06/22 FINAL REPORT

Selection methods for the improvement of drought tolerance and water use efficiency in sugar beet

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Executive Summary

Achievements and progress, 2006

Characterisation of genetic resources

In 2006, 27 genotypes were tested under irrigated and managed drought conditions using the polytunnel system. These genotypes consisted of six 'core' hybrids that have been tested over several years and are known to contrast for drought tolerance and water use efficiency. The best of these (KWS4-03) showed greater drought tolerance than the benchmark variety Cinderella. However, in a bid to discover new germplasm with a greater range of responses, 200 testcross hybrids from KWS were tested in observation plots 2004. A subset of these was tested in replicated plots in 2005, and a further subset was tested a second year in 2006. The result, shown here, is 11 testcross genotypes that show low and high drought tolerance, including some that show significantly greater drought tolerance than genotypes studied previously. In addition, ten genotypes from Strube Dieckmann were tested. None of these had been evaluated for drought tolerance previously. Some of these genotypes showed exceptional drought tolerance and yield potential. Characterisation of these materials help us learn more about what characters contribute to drought tolerance, and inform the breeder which germplasm tends to be more successful.

Water use efficiency

The differences between genotypes in the way they use water to form yield (the water use efficiency, or WUE) are related to growth potential more than differences in actual water use. Hence, genotypes that yield poorly, but use similar amounts of water to other more high yielding genotypes, essentially waste water. Breeders would like to eliminate these types from their advanced breeding pool. Carbon isotope discrimination ratio (CID) was inversely related to WUE, but the correlation was not as strong as that observed in previous experiments. Data from a lysimeter experiment designed to measure water use efficiency more accurately than in field experiments were not reliable because plants became too stressed during unusually hot weather in July. However, much was learned about how to do these types of experiments.

Secondary traits

Traits that are associated with drought tolerance and water use efficiency, but are easier and cheaper to measure, could be useful selection criteria in breeding programmes. We studied a number of morphological and physiological traits that have shown promise in previous studies. New ways of measuring stomatal conductance and specific leaf weight were investigated, and improvements in techniques were made. We field-tested and calibrated a new viscous flow porometer, which should be a useful tool for assaying transpiration capacity, as measurements can be made rapidly on a large number of plants.

Conclusions

These experiments fulfilled the objectives of confirming the ranking of an extended and more diverse set of genotypes than had previously been examined. These genotypes will be useful tools in on-going studies. The relationships between secondary traits and water use efficiency were further elucidated, but the strength of the correlations are not yet strong enough to warrant their immediate use by breeders as indirect selection criteria for drought tolerance or water use efficiency. We have discovered extensive genotypic variation for carbon isotope discrimination ratio, and this trait could be directly applied in breeding programmes for water use efficiency.

Future directions

KWS and Strube Dieckmann have been instrumental partners with the BBRO in this work, and have shown a solid commitment to the research, knowing that improvements in drought tolerance will be vital for their markets not only in the UK but in eastern Europe and other drought-prone regions. The key to continued progress is to supply breeders with the tools necessary to begin making selections for enhanced drought tolerance in early stages of the breeding programme. The next phase of research is aimed at refining the techniques for field screening and phenotyping mapping populations. Current work also begins to address the ability of sugar beet to recover from drought. This aspect has not yet been considered and there may be considerable genotypic differences that breeders can exploit.

Knowledge and technology transfer

Findings from the research have been communicated through refereed journals, articles in the popular and farming press, and in talks and posters given at scientific meetings (see below). In addition, there have been information displays at Open Days and farming events (e.g. Cereals), and direct conversations between the research team and growers. Regular meetings have been held with breeders, involving in-depth analysis of data and discussion of research ideas.

Project résumé: Expenditure and scientific staff input

What was the planned expenditure?	£41,827
What was the actual expenditure?	£43,455
What was the planned scientific input in staff years?	0.64 (140 days)
What was the actual scientific input in staff years?	0.66 (146 days)

Has there been any outputs this year?	YES
Have any opportunities for IP rights been identified?	NO
Are there any scientific opportunities arisen not mentioned?	NO

Project résumé: collaborations

KWS, Drs. Rudolf Jansen, Günter Diener, Hinrich Harling, Bernd Truberg (seed, breeding advice, financial support)

Strube Dieckmann, Dr. Axel Schechert

Sugar Beet Seed Institute of Iran, Dr Y Sadeghian, Abouzar Rajabi (seed and studentship)

Cambridge University, Prof. Howard Griffiths (expertise with CID; co-supervision of Mr. Rajabi)

Project résumé: Publications, presentations and dissemination of results

1. Rajabi A, Griffiths H, Ober ES, Kromdijk W (2007) Genetic characteristics of water-use related traits in sugar beet. Euphytica (in press).
2. Ober ES, Sharp RE (2007) Regulation of root growth responses to water deficit. In, MA Jenks, PM Hasegawa, SM Jain (eds) Advances in molecular-breeding toward drought and salt tolerant crops. Springer (in press).
3. Rajabi A (2006) Carbon isotope discrimination and selective breeding of sugar beet (*Beta vulgaris* L.) for drought tolerance. PhD thesis, Cambridge University (co-supervised with Prof. Howard Griffiths).
4. Ober ES, Rajabi A (2006) Evidence for genotypic differences in water use efficiency (WUE) during drought, and how to improve WUE through selection. Proceedings of the 69th IIRB Congress, Brussels.
5. Ober ES (2006) Evaluation of differential rooting and water use characteristics of sugar beet genotypes under field drought conditions. ECP Genetic Resources Working Group on *Beta* and World *Beta* Network Meeting, Tenerife, March, 2006.

Selected Key Results

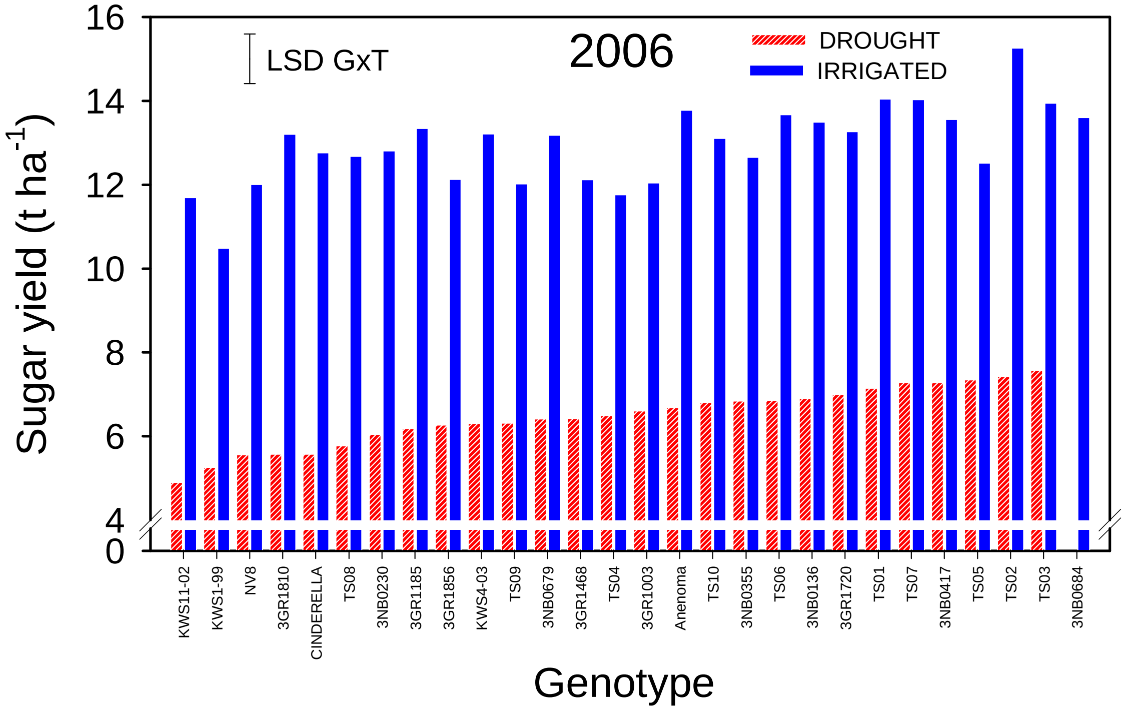


Fig. 1. Sugar yields obtained in 2006, comparing 6 'core' hybrids, the 11 most contrasting KWS testcross populations (3GR…3NB…) taken from the 2005 trial, and 10 new Strube genotypes tested for the first time. Overall, a 50% yield loss was caused by the managed drought conditions. Note that compared with Cinderella and Anenoma, several genotypes showed better yields under droughted conditions. (3NB0684 was only tested under irrigated conditions.). Drought was imposed using polythene tunnels as described previously, and irrigated plots were maintained in a separate experiment adjacent to the droughted plots. Irrigated plots were used to establish yield potential of genotypes.

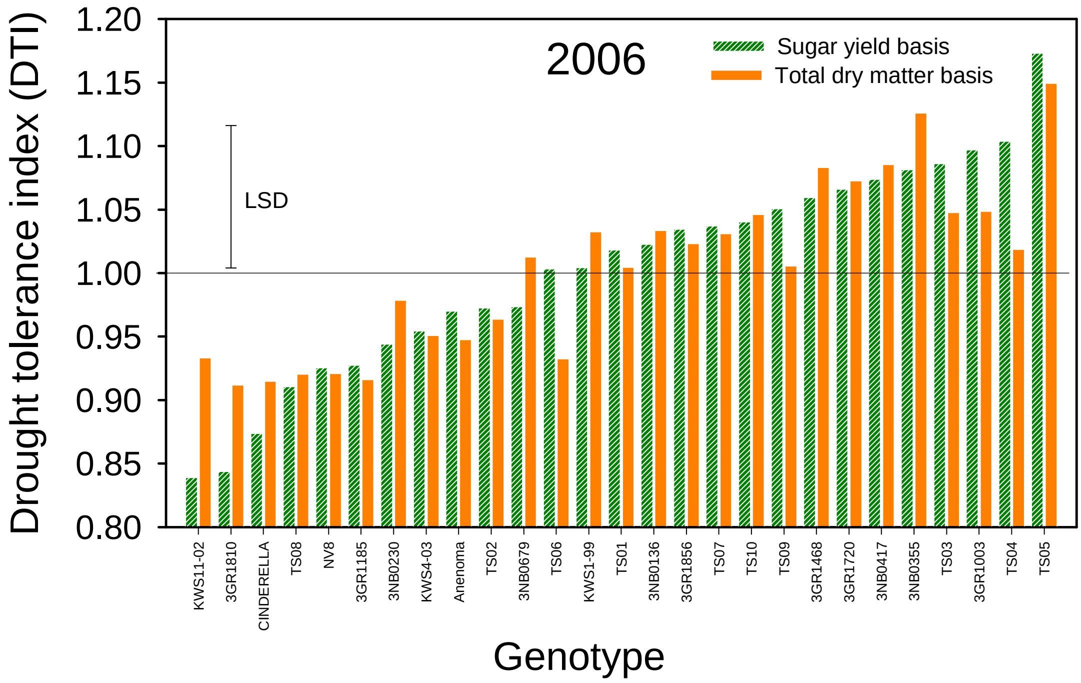


Fig. 2. Drought tolerance index of genotypes tested in 2006, computed on a sugar yield or total dry matter basis. Note that some new genotypes showed significantly greater drought tolerance than Cinderella and Anenoma.

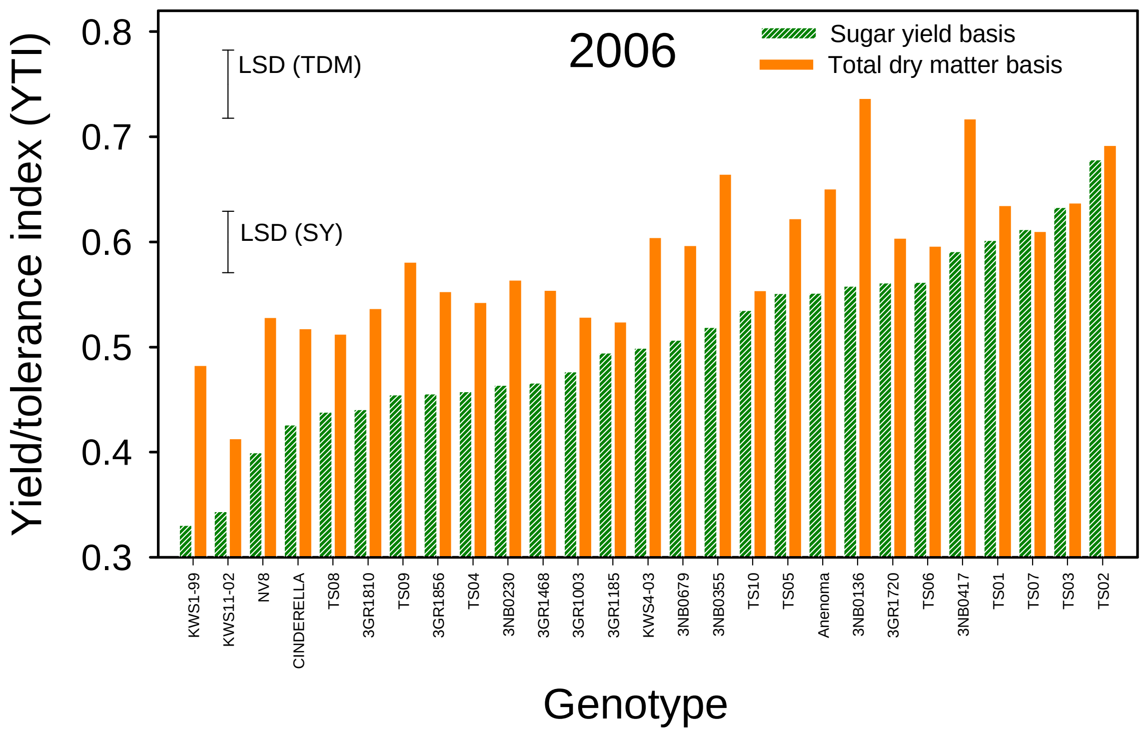


Fig. 3. Yield/tolerance index of genotypes tested in 2006, computed on a sugar yield or total dry matter basis. Genotypes with high YTI combine good yield stability with yield potential.

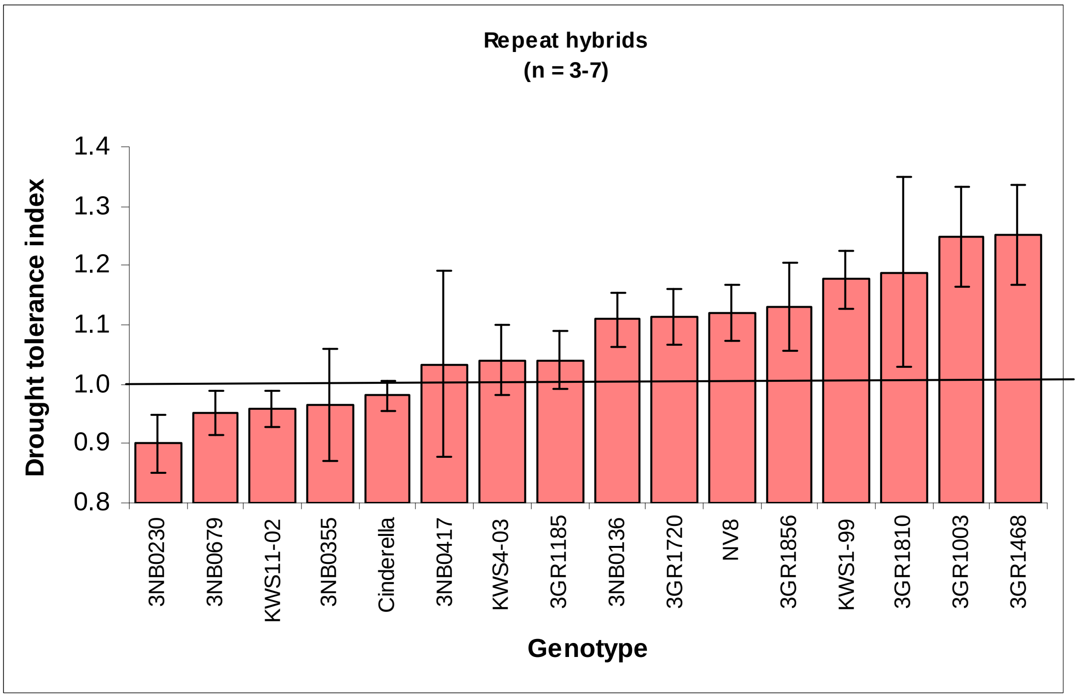


Fig. 4. Drought tolerance (sugar yield basis) of hybrids tested under field drought and irrigated conditions over several seasons (n = 3-7). This shows the repeatability and consistency of the genotype rankings. KWS testcross hybrids (3NB…3GR…) showed smaller and greater drought tolerance than benchmark varieties and the 'core' hybrids used previously. Mean drought tolerance index of all genotypes is 1.0.

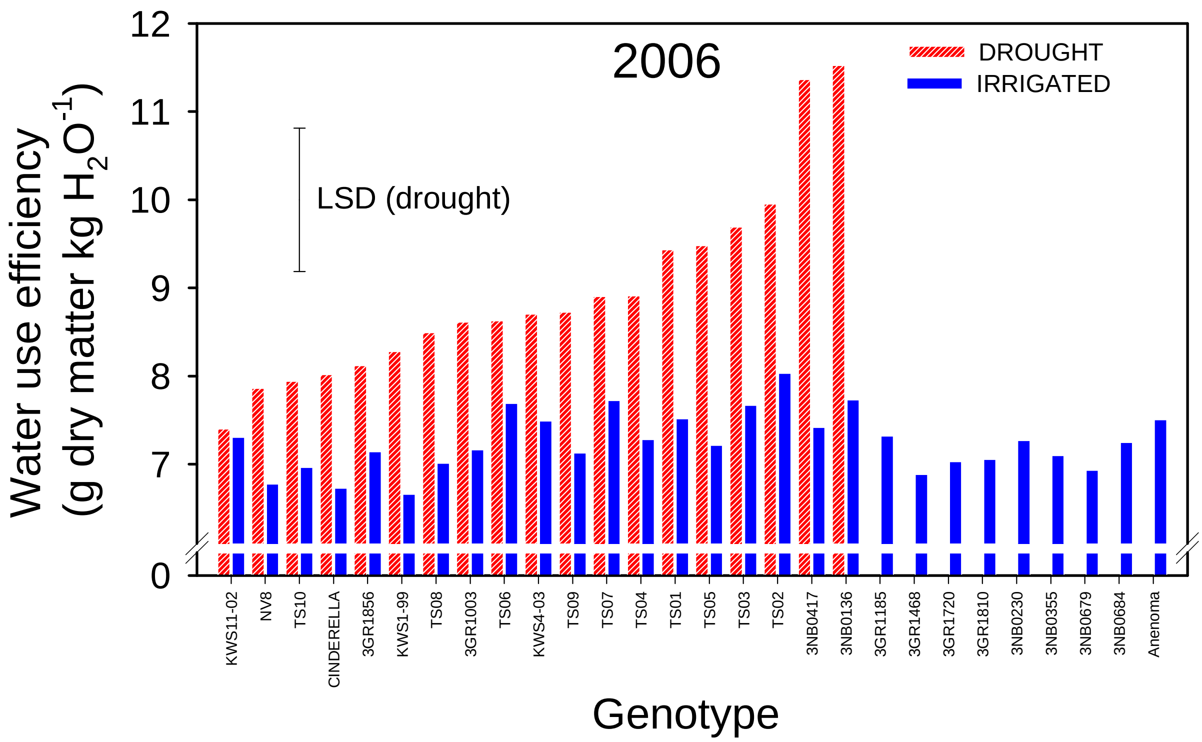


Fig. 5. Water use efficiency (WUE) differed significantly between genotypes that yielded well (e.g. 3NB0417) and genotypes that yield poorly (e.g. KWS11-02) under droughted conditions. A subset of 19 genotypes were measured under droughted conditions. Genotypes also differed in WUE under irrigated conditions. WUE was estimated in irrigated plots by adjusting calculated Et for plot differences in crops cover throughout the season.

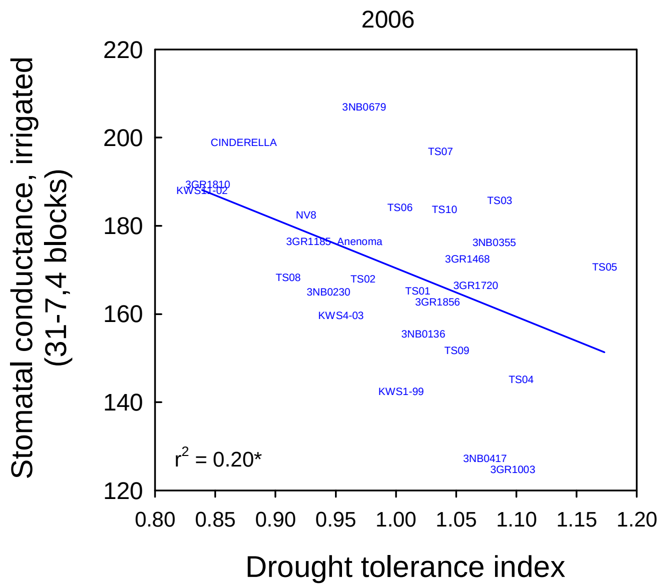


Fig. 6. Scatter plots of physiological/morphological traits with drought performance indicators from field experiments. Genotypes that showed greater stomatal conductance tended to be less drought tolerant, perhaps through early exhaustion of soil water. This result will be re-tested.

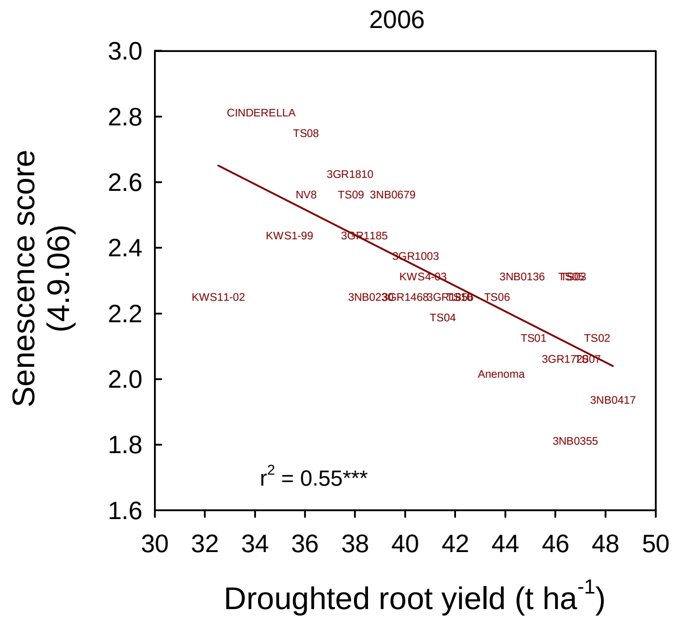


Fig. 7. Senescence of the lower canopy in late summer after significant drought stress is a good indicator of poor drought tolerance.

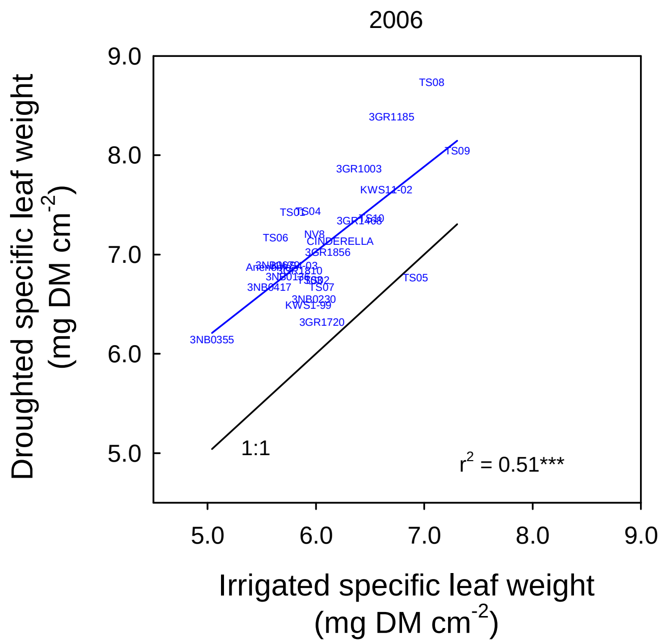


Fig. 8. Specific leaf weight is negatively related to drought tolerance, but shows little GxE interaction: the ranking of genotypes changes little under droughted and irrigated conditions, although leaves become thicker under drought. Faster, more accurate ways to assess specific leaf weight are being sought.

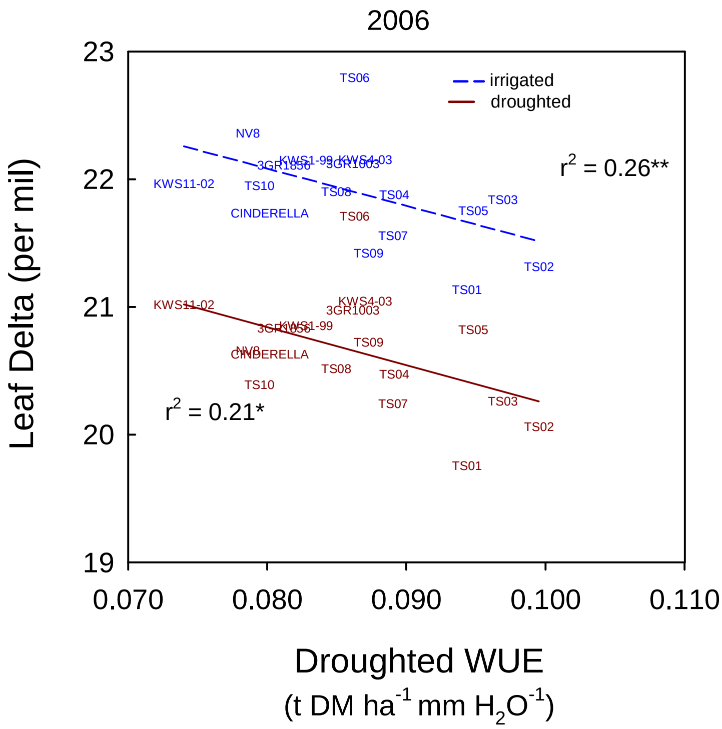


Fig. 9. Correlation between carbon isotope discrimination ratio (Delta), measured in leaves under droughted and irrigated conditions, and season-long water use efficiency (WUE) measured under droughted conditions. Selection against genotypes with high Delta could be used to cull genotypes with low WUE.

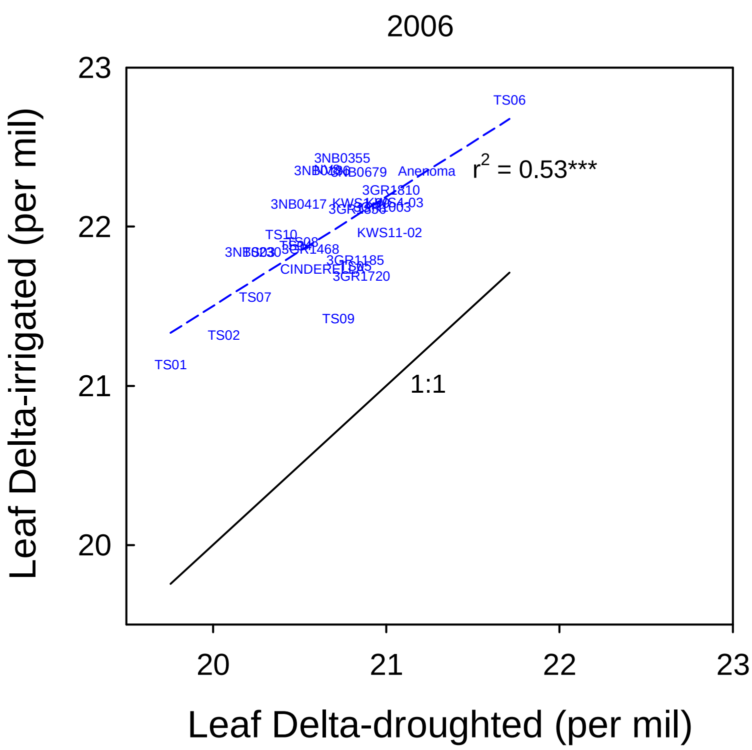


Fig. 9. Correlation between Delta measured under irrigated and droughted conditions. The good correlation indicates that measurements made without managed drought conditions are sufficient to establish genotype rankings. Delta is shifted to smaller values under drought because stomatal conductance is limited.

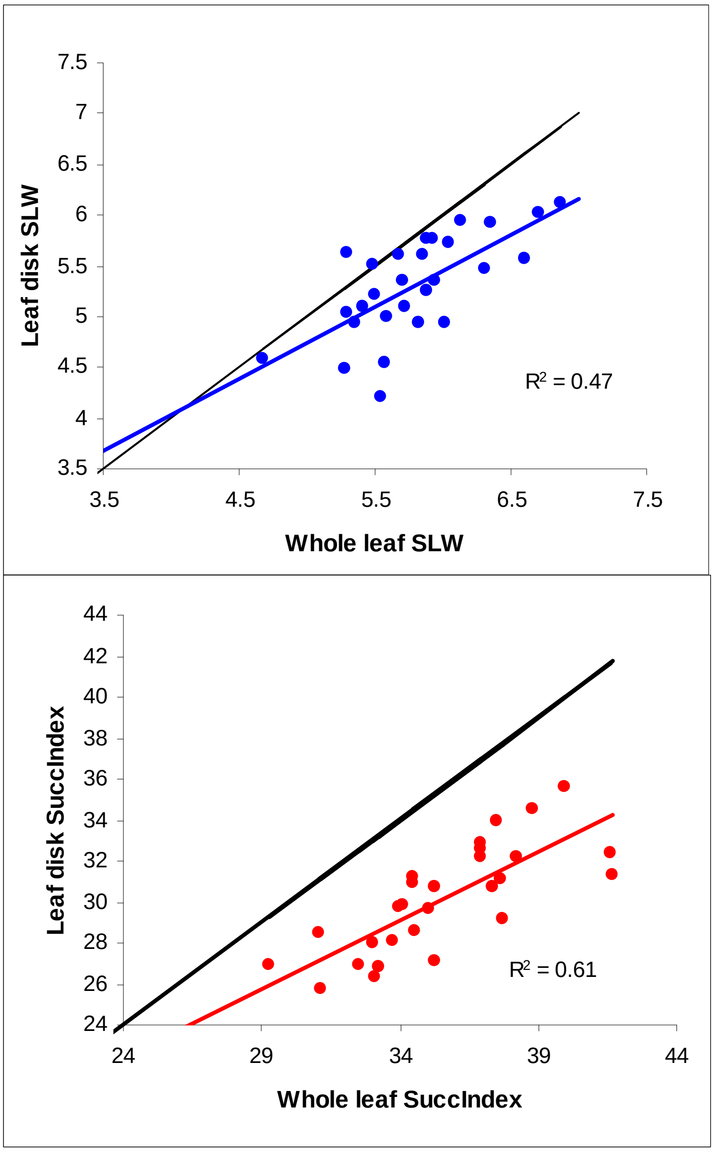
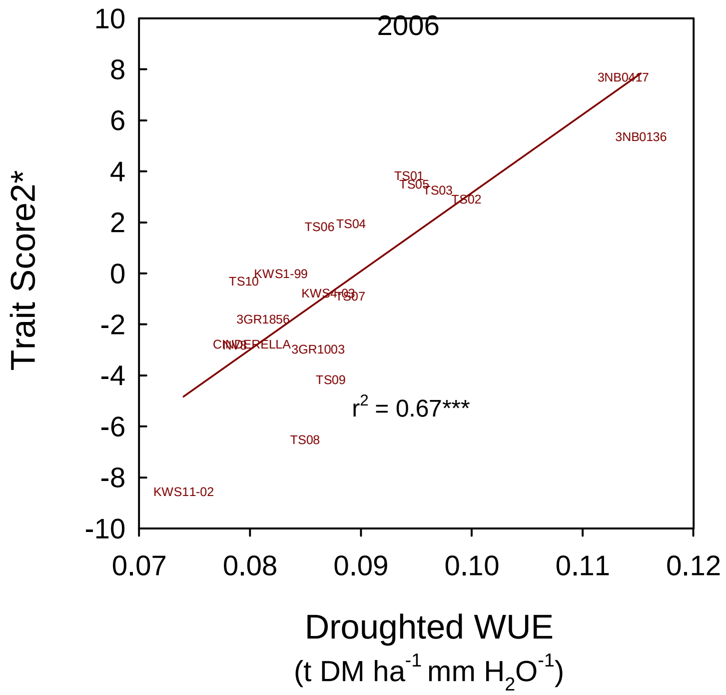
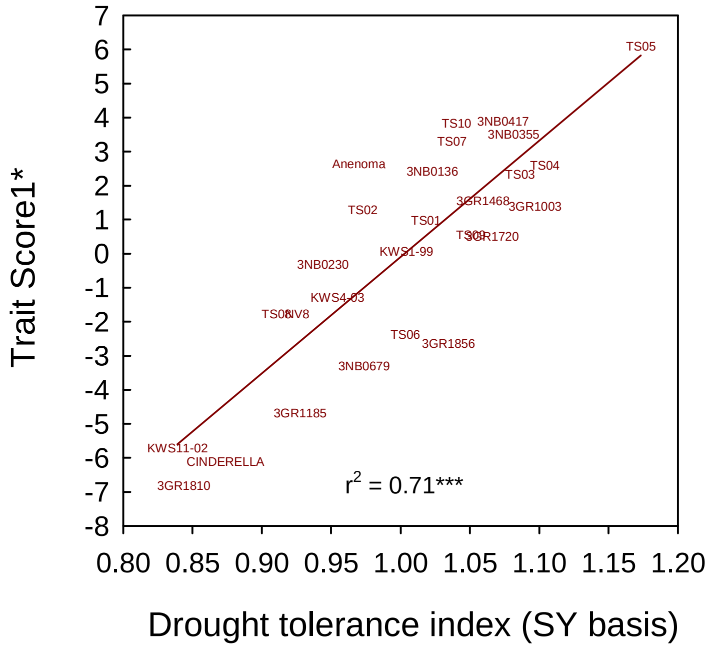


Fig. 10. Correlation between two methods of measuring leaf morphological traits. Whole-leaf measurements slightly underestimate the value obtained using leaf disks, but the correlation is high. This indicates that the faster, easier whole-leaf method is an acceptable alternative approach better suited to high-throughput situations.



Trait Score2 = sum of standardised values for: D_Sugar%DW, D_Root%WC, 1/I_LeafThick

1/Sen4-9; 1/I-ln VFP; I-CanTemp5_8; D_Cov15-6



Trait Score1 = sum of standardised values for: D_Sugar%DW, D_leaf%WC, 1/I-Trans31-7

1/Sen4-9; 1/I-ln VFP; I-C:N; D_Cov25-7

Fig. 11. Relationship between water use efficiency (WUE) and a composite trait score based on standardised values for sugar concentration (dry weight basis), root water content, leaf thickness, senescence, canopy temperature and leaf porosity (indicators of transpiration rate), and green canopy cover. The same composite trait score must be tested in a different environment to assess its general utility. A similar trait score was used to describe drought tolerance (bottom panel).

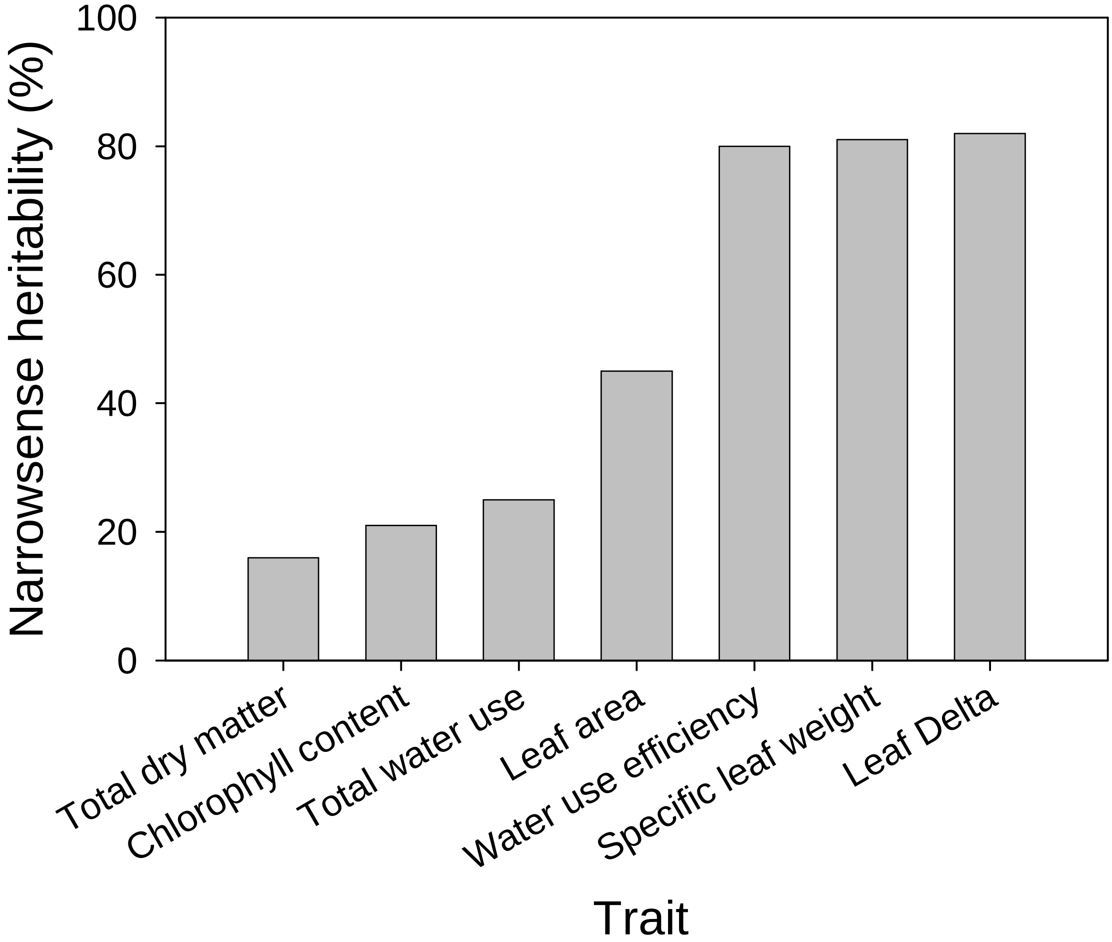


Fig. 12. Narrow-sense heritability of traits measured in a pot experiment of 25 genotypes resulting from a diallel cross of 5 inbred parental lines. High heritabilities for Delta and specific leaf weight indicate that breeding for these traits should result in high genetic gain and therefore improvements in the associated target traits (drought tolerance and water use efficiency).

Appendix. Drought tolerance definitions

Tolerance index (DTI)

The tolerance index (DTI) is similar to the drought susceptibility index (Fischer and Maurer, 1978), except that the percentage yield remaining rather than lost was used as a basis:

$$DTI = \frac{Y_D / Y_I}{\overline{\overline{Y_D}} / \overline{\overline{Y_I}}}$$

where Y_D is the yield under drought and Y_I is the genotype mean yield under irrigation. The denominator is the drought intensity index based on the mean droughted and irrigated yields across all genotypes within a trial.

Yield/tolerance index (YTI)

The other index combines the relative performance of a genotype under drought with its potential yield under irrigated conditions (Ehdaie et al., 2003; Fernandez, 1992).

$$YTI = \left(\frac{Y_D}{\overline{\overline{Y_D}}}\right) \cdot \left(\frac{Y_I}{\overline{\overline{Y_I}}}\right) \cdot \left(\frac{\overline{\overline{Y_D}}}{\overline{\overline{Y_I}}}\right) = \frac{Y_D \cdot Y_I}{\left(\overline{\overline{Y_I}}\right)^2}$$

To differentiate this index from TI, it has been given the notation YTI since it joins measures of yield and tolerance.

Table 1. Summary ANOVA terms for yield traits from the 2006 experiments conducted under irrigated and managed drought conditions. Drought tolerance index on a sugar yield basis (DTI-SY) and yield/tolerance index (YTI) were calculated as shown in the Appendix. Top yield and harvest indices are expressed on a dry matter (DM) basis. The F probabilities for genotype (G), irrigation treatment (T) and interaction (GxT) effects are shown, including the LSD for the GxT term (or the G term for drought indices); values less than 0.05 are statistically significant. Precision (R^2) estimates are based on the ratio of residual and total sum of squares.

Variate	Genotype	Treatment	GxT	LSD (GxT)	R^2	Grand mean	Droughted mean	Irrigated mean	units
SugarYield	<0.001	<0.001	0.086	1.2	0.95	9.7	6.5	12.9	t ha^{-1}
Total DryMatter	0.001	<0.001	0.116	1.8	0.95	17.4	12.8	22.0	t ha^{-1}
Clean RootYield	<0.001	<0.001	0.053	7.4	0.94	59.4	41.5	77.4	t ha^{-1}
TopYield	<0.001	<0.001	0.475	0.95	0.75	4.8	4.0	5.5	t ha^{-1}
Sugar:total DM	<0.001	<0.001	0.324	0.04	0.78	0.55	0.5	0.59	-
Root:top	<0.001	<0.001	0.494	0.58	0.72	2.7	2.3	3.1	-
DTI-SY	<0.001	-		0.11	0.65	1.0			-
YTI-SY	<0.001	-		0.06	0.86	0.5			-

Table 2. Summary ANOVA terms for water use efficiency (WUE) and water use parameters from the 2006 experiments conducted under irrigated (I) and managed drought(D) conditions. The F probabilities for genotype (G) effects are shown; values less than 0.05 are statistically significant. Precision (R^2) estimates are based on the ratio of residual and total sum of squares. Potential evapotranspiration is the Penman-Monteith Et based on weather variables measured in the open at Broom's Barn.

Variate	Genotype	LSD	R^2	Grand mean	units	legend
I_WUE	0.21	0.007	0.4	0.07	t DM ha^{-1} mm H_2O^{-1}	water use efficiency (total dry matter [DM] basis), irrigated
D_WUE	<0.001	0.02	0.57	0.09	t DM ha^{-1} mm H_2O^{-1}	water use efficiency (total dry matter [DM] basis), droughted
I_WU	<0.001	22	0.48	304	mm H_2O	total seasonal water use estimated from Et and plot crop cover
D_WU	0.148	20	0.32	144	mm H_2O	WU estimated from Et and changes in soil moisture content
D_Ta/Tp	0.148	0.05	0.31	0.33	-	ratio of actual to potential evapotranspiration
WU 70 cm	0.012	2.8	0.45	6.1	mm H_2O	total water removed from the layer 65-75 cm from the soil surface

Table 3. Summary ANOVA terms for morpho-physiological traits from the 2006 experiments. Variates are prefixed according to treatment (irrigated (I) or managed drought (D) conditions), and followed by the measurement date in some cases. Where applicable, the F probabilities for genotype (G), irrigation treatment (T) and interaction (GxT) effects are shown, including the LSD for the GxT or G term in single treatment experiments. Precision (R^2) estimates are based on the ratio of residual and total sum of squares.

Variate	Genotype	Treatment	GxT	LSD (GxT)	R^2	Grand mean	Droughted mean	Irrigated mean	units	legend
I_SPAD	<0.001	-		2.7	0.25		-	44.2	-	leaf chlorophyll content
I_ETR	0.016			120	0.51			256	-	electron transport rate
D_ETR	0.500			131	0.31		233		-	
wilt30-6	0.018			0.95	0.53		2.6		-	visual wilt score
wilt5-8	0.082			0.65	0.53		2.7		-	
I_CanopyTemp	0.191			1.2	0.55			21.1	ºC	canopy temperature
D_CropCover25-7	<0.001			5.7	0.73		40		%	green canopy cover
I_CropCover25-7	0.092			10	0.63			63.5	%	
I_Gs31-7	0.765			58	0.54			172	mmol m^{-2} s^{-1}	stomatal conductance
I_LeafThick	<0.001			0.04	0.21			0.42	mm	leaf thickness
SLW	<0.001	<0.001	0.235	0.8	0.80	6.6	7.1	6.1	mg DM cm^{-2}	specific leaf weight
SuccIndex	<0.001	<0.001	0.445	4.5	0.67	31.3	28.5	34.0	mg H_2O/cm^{-2}	leaf succulence index
DeltaC	<0.001	<0.001	0.254	0.49	0.88	21.1	20.5	21.8	per mil	$^{13}C/^{12}C$ discrimination ratio
DeltaN	0.025	<0.001	0.622	0.83	0.74	4.5	3.8	5.2	per mil	$^{15}N/^{14}N$ discrimination ratio
C:N	<0.001	<0.001	0.003	0.8	0.71	9.4	8.9	9.8	-	leaf carbon:nitrogen ratio

Table 4. Summary ANOVA terms for quality yield traits from the 2006 experiments conducted under irrigated and managed drought conditions. Sugar concentrations are expressed on a fresh weight (FW) and dry weight (DW) basis. The water content (WC) of roots and tops at harvest are shown. The F probabilities for genotype (G), irrigation treatment (T) and interaction (GxT) effects are shown, including the LSD for the GxT term. Precision (R^2) estimates are based on the ratio of residual and total sum of squares.

Variate	Genotype	Treatment	GxT	LSD (GxT)	R^2	Grand mean	Droughted mean	Irrigated mean	units
Amino N	0.003	<0.001	0.295	2.9	0.78	12.2	15.0	9.3	mg/ 100g beet FW
K	0.002	<0.001	0.002	16	0.78	179	164	194	mg/ 100g beet FW
Na	<0.001	<0.001	0.182	6.1	0.79	17.8	23.6	12.0	mg/ 100g beet FW
Sug%DW	<0.001	<0.001	0.010	2.33	0.77	76.2	74.2	78.2	%
Sug%FW	<0.001	<0.001	0.551	0.72	0.69	16.4	15.9	16.9	%
RootWC	<0.001	0.274	0.649	0.7	0.69	78.5	78.5	78.4	%
TopWC	<0.001	<0.001	0.270	2.2	0.62	83.2	82.3	84.1	%