



**PHYSIOLOGICAL TRAITS FOR SCREENING
DROUGHT RESISTANCE IN BARLEY**

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LIST OF ABBREVIATIONS

CL	Coleoptile length
DGS	Decimal growth stage
DH	Days to heading
DIF	Difference between CL in control and CL in water stress
DM	Dry matter
dry-wt	Dry weight
DSI	Drought susceptibility index
EV	Early vigour
G × E	Interaction between genotype and environment
GP	Germination percentage
GY	Grain yield
h	Hours
HI	Harvest index
IWC	Initial water content
L	litre
L.s.d.	Least significant difference
LAC	Leaf ash content
mg	milligram
min	minute
mL	millilitre
mm	millimetre
MPa	Mega Pascal
PEG	Polyethylene glycol
QTL	Quantitative trait loci
RCL	Relative coleoptile lengths
RWL	Rate of water loss
S.e.d.	Standard error of differences of means
SLA	Specific leaf area
t	tone

TE	Transpiration efficiency
WUE	Water use efficiency
Δ	Carbon isotope discrimination
ψ_l	Leaf water potential

SUMMARY

Barley is an important winter cereal grown in southern Australia, second only to wheat. It is mostly grown under dryland conditions, where yield is frequently limited by drought caused by low and variable rainfall. Therefore, increasing the yield and yield stability of barley under water limited conditions has been a major aim of barley breeding programs since the inception of modern plant breeding. In most cases, selection has been based largely on yield, but it has frequently been suggested that yield *per se* is a poor indicator of drought resistance and that greater progress could be made by selecting for other attributes specifically related to drought resistance. Many physiological traits have been proposed as selection criteria for screening cereal germplasm over the last few decades, however, except for rare studies, such tests have been ignored by plant breeders because it has seldom been demonstrated that indirect selection for increased yield via these criteria is more effective than direct selection for yield. Moreover, some of the traits that have been suggested are very complicated, time consuming and expensive to measure. Hence, the aims of the present study were to evaluate critically a number of physiological traits which may be related to drought resistance in cereals, and to examine the feasibility of using these screening techniques in selecting more drought resistance genotypes of barley for South Australia.

A number of laboratory, glasshouse and field experiments were conducted. The experiments used a set of eighteen barley varieties including twelve varieties which are commercially grown in South Australia, and six varieties which are parents of doubled haploid populations. These varieties showed differences in drought susceptibility index (DSI; Fischer and Maurer, 1978) and grain yield (GY) in a severe drought year (1994).

The traits examined were germination percentage in osmotic solutions (GP), elongation of coleoptiles in osmotic solutions (CL), initial water content (IWC) and rate of water loss (RWL) of excised leaves, early vigour (EV), date of heading (DH) and transpiration efficiency (TE) predicted by carbon isotope discrimination (Δ), specific leaf area (SLA), and leaf ash content (LAC).

Water stress was induced by a range of methods. Solutions of polyethylene glycol (PEG) at water potentials of -0.7 MPa and -1.0 MPa were used in the germination tests and coleoptile elongation tests in laboratory. In the glasshouse, post-anthesis water stress was induced by supplying only 50% of water applied in the well-watered treatment. In the field, plants were grown under irrigated and non-irrigated conditions.

The response to stress at the germination stage was evaluated by measuring GP and CL. In glasshouse and field experiments, EV, IWC and RWL were assessed at the five leaf stage, while Δ , SLA, LAC were measured on leaf samples taken at flowering. GY and DSI were also calculated for these experiments. The usefulness of each trait and the feasibility of screening techniques are briefly discussed in the following sections.

In an osmotic solution of -0.7 MPa, barley varieties differed significantly in GP although they had similar GP in the control treatment. This finding provided evidence that GP of barley under water stress may be under genetic control. GP was found to be positively correlated with GY of barley grown under water stress in the glasshouse, but it was not correlated with either GY or DSI of barley grown under severe drought in 1994 and non-irrigated conditions in the field in 1996. This suggests that GP may not be generally associated with GY of barley in the environments of South Australia.

Coleoptile length in osmotic solution (-1.0 MPa), an indicator of osmoregulation in cereals (Morgan, 1990; 1991), differed significantly among barley genotypes. The genotypic ranking in CL among genotypes was consistent over experiments. Interestingly, CL was positively correlated with GY of barley grown under water stress in the glasshouse and strongly correlated with GY and DSI of barley grown in the severe drought year of 1994. These findings lend support to the hypothesis that elongation of coleoptiles under osmotic stress may be a reliable indicator of drought tolerance in barley. Therefore, this technique could be used to screen a large population using a simple, rapid and inexpensive procedure.

A large variation in IWC and RWL from excised leaves of barley varieties grown under both glasshouse and the field conditions was demonstrated. There was no interaction between variety and environment for both IWC and RWL, but only genotypic ranking of IWC was consistent between glasshouse and the field. However, RWL was only correlated with GY under water stress conditions in the glasshouse, where severe drought was imposed on the plants from flowering to maturity ($r = -0.63$, $P < 0.01$). No correlation was found with yield under rainfed conditions in the field, where the level of stress was less. These results suggest that RWL may be beneficial for GY in severe drought conditions, where cuticular water loss may be an important factor in reducing severe desiccation. This technique is quite labor intensive, and further development would be required before implementation in a breeding program.

Large differences among varieties in TE, Δ , SLA and LAC were detected under different water treatments in both glasshouse and field experiments. The experimental evidence showed that Δ , SLA and LAC were negatively correlated with TE in barley, and SLA and LAC were strongly and positively correlated with Δ . These relationships and the

genotypic ranking for SLA and LAC were consistent over glasshouse and the field conditions. The findings suggest that while measuring Δ is very expensive and complicated, the simpler and cheaper measurements of SLA and LAC could be used as surrogates for Δ in predicting TE in barley. However, the relationships between these traits and GY were inconsistent. Δ , SLA and LAC were negatively correlated with GY under well-watered conditions, but not correlated with GY under water stress conditions. The results suggest that selection for TE alone may not be sufficient to improve yield under drought, but may be incorporated along with other traits such as EV and early heading to improve yield under water-limited conditions.

Consistent genotypic differences in EV and DH were found in both glasshouse and field experiments. EV was negatively correlated with DSI, and DH was positively correlated with DSI of barley grown in the field conditions. This provides evidence that under terminal drought conditions, like that experienced in South Australia, high EV and early heading are crucial traits contributing to drought resistance in barley. However, in this study, there was an association between EV and DH. As well, EV was positively correlated with Δ , SLA and LAC indicating that EV was negatively correlated with TE. Δ , SLA and LAC were also found to be negatively correlated with DH implying that DH may be positively correlated with TE. These correlations were observed in both glasshouse and field experiments and they suggest that the associations between TE and EV or DH may constrain the development of lines which have higher TE, high EV and early heading.

Among the traits investigated, CL of barley in water stress induced by osmotic solutions was the most promising because it was correlated with GY and DSI of barley grown under water stress conditions. CL was measured on 150 lines from the doubled haploid mapping

population of Clipper × Sahara to identify possible quantitative trait loci (QTLs) for this trait in barley. Forty one percent of the variation in CL in water stress was explained by 6 QTLs which are located near to the RFLP markers of X7GLOB on the chromosome 1H ($P = 0.023$), 2R/6R on the chromosome 2H ($P = 0.025$), WG405 on the chromosome 3H ($P = 0.0087$), PSR163 on the chromosome 4H ($P = 0.0055$), ksuA1 on the chromosome 5H ($P = 0.0017$) and PSR167 on chromosome 6H ($P = 0.01$). The QTLs for CL in water stress was independent with the QTLs controlling CL in non-water stress. This suggests that CL in water stress is a genetically independent trait, which is not related to CL in non-water stress.

In conclusion, a wide range of genotypic differences in GP, CL, IWC, RWL, TE, Δ , SLA, LAC, EV and DH were detected among the eighteen barley varieties. Among them, CL was found to be the most promising for indirect selection criterion for GY under terminal water stress conditions. CL is controlled by a number of genes and the screening technique is rapid, inexpensive and can be used at the early stage of the development of plant. IWC and RWL indicated some degrees of drought resistance in barley, but the screening technique is very labor intensive and therefore it is not suitable for routine screening of breeding populations. The strong and consistent relationship between SLA and LAC with Δ and then TE lend support to the hypothesis that SLA and LAC could be used as surrogates for Δ in predicting TE in barley; the measurements are also simpler and cheaper than analysing Δ . However, these traits were not related to barley GY under drought conditions either in the glasshouse or the field. This suggests that improving TE through selecting for SLA or LAC should be considered in the context of an entire plant ideotype for drought environments, where TE should be incorporated with other drought resistant traits. Both EV and early heading appeared to be the most important traits

determining DSI in barley in the field. Screening either for EV or DH is quite simple and can be carried out in various environments. However, to avoid the low yield potential of early maturing varieties in favourable seasons, the plant ideotype of barley in South Australia should be the combination between high EV and early to mid-season maturity.

DECLARATION

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

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

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