stressed for 48 h. followed by 5 days recovery after rewatering. Measurements made during the dark period are indicated (■) and the recovery period is shown (□). Significant differences are indicated by * (P<0.05) and ** (P<0.01).

(continued)

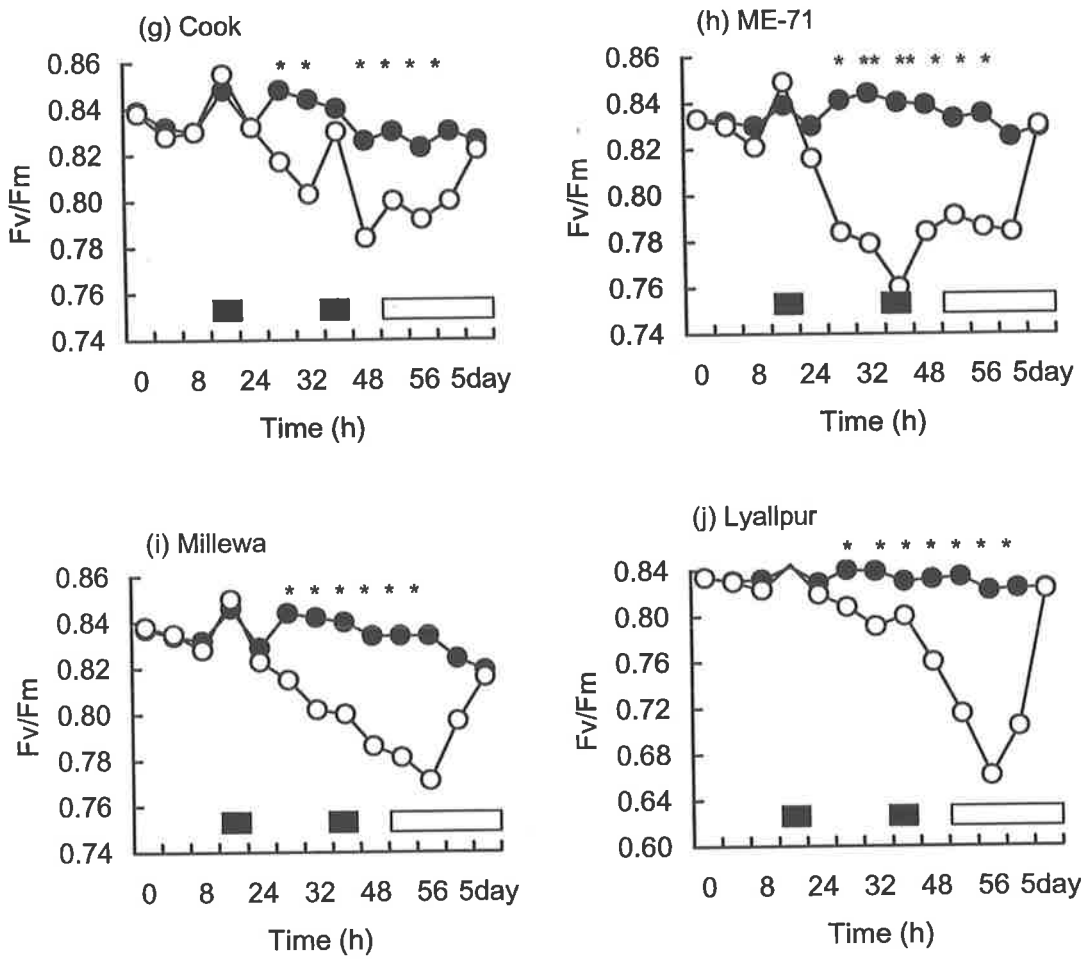


Figure 5.5 (continued). The changes in Fv/Fm in 10 varieties of wheat when seedlings were stressed for 48 h. followed by 5 days recovery after rewatering. Measurements made during the dark period are indicated (■) and the recovery period is shown (□). Significant differences are indicated by * (P<0.05) and ** (P<0.01).

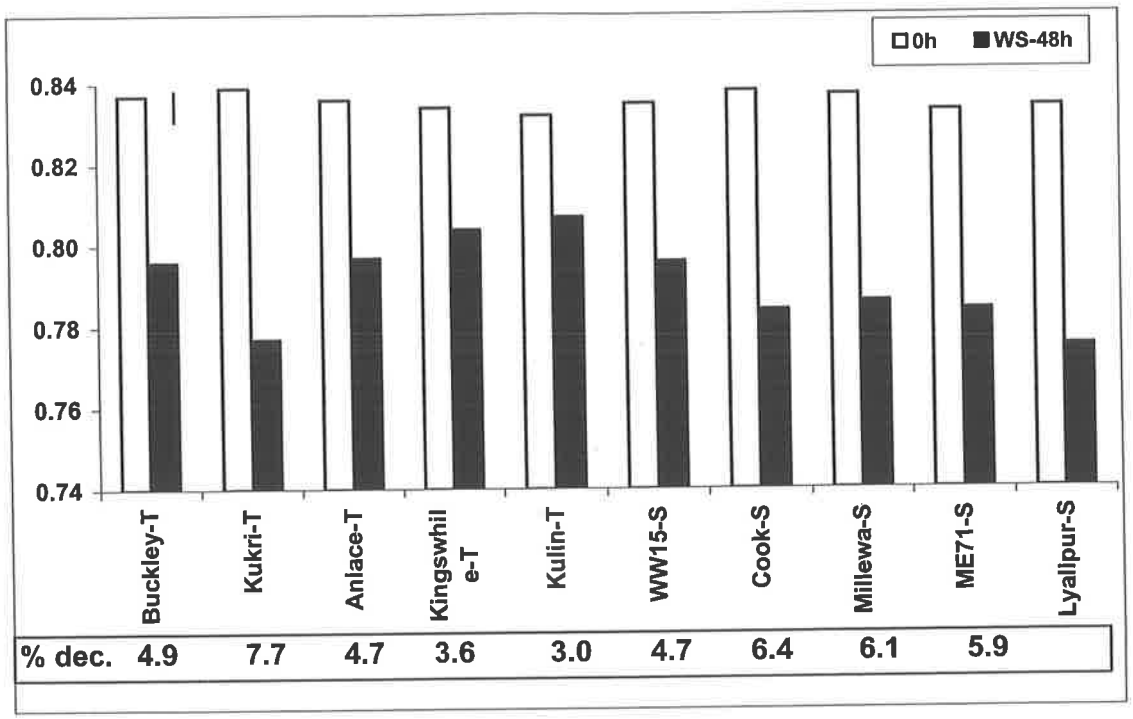


Figure 5.6: The effect of 48 h. of water stress on the ratio of Fv/Fm in wheat seedlings of heat tolerant (T) and sensitive (S) genotypes. Vertical bar is the Lsd at 5% (Var. x Trt.)

Two heat-sensitive genotypes, WW-15 and Cook, also showed almost similar responses during the recovery phases, as a gradual recovery was evident in these genotypes. There was a delayed recovery in the photoefficiency of the heat-sensitive genotypes Millewa, ME71 and Lyallpur. In particular, the heat-sensitive genotype Lyallpur showed a considerable response to 48 h. of drought stress and during the recovery phases. This decline in Fv/Fm was evident even during recovery periods from 52 h. to 56 h. and then a delayed recovery was observed from 80 h. to 5 days. Hence, the extreme response of heat sensitive genotype seedlings and particularly genotype Lyallpur shows that drought stress at 48h severely affected the maximum efficiency of PSII and the recovery responses in most heat-sensitive genotypes were delayed.

Percent decreases in Fv/Fm values after 48 h. of drought stress in heat-tolerant and sensitive genotypes seedlings are shown in Figure 5.6. The smallest decreases in Fv/Fm

were observed in two heat tolerant genotypes Kulin and Kingswhite, which declined by 3.0 and 3.6 %, respectively, while Kukri was the only heat-tolerant genotype showing a large reduction in Fv/Fm ratio (7.7%) followed by the heat-sensitive genotype Lyallpur-S (7.1%). Other tolerant and sensitive genotypes have shown moderate responses after 48 hours of drought stress.

5.4 EXPERIMENT 3

Photosynthetic efficiency of thermo-tolerant and sensitive seedlings of wheat genotypes under low, moderate and severe moisture-stresses.

5.4.1 Introduction

The study was conducted to examine the physiological responses to three different drought stresses of heat tolerant and heat sensitive genotypes at the seedling stage. The genotypes were selected from 100 genotypes screened for heat tolerance (Chapter 4). Some of genotypes were the same as those used in Experiment 2. These genotypes are mentioned in Materials and Methods (5.4.2) below. An earlier study by Balota and Lichtenthaler (1999) suggested that the chlorophyll fluorescence parameters were not affected by mild water stress (63% of the control soil moisture content), however severe water-stress (38% of the control soil moisture content) reduced the chlorophyll fluorescence parameters only in drought sensitive wheat seedlings. Therefore, this study was conducted to see the effect of three different moisture regimes on chlorophyll fluorescence parameters: initial fluorescence (Fo), variable fluorescence (Fv), maximum fluorescence (Fm) and Tm values. Three levels of drought stresses were low, moderate and severe drought stress applied 28 days after germination. Recovery in the fluorescence parameters was not monitored during this study.

5.4.2 Materials and Methods

Ten genotypes were selected from 100 genotypes screened previously (Chapter 4): the tolerant genotypes Anlace and Meering, the moderately tolerant genotypes, Halberd, Krichauff, Machete, Condor and Kukri, the moderately sensitive genotype, and the sensitive genotypes Lyallpur, Oxley and Millewa (Table 4.3). The seedlings were grown for 28 days at 20/15°C (day/night temperature) and 8 h./16 h. (day/night). A slightly larger pot size (approximately 500 mL capacity) was used in this experiment.

Sixty plastic pots were filled with 400g of University of California (1.6:1 soil sand:peat mix with complete nutrients and pH 6.8) and five to six seeds of each genotype were sown in each pot. This soil mix has a higher moisture holding capacity than the soil used previously and it was anticipated that it would dry less quickly. Five days after germination, seedlings were thinned to 3 seedlings per pot. The experimental design was a RCBD with three replications.

Seedlings were grown for 22 days at a moisture level of 27% (w/w), which represents the drained limit for this soil mix, and then watering was stopped in half of the pots in which the moisture level was gradually reduced to 18% (w/w) over a 6-day period. In the following 3 days, the plants dried the soil water content 13% (w/w) and over the next 3 days, moisture level was further reduced to 7% (w/w). As no wilting was observed under 18% and 13% water contents, these levels of stress were considered low and moderate water stresses, respectively. Wilting was observed in seedlings when the soil moisture consent had reached 7%. This was considered as severe moisture. Chlorophyll fluorescence was recorded on 28-day old seedlings of control and drought stressed plants, after 15 minutes dark adaptation as described in Section 3.5.

Chlorophyll fluorescence parameters was analysed for low, moderate and severe drought stresses separately using Genestat 5.

5.4.3 Results

Changes in fluorescence parameters depended on the level of drought stress. Severe drought stress significantly affected ($P< 0.001$) initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), Fv/Fm, but not Tm values (Table 5.2). The Genotype x Treatment interaction was significant for Fv/Fm and Tm. Moderate drought stress also significantly ($P<0.01$) affected the fluorescence parameters including Fo, Fv, Fm and Fv/Fm values except Tm, whereas the Genotype x Treatment interaction was significant for Fv and Tm only. Low drought stress did not affect any of the fluorescence parameter. This shows that only severe drought stress showed the best discrimination between genotypes for Fv/Fm ratios and Tm values.

Responses in Fv/Fm ratios of individual genotypes under three drought stresses are presented in Figure 5.7. Under severe drought stress, the greatest reductions in Fv/Fm, were observed in heat-sensitive wheat seedlings of Oxley (12%), Lyallpure (13%) and Millewa (15%) followed by a moderately heat-tolerant and moderately heat- sensitive genotypes Halberd (9%) and Condor (9%), while two heat-tolerant wheat seedlings Kukri and Anlace showed the smallest decreases (0.7% and 2%). Under the moderate and low drought stresses, non-significant differences in Fv/Fm were observed for all genotypes. These results are in agreement with the previous experiment, which showed that photoefficiency of heat-sensitive genotypes was more affected after 48 h. of drought stress than the heat tolerant genotypes. Low and moderate drought stresses did not affect the Fv/Fm ratios significantly in any genotype.

Table-5.2 Mean squares values for the chlorophyll fluorescence parameters Fo, Fm, Fv and Fv/Fm under different levels of drought stress in wheat seedlings. The different levels of drought stress were achieved by gradual drying of the soil over a 6-day period.

A. Severe drought-stress (7% w/w)

SOV.	d.f	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	967	344618	350232	0.0013851	2338
Variety (Var)	9	2313ns	170297*	187462*	0.0022951*	2069ns
Treatment (Trt)	1	85428**	4471740**	8501394**	0.0670673**	1490ns
Var. x Trt.	9	2840ns	132667ns	159221*	0.0023057*	3684*
Residual	38	1753	71509	73355	0.0009324	1526
Total	59					

B. Moderate drought -stress (13% w/w)

SOV	d.f	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	519	20468	14472	0.00001	166.5
Variety (Var)	9	950ns	35612ns	26928ns	0.0000631ns	1774*
Treatment (Trt)	1	36952**	1538241**	2052020**	0.0218886**	9.6ns
Var. x Trt.	9	1678ns	47894ns	38926ns	0.0001830ns	2173*
Residual	38	1711	23424	16763	0.0001159	748.8
Total	59					

C. Low drought -stress (18% w/w)

SOV	d.f	Fo	Fm	Fv	Fv/Fm	Tm
Replication	2	655	29611	20713	0.00000	2153
Variety (Var)	9	2117ns	69061ns	55159ns	0.00016ns	3353ns
Treatment (Trt)	1	194ns	614ns	38ns	0.00002ns	184ns
Var. x Trt.	9	384ns	53456ns	48165ns	0.00011ns	1271ns
Residual	38	1393	22738	21110	0.00017	1214
Total	59					

***, ** Significant (P < 0.05 and 0.001) ns= not significant**

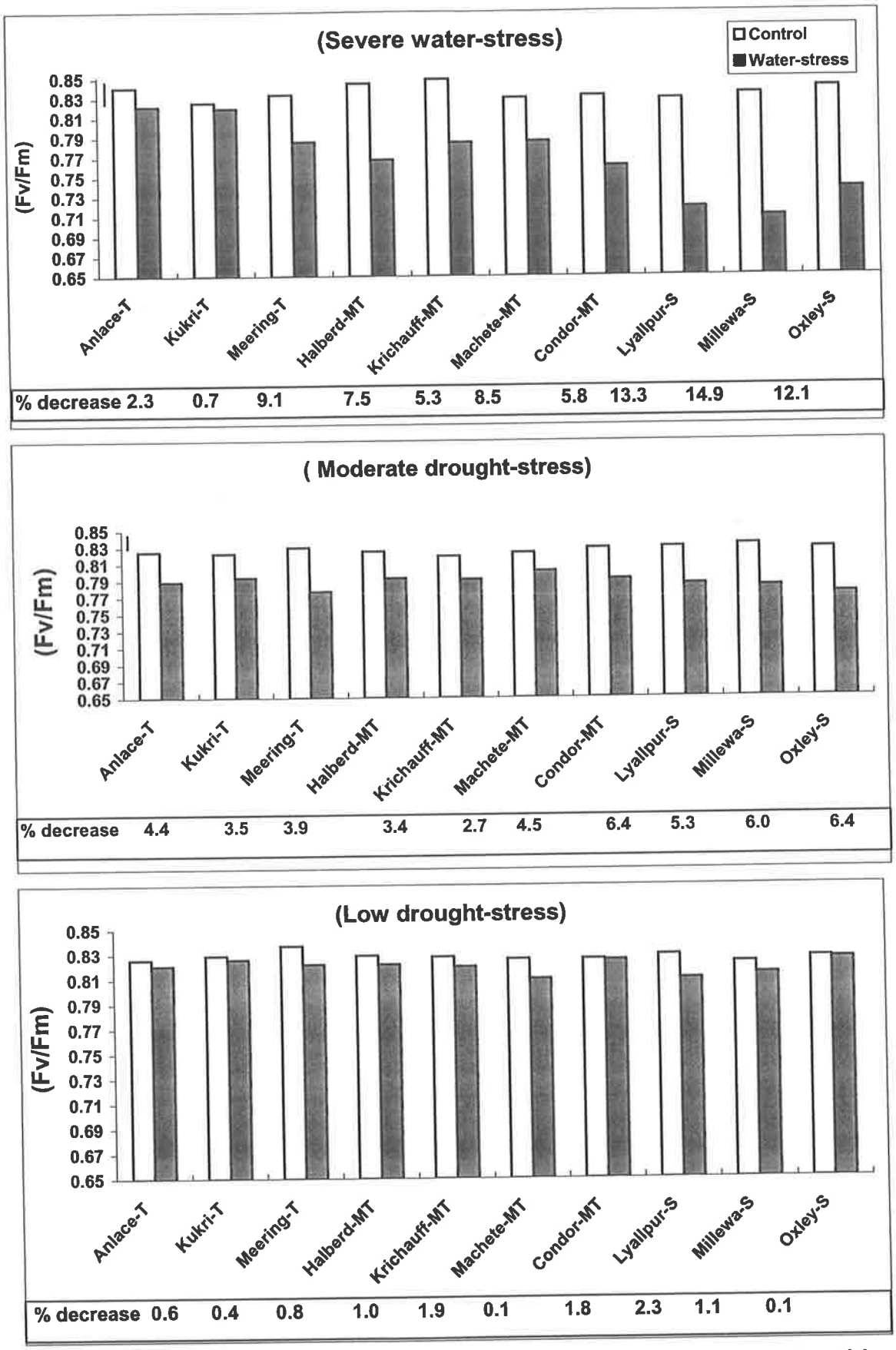


Figure 5.7: Effects of drought-stress on Fv/Fm values in heat tolerant and sensitive wheat seedlings. Vertical bar = Lsd at 5% (Var. x Trt.)

The effects of low, moderate and severe drought stresses on Fo, Fv, Fm and Tm are presented in Figure 5.8. Slight increases and decreases in different parameters among genotypes were observed especially under severe drought stress. For two parameters, Tm and Fo, values slightly increased in response to moderate and severe drought stresses but the differences were non-significant among genotypes, whereas Fv and Fm values decreased in response to severe drought stress. Therefore Fv/Fm ratios also decreased proportionally to the severity the drought stress (Fig.5.7).

Considerable decreases in Fv values were found in seedlings of moderately heat-tolerant genotypes Machete (42%), Condor (34%), and heat-sensitive genotypes Meering (33%), Lyallpur (36%), Millewa (35.5%) and Oxley (35%), while the smallest decreases in Fv values were observed in heat-tolerant Anlace (15%), Kukri (15.5%) and moderately tolerant genotypes Halberd (13%) and Krichauff (12.5%). Greatest decreases in Fm values were found in heat-sensitive genotypes Meering (25%), Lyallpur (22%), Millewa (21.5%) and Oxley (22%), while the range in percent reduction Fm in tolerant and moderately tolerant genotypes was 12% to 18%. The values of Tm also varied considerably among three moistures stresses and genotypes as well, but there were no consistent increases or decreases found in Tm values among low, moderate and severe drought stresses and between genotypes (Figure 5.8).

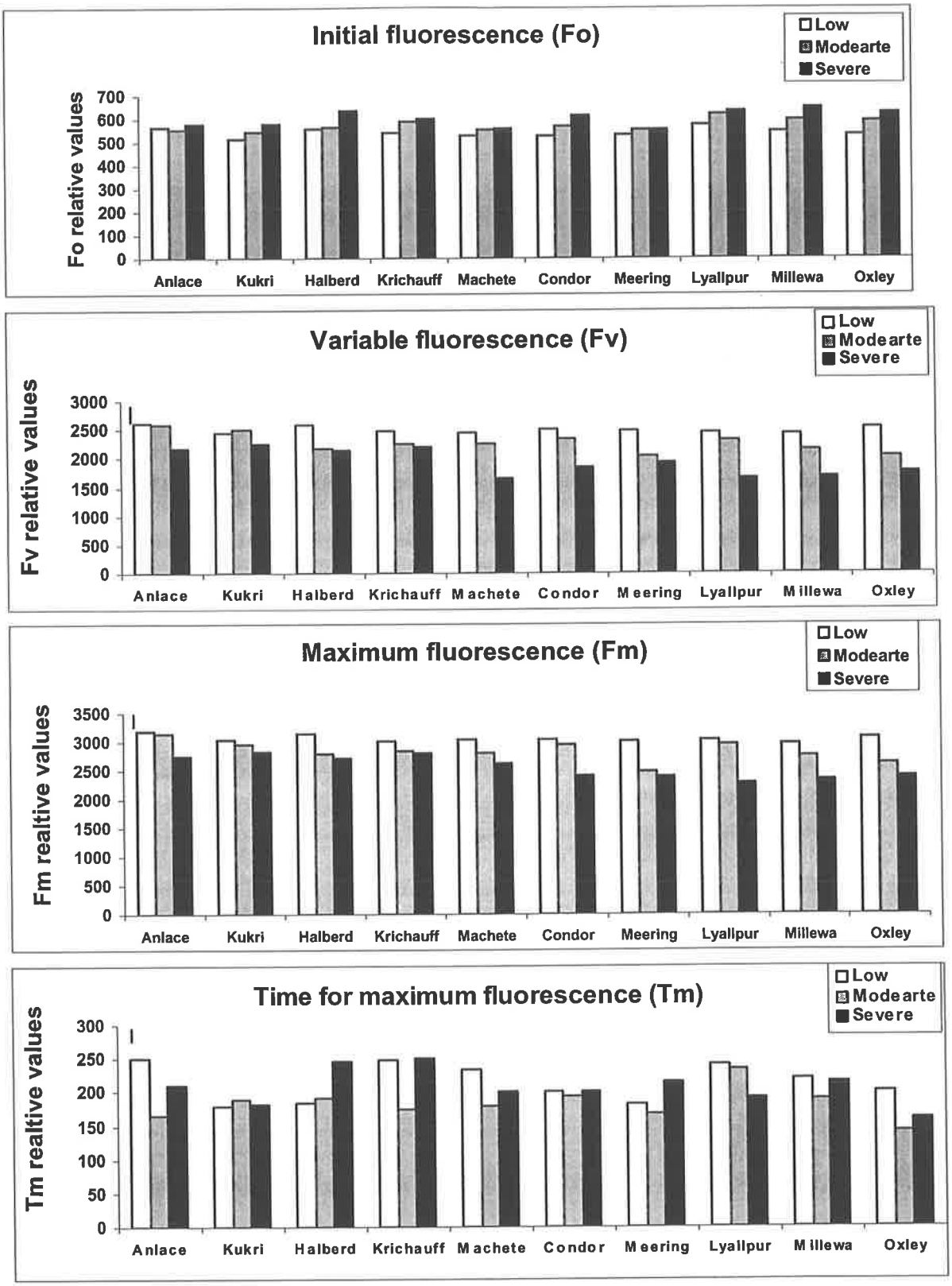


Figure 5.8: Effect of low, moderate and severe water stress on Fo, Fv, Fm and Tmin wheat seedlings differing in heat-tolerance. Vertical bar = Lsd at 0.05%.

5.5 EXPERIMENT 4

Transpiration efficiency and morpho-physiological trait of heat tolerant and heat sensitive wheat genotype seedlings under well-watered and gradual drought stress.

5.5.1 Introduction

Transpiration efficiency (TE) is the dry matter produced per unit of water transpired. Relationships between traits such as TE, photochemical efficiency (Fv/Fm ratio), leaf area, specific leaf area, dry weight and chlorophyll contents have been found under well-watered and water-limited conditions in a range of different crops, including cotton (Zhemmin *et al.*, 1997), sorghum (Henderson *et al.*, 1998), chickpea (Dalal *et al.*, 1998), soybean, maize and sunflower (Sadras and Calvino, 2001), wheat (Lu and Zhang, 1998., Angus and van Herwaarden., 2001). The objective of this study were to:

i) Investigate the responses of seedlings of heat-tolerant and heat-sensitive genotype under gradual water-stress.

ii) Examine the relationship between TE, photochemical efficiency (Fv/Fm), leaf area, specific leaf area, dry weight and chlorophyll contents in seedlings of a range wheat genotypes.

iii) Investigate whether; drought-tolerant and drought-sensitive genotypes could be assessed at the seedling stage based on these simple traits.

5.5.2 Material and Methods

The same genotypes that were previous used in Experiment 3 were selected. Small plastic pots (500 ml) were filled with 400g of oven dried University of California soil. Each pot was lined with plastic bag to avoid any drainage of water from the pots. Six seeds were sown in each pot and 5 days after emergence they were thinned to 3 healthy seedlings per pot. To minimise any surface evaporation from the pots, a 1.5 cm

thick layer of acid-washed black plastic beads was applied to the surface of the soils. Water use by seedlings was measured by regularly weighing the pots. Plants were grown in a growth room maintained at 20/15°C (day/night) under day length of 8 h./16 h. (day/night). A soil moisture level of 27% w/w was maintained in all pots until 28 days after germination, when the seedlings had 3-4 fully emerged leaves.

Sixty pots containing 28-day old seedlings were arranged in a split plot design with three replications. The water stress treatment (control *vs* stress) was the whole plot and varieties were the subplots. Three pots, prepared as described above but without any plants, were used to estimate evaporation from a bare pot. It may overestimate the actual soil evaporative loss because the seedling does not shade the surface. Their weight was measured daily at the same time as the other pots and they were randomly placed among the other pots. Gradual drought stress was imposed on half of the pots of each cultivar 28 days after germination. Water was withheld and the pots allowed to dry to the following moisture contents (w/w) over 12 days: 25.5%, 23.5%, 22.5%, 21.5%, 20.5%, 18.5%, 17.5%, 15.5%, 12.5% and 7.5%. The pots were weighed daily and if pots dried below the target weight then water was added to maintain them at the specified soil moisture content.

5.5.3 Measurements

Total water use (TWU)

Total water use (g) of each genotype was calculated by adding the daily water used by three seedlings.

Leaf area of youngest expanded leaf (LA-YEL)

Three seedlings were harvested by cutting the stems at the soil surface. Leaf and stems were separated. The leaf area of the youngest fully-expanded leaf from control well-watered and water-stress was measured as described by Wilhelm and Mielke, (1988):

$$LA\ (cm^2) = (length \times width \times 0.76)$$

Dry weight (DM)

Shoots (leaves and stem) from the three seedlings were oven-dried at 65°C for 48h and weighted. Values are expressed as dry matter per plant (mg).

Specific leaf area (SLA)

SLA was estimated as the ratio of the area of a sample of fully expanded leaf (cm^2) to the sample dry weight (mg):

$$SLA(cm^2/mg) = \frac{\text{leaf area}}{\text{leaf dry weight}}$$

Transpiration efficiency (TE)

Transpiration efficiency was calculated as total shoot dry weight (mg) of three seedlings divided by the water use (mL) by these seedlings. Total water use was corrected for loss directly from soil by subtracting evaporative water loss from bare pots.

$$TE\ (mg/mL) = \frac{\text{seedling dry weight}}{\text{(water use -evaporation from empty pot)}}$$

Total chlorophyll contents (SPAD)

Total chlorophyll content of the second fully-expanded leaf of each of the three seedlings per pot was measured before harvesting the seedlings. A Minolta SPAD-502 meter was used to record the chlorophyll contents and values are expressed as SPAD units.

Photoefficiency of seedlings (Fv/Fm)

Before harvesting the seedlings, photoefficiency (Fv/Fm) was measured on a fully-expanded leaf in all pots as described in section 3.5.

5.5.4 Results

Drought stress significantly ($P<0.01$) affected most of the morpho-physiological traits in wheat seedlings except the youngest expanded leaf area (Table 5.3). The most traits most affected by water stress were total water use (TWU), water use under stress (WU-stress), transpiration efficiency (TE), dry weight of seedling (DW), specific leaf area (SLA), total chlorophyll content (SPAD) and photosynthetic efficiency (Fv/Fm ratio) (Table 5.4). Variety x Treatment interactions were significant for TWU, WU-stress, TE, SPAD and Fv/Fm ratios (Table 5.3). The significant interactions suggested that genotypes showed a differential response to the severity of water-stress for these morph-physiological traits.

Table 5.4 shows that the maximum total water use (TWU) was observed for the seedlings of Lyallpur under both watering treatments: 942 mL (well-watered) and 742 mL (gradual water-tress). This was followed by the genotypes Kukri (917 mL and

Table-5.3 Mean squares values for the total water use (TWU), water use under stress (WU-stress), transpiration efficiency (TE), dry weight (DW), leaf area of youngest expanded leaf (LA-YEL), specific leaf area (SLA), SPAD values and photoefficiency (Fv/Fm) of wheat seedlings under well-watered and moisture-stress treatments.

SOV.	d.f	TWU (mL)	WU-stress (mL)	TE (mg/mL)	DW (mg)	LA-YEL (cm^2)	SLA (cm^2/mg)	SPAD	Fv/Fm
Rep.	2	14207	1641	0.0516	0.16613	3.720	1621	26.573	0.0014
Trt.	1	314732**	142984**	1.6353**	0.28057*	1.788ns	9943**	1567.74**	0.0670**
Resid	2	2693	1532	0.08527	0.06955	2.389	1027	0.855	0.00065
Var.	9	19122**	5452**	0.1167ns	0.1677**	8.002ns	6597*	68.420**	0.0023*
Var. x Trt.	9	13652*	3459*	0.15563*	0.0441ns	8.774ns	3016 ns	37.763**	0.0023*
Resi.	36	4669	1277	0.06140	0.03561	6.921	1877	6.772	0.0009
Total	59								

* = Significant ($P < 0.05$) ** = Highly significant ($P < 0.01$)

750 mL) and Anlace (908 mL and 654 mL). In the well-watered treatment, minimum water was used by the seedlings of Oxley (646 mL) followed by Meering (654 mL) and Krichauff (736 mL), while under water stress the lowest amount of water was used by Meering (589 mL) and Millewa (660 mL).

Transpiration efficiency did not change significantly under water stress except in two cultivars Anlace and Condor, where TE increased (Table 5.4). Water stress increased the SLA and there were also significant differences between genotypes. Chlorophyll content fell under water stress, with the biggest reductions occurring in Lyllapur, and Millewa. Apart from the large reduction in the SPAD reading in these two genotypes, significant reduction in the efficiency of photosystem-II (Fv/Fm 0.719 and 0.709) under the water-stress treatment showed that water-stress had drastically affected the photosynthesis. The drastic decline in total chlorophyll contents (low SPAD values) there was also a indicated that in the leaves of sensitive genotypes total chlorophyll

Table-5.4: Total water use (TWU), transpiration efficiency (TE), dry weight (DW), leaf area of youngest expanded leaf (LA-YEL), specific leaf area (SLA), SPAD values and photosynthetic efficiency (Fv/Fm) in wheat seedlings under well-watered (WW) and gradual drought stress (WS)

Genotype	TWU (ml)		TE. (mg/ml)		DW (mg)		LA-YEL (cm^2)		SLA (cm^2/mg)		SPAD		Fv/Fm	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Anlace	908	654*	1.95	2.79*	1.76	1.83ns	15.74	17.21ns	232.3	236.7ns	34.27	31.40ns	0.841	0.823ns
Kukri	917	750*	2.73	2.86ns	2.62	2.51ns	18.34	18.01ns	262.6	232.2ns	36.81	31.82ns	0.826	0.820ns
Meering	801	589*	2.46	2.67ns	1.97	1.57ns	16.96	13.69*	272.2	286.5ns	30.61	24.49*	0.834	0.786ns
Machete	881	732*	2.24	2.58ns	1.97	1.89ns	17.67	16.11ns	267.1	293.4ns	30.40	21.11*	0.830	0.786ns
Krichauff	736	697ns	2.63	2.51ns	1.93	1.74ns	17.34	15.05*	217.3	225.8ns	36.82	27.88*	0.849	0.785*
Halberd	862	705*	2.28	2.67ns	1.97	1.88ns	15.33	14.07ns	229.6	239.1ns	37.22	23.80*	0.845	0.768*
Condor	811	634*	2.24	3.11*	1.81	1.97ns	15.90	17.92*	256.1	269.5ns	35.71	20.27*	0.832	0.761*
Oxley	646	722ns	2.50	2.50ns	1.89	1.81ns	18.47	15.28*	253.0	253.3ns	30.41	22.35*	0.840	0.738*
Lyallpur	941	742*	2.45	2.55ns	2.12	1.89ns	16.23	20.29*	245.8	341.0*	34.91	17.08*	0.829	0.719*
Millewa	832	660*	2.19	2.55*	1.83	1.68ns	17.30	18.2ns	272.7	389.8*	32.55	17.25*	0.838	0.709*

* =Significant differences: ns = non-significant; * - P<0.05; ** - P<0.01.

Table-5.5 Correlation coefficients between transpiration efficiency (TE) and morpho- physiological parameters under well-watered and gradual drought stress in wheat seedlings (n=20)

	TE	TWU	WU-stress	LA-YEL	SLA	Fv/Fm	DW	SPAD
TE	1.000							
WU	-0.567**	1.000						
WU-stress	-0.585**	0.890**	1.000					
LA-YEL	0.302**	0.057	0.280	1.000				
SLA	0.057	-0.224	-0.272	0.369	1.000			
Fv/Fm	-0.324	0.503**	0.484**	-0.074	-0.698**	1.000		
DW	0.384*	0.463**	0.381*	-0.467**	-0.199	0.280	1.000	
SPAD	-0.299	0.629**	0.611**	-0.056	-0.699**	0.920**	0.421**	1.000

Significance: * = P<0.05; ** = P< 0.01)

contents would have been severely damaged, which caused a severe decline in photosynthetic efficiency. Under stress conditions Fv/Fm and the SPAD readings were significantly correlated (r=0.927, P<0.01, n=10), whereas under non stress conditions, there was no correlation (r=0.15, n.s.).

Correlation coefficients between different morpho-physiological traits under well watered and stressed conditions combined are shown in Table 5.5. There were highly significant negative correlations between TE and total water use (r =-0.567), water use under moisture-stress (r = -0.585**) and a positive correlation with leaf area of youngest expanded leaf (r=-0.302**) seedling dry weight (r=0.384*). Highly significant positive correlations were apparent between total water use (r=0.890**) and water use under stress. Positive correlations were found between total water use in wheat seedlings and Fv/Fm (r=0.503**), dry weight of seedlings (r=0.463**) and SPAD values (r=0.629**). Similarly, water use under stress showed a strong positive correlation with photosynthetic efficiency (r=0.484**) and SPAD (r=0.611**) and dry weight of seedlings (r=0.381*). Specific leaf area had strong negative associations with Fv/Fm (r=-0.698**) and SPAD values (r=-0.699**). Strong positive correlation (r=0.920**) between photosynthetic

efficiency and SPAD values had also positive effect on the dry weight of seedlings (r=0.421**).

5.6 Discussion

5.6.1 General effects of drought-stress on chlorophyll fluorescence Fv/Fm and other parameters.

Generally, moderate and severe drought stresses (Experiments 2, 3) reduced Fv/Fm in all genotypes but there was no significant reduction under a low water-stress (Experiment 3). Results in Experiment-2 showed significant variation among genotypes at different time intervals under the moisture-stress treatment. In particular, drought stress at 48 h. considerably affected the Fv/Fm values including the recovery periods from 52 h. to 80 h. (Table 5.1). Significant interactions between genotypes and drought stress during 48 h. (day time), as well as dark periods (12 h. and 36 h.) including two recovery periods, 56 h. and 80 h. suggested that genotypes have shown considerable differentiation during these times of drought stress (Table 5.1). Similarly, different water regimes i.e., particularly moderate and severe drought stresses significantly affected the Fv/Fm ratios including other fluorescence parameters, Fo, Fv, Fm and Tm values (Table 5.2). Significant decrease in photoefficiency of PSII was observed under the severe water-stress in 8 days old seedlings of two winter wheat genotype (Balota and Lichtenthaler 1999).

After rewatering the stressed seedlings at 48 h., the recovery of the photochemical efficiency was observed in all genotypes (Experiment 2; Figure 5.5). However, different recoveries were observed in genotypes that showed differences in heat tolerance in the intial screening experiments (Chapter 4). During the first 24 hours of the recovery phase (from 52 h. to 80 h.), further decreases in photoefficiency of PSII were observed in the most sensitive wheat seedlings, Lyallpur and Millewa. Furthermore, Lyallpur showed a

prolonged delay in its recovery up to 5 days, which demonstrates that efficiency of PSII was severely damaged in this genotype. Moreover, prolonged recovery of PSII efficiency after rewatering at 48 h., especially in heat-sensitive genotype seedlings (Millewa, ME-71 and Lyallpur), suggested that thyalkoid membranes may have severely damaged, which might have subsequently affected the electron transport in the light-harvesting complexes (LHC-II) of photosystem-II in these genotypes (Di-Marco *et al.*, 1988; Krause and Weis., 1991). However, a gradual improvement in PSII efficiency was observed in all heat-tolerant genotype seedlings (Anlace, Buckley, Kukri, Kulin, Kingswhite) and some of the sensitive genotypes (Cook, WW15 and ME71; Figure 5.5).

Water deficit causes stomatal closure, which ultimately causes significant decreases in CO_2 uptake and a reduction in photosynthesis rate. However, the light energy normally trapped and used in the light reaction still needs to be dissipated, otherwise damage to thylakoid membranes from reactive oxygen species may occur. Therefore the dynamics of the recovery processes in tolerant and sensitive genotype seedlings under drought stress may reflect changes in CO_2 intake and other metabolic processes. Therefore, faster recovery of PSII efficiency in tolerant genotype seedlings after rewatering could be due to immediate recovery of CO_2 uptake and stomatal opening as compared to sensitive genotype seedlings. The roles of carotene, xanthophylls and photosynthetic proteins (D1 & D2) have also been suggested by various studies (Dannehl *et al.*, 1996; Demmig and Adams, 1996). Dannehl *et al.*, (1996) reported the role of D1 and D2 proteins in the recovery of PSII photosystem-II in spinach leaves. They reported that during first 24 hours of recovery phase, the thyalkoid proteins (D1) increased significantly and after 2 days of recovery phase, the level of other thyalkoid proteins such as D2, CP29, CP43 and CP47 increased. This may be an explanation for the particularly rapid recovery of Fv/Fm in the tolerant genotypes Buckley, Kukri, Anlace, Kingswhite, and Kulin.

Results of the Experiment 3 suggested that only severe drought stress significantly affected Fv/Fm in wheat seedlings (Table 5.2). Greater decreases in the Fv/Fm were observed under the severe drought stress than the in moderate and low drought stresses (Figure 5.7). The Fv/Fm ratio decreased drastically in heat-sensitive wheat seedlings (Lyallpur, Millewa and Oxley) compared to the seedlings of heat-tolerant genotype (Anlace and Kukri). Balota and Lichtenthaler (1999) have also suggested that mild water-stress had no effect on the chlorophyll fluorescence parameters in 8 to 20 day old wheat seedlings of both tolerant and sensitive wheat genotypes. It may be because wheat seedlings were able to adjust their photosynthetic functions under the moderate drought stress. Interestingly, the results of the present experiments also showed that low and moderate water-stress have no significant effect on Fv/Fm (Figure 5.7) and seedlings of two heat-tolerant genotypes (Anlace and Kukri) showed a smaller drop in Fv/Fm under severe drought stress when compared to the moderate drought stress. As in these experiments, drought stress was increased from low to moderate and severe within 10-12 days period, it seems that heat-tolerant genotype seedlings of Anlace and Kukri might have adapted to the stress accordingly (Figure 5.7). The development of stress was quite rapid and so adaptations such as osmotic adjustment may not have been significant. This may indicate that under the particular conditions of the experiment, structural rearrangements in the chloroplasts and thyalkoid membranes could have occurred during moderate drought stress particularly in the tolerant genotypes (Blum *et al.*, 1989). The importance of this under field conditions needs to be explored further.

Other fluorescence parameters including Fo, Fv, Fm and Tm values were also determined under low, moderate and severe drought stresses (Figure 5.8). Variable fluorescence is the most important component of the Fv/Fm ratio as it reflects the balance

of energy between the antenna and reaction centres of PSII and Fm is associated with quantum efficiency of photosystem-II. Therefore, the drastic decreases in these components indicate that the efficiency of photochemistry is drastically reduced under water stress, particularly in heat-sensitive genotypes when compared with the heat-tolerant genotypes. However, Figure 5.8 showed that low, moderate and severe drought stresses have no significant effect on Fo values. As severe decreases in Fo indicate the physical dissociation of antenna complexes from PSII centres, these results suggests that there could be no physical dissociation of antenna from PSII. Havaux (1998) also reported that usually water-stress doesn't modify the Fo levels significantly.

5.6.1.1. Conclusions

i) Moisture-stress of 48 h. affected the photochemical efficiency of PSII in all wheat genotype seedlings irrespective of their heat-tolerances and susceptibility.

ii) However, heat-tolerant genotypes showed a smaller drop in their photoefficiency (Fv/Fm ratios) as compared to heat sensitive genotypes.

iii) Similarly, seedlings of heat-tolerant and moderately tolerant cultivars showed rapid recovery in their photosynthetic efficiency after removing the moisture-stress.

iv) Two of the heat sensitive genotype seedlings (ME71 and Lyallpur) demonstrated drastic decreases in Fv/Fm ratios after 48 h. of moisture-stress.

v) A very drastic response in the recovery of the PSII efficiency was observed in a heat-sensitive genotype Lyallpur-S, which showed a severe drop in photoefficiency of PSII even after rewatering the pots at 48 h. This suggests that 48 h. of drought stress might have severely damaged the photosynthetic apparatus involving chloroplasts and thyalkoid membranes particularly in this genotype.

vi) Genotypes such as Buckley, Anlace, Kukri, Kingswhite and Kulin, which had small reductions in Fv/Fm ratios during heat-stress (Chapter 4) and also had small reductions in Fv/Fm under moisture-stress (Chapter 5), as an immediate recovery of photochemical efficiency. This suggests there is a physiological link between tolerance to heat and water stress.

5.6.2 Transpiration efficiency and photoefficiency in wheat seedlings.

Plant physiologists and breeders had considered improving the drought tolerance in crops based on morpho-physiological traits extensively for many years. However, according to Richards and Condon (1994), increasing the TE for biomass production might increase yield especially, under drought conditions. There is a considerable volume of literature on the use of these traits to improve drought tolerance and yield in cereals, especially on latter growth stages such as at flag leaf emergence, booting, grain growth and grain yield. However, very little research had been conducted so for using seedling stage. Therefore the present study was conducted to study the effect of gradual water-stress on these morpho-physiological traits at the seedling stage in wheat.

Results in Table 5.3 showed that the gradual water-stress affected most of the morph-physiological traits but there were no Variety x Treatment interactions for

morphological traits such as dry weight of seedlings, leaf area of youngest expanded leaf and specific leaf area. This suggests that cultivars did not respond greatly for morphological traits under gradual water stress treatment.

Table 5.4 shows that gradual water-stress had considerable effects on seedlings of wheat cultivars for different physiological traits. Differences in total water use, water use under stress and transpiration efficiency under well-water and gradual water-stress treatments could be found for different cultivars. The seedlings of cultivar Lyallpur, Kukri and Anlace used the greatest amounts of water, while only one cultivar, Anlace, had a significantly higher TE. Sarim *et al.* (1997) have also reported durum wheat varieties differed in their response for transpiration efficiency under irrigated and dry land conditions.

When the other morphological traits of cultivar Anlace were considered under well-watered and gradual water-stress, there was a slight increase in dry matter from 1.76 to 1.83mg, leaf area of youngest expanded leaf (15.7 to 17.2 cm^2), SLA (232.2 to 236.7 cm^2/mg) under the gradual stress, while a minimum decreases were observed for physiological traits such as SPAD values (34.3 to 31.4) and photoefficiency (0.841 to 0.823). In comparison, the seedlings of cultivar Lyallpur despite using the greatest amount of water under both treatments, as well as increases in leaf area (16.23 to 20.29 cm^2) and specific leaf weight (245 to 341 cm^2/mg) Table 5.4, showed considerable decreases in dry weight (2.12 to 1.89mg), chlorophyll contents (34.9 to 17.1 SPAD values), and Fv/Fm ratios (0.829 to 0.719). This shows that cv. Lyallpur was sensitive to the gradual water-stress treatment when compared to cv. Anlace and Kukri. Flagella *et al.* (1996) also reported that drought tolerant and sensitive durum wheat cultivars showed differential responses under two water regimes, including moderate and severe drought

stresses. They suggested 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 photoefficiency under severe drought stress. Rhodes and Hanson (1993) also suggested that maintenance of turgor by osmotic adjustment provides a major physiological mechanism to minimise the detrimental effects of drought stress in plants. This means that good osmotic adjustment and leaf water potential aren't the only traits used by plants to maintain photosynthetic efficiency. Fv/Fm was positively correlated with SPAD values and negatively with SLA, indicating thicker leaves with a higher chlorophyll content had a greater photosystem efficiency. Retention of chlorophyll under stress is therefore an important physiological trait for drought resistance.

Although no other physiological traits were measured in this study, the results suggests that two cultivars Anlace and Kukri could be drought tolerant as they showed a minimum decline in SPAD values along with lesser decrease in Fv/Fm ratios (Table 5.4). Gummuluru *et al.*, (1989) have also suggested that wheat genotypes tolerant to water deficit maintained higher total chlorophyll contents comparing to susceptible genotypes. Also, the results in Table 5.5 supports this supposition showing the higher positive correlations between SPAD values and Fv/Fm ratios, which suggest that maintenance of total chlorophyll contents could be used to indirectly select drought tolerant genotypes (Reynolds *et al.*, 1994). SPAD values have also shown strong positive associations with total water use ($r = 0.629^{**}$) and water use under stress ($r = 0.611^{**}$) again suggesting that SPAD values might be used to identify drought tolerant and susceptible genotypes under well water and drought stress conditions.

5.6.2.1 Conclusions

i) Gradual water stress affected the different morpho-physiological characteristic of wheat seedlings including total water use, water use during stress, TE, dry weight of seedlings, SPAD values and photochemical efficiency.

ii) Genotypic variations were also observed for most of the physiological traits except TE, while genotype x drought stress treatment shows that genotypes responded only for physiological traits to gradual water-stress and not for morphological traits.

iii) Although, the effect of gradual water-stress was not highly significant on different morphological and physiological traits, some genotypes such as Anlace, Kukri, Lyallpur and Millewa showed tolerance and susceptibility to gradual water-stress on the basis of some physiological traits.

iv) Photosynthetic efficiency (Fv/Fm ratios) and total chlorophyll contents (SPAD) values and their strong association might have suggested their possible role in selecting drought tolerant genotypes at the seedling stage.

CHAPTER 6
EFFECT OF HIGH TEMPERATURE STRESS ON SEEDLING EMERGENCE AND COLEOPTILE LENGTH IN WHEAT GENOTYPES DIFFERING IN THERMOTOLERANCE

6.1 Introduction

Air and soil temperatures at sowing determine the rate and success of seed germination and seedling emergence. In many parts of the world, especially the tropics, subtropics, arid and semi-arid regions, temperature at the time of sowing and during emergence of wheat can rise above the optimum for germination and emergence (Smith *et al.*, 1989; Abayomi and Wright, 1999). Peterson (1965) reported the optimum temperature range of wheat germination to be 20-25°C, while Andoh and Kabota (2001) reported an optimum temperature of 23°C for wheat germination and seedling emergence. However, in some parts of Pakistan, especially in South-eastern and South-western areas, the air temperature can rise to 35-45°C during the months of September-November when wheat is sown (Figure 2.2). Soil temperatures may be higher. These temperatures can adversely affect seed germination and seedling establishment (Bajwa, 1984). Moreover, due to the problem of high temperatures, farmers don't plant the wheat until late November and early December and because of this delay, the grain filling and maturity periods are also delayed until April and May, which adversely effects the grain development due to high temperature (Wardlaw *et al.*, 1989).

A number of studies in various crop species have shown that poor stand establishment can be caused by high temperature stress (Radford, 1987; Radford and Key, 1993). This is because high temperature can reduce final percentage germination and coleoptile

length in many crop species including wheat (Radford, 1987; Schillinger *et al.*, 1998), pearl millet (Smith and Hoveland, 1986) and sorghum (Radford and Henzell, 1990). Therefore, improving the ability to germinate and of the coleoptile to elongate at supra-optimal temperatures will improve the establishment of the crops.

Genetic variability has been established for seedling emergence and coleoptile length in wheat (Allan *et al.*, 1962). Lafond and Baker (1986) reported that wheat cultivars showed differential responses to different temperatures ranging from 5 to 30°C. They also observed that median germination time of various cultivars differed by as much as 7 hours at 20°C. Radford (1987) investigated the effect of different temperatures on coleoptile length in wheat cultivars and found genetic variability in mean coleoptile length. Coleoptile length has found to be associated with emergence capability in tall and semi-dwarf wheat cultivars due to the presence of the Rht1 or Rht2 dwarfing genes (Schillinger *et al.*, 1998). Hence all these studies showed that wheat cultivars differ in seedling emergence rate and coleoptile length, however the effect of high temperatures on seedling emergence and coleoptile length in heat-tolerant and heat-sensitive genotypes have not been reported so far. Therefore, the present study was conducted with the following aims:

i) To examine the effect of high temperature stress on seedling emergence and coleoptile length of heat-tolerant and heat-sensitive wheat cultivars as assessed using chlorophyll fluorescence of seedlings.

ii) To investigate whether heat-tolerant wheat cultivars can be selected using the simple technique of seedling emergence and coleoptile length.

6.2 Material and Methods

Five heat-tolerant and five heat-sensitive wheat genotypes were selected after screening 100 genotypes (Chapter 4). Genotypes selected were heat-tolerant (Anlace, Kukri, Meering), moderately heat-tolerant (Krichauff, Halberd, Machete), moderately heat-sensitive (Oxley) and heat-sensitive (Lyallpur-73, Millewa). Two constant temperatures, 20°C and 38.5°C were used in the experiment. The experiment was conducted in the dark in a growth room where the maximum temperature attainable was 38.5°C, slightly lower than the 40°C used in previous studies. The seeds were germinated in free-draining plastic trays (39 x 28 x 11 cm^3) with one tray per variety. Trays were filled with 8.5 kg of University of California soil. Twenty seeds were sown in 2 rows, 20 cm apart. Seeds of same size of a genotype were sown at a depth of 3 cm. RO water was added to each tray 24 hours prior to seed sowing to a moisture content of ~30% moisture content, allowing it to drain to approximately field capacity. Growth room temperatures (20°C or 38.5°C) were set 24 hours prior to sowing the experiment. To minimise water stress during high temperature stress (38.5°C), the soil moisture level was maintained by adding RO water with a watering can each morning and evening if necessary until excess moisture drained from the bottom of each tray. The free-draining nature of the soil meant that waterlogging did not occur.

The experimental design was a completely randomised block design with 4 replications. The experiment was repeated twice because only 20 trays (2 replicates) could be placed in the growth room at one time. Two growth rooms were used for each temperature, one at 20°C and another one at 38.5°C. Twenty trays were placed in each growth room set at constant temperatures (20°C or 38.5°C) with lights off to make the growth room completely dark. The growth rooms were swapped during the second part of the experiment (that is, for replicates 3 and 4). Data for number of seedling emerged was

recorded every 6 hours after the first emergence of seed. The emergence was first recorded when a least one coleoptile had emerged above the soil surface. Emergence was recorded under dim blue light. Emergence counts were continued until complete emergence had occurred. Coleoptile length (mm) and first leaf length (mm) were recorded when emergence was completed by all of the genotypes.

6.1.3 Data analysis

Data for the final seedling emergence percentage was analysed by standard analysis of variance techniques using Genstat 5. The change in emergence over the time was described by fitting a logistic curve to the mean cumulative data of percentage final emergence. Emergence percentage was based on the maximum potential emergence, that is 20 seedlings = 100% emergence. Time was converted to degree hours (°C hours: 1 °C day = 24 °C hours) and a base temperature of 0° C was assumed. The logistic equation used was,

$$Y = a + \frac{c}{1 + \exp[-b\,(X-m)]}$$

Where, X is the time in °C hours, Y is the cumulative seedling emergence percentage of each genotype, and m, a, b, and c are constants. Logistic curves were fitted for each replicate and the following parameters derived:

(i) m, the point of inflection of the curve;

(ii) the maximum rate of germination which occurs at X=m and which was calculated from bc/4

(iii) the time for 50% (t_{50}) and 90% (t_{90}) emergence rate, which were determined as follows;

$$t_{50} = m - 1/b \log\left[\left(\frac{c}{(50-a)}\right) - 1\right]$$

and $t_{90} = m - 1/b \log [(\frac{c}{(90-a)}) - 1]$

6.2 Results

6.2.1 Seedling emergence

Results in Table 6.1 showed that temperature had a highly significant ($P < 0.001$) effect on seedling emergence percentage and also there was a significant difference between varieties. However, the Variety x Temperature interaction was non-significant showing no significant differences in responses of genotypes to high temperature. Boubaker and Yamada (1991) also observed non-significant Genotype x Temperature interactions in wheat seedling emergence under supra-optimal temperatures.

Table 6.1. Analysis of variance for final seedling emergence percentage of wheat genotypes at constant temperatures of 20°C and 38.5°C.

SOV	d.f	m.s.
Replication	3	618.6
Variety (Var.)	9	861.1**
Temperature (Temp).	1	7702.8**
Var. x Temp.	9	322.3
Residual	57	220.4
Total	79	

** Highly significant P >0.001

Generally more than 90% emergence was observed under optimum temperature (20°C) in all genotypes except Cook (Table 6.2). On average, final germination was reduced from 94% to 74% by high temperature. The lowest seedling emergence occurred in

Table 6.2. Seedling emergence (%) of heat tolerant and sensitive wheat genotype seedlings at two constant temperatures and the relative reduction in emergence

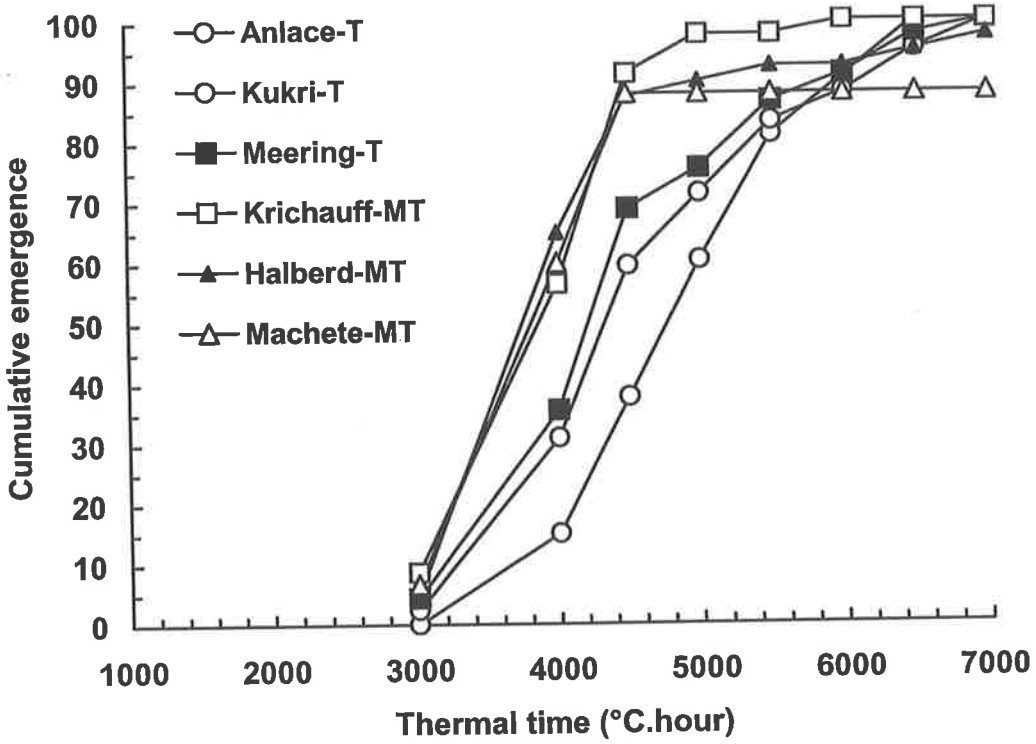
Genotype	Thermotolerance[A]	Seedling emergence (%)			
		20°C	38.5° C	Mean	Reduction at 38.5°C (%)
Anlace	T	100	85	93	15
Kukri	T	100	75	88	25
Meering	T	100	85	93	15
Krichauff	MT	100	76	88	24
Halberd	MT	96	76	86	21
Machete	MT	88	83	86	6
Oxley	MS	100	88	94	12
Lyallpur-73	S	93	72	83	23
Millewa	S	100	66	83	34
Cook	S	67	51	59	24
Mean		94	74		21
LSD (P=0.05) (Var)				15	

cultivar Millewa followed by Cook. The greatest reduction in emergence percentage was observed in genotype Millewa (34%) and the smallest decreases in the seedling emergence percentage occurred in Machete (6.0%) followed by the Oxley (21%).

Cumulative emergence rates for the different genotypes at the two constant temperatures, 20° C and 38.5° C, are presented in Figure 6.1 and Figure 6.2. Heat-tolerant and heat-sensitive genotype seedlings started to emerge at approximately 3900 °C-hour thermal time at 20°C (Figure 6.1). Fifty percent emergence was completed close to 3900 °C-hour thermal time by the three moderately heat-tolerant genotypes, Krichauff, Halberd and Machete, while the heat-tolerant genotypes Anlace and Kukri reached 50% emergence at 4700 and 5100 °C-hour (Figure 6.1). Almost 100%

cumulative emergence rate was observed in all heat-tolerant and moderately heat-tolerant genotypes around 6000 °C-hour at 20°C temperature. The only genotypes where there was not more than 90% emergence were Lyallpur-73 and Cook. The lag phase for germination for these genotypes was about 4100 °C-hour, which was longer than the other genotypes (Figure 6.1),

First emergence at 38.5°C occurred at 3800 °C-hour and then increased slowly until about 4600 °C-hours, with initial emergence tending to be slower in moderately sensitive and sensitive genotypes (Figure 6.2). Time to 50% emergence was less in the heat-tolerant genotype Anlace, at around 5000°C-hour. All other heat-tolerant genotypes including Krichauff, Halberd, Machete and Kukri reached 50% cumulative emergence from 5400 to 5600 °C-hour. Final emergence varied between varieties. Anlace, Machete, Krichauff and Halberd completed more than 85% to 98% emergence, while genotype Kukri completed nearly 70% emergence. The heat- sensitive genotypes, Oxley, Lyallpur-73, Cook, Millewa completed approximately 80% emergence while only one moderately heat-sensitive genotype Meering completed more than 90% emergence under high temperature 38.5°C (Figure 6.2). Figures 6.2 showed that under the high temperature stress, final emergence in heat- tolerant and heat-sensitive wheat genotype seedlings occurred a little before 7000 °C-hour while for the moderately sensitive and sensitive genotypes, maximum emergence occurred at or after 7000°C-hours. In summary, high temperature stress tended to delay emergence and slow the rate of emergence and this effect tended to be greater in the more thermosensitive genotypes. Final emergence was reduced in some genotypes, but this was not consistently related to their thermotolerance.



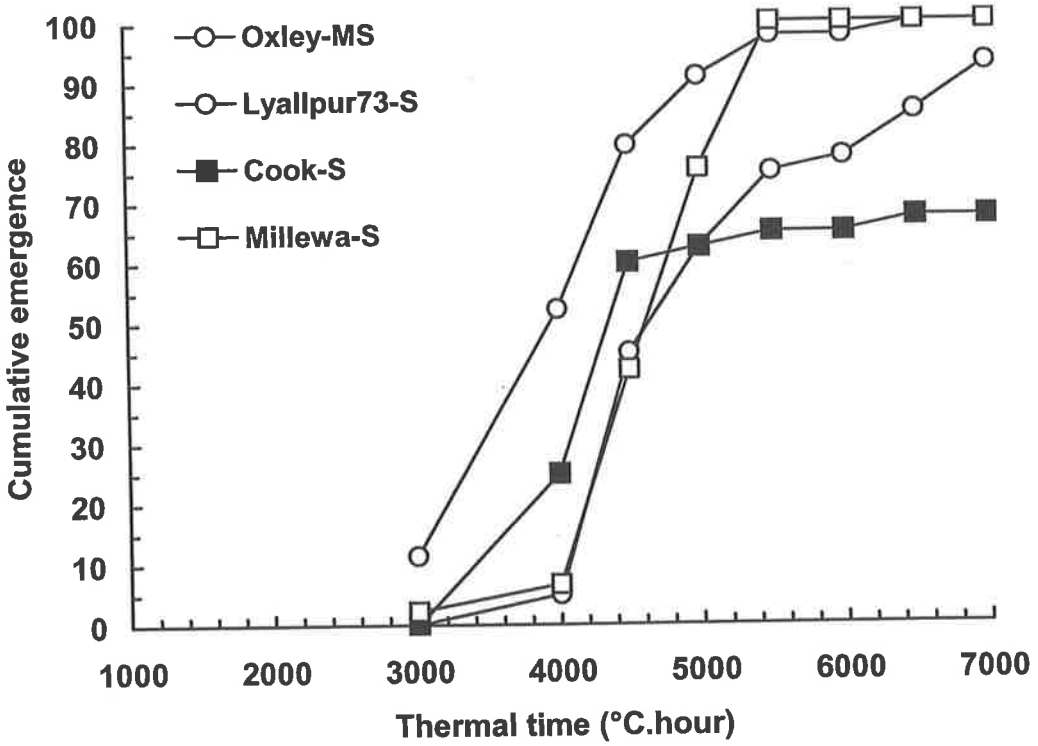


Figure 6.1. Cumulative emergence rate at 20°C in wheat genotype seedlings varying in thermotolerance: T – tolerance; MT – moderately tolerant; MS- moderately sensitive; S - sensitive

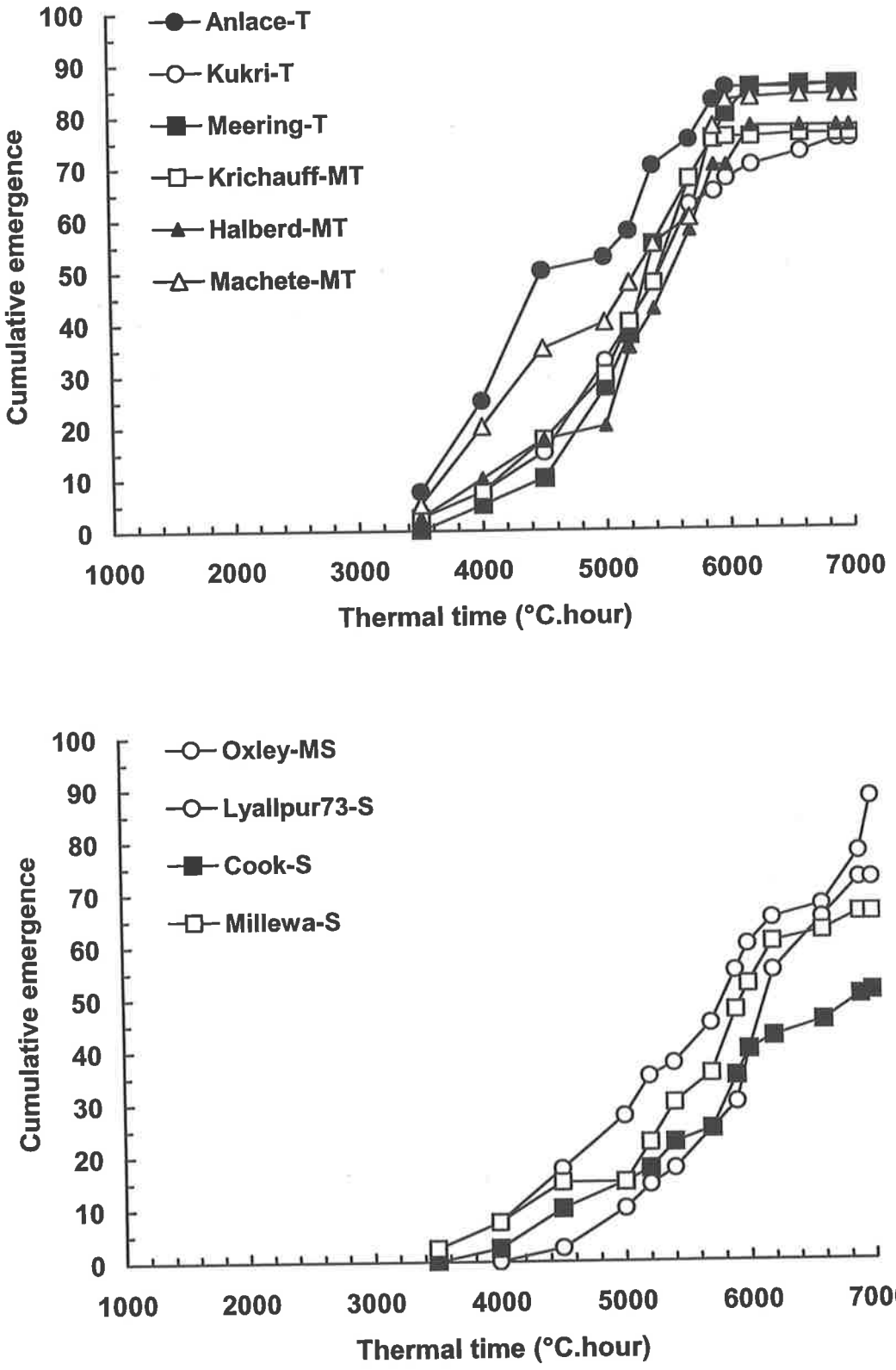


Figure 6.2. Cumulative emergence rate at 38.5°C in wheat genotype seedlings varying in thermotolerance: T – tolerance; MT – moderately tolerant; MS- moderately sensitive; S - sensitive

Table 6.3 Thermal time for start of emergence, 50% (t_{50}) and 90% (t_{90}) final emergence of wheat genotype seedlings with different levels of thermotolerance under low (20°C) and high temperature stress (38.5°C)

Temperature	Tolerant and moderately tolerant genotypes			Sensitive and moderately sensitive genotypes		
	Start	t_{50}	t_{90}	Start	t_{50}	t_{90}
	°C-hour					
20°C	3900	4900	6200	3900	4800	6100
38.5°C	3600	5400	6800	3600	6200	7000

Table 6.3 shows thermal time to complete 50% and 90% emergence in heat-tolerant and heat-sensitive genotypes seedlings. At 20°C, heat-tolerant and heat-sensitive genotype seedlings started to emerge at 3900 °C hour, while they reached 50% emergence at about the same thermal times, 4800-4900 °C hour. At 38.5°C both heat-tolerant and sensitive genotype seedlings started to emerge at 3600 °C hour, while heat-tolerant genotypes reached 50% emergence at 5400 °C hour, which was earlier than heat-sensitive genotype seedlings which reached 50% emergence at 6200 °C hour. Whereas,

Table 6.4. Mean square values for coleoptile length and first leaf length of different wheat genotype seedlings at constant temperatures 20°C and 38.5°C.

SOV	d.f	Coleoptile length (mm)	First leaf length (mm)
Replication	3	282.89	660.7
Variety (Var.)	9	588.67**	345.9ns
Temperature (Temp.)	1	20160.30**	213.9ns
Var. x Temp.	9	206.91**	174.1ns
Residual	57	51.50	132.5
Total	79		

Significance: ** Highly significant (P <0.001); ns = not significant

Table-6.5. The effect of temperature during germination and emergence on the coleoptile length of wheat seedlings.

Genotype	Thermotolerance	Coleoptile length (mm)		Reduction (%)
		20°C	38.5°C	
Anlace	T	51	31	39
Kukri	T	65	19	71
Meering	T	55	29	47
Krichauff	MT	74	31	58
Halberd	MT	81	35	57
Machete	MT	56	33	41
Oxley	MS	56	33	41
Lyallpur-73	S	71	36	49
Millewa	S	54	22	59
Cook	S	39	16	60
Mean		60	29	52
LSD (Var. x Trt.) (P=0.05)		7		

90% emergence under the was reached almost at the same thermal time (6100 to 6200 °C hours) at 20 °C by both tolerant and sensitive cultivars, but at 38.5°C, heat-tolerant genotypes reached 90% emergence 200°C-hours earlier than the sensitive genotypes.

6.2.2 Coleoptile length and first leaf length

Significant differences in coleoptile length were observed in all genotypes (Table 6.4). The temperature effect was highly significant, and there was a significant Genotype x Temperature interaction. This shows the differential response of genotypes to the temperature treatments for coleoptile elongation. However, high temperature did not affect the length of first leaf of seedlings. The relative decrease in mean coleoptile length is shown in Table 6.5. Greatest coleoptile length (81 mm) was recorded with

Halberd under the 20°C temperature. This was followed by genotypes Krichauff (74mm) and Lyallpur-73 (71 mm). Maximum coleoptile length under high temperature stress (38.5°C) was observed in Lyallpur-73 (36 mm) and minimum coleoptile length was observed in Cook (16 mm). The greatest percent decrease in coleoptile length was in Kukri (71%) followed by Cook (60%) and Millewa (59%). Figure 6.3 also shows that there is significant linear relationship between the reduction in final emergence and the reduction in coleoptile length, but there is no clear separation of genotypes based on thermotolerance of the seedlings.

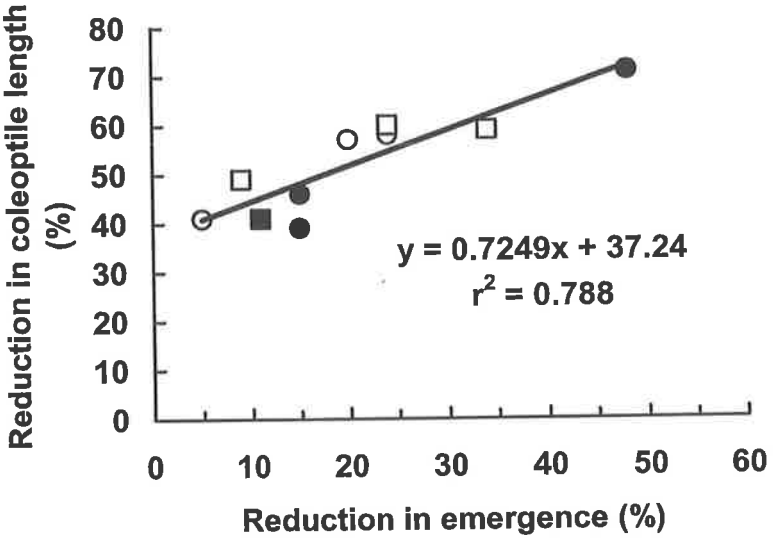


Figure 6.3: The relationship between percent reduction in seedling emergence and coleoptile length in wheat for heat tolerant (•), moderately heat tolerant (○), moderately heat sensitive (■) and heat sensitive (□) genotypes. Thermotolerance classification is based on the screening of seedlings based on chlorophyll fluorescence (Chapter 4).

6.3 Discussion

The effect of two constant temperatures, an optimum temperature (20°C) and high temperature stress (38.5°C) was investigated on the seedling characteristics such as seedling emergence percentage, cumulative seedling emergence rate at 50% and 90%,

coleoptile length and first leaf length in seedlings of heat-tolerant and heat-sensitive wheat genotypes. Generally, high temperatures affected the emergence and coleoptile length in all heat-tolerant and heat-sensitive genotypes (Table 6.2 and Table 6.4). Although the Genotype x Temperature interaction for final seedling emergence was non-significant (Table 6.1), it was significant for coleoptile length (Table 6.4). The reduction in coleoptile length was associated with a reduction in emergence. Allan *et al.*, (1961 and 1965) also found a positive correlation between coleoptile length and seedling emergence in wheat. However, coleoptile length in wheat is strongly associated with genetic factors, especially the presence or absence of Rht1 and Rht2 genes that control the coleoptile elongation (Allan *et al.*, 1962). Allan (1980) found that semi-dwarfism, particularly when both genes (Rht1 and Rht2) present, generally reduced the stand establishment in wheat.

Radford (1987) observed that coleoptiles in wheat were longest at 10 to 15°C temperature and decreased as the temperature increased from 15 to 40°C. He also demonstrated that tall cultivars had the longest coleoptile whereas semi-dwarf had the shortest coleoptile lengths. In this experiment the tall cultivars such as Halberd and Lyallpur-73 had the longest coleoptile length at 20°C constant temperature (Table 6.5) while cultivars such as Anlace, Kukri, Oxley, Millewa and Cook, which are semi-dwarf wheat varieties, produced shorter length coleoptiles.

Results in Table 6.2 and Table 6.5 showed the greatest percent reduction (71%) in coleoptile length as well as greatest reduction in emergence (48%) was found in Kukri, which was a semi-dwarf cultivar. Cultivars Lyallpur-73 and Machete showed the smallest decreases in emergence rate (9% and 5%) under high temperature (38.5°C)

while there was a moderate reduction in their coleoptile lengths (49% and 41%) under high temperature stress. Overall, these results showed that high temperature (38.5°C) delayed emergence, which was most probably the effect of high temperature stress in reducing the coleoptile length in wheat seedlings (Fig. 6.3). Various studies have shown a direct relationship between reductions in coleoptile length and delayed emergence in wheat seedlings (Allan *et al.*, 1962; Kaufmann, 1968; DeJong and Best, 1979). Similarly, the coleoptile length is considered to be significantly related to fast emergence ability in wheat (Allan *et al.*, 1965), while Schillinger *et al.*, (1998) established that the coleoptile length in wheat is a better indicator of seedling emergence than the first leaf length and the results of present study (Table 6.4) also confirmed this.

Coleoptile length was not associated with the thermotolerance of the genotype; however, interestingly cumulative emergence rate showed some association with heat-tolerance and susceptibly of the genotypes (Figures 6.1 and 6.2). Likewise, differences in 50% and 90% cumulative emergence rate were found in heat-tolerant and heat-sensitive genotypes (Table 6.3). Under the optimum temperature (20°C), both heat-tolerant and heat-sensitive genotype seedlings started to germinate at the same thermal time (3900 °C hour) while they have completed t_{50} and t_{90} emergence almost at the same time (4900 and 4800 °C hour, respectively). However, under high temperature stress, there was a marked difference between t_{50} emergence of heat-tolerant and heat-sensitive genotype seedlings. Heat-tolerant genotype seedlings completed 50% cumulative emergence 1700 °C hour earlier than the heat-sensitive genotype seedlings (Table 6.3) while there was not a marked difference to complete 90% cumulative emergence. Khan *et al.*, 1986 reported that the rate of seedling emergence has a

relationship with very low or very high temperature regimes and this study also showed similar results.

6.4 Conclusions

(i) High temperature (38.5°C) reduced seedling emergence and coleoptile length both in heat-tolerant and heat-sensitive genotype seedlings.

(ii) Seedling emergence was reduced due to the reduction in coleoptile length under the high temperature stress.

(iii) A strong association was found between reduction in coleoptile length and reduction in seedling emergence in response to heat stress.

(iv) The association between heat-tolerance and coleoptile elongation was not established under this study

(v) Cumulative emergence rate showed a relationship between cumulative seedling emergence and heat-tolerance under high temperature stress.

CHAPTER 7

GENERAL DISCUSSION

Heat-stress and water-stress reduce the yield and grain production of wheat in Australia and Pakistan. These stresses affect a number of physiological and metabolic processes in wheat during seedling emergence, establishment, seedling growth and during grain filling (Al-Khatib and Paulsen, 1984; Radford, 1987; Flagella *et al.*, 1996; Schillinger *et al.*, 1998). A series of experiments was conducted to examine the effect of heat and drought stresses on the photosynthetic efficiency and seedling emergence in wheat.

7.1 Screening of 100 genotypes for heat-tolerance

Initially 100 wheat genotypes of diverse genetic background, which represent genotypes used in breeding programmes in different agro-climatic zones of Australia and other world regions, were screened. Many of these genotypes have been tested previously for heat-tolerance at anthesis or grain filling (Wardlaw *et al.*, 1989; Stone and Nicolas, 1994 & 1995, Blumenthal *et al.*, 1994), which allowed a comparison in the responses at different growth stages.

Generally, results showed that 6 hours of heat-stress (40°C) affected the *in vivo* chlorophyll fluorescence in all wheat genotype seedlings. A range of responses was observed among the 100 genotypes on the basis of Fv/Fm ratios after 6 hour of heat-stress. Considerable reductions were observed in Fv/Fm ratios in ME-71, Lyallpur, Millewa, Cook, Bodallin, Lark, Bayonet and WW15, and these were considered to be heat senstivie. There were smaller reductions in Fv/Fm ratios in a number of genotypes including Kulin, Anlace, Buckley, Mira, Meering, Krichauff, Kukri, Amery, Halberd,

Goldmark, Yitpi and Hartog and these were considered to be heat tolerant. Hierarchical cluster analysis was used to group the genotypes and 6 distinct clusters were evident. Heat-tolerant and moderately heat-tolerant genotypes grouped together in clusters 1 to 4 while cluster 5 consisting some of moderately heat-sensitive and moderately heat-tolerant genotypes and cluster 6 consisting only one heat-sensitive genotype ME-71.

Many of the genotypes, which were grouped together in clusters 5 and 6 were heat-sensitive genotypes that had a high proportion of CIMMYT genotypes in their genetic background. Some of these varieties and their pedigrees were:

1) Oxley (Penjamo 62/4* 56/2/Tezanospintos Precoznainari 60/4/2*Lerma Rojo/2/Noin10/Brevor//3/3*Andes)
2) WW15 (Penjamo 62/4* 56/2/Tezanospintos Precoznainari 60/4/2*Lerma Rojo/2/Noin10),
3) Machete (Sonora64/2/Tezanospintosrecoz/Yaqui 54/3/*Gabo/4/Madden),
4) Millewa (Sonora64/Yaqui 50e/2/Gaboto/Mexico 8156).

Moreover these lines have also been proved to be heat-sensitive at the post anthesis stage in several studies in Australia (Wardlaw *et al.*, 1989; Blumenthal *et al.*, 1995; Stone and Nicolas, 1994,1995, 1998). These results suggest that perhaps the heat sensitivity of these genotypes may be related to the influence of many of the CIMMYT lines, but the heat tolerance of these Mexican lines would need to be determined to substantiate this idea

Genetic similarities between genotypes were examined using the coefficient of parentage (COP) for those genotypes where information was available. The analysis

showed strong association between different genotypes again showing similar genetic background as many of them from CIMMYT. For example the high COP values of WW15 and Cook (0.526), Condor and Cook (0.591), Condor and WW15 (0.691) is a result of their similar pedigrees:

- Condor: Penjamo62/4*Gabo56/2/TezanosPintos Precoznainari60/4/2*Lerma Rojo /2/Norin 10/3/3*Andes
- WW15: Penjamo 62/4*Gabo 56/2/Tezanos Pintos Precoz /Nainairo 60/4/2* Lerma Rojo/Norin
- Cook: Timgalen/Condor 'S'/2/Condor

Similarly, Meering, Condor and Oxley were the siblings of the same cross (Penjamo62/4*Gabo56/2/Tezanos Pintos Precoznainari60/4/2*Lerma Rojo/2/Norin 10/3/3*Andes) and had higher COP values of 0.691, 0.591, respectively.

Percent reduction in Fv/Fm ratios after 6 hours of heat-stress (40°C) along with grouping of many genotypes in clusters as well as higher COP values of some of the heat-sensitive genotypes have shown their origin and genetic back ground originated from CIMMYT, Mexico. Therefore, this study showed that the thermo-sensitivity of a number of genotypes could be traced to the sensitivity of a number of the CIMMYT genotypes in their pedigree.

7.1.1 Comparison of heat-tolerance in wheat genotypes at grain filling and seedling stages

Previously, Stone and Nicolas (1995) reported the effect of heat-stress at the grain filling stage (10 or 30 DAA) on yield and quality traits in 75 wheat genotypes. They concluded that the effects of heat-stress on grain yield were mainly due to the percent reductions in individual grain mass and that there was considerable variation among the genotypes surveyed. Their results allow a comparison to be made between responses to heat stress in seedlings (Chapter 4) and during grain filling (Figures 7.1 and 7.2; Appendix 2) for the genotypes that were common in both studies.

Overall, there was a weak negative correlation between the responses during grain filling at 10 DAA ($r=-0.303$, $P<0.05$, $n=41$), at 30 DAA ($r=-0.244$, $P<0.05$, $n=41$) and at the seedling stage, indicating there was a poor relationship between tolerance to heat stress at the two stages. However, some genotypes showed a distinct response to heat-stress both at the post-anthesis stage and seedling stage. In particular, Kulin appeared to be heat tolerant at both the seedling stage and at 10 DAA and 30 DAA. Genotypes such as Tincurrin, Bodallin, Lyallpur, Kalyansona, ME-71, and Millewa showed heat sensitivity at the seedling stage, but they were heat tolerant at post-anthesis stage. Genotype ME-71 showed heat-susceptibility at the seedling stage as well as post-anthesis stage after 10 DAA heat stress, whereas, it showed better heat tolerance at the grain filling stage after 30 DAA. However, Cook and Bayonet appeared to be heat sensitive at both seedling stage and after 10 DAA and 30DAA. Furthermore, some genotypes that have been reported to be heat-susceptible at the post anthesis stage such as Cook, Oxley, ME-7, were also heat-susceptible at the seedling stage in this study (Wardlaw *et al.*, 1989; Blumenthal *et al.*, 1994; Blumenthal *et al.*, 1995; Stone and Nicolas (1995).

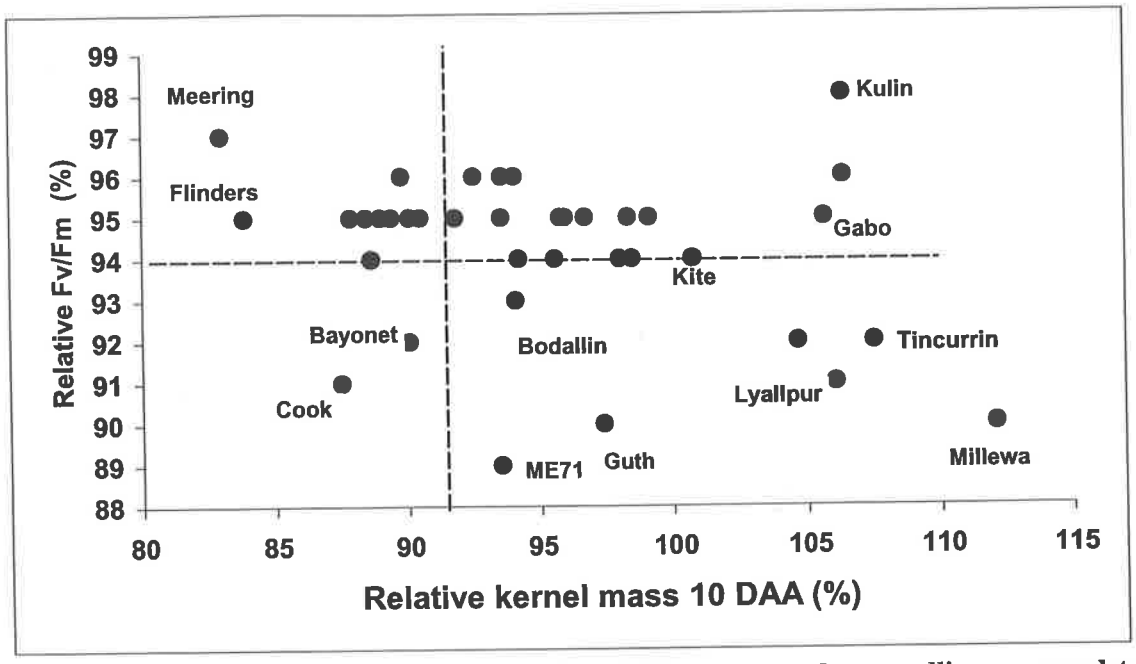


Figure 7.1. Relationship between the relative Fv/Fm measured on seedlings exposed to 40°C for 6 hours (Experiment-1, Chapter-4) and the relative kernel mass after exposure to 40°C at 10 days after anthesis. The data for reductions in kernel mass were obtained from Stone and Nicolas (1995). Horizontal and vertical dashed lines presenting the mean values for the relative values of Fv/Fm and relative kernel mass.

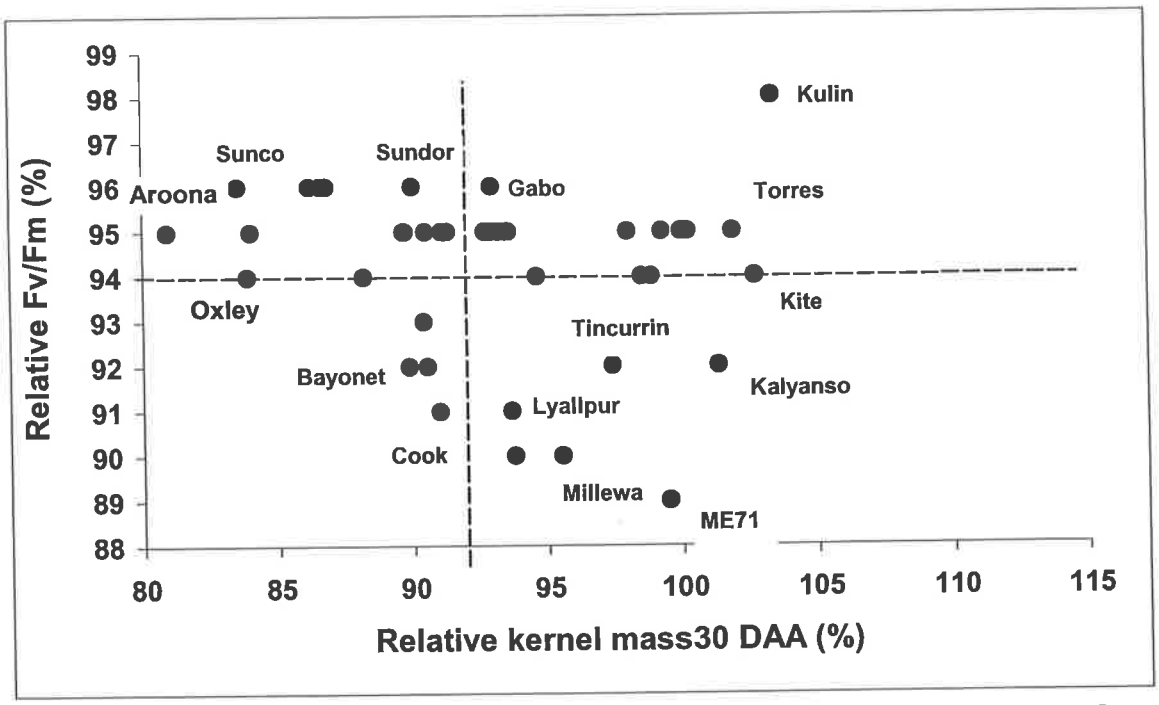


Figure 7.2. Relationship between the relative Fv/Fm measured on seedlings exposed to 40°C for 6 hours (Experiment-1, Chapter-4) and the relative kernel mass after exposure to 40°C at 30 days after anthesis. The data for reductions in kernel mass were obtained from Stone and Nicolas (1995). Horizontal and vertical dashed lines are the mean values for the relative values of Fv/Fm and relative kernel mass.

However, some genotypes showed opposite responses and apparently were tolerant at the seedling stage and sensitivity during grain filling. Thus according to the information from Figure 7.1 and Figure 7.2, the heat-tolerance at the seedling stages is not correlated strongly with the post anthesis heat-tolerance. Considering the different stages of the crop development (seedling stage and grain filling stage) these differentiated response of some of the genotypes were perhaps not unexpected, as the presence of various other physiological and metabolic factors could also be involved for their differential responses at seedling and post anthesis stages. As seedlings, the main effect of heat stress will be on the photosynthetic apparatus and it is expressed as changes in fluorescence characteristics. During grain filling however, the main effect of heat stress is on the activity of the enzymes involved in starch synthesis, especially soluble starch synthase, which is seen in changes in kernel weight (Stone and Nicolas 1995b, 1998a, b). However there are some genotypes that displayed a high level of heat tolerance at both stages, indicating that it is possible to combine tolerance to early and late heat stress. In environments where heat stress can occur both early and late in the growing season, such varieties will be useful.

7.1.2. Recovery of the photosynthetic efficiency after heat-stress in wheat seedlings

In Chapter 4, it was found that there was wide variability among the 100 genotypes in the recovery of photoefficiency from heat stress in seedlings. Results of replicated experiments using representative genotypes confirmed the faster recovery of PSII efficiency in heat-tolerant genotypes than in heat sensitive seedlings (Experiments 2 to 4, Chapter 4). Various studies have shown fast or slow recovery of photoefficiency in various crops and evergreen plants after many abiotic stresses (Bruggermann *et al.*,

1992; Srinivasan and Strasser 1996; Verhoeven *et al.*, 1996). Srinivasan and Strasser (1996) reported recovery of *in vivo* chlorophyll fluorescence Fv/Fm ratios in grain legumes such as groundnut, soybean, pigeonpea and chickpea after heat-stress. They reported that the recovery of PSII efficiency was faster in groundnut than any other grain legume crop which correlated with the reported greater heat tolerance in this species. However, no comparable studies have been reported so far in wheat seedlings.

Recovery of PSII efficiency after heat-stress is a very important factor involved in the tolerance of a genotype to heat stress. (Srinivasan and Strasser 1996). Heat tolerant genotypes recovered the function of photoefficiency relatively more quickly which means that the damage of PSII was reversible. Moreover, the present study showed that recovery of the PSII efficiency in wheat seedlings might be more important for stress tolerance than the reduction of Fv/Fm ratios during heat stress. Under field conditions crops can confront a wide range of fluctuations in heat or water stress. Therefore fast recovery of PSII efficiency shows that heat tolerant genotypes can effectively endure these stresses in the field and recover quickly.

7.1.3 Effect of heat-stress on initial fluorescence, variable fluorescence, maximum fluorescence, Fv/Fm and Tm values

Heat-stress had multiple effects on the photosynthetic efficiency of wheat seedlings affecting all fluorescence parameters including Fo, Fv, Fm, Fv/Fm and Tm values (Chapter 4). Fast changes in all these parameters during heat-stress and then gradual recovery of these parameters after removing heat-stress suggested various physiological and structural changes in the chloroplast and thyalkoid membranes.

Variable fluorescence is an important component of the Fv/Fm ratio because it indicates the balance of energy between reaction centre and antenna of the PSII system and the photochemical processes use this energy balance. Therefore any stress that causes drastic changes in Fv will ultimately reduce the PSII efficiency. Results of this study have shown that drastic decreases in Fv/Fm ratios were associated with severe decreases in Fv as well as Fm values (Chapter 4). Similarly, large increases in Fo values in all genotypes even after heat-stress showed a possible dissociation of light harvesting pigment systems of PSII (Havaux, 1993). The changes in Fv/Fm ratios indicate the maximum quantum yield of PSII efficiency was reduced drastically in response to heat stress due to a decrease in Fv values. This was more apparent in heat-susceptible genotype seedlings than heat tolerant genotypes. The reductions in the Fv/Fm ratios could be used as indicators of heat tolerance (Sayed *et al.*, 1989). Some earlier studies have suggested that for healthy leaves the ratios of Fv/Fm should be in the range of 0.820 to 0.830 and these were confirmed by measuring these ratios on large number of plants (Bjorkman and Demming, 1987). Results of the present study substantiated that before heat and drought stresses, the Fv/Fm ratios in healthier leaves were almost in the same range. As well, decreases in ratios of Fv/Fm approximately below the range of 0.760 to 0.750 especially in heat-sensitive genotypes such as Lyallpur and ME-71 did not recover quickly, whereas, these ratios were observed around 0.780 in heat tolerant genotypes after heat stress such as Anlace, Buckley and Kulin and they recovered more quickly. Hence, it appears that in heat sensitive genotypes, the Fv/Fm ratio is reduced to less than 0.760 or 0.750 in wheat leaves and the recovery after the stress is slow.

In the same way, severe reductions in Fv, Fm and Fv/Fm during time course of heat-stress (Table 4.7, Chapter 4) indicated the severe decreases in the activity of oxygen

evolving complexes and also inhibition of electron transport at the acceptor side of PS-II (Strasser, 1997). This showed that heat-stress, even after one hour, had drastically reduced the stability of the thyalkoid membranes and relative efficiency of electron transport from PSII to PSI, especially in heat-susceptible genotype seedlings (Experiment 4, Chapter 4). As mentioned above that fast recovery from the damage cause by heat-stress is more important after removing the heat-stress, which indicate the thermo-stability of PSII in heat-tolerant genotype seedlings.

7.2 Effect of drought stress on *in vivo* chlorophyll fluorescence in wheat seedlings

Drought stress also affected the *in vivo* chlorophyll fluorescence measurements in wheat seedlings (Chapter 5). Results of water-stress experiments have shown that drought-stress affected the photosynthetic efficiency of PSII almost in the same way as heat-stress affected the PSII efficiency. This is probably because water deficit usually inhibits the photosynthetic carbon metabolism and also causes damage to the electron transport chain in the chloroplast, which ultimately increases the proportion of absorbed light energy that is not utilized by the reaction centres and hence causes heat increase inside the leaf (Reynolds *et al.*, 1994). The results showed that usually the quantum yield of photosynthetic efficiency (Fv/Fm ratios) was reduced gradually after 24 to 36 hours of drought-stress (Experiments 1 and 2, Chapter 5). However, 48 hours of drought-stress and severe drought-stress affected the PSII efficiency (Fv/Fm ratios) drastically in all genotypes showing a comparable severe damage to chloroplast and thyalkoid membranes as caused by the heat stress (Chapter 4 and 5).

Flagella *et al.*, (1995) suggested that changes in the chlorophyll fluorescence measurements could be used as drought-tolerance test in wheat. Therefore an objective

of the present study was to investigate the fluorescence characteristics under water stress and to investigate whether the seedlings of heat-tolerant genotypes were also more tolerant to water-deficit. When under water stress, transpiration decreases and leaf temperature can increase, although not always to levels that may induce heat stress. Whether a degree of thermotolerance is also important in tolerance to water stress has not been investigated. In general the results suggested that the heat-tolerant genotypes were also more tolerant to water-deficit, based on the responses in chlorophyll fluorescence (Experiments 2 and 3, Chapter 5). Since, there were comparatively smaller reductions in Fv/Fm ratios in heat-tolerant genotype seedlings than heat-sensitive seedlings after 48h of drought-stress or under severe water-stress (Experiment 2 and 3, Chapter 5), the data of Experiment 3 (Chapter 4) and Experiment 2 (Chapter 5) were compared (Figure 7.3).

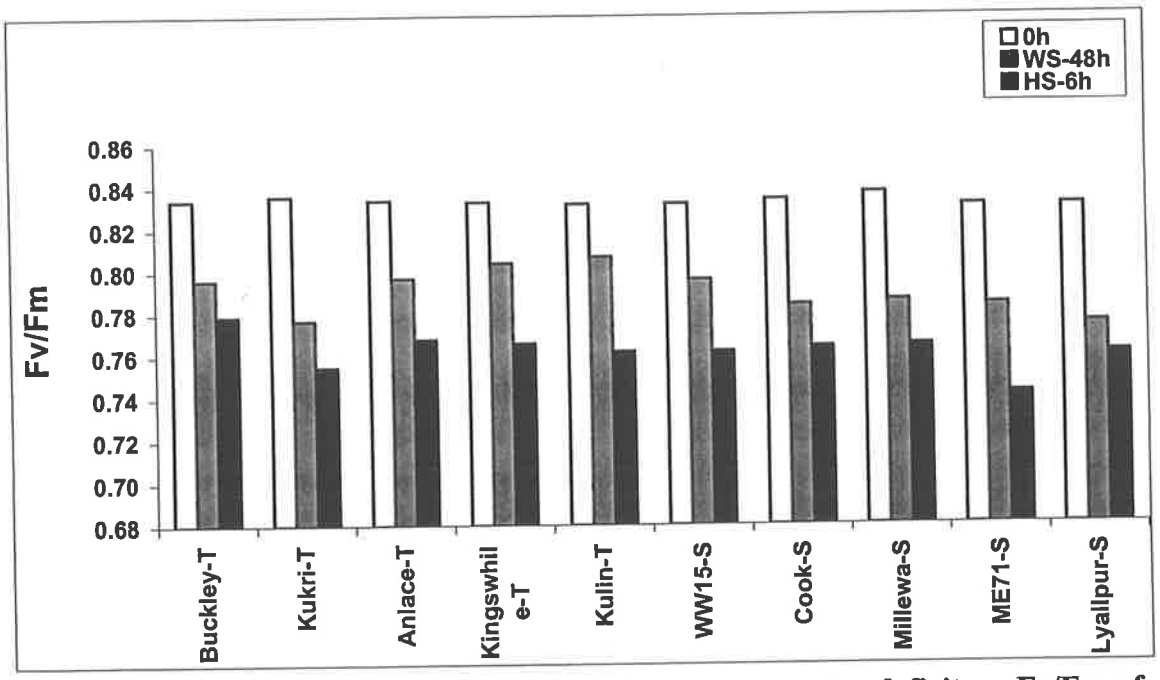


Figure 7.3. Comparison of the effects of heat stress and water deficit on Fv/Fm of seedlings of varieties either tolerant (T) or susceptible (S) to heat stress. Data of Experiment 3 (Chapter 4) and Experiment 2 (Chapter 5) was included. Seedlings were exposed to 40°C for 6 hours or were subject to water stress for 48 hours.

Six hours of heat-stress (40°C) reduced the PSII efficiency more drastically than water-stress of 48 hours. These results are in agreement with many previous studies, which reported that generally high temperature stress affects quantum yield of PSII more severely than water-deficit (Krause, 1988; Havaux *et al.*, 1988; Yang *et al.*, 1996).

Recovery of PSII efficiency was also observed in these seedlings after two water stresses including 48h of gradual drought stress and severe drought stress (Chapter 5). A steady and relatively fast recovery in PSII efficiency was observed in heat-tolerant genotype seedlings Buckley, Anlace, Kukri, Kulin, and Kingswhite including one heat-sensitive genotype WW15 (Figure 7.4, Chapter 5), whereas, a gradual and relatively

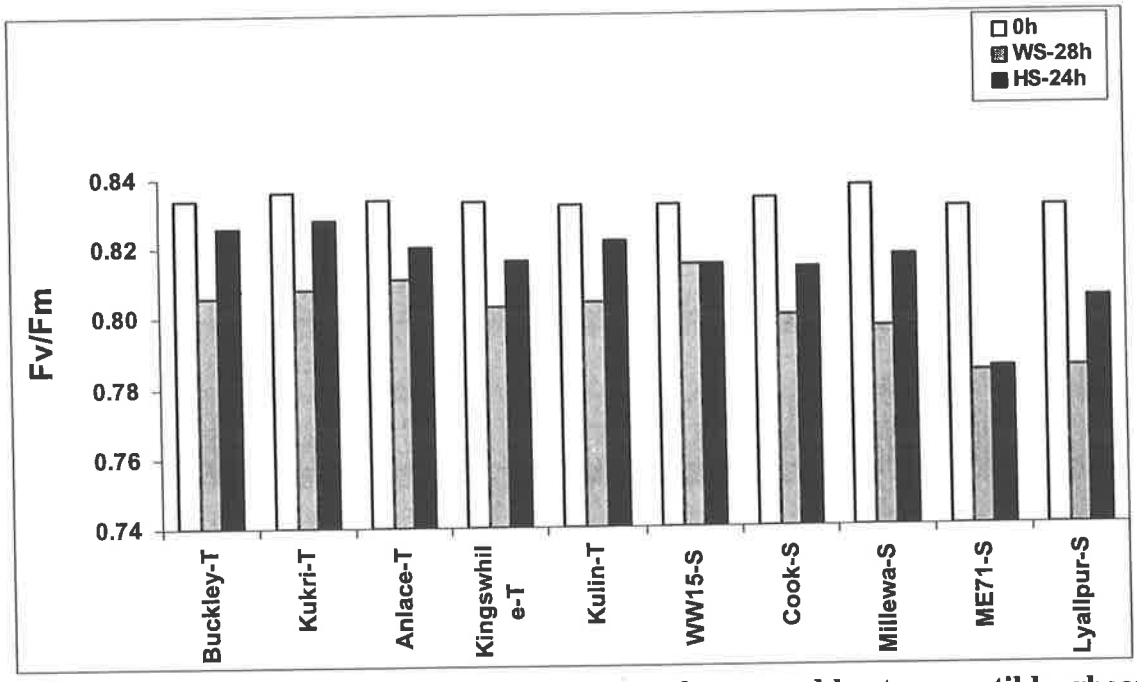


Figure 7.4. Recovery of PSII efficiency in heat-tolerant and heat-susceptible wheat seedlings after 6h of heat-stress (40°C) or 48h of water-stress.

slow recovery in PSII efficiency was observed in heat susceptible genotypes such as Cook, Millewa and ME71. One of the heat-sensitive genotypes, Lyallpur, showed a very drastic reduction in PSII efficiency, even during a recovery phases that included 52

h, 56 h, 80 h and 5-days recovery periods (Figure 5.4 H-J, Chapter 5). The recovery in Fv/Fm after 6h of heat-stress (Experiment 3, Chapter 4) and water stress (Experiment 2, Chapter 5) were compared (Fig 7.4). The recovery in Fv/Fm from drought-stress was smaller in all genotype seedlings except WW15 and ME71. Krause (1991) suggested that recovery of PSII photosystem proceed via a PSII repair cycle, involving the transformation of damaged PSII reaction centres as they were restructured by the D1 protein degradation and replacement of new synthesised proteins. Therefore repairing the damaged structure of PSII was better in heat-tolerant seedlings as compared to heat-sensitive seedlings.

The statistical analysis of the correspondence between heat and water stress tolerance (Figures 7.3 and 7.4) indicates that the two are not strongly correlated. The Spearman's rank correlation (r_s) for Fv/Fm between heat and water stress were:

Absolute values under stress: $r_s = 0.582$, P=0.080

Values relative to the control, under stress: $r_s = 0.531$, P=0.116

Although there are some genotypes that show both heat and water stress tolerance, overall the relationship is not strong. However, there was a stronger and significant relationship between the genotypic differences in the ability to recover from stress: r_s=0.624, (P=0.054). Also the values after recovery were not related to the values under stress for heat stress (r_s = 0.385, non-significant) or water stress (r_s= 0.434, non-significant). This suggests that the recovery was not related to the level of damage under stress; in other words, varieties that were better able to recover from water stress,

also tended to recover better from heat stress, irrespective of the relative differences in maximum damage.

Havaux *et al.*, (1988) reported a strong correlation between heat tolerance and drought tolerance in wheat and other cereal crops based on Fo values and fluorescence quenching. In the present study, fluorescence quenching was not measured, but generally the results of the drought-stress experiments suggested that heat-tolerant genotypes like Buckley, Anlace, Kulin, Kingswhite were also water-deficit tolerant. Likewise one of the heat-sensitive genotype WW15 showed a moderate drought-tolerance. Araus *at al.*, (1998) reported positive genetic correlations between chlorophyll fluorescence parameters and grain yield in wheat. These parameters included Fo (r= 0.88), Fm (r=0.74), Fv (r=0.71) and t ½ (r = -0.92). Therefore, it is suggested that further studies involving more genotypes and considering the other fluorescence measurements at the seedling stage and grain yield be conducted to examine the relationship between two tolerance mechanisms and should be conducted for the confirmation of the results.

The results of the drought experiments need to be viewed within the context of the experimental methods used. The seedlings were grown in small pots and the water stress developed quickly once watering was stopped. Later experiments used different soils and methods of watering to slow the rate of drying and allow a more gradual development of water stress. However, the experimental procedure probably meant that factors such as osmotic adjustment and the ability for root growth to maintain water uptake would not have been fully expressed. The fluorescence characteristics under drought of the varieties tested therefore needs to be confirmed using large soil volumes

and more gradual stress. The fact that under the conditions of the experiment, plants were wilted at the end of the stress period suggests that the differences measured would be most likely to be valid under severe stress in the field, when the plants have become dehydrated. This can occur after prolonged stress of when the plants are exposed to severe atmospheric drying conditions (eg hot, dry winds) even when soil moisture is relatively high.

7.3 Transpiration efficiency and photosynthetic efficiency in wheat seedlings

Gradual water-stress had moderate affects on different morpho-physiological traits in wheat seedlings (Chapter 5). This study showed that although gradual water-stress affected the different morpho-physiological components of wheat seedlings, the effect of gradual water-stress on TE, DW and SLA was not highly significant. Doyle and Fischer (1979) have also shown that water-stress may increase the transpiration efficiency a little but not significantly in wheat under the field conditions. A similar observation was reported by Gifford (1978), who showed that under slow drought-stress, a 70% reduction in transpiration occurred but there was only a 20% increase in TE. Various studies suggested that TE is related to the carbon use efficiency and photoefficiency of plants especially under water-limited environments such as arid and semi-arid regions. Leaf growth can be affected by water-stress but this study showed that gradual water-stress had not affected the leaf area and SLA of most of the genotypes except two, Lyallpur and Millewa, that showed higher SLA (Table 5.4, Chapter 5). The observed differences in SLA of these two genotypes might be attributed to the slight increase in the leaf area of youngest expanded leaf in these cultivars. There are a number of possible explanations for this, but most probably, as various studies have shown, is that sometimes mild water-stress can affect the leaf anatomical and structural features in some plant species- (Masojidek and Hall, 1992).

These changes in leaf anatomy and structures are considered to be structural adjustments or adaptive mechanism for gradual water-deficit tolerance, Flagella *et al.* (1996). However, these genotypes also showed significant decreases in total chlorophyll contents (SPAD values) and photoefficiency (Fv/Fm ratios) despite increases in specific leaf area. Leidi *et al.*, (1999) observed in cotton that despite increase in specific leaf area other drought tolerance traits such as leaf area, osmotic potential and water contents decreased under drought-stress.

Morpho-physiological traits of seedlings of genotype Anlace and Kukri particularly SPAD values and photosynthetic efficiency (Table 5.4, Chapter 5), suggested that Anlace and Kukri might be drought tolerant cultivars comparing with cv. Lyallpur and Millewa. Flagella *et al.*, (1996) have shown a minimum decline in photosynthetic efficiency while increase in osmotic adjustment and leaf water potential in drought tolerant durum wheat cultivars under moderate and severe drought stresses compared with a drought susceptible genotype. The results of the current study also suggested that seedlings of genotype Anlace and Kukri might be drought tolerant as they have shown minimum decreases in photosynthetic efficiency and SPAD values. The recovery of the PSII efficiency was also relatively quicker after removing the water-stress in these genotypes (Experiment 3, Chapter 5). The same genotypes also recovered relatively quicker after removing the heat stress, which suggested that there is a possibility of an association between heat and drought tolerance in these cultivars.

Correlation between TE and other parameters have shown significant negative associations between TE and total water-use, water use under stress and leaf area of the youngest expanded leaf, while SPAD values had strong positive correlations with

Fv/Fm ratios (Table 5.5, Chapter 5). At the end of gradual water-stress, a drastic decline of SPAD values was observed with yellowing of leaves especially in seedlings of Lyallpur and Millewa, showing that stress had affected the total chlorophyll contents and there might be damage to the stromal structure causing a severe decrease in photochemical efficiency. These results also suggested that this could be an accelerated leaf senescence occurred in these genotypes particularly under water-stress. Various studies have reported accelerated leaf senescence in various crops under drought-stress at flag leaf stage (Baker *et al.*, 1997; Ommen *et al.*, 1999). Although, we have not recorded SPAD values in other experiments, the strong association of SPAD with Fv/Fm ratios suggested that these parameters might be used for screening drought tolerant and as well as heat tolerant genotypes at the seedling stage.

7.4 Effect of heat-stress on seedling emergence and coleoptile length in wheat seedlings

High temperature stress at the time of wheat sowing can occur in many parts of the world especially in tropical and sub-tropical regions in Australia and Pakistan. Therefore, the present study was conducted to examine the effect of high temperature (38.5°C) stress on seedling emergence, coleoptile length and thermal time required by the heat-tolerant and heat-sensitive genotypes.

High temperature stress affected the seedling emergence, coleoptile length as well as the thermal time to emergence (Chapter 6). Although there was no significant interaction observed between genotype and temperature, however a significant linear relationship was observed between percent reduction in seedling emergence and percent reduction in coleoptile length (Figure 6.3). The strong correlation between emergence and coleoptile length had been reported previously (Allen *et al.*, 1965; DeJong and Best, 1979;

Schillinger *et al.*, 1998). Similarly, fast seedling emergence is considered to relate not only with good emergence in the field but also has a good relationship between stand establishment and seedling vigour especially under Mediterranean climates in Australia (Richards, 1991). The present study has also confirmed that first leaf length is not associated with seedling emergence and coleoptile length (Schillinger *et al.*, 1998)

Although the present study had not established the relationship between coleoptile length and heat-tolerance of the seedlings, there appeared to be a relationship between 50% and 90% cumulative emergence rate with heat-tolerance and heat susceptibility of the seedlings (Figures 6.1 and 6.2) and this has not been reported before. Generally, both heat-tolerant and heat-sensitive wheat seedlings started to emerge at the same thermal time under the optimum temperature 20°C and high temperature 38.5°C, but the completion of 50% and 90% emergence was different for heat-tolerant and heat-sensitive genotype seedlings (Table 6.3). Seedlings of tolerant and sensitive genotypes started to emerge at 3900°C hour under the optimum temperature, however the t_{50} and t_{90} in heat-tolerant seedlings were 1000°C hour and 2300°C hour respectively, which was similar to the t_{50} and t_{90} for heat-sensitive seedlings (900 and 2200°C hour). Under heat stress, both heat-tolerant and sensitive genotype seedlings started to emerge at 3600°C, but the rate of emergence was quicker in the heat tolerant genotypes. The t_{50} was 1800 °C.hours for tolerant genotypes and 2600°C.hours for sensitive genotypes, and t_{90} in heat-tolerant was 3200°C.hour compared to 3400°C.hours in the sensitive genotypes. Delayed seedling emergence in wheat especially in arid and sub-arid regions of Pakistan could adversely affect the total yield in these regions.

This study showed that heat-stress delayed the seedling emergence, however, heat-tolerant genotypes were relatively less affected than heat-susceptible genotypes. However, more detail studies involving more heat-tolerant and sensitive genotypes as well as other temperature and drought regimes before making any conclusions to establish relationship between heat-tolerance and emergence rate is suggested.

7.5 Chlorophyll fluorescence as a selection tool for heat and drought tolerance

Using the screening methods and experimental conditions described in Chapter 4, a wide range of genotypic variation in wheat genotypes at the seedling stage has been detected by *in vivo* chlorophyll fluorescence. Genetic material used in this study included many cultivars which have been tested for their thermo-tolerance at post anthesis stage and the present study more or less confirmed the heat susceptibility of some the genotypes at the seedling stage such as Lyallpur 73, Millewa, Cook, WW15 and ME71. Detailed experiments had also comparatively revealed the heat-tolerance and drought-tolerance of some of the selected genotypes.

In general, heat-tolerant cultivars were considered those which showed a minimum decrease in Fv/Fm ratios after 6 hours of heat-stress of 40°C, while heat-susceptible genotypes showed maximum decreases in Fv/Fm ratios after the heat stress. This ratio was approximately 0.820 to 0.830 in the leaves of seedling before heat-stress. After the heat-stress, it usually decreased in the range of 0.795 to 0.780 in heat-tolerant genotypes, while it reduced more than 0.760 to 0.750 in heat-sensitive genotype seedlings, commonly. However, detailed experiments have revealed that recovery of Fv/Fm ratios was also important factor in determining the heat-tolerance or heat-sensitivity of the genotype. The Fv/Fm ratios recovered relatively more quickly in heat-tolerant genotype

seedlings than sensitive seedlings and this was also observed both for heat-tolerance and drought-tolerance. Therefore, Fv/Fm ratios were found to be a useful criterion for selecting heat-tolerant or drought-tolerant wheat cultivars at the seedling stage under control growth room conditions. In general, this study provided a suitable methodology and experimental conditions to evaluate or screen large number of wheat genotype seedlings for heat or drought tolerance.

7.6 Implications for breeding and future strategies

The present study showed that a large number of genotypes could be evaluated for heat or drought tolerance relatively quickly and inexpensively under the control growth room conditions by *in vivo* chlorophyll fluorescence. However, water-deficit and high temperature usually occur together under the field conditions and will exert a substantial influence on all plant growth stages from seedling emergence to final yield. Moreover, plant breeders generally are more interested in the final yield and therefore much of the work is still focused on improving the yield potential of the crops under water limited conditions.

Plant breeders have improved the performance of crops for heat and drought resistance or tolerance for better yield and quality using different empirical selection criterion and methodologies. However, several studies have attempted to identify resistance or tolerance mechanisms, which can be used in the development of an earlier and economical selection technique. This study suggested the use of *in vivo* chlorophyll fluorescence to screen large number of genotypes rapidly and economically is feasible, however, selection and evaluation for potential heat and drought tolerance at the seedling stage still needs to address at the final stage of yield improvement and stability. Since the

final objective is to develop high yielding varieties for stressed environments, plant breeders have to rely on relationship between the potential yield as well as heat and drought tolerance in crops at various growth stages. Heat and drought stresses can occur at any crop growth stage, therefore, more detailed studies still need to incorporate various physiological, morphological, bio-chemical and molecular mechanisms to evaluate plants for these stresses and to improve the yield stability. In future, the plant breeders, plant physiologists and molecular biologists need to work to screen large numbers of crop seedlings relatively quickly and inexpensively to evolve the relative heat and drought tolerant cultivars with high yielding capacity.

7.7 Conclusions

1. A wide range of genotypic variability was observed in 100 wheat genotype seedlings for heat-tolerance on the basis of *in vivo* chlorophyll fluorescence.

2. Heat-stress reduced the PS-II efficiency more severely than the drought stress in wheat seedlings.

3. Recovery of PSII efficiency was relatively quicker in heat-tolerant genotypes comparing to heat-sensitive genotypes after removing the heat-stress and drought- stress.

4. Both heat and drought stresses affected all chlorophyll fluorescence parameters including initial fluorescence (Fo), variable fluorescence (Fv), maximum fluorescence (Fm), and ratio of variable to maximum fluorescence (Fv/Fm) and time to reach maximum fluorescence (Tm) values.

5. Drastic increases in Fo values after heat-stress showed that PSII photo-reduction process was inhibited severely even after one hour of heat-stress in wheat seedlings.

6. In general, results have suggested the potential value of Fv/Fm ratios for screening the large numbers of wheat seedlings for heat-tolerance and drought-tolerance.

7. The strong relationship between photosynthetic efficiency (Fv/Fm ratios) and total chlorophyll contents (SPAD) values have suggested their possible role in selecting drought tolerant genotypes at the seedling stage.

8. Heat-stress also affected the seedling emergence and coleoptile length in heat-tolerant and heat-sensitive genotype seedlings.

9. A strong relationship was observed between reductions in coleoptile length and seedling emergence in wheat genotypes.

10. Differences in cumulative emergence rate indicated a possible relationship with tolerance to heat stress in seedlings.

11. This study provided in general, a suitable methodology and experimental conditions to evaluate large number of wheat genotypes at the seedling stage

for heat and drought tolerance relatively more quickly and in-expensively using chlorophyll fluorescence Fv/Fm ratios.

Although this study had demonstrated that it is possible to identify genotypic differences by screening a large number of wheat genotypes for heat and drought stresses effectively and quickly, further studies are needed to test these genotypes under the field conditions and also to measure grain yield to see if there is improved growth and yield.

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APPENDICES

APPENDIX 1

Summary of the analyses of variance for logistic curves used to describe genotypic differences in seedling emergence (Chapter 6). Curves were fitted to data from each replicate and the derived parameters were then analysed by ANOVA. There were no significant effects of heat stress, variety of variety x heat stress interaction.

Table A6.1 Analysis of variance of components of logistic curve.

		Parameter of logistic curve						
SOV	**df**	**a**	**b**	**c**	**bc/4**	**m**	**t_{50}**	**t_{90}**
		Mean squares						
Rep	3	62339	0.03044	59126	2.56	953	34.5	205.8
Variety (Var.)	9	30898	0.05261	31779	23.1	1037	133.8	202.5
Treatment (Trt.)	1	12453	0.00159	15093	0.62	2710	388.4	147
Var. Trt	9	32021	0.03465	32925	16.8	1104	102.4	133.2
Residuel	57	40415	0.05181	42825	18.11	1474	314.1	451.1
Total	79							

Table A6.2. Distribution of sums of squares for the analysis of variance of components of logistic curves shown in Table A6.1

		Parameter of logistic curve						
SOV	**df**	**a**	**b**	**c**	**bc/4**	**m**	**t_{50}**	**t_{90}**
		Sums of squares (%)						
Rep	3	6.1	2.4	5.5	0.5	2.6	0.5	2.1
Variety (Var.)	9	9.1	12.4	8.9	14.9	8.6	5.9	6.2
Treatment (Trt.)	1	0.4	0.0	0.5	0.0	2.5	1.9	0.5
Var. Trt	9	9.4	8.1	9.2	10.8	9.1	4.5	4.1
Residuel	57	75.1	77.1	75.9	73.8	77.2	87.2	87.2
Total	79	100.0	100.0	100.0	100.0	100.0	100.0	100.0

APPENDIX 2

Table 7.1 Individual kernel mass of wheat genotypes exposed to heat stress 10 DAA and 30 DAA (Stone and Nicolas, 1995) and photosynthetic efficiency of seedlings after 6 hours at 40°C.

		Stone and Nicolas data					This study		
	Variety	Kernel wt (mg)			Relative reduction in kernel wt. (%)		Fv/Fm		Reduction
		Control	10 DAA	30 DAA	10 DAA	30 DAA	0 hour	6h HS	%
1	Aroona	47.7	46.9	38.6	98.3	80.9	0.825	0.784	95
2	ME71	39.6	40.1	38.4	93.5	99.5	0.834	0.745	89
3	Bass	38.6	37.0	36.0	95.8	98.0	0.829	0.791	95
4	Bayonet	36.9	37.0	37.5	90.1	90.5	0.836	0.773	92
5	Bodallin	40.2	40.2	36.8	95.5	89.9	0.829	0.762	92
6	Canna	35.5	33.4	32.1	94.1	90.4	0.834	0.775	93
7	Condor	31.6	28.6	28.6	90.5	90.5	0.834	0.792	95
8	Cook	40.0	35.0	36.4	87.5	91.0	0.829	0.757	91
9	Cranbrook	35.9	32.1	32.2	89.4	89.7	0.833	0.792	95
10	Dagger	37.1	32.6	33.8	87.9	91.1	0.829	0.789	95
11	Darkan	42.4	41.0	42.1	96.7	99.3	0.836	0.794	95
12	Dundee	36.2	32.6	36.2	88.6	98.5	0.836	0.787	94
13	Flinders	42.7	35.8	39.8	83.8	93.2	0.827	0.786	95
14	Gabo	29.1	27.4	29.2	106.4	93.0	0.832	0.798	96
15	Gamut	31.6	30.2	29.9	95.6	94.6	0.832	0.781	94
16	Gutha	30.5	29.7	28.6	97.4	93.8	0.837	0.756	90
17	Halberd	38.8	35.3	36.6	94.0	86.6	0.821	0.787	96
18	Hyden	30.7	31.5	29.3	90.1	92.7	0.835	0.793	95
19	Jacup	34.5	30.7	31.5	89.0	91.3	0.827	0.787	95
20	Kalyanson a	30.2	31.6	30.6	104.6	101.3	0.829	0.762	92
21	King	45.8	36.3	45.9	79.3	100.2	0.837	0.796	95
22	Kite	26.4	26.6	27.1	100.8	102.7	0.831	0.782	94

(continued)

Table 7.1 (continued)

23	Kulin	42.9	41.1	41.0	106.4	103.3	0.827	0.808	98
24	Lance	24.4	25.6	25.2	98.5	88.2	0.838	0.788	94
25	Lyallpur 73	34.7	36.8	32.5	106.1	93.7	0.831	0.757	91
26	Matong	36.9	35.4	34.5	95.9	93.5	0.833	0.791	95
27	Meering	34.1	28.3	26.4	83.0	77.4	0.812	0.788	97
28	Mendos	35.5	37.5	33.0	105.6	93.0	0.833	0.795	95
29	Miling	34.3	34.0	32.1	99.1	93.6	0.829	0.786	95
30	Millewa	43.5	41.8	38.3	112.0	95.5	0.832	0.748	90
31	Mokoan	34.9	33.2	32.8	93.5	89.7	0.823	0.786	95
32	Olympic	40.2	36.1	34.9	89.8	86.8	0.833	0.795	95
33	Osprey	63.5	53.8	59.2	88.4	84.0	0.832	0.790	95
34	Oxley	32.1	31.8	30.9	98.0	83.9	0.827	0.781	94
35	Quarrion	30.9	26.3	25.4	94.2	98.9	0.838	0.791	94
36	Spear	35.8	33.5	29.9	78.4	86.2	0.827	0.796	96
37	Sunco	32.4	30.9	32.6	93.6	83.5	0.823	0.788	96
38	Sundor	38.7	41.6	37.7	92.5	90.0	0.827	0.795	96
39	Tincurrin	32.8	27.6	32.4	107.5	97.4	0.832	0.763	92
40	Torres	31.5	31.3	30.8	77.6	101.9	0.839	0.796	95
41	Vulcan	34.2	31.4	34.2	91.8	100.0	0.834	0.788	94
	Mean	36.5	34.4	34.2	94.2	92.7	0.831	0.783	94