Molecular genetics of tolerance to high soil boron and drought in Australian wheat and barley germplasm

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Abbreviations

°C	Degree Celsius
A$	Australian dollar
A^m	A-genome from *Triticum monococcum*
ANU	Australian National University in Canberra
BAC	Bacterial Artificial Chromosome
BC	Backcross
BLAST	Basic Local Alignment Search Tool
CIMMYT	*span*. Centro Internacional de Mejoramiento de Maíz y Trigo
cM	centi Morgan
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DH	doubled haploid
DNA	Deoxyribonucleic Acid
e.g.	For example
ESTs	Expressed Sequence Tags
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Statistical database of the FAO
i.e.	*lat*. "*id est*"; that is, that means
IWGSC	International Wheat Genome Sequencing Consortium
ha	hectare
kb	kilo base pairs
MAS	Marker-Assisted Selection
Mio	Million
PCR	Polymerase-Chain-Reaction
Ppd-D1	Photoperiod sensitivity locus of the D-genome
QTL	Quantitative Trait Locus
RFLP	Restriction Fragment Length Polymorphism
Rht	Reduced height gene
ROS	Reactive Oxygen Species
SNP	Single Nucleotide Polymorphism
t	tons
TaFT-B1	*Triticum aestivum FLOWERING LOCUS T* of the B-genome
Vrn-B1	Vernalization locus of the B-genome

Contents

Introduction and Problem

"... I'd begun wondering too about the aftereffects of empty stomachs. Obviously, peace and prosperity couldn't survive without food; even the most serene society could turn violent. What have hungry people to lose? ... Hungry people are angry people."

Norman Borlaug
(in: Borlaug, Volume 1, page 134, by Noel Vietmeyer 2008)

The worldwide acreage of harvested wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) crops in 2011 accounted for more than 268 Mio ha (FAOSTAT February 2013; http://faostat.fao.org). Among the world food crops wheat ranked number one with 220 Mio ha followed by maize (*Zea mays* L.; 170 Mio ha), rice (*Oryza sativa* L.; 164 Mio ha) and barley (48 Mio ha). Although wheat is the most widely grown crop on a global scale, barley has been well known as being adapted to a very broad ecological range, in particular to more marginal production areas, where wheat cultivation often becomes uneconomical or infeasible (Fischbeck 2002; Stanca et al. 2003). In industrialized countries, barley has lost its status as a staple food, where grains are mostly used for animal feed and malting; human consumption is mainly restricted to subsistence farmers in developing countries (Fischbeck 2002). However, wheat is one of the prime staple foods of a globalized world, wherein wheat prices are traded at stock markets globally and wheat harvests of entire countries can influence global demand and price development (Henn 2011; Williams 2011). Both todays, and future global food security will rely heavily on sufficient availability of maize, rice, wheat and barley crops to keep up with the projected demand from population growth to nine billion people in 2050 (Godfray et al. 2010; Roberts 2011; Ray et al. 2012). Most importantly, this increasing demand must be met simultaneously by increased production from an almost unchanged area of arable land (Lambin 2012), using more sustainable agricultural systems. Notably, the required agricultural intensification must mitigate soil degradation, habitat pollution or

loss, preserve water resources and socio-economic aspects (Evans 2003; Schmidhuber and Tubiello 2007; Lal 2007, 2009). Moreover, projected crop yields globally are threatened by climate change and compete for land with bioenergy and animal feed crops, thus putting another level of uncertainty to future food security (Lobell et al. 2011; Lobell and Gourdji 2012; Valentine et al. 2012). Regional variations due to climate change are anticipated (Lobell and Gourdji 2012) but may often worsen food supply particularly in developing countries as they have usually been associated with already disadvantaged climatic conditions (Vermeulen et al. 2012).

Climate change has also been affecting the Australian continent, which has become warmer (~ +0.8°C) in the last century. In addition, rainfall patterns have become more variable, with increased precipitation in the North, East and South (particularly during summer) but decreased in the West (Hughes 2003; Suppiah et al. 2007). Projections for Australia's future climatic changes vary greatly depending on models and conditions used. However, the most probable annual average temperature increases (relative to 1990) may reside within the range of 0.4 to 2.0°C by 2030 and 1.0 to 6.0°C by 2070 (Hughes 2003). There is an expected large increase in the number of days above 35 and 40°C. However, coastal regions, where a high proportion of the countries' population reside, show lower predicted warming compared to inland regions (outback; Suppiah et al. 2007; Alexander and Arblaster 2009). Expected changes in rainfall patterns will be more complex than temperature alterations but with an overall trend of lower precipitation in the south in winter and spring (Suppiah et al. 2007). Precipitation in Northern Australia may increase due to heavier rain events and cyclone activities whereas the southern Australian climate has been traditionally influenced by the Southern Pacific Oscillation, i.e. El Niño, which is associated with periodic drought events (Hughes 2003). Importantly, most of Australia's cereal belt falls within this southern region (Figure 1) and is hence periodically affected by El Niño events. These events are associated with severe drought damage and even complete crop failure; the projected increased frequency of these events due to climate change may even worsen the situation of many farm-based, rural communities in the future

(Anwar et al. 2007; Hanna et al. 2011). Climatic factors impose major challenges upon the Australian society and the sustainability of its agricultural production system; but soil quality is equally important for an efficient and economically viable agriculture (Rengasamy 2002). The so called 'Dryland Mediterranean Farming System' has been adopted in the majority of Australia's cereal belt, extending from Western Australia across southern Australia into western and northern Victoria. This region is characterized by cool, wet winters and, hot, dry summers with annual rainfall ranging from 300 to 600 mm. In the dryland farming zone, the cereal-livestock farming system pre-dominates and accounts for approximately one third of Australia's total agricultural production (Rovira 1992).

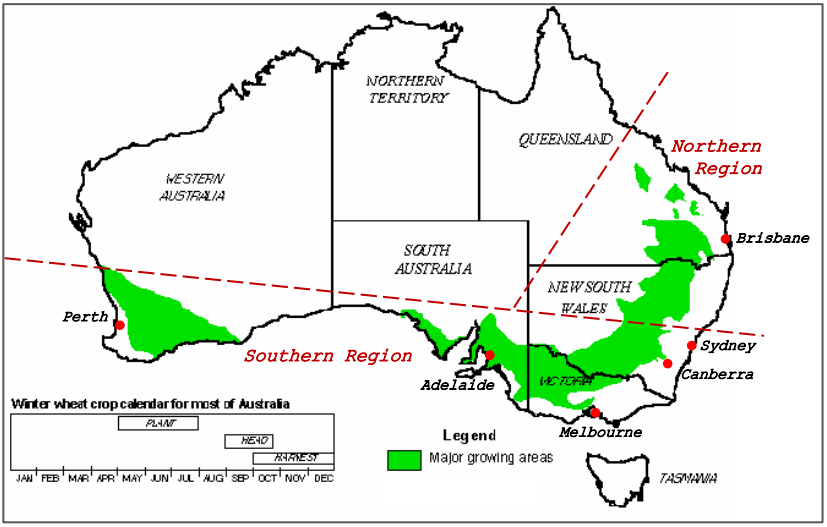

Figure 1: The major growing areas of the Australian cereal belt. Based upon latitude, soil characters and rainfall pattern two largely representative growing regions can be classified. The northern region is mainly characterized by relatively deeper soils with fewer subsoil constraints and predominately summer rainfall, frequently resulting in low post-anthesis water availability. Grain yields mainly rely upon the stored amount of water in the soil after plants had reached anthesis. The southern growing regions often exhibit shallow soils with compact subsoils containing toxic or deficient amounts of nutrients, accompanied by intermittent and variable rainfall events starting during pre-anthesis through to post-anthesis development. Here yields mainly depend upon the frequencies and quantities of irregular rain events. For a more refined characterization of the Australian cereal belt please see Chenu et al. (2013). Also, please note that Australians grow winter cereal crops which get sown under short-day conditions in fall or winter (May to July) and depending upon region get harvested in spring or summer (October to December).

Soils in this region are deep, coarse-textured sands and sandy loams, duplex soils with coarse-textured sands over clay (generally low in nutrients and organic matter), and fine-textured red-brown earths of low hydraulic conductivity. The main production constraints associated with these soils are sodicity (i.e. saline or alkaline soils with excessive sodium chloride (NaCl) or sodium carbonate (Na_2CO_3), respectively), salinity, soil structural degradation, acidity, alkalinity, water repellence, waterlogging, nutrient deficiencies and toxicities due to boron, carbonate, aluminate, and root diseases (Rovira 1992; Rengasamy 2002). Most of these subsoil-derived (i.e. $\geq$ ~50cm) stressors impose production limits up to 50% potential yield and are not accessible for normal soil management practices (Rengasamy 2002; Adcock et al. 2007). Latest analyses estimating the contribution of individual subsoil stressors on yields of bread wheat crops across the Australian cereal belt (12 years!!; 233 field trials!!!) revealed that salinity ranked number one explaining up to 34% of the genetic variation of yield followed by aluminium (26%) and boron (21%) (McDonald et al. 2013). The fact that boron plays an important role for root health and adaptation among the Australian crops had been well established and dates back to the early 1980s, with the discovery that many soils along the cereal belt contained high amounts of plant-available boric acid (H_3BO_3) and other boron-containing salts (Cartwright et al. 1983), such as borax ($Na_2B_4O_7$) or potassium aluminium borate ($K_2Al_2B_2O_7$), that negatively affected growth of barley crops, particularly in South Australia (Cartwright et al. 1984; Cartwright et al. 1986). It is evident that intact and healthy roots are fundamental for high crop yields. This is particularly important in Mediterranean environments, where water is scarce and plant performance depends on the water capture capacity of the root (Rengasamy et al. 2003).

Despite its harsh climatic conditions and subsoil-derived production constraints, Australia has developed an economically competitive agricultural sector that was valued between A$25-30 billion from 2005 to 2010, achieving 93% self-sufficiency with domestic food supply whilst meeting increasing export demands from overseas countries. Wheat ranked number one among the plant-based agricultural commodities with an average annual value of A$5.2

billion (Millar and Roots 2012). Australian cereal production is almost exclusively based on rainfed agriculture, particularly in the southern cereal belt where wheat and barley yields average 1.6 and 1.8 t/ha, respectively (years 2007 to 2011; FAOSTAT 2013). This is clearly below global average yields of wheat (3.0 t/ha) and barley (2.7 t/ha); and substantially lower compared to, for example, yields of German wheat (7.4 t/ha) and barley (6.0 t/ha) (years 2007 to 2011; FAOSTAT 2013). Despite very low average yields, Australian wheat production (total 21.2 Mio t per year; from 2007 to 2011) is almost as productive as wheat production in Germany (23.8 Mio t; FAOSTAT 2013) due to higher wheat acreage (13.5 Mio ha compared to 3.2 Mio ha in Germany) (FAOSTAT 2013). Yield limits in the Australian dryland farming system are usually a result of a combination of abiotic stressors such as drought, subsoil constraints, heat or frost (Rengasamy et al. 2003; Doherty et al. 2010; Passioura and Angus 2010; Zheng et al. 2012). Clearly, investing in a secure and sustainable cereal production is pivotal for the Australian economy and society, particularly in light of the current predicted threat of climate change (Millar and Roots 2012).

Mitigating production constraints can be achieved through various avenues. Genetic improvement of wheat and barley cultivars, with improved adaptation to these constraints appears to be one of the most promising approaches (Rengasamy et al. 2003; Passioura 2006; Adcock et al. 2007; Zheng et al. 2012). To facilitate more rapid genetic gains through well-informed, targeted breeding decisions using marker-assisted selection (MAS) or genomic selection (GS) necessitates a better understanding of the underlying genes or chromosomal regions conferring tolerance to important abiotic stressors. The outlined research presented here has been performed in this spirit and hence has already been providing decisive genetic information and diagnostic markers for breeding programs. Yet results from these studies may also contribute towards a deeper biological insight into the mechanisms providing tolerance to high soil boron and drought in temperate cereals.

The present habilitation thesis comprises 11 peer-reviewed articles covering the areas of adaptation and tolerance to high soil-borne boron levels and drought in Australian wheat and barley germplasm. The main goals of the presented studies were:

(A) To uncover the molecular-genetic base of tolerance to high soil boron in selected highly tolerant wheat and barley germplasm (A1 – A6).

(B) To better understand the physiological, developmental and genetic basis of well-adapted, drought-tolerant Australian bread wheat germplasm (A7 – A11).

2 Peer-reviewed Articles (A1 – A11)

2.1 Boron Studies (A1 – A6)

A1 Schnurbusch T, Hayes J and T Sutton (2010) Boron toxicity tolerance in wheat and barley: Australian perspectives. *Breed Sci* 60: 297–304

A2 Schnurbusch T, Collins NC, Eastwood RF, Sutton T, Jefferies SP and P Langridge (2007) Fine mapping and targeted SNP survey using rice-wheat gene colinearity in the region of the *Bo1* boron toxicity tolerance locus of bread wheat. *Theor Appl Genet* 115: 451–461

A3 Schnurbusch T, Langridge P and T Sutton (2008) The *Bo1*-specific marker AWW5L7 is predictive of boron tolerance status in a range of exotic durum and bread wheats. *Genome* 51: 963–971

A4 Sutton T, Baumann U, Hayes J, Collins NC, Shi B-J, Schnurbusch T, Hay A, Mayo G, Pallotta M, Tester M and P Langridge (2007) Boron-toxicity tolerance in barley arising from efflux transporter amplification. *Science* 318: 1446–1449

A5 Schnurbusch* T, Hayes* J, Hrmova M, Baumann U, Ramesh SA, Tyerman SD, Langridge P and T Sutton (2010) Boron toxicity tolerance in barley through reduced expression of the multifunctional aquaporin HvNIP2;1. *Plant Physiol* 153: 1706–1715 **joint first-authorship*

A6 Pallotta* M, Schnurbusch* T, Hayes J, Hay A, Baumann U, Paull JG, Langridge P and T Sutton (2014) Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. *Nature* 514: 88-91 **joint first-authorship*

2.2 Drought Studies (A7 – A11)

A7 Izanloo A, Condon AG, Langridge P, Tester M and T Schnurbusch (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J Exp Bot* 59: 3327–3346

A8 Bowne JB, Erwin TA, Juttner J, Schnurbusch T, Langridge P, Bacic A and U Roessner (2012) Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. *Mol Plant* 5: 418–429

A9 Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T and P Langridge (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. *Theor Appl Genet* 124: 697–711

A10 Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P and T Schnurbusch (2012) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. *Theor Appl Genet* 125: 255–271

A11 Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P and T Schnurbusch (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. *Theor Appl Genet* 125: 1473–1485

2.1 Boron Studies (A1 – A6)

A1 Schnurbusch T, Hayes J and T Sutton (2010) Boron toxicity tolerance in wheat and barley: Australian perspectives. *Breed Sci* 60: 297–304 (doi:10.1270/jsbbs.60.297)

Abstract

Boron (B) toxicity is a significant constraint to cereal production in regions worldwide, including parts of southern Australia. In recent years, much progress has been made by research groups investigating the molecular and physiological mechanisms involved in B toxicity tolerance in both barley (*Hordeum vulgare* L.) and wheat (*Triticum* sp. L.). In barley, genes have been identified controlling B tolerance at two of the four known B toxicity tolerance loci, both of which encode B transporters. Progress has also been made towards the identification of genes involved in B toxicity tolerance in wheat. Here we describe the current status of this work, in the context of B toxicity tolerance research in Australia and internationally. We also summarize prospects for breeding new cereal varieties with B toxicity tolerance in the future.

Received 7 September 2010. Accepted 20 October 2010.

2.1 Boron Studies (A1 – A6)

A2 Schnurbusch T, Collins NC, Eastwood RF, Sutton T, Jefferies SP and P Langridge (2007) Fine mapping and targeted SNP survey using rice-wheat gene colinearity in the region of the *Bo1* boron toxicity tolerance locus of bread wheat. (doi:10.1007/s00122-007-0579-0)
Theor Appl Genet 115: 451–461

Abstract

Toxicity due to high levels of soil boron (B) represents a significant limitation to cereal production in some regions, and the *Bo1* gene provides a major source of B toxicity tolerance in bread wheat (*Triticum aestivum* L.). A novel approach was used to develop primers to amplify and sequence gene fragments specifically from the *Bo1* region of the hexaploid wheat genome. Single-nucleotide polymorphisms (SNPs) identified were then used to generate markers close to *Bo1* on the distal end of chromosome 7BL. In the 16 gene fragments totaling 19.6 kb, SNPs were observed between the two cultivars Cranbrook and Halberd at a low frequency (one every 613 bp). Furthermore, SNPs were distributed unevenly, being limited to only two genes. In contrast, RFLP provided a much greater number of genetic markers, with every tested gene identifying polymorphism. *Bo1* previously known only as a QTL was located as a discrete Mendelian locus. In total, 28 new RFLP, PCR and SSR markers were added to the existing map. The 1.8 cM *Bo1* interval of wheat corresponds to a 227 kb section of rice chromosome 6L encoding 21 predicted proteins with no homology to any known B transporters. The co-dominant PCR marker AWW5L7 co-segregated with *Bo1* and was highly predictive of B tolerance status within a set of 94 Australian bread wheat cultivars and breeding lines. The markers and rice colinearity described here represent tools that will assist B tolerance breeding and the positional cloning of *Bo1*.

Received 11 January 2007. Accepted 21 May 2007. Published online 15 June 2007.

2.1 Boron Studies (A1 – A6)

A3 Schnurbusch T, Langridge P and T Sutton (2008) The *Bo1*-specific marker AWW5L7 is predictive of boron tolerance status in a range of exotic durum and bread wheats. *Genome* 51: 963–971 (doi: 10.1139/G08-084)

Abstract

High soil boron (B) constitutes a major soil problem in many parts of the world, particularly in low-rainfall areas and land under irrigation. Low accumulation of B in the shoot or grain of cereal crops is correlated with the maintenance of biomass production and grain yield under high B conditions, suggesting that this trait is an important component of field tolerance. A novel screening protocol to measure B accumulation in aerated and supported hydroponics was validated using a set of known and exotic bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) accessions. Furthermore, B accumulation in two *Triticum urartu* Tumanian ex Gandilyan and 54 *Triticum monococcum* L. accessions was measured and showed considerable phenotypic variation. However, B accumulation in these lines was higher than that observed in the most tolerant durum or bread wheats. Mapping of high B tolerance in the durum population AUS14010/Yallaroi revealed a locus possibly allelic to *Bo1*, a major source of B toxicity tolerance previously identified in bread wheat. Here, we show that the *Bo1*-specific codominant PCR marker AWW5L7 is predictive of B tolerance status among exotic durum and bread wheat accessions. All tolerant durum accessions assayed carried very similar AWW5L7 marker fragments, indicating wide distribution of this allele among tolerant durum wheats. Three bread wheat accessions had tolerance that was independent of *Bo1* and is probably located on chromosome 4A. These lines represent a valuable genetic resource for B toxicity tolerance breeding in wheat.

Received 15 May 2008. Accepted 13 September 2008. Published online November 17 2008.

2.1 Boron Studies (A1 – A6)

A4 Sutton T, Baumann U, Hayes J, Collins NC, Shi B-J, Schnurbusch T, Hay A, Mayo G, Pallotta M, Tester M and P Langridge (2007) Boron-toxicity tolerance in barley arising from efflux transporter amplification. (doi: 10.1126/science. 1146853) *Science* 318: 1446–1449

Abstract

Both limiting and toxic soil concentrations of the essential micronutrient boron represent major limitations to crop production worldwide. We identified *Bot1*, a *BOR1* ortholog, as the gene responsible for the superior boron-toxicity tolerance of the Algerian barley landrace Sahara 3771 (Sahara). *Bot1* was located at the tolerance locus by high-resolution mapping. Compared to intolerant genotypes, Sahara contains about four times as many *Bot1* gene copies, produces substantially more *Bot1* transcript, and encodes a Bot1 protein with a higher capacity to provide tolerance in yeast. *Bot1* transcript levels identified in barley tissues are consistent with a role in limiting the net entry of boron into the root and in the disposal of boron from leaves via hydathode guttation.

Received 21 June 2007. Accepted 9 October 2007. Published 30 November 2007.

2.1 Boron Studies (A1 – A6)

A5 Schnurbusch* T, Hayes* J, Hrmova M, Baumann U, Ramesh SA, Tyerman SD, Langridge P and T Sutton (2010) Boron toxicity tolerance in barley through reduced expression of the multifunctional aquaporin HvNIP2;1. (doi: 10.1104/pp.110.158832) *Plant Physiol* 153: 1706–1715 **joint first-authorship*

Abstract

Boron (B) toxicity is a significant limitation to cereal crop production in a number of regions worldwide. Here we describe the cloning of a gene from barley (*Hordeum vulgare*), underlying the chromosome 6H B toxicity tolerance quantitative trait locus. It is the second B toxicity tolerance gene identified in barley. Previously, we identified the gene *Bot1* that functions as an efflux transporter in B toxicity-tolerant barley to move B out of the plant. The gene identified in this work encodes HvNIP2;1, an aquaporin from the nodulin-26-like intrinsic protein (NIP) subfamily that was recently described as a silicon influx transporter in barley and rice (*Oryza sativa*). Here we show that a rice mutant for this gene also shows reduced B accumulation in leaf blades compared to wild type and that the mutant protein alters growth of yeast (*Saccharomyces cerevisiae*) under high B. HvNIP2;1 facilitates significant transport of B when expressed in *Xenopus* oocytes compared to controls and to another NIP (NOD26), and also in yeast plasma membranes that appear to have relatively high B permeability. We propose that tolerance to high soil B is mediated by reduced expression of HvNIP2;1 to limit B uptake, as well as by increased expression of Bot1 to remove B from roots and sensitive tissues. Together with Bot1, the multifunctional aquaporin HvNIP2;1 is an important determinant of B toxicity tolerance in barley.

Received 4 May 2010. Accepted 21 June 2010. Published online 25 June 2010.

2.1 Boron Studies (A1 – A6)

A6 Pallotta* M, Schnurbusch* T, Hayes J, Hay A, Baumann U, Paull JG, Langridge P and T Sutton (2014) Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. (doi: 10.1038/nature13538) **joint first-authorship* *Nature* 514: 88–91

Abstract

Environmental constraints severely restrict crop yields in most production environments, and expanding the use of variation will underpin future progress in breeding. In semi-arid environments boron toxicity constrains productivity, and genetic improvement is the only effective strategy for addressing the problem[1]. Wheat breeders have sought and used available genetic diversity from landraces to maintain yield in these environments; however, the identity of the genes at the major tolerance loci was unknown. Here we describe the identification of near-identical, root-specific boron transporter genes underlying the two major-effect quantitative trait loci for boron tolerance in wheat, *Bo1* and *Bo4* (ref. 2). We show that tolerance to a high concentration of boron is associated with multiple genomic changes including tetraploid introgression, dispersed gene duplication, and variation in gene structure and transcript level. An allelic series was identified from a panel of bread and durum wheat cultivars and landraces originating from diverse agronomic zones. Our results demonstrate that, during selection, breeders have matched functionally different boron tolerance alleles to specific environments. The characterization of boron tolerance in wheat illustrates the power of the new wheat genomic resources to define key adaptive processes that have underpinned crop improvement.

Received 30 October 2013. Accepted 28 May 2014. Published online 02 July 2014.

2.2 Drought Studies (A7 – A11)

A7 Izanloo A, Condon AG, Langridge P, Tester M and T Schnurbusch (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J Exp Bot* 59: 3327–3346

A8 Bowne JB, Erwin TA, Juttner J, Schnurbusch T, Langridge P, Bacic A and U Roessner (2012) Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. *Mol Plant* 5: 418–429

A9 Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T and P Langridge (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. *Theor Appl Genet* 124: 697–711

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A7 Izanloo A, Condon AG, Langridge P, Tester M and T Schnurbusch (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. (doi: 10.1093/jxb/ern199) *J Exp Bot* 59: 3327–3346

Abstract

In the South Australian wheat belt, cyclic drought is a frequent event represented by intermittent periods of rainfall which can occur around anthesis and post-anthesis in wheat. Three South Australian bread wheat (*Triticum aestivum* L.) cultivars, Excalibur, Kukri, and RAC875, were evaluated in one greenhouse and two growth-room experiments. In the first growth-room experiment, where plants were subjected to severe cyclic water-limiting conditions, RAC875 and Excalibur (drought-tolerant) showed significantly higher grain yield under cyclic water availability compared to Kukri (drought-susceptible), producing 44% and 18% more grain compared to Kukri, respectively. In the second growth-room experiment, where plants were subjected to a milder drought stress, the differences between cultivars were less pronounced, with only RAC875 showing significantly higher grain yield under the cyclic water treatment. Grain number per spike and the percentage of aborted tillers were the major components that affected yield under cyclic water stress. Excalibur and RAC875 adopted different morpho-physiological traits and mechanisms to reduce water stress. Excalibur was most responsive to cyclic water availability and showed the highest level of osmotic adjustment (*OA*), high stomatal conductance, lowest ABA content, and rapid recovery from stress under cyclic water stress. RAC875 was more conservative and restrained, with moderate *OA*, high leaf waxiness, high chlorophyll content, and slower recovery from stress. Within this germplasm, the capacity for osmotic adjustment was the main physiological attribute associated with tolerance under cyclic water stress which enabled plants to recover from water deficit.

Received 30 April 2008. Accepted 23 June 2008. Published online 13 August 2008.

2.2 Drought Studies (A7 – A11)

A8 Bowne JB, Erwin TA, Juttner J, Schnurbusch T, Langridge P, Bacic A and U Roessner (2012) Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. (doi: 10.1093/mp/ssr114) *Mol Plant* 5: 418–429

Abstract

Drought has serious effects on the physiology of cereal crops. At the cellular and specifically the metabolite level, many individual compounds are increased to provide osmoprotective functions, prevent the dissociation of enzymes, and to decrease the number of reactive oxygen species present in the cell. We have used a targeted GC–MS approach to identify compounds that differ in three different cultivars of bread wheat characterized by different levels of tolerance to drought under drought stress (Kukri, intolerant; Excalibur and RAC875, tolerant). Levels of amino acids, most notably proline, tryptophan, and the branched chain amino acids leucine, isoleucine, and valine were increased under drought stress in all cultivars. In the two tolerant cultivars, a small decrease in a large number of organic acids was also evident. Excalibur, a cultivar genotypically related to Kukri, showed a pattern of response that was more similar to Kukri under well-watered conditions. Under drought stress, Excalibur and RAC875 had a similar response; however, Excalibur did not have the same magnitude of response as RAC875. Here, the results are discussed in the context of previous work in physiological and proteomic analyses of these cultivars under drought stress.

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2.2 Drought Studies (A7 – A11)

A9 Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T and P Langridge (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. (doi: 10.1007/s00122-011-1740-3) *Theor Appl Genet* 124: 697–711

Abstract

In southern Australia, where the climate is predominantly Mediterranean, achieving the correct flowering time in bread wheat minimizes the impact of in-season cyclical and terminal drought. Flag leaf glaucousness has been hypothesized as an important component of drought tolerance but its value and genetic basis in locally adapted germplasm is unknown. From a cross between Kukri and RAC875, a doubled-haploid (DH) population was developed. A genetic linkage map consisting of 456 DArT and SSR markers was used to detect QTL affecting time to ear emergence and Zadoks growth score in seven field experiments. While ear emergence time was similar between the parents, there was significant transgressive segregation in the population. This was the result of segregation for the previously characterized *Ppd-D1a* and *Ppd-B1* photoperiod responsive alleles. QTL of smaller effect were also detected on chromosomes 1A, 4A, 4B, 5A, 5B, 7A and 7B. A novel QTL for flag leaf glaucousness of large, repeatable effect was detected in six field experiments, on chromosome 3A (*QW.aww-3A*) and accounted for up to 52 percent of genetic variance for this trait. *QW.aww-3A* was validated under glasshouse conditions in a recombinant inbred line population from the same cross. The genetic basis of time to ear emergence in this population will aid breeders' understanding of phenological adaptation to the local environment. Novel loci identified for flag leaf glaucousness and the wide phenotypic variation within the DH population offers considerable scope to investigate the impact and value of this trait for bread wheat production in southern Australia.

Received 15 May 2011. Accepted 18 October 2011. Published online 2 November 2011.

2.2 Drought Studies (A7 – A11)

A10 Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P and T Schnurbusch (2012) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. (doi: 10.1007/s00122-012-1831-9) *Theor Appl Genet* 125: 255–271

Abstract

In the water-limited bread wheat production environment of southern Australia, large advances in grain yield have previously been achieved through the introduction and improved understanding of agronomic traits controlled by major genes, such as the semi-dwarf plant stature and photoperiod insensitivity. However, more recent yield increases have been achieved through incremental genetic advances, of which, breeders and researchers do not fully understand the underlying mechanism(s). A doubled haploid population was utilised, derived from a cross between RAC875, a relatively drought-tolerant breeders' line and Kukri, a locally adapted variety more intolerant of drought. Experiments were performed in 16 environments over four seasons in southern Australia, to physiologically dissect grain yield and to detect quantitative trait loci (QTL) for these traits. Two stage multi-environment trial analysis identified three main clusters of experiments (forming distinctive environments, ENVs), each with a distinctive growing season rainfall patterns. Kernels per square metre were positively correlated with grain yield and influenced by kernels per spikelet, a measure of fertility. QTL analysis detected nine loci for grain yield across these ENVs, individually accounting for between 3 and 18% of genetic variance within their respective ENVs, with the RAC875 allele conferring increased grain yield at seven of these loci. These loci were partially dissected by the detection of co-located QTL for other traits, namely kernels per square metre. While most loci for grain yield have previously been reported, their deployment and effect within local germplasm are now better understood. A number of novel loci can be further exploited to aid breeders' efforts in improving grain yield in the southern Australian environment.

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2.2 Drought Studies (A7 – A11)

A11 Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P and T Schnurbusch (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. (doi: 10.1007/s00122-012-1927-2)
Theor Appl Genet 125: 1473–1485

Abstract

A large proportion of the worlds' wheat growing regions suffers water and/or heat stress at some stage during the crop growth cycle. With few exceptions, there has been no utilisation of managed environments to screen mapping populations under repeatable abiotic stress conditions, such as the facilities developed by the International Wheat and Maize Improvement Centre (CIMMYT). Through careful management of irrigation and sowing date over three consecutive seasons, repeatable heat, drought and high yield potential conditions were imposed on the RAC875/Kukri doubled haploid population to identify genetic loci for grain yield, yield components and key morpho-physiological traits under these conditions. Two of the detected quantitative trait loci (QTL) were located on chromosome 3B and had a large effect on canopy temperature and grain yield, accounting for up to 22 % of the variance for these traits. The locus on chromosome arm 3BL was detected under all three treatments but had its largest effect under the heat stress conditions, with the RAC875 allele increasing grain yield by 131 kg ha^{-1} (or phenotypically, 7 % of treatment average). Only two of the eight yield QTL detected in the current study (including linkage groups 3A, 3D, 4D 5B and 7A) were previously detected in the RAC875/Kukri doubled haploid population; and there were also different yield components driving grain yield. A number of discussion points are raised to understand differences between the Mexican and southern Australian production environments and explain the lack of correlation between the datasets. The two key QTL detected on chromosome 3B in the present study are candidates for further genetic dissection and development of molecular markers.

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3 General Discussion

The following general discussion shall summarize the most important findings of the peer-reviewed articles A1 to A11 and discuss the obtained results in a broader context. A detailed discussion of specific findings has been avoided. Specific aspects have already been part of the original articles and hence shall be referred to. Moreover, background information to the individual studies, including objectives, materials and methods as well as a detailed presentation of the results shall be found in the original articles.

3.1 Selection for root health as a key adaptive trait for the Australian cereal germplasm

The history of cereal production on the Australian continent is rather recent and started only 225 years ago with the first British settlement on 26 January 1788 at a site close to what nowadays is better known as Sydney. Although the continent had been inhabited for more than ~40.000 years by aboriginal people, the Australian landscape had never seen wheat or barley plants before. Expectedly, first cultivar imports into Australia originated predominantly from the UK and other western European countries and aimed at examining the cultivars' abilities for the Australian growing conditions (Spennemann 2001). First farmers quickly realized that Australian soil and climate conditions were quite different from those in Europe. As a consequence, seed suppliers started to buy seed from many different parts of the world to search for better adapted varieties (Spennemann 2001). This *trial-and-error* approach was met with limited success for the first hundred years of the British settlement. The situation significantly improved after 1889 through the introduction of cross-breeding by William J. Farrer (1845 – 1906) and Hugh Pye (1860 – 1942) the two pioneer breeders of the Australian wheat breeding (Wrigley 1981; Sims 1988). Through experimenting, Farrer had found that Indian wheat cultivars matured early enough to avoid hot, dry winds and terminal drought, were rust resistant and short-stature; whereas Canadian cultivars possessed excellent baking qualities but were too late in maturity. Cross-hybridizations

and selections of these types produced his first commercially successful cultivar 'Yandilla' at around 1900 (Wrigley 1981). A 'Yandilla'-derived selection resulted in the development of the cultivar 'Federation' (pedigree: 'Yandilla'/14A, a 'Purple Straw') which was released in 1901. Due to 'Federation's exceptional yield performance it became the most widely grown bread wheat variety in the whole continent between 1910 and 1925 (Wrigley 1981). Pye, who worked as a schoolteacher and college principle, started cereal cross-breeding autodidactically. It was only after contact with Farrell in 1889 that he fully committed to wheat breeding (Sims 1988). Using a back-crossing strategy Pye released his first wheat cultivar 'Improved Steinwedel' in 1899 which was followed by several other breeds until 1912 when he released 'Currawa', his most successful bread wheat variety which became number two behind 'Federation' in the mid-1920s (Sims 1988). 'Currawa' was an interspecific hybridization between 'True Club' (hexaploid) and durum wheats (tetraploid) with a view to combining high yield with adaptation to moderate to low rainfall areas (Sims 1988). Early growers and breeders thus realized that cereal breeds for the Australian continent required special adaptation in order to perform and that targeted breeding resulted in adapted cultivars with substantially improved yields.

Further developments in the fields of geology, soil sciences, agronomy and plant nutrition moved soil fertility in the spotlight, revealing that most Australian soils were old, leached and deficient in particular for micro-nutrients and phosphorus (Stephens and Donald 1959). However, despite greatly improved agronomic practices, in terms of fertilizer application and nutrient availabilities within the second half of the 20th century, many crop yields were still low and remained under their yield potential, mainly as a result of severe subsoil constraints (Rengasamy et al. 2003). Figure 2 (see below) illustrates the extent of subsoil constraints within the dryland farming system of the Australian cereal belt, highlighting the physical, chemical and biological impediments faced by plant roots. Most problematic is that many of these stressors occur together in the same field with stark spatial variation within and across sites, thus leading to the conclusion that breeding for tolerances against such

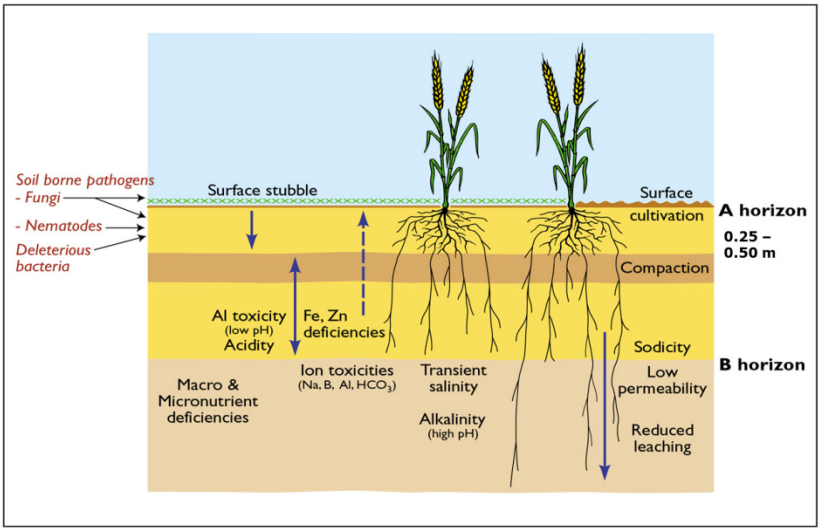

Figure 2: Root zone soil constraints limiting productivity in the drylands of the Australian cereal belt. Conceptual outline of physical (i.e. compaction, crusting, hardsetting), chemical (salinity, sodicity, acidity, alkalinity, ion deficiency and toxicity) and biological (soil-borne pathogens) subsoil factors impeding root growth; modified from Rengasamy et al. (2003).

stressors should be best performed in selected stress-specific field trails to maximize selection gains (Rengasamy et al. 2003).

Boron plays an important role for root health and adaptation among the Australian crops (Rengasamy et al. 2003; McDonald et al. 2013), particularly in South Australia (Cartwright et al. 1984; Cartwright et al. 1986). Alerted by these findings, Nable (1988) and Paull et al. (1988, 1990) searched for phenotypic variation of tolerance to high soil boron within the bread wheat gene pool and found that the Australian cultivar 'Halberd', released in 1969, as well as one accession from Greece, were among the most tolerant genotypes. Results obtained from pot experiments showed an overall good agreement with analyses from field-grown wheats, establishing the basis for an efficient greenhouse and seedling screen (Paull et al. 1988; Paull 1990). Later, seedling tests and root length assays were performed on filter paper and in solution culture using high boron

supply (Chantachume et al. 1995). Detailed inheritance studies among contrasting bread wheat genotypes, including 'Halberd' and Greek, revealed that the 'Halberd' and Greek derived tolerance sources were genetically different and that tolerance to high boron followed a simple monogenic distribution with two major loci on chromosomes 4A and 7B (Paull 1990; Paull et al. 1991; Paull et al. 1992). Related results were also obtained for durum wheats, which showed very similar levels of tolerance with a simple genetic basis on chromosome 7B (Jamjod 1996). Here, the Chinese durum accession AUS14010 'Lingzhi' showed the highest level of tolerance and its tolerance locus was transferred into the first boron tolerant Australian durum cultivar 'Kalka', released in 2003, bred by A.J. Rathjen.
In his paper from 1988, Nable also described the highly boron tolerant barley accession 'Sahara' among landraces from Northern Africa. However, due to inconsistencies in the naming of various accessions from the Sahara region, it is not clear which of these accessions later became 'Sahara 3771'. In this article from 1988, the name 'Sahara 3771' appeared only once at the bottom of page 46; whereas all 'Sahara' accessions in tables had different numbers. Since accession 'Sahara 3763' appeared phenotypically superior to the other two accessions it seems most likely that the most boron tolerant accession had been selected for further work. Yet, studies in barley and wheat consistently found that tolerance to high boron conditions was mainly conferred through the ability to exclude boron at the root level (Nable 1988, 1991; Hayes and Reid 2004).

Through progress in molecular-genetic methods and the introduction of molecular markers made from DNA sequences in the 1990s, more detailed genetic studies, including quantitative trait locus (QTL) analyses became available. Boron research was still on the agenda since anecdotal evidence among breeders and farmers implicated that "*commercially successful cultivars must have a certain degree of adaptation to high soil boron*", particularly for South Australia. With this in mind, Peter Langridge and colleagues developed experimental mapping populations and were able to perform the first QTL analyses on the tolerance to high boron in the wheat 'Cranbrook' x 'Halberd' doubled haploid (DH) population (Jefferies et

al. 2000) and the barley 'Clipper' x 'Sahara' DH population (Jefferies et al. 1999). For barley, Jefferies et al. (1999) identified four significant QTL on chromosomes 2H, 3H, 4H and 6H with the 4H QTL having a major effect on root length, shoot weight and boron uptake. The genetics of tolerance to high soil boron in wheat appeared less complex and confirmed the previous position of the *Bo1* locus in 'Halberd' on the long arm of chromosome 7B (Paull 1990; Jefferies et al. 2000). For the major tolerance loci in both species, closely link molecular markers were identified and for the first time it became possible to apply marker-assisted selection (MAS) in breeding material using RFLPs.

At the time, RFLPs were rather costly, laborious, low-throughput and not cost effective compared to phenotypic seedling assays for boron tolerance in breeding programs. Hence, PCR-based molecular markers were in strong demand and the articles A2 and A3 of the present habilitation thesis deal with the development and usage of a gene-derived PCR marker closely linked to the *Bo1* locus in bread and durum wheats. The study in A2 builds upon work established by Jefferies et al. (2000) and used the 'Cranbrook' x 'Halberd' DH population for further fine mapping of the *Bo1* genomic region as a monogenic trait using RFLP and PCR-based markers. There was a lack of genomic sequence information in polyploid wheats, so the development and fine mapping of both marker types relied solely upon syntenic relationships with the recently sequenced rice genome (Feng et al. 2002; Sasaki et al. 2002; Matsumoto et al. 2005), as well as upon the availability of sufficient wheat ESTs (Hossain et al. 2004; Lazo et al. 2004; Qi et al. 2004). Further mapping of RFLP, microsatellite and PCR-based, gene-derived markers localized *Bo1* to a 1.8 cM genetic interval corresponding to an approximately 227 kb syntenic genomic region on rice chromosome 6L, encoding 21 predicted rice proteins but with no homology to any known boron transporter. Putative candidate proteins for boron transport had been obtained from the *Arabidopsis* BOR1 transporter, showing polypeptide similarities to bicarbonate transporters in animals; AtBOR1 showed responsible for boron uptake and xylem loading in the root under boron deficient conditions (Takano et al. 2002; Takano et al. 2005). A second putative candidate class of boron

transporters belonged to the large family of major intrinsic proteins (MIPs) within the subclass of the NOD26-like intrinsic proteins (NIPs); AtNIP5;1 represented a boron channel whose expression became inducible in young root parts upon boron deprivation (Takano et al. 2006; Tanaka et al. 2011). Our study also showed that RFLP markers were much more polymorphic compared with the PCR-based, gene-derived sequences of the B genome. The very low level of sequence-polymorphism between the two mapping parents in the B genome-derived gene fragments and difficulties in generating B genome-specific amplification were the main reason why this PCR approach yielded a low number of mappable markers; similar results had been obtained by Bryan et al. (Bryan et al. 1999) and Ravel et al. (Ravel et al. 2006). However, at least one of the two PCR-based, gene-derived markers, AWW5L7, co-segregated with *Bo1* in 161 DH individuals and appeared to be near diagnostic in a set of 94 Australian hexaploid bread wheat cultivars. The results from the germplasm screen also revealed that tolerance to high soil boron in Australian bread wheat germplasm relied almost entirely on this single source of tolerance. Findings in article A3 clearly demonstrated that in hexaploid wheat, *Bo1* was homoeo-allelic to the high boron tolerance locus in durum wheats using F2 progeny of AUS14010 x 'Yallaroi'. In this durum cross, the closely linked PCR marker AWW5L7 co-segregated again with root length and boron uptake phenotypes in 135 F2s. This indicated that the marker would be very useful for MAS in both wheat species. In fact, due to its accuracy, AWW5L7 has become an inherent part of MAS programs at Australian Grain Technologies (AGT) in Adelaide (H. Kuchel, pers. comm.) and at CIMMYT (S. Dreisigacker, pers. comm.), to monitor and enrich populations segregating for the *Bo1*-derived tolerance allele in bread and durum wheats. Moreover, in this study a supported hydroponics screen for seedlings was established which allowed more efficient high-throughput phenotypic screens for boron tolerance status compared to previous assays (Chantachume et al. 1995). With the availability of such a convenient screening system it became possible to phenotype large numbers of accessions, from diverse origins. Among these, 56 diploid einkorn (*T. monococcum* L.) wheats were tested to determine their level of tolerance to high boron supply. Unexpectedly none of the einkorn accessions reached

similar low boron accumulation as the tolerant hexa- or tetraploid check cultivars, perhaps suggesting that genes/alleles conferring high levels of tolerance were absent from the A^m genome.

3.2 Isolation, allelic diversity and functional analyses of transporters conferring tolerance to high soil boron in barley and wheat

Technological Breakthroughs in sequencing technology within the first decade of the 21st century had seen a very rapid adoption of latest methodologies in the medical and biological sciences. Today this is much better known as 'Next-Generation-Sequencing', such as 454 pyrosequencing and Illumina™ sequencing technology (Shendure and Ji 2008; Lam et al. 2012), which sparked an unprecedented viability to sequence large genomes (Coombs 2008; Shendure and Aiden 2012). In 2006 the barley community utilized these evolving opportunities and established the International Barley Sequencing Consortium (IBSC; http://barleygenome.org) to further develop barley genomic resources (Stein 2007; Schulte et al. 2009). Unlike wheat, barley had always been more amenable to genetic and genomic approaches. For example, simply due to the diploid genome structure barley research was able to produce a large number of collections of historical mutants for functional gene studies (Druka et al. 2011), to develop whole-genome SNP marker platforms (Stein et al. 2007; Close et al. 2009), and to readily allow transgenic approaches (Shrawat and Lorz 2006; Hensel et al. 2011). Through the new sequencing methods, other essential resource developments in barely gained momentum, which resulted in the generation of BAC libraries, low-priced BAC sequencing (Wicker et al. 2006; Steuernagel et al. 2009; Schulte et al. 2011) and finally physical mapping of the entire barley genome (Mayer et al. 2012). Therefore, the isolation of a large-effect QTL using a map-based approach in a big genome such as barley (5.1 Gb) appeared ambitious but achievable. Results presented in article A4 and A5 have to be considered in this context and followed up on research previously presented by Jefferies et al. (1999), who detected the major QTL for boron accumulation, shoot biomass and root length on

chromosome 4H and 6H in the barley 'Clipper' x 'Sahara' mapping population. Article A4 describes work related to isolating the major boron tolerance QTL on the long arm of chromosome 4H. To "Mendelize" this QTL for further high-resolution mapping, two carefully selected DH lines were crossed which carried contrasting chromosomal segments at the 4HL QTL but were fixed for the other three QTL alleles (2H, 3H, 6H). The obtained F2 and F3 progenies of this cross mapped the tolerance locus into a 0.15 cM genetic interval using 3360 F2s. Syntenic protein prediction for this interval in rice and Brachypodium indicated two to three putative genes but no known transporters (i.e. homologs of AtBOR1, AtNIP5;1). However, candidate gene mapping of these putative boron transporters in barley produced genetic co-localization between a barley *BOR1* homolog and the 4HL QTL. This putative barley boron transporter, *Boron transporter 1* (*HvBot1*), possessed two nucleotide substitutions, differentiating the 'Clipper' (intolerant) and 'Sahara' (tolerant) alleles; both resulting in amino acid changes of the predicted protein. Using a heterologous expression system in yeast (*Saccharomyces cerevisiae*) it was shown that under high boron supply, boron efflux from yeast cells was more effective in cells expressing 'Sahara' *HvBot1* compared with 'Clipper' *HvBot1*. This suggested that structural differences in the two proteins had influenced boron efflux transport activity. Moreover, *HvBot1* was constitutively expressed in the roots of barley plants but to a much higher extent in the boron tolerant accession 'Sahara'; higher expression of 'Sahara' *HvBot1* was found to be associated with higher copy number of this gene (~4 times higher). Work in this study revealed for the first time the genetic basis of tolerance to high soil boron in plants; but similarly provided opportunity to introgress this exotic source of tolerance into the Australian barley breeding material using MAS. Hence, Emebiri et al. (2009) developed BC6-derived barley introgression lines carrying the 'Sahara' *HvBot1* allele in two elite Australian two-rowed cultivars 'Sloop' and VB9104 in order to evaluate its effect in germplasm relevant for Australia. Further selections from these BC-lines produced the first moderately boron tolerant malting barley variety for the domestic market, 'Sloop Vic' (Sahara/WI2723//Chebec(VB9743) /2*/Sloop), released in 2002 and bred by D. Moody. However, results from several field studies

also demonstrated that the possession of the 'Sahara' *HvBot1* and 'Sahara' 2H alleles were effective in reducing boron accumulation in the shoot but may have also negatively affected other traits, such that depending on site-specific soil conditions, yield improvements were non-significant to modest (Emebiri et al. 2009; McDonald et al. 2010).

Following a candidate approach, the gene underlying the 6H boron tolerance QTL had been identified; work related to the functional characterization of this gene was described in article A5. That boron (boric acid, H_3BO_3) and silicon (silicic acid, H_4SiO_4) share very similar transport properties simply because of their molecule structure and behavior in aqueous solutions had already been known for some time (Nable et al. 1990). The identification of a silicon influx transporter in a rice mutant, *Low silicon rice 1* (*Lsi1*), belonging to the NIP subclass of aquaporin channels (Ma et al. 2006), in conjunction with the fact that AtNIP5;1 was able to facilitate boron transport in plants (Takano et al. 2006) concentrated efforts on an in-silico comparative analysis of NIP proteins between rice and barley. BLAST similarity searches established that only rice *OsNIP2;1* (=*Lsi1)* was co-locating in a syntenic position overlapping with a previously identified QTL for boron uptake on chromosome 6H in barley (Jefferies et al. 1999). Genetic mapping of EST-derived sequences of the putative orthologous gene in barley, *HvNIP2;1*, confirmed its location beneath the peak of the 6H QTL. Further analyses of the HvNIP2;1 protein in yeast clearly demonstrated that it transported not only boron but also water (H_2O), germanium (Ge) and arsenic (As), which was supported from structural protein modeling of its pore (article A5). Multifunctional transport activities were also obtained for OsNIP2;1, including selenite (Se), urea and boron (Mitani et al. 2008; Li et al. 2009; Zhao et al. 2010); similarly it was shown that HvLsi (=HvNIP2;1) was a silicon influx transporter in barley (Chiba et al. 2009). Root zone expression patterns of *HvNIP2;1* under high boron supply confirmed previous observations for silicon (Chiba et al. 2009), where higher mRNA expression in more basal roots (>10 mm from the tip) was consistently associated with increased uptake of substrates. Interestingly, cell-type specific localization and expression of *HvNIP2;1* in barley appeared to be

different to *OsNIP2;1* in rice (Chiba et al. 2009), suggesting that HvNIP2;1 mediates radial transport of substrates through epidermal and cortical cells of the basal root in barley. Taken together, the observed transport activities for HvNIP2;1 appeared to be consistent with the view of a multifunctional aquaporin channel important for radial influx of solutes, including boron, predominantly in more basal roots of barley. These results had established HvNIP2;1 as a boron transporter, and thus as a promising candidate gene for the 6H boron tolerance QTL. However, 'Clipper' and 'Sahara' did not reveal any sequence polymorphism for the HvNIP2;1 protein which could have been responsible for boron uptake differences between both genotypes. In fact, significant differences in *HvNIP2;1* mRNA levels in basal roots of 'Clipper' and 'Sahara' was shown to be causative for the boron uptake phenotype. The observation of lower boron uptake in 'Sahara' was consistent with significantly lower *HvNIP2;1* expression in basal roots, supporting the hypothesis that radial root permeation of boron in basal roots was reduced in 'Sahara'.

Findings of articles A4 and A5 were cumulated and combined in a working model of how tolerance to high soil boron in 'Sahara' barley may function in relation to the major boron uptake QTL on chromosome 4H and 6H. This model was proposed in the review article A1. That progeny carrying both 'Sahara' alleles on 4H (*HvBot1*) and 6H (*HvNIP2;1*) had strongly reduced boron accumulation of the shoot had already been shown in article A5. Lower boron accumulation in the shoots may have been a result of the lower permeability of the root, which in turn may decrease xylem loading in the root and boron upward translocation. The decreased permeability at the root cell level appears as a direct result of the antagonistic actions of both transporters. 'Sahara' exhibits a higher abundance of the boron efflux transporter HvBot1 in conjunction with a lower abundance of the boron influx channel HvNIP2;1, thus leading to a lower net entry of boron into the root under high boron conditions. Because of reduced boron levels in the inner root cells, meristematic activity and root growth becomes less severely affected in a boron tolerant genotype (Aquea et al. 2012). In this context it has to be noted that both tolerance alleles have never been tested together under real-world conditions, in any barley breeding program

until today. One reason may be that the source of boron tolerance comes from an exotic six-rowed African landrace ('Sahara') with poor agronomic performance, and hence breeders have been hesitant to use it as crossing parent for the Australian two-rowed malting barley breeding programs. Another reason could be the rather modest and disappointing 'Sahara' *HvBot1* effect in the BC-derived lines of VB9104 and 'Sloop' (Emebiri et al. 2009; McDonald et al. 2010). However, that tolerance to high soil boron plays an important role in the adaptation to Australian soils had been clearly demonstrated for wheat (McDonald et al. 2013); so similar trends may also hold true for barley.

With a clearly defined mapping interval for *Bo1* in wheat (see article A2) the prerequisites to pursuing a map-based cloning approach seemed promising. The rather telomeric localization of *Bo1* on the long arm of chromosome 7B also implied that it may lie in a gene-rich, recombination-friendly region (Erayman et al. 2004), while the lack of any known candidate transporters in the syntenic region fed the hope of discovering something novel (see article A2). However, the sheer size of the hexaploid wheat genome (17 Gb) in conjunction with its polyploid nature made bread wheat less amenable to developing genomic resources especially for SNP-based marker development, the generation of mutants and transformation technology; which are all essential tools for functional gene analyses (Lagudah et al. 2001). Nevertheless, the International Wheat Genome Sequencing Consortium (IWGSC; http://www.wheat genome.org) had launched in 2005 (Feuillet and Eversole 2007) and developed many essential, publicly available wheat genomic resources such as BAC libraries (Allouis et al. 2003; Cenci et al. 2003; Nilmalgoda et al. 2003; Chalhoub et al. 2004; Safar et al. 2004; Akhunov et al. 2005), physical maps (Feuillet and Eversole 2007; Paux et al. 2008) and flow cytometry of individual chromosome arms in combination with latest sequencing technologies (Dolezel et al. 2007; Wicker et al. 2008; Dolezel et al. 2012). Moreover, the recent emergence of *Brachypodium distachyon* (L.) P. Beauv. as a new model species for the temperate grasses (Bossolini et al. 2007; Garvin et al. 2008; Vogel et al. 2010) supported the idea that map-based gene isolation in hexaploid wheat may become reality. Thus,

findings described in article A6 have only been possible due to efforts of dedicated people from the international wheat research community, who advanced genomic resources. So, *Bo1* was identified using a combined approach between map-based cloning and candidate mapping. Initially, 2100 F2 plants from a 'Cranbrook' x 'Halberd' cross were used for high-resolution mapping delineating a 0.05 cM genetic interval. Using markers from this genetic interval, a physical contig in diploid *Aegilops tauschii* (Akhunov et al. 2005) was identified. Corresponding BAC clones from this contig were hybridized with probes of candidate transporters (i.e. *HvBot1* and *HvNIP2;1*), using simple Southern dot blot analysis which indicates presence or absence of candidate genes on specific BAC clones. Positive radiolabelled signals were only obtained using the full-length cDNA probe of *HvBot1*, clearly suggesting that a non-syntenic *HvBot1*-like gene was located in the *Bo1* interval. Subsequent mapping of markers derived from this putative efflux transporter, and co-segregation with *Bo1,* confirmed its physical location. Further sequence analyses of the corresponding *Ae. tauschii* BAC clones (D genome) revealed that *Bo1* indeed belonged to a related class of boron efflux transporters which were only present in the B and D genomes but absent in the A genome of tetra- and hexaploid wheats. Moreover, phylogenetic analyses even showed that there is no direct orthologous sequence of *Bo1* in einkorn wheat (*T. monococcum*; A^m genome), barley, Brachypodium, rice, sorghum or Arabidopsis. In fact, the presence of this transporter in *Ae. tauschii*, as well as in the B and D genomes of polyploid wheats, clearly illustrated that *Bo1* belonged to a genus-specific group of boron efflux transporters implicated of being involved in the evolution of *Aegilops* species. Further work related to the identification of two independent EMS-induced mutant alleles of *Bo1* and heterologous expression studies in yeast confirmed its role in conferring tolerance to high soil boron. While studying *Bo1*-related sequences in diverse wheat germplasm (see articles A2 and A3) nine different alleles of *Bo1* could be detected showing different levels of tolerance to high soil boron. Cultivars carrying a null allele of the transporter (e.g. 'Cranbrook', 'Langdon') displayed the lowest level of tolerance followed by cultivars which possessed the reference allele of the efflux transporter (e.g. 'Chinese Spring'), whereas cultivars with the

tolerant *Bo1* allele on the long arm of chromosome 7B (e.g. 'Halberd', 'Currawa', 'Lingzhi') had the highest level of tolerance. The tolerance *Bo1* alleles in tetra- ('Lingzhi') and hexaploid wheats ('Halberd', 'Currawa') differed by only one synonymous SNP but showed 16 SNPs compared with the 'Chinese Spring' allele, further supporting the notion that *Bo1* most probably originated from 'Currawa's' durum wheat parentage (Sims 1988), which was transferred through to 'Halberd'. Further analysis of the Australian wheat germplasm pool found that the 'Halberd'-derived allele was present in all bread wheat cultivars which were of historical importance in southern Australian farming systems, such as 'Frame', 'Insignia', 'Spear', 'Krichauff', 'Yitpi', 'Gladius' and most recently, 'Mace'. Moreover, all *Bo1*-derived tolerance alleles also shared significantly higher transcript levels of the *Bo1* transporter, indicating that also increased active boron efflux from root cells is part of the tolerance mechanism in wheat.

It appears that the *Bo1* tolerance locus in wheat is rather a success story. Breeding for adaptation in historical germplasm resulted early on in the selection of one superior allele, which has been repeatedly selected in different cultivars until today. Among wheat breeders, there is little doubt that *Bo1* is of importance, particularly in those sites where boron is limiting production. However, results obtained in wheat seem to contradict results in barley, where introgressions of boron tolerance alleles did not fulfill expectations (McDonald et al. 2010). In fact, by realizing the findings from article A6 it might become clearer that the lacking boron transporter on chromosome 7H in barley may, at least partially, explain this difference in performance. In wheat it appears that the presence of the boron transporter *per se* confers some level of adaptation to high soil boron, whereas the presence of the *Bo1* allele lifts the tolerance to a higher level. Hence, transferring the *Bo1* allele from wheat into barley might overcome this problem, and it would be very interesting to learn what levels of boron tolerance are achievable in barley using this system.

The work related to boron tolerance in wheat had already been proposed as being a showcase for work on other soil constraints which limit wheat production in Australia (Rengasamy et al. 2003).

Therefore, this concept lead to the establishment of the Australian Center for Plant Functional Genomics (ACPFG; est. Dec 2002), whose research is nowadays primarily focusing on salinity and drought related problems in cereals.

3.3 Tell me your drought and I'll tell you…!

Based upon latitude, soil characters and rainfall pattern, the Australian cereal production area can be largely divided into two regions (see Figure 1). The northern region is mainly characterized by relatively deeper soils with fewer subsoil constraints and predominately summer rainfall, frequently resulting in low post-anthesis water availability. As a consequence grain yields in the North mainly rely upon the stored amount of water in the soil after anthesis. The southern growing regions often exhibit shallow soils with compact subsoils containing toxic or deficient amounts of nutrients, accompanied by intermittent and variable rainfall events usually starting during pre-anthesis through to post-anthesis development. Therefore, yields in the southern environments mainly depend upon the frequencies and quantities of these irregular rain showers. In reality the occurrence of drought is frequent in both regions and had been clearly exemplified in the years 2001 to 2007 for South East Queensland (http://www.longpaddock.qld.gov.au/about/publications/pdf/seq_drought_2007.pdf); but similarly from 2003 to 2008 in the southern region, which appeared to be one of the worst droughts on record (Chenu et al. 2013). Coincidently with these severe drought events, 'Drysdale' one of the first proven drought-tolerant Australian bread wheat cultivars was released in 2002; bred by Richard A. Richards (CSIRO Plant Industry, Canberra), Graham D. Farquhar (ANU, Canberra) and colleagues in collaboration with the Australian Wheat Board (AWB), Grains Research and Development Corporation (GRDC), Syngenta and New South Wales Agriculture. 'Drysdale' was developed and selected using the carbon isotope discrimination (CID) method described by Farquahr et al. (Farquhar et al. 1982). CID allowed selection for higher transpiration efficiency (Condon et al. 1987; Condon and Richards 1992), and thus water use per unit biomass

was lowered; i.e. wheat plants improved their water-use efficiency (WUE) following the idea of getting "*more crop per drop*" (Condon et al. 2004; Morison et al. 2008). 'Drysdale' became a success in the dry areas of the northern growing regions, where it yielded up to 10% better than other adapted varieties (Condon and Richards 2003). However, this success was not replicated in the dry environments of the south, suggesting that this mechanism of drought tolerance was less effective, despite its higher WUE. This observation made by Steve P. Jefferies, Gill J. Hollamby, Haydn Kuchel and colleagues from Australian Grain Technologies (AGT) in 2003/2004 triggered a series of events which lead to a collaborative effort together with scientists from ACPFG (P. Langridge, N. Collins, T. Schnurbusch; meeting on 25 March 2004) to elucidate the physiological and genetic basis of well-adapted, drought-tolerant South Australian bread wheat germplasm. Staff from AGT proposed to investigate two drought-tolerant genotypes which both performed exceptionally well during the severe drought of 2003 and yielded 10 to 40% higher than current elite varieties, including 'Drysdale' (S.P. Jefferies, pers. comm.). These genotypes were cultivar 'Excalibur', released in 1991 and a breeder's line, called RAC875. Both were proposed to be crossed to a drought-intolerant cultivar, 'Kukri', released in 1999. All three genotypes shared a similar yield potential under favorable conditions but clearly differed in very low to medium-low rainfall environments. To further avoid foreseeable complications of future analyses all genotypes shared the *Rht2* semi-dwarfing gene and very similar maturity. All proposed genotypes had been bred by the publicly funded wheat breeding program of the University of Adelaide which was located at Roseworthy, North of Adelaide, and hence, should possess good adaptation for the southern regions. Moreover, few progeny from crossings among the three genotypes, including cultivar 'Krichauff', similarly performed very well in the drought of 2003, supporting the view that this '*drought adaptation*' was inheritable and had a genetic basis.

To gain deeper insight into this '*drought adaptation'* of the two drought-tolerant genotypes, detailed physiological, metabolic and genetic analyses were conducted and described in articles A7 to A11. However, prior to commencing these experiments there were

two major points of concern related to this germplasm and its assumed '*drought adaptation*'. These points shall be briefly outlined here: (1) what, if this observed '*drought adaptation*' was not linked at all to tolerance to water deficit but rather related to other adaptive traits such as tolerance to salinity, high boron, high pH, low zinc, yet unknown nematode resistance or a combination of these factors. Secondly, if it were tolerance to water deficit, how could it be shown? In the latter case, the challenge was how to find a controlled environment assay which closely resembled outdoor growing conditions, culminating in the basic research question: would it be possible to simulate a complex environment like drought for a wheat crop under semi-realistic, more controlled growing conditions to study the multi-factorial trait grain yield. Finding such a design was one of the major outcomes of article A7 and occurred as a result of a complexity-reduction-approach while looking closely at the characteristics of the dry environments found in the southern growing areas (see Figure 3). In principle two decisive factors of variation—

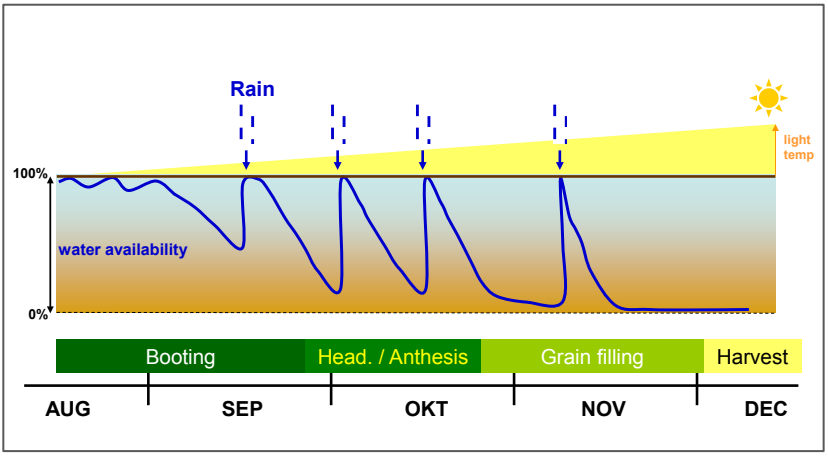

Figure 3: Progression of water deficit during important stages of yield establishment in the dry environments of the southern regions. Pre- and post-anthesis crop development, and consequently harvestable grain yield, solely relies upon rainfall frequency and quantity. The yellow arrow indicates that light intensity, temperature and thus evapotranspiration will increase towards summer.

soil parameters and water availability—had to be controlled in such a way that many potential confounding effects could be eliminated. For example by using pre-analyzed, defined soil from one field site close to Adelaide, many of these confounding factors could be indeed controlled or completely eliminated. To this end, the soil had to have an acceptable pH-level, non-toxic saline or boron concentrations, sufficient zinc (which could be added, if required), and no nematodes. Using natural soil also favored several other, more advantageous factors during the ongoing drought experiment. Wheat plants growing under such conditions would also show normal root development; and soil drying would develop more naturally, giving plants the chance to acclimate to the drought. Secondly, the watering regime had to be controlled and adjusted in a way that it resembled the intermittent pulses of rainfall during the life cycle of the wheat plants starting from around heading time. Moreover, a drying period had to be stopped, and watering had to re-occur when the drought-intolerant cultivar showed signs of wilting. To this end, a cyclical watering pattern of drying and re-watering followed by another round of drying and re-watering and so on, was pursued until mid of grain-filling. This experimental design described above in conjunction with sufficient light conditions and a programmable temperature regime of the growth cabinet was crucial and a prerequisite while performing the drought experiments. However, more details related to the experimental set up can be found in article A7. Following the experimental approach outlined above, utilizing two independent growth room experiments under severe and mild drought, it was clearly demonstrated that both drought-tolerant genotypes possessed some level of drought tolerance compared to intolerant ‘Kukri’. The higher tolerance to water deficit was illustrated through significantly higher grain yields per plant, in particular under severe water-limiting conditions, where both drought-tolerant genotypes produced on average 18 to 44% more grains than ‘Kukri’. However, there were no differences in control plants from the same experiments, which usually yielded up to five times more grains than the drought-exposed wheat plants, indicating that the observed differences in yield were due to water deficit and not some other factor. These results also meant that yield trends found in dry field environments of the southern regions were at least partially

transferable to controlled conditions, if environment-specific (cyclical water availability) and development-specific (reproductive phase) stress factors were considered in the experimental set up. Eventually it was concluded that the experimental design chosen in article A7 should form the basis of all subsequent physiological, metabolic and proteomic analyses of these genotypes.

From previous work by Morgan, Blum and colleagues (Morgan 1977, 1995; Blum 1996; Blum et al. 1999; Zhang et al. 1999; Morgan 2000), it was proposed that osmotic adjustment may play a role in conferring some level of drought tolerance under the observed conditions in the southern regions of Australia. Briefly, osmotic adjustment can play a major physiological role during the desiccation and recovery process of the plant under water stress. To verify this hypothesis all three genotypes were grown in an independent greenhouse experiment, where water was withheld starting from flag leaf emergence. While closely monitoring water status and osmotic potential of flag leaves over a period of two weeks, differences in osmotic adjustment among genotypes became evident. Both drought-tolerant genotypes clearly showed the capability to osmotically adjust their flag leaf cells, thus possibly providing an explanation for better performance under cyclical water deficit. Obtained results from previous growth room experiments for physiological traits such as stomatal conductance, chlorophyll content/fluorescence, abscisic acid (ABA) levels, water-soluble carbohydrates, CID and WUE were most consistent with the idea of osmotic adjustment being at work (see article A7). Results obtained in article A8 strengthened this view. Following the scheme of cyclical water deficit flag leaf samples of the three genotypes were taken at five different time points during drought progression for metabolite analysis. In principle, both water treatments (control and cyclical water deficit) could be clearly differentiated based upon their amino acid accumulation; notably proline, known as a potent osmoprotectant (Morgan 1992; Zhang et al. 1999), increased in all genotypes under water deficit. However, stress-specific responses of the drought-tolerant genotypes were mainly seen in other amino acids such as higher tryptophan (RAC875), or tyrosine, phenylalanine levels (‘Excalibur’). Decreased levels of sugar or

organic acids were observed at around wilting point, suggesting that these differences may relate to adjusting cellular responses and metabolism under water-limiting conditions. For example, the pool of free chloroplastic tryptophan may capture photosynthesis-derived reactive oxygen species (ROS) produced in the chloroplast to protect proteins against oxidation, thus reducing the cellular oxidative stress level, without large detrimental growth effects. Observations made on the metabolite level (aromatic amino acids) agreed with proteomic work performed on the same samples from this experiment (Ford et al. 2011). Ford et al. found that proteins involved in scavenging ROS (e.g. superoxide dismutase, catalase) were more highly expressed in drought-stressed plants, whereas proteins for photosynthesis and Calvin-cycle significantly decreased. The observed metabolic and proteomic signatures of both drought-tolerant genotypes were clearly different, thus further supporting the notion from previous growth room experiments (see article A7) that both drought-tolerant genotypes possess different mechanisms of adaptation to drought. Among the three genotypes, RAC875 showed the most obvious alterations in metabolic and proteomic profiles under cyclical water deficit. However, for the physiological reaction, i.e. osmotic adjustment of the flag leaves, 'Excalibur' provided the highest capacity. Future work needs to elucidate how these different responses can be related to increased drought tolerance in the individual cultivars.

Work described above identified osmotic adjustment as being one putative, potent source of drought tolerance in the South Australian cultivars. The ability to osmotically adjust cellular responses is not only beneficial under water stress but similarly under salinity and high pH (Zhang et al. 1999; Chen and Jiang 2010; Chen et al. 2011). All these stressors occur frequently in the southern growing areas of Australia's cereal belt and it could be assumed that breeding in these environments favors or selects for drought tolerance mechanisms utilizing osmotic adjustment. However, osmotic adjustment can be manifested physiologically through many pathways but in principle, it can be achieved through two types of osmolytes. Firstly, organic solutes such as amino acids, sugars or organic acids; secondly inorganic ions, mainly potassium (K^+), sodium (Na^+), chloride (Cl^-), or

calcium (Ca^{2+}). Depending upon the type of stress it seems that different osmolytes can be utilized for different purposes. Cotton plants responded differently to different types of osmotic, saline or alkali stress, where for example, organic acids were the major contributor under alkaline conditions (Chen et al. 2011). Work conducted by Morgan showed that higher accumulation of potassium (K^+) and amino acids may play a major role for the *osmoregulation* (*or*) gene in conferring osmotic adjustment to leaves and pollen of wheat plants under water stress (Morgan 1992, 1999b). Coarse mapping in F2s using RFLPs located the recessive *or* gene on chromosome 7A, close to the centromere (Morgan 1991; Morgan and Tan 1996). Morgan developed a pollen assay which allowed selection for *or* and was well correlated with osmoregulation of flag leaves under water stress (Morgan 1999b). Moreover, in 39 field experiments over eight seasons along the wheat belt he found that selected breeding lines carrying the *or* gene had higher grain yields in response to reduced rainfall and increased evaporative demand, when climatic, soil and developmental factors were considered (Morgan 2000). His evaluations let him conclude that, more notably in dry marginal areas, where soils had lower water-holding capacity, largest effects from osmoregulation could be anticipated (Morgan 2000). However, most surprisingly, there has been no follow-up work on Morgan's well laid out conceptual framework of the effect of osmoregulation on water-stressed wheat plants in arid environments. Anyhow, the two drought-tolerant genotypes evaluated in the growth room experiments of article A7 showed that under water stress, there was significantly higher accumulation of potassium (K^+) and sodium (Na^-) in flag leaves compared to 'Kukri' (Izanloo, unpublished data). Results obtained from controlled conditions, however, were not confirmed in two field sites (Roseworthy, Minnipa) in 2006 (Izanloo, unpublished data), possibly suggesting that a simple determination of osmolyte concentrations of flag leaves remains inconclusive without careful consideration of water availability, temperature course, soil characters and sampling time point (Morgan 2000).

3.4 Tell me your drought QTL and I'll tell you…!

Performing a genetic analysis of the multi-factorial trait grain yield in an admittedly complex, highly variable field environment, including water stress, requires a carefully arranged experimental set up, dedicated researchers and well-selected, contrasting germplasm for the trait of interest. If '*drought tolerance*', i.e. grain yield performance under water stress, is of interest, then unrelated confounding factors preferably need to be eliminated, or at least reduced. Unavoidable confounding factors need to be monitored and preferably measured to help interpreting results. For an experiment under southern Australian growing conditions, this meant monitoring of all possible trait-relevant confounding factors. These factors could be basically divided into three groups, (1) uncontrollable physicochemical effects such as rainfall pattern and amount, temperature development, including frosty nights and hot winds, or, e.g. days above 30°C (Kuchel et al. 2007), and day length; (2) confounding soil effects, which were mainly related to soil constraints, toxicities or nematodes, but could be bypassed through previous knowledge about adverse soil quality, e.g. predominance of salinity; or through usage of so called '*indicator lines*', i.e. usually cultivars with known susceptibilities or resistances to e.g. nematodes, which can be supportive in less characterized sites; and finally (3) confounding genetic factors, which could be e.g. dissimilar semi-dwarfing genes (*Rht*); or dissimilar genes/alleles contributing towards heading time. Time to heading is probably the most difficult factor to control for the development of bi-parental populations, unless diagnostic markers for specific major genes such as *Ppd-1* have already been available to better match parent selection (Reynolds et al. 2009; Eagles et al. 2010). Yet due to the inherent nature of heading time as a quantitative trait, many genes/alleles with medium or small effects may still segregate and thus produce phenotypic differences of one to two weeks. Therefore, some variation is almost unavoidable; unless one could select a smaller subset of e.g. ˜200 lines from a very large population of lines (> 2,000 lines) to match heading time in a very narrow time window. But still, why could large phenotypic differences (i.e. more than two weeks) in time to heading create confounding effects under water stress? Under field conditions water

deficit usually starts to occur at a specific time point during the life cycle of the plant. If stress hits plants at different developmental stages, they may experience different degrees of damage, thus leading to more variable performances. Commonly, later maturing plants are more negatively affected than earlier maturing plants simply due to the fact that early plants experience the onset of stress relatively late in their life cycle; i.e. if decisive yield-establishing stages have already passed, yield penalties become less severe. Thus, the relative lower performance of later maturing plants confounds QTL analysis because favorable alleles of drought tolerance may get low phenotypic values simply because plants are late. Conversely, unfavorable alleles of drought tolerance receive relatively higher phenotypic values simply because plants are early. This discrepancy is problematic since every genetic analysis clearly relies on exact phenotypic and genotypic evaluations, while confounding factors decrease the true genetic component of traits; they lower the heritability but increase erroneous variation. Fleury et al. (2010) and Tuberosa (2012) came to similar conclusions in their recently published review articles.

All these considerations outlined above formed the basis for the analysis of experiments described in articles A9 and A10. Both data sets have been obtained from the same southern Australian field experiments and detected QTL are strongly interrelated. Hence for practicality, findings from these data sets shall be jointly discussed. Under investigation was the F1-derived, doubled haploid mapping population between drought-tolerant RAC875 and drought-intolerant cultivar ‘Kukri’. The population comprised 368 individuals and provided field data from four growing seasons in 16 field sites. Correlation analysis between sites for grain yield allowed a more detailed site analysis in terms of prevalent climatic conditions during the growing cycle. In this context it has to be noted that the years 2006, 2007 and 2008 were generally identified as being severe drought years throughout southern Australia and that average yields at each site were highly correlated with the amount of rainfall received. According to these analyses, three major environmental classes (ENV1, ENV2, ENV3) became apparent which could be mainly divided based upon the amount of rainfall received after

plants had reached anthesis. From the total amount of rainfall received during the entire growing season of ENV1 only 8% of it had fallen after anthesis; in ENV2 ca. 20% and in ENV3 ca. 30%. Moreover, due to temperature differences experienced in ENV2 and ENV3 each class could be further subdivided into hotter and cooler environments. From grain yield analyses of the 16 sites it became evident that RAC875 had significantly out-performed 'Kukri' in 14 of these sites. There was a fair variation for grain yield in the population, with kernel number per square meter (KPM^2) being the major driver for variation in grain yield. Higher KPM^2 were well correlated with higher kernel number per spikelet (KPSL) and kernel number per spike (KPS), suggesting increased floret survival under water-limiting conditions. Due to variation in heading time, QTL for grain yield were often co-locating with QTL for heading time. As major drivers of heading time variation loci on chromosomes 2BS (*Ppd-B1*), 2DS (*Ppd-D1*), 5BL (*Vrn-B1*), 4AS, 4BS (possibly homoeo-allelic *earliness per se*, *eps*, loci), 7BS (possibly wheat *FLOWERING LOCUS T*; *TaFT-B1*) and a cluster of QTL around the centromere of chromosome 7A (possibly including *TaFT-A1*) were identified; virtually covering all classes of known flowering time genes in cereals (Snape et al. 2001). Very similar results for heading time QTL had also been found by other authors using Australian bread wheat germplasm for the 'Trident x Molineux' (Kuchel et al. 2006) and 'Excalibur x Kukri' populations (Edwards 2011). After using heading time as co-factor and applying covariate analysis, grain yield QTL independent of heading time became detectable. Three of these grain yield QTL, on chromosomes 2B and two on 7A, appeared most promising because all contributed positively to grain yield, mainly through higher KPM^2, whereas none of these were associated with an expected smaller grain size. Interestingly, one of the 7A grain yield QTL close to the centromere had very consistent positive effects on floret survival or spikelet fertility, KPM^2, KPS and harvest index especially in hotter environments (ENV2-hot). The trait leaf waxiness (i.e. glaucousness) also clearly segregated in this mapping population. Drought-tolerant RAC875 expressed this trait most strongly thereby producing the characteristic greyish-silvery appearance of this cultivar in the field; whereas 'Kukri' only showed light glaucousness. Since glaucousness had been implicated in

being involved with providing some level of drought tolerance in durum wheat (Johnson et al. 1983; Richards et al. 1986), this was a good opportunity to verify whether glaucousness from this germplasm had any positive effects on drought tolerance. Overall, 11 QTL for glaucousness were detected, of which seven were derived from the highly glaucous RAC875. Six of these had only minor phenotypic effects. However, one major QTL for glaucousness on chromosome 3A explained up to 52% of the genetic variance of this trait. In comparison with previously known QTL for glaucousness, almost all QTL appeared to be different in this germplasm, including the major QTL on 3A. Most interestingly, none of these QTL for glaucousness showed any association with grain yield QTL under drought, clearly demonstrating that the glaucousness-effect did not positively contribute to higher grain yield under water deficit in this population.

Work described above identified very useful genetic information from the RAC875 x 'Kukri' population in terms of adaptation to heading time and grain yield under drought for southern Australia. In this regard the QTL region around the centromere on chromosome 7A stands out since it showed major effects on both traits (even on grain yield after the heading time effect had been remove). Very similar QTL on 7A had also been seen in the other drought mapping population 'Excalibur x Kukri'. Here, a major QTL for heading time from the drought-tolerant cultivar 'Excalibur' appeared to be in very close linkage to a sodium (Na^+) exclusion QTL detected in hydroponics and field grown plants (Edwards 2011). Most interestingly, the Na^+ exclusion QTL provided a 6 to 20% yield advantage, especially in the driest environments, which had medium to high soil Na^+ (Edwards 2011). Confirming this data, the same QTL region for Na^+ exclusion was also independently found in hydroponics, in the 'Cranbrook x Halberd' wheat population (Shavrukov et al. 2011). Moreover, mapping of *HvNax3*, *Na^+ exclusion locus 3* in barley, revealed a related chromosomal region on barley chromosome 7HS close to the centromere, clearly showing that *HvNax3* mapped only 6.35 cM proximal to barley *FLOWERING LOCUS T* (*HvFT*) and 2.4 cM distal to another flowering time gene, *HvVRT2* (Shavrukov et al. 2010). Assuming very close syntenic

relationships in these regions between wheat and barley one could postulate that the seen heading time effect in wheat could be due to *FT* and/or *VRT2*; whereas the found Na^+ exclusion QTL in both species might be related to *Nax3*. According to latest analysis, *HvNax3* most likely underlies a vacuolar H^+-pyrophosphatase (*HVP10*), where higher, induced expression of this gene in roots and shoots may confer tolerance to salinity (Shavrukov et al. 2013). Taken together, obtained results from wheat conclusively showed that the QTL cluster on 7A appears to be one essential part of the southern Australian wheat germplasm in adapting to harsh and hostile environments. Whether any of these 7A QTL correspond to the ominous *or* (*osmoregulation*) locus found by Morgan (2000) needs still to be shown. In fact, in his backcross lines Morgan observed that *or*-carrying lines reached heading time earlier but similarly showed a negative association with lower dough strength due to close linkage with the *Per-A4* locus on 7A (Morgan 1999a; Erayman et al. 2004). He concluded that possibly the association with unfavorable baking quality may have prevented a broader adoption of *or* in the Australian wheat germplasm (Morgan 2000). Later analysis in this context suggested, however, that *or*'s negative association with baking quality can be overcome in a wheat breeding program (Neacsu et al. 2009).

QTL results obtained from southern Australian drought-stressed environments clearly showed that this germplasm contained favorable, drought-tolerant alleles. To test this population under other drought environments, a collaboration with Matthew Reynolds from CIMMYT, Obregon, Mexico was developed. CIMMYT's site in Obregon allows flood- or drip-irrigation and hence different water availability regimes permit to simulate specific drought occurrences starting from pre- or post-anthesis. Moreover, besides water stress, heat stress can also be invoked simply through later sowing dates. To utilize these facilities, it appeared interesting to ask the question whether this germplasm may also carry useful heat tolerance alleles. Some tolerance to heat, i.e. tolerance to high temperature above 30°C (Kuchel et al. 2007), is also one important abiotic factor in the southern Australian drought-prone environments. Considering the projected climate scenario for southern Australia, which forecasts

higher temperature in combination with lower rainfall, possessing tolerance to heat may become even more relevant in the near future (Chapman et al. 2012; Nuttall et al. 2012). Article A11 summarizes the results obtained in the managed drought and heat environments from Obregon in 2007, 2008 and 2009 for the RAC875 x 'Kukri' population. Soils around Obregon are usually deep and fertile with almost no subsoil constraints rather resembling those conditions from the northern growing areas of Australia. For three different treatments (full-irrigation, drought, heat) and six field experiments, grain yield and its components, heading time and physiological traits, including canopy temperature, were taken. The drip-irrigation regime in the drought treatment simulated a terminal, post-anthesis drought, whereas the full-irrigated treatment received non-limiting water. The heat treatment differed by a delayed planting date but received full irrigation. As planned, due to the late sowing, the number of days above 30°C was highest in the heat experiment (~60 days) compared with the other two experiments (drought ~11 days; irrigated ~21 days). Average population means for grain yield clearly showed how effective different stress treatments reduced yields in the heat (1.9 t/ha) and drought (1.9 t/ha) experiments compared to full-irrigation (5.4 t/ha) in this population. In total, across all treatments, eight QTL for grain yield were detected with two major QTL on chromosome 3BS and 3BL, one mid-sized QTL close to the centromere of 7A and five minor QTL on 3A, 3D, 4A, 4D and 5B. The 3BL QTL showed the largest and most consistent effect on grain yield under drought and heat, where the RAC875 allele was advantageous. Very similar allele effects and yield trends were obtained for the same locus in a QTL-validation study, further providing evidence that the RAC875 allele yielded on average 12.5% more under drought and heat. However, it was found that the 'Kukri' allele produced 9% more grain yield under more favorable conditions (Bonneau et al. 2013). The other major QTL on 3BS was only found under well-watered and heat treatments but most interestingly both major QTL co-located with QTL for canopy temperature during vegetative development and grain-fill. The mid-sized QTL on 7A close to the centromere only appeared under the drought treatment and co-located with QTL for yield components and heading time. Distally linked to this QTL on 7AS, one heading time QTL became

apparent which also overlapped with QTL for canopy temperature during vegetative development. Both QTL from 7A were located close to previously identified QTL in the southern Australian environments. Interestingly, but not unexpectedly, all other detected grain yield QTL in Obregon were not coinciding with detected QTL in southern Australia, except for 4D.

The low correlation between grain yield QTL in both sites (southern Australia and CIMMYT, Obregon, Mexico) has not been too surprising since CIMMYT's managed environments in Obregon rather resemble growing areas in northern Australia. Historically, CIMMYT-derived germplasm had been utilized in breeding efforts for all growing areas of Australia. However, there seemed to be a better adaptation of this material to the northern regions (Brennan and Fox 1998; Brennan and Quade 2006; Mathews et al. 2007). One possible explanation for this trend could have been that southern Australian sites are more closely related to the mega-environments 4A and 9 (Braun et al. 1996), which are only weakly selected for at Obregon (Mathews et al. 2007). Nevertheless, the major QTL detected on chromosome 3B would be very useful breeding targets for the germplasm of the northern regions of Australia. Efforts to further elucidate the molecular basis of one of these QTL are already underway and may shed new light upon the underlying heat and drought mechanisms (Bonneau et al. 2013). The QTL cluster on 7A appeared to be effective in all tested environments, where it provided an adaptive advantage under water-limiting and heat conditions. Due to chromosome 7A's outstanding significance for heading time, drought, salinity and other agronomically important traits (e.g. nematode resistance) for all growing areas it became strategically important for the Australian wheat community to build a physical map of this chromosome as part of the IWGSC and start sequencing it (http://www.wheatgenome.org/content/view/full/576). Though the genetic nature of the QTL cluster detected on 7A seems complex, and close linkage between traits may hamper rapid progress, future work will reveal the underlying genes/alleles linked to different traits. This newly gained knowledge may entail improved future germplasm with better adaptability, higher grain yield potential and adequate baking quality. Breeders' knowledge from the present studies has

already yielded new varieties which are direct derivatives of RAC875, ‘Excalibur’ and ‘Kukri’. For example, bread wheat cultivars ‘Axe’ and ‘Gladius’, both released in 2007, came out of selections made in the terrible drought years of 2003, 2004 and later.

The physiological, genetic, metabolic, proteomic, and phenotypic data collected on this germplasm, presented in articles A7 to A11, make it possibly one of the most thoroughly studied set of drought-tolerant wheat germplasm in the world. The approaches utilized, results obtained and useful results from the present studies may hopefully encourage others as a leading example of how to tackle difficult traits in other areas of the world, where production is limited by drought, salinity or other abiotic stressors.

4 Summary (English and German)

The present thesis contains 11 peer-reviewed articles covering two major areas of research: (i) adaptation to high levels of soil-borne boron, and (ii) tolerance to drought in Australian wheat and barley germplasm.

Approximately 200 years ago first settlers and farmers quickly realized that cereal production under Australian conditions required especially adapted cultivars in order to yield. Soil conditions turned out to be fairly different compared to, for example European soils. Australian soils were found to be old, leached and impoverished of nutrients. Moreover, soils often possessed root zone constraints, such as high soil boron (boric acid; H_3BO_3), which strongly impeded root growth and root health. The fact that a toxic level of boron limited cereal production, particularly in southern Australia, had already been discovered in the early 1980s. Subsequent germplasm screens and breeding efforts identified the first boron-tolerant germplasm in barley and wheat, leading to the first generation of experimental mapping populations with contrasting levels of tolerance to high boron. Molecular-genetic analyses in bread and durum wheats located a major QTL for tolerance to high soil boron, *Bo1*, on the distal long arm of chromosome 7B. A closely linked, almost diagnostic PCR-based molecular marker was developed in bread and durum wheats which allowed for the first time MAS for high-boron tolerance in breeding programs. Later analyses found that *Bo1* underlies a boron efflux transporter with similarities to bicarbonate transporters in animals that was only present in the B and D genomes of polyploid wheats but was lacking in other grass genomes such as einkorn wheat, barley, Brachypodium, sorghum or rice. Nine *Bo1*-related alleles were identified, of which only one tolerance allele appeared to be prevalent among historically important bread wheat cultivars from southern Australia.

Initial mapping of tolerance to high soil boron in barley located four QTL on chromosomes 2H, 3H, 4H and 6H. The two QTL on 4H and 6H had the highest effect on lowering boron uptake of which the 4H QTL showed the largest reduction. Later analyses of this QTL demonstrated that it was the first boron efflux transporter discovered

in plants which conferred tolerance to high soil boron. The gene underlying the 6H QTL belonged to the large family of major intrinsic proteins within the subclass of the NIP proteins. The multifunctional aquaporin channel HvNIP2;1 showed significantly lower transcript levels in basal roots of the tolerant accession, suggesting that radial root permeation of boron in basal roots was reduced. A working model including both loci is presented hypothesizing how tolerance to high soil boron in barley may function at the root cell level.

Based upon latitude, soil characters and rainfall pattern, two largely representative growing areas of the Australian wheat belt can be classified. The northern region is mainly characterized by relatively deeper soils with fewer subsoil constraints and predominately summer rainfall, frequently resulting in low post-anthesis water availability. The southern growing regions are characterized by shallow soils with compact subsoils, containing toxic or deficient amounts of nutrients, accompanied by intermittent and variable rainfall events starting during pre-anthesis through to post-anthesis development. As a consequence well-adapted germplasm from the North rarely performs well in the South and vice-versa. This became particularly evident in the southern Australian droughts of 2003 and 2004, when drought-tolerant bread wheats from the North were definitely out-performed by more drought-tolerant southern Australian breeds, suggesting that the two regions may favor separate mechanisms of adaptation to drought. Based upon grain yield results, breeders identified well-adapted, drought-tolerant and intolerant bread wheat germplasm from southern Australia for further analyses. To simulate a characteristically cyclical drought event prevalent for the southern regions in controlled conditions, an experimental design was developed using repeated cycles of drying and re-watering. Following this approach similar grain yield differences compared to field-grown wheat crops became apparent among wheat cultivars under these controlled, water-limiting conditions. Further analyses suggested that an increased capacity for osmotic adjustment under water deficit may be the major physiological driver of tolerance to drought in the high-yielding cultivars. Metabolite analyses using a cyclical watering regime in controlled conditions supported this view and mainly found altered

pools of amino acids, sugars and organic acids. Genetic analyses were performed in the doubled haploid mapping population between drought-tolerant RAC875 and drought-intolerant cultivar 'Kukri'. The population comprised 368 individuals and provided field data from four growing seasons and 16 field sites in southern Australia. In this population, variation for grain yield was mainly determined by variation in heading time. However, independent of heading time two grain yield QTL regions on chromosomes 2B and 7A centromeric were consistently detected. Field-testing of this population at CIMMYT's managed drought and heat environments in, Obregon, Mexico, consistently revealed two major QTL on 3B but similarly the QTL cluster on 7A centromeric. Thus, the QTL cluster on 7A appeared to be effective in all tested environments, where it provided an adaptive advantage under water-limiting and heat conditions. Interestingly, this chromosomal region appeared to be associated with *osmoregulation* (*or* locus) and tolerance to salinity under southern Australian growing conditions. The physiological, genetic, metabolic, proteomic, and phenotypic data collected on this germplasm make it possibly one of the best examined drought-tolerant wheat germplasm in the world.

Zusammenfassung Deutsch

Die vorliegende Schrift umfasst 11 begutachtete Forschungsartikel, die im Wesentlichen zwei Forschungsschwerpunkte beschreiben. Diese sind einerseits (i) die Anpassung an hohe bodenbürtige Borgehalte, und andererseits (ii) die Toleranz gegenüber Dürre im australischen Weizen- und Gerstengenpool.

Vor ca. 200 Jahren hatten bereits die ersten australischen Siedler und Bauern herausgefunden, dass der Getreideanbau unter australischen Bedingungen besonders angepasste Sorten benötigte, um gute Ernten zu ermöglichen. Insbesondere die vorherrschenden australischen Bodenbedingungen erschienen sehr verschieden im Vergleich zu z.B. den fruchtbaren Böden Europas. Australische Böden sind dagegen überwiegend geologisch alt, ausgewaschen und nährstoffarm. Oft sind sie durch Bodenstörungen gekenn-

zeichnet, wie z.B. toxische Borgehalte (Borsäure; H_3BO_3), die sich schädlich auf Wurzelwachstum und Wurzelgesundheit auswirken. Dass toxische Borgehalte die Getreideproduktion vor allem im südlichen Australien einschränken können, ist schon seit den frühen 1980-er Jahren bekannt. Sich daran anschliessende Züchtungsbemühungen identifizierten die ersten hoch-Bor-toleranten Genotypen in Gerste und Weizen, welche zur Erstellung von experimentellen Kartierungspopulationen verwendet wurden. Molekular-genetische Analysen in Durum- und Brotweizenpopulationen lokalisierten einen QTL-Haupteffekt, *Bo1*, auf den distalen langen Arm von Chromosom 7B. Ein sehr eng gekoppelter, fast diagnostischer PCR-basierter molekularer Marker wurde für diesen Lokus in Durum- und Brotweizen entwickelt, der es zum ersten Mal ermöglichte, effizient markergestützte Selektion in Züchtungsprogrammen für Toleranz gegenüber hohen Borgehalten durchzu-führen. Spätere Analysen ergaben, dass *Bo1* für einen Bor-Efflux-Transporter kodierte, der sehr hohe Ähnlichkeit zu Bi-Karbonat-Transportern aus Tieren aufwies, und dass dieser Transporter nur in den B- und D-Genomen polyploider Weizen vorhanden war, aber in anderen Gräserarten wie z.B. Einkornweizen, Gerste, Brachypodium, Sorghum oder Reis fehlte. Insgesamt konnten neun *Bo1*-Allele nachgewiesen werden, von denen aber nur eines der toleranz-vermittelnden Allele überwiegend in historisch bedeutenden Brotweizensorten aus Süd-Australien vorkam. In ersten QTL-Analysen zur Toleranz gegenüber hohen Borgehalten in Gerste wurden vier Chromsomenregionen auf 2H, 3H, 4H und 6H identifiziert. Die QTL auf 4H und 6H zeigten die stärkste Wirkung bzgl. verminderter Bor-Aufnahme, wobei der Haupt-Effekt für niedrigere Borgehalte vom QTL auf 4H stammte. Spätere Analysen zeigten, dass dieser Bor-Efflux-Transporter der erste in Pflanzen entdeckte Transporter mit toleranz-vermitteln-der Wirkung gegenüber hohen Borgehalten war. Das Gen welches dem 6H QTL zugrunde lag, gehörte zu der grossen Familie der Major Intrinsic Proteins und dort zur Unterklasse der NIP-Proteine. Dieser multi-funktionale Wasserkanal (Aquaporin) *HvNIP2;1* zeigte signifikant niedrigere Transkripte in basalen Wurzelteilen der toleranten Akzession, was vermutlich die radiale Wurzelpermeation mit Bor in den basalen Wurzelteilen vermindert hat. Ein

Arbeitsmodell unter Einschluß beider Toleranz-Loci wird vorgestellt, um darzustellen, wie Toleranz gegenüber hohen Borgehalten auf Ebene der Wurzelzelle funktionieren könnte.

Basierend auf Breitengrad, Bodeneigenschaften und Nierderschlagsverteilung können grundsätzlich zwei unterschiedliche Anbauregionen innerhalb des australischen Weizengürtels unterschieden werden. Die nördlichen Anbauregionen sind hauptsächlich durch relativ tiefgründigere Böden mit nur wenigen Bodenstörungen und niederschlags-reichen Sommern gekennzeichnet, was beim Weizen häufig zu Wassermangelerscheinungen während der Nachblütephase führt. Die südlichen Gebiete kennzeichnen sich eher durch flache Böden mit Bodenstörungen (u.a. Bodenverdichtungen, Nährstofftoxizität oder –mangel) und variabel auftretenden Niederschlagsereignissen aus, die ab der Vorblüte- bis Nachblütephase auftreten können. Als eine Folge dieser unterschiedlichen Anbaubedingungen ergaben sich häufig verminderte Ertragsleistungen gut angepasster Sorten aus dem Norden, wenn sie im Süden angbaut wurden und umgekehrt. Dies wurde in den süd-australischen Dürrejahren 2003 und 2004 besonders deutlich, als dürre-tolerante Weizensorten aus dem Norden viel weniger dürre-tolerant erschienen als Sorten aus dem Süden, was wiederum die Vermutung aufkommen liess, dass die zwei unterschiedlichen Anbau-regionen möglicherweise auch unterschiedliche pflanzliche Anpassungs-mechanismen gegenüber Dürre erfordern. Basierend auf Ertragsdaten aus diesen Dürrejahren wurden gut angepasste, dürre-tolerante und -intolerante süd-australische Weizensorten durch Züchter ausgewählt und für weitere Analysen bereitgestellt. Für die Simulation einer typisch süd-australischen Dürre unter kontrollierten Bedingungen wurde hierbei ein zyklisch-verlaufender experimenteller Versuchsaufbau mit sich abwechselden trockenen und feuchten Perioden entwickelt. Nach diesem Ansatz konnten unter kontrollierten, wasser-limitierenden Bedingungen Ertragsunterschiede zwischen toleranten und intoleranten Genotypen ähnlich wie in Feldstudien gefunden werden. Weitere Analysen ergaben, dass die erhöhte Fähigkeit sich unter Wassermangel besser osmotisch Anzupassen möglicherweise ein physiologischer Hauptgrund dafür sein könnte, warum dürre-tolerante Weizengenotypen höhere

Erträge lieferten. Metabolit-Analysen dieser Genotypen unter kontrollierten Anbaubedingungen und zyklischer Wasserverfügbarkeit unterstützten diese Hypothese, da hauptsächlich veränderte Gehalte an Aminosäuren, Zuckern und organischen Säuren gefunden wurden. Die genetischen Untersuchungen wurden an nur einer doppelt-haploiden (DH) Kartierungspopulation, bestehend aus der dürre-toleranten Linie RAC875 und der dürre-intoleranten Sorte 'Kukri', durchgeführt. Die DH-Population umfasst 368 Individuen und lieferte süd-australische Feld-Daten aus vier Anbaujahren und 16 Umwelten. Die gefundene Variation für Kornertrag wurde in dieser Population v.a. durch die starke Variation des Merkmals Ährenschieben beeinflusst. Es wurden jedoch unabhängig vom Merkmal Ährenschieben konsistent zwei QTL-Regionen auf Chromosom 2B und 7A zentromerisch detektiert. Feldanbau der selben Population unter CIMMYT's steuerbaren Dürre- und Hitze-Umwelten in Obregon, Mexico, ergab die konsistente Detektion von zwei QTL mit Haupt-Effekten auf Chromosom 3B sowie das QTL Cluster auf 7A zentromerisch. Das QTL Cluster auf 7A wurde damit in allen getesteten Umwelten nachgewiesen und vermittelt daher eine verbesserte Anpassung sowohl unter Dürre- als auch unter Hitzestress. Interessanterweise zeigten andere Genotypen in der selben Chromsomenregion auf 7A QTL für *Osmoregulation* (*or* Lokus) und verbesserte Toleranz gegenüber hohen Salzgehalte unter süd-australischen Anbaubedingungen. Die hier gezeigten physiologischen, genetischen, metabolischen, proteomischen und phänotypischen Daten dieser Genotypen machen sie möglicherweise zu einen der bestuntersuchten dürre-toleranten Genotypen weltweit.

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Peer-reviewed Publications

1. PALLOTTA*, M., **T. SCHNURBUSCH***, J. HAYES, A. HAY, U. BAUMANN, J.G. PAULL, P. LANGRIDGE and T. SUTTON **(2014)** Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. ***Nature* 514:** 88-91 (*joint first-authorship)

2. POURSAREBANI, N., T. NUSSBAUMER, H. ŠIMKOVÁ, J. ŠAFÁŘ, H. WITSENBOER, J. VAN OEVEREN, INTERNATIONAL WHEAT GENOME SEQUENCING CONSORTIUM (IWGSC), J. DOLEŽEL, K.F.X. MAYER, N. STEIN, and **T. SCHNURBUSCH (2014)** Whole genome profiling (WGP™) and shotgun sequencing delivers an anchored, gene-decorated, physical map assembly of bread wheat chromosome 6A. ***Plant J* 79: 334-347**

3. YOUSSEF, H.M., R. KOPPOLU, T. RUTTEN, V. KORZUN, P. SCHWEIZER and **T. SCHNURBUSCH (2014)** Genetic mapping of the *labile* (*lab*) gene: a recessive locus causing irregular spikelet fertility in *labile*-barley (*Hordeum vulgare* convar. *labile*). ***Theor Appl Genet* 127:** 1123-1131

4. GAWROŃSKI, P., R. ARIYADASA, A. HIMMELBACH, N. POURSAREBANI, B. KILIAN, N. STEIN, B. STEUERNAGEL, G. HENSEL, J. KUMLEHN, S.K. SEHGAL, B.S. GILL, P. GOULD, A. HALL and **T. SCHNURBUSCH (2014)** A distorted circadian clock causes early flowering and temperature-dependent variation in spike development in the *Eps-3A*^{m} mutant of einkorn wheat. ***Genetics* 196:** 1253-1261

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6. KOPPOLU, R., N. ANWAR, S. SAKUMA, A. TAGIRI, U. LUNDQVIST, M. POURKHEIRANDISH, T. RUTTEN, C. SEILER, A. HIMMELBACH, R. ARIYADASA, H.M. YOUSSEF, N. STEIN, N. SREENIVASULU, T. KOMATSUDA and **T. SCHNURBUSCH (2013)** *Six-rowed spike4* (*Vrs4*) controls spikelet determinacy and row-type in barley. ***Proc Natl Acad Sci U S A* 110:** 13198–13203

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