

Genetic Diversity of Barley and Wheat for Waterlogging Tolerance in Western Australia

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Abstract

Barley and wheat cultivars from State and National breeding programs were screened for waterlogging tolerance in the field at Esperance, WA. Results demonstrate a genetic diversity in waterlogging tolerance of barley and wheat exposed to intermittent waterlogging over 4 weeks. Environmental measurements in the field demonstrate that waterlogging is highly variable in time and space with up to 400-fold differences in waterlogging duration/intensity occurring over 50 m.

Growth measurements demonstrate a genetic diversity for waterlogging tolerance based on grain yield and plant health using leaf chlorosis. However, just because plants are green does not indicate that they are high yielding following waterlogging. Generally barley cultivars became more chlorotic than wheat during waterlogging. Grain yields of waterlogged barley were reduced 50 to 85% relative to non waterlogged plants; Stirling and Fitzgerald were identified as the most tolerant cultivars. Grain yields of waterlogged wheats were reduced 20 to 80%, with Champel, Currawong and Carnamah identified as the most tolerant cultivars. Other data demonstrate that cultivars with moderate waterlogging tolerance at the vegetative stage often have little waterlogging tolerance at the seed germination stage.

Physiological traits evaluated for waterlogging tolerance include root traits (aerenchyma, suberisation, nodal/seminal roots), shoot/root carbohydrates and phenology. For spring wheat cultivars, yield is positively correlated to percent nodal root aerenchyma ($r^2=0.50$); while for barley there is little or no correlation to root aerenchyma. Collaborative international research is aimed at improving tolerance of barley and wheat to waterlogging through further evaluations and selection, and a mechanistic approach to germplasm improvement.

Introduction

Transient waterlogging occurs extensively in both irrigated and dryland (rainfed) agriculture on clay flats and duplex soils in Southwestern Australia (WA; McFarlane, 1990) and in Southeastern Australia (Victoria; Fried and Smith, 1992). In WA, waterlogging occurs over a cereal crop area of 500,000 ha/y, i.e. on 8% of the total cropland, with an additional 1.3 million ha/y of pastures effected (Department of Agriculture, 1991). This is a minimum cropping area affected in WA because, since these data were collected, cropping intensity has increased 2-3 fold in many shires due to the downturn in wool and meat markets (Stephens, unpublished).

For barley, waterlogging is estimated to reduce yields by 20-25% overall with a range of 0-53% for waterlogging at different stages of development; no information is available on the effects of waterlogging on grain quality (Belford,1995). The barley cropping area in WA is

about 250,000 ha. Assuming waterlogging in 2 out of 5 years, the cost of waterlogging is therefore $250,000 \text{ ha} \times 2/5 \times 20\% \times 1.9\text{t/ha} \times \$180/\text{t} = \$6.84 \text{ million /year}$.

Mechanisms of survival during waterlogging may be important at the seed germination stage or during vegetative growth. These mechanisms may involve avoidance of waterlogging effects, e.g. by phenological development; adaptation to waterlogging, e.g. by aerenchyma or metabolic change; and recovery mechanisms following waterlogging (Greenway *et al.*, 1994). One or all of these mechanisms may be used by plants for waterlogging avoidance / tolerance in WA. The particular mechanism used in cereals may vary depending on the crop (barley versus wheat), the growth habit of the cultivar (long versus short season, or spring versus winter growth habit) and the duration of waterlogging (long term versus short term or intermittent waterlogging). Evaluation of the genetic diversity of barley and wheat will help define:

- (1) existing mechanisms of waterlogging tolerance in species that may already be in use, as selection criteria for crop improvement (Blom, 1999), and
- (2) novel mechanisms which are not available or have not yet been discovered in current germplasm. The latter strongly supports a research approach linked to collaborative national and international research.

Methods

Eight barley and sixteen wheat cultivars from the state and national breeding programs were grown at “The Oaks” farm at Esperance, WA, in 1998. The gravelly sand, duplex soil at this site has been extensively classified (Seymour, 1999), with a topsoil EC of 20 mS/m; pH (CaCl₂) of 4.5; organic carbon of 1.2% and total N of 0.13%. Cultivars were planted in plots 100 m long and 1.8 m wide on this naturally waterlogged site in a randomised block with 3 replicates. Waterlogging was assessed over the season using dipwells placed every 10 m in a grid pattern and by calculating the daily Sum of Excess Water in the profile above 30cm soil depth for each dipwell (SEW₃₀; McFarlane *et al.*, 1989). Waterlogging (SEW₃₀) contours were then plotted using a contouring software package: Surfer for Windows.

Plants exposed to similar waterlogging (SEW₃₀ values; see dashed lines in Fig. 1) were collected; and measurements made of plant dry weight, yield and physiological traits. Chlorosis was rated visually. For estimates of aerenchyma in the cortex, transverse root sections were taken 2 cm from the base of the stem on 1st October 1998, at the end of waterlogging period. The sections were fixed in the field, and later samples were viewed under a fluorescence microscope. Light and TEM electron microscopic examination of at least 3 sections per 3 replicate plants were used to make estimates of aerenchyma and suberisation in the hypodermis. Other root traits such as the ratio of nodal to seminal roots, and the number of nodal roots were noted.

Results

Characterising waterlogging in the field.

Values of the Sum of Excess Water (SEW₃₀) integrate the waterlogging that occurs each day at different depths in the top 30cm of soil, over the whole season. The occurrence of waterlogging in the field was highly variable and changed up to 400 fold over a distance of 50m (Fig. 1). Considerable variation in waterlogging location and severity also occurred in different years at this site (data not presented). The variability was not related to the

topography of the site as the area was uniformly flat with a slope of $< 0.5\%$. In experiments presented here, the “Waterlogged” site had a SEW_{30} of 160 cm.d., and the “Non waterlogged” site had a SEW_{30} of 40 cm.d. A SEW_{30} value of 160 cm.d. is equivalent to various possible conditions including (i) continuous waterlogging to the soil surface, i.e. over the entire profile in the top 30cm of soil, for 5.3 days ($5.3 \times 30 = 160$); or (ii) waterlogging at 20cm soil depth, i.e. over 10cm soil in the top 30cm, for a period of 16 days ($10 \times 16 = 160$).

Germplasm Evaluation.

Crop species were quite disparate in the response of plant growth and health during waterlogging, and yield after waterlogging. Barley cultivars became more than twice as chlorotic as wheat cultivars (Table 1); and barley showed a greater reduction in dry weight (up to 75%) during waterlogging compared with wheat (up to 59%; Table 1). These adverse effects of waterlogging on plant health were reflected in the larger reductions in yields in barley relative to wheat (51-84% and 19-82% respectively; Table 1). Despite the larger reductions in yield due to waterlogging, many barley cultivars had higher yields than wheat cultivars after waterlogging because they had up to 60% higher yield in non-waterlogged areas of this site (Table 1).

The Oaks 98ES70 & 98ES84 SEW_{30} 1998

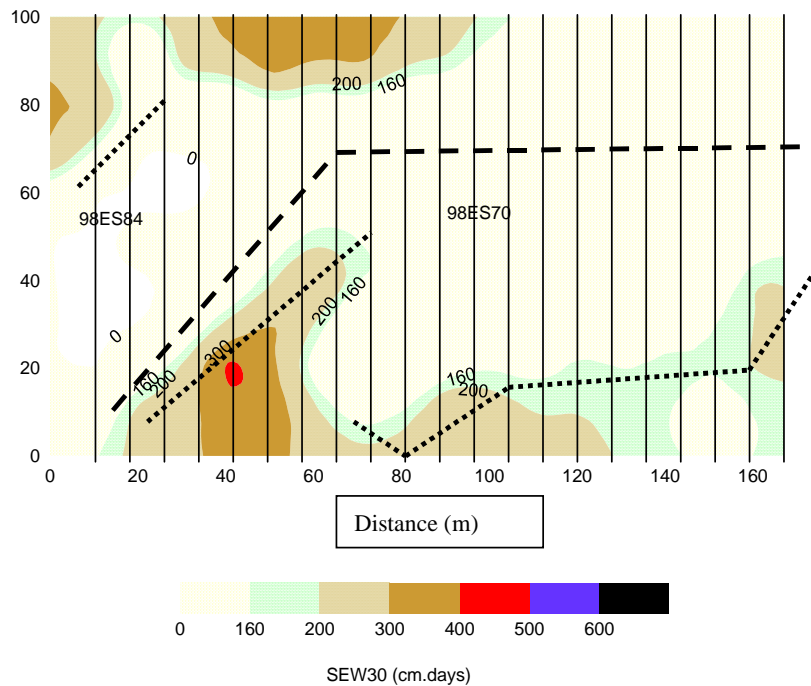


Figure. 1. Variation in waterlogging at The Oaks field site at Esperance, WA. Waterlogging throughout the season is integrated using the Sum-of -Excess-Water (SEW_{30}) values which integrate the time and severity of waterlogging by the days soil is waterlogged within the top 30 cm of soil throughout the season. Vertical lines represent plot lengths (100m). Dashed lines indicate sampling areas used for “Non waterlogged” ($SEW_{30}=0-40$ cm.d; - - -) and “Waterlogged” regions ($SEW_{30}=160-$

200 cm.d;). Some of the contours (available in 30 cm-d intervals) and plot indicator lines are removed to simplify the diagram.

Barley.

Grain yield of barley cultivars was reduced by 51 to 84% of non waterlogged plants (Table 1). Stirling had the lowest reduction in yield (51%), however it also had the lowest yield of the barley cultivars when not waterlogged. Fitzgerald and Molloy gave the highest yields, even above Stirling, however this was a consequence of exceptionally high yields for non-waterlogged plants (Table 1).

Waterlogging tolerance was assessed using leaf chlorosis following waterlogging. For example, Franklin was chlorotic following waterlogging, and grain yield was reduced by 84% (Table 1) - identifying Franklin as not tolerant to waterlogging. However, Stirling had a similar chlorosis, and it had almost a 3-fold greater yield than Stirling (Table 1).

Table 1. Dry weight, yields and chlorosis of barley and wheat cultivars grown at Esperance on a naturally waterlogged site (Fig. 1). Dry weights were measured on 1/10/98 which was at the end of waterlogging events for that year. Yields and chlorosis were from specific areas in plots that were exposed to “Waterlogged” (SEW₃₀=160cmd) or to “Non waterlogged” soil (SEW₃₀=40cmd).

Cereal	Variety	DW –	Yield (kg/ha) -	Yield (kg/ha) -	% Yellowing -
		[% Waterlogged / Non waterlogged]	Non waterlogged	Waterlogged [% Non waterlogged]	Waterlogged
Barley	Stirling	60	2677	1324[49]	26
	Fitzgerald	32	3914	1825[47]	22
	Molloy	79	4170	1847[44]	26
	Skiff	33	3731	1290[35]	19
	Gairdner	25	3381	1140[34]	15
	Onslow	47	3338	986[30]	17
	Harrington	38	2777	803[29]	24
	Franklin	100	3417	556[16]	26
Wheat	Champtel	54	1651	1344[81]	18
	Currawong	41	2842	735[59]	7
	Carnamah	72	2883	1532[53]	7
	W486175	67	2463	1130[45]	8
	WAWHT2153	46	2965	1176[40]	6
	Cunderdin	54	3282	1217[37]	8
	Spear	68	2879	956[33]	6
	Eradu	90	2657	841[32]	9
	Brookton	69	3427	995[29]	5
	Gamenya	96	2316	615[27]	9
	Sunmist	56	2684	643[24]	20
	Stiletto	59	2703	622[23]	6
	Cascades	58	2992	699[23]	6
	Amery	80	2863	646[23]	6
	Ajana	87	3476	714[21]	6
	Cadoux	52	2591	453[18]	6

Wheat

Within wheat cultivars there was, similarly, a large variation in waterlogging tolerance (Table 1). There was no strong link between leaf chlorosis and yield in wheat cultivars. For example, Sunmist and Stiletto had a similar yield, however they differed in chlorosis by more than 3-fold. Furthermore, Ajana had little chlorosis after waterlogging, yet yield was reduced by 79% (Table 1). Hence, just because plants were green after waterlogging, did not mean that they would be high yielding.

Root Traits

Assessment of root traits thought to impart some waterlogging tolerance showed that not all traits of adaptive value may be present at the same time in barley and wheat. No significant variation was observed between or within species in the number of nodal and seminal roots, with all root systems being made up equally of nodal and seminal roots (data not presented). Microscopic observation of roots collected from plants grown in the field showed that there was no significant suberisation of the hypodermis or the epidermis of either barley or wheat (data not presented). There was variation in the % aerenchyma in the mid cortex of nodal roots of both barley and wheat, with the range of aerenchyma being 7 to 63% and 10 to 81% respectively. Furthermore, there is a positive correlation between yield of Spring wheat grown under these waterlogging conditions and the % aerenchyma in nodal roots (Fig. 2, $r^2=0.50$), but this relationship did not hold for barley (data not presented).

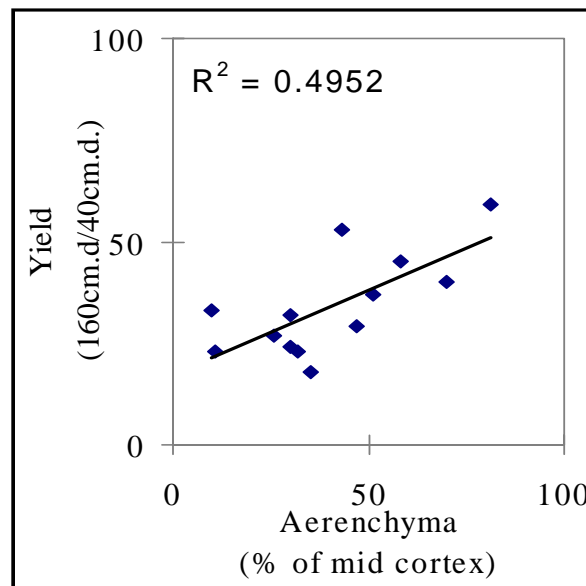


Figure 2. Relationship between yield of Spring wheat cultivars grown under waterlogged conditions and aerenchyma (% of mid cortex of nodal roots of waterlogged plants). Plants were grown at Esperance, WA, under intermittent waterlogged. Yield is estimated as the percentage of ["Waterlogged" ($SEW_{30} = 160\text{cm.d.}$) / "Non waterlogged" ($SEW_{30} = 40\text{cm.d.}$)].

Discussion

The highly variable nature of the waterlogging in the field, in both space and time, emphasises the complexity of the problems of screening germplasm in the field. This necessitates a detailed characterisation of the field environment including use of specific approaches, e.g.

specific site sampling or use of characterised waterlogging gradients, to relate yields to localised conditions for obtaining accurate assessments of genetic diversity. These conditions, and the transient nature of waterlogging in the field, make it obvious that simulation of field conditions in pot or controlled experiments requires more than a simple flooding of a pot.

Waterlogging events in the field in Western Australia are generally intermittent not continuous, under low irradiance, they vary greatly in duration and intensity, and they are often associated with sandy duplex soils with low mineral nutrition. These conditions could easily affect the waterlogging tolerance of cultivars relative to locations in other regions of the world where there is continuous waterlogging, at high light, high nutrition, and in heavy clay soils. Further work in WA is therefore clearly required to characterise the environment, particularly changes in the nutritional status of soils and plants during waterlogging in the field.

A difference in waterlogging duration and intensity may explain the variation in results that have come from trials with international wheat cultivars from CIMMYT grown in WA (Condon, 1999); where cultivars overseas are selected after several weeks of continuous waterlogging at high irradiance and high nutrition (van Ginkel et al 1992).

It is possible that the intermittent nature of the waterlogging in WA may influence different strategies for waterlogging tolerance of plants. Short term or intermittent waterlogging primarily requires plants to maintain processes associated with survival; while growth is a secondary priority. Strategies that could be used include diverse traits such as high rates of alcoholic fermentation to overcome anoxia, high carbohydrate concentrations to sustain alcoholic fermentation, reduced metabolite leakage, efficiency of nutrient uptake, and damage due to oxygen free radicals associated with return to aerobic conditions following waterlogging events. Tolerance to long term waterlogging requires plants not only to 'survive' but also to grow during the waterlogging event(s). The key strategy used for long term waterlogging is the development of aerenchyma in roots to facilitate gas diffusion (Armstrong, 1979; Blom, 1999; Jackson and Armstrong, 1999). Other important traits in long term adaptation include suberisation of nodal roots which contributes to "effective" aerenchyma development. There is no published information on this trait in commercial cereal cultivars.

Recent results with a wild relative of barley (*Hordeum marinum*) at The University of Western Australia exhibits the important trait of a barrier to radial O₂ loss (ROL) which has never before been found in dryland crops or their relatives (M. McDonald, T. Colmer and M. Galwey, unpublished data). A barrier to ROL prevents the passive leakage from the roots to the O₂ deficient soil thus enhancing the supply of O₂ to the growing root tip, i.e. making aerenchyma more effective as in rice. Whether this barrier to ROL is due to root suberisation or to other root tissue permeability traits is unclear.

Results presented here demonstrate that it is important to define waterlogging tolerance. The use of chlorosis during waterlogging is not be an accurate working definition, as green plants following waterlogging often do not have high yields (Table 1). This observation also applies to the traditional belief that oats are tolerant to waterlogging, since plants often appear green. However, significant yield reductions may occur in oats due to waterlogging, even at waterlogging intensities examined here (data not presented). Grain yield remains the best criterion for waterlogging tolerance in the present field locations in WA. Further research will be directed to (1) evaluation of a wide range of national and international cereal

germplasm for waterlogging tolerance through support from the Australian Centre for International Agricultural Research (ACIAR), and (2) developing a molecular approach to selecting for adaptive root traits for waterlogging tolerance.

If molecular markers can be developed for traits such as aerenchyma development, this could be used to assess a large number of germplasm quickly without the constraints of field variability shown in Fig. 1. It would be unlikely to find a single gene that relates to such a complex physiological trait such as aerenchyma development, however a transduction signal could initiate a gene cascade which would make such traits easy to monitor in a breeding program. Previous research on rice suggests that a transduction factor may be responsible for initiating a gene cascade for adaptation of tolerant cultivars to flooding (Setter et al., 1997).

References

- Armstrong, W. (1979) *Advances in Botanical Research*, Vol. 7, pp. 225-332.
- Belford, R.K. (1995) pp. 17-22 In: K. Young, Ed, *The Barley Book*. Dept. of Agriculture, Western Australia.
- Blom, C.W.P.M. (1999) *Plant Biol.* 1: 261-273.
- Condon, A.G. (1999) *Australian Plant Breeding Conference*, Adelaide. 19-23, April, 1999.
- Department of Agriculture (1991) *Situation statement: soil and land conservation programme in Western Australia*. WA Dept of Agriculture and Water Authority of WA. 66 p.
- Fried A and Smith N (1992) *Soil structure deficiency in extensive croplands of northern Victoria*. Soil and Water Conservation Association of Victoria. January, 1992. 52 pp.
- Greenway, H., Gibbs, J. and Setter, T. (1994). *Mechanisms of tolerance to waterlogging and submergence*. IRRI, The Philippines. UWA and IRRI, Publ. 136 pp
- Jackson, M.B. and Armstrong, W. (1999) *Plant Biol.* 1 274-287.
- McFarlane DJ (1990) *Land and Water Research News* 7: 5-8.
- McFarlane DJ, Barrett-Lennard EG and Setter TL(1989) *Proceedings of the Fifth Australian Agronomy Conference*. Perth, Western Australia. pp.74-83.
- Setter, T.L. Ellis, M., Laureles, E.V., Ella, E.S., Senadhira, D., Mishra, S.B., Sarkarung, S. and Datta, S. (1997) *Annals of Botany* 79:67-77.
- Seymour, M. (1999) *Survey of the soil types, nutrient status and variability of a proposed surface drainage site – “The Oaks”, Esperance Western Australia*. Report of Agriculture Western Australia, Perth. 16 April, 1999.
- vanGinkel M., Rajaram, S. and Thijssen, M. (1992) pp. 115-124. In: *The Seventh Wheat Workshop for Eastern, Central and Southern Africa*. Nakuru, Kenya, Sept. 16-19, 1991.