THE UNIVERSITY OF ADELAIDE
SCHOOL OF AGRICULTURE, FOOD & WINE

# "PHYSIOLOGICAL ATTRIBUTES OF DROUGHT-ADAPTATION AND ASSOCIATED MOLECULAR MARKERS IN THE SERI/BABAX HEXAPLOID WHEAT (*Triticum aestivum,* L.) POPULATION"

THESIS PRESENTED FOR THE AWARD OF THE PH.D. DEGREE TO

**JUAN JOSÉ OLIVARES-VILLEGAS**

# DEDICATORY

To Sofía, my wife, for your love, everlasting trust and wholehearted support through the marvelous adventure of having embarked into the luminosity of scientific and spiritual discovery.

A mi Mamá Juanita, porque con tu luminoso amor, ejemplo, cariño y cuidados me has enseñado la dicha de la vida y has educado para aprender, respetar, amar y soñar.

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To Norman E. Borlaug, lifelong mentor, friend and guide on the moral obligation of contributing through scientific work to the betterment of humankind.

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# ABSTRACT

Agronomic and physiological traits associated with drought adaptation were assessed within the Seri/Babax recombinant inbred line population, derived from parents similar in height and maturity but divergent in their sensitivity to drought. Field trials under different water regimes were conducted over three years in Mexico and under rainfed conditions in Australia.

Under drought, canopy temperature (CT) was the single-most drought-adaptive trait contributing to a higher performance ($R^2$= 0.71, $p$<0.0001), highly heritable ($h^2$= 0.65, $p$<0.0001) and consistently associated with yield phenotypically ($r$= –0.75, $p$<0.0001) and genetically [$R(g)$= –0.95, $p$<0.0001]. CT epitomises a mechanism of dehydration avoidance expressed throughout the growing season and across latitudes, which can be utilised as a selection criteria to identify high-yielding wheat genotypes or as an important predictor of yield performance under drought.

Early response under drought, suggested by a high association of CT with estimates of biomass at booting ($r$= –0.44, $p$<0.0001), leaf chlorophyll ($r$= –0.22, $p$<0.0001) and plant height ($r$= –0.64, $p$<0.0001), contrast with the small relationships with anthesis and maturity (averaged, $r$= –0.10, $p$<0.0001), and with osmotic potential ($r$= –0.20, $p$<0.0001). Results suggest that the ability to extract water from the soil under increasing soil water deficit is a major attribute of drought adaptation.

Ample genetic variation and significant transgressive segregation under drought suggested a polygenic governance feasible of dissection via molecular markers of CT and associated physiological and agronomic traits. Bulked segregant analysis of selected secondary traits was utilised as an alternative to complete genome mapping, due to a low polymorphism (27%) within the cross and limited chromosomic linkage of loci. The assessment of the extremes of expression in a genotypic subset with a composite molecular database of 127 markers (PCR-based and AFLPs) allowed evaluation of the three hexaploid wheat genomes and coverage of all chromosomic groups, except 3D. One-way analysis of variance indicated significant associations of loci explaining phenotypic variance under drought and rainfed conditions, of 20-70% in Mexico and 20-45% in Australia ($F \geq 5.00$, $p$<0.05). Significant loci were established in both latitudes for all physiological and agronomic

traits assessed via BSA, with CT being the trait with the most numerous associations (in Mexico, 34 loci; in Australia, 24).

Results demonstrate an efficient development of molecular markers associated to physiological traits under specific soil water conditions in Mexico and Australia, and suggest further genomic and transcriptomic studies be conducted for unravelling the complex relationship between drought adaptation and performance under drought.

## DECLARATION

This Ph.D. Thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been stated in the text.

I hereby give consent to this copy of my Ph.D. Thesis being made available in the University Library.

**Juan José Olivares-Villegas**

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# INTRODUCTION

Drought stress is a permanent constraint to wheat (*Triticum aestivum*, L.) production on at least 40 million ha in the developing world and in *ca.*25 million ha in industrialised nations (Morris *et al.* 1991; Byerlee & Moya 1993). Modelling exercises suggest that yield in marginal wheat growing environments is typically reduced by between 50 and 90% of their theoretical irrigated yield potential due to factors associated with water-limited environments (Morris *et al.* 1991). As water resources are likely to decline in the coming decades (World Meteorological Organisation 1997), the areas devoted to wheat production will be increasingly threatened by water availability. Hence, improving wheat adaptation to drought will acquire a greater socioeconomic importance across the globe than it currently has.

Breeding for drought adaptation in wheat has been largely empirical to date, based on drought escape (phenology modification for hydration maintenance) or selection for traits contributing to improved water use efficiency indirectly, such as enhanced tolerance to soil toxicities or deficiencies, or resistance to root diseases (Richards 1996; Trethowan & Pfeiffer 2000). More strategic approaches have been advocated which target one or more specific drought-adaptive (physiological) traits consistently related to yield (Morgan 1983; Ludlow & Muchow 1990; Loss & Siddique 1994; Richards 1996).

Even though drought escape is recognised as an effective strategy in some wheat cultivars for overcoming the pernicious effects of drought stress by phenophasic modification (Ludlow & Muchow 1990; Richards 1991; Loss & Siddique 1994), it compromises yield potential to an extent dependent on the timing of the stress during the crop cycle (Blum 1996). In contrast, adaptation to a drought-stressed environment via a number of bioenergetic (efficiency of the photosynthetic and respiratory systems for carbon fixation and energy consumption), metabolic (nutrient utilisation and assimilates distribution) and physiological (complex regulatory networking and environmentally responsive systems) mechanisms is conducive to maintaining a high level of hydration in varying moisture environments (Bálint 1984; Blum 1988; Goggin & Setter 2004; Xue *et al.* 2006). This is achieved either by reducing transpiration rate (thereby increasing water use efficiency) or by managing transpiration via attributes that sustain *in planta* availability of water resources

(Turner & Begg 1981; Blum 1988). When transpiration is reduced by stomatal closure, gas exchange is affected and carbon fixation is reduced (Turner & Begg 1981; Molnár *et al.* 2004). In contrast, when hydration is maintained through water expenditure, the plant is known to be characterised by a lower canopy temperature, a more open stomata and a higher carbon isotope discrimination ($\Delta^{13}C$) (Araus *et al.* 2002), altogether associated with increased carbon fixation, biomass and yield (Condon *et al.* 1987).

Physiological traits that are integrative, either in time or at an organisational level (Araus *et al.* 2002), constitute ideal selection criteria for drought adaptation. In recent years they have acquired increased importance in breeding programmes largely due to a greater understanding of their relative contribution to yield (Blum *et al.* 1982; Richards *et al.* 2001; Araus *et al.* 2002; Rebetzke *et al.* 2002; Reynolds *et al.* 2005). An ample portfolio of novel indirect selection methodologies that assist in evaluating such integrative traits (Araus 1996; Araus *et al.* 2002) are not only practical, but increasingly cost-efficient tools that can support breeders in screening, early generation or advanced-line selection (Blum *et al.* 1982; Araus *et al.* 2001, 2002; Richards *et al.* 2001; Reynolds *et al.* 1994, 2005). However, there has been little systematic evaluation of these traits in large populations of sister lines over a range of environments and varying drought intensities.

While conventional or physiological breeding strategies have utilised genetic diversity at its various levels of expression for developing drought-adapted genotypes for increased crop productivity, they have encountered limitations in dissecting the complex polygenic interactions associated to the quantitative genotypic response through phenotype selection (Cushman & Bohnert 2000; Ribaut *et al.* 2001). With the advent of an increased diversity of molecular markers (Liu 1998), it has been possible to develop comprehensive genetic maps of virtually any crop species, permitting the location of the genetic factors responsible for subtle, quantitative differences (Sax 1923) or quantitative trait loci (QTLs) (Geldermann 1975), in specific genetic pools. However, the application of QTL mapping to the dissection of drought-adaptive physiological traits in hexaploid wheat has been limited not solely because of a restricted access to markers for map saturation, but as a consequence of the difficulty of obtaining a low repeatability when phenotyping under stressed conditions and, mainly, on the virtual inexistence of suitable genetic pools in where the response is amply diverse and significantly inherited.

## AIMS

*i*. To investigate and understand the physiological and molecular basis for genotypic differences in wheat performance under drought.

*ii*. To evaluate a number of traits linked to drought adaptation in the Seri/Babax recombinant inbred line population, which is characterised by a relatively low variation for phenology and height, factors that might obscure those drought-adaptive traits that directly contribute to an increased performance under drought.

*iii*. To identify genome regions in selected Seri/Babax recombinant inbred lines associated with physiological traits controlling drought adaptation via molecular markers.

*iv*. To develop a better understanding of the genetics and physiology of drought adaptation in the Seri/Babax hexaploid wheat population.

## OBJECTIVES

*i*. To evaluate the genetic diversity for drought adaptation in the Seri/Babax population under drought stress in a number of environments in terms of yield, phenology and physiological attributes.

*ii*. To establish the traits best associated with yield under drought.

*iii*. To discern the drought-adaptation strategy and inherent mechanisms contributing to a higher performance under drought.

*iv*. To assess the feasibility of utilising secondary selection criteria to identify high-yielding hexaploid wheat genotypes.

*v*. To examine the possibility of efficiently develop molecular markers for locating genomic regions explaining the phenotypic variation of selected traits in a number of environments.