Drought Tolerance Selection of Sugarbeet Hybrids

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ABSTRACT

Increased water demands and drought have resulted in a need to identify crop hybrids that are drought tolerant, requiring less irrigation to sustain yields. This study was conducted to assess differences in drought tolerance among a group of genetically diverse sugarbeet hybrids. The study was conducted over three consecutive growing seasons (2008-2010) at the USDA Northwest Irrigation and Soils Research Laboratory in Kimberly, ID on a Portneuf silt loam soil (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid). Drought tolerance was evaluated by measuring sucrose yield ha^{-1} of six experimental hybrids of KWS SAAT AG and one commercial hybrid (Betaseed Inc.) under six water input treatments (irrigation + precipitation). Hybrid drought tolerance was evaluated by linear regression analysis (slope and intercept) of yield versus water input, calculation of a drought stress index (DSI), and comparison to yield potential under full irrigation. The water input treatments were based on a percentage of estimated crop evapotranspiration (ETc). Water input treatments were 125% ETc (W1), 100% ETc (W2), 75% ETc (W3), 50% ETc (W4), 25% ETc (W5) and rain-fed (W6). There were significant differences in overall yield potential and in the sucrose yield response to water among hybrids. Greater drought tolerance or greater difference in sucrose yield among hybrids was seen at the lowest water input treatment (intercept difference). Significantly greater drought tolerance was observed between the hybrid, KWS-05, and the commercial hybrid. Based on these results it was concluded that there is genetic diversity for drought tolerance among existing sugarbeet experimental hybrids.

In many sugarbeet production areas of the U.S., increased water demands and drought have resulted in concern that water supplies will be inadequate to meet sugarbeet evapotranspiration (ET) requirements. Water deficit stress negatively affects plant physiology and metabolism (Zhu, 2002). The severity of water deficit stress on plant function can range from mild to severe depending on the degree and extent of the stress (Jaleel and Llorente, 2009). Water deficits can limit growth and influence a host of physiological functions in plants to a greater extent than any other environmental factor (Jaleel and Llorente, 2009; Cattivelli et al., 2008). Thus, considerable research effort has been undertaken to improve crop production under deficit conditions (Cattivelli et al., 2008; Wang et al., 2003).

Research into molecular mechanisms affecting abiotic stresses has shown promise for genetic modification of drought tolerance in agricultural crops (Wang et al., 2003). In sugarbeet, drought tolerance research has been limited because the extent of the problem of water stress was not fully comprehended; there were thought to be few differences among cultivars, and it was difficult to design appropriate selection methods in breeding programs (Ober et al., 2004; Ober and Rajabi, 2010). However, limited research has shown that there exists a large variation in sugarbeet yield and quality due to drought tolerance within commercial sugarbeet cultivars (Bloch and Hoffmann, 2005; Ober et al., 2004; Pigeon et al., 2006). Bloch and Hoffmann (2005) found that four sugarbeet cultivars differed significantly in root and leaf dry matter mass and concentration of sucrose, potassium (K), Sodium (Na), and α-amino acid nitrogen (N) in the root as a result of water deficit stress conditions. Ober et al. (2004) and Pidgeon et al. (2006) found significant variation in sucrose yield among several hybrids grown in water deficit conditions. Ober et al. (2004) concluded that the germplasm available to breeders contains the genetic variation needed to increase drought tolerance in sugarbeet. Under water stress conditions, sugarbeet sucrose storage has been found to be reduced as a result of the accumulation of ions and solutes (Hoffmann, 2010). Continued research is needed to better select for drought tolerant hybrids, improve production under water deficit conditions, and understand the physiological processes of drought tolerance in sugarbeet (Bloch et al., 2005).

Crop yield over a range of environments is considered the best indicator for assessing drought tolerance (Cattivelli et al., 2008). Regressing crop yield (dependent variable) against a host of environmental indices (independent variables) is an effective method for evaluating genotype adaptability to drought (Cattivelli et al., 2008). Many environmental indices have been derived from measurements such as canopy temperature, water potential, soil water availability, water input, and transpirable soil water (Idso et al., 1981; Motzo et al., 2001; Araus et al., 2003; Rizza et al., 2004). Utilizing slope and intercept data of the regression between crop yield and other dependent variables over a range of water deficit is an adequate method to compare hybrids (Cattivelli et al., 2008). The ideal drought tolerant genotype would be one with the highest intercept (highest yield under lowest water input) and lowest slope (lowest sensitivity to water deficits) (Cattivelli et al., 2008). These relationships have been utilized to assess drought tolerance in sugarbeet (Pidgeon et al., 2006; Ober and Rajabi, 2010), and differences among hybrids have been found to exist. Another common method of identifying plant hybrids that have yield stability under limited water conditions is the drought susceptibility index (DSI, Siahpoosh et al., 2011). The DSI compares yield differences under water stressed and optimum water levels.

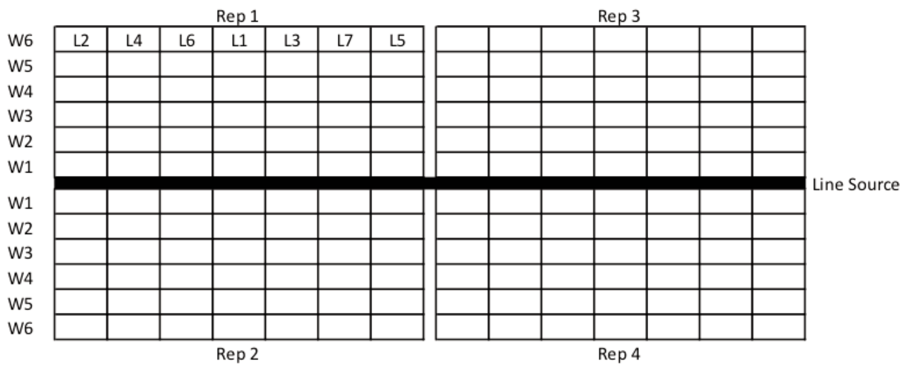
The objective of this research was to produce water production functions of selected genetically diverse advanced KWS SAAT AG experimental hybrids using a line source sprinkler irrigation system, and use these data to compare potential drought tolerance among the experimental hybrids to guide future breeding efforts.

MATERIALS AND METHODS

This study was conducted over a three year period (2008 – 2010) at the Northwest Irrigation and Soils Research Laboratory in Kimberly, ID on a Portneuf silt loam soil (coarse-silty mixed, superactive, mesic Durinodic Xeric Haplocalcid) to assess the yield of six KWS SAAT AG experimental hybrids and one Betaseed Inc. commercial hybrid under a range of water inputs [rain-fed (W6) and approximately 25% (W5), 50% (W4), 75% (W3), 100% (W2) and 125% (W1) of estimated crop evapotranspiration (ETc)]. The six KWS SAAT AG hybrids were monogerm, experimental genotypes. They represented materials within the KWS sugarbeet breeding pool that have a diverse genetic background and various resistance/tolerance backgrounds for Rhizomania, Curly Top and nematodes. Additionally, all six hybrids were selected based on preliminary information about variation under drought stress conditions and a close relationship to currently available commercial hybrids in the Western U.S.. However, no detailed research has been conducted to understand the hybrid by water input interactions. The commercial hybrid was selected based on popularity among producers in the growing area.

The experimental design was established based on the use of a line source irrigation system (Figure 1). Impact nozzles (Weather Tec G50V 23 degree; nozzle size and type = 6.5 MPS; flow rate = 12.4 Lpm) were spaced at 6.1 m intervals down the line to provide uniform irrigation distribution for a given perpendicular distance from the line. The sprinkler spacing and nozzle type were selected to ensure uniform water application parallel to the line source at any given distance from the line source. Tests were conducted to validate uniformity (data not shown). Water treatments were based on applying irrigation to supplement precipitation to match 100% crop water requirement (W2), calculated using the 1982 Kimberly-Penman Reference Evapotranspiration Model and daily crop coefficients (Wright, 1982) using data from an Agrimet weather station (U.S. Bureau of Reclamation, Boise, ID). The remaining

Figure 1. Diagram of experiment design. Irrigation levels (W1-W6) ran parallel to line source. Sugarbeet hybrids (L1-L7) were randomized within each combination of irrigation level and replication (example shown for replication one, W6). Research was conducted from 2008-2010 in Kimberly, ID.

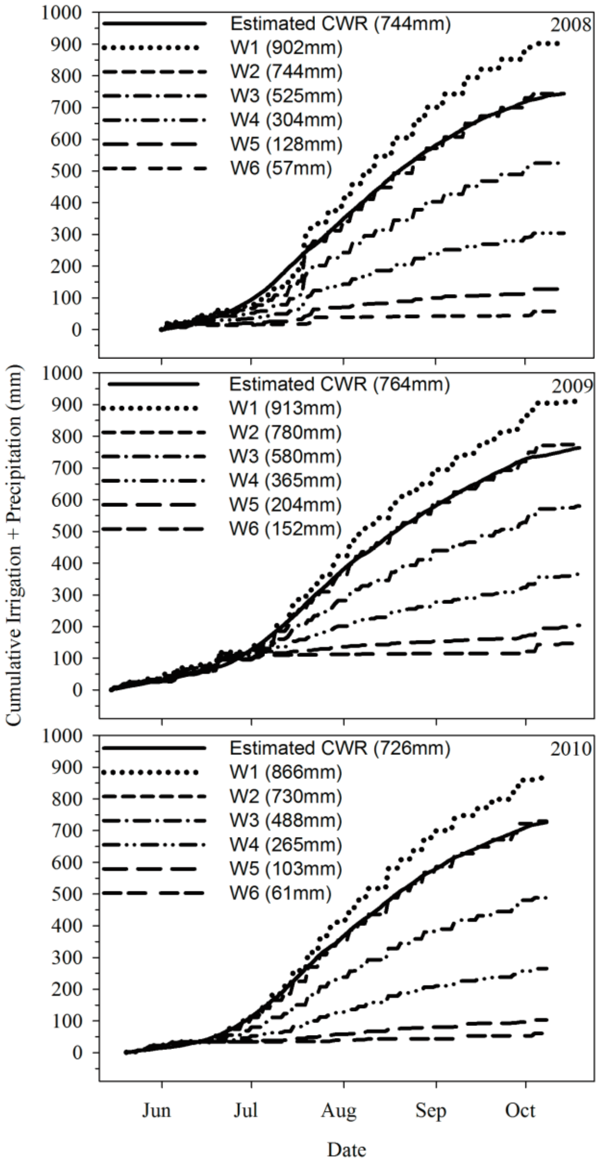


treatments received an amount of water based on the distance from the line source. Irrigation depth decreased as perpendicular distance from the line increased. Figure 2 shows the cumulative irrigation and precipitation, and cumulative estimated ETc over time for each treatment in 2008, 2009, and 2010. The 10 year average growing season (April 1 – October 15) precipitation in Kimberly, ID is 111 mm. Within each irrigation level, the seven sugarbeet hybrids were randomized. Each plot was 9.1 m long and 2.2 m wide (4 rows). Each irrigation amount and experimental hybrid combination was replicated 4 times in blocks.

Weed control consisted of pre emergence broadcast application of Nortron SC (ethofumasate) at a rate of 1.3 kg a.i. ha^{-1} incorporated with a roller harrow and hand weeding as needed during the season.

Prior to planting, six soil cores (4.4 cm diameter) were collected in 0.3 m depth increments to an overall depth of 0.9 m across the study site. Soil cores were composited at each depth increment across the study area. Soil samples were analyzed for nitrate-N (NO_3-N) and ammonium-N (NH_4-N) after extraction in 2M KCl (Mulvaney, 1996) using a flow injection analyzer, sodium bicarbonate extractable P and exchangeable K (Olsen et al., 1954). Based on the analysis, the study site was fertilized uniformly based on University of Idaho recommendations. After fertilizer was applied, the study site was tilled using moldboard plow followed by roller harrowing and bedding. Plots were planted at a population of approximately 352,000 seeds ha^{-1} and thinned to 117,000 plants ha^{-1} at the two leaf stage. Prior to planting, all sugarbeet hybrid seeds were treated with Poncho Beta (60 g a.i. clothianidin and 8 g a.i. beta-cyfluthrin per 100,000 seeds). After planting, approximately 50 mm of water was uniformly applied to all plots using sprinklers to en-

Figure 2. Annual cumulative crop water requirement (CWR) and water added for each irrigation rate treatment from plant emergence to harvest based on Agrimet weather data. Research was conducted from 2008-2010 in Kimberly, ID.



sure even plant emergence.

Irrigation water was applied two times a week during the early and late parts of the growing season and three times a week during the middle of the growing season. Irrigation depth was measured during each irrigation event using a transect of rain gages placed in plots within each replication in the center of each irrigation treatment.

To monitor effects of water input treatments on soil water, volumetric soil water content at depths of 0-0.15, 0.15-0.30, 0.30-0.45, 0.45-0.61, 0.61-0.76, 0.76-0.91, 0.91-1.07 and 1.07-1.22 m was measured weekly from three replications in W1, W2, W3, W4, and W6 water treatments from emergence to harvest using the neutron probe method (Evett and Steiner, 1995). Volumetric soil measurements were multiplied by soil depth to obtain soil water depth. Soil water depths for each soil depth increment were summed to determine total profile water content at each measurement date. Plant available water was determined based on estimated water content at field capacity (0.32 m^3 m^{-3}) and water at permanent wilting point (0.14 m^3 m^{-3}). A management allowable depletion (MAD) level of 55% was set as the depletion level above which the crop would be water-stressed (Jensen et al., 1990). Due to the interaction with the atmosphere above the soil surface and the neutron-probe function, a separate calibration curve was used for the 0-0.15 m depth to account for the loss of neutrons from the soil surface (Evett and Steiner, 1995).

In October, roots in the center two rows of each plot were counted and harvested. Total root yield was determined from each plot using a load cell-scale mounted on a plot harvester. From each plot a 20-root sample was collected and sent to the Betaseed tare lab for analysis of percent sucrose and impurities. From these data, sucrose and root yields were determined.

Drought susceptibility index (DSI) is defined as a criterion to evaluate hybrids performance under water-stress (Siahpoosh et al., 2011). The DSI compares yield differences under water stressed and optimum water levels and is calculated as:

$$DSI = \frac{1 - \left(\frac{Xs}{Xp}\right)}{1 - \left(\frac{Ys}{Yp}\right)}$$

Where, Xs is the sucrose yield of selected hybrid under water stress, Xp is the sucrose yield of selected hybrid under optimum water level, Ys is the average sucrose yield of all hybrids under water stress, and Yp is the average sucrose yield of all hybrids under optimum water level.

Sucrose yields at the W2 water level (100% ETc) and DSI from each sugarbeet hybrid were compared statistically using an ANOVA randomized complete block model in Statistix 8.2 (Analytical Software, Tallahassee, FL). Mean separations were carried out using the least significant difference (LSD) method. Significance was determined at the 0.05 probability level for all analyses.

The slope and intercept of the linear relationships between the sucrose and root yield and deficit water inputs (irrigation levels W1 to W4) among hybrids were determined and compared using SAS Proc MIXED (SAS Institute, Cary, NC). In the model statement, the fixed effects were experimental hybrid and the experimental hybrid by water input interaction. The random effects were block and block by water input interaction. In the model statement, the denominator degrees of freedom were calculated using the DDFM=KENWARDRODGER option. Confidence intervals (95%) were established for the comparison of experimental hybrid linear regression slopes and intercepts.

Drought susceptibility index values for each sugarbeet hybrid were compared statistically using a randomized complete block model in Statistix 8.2. Mean separations were carried out using the LSD method. Significance was determined at the 0.05 probability level for all analyses.

RESULTS

Sucrose Yield

Mean hybrid sucrose yields by water input treatments are shown in Figure 3. Sucrose yields at W2 (approximately 100 % ETc) for sugarbeet hybrids are shown in Table 1. Hybrid treatment sucrose yield differences were the same across years as indicated by the significant hybrid main effect and non-significant year by hybrid interaction. The year main effect was significant with sucrose yields being greater in 2008 (14.1 Mg ha^{-1}) compared to 2010 and 2009 (12.8 and 12.7 Mg ha^{-1}). At the W2 water input level, experimental hybrid KWS-01 had a significantly higher average sucrose yield across all years than all sugarbeet hybrids except KWS-02.

Linear Regression Slope and Intercept Comparisons

In 2008, the intercepts for experimental hybrids KWS-01, KWS-04, and KWS-05 were significantly greater than KWS-03 and for the commercial hybrid, and KWS-04 was greater than KWS-06 (Table 2). In 2008, there were no differences in slopes among sugarbeet hybrids. In 2009, the intercept and slope for experimental hybrid KWS-05 was significantly greater than the commercial hybrid. In 2010, the intercept for experimental hybrid KWS-05 was significantly greater than the commercial hybrid.

Drought Susceptibility Index

Over all years, the DSIs calculated for W6 relative to W2 for experimental hybrid KWS-02 and the commercial hybrid were significantly greater than the DSIs for experimental hybrids KWS-03, KWS-04, and KWS-05 (Table 3). The DSI calculated for W5 relative to W2 for the commercial hybrid was significantly greater than the DSIs for KWS-03, KWS-05, and KWS-06. The DSIs for the experimental hybrids KWS-01, KWS-02, and KWS-04 were significantly greater than the DSI for

Figure 3. Mean sucrose of experimental hybrids versus growing season irrigation and precipitation in 2008, 2009, 2010. Research was conducted from 2008-2010 in Kimberly, ID.

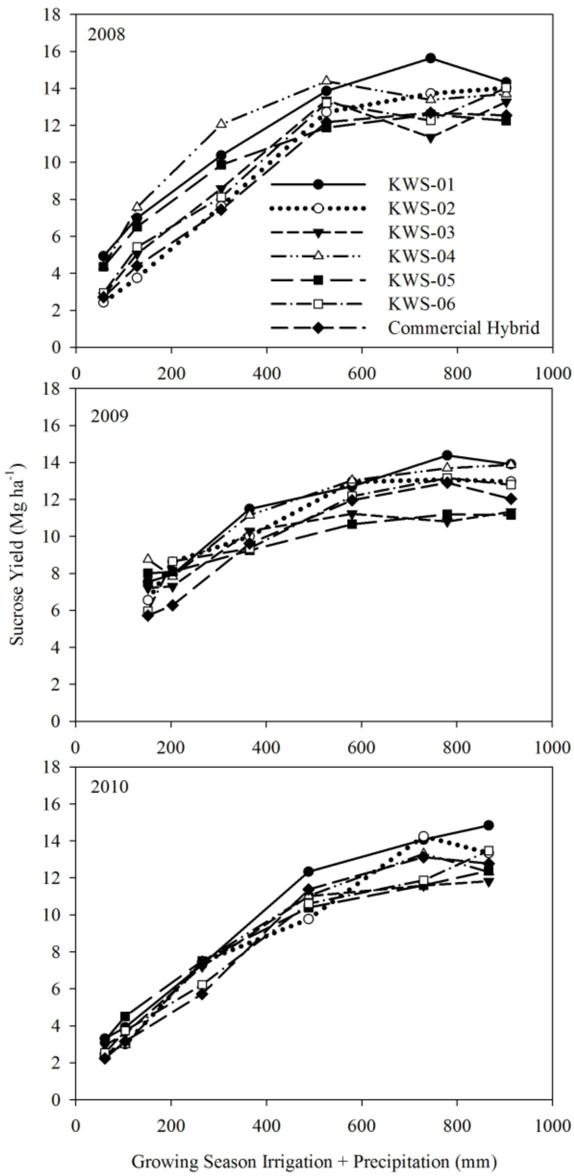


Table 1. Analysis of variance of sucrose yields at all irrigation inputs and comparison of sugarbeet hybrids sucrose yields. Values are averaged over years. Treatment means with the same letter are not significantly different p = 0.05 based on LSD. Research conducted from 2008-2010 in Kimberly, ID.

ANOVA	**Water Input Treatment**					
	W1	**W2**	**W3**	**W4**	**W5**	**W6**
Hybrid (H)	<0.001	<0.001	<0.001	<0.001	0.008	<0.001
Year (Y)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
H × Y	0.6228	0.771	0.046	0.110	0.035	0.3931
Hybrid	**Mg ha^{-1}**					
KWS-01	14.7 a	15.1a	13.3	10.0 ab	6.5	5.4 a
KWS-02	13.8 ab	14.0ab	12.1	8.5 cd	5.2	3.9 b
KWS-03	12.6 cd	11.7e	12.2	9.0 bcd	5.4	4.4 ab
KWS-04	13.7 abc	13.8bc	13.2	10.6 a	6.4	5.4 a
KWS-05	12.2 d	12.1de	11.2	9.1 bc	6.5	5.3 a
KWS-06	13.8 ab	12.7cde	12.3	8.2 cd	6.1	3.8 b
Commercial Hybrid	12.8 bcd	13.2bcd	12.1	7.8 d	4.7	3.7 b
Year	**Mg ha^{-1}**					
2008	14.5 a	14.1 a	14.0	9.9 a	6.1	3.7 b
2009	12.6 b	12.7 b	12.1	10.2 a	7.8	7.1 a
2010	13.0 b	12.8 b	10.9	7.0 b	3.6	2.8 c

KWS-05. The DSI calculated for W4 relative to W2 for the commercial hybrid was significantly greater than the DSIs for KWS-03, KWS-04, and KWS-05. The DSIs for the experimental hybrids KWS-01, KWS-02, and KWS-06 were significantly greater than the DSI for KWS-03.

DISCUSSION

Our experimental design had some advantages over other research studies assessing similar objectives. For example, due to our dry growing season, we did not need to use precipitation exclusion covers to ensure reduced water input (Ober et al., 2004). Ober et al. (2004) stated that the polythene covers used in their study had a significant effect on the microclimate (decreased windrun, photosynthetic active radiation and ET) compared to the uncovered treatment. Each plot in our study was exposed to the natural atmosphere, which eliminated the experimental error associated with the covers.

Water input treatments affected soil water in the 1.2 m root zone depth. In general, the depth of water in the soil decreased as the water input decreased (Figure 4). The soil water depth of the W4 and W6 treatments were close to the permanent wilting point during all years;

Table 2. Comparison of linear model constants among hybrids for sucrose yield versus growing season deficit irrigation and precipitation depths in 2008, 2009 and 2010. For each year, intercepts and slopes within each column are not significantly different (p = 0.05). Research was conducted from 2008-2010 in Kimberly, ID.

Year	Sugarbeet Hybrid	Intercept	Slope
2008	KWS-01	4.3 ab	0.019 a
	KWS-02	NS[1]	NS
	KWS-03	1.9 c	0.022 a
	KWS-04	4.7 a	0.020 a
	KWS-05	4.2 ab	0.016 a
	KWS-06	2.2 bc	0.021 a
	Commercial Hybrid	1.6 c	0.020 a
2009	KWS-01	5.6 ab	0.013ab
	KWS-02	5.2 ab	0.013 ab
	KWS-03	5.6 ab	1.11 ab
	KWS-04	6.2 ab	1.12 ab
	KWS-05	6.9 a	0.006 b
	KWS-06	5.1 ab	0.012 ab
	Commercial Hybrid	3.4 b	0.015 a
2010	KWS-01	1.8 ab	0.021 a
	KWS-02	1.5 ab	1.18 a
	KWS-03	1.7 ab	1.19 a
	KWS-04	1.2 ab	0.021 a
	KWS-05	2.7 a	0.016 a
	KWS-06	1.6 ab	0.018 a
	Commercial Hybrid	0.9 b	0.021 a

[1]NS = Not significant

while the W1 and W2 treatments were between the 55% management allowed depletion (MAD) and field capacity.

The relationships between sugarbeet sucrose yield and water input during the three years of the study were similar to many reported yield responses over a range of a yield limiting input factors (e.g., nutrients) (Dobermann et al., 2011). The response is visually defined as a linear increase at the inputs deficient range, decreasing rate of increase as the input reaches a sufficient amount and a plateau or decline when the input is sufficient and in excess.

The results from our study differed from Ober et al. (2004) in that hybrids with the highest water input yields did not necessarily have the highest yields under deficit water inputs. Ober et al. (2004) compared

Table 3. Analysis of variance of drought susceptibility index (DSI) calculated at W4, W5, and W6 water input treatments and comparison of sugarbeet hybrids DSIs. Drought susceptibility indexes were calculated at the selected water input treatments relative to the W2 water input treatment. Values are averaged over year. Treatment means within each water input treatment with the same letter are not significantly different (p = 0.05) based on LSD. Research was conducted from 2008-2010 in Kimberly, ID.

ANOVA		**P>F**	
Year (Y)	0.024	0.033	0.904
Sugarbeet Hybrid (SH)	0.006	0.020	<0.001
Y × SH	0.833	0.332	0.050
		DSI	
Sugarbeet Hybrid	W4	W5	W6
KWS-01	0.49ab	0.89ab	1.00abc
KWS-02	0.58a	0.91ab	1.10a
KWS-03	0.23c	0.76bc	0.86cd
KWS-04	0.35bc	0.87ab	0.93bcd
KWS-05	0.35bc	0.67c	0.81d
KWS-06	0.57ab	0.79bc	1.09ab
Commercial Hybrid	0.61a	0.99a	1.14a

the relationship between sugar yields under irrigation and sugar yields under drought. The relationship was significant ($P < 0.001$) with an r^2 of 0.64. In our study the relationship was not significant for each year (Figure 6), indicating that hybrids with highest sucrose yields under full irrigation were not necessarily the highest yields under deficit irrigation (approx. 25% ETc).

Evaluating sucrose yields near the maximum yield (W2 treatment) also was necessary to correctly interpret linear model intercept and slope data over the deficit water input range. In general, a sugarbeet hybrid with a greater intercept compared to another hybrid indicates greater yield under rain-fed conditions, and a greater slope indicates a greater rate of yield increase as water input amount increases. Potential intercept, slope, and maximum yield situations listed below and in Figure 5 help clarify the concept. Situations below refer to the comparison of hypothetical hybrids X and Y.

a. Hybrid X is more drought tolerant than Hybrid Y at low water inputs but differences in tolerance decrease as water increases. At optimum water inputs, yield of Hybrid Y is greater than Hybrid X.
b. Hybrid X is more drought tolerant than Hybrid Y at low water deficit inputs. As water input increases, the differences between hybrids decrease, but at optimum water inputs, yield of Hybrid X is still greater than Hybrid Y.

Figure 4. Soil water in the 1.2 m soil profile over time for the W1, W2, W3, W4, and W6 water treatments in 2008, 2009, and 2010. Each value is the average of three replications. Solid, dotted, and dashed horizontal hybrids represent water depth at field capacity, 55% of plant available water (management allowed depletion), and permanent wilting point, respectively. Research was conducted from 2008-2010 in Kimberly, ID.

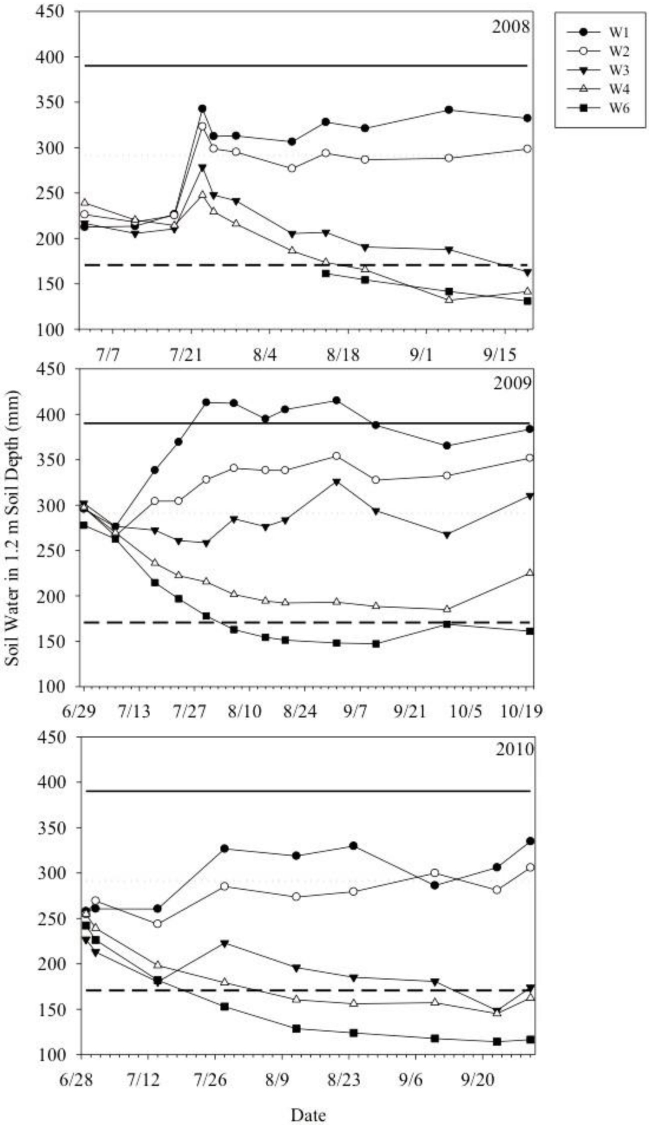


Figure 5. Example of potential intercept, slope, and maximum yield situations for two hypothetical sugarbeet hybrids. * = statistically significant, NS = not statistically significant. Research was conducted from 2008-2010 in Kimberly, ID.

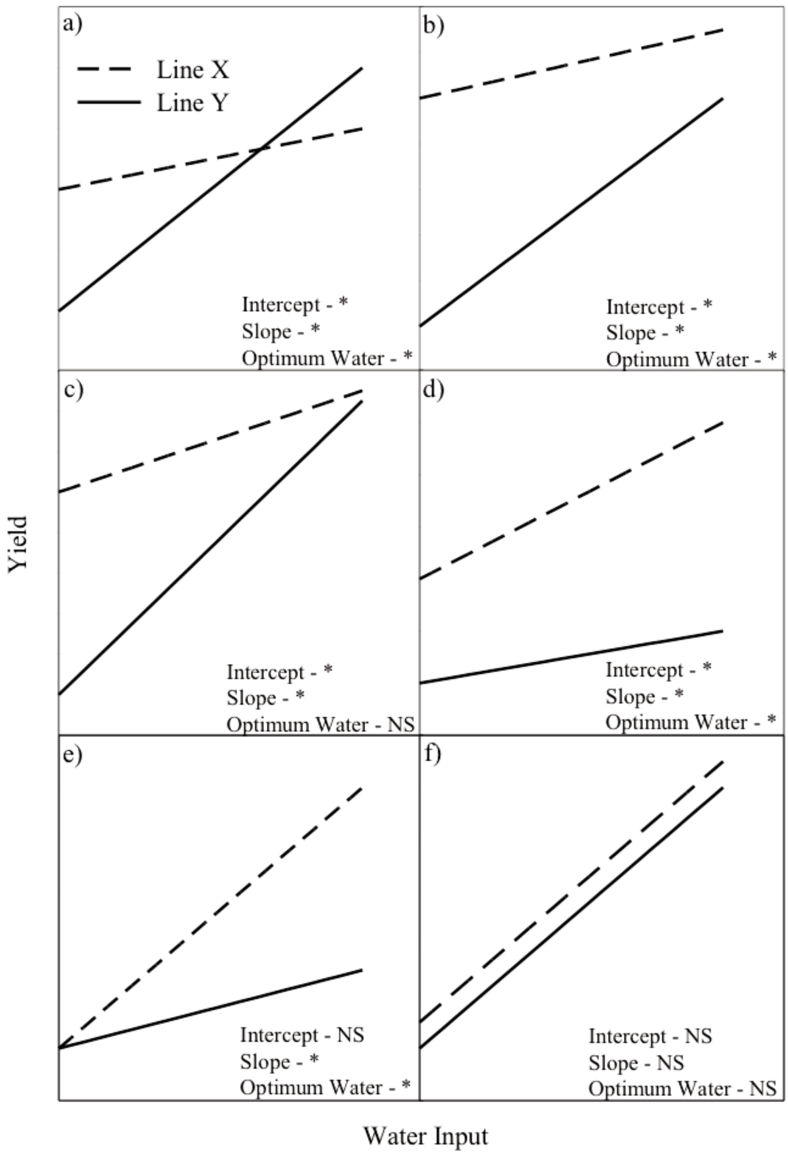
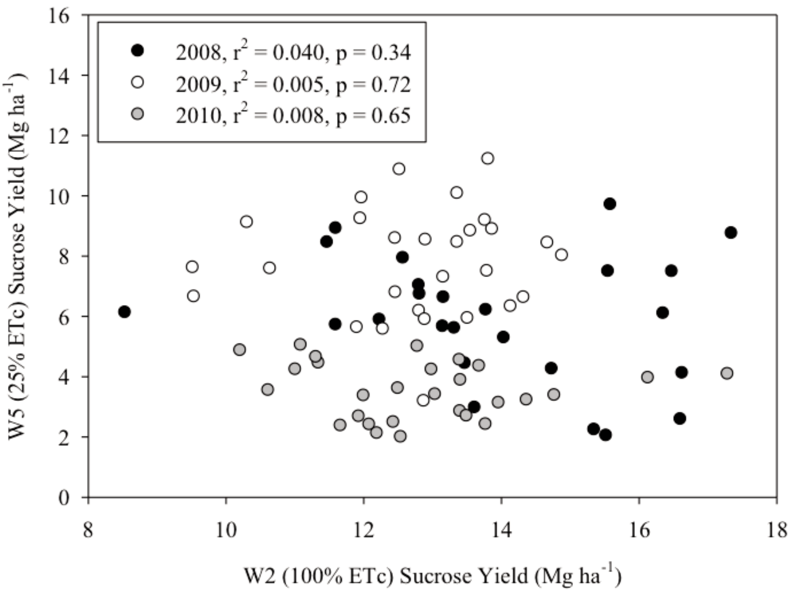


Figure 6. Sucrose yields under approximately 25% ETc (W5) plotted against sucrose yields at 100% ETc (W2) for all hybrids in each study year. Each data point represents the relationship for each water input treatment combination in a replication. For each year linear regression was fitted to the data points. Significance of linear relationships were determined at p = 0.05. Research was conducted from 2008-2010 in Kimberly, ID.



c. Hybrid X is more drought tolerant than Hybrid Y at low water deficit inputs but differences in tolerance decrease as water in- creases. At optimum water inputs, yields are similar between the two hybrids.
d. Hybrid X is more drought tolerant than Hybrid Y at low water inputs and the yield difference increases as water input increases.
e. Hybrid X and Hybrid Y have the same yield under low water deficit inputs. As water inputs increase the yield of Hybrid X increases over Hybrid Y.
f. Hybrid X and Hybrid Y have the same yield under low water deficit input and optimum water inputs.

Linear regression and DSI analysis showed that the sugarbeet hybrids used in this study had differences in their response to water input quantity from deficit to full irrigation. Among the six KWS experimental hybrids, intercepts and slopes were statistically similar with the only statistically significant differences measured in sucrose yield at W2. Taking into account linear regression intercepts and slopes for the relationships between sucrose yield and water input, and sucrose yields at the W2 treatment over the three years, experimental hybrid KWS-05 was more drought tolerant than the commercial hybrid, but they both had similar sucrose yields under full irrigation (Tables 1 and 2). This indicated the greatest difference in sucrose yield between these two hybrids was at the lowest water input and the difference decreased as water input increased. Situation C (Figure 5) best described KWS-05 compared with the commercial hybrid. The DSI calculated at various levels of deficit irrigation also showed KWS-05 was less susceptible to drought than the commercial hybrid (Table 3). Over all water input DSIs and sucrose yield at W2, KWS-03 was similar to KWS-05.

Experimental hybrid KWS-01 had the highest yields over the study period but for two out of three years, the intercept of KWS-01 was not different from that of the commercial hybrid. There also were no differences in DSI between KWS-01 and the commercial hybrid at all deficit water levels relative to W2 (Table 3). These data shows that KWS 01 had higher yields under full irrigation (W2) but not under the greatest deficit irrigation amount (W6).

CONCLUSIONS

There were differences in sugarbeet sucrose yield responses to water for the sugarbeet hybrids used in this study. The differences were demonstrated by comparing sugarbeet hybrids sucrose yield using linear regression slope and intercept comparisons over the range of deficit water inputs, DSI, and near maximum yield at the W2 water input treatment (100% ETc). Greater drought tolerance or greatest difference in sucrose yield between hybrids was seen at the lowest water input treatment (intercept difference). Linear regression analysis and DSI support the conclusion that KWS-05 exhibited greater drought tolerance compared to the commercial hybrid. There also were differences in overall yield potential among hybrids. This study showed that differences in sugarbeet drought production response to water input exist among hybrids. Therefore, this genetic diversity can potentially be utilized to develop commercial varieties that produce high sucrose yields under various water input conditions. The use of screening procedures, such as the line source method utilized in this study, can be an effective way to evaluate drought tolerance among hybrids.

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