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 seeding emergence of wheat as well as grain filling stage. These stresses adversely affect the seeding emergence, coleoptile length, seeding growth and photosynthesis in wheat (Alkatib 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, seeding emergence, coleoptile length and different morpho-physiological traits in wheat seedlings. The main objective was to examine tolerance to these stresses during seeding 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 Nicola, 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 Analyzer (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, Anlase, Krichauff, Kukri, Ianz, Excalibur, Molineux, Aroona and Bindavara, 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, Anlase, Buckley, Kulin, Meerag 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 Beckley, 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 as 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 photoysystems. 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 *in vivo* 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.