

# A Midwestern USA Perspective on Water Use Efficiency and Drought Tolerance in the Soybean

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## Abstract

The response of soybean biomass and seed yield to variable seasonal water is linear. Discerning the genetic basis of water use efficiency on a seed yield basis (i.e., WUEy, but termed yield *beta* in this report) has long been a key research objective. Yield *beta* data have been collected on five RIL mapping populations of 250-300 members each, but RIL marker-genotyping is incomplete in three. A few yield *beta* QTLs have been detected to date, but their map positions have coincided with QTLs governing genotypic mean yield, and/or with genes governing plant growth habit and maturity. A carbon isotopic composition ( $\delta^{13}\text{C}$ ) estimation of transpiration efficiency (TE), conducted in two populations, identified a strong  $\delta^{13}\text{C}$  QTL, but its map position was coincident with that of the growth habit gene. In all five populations, the yield of a genotype in a water-abundant environment was highly correlated with its yield in a water-scarce environment, which is expected when genotypic yield *beta* and mean yield are governed in a coupled manner by either linked or pleiotropic QTLs, as seems to be the case. If so, then genetic yield advances in water-abundant environments are expected to generate concomitant yield advances in water-scarce environments (though to a lesser degree). Thus, genetic yield improvement for rainfall-variable soybean production areas (i.e., Midwestern USA), where extremes in water-abundance and water-scarcity are equally probable, is probably best attained by elevating the slope of the yield-to-water response (i.e., WUEy), while concurrently limiting the potential for such elevation to cause a downward slippage of the low end of the response slope.

## Media summary

Reported here are conclusions drawn from Midwestern USA research on the genetics of soybean WUE

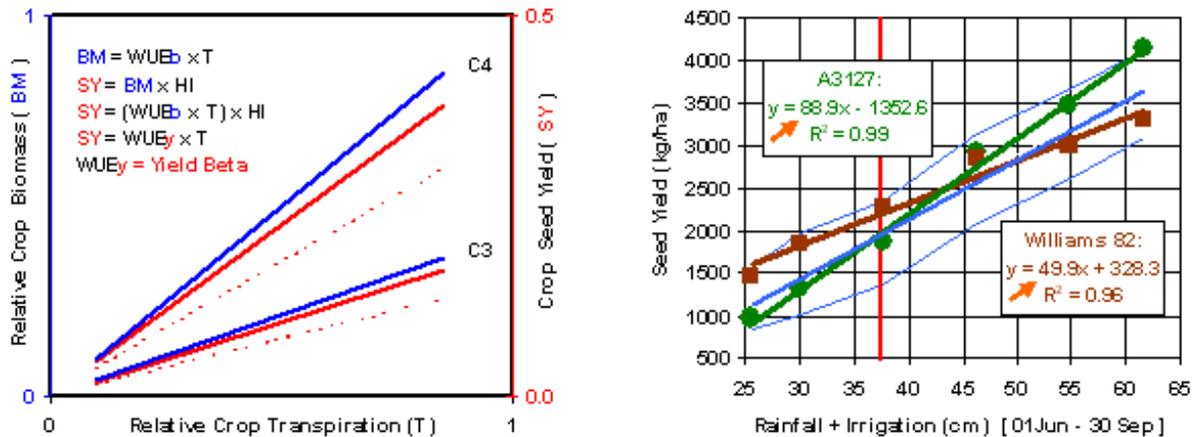
## Key Words

Irrigation, water stress, low rainfall, harvest index.

## Introduction

Water, when it is scarce, is often viewed as being an abiotic stressor. However, water should really be treated as just another resource (i.e., light, CO<sub>2</sub>, etc.) that must be efficiently exploited – even more so when crop productivity is constrained by its scarcity. Crop biomass (BM) is a highly linear function of the amount of water transpired (T) by the crop. Simply put, plants must exchange water for carbon. The proportionality of that exchange is determined by a biomass-based water-use efficiency coefficient (WUEb, defined as  $\Delta\text{BM} / \Delta\text{T}$ ), to wit:  $\text{BM} = \text{T} \times \text{WUEb}$ . Seed yield (SY) is similarly related to T, if harvest index (HI) - the seed fraction of total biomass - is incorporated into the equation, to wit:  $\text{SY} = \text{T} \times \text{WUEb} \times \text{HI}$  (Fig. 1, left). This equation was first postulated by Passioura (1977). Because  $\text{WUEb} \times \text{HI} = \text{WUEy}$  (a yield-based WUE, defined as  $\Delta\text{SY} / \Delta\text{T}$ ), the equation translates into:  $\text{SY} = \text{T} \times \text{WUEy}$ . This equation clearly shows that if soybean breeders are to increase SY (beyond any genetic improvement still possible in the asymptotically limited HI parameter), genotypes must be selected that will (1) *transpire more of the available annually rechargeable soil water*, and/or (2) *exhibit a greater WUEy, which means acquiring more carbon per unit of exchanged water* (Purcell and Specht, 2004). Specht et al (1986) evaluated the seed yield response of 16 soybean cultivars to six seasonal water amounts by replenishing potential crop evapotranspiratory (ET) loss on a weekly basis at levels ranging from 0% to 100%. A severe drought fortuitously occurred in both test seasons. Regression of final cultivar seed yield on seasonal water amount demonstrated rather convincingly that seed yield was a linear function of seasonal water amount (E+T). The cultivar regression coefficients (termed genotypic yield *betas* by the author) were season-

specific estimators of the cultivar  $WUE_y$ . Notably, the cultivars A3127 and Williams 82 had the most extreme yield  $\beta$  values (Fig. 1, right), and this was consistent with anecdotal perceptions relayed to agronomists by producers as to the performance of these two cultivars in water-abundant and water-scarce production environments. Discerning the genetic basis of yield  $\beta$  – a quantitatively inherited trait – was of great interest, but applying classical quantitative genetics tools was unappealing. Instead, the focus shifted to the development of a soybean molecular marker linkage map and mapping populations of recombinant inbred lines (RILs). With these tools, the genetics of yield  $\beta$  could be examined at a sub-genomic level, via marker-facilitated, quantitative trait locus (QTL) analyses.

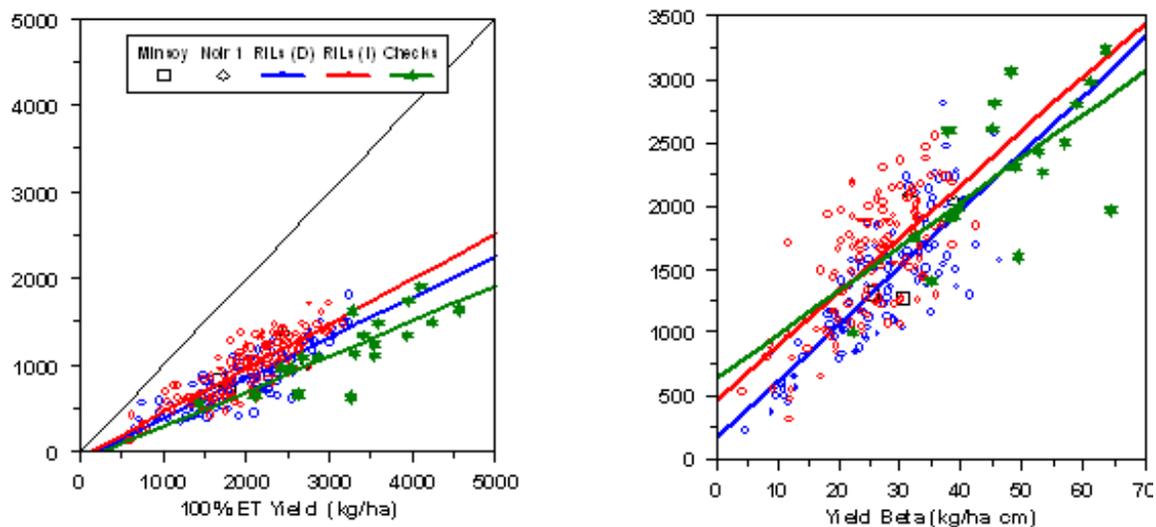


**Figure 1. Left Panel:** A graphical depiction of the Passioura (1977) thesis that seed yield (SY) is a function of the amount of water transpired (T), a biomass (BM)-based water-use efficiency ( $WUE_b$ ) coefficient, and a harvest index (HI) coefficient reflecting the seed fraction of total crop BM. Note that  $WUE_b \times HI = WUE_y$ . The  $WUE_b$  and  $WUE_y$  linear regression coefficients for the BM and SY trend lines reflect what a C4 or C3 photosynthetic crop species might display given theoretical  $c_i/c_a$  values of 0.7 and 0.3 respectively (Sinclair, 1994). The SY trend lines reflect an HI = 0.45 (solid) or HI = 0.35 (dashed). The SY (right) axis is scaled to be 0.5 of the BM (left) axis. **Right Panel:** Measured yield responses of soybean cultivars to a 0, 20, 40, 60, 80, or 100% weekly replenishment of all potential evapotranspiratory (ET) water loss. Regression data shown only for cultivars A3127 (highest yield  $\beta$ ) and Williams 82 (lowest yield  $\beta$ ); both had near-similar HI values. The 16-cultivar mean value and bracketing max/min range values are plotted at each of the six water levels. The June-September rainfall of 24.9 cm at the test site was ca. 66% of the rainfall norm (vertical line). The plotted data are from Specht et al (1986).

#### Yield Beta as an Indicator of $WUE_y$

When the first RIL mapping population became available, the 267 RILs, two parents, and 19 elite (check) cultivars were evaluated for yield  $\beta$ . A severe drought occurred in one of the test seasons (Specht et al, 2001). A coordinate graph of yields of the 290 genotypes in the 0%ET vs. 100% ET treatments provided an uncluttered means of visualizing the differences among 290 genotypes in terms of their yield response to water-scarce and water-abundant conditions (Fig. 2, left). The following observations were derivable from the yield  $\beta$  data. (1) The genotypic yield range in the 0% ET treatment (i.e., ca. 0 to 2000 kg/ha) was significantly constrained compared to that of the 100% ET treatment (i.e., ca. 250 to 4500 kg/ha). Rossielle and Hamblin (1981) noted some 20 years ago that a given set of genotypes will ordinarily exhibit a smaller genetic variance in stress environment than that same set will exhibit in a non-stress environment. Breeders know well that if genetic yield variance is restricted, genetic yield gains from selection will also be lessened. (2) Genotypes occupied graph positions farther from the 1:1 diagonal line the greater their yield potential, which is consistent with the Rossielle and Hamblin theorization that selection aimed at *genetically reducing the difference* between non-stress and stress yield would almost certainly lead to lower rather than higher yielding genotypes. (3) Genotypic yields in the water-abundant

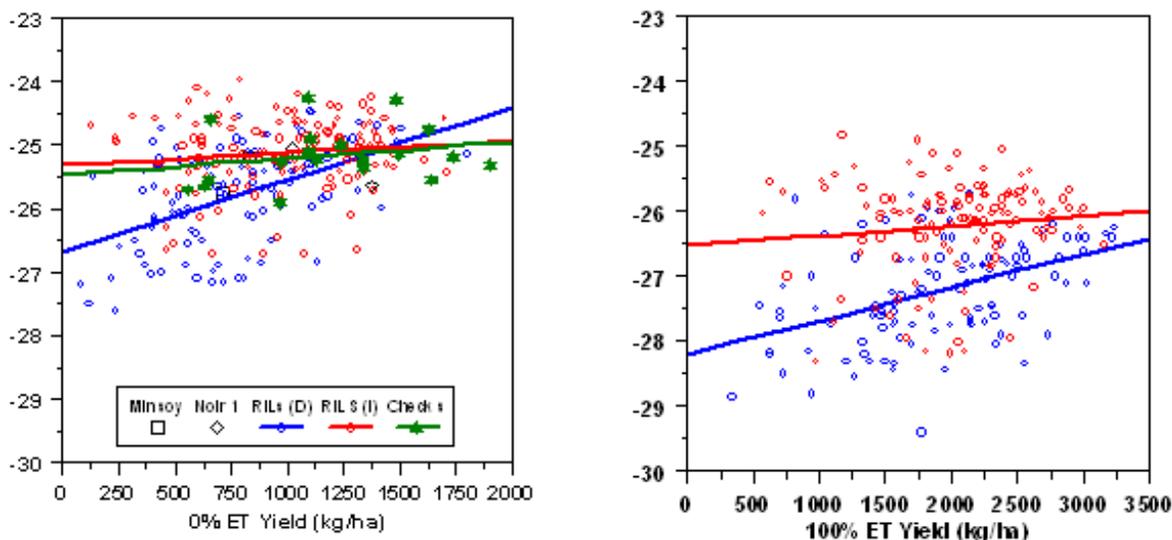
and in the water-scarce treatments were highly correlated ( $r = 0.85$ ), indicating that direct selection for yield improvement in the former will certainly *not* be incompatible with a concordant indirect improvement of yield in the latter. (4) The yield *beta* values of 40-65 kg/ha cm that were estimated for the early-maturing elite cultivars in this test (Fig 2, right), were somewhat lower than the 50-89 kg/ha cm values detected for the later-maturing elite cultivars in the earlier test (Fig. 1, right), indicating that shortening the days to maturation lessens yield *beta*. (5) As shown in the Fig. 2 (right) graph, the yield *beta* of a genotype correlated well with its mean yield performance over all six environments ( $r = 0.71$ ). Though not graphed here, the yield *beta* correlation with yield in the 100% ET treatment ( $r = 0.84$ ) was higher than that found in the 0% ET treatment ( $r = 0.48$ ) (Specht et al, 2001), because the *magnitude* of genotypic yield-to-water *slope* was dependent upon the genetic yield variance at the slope ends, and (as already noted above) that variance was smaller at the lower end of the slope. The foregoing generalizations have been applicable to the yield *beta* data collected in four other mapping populations (data not yet published because marker genotyping is not yet complete). A preliminary analysis of yield *beta* data collected in Nebraska in 2003 (a severe drought year) on 42 elite cultivar checks and 350 germplasm bank accessions (from drought-prone or highly productive regions of China) now suggests that those generalizations may, in fact, extend to populations of unrelated genotypes.



**Figure 2. Left Panel:** A graph of non-irrigated (0% ET) and fully irrigated (100% ET) seed yields of the Minsoy and Noir 1 cultivars, their (267) RIL progeny of the determinate (D) or indeterminate (I) stem growth habit type, and 19 unrelated commercial cultivars. Trend lines provided for the two RIL progeny types and cultivar checks. **Right Panel:** A graph of mean yield versus yield *beta*. See Specht et al (2001) for additional details.

#### Soybean Leaf $\delta^{13C}$ Values – Relationship with Yield Beta

A carbon isotopic composition ( $\delta^{13C}$ ) analysis procedure was used to evaluate transpiration efficiency (TE) in the first soybean RIL population (Specht et al, 2001), and also in a second population (manuscript in preparation). Both data sets resulted in the same generalizations, so only the first set of leaf  $\delta^{13C}$  data are presented (Fig. 3). Note that that leaf  $\delta^{13C}$  values were more positive (i.e., indicative of greater TE) in the 0% ET treatment, suggesting that soybean TE increases naturally in response to an increasing soil water deficit. Still, the correlation between the leaf  $\delta^{13C}$  value of a genotype and its yield was low in both water regimes. The  $\delta^{13C}$  values for the determinate RILs were likely artefacts, since juvenile (carbon-importing) leaflets were not ontologically available on determinate stem tips on the sampling date (i.e., older carbon-exporting leaflets were inadvertently sampled instead). Leaf  $\delta^{13C}$  (and thus TE) was positively correlated with yield *beta*, but not to the extent ( $r = 0.26$ ) of enthusing soybean breeders to use it as a selection proxy for elevating TE or yield *beta*, despite the successful use of  $\Delta^{13C}$  for TE in wheat (Condon et al, 2002; Richards et al, 2002).



**Figure 3. Carbon isotope composition ( $\delta^{13}\text{C}$ ) values for leaf tissue collected (mid-July) from stem tips of the Minsoy and Noir 1 cultivars, their (267) RIL progeny of the determinate (D) or indeterminate (I) stem growth habit type, and 19 unrelated check cultivars. Leaf  $\delta^{13}\text{C}$  values plotted against the seed yield of those genotypes. The more positive the  $\delta^{13}\text{C}$  value, the greater the TE (Condon et al, 2002). Left Panel: 0% ET (non-irrigated) data. Right Panel: 100% ET (fully irrigated) data (check data lost). See Specht et al (2001) for other details.**

#### QTL Analyses – Yield Beta and Leaf $\delta^{13}\text{C}$

To improve soybean WUEy, breeders need to identify genotypes whose yield-to-water response (i.e., yield *beta*) is steeper. However, selection for a higher yield *beta* is difficult, not only because of its quantitatively inherited nature, but also (and more importantly) because of the low probability of encountering drought seasons routinely enough in the specific breeder-targeted regions to effectively practice such selection. The identification of marker-flanked favourable alleles at QTLs for yield *beta* QTLs would bypass those difficulties, since marker-assisted selection (MAS) could be used to rapidly introgress those favourable alleles into elite cultivars. Still, one must take care to know just exactly what is being introgressed. In the Specht et al (2001) report, QTLs detected as controlling significant amounts of variation in yield *beta* and yield mean were in fact pleiotropic expressions of existing QTLs (or genes) known to control days to maturation and stem habit. Indeed, a QTL detected on soybean linkage group (LG) L controlled 25% of the phenotypic variation in leaf  $\delta^{13}\text{C}$  and its additive effect on leaf  $\delta^{13}\text{C}$  was a huge 0.405‰, but it mapped to the genomic position of a stem determinancy locus. As noted in a prior section, this leaf  $\delta^{13}\text{C}$  QTL was effectively an artefact arising from the fact that appropriate leaf tissue for the  $\delta^{13}\text{C}$  analyses could not be effectively sampled from the determinate RILs. Although a leaf  $\delta^{13}\text{C}$  QTL was detected on LG C2, and it had a lesser additive allelic effect of 0.204‰, this QTL accounted for only 7% of the leaf  $\delta^{13}\text{C}$  phenotypic variation. In QTL mapping experiments, selection bias (i.e., the overestimation of the effects attributable to moderate or minor QTLs) is common for QTLs controlling less than 10% of the phenotypic variation making it necessary for the researcher to confirm the QTL effect in an independently derived population. Moreover, the true additive effect really needs to be large enough to warrant the time, effort, and cost of manipulating the favourable alleles at the given QTL in a MAS program. So far, we have not yet observed a yield *beta* or leaf  $\delta^{13}\text{C}$  QTL in the currently genotyped RIL populations that would be worthy of such attention.

#### Concluding Remarks and Future Prospects

Our published (Specht et al, 1986, 2001) and unpublished results have confirmed a close association of soybean seed yield with transpiration. Moreover, the close association between genotypic yield *beta* (a

field-based proxy for WUE<sub>y</sub>) and genotypic mean yield provides credence to the postulation that soybean WUE<sub>y</sub> (and thus drought tolerance *per se*) is improved coincidentally by simply selecting for higher genotypic mean yield. It was also clear in each mapping population that the highest yielding entries when water was abundant (100% ET) were also the highest yielding entries when water was scarce (0% ET). This was consistent with the observation that most of the QTLs detected for yield *beta* WUE<sub>y</sub> in the now marker-genotyped RIL populations were positionally coincident (in terms of genome position) with the QTLs that we detected for yield *per se*. Whether this coincidence turns out to be linkage or pleiotropy is probably irrelevant, given that the highly positive correlation between two desirable traits is probably not going to limit the success of breeders trying to improve both traits when developing cultivars targeted for use in the Midwestern USA. Future attempts to wring out a large increase in soybean WUE<sub>b</sub> or WUE<sub>y</sub> would have to focus on upping the  $\Delta$ BM or  $\Delta$ SY numerators of these WUE parameters. Indeed, the evolution of some plant species from a C3 to a C4 form of photosynthesis provides an ecological precedent for WUE improvement efforts (Fig. 1, left).

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