Final report for the project

Reducing the impact of soil water deficits on soybean yields in Ontario

(S2011GE03)

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1. Executive Summary

A three-year GFO- and AAC--funded project completed in 2011 demonstrated that under typical Ontario growing conditions soybean yields are often limited by soil water availability late in the growing season, even in years where precipitation appears to be adequate and there are no outward symptoms of water stress. Improved tolerance of such mild, late-season water stresses should be a target for breeding high-yielding soybean varieties for this region. One trait that is hypothesized to be beneficial under this type of stress scenario is increased crop water use efficiency (WUE, the amount of crop dry matter produced per unit water used). Objectives of the present study are 1) to identify the breadth of variation for WUE among commercial soybean varieties adapted to the 2700 HU zone, and 2) to ascertain how differences in WUE affect variety susceptibility to yield loss under naturally occurring soil water deficits in the field.

In the first year of the current project (2011) we acquired 23 current commercial soybean varieties of similar maturity and compared them for vegetative-stage WUE in a greenhouse experiment. The greenhouse trial demonstrated a broad range (17%) of whole-plant WUE among these 23 varieties, as we expected based on past screening studies.

In the first field season (2011) we compared 20 of these 23 varieties for responses to irrigation in a field trial with three replications. In 2012 and 2013 the number of entries was reduced to 15, and the number of replications was increased to four. Averaged across the varieties tested, the yield response to irrigation ranged from a low of 1.9% in 2011 (not statistically significant) to 15.8% in 2012. Our results indicate that, across the three years and all varieties, the average yield loss due to water stress was small (6.5%) but statistically significant.

Variety responses to water stress differed significantly, and provided insight into the mechanistic basis of genotypic difference in susceptibility to yield loss under typical rainfed conditions. For example, in all three years, varieties that increased their total crop biomass the most under irrigation also saw the largest yield response to irrigation. By contrast, increased harvest index (the fraction of total crop biomass allocated to the seed) was not consistently associated with yield increases under water stress; indeed, harvest index was quite stable across years, treatments and varieties. Additionally, we found that some varieties were more susceptible than others to reductions in pod numbers under water stress. Some varieties utilized the extra water in the irrigation treatment to produce larger pods (more 3-seeded pods) without greatly increasing pod numbers, while others produced a larger number of pods under irrigation (mostly additional 2-seeded pods); the latter strategy was generally more beneficial.

While there was significant variation for WUE among the varieties tested, we did not find strong evidence that this trait imparted enhanced drought tolerance in the field. Importantly, the greenhouse screening study revealed that high WUE among these varieties was not associated with soil water conservation, but rather with aggressive growth and somewhat **higher** rates of water consumption. In the field, this sometimes translated into higher yields under water-replete (irrigated) conditions, but was detrimental to yield under the relatively dry conditions of the rainfed treatment in 2012. This result points to the importance of understanding the physiological basis of high WUE when it occurs. Since WUE is the ratio of biomass production to water use, it can be increased by either robust growth or by conservative use of soil water. In the present study the high WUE varieties were of the former type, and so had high yield potential when water was plentiful but were slightly more susceptible to yield loss under severe soil water deficits.

In summary, WUE was not found to be an important trait for enhancing yields under naturally occurring soil water deficits typical of Ontario. However, the fact that these commercial varieties differed significantly for their yield responses to water stress indicates that there is genetic variation for real-world drought tolerance among Ontario-adapted soybean lines, and so the opportunity for additional genetic improvement exists.

2. Project Description

Project Objectives

The objectives of this project, as originally proposed, were:

1. **To screen a selection of Ontario-adapted commercial soybean varieties of similar maturity for differences in water use efficiency** (WUE, the amount of whole plant biomass produced per unit water used). The screening was to be conducted using established methods, in the greenhouse, in the vegetative stage. It was important to use varieties of similar maturity so that in the field trials they would experience naturally-occurring water deficits at approximately the same developmental stage.

2. **To determine if variety differences in WUE (as measured in the greenhouse screening study) result in differences in susceptibility to yield loss under naturally-occurring water stress in the field.** This was to be accomplished in replicated small-plot field trials, one in each year of the study. The design was a split-plot, with the irrigation treatment (rainfed or water-replete) as the main plot factor, and variety as the sub-plot factor. The overall hypothesis was that there would be significant treatment x genotype interactions for yield, and that varieties with higher WUE would be the ones that were least susceptible to yield loss under naturally occurring water deficits. If this hypothesis were supported, it would identify WUE as a trait to be selected for to increase drought tolerance of Ontario soybean under “real world” conditions.

In addition to yield, we also measured related traits in the field trial, to better understand the physiological basis of yield reductions under water stress and, especially, variety differences for these effects.

Activities Accomplished

All aspects of the project, as originally proposed, were successfully accomplished.

We selected 24 soybean varieties of similar maturity, based on the Days to Maturity ratings in the OOPSCC soybean brochure. These were from 14 different public- and private-sector organizations (University of Guelph Ridgetown, Agriculture and Agrifood Canada Ottawa, SeCan, PRO Seeds, Prograin, Syngenta, Woodrill Farms, Hensall District Coop, Maizex Seeds, Mycogen, Hendrick Seeds, Bramhill Seeds, Coop Federee, Hyland Seeds). All of the organizations contacted agreed to provide the varieties requested, and only one of the varieties

(Madison, from Hyland Seeds) failed to arrive. This left us with 23 entries. This collection represents a large fraction of all of the available varieties within the target maturity range, with the exception of those sold by Monsanto and Pioneer. We avoided those companies only because they require Material Transfer Agreements that would have unduly delayed communication of the results of this project.

The greenhouse screening trial was completed ahead of schedule. In the original timeline, we had anticipated completing this by December 2012, not being certain how large the variety differences would be, how many replications we would require, or if we would encounter any technical difficulties or setbacks. As it turns out, we had all of the data for 23 varieties and six replications in hand by the end of September 2011.

The milestones with respect to all three years of the field trial were also met. We purchased and installed two 5000-gallon water tanks for onsite water storage in the spring of 2011 as planned. We had originally intended to test approximately 12 varieties in the field in 2011, then increase that to about 24 in 2012 and 2013. Instead, we opted to test 20 varieties in 2011, with a reduced number of replications (3 instead of 4). Based on those results (described below), we decided to reduce the number of entries to 15 for 2012 and 2013, since that is the maximum number we could fit under our irrigation system with four complete replications.

Project Inputs Utilized to Date

In order to have access to the required land at the Elora Research Station, we submitted a Tier II application to the University of Guelph / OMAFRA Research Program. This was granted, to provide access to 2 acres of land for each year of the project. The Program calculates the value of this access to be $4150 per year, with 8% payable by the partner (GFO), and the remaining $3818 sponsored by the Program.

Over the duration if the project, the following additional expenses were incurred (amounts are approximate – see project financial statement from University of Guelph for exact amounts):

$ 9256 – to purchase water tanks and associated hardware, other minor supplies
$ 4473 – charges for use of University vehicle
$ 2564 – greenhouse access fees
$ 28 326 – partial salary + benefits for contract technician
$ 38 997 – graduate student stipend
$ 1245– research station access fees
$ 11040 – University overhead charge

Contract technicians Laxhman Ramsahoi and Li Guo spent approximately 15% of their time on this project, between May 2011 and October 2013. In addition, several summer students assisted with field measurements on a weekly basis from July to September in all three years of the field study.

3. Results

Greenhouse Screening for WUE

The greenhouse screening for WUE was completed in 2011. We completed 6 replications with all 23 varieties. The replications were planted sequentially, with a spacing of 1 to 2 weeks. We found strong differences for WUE amongst these current, commercial varieties adapted to Ontario (Table 1). We had hypothesized that there would be such a range within this maturity group, based on prior (GFO-funded) screening efforts that utilized Ontario-adapted varieties from across a broad range of maturity zones.

These results were entirely in line with our expectations and provided the basis for the remainder of the project – investigating how these differences in WUE affect (or don't affect) relative variety susceptibility to naturally occurring water deficits in the field.

A surprise in the greenhouse study was the finding that high WUE was significantly correlated with both high biomass production and **increased** water use. (Figure 1). High WUE is more commonly associated with soil water conservation and, in some cases reduced biomass production.

Field Trials, 2011 to 2013

Although we included 20 varieties (with three replications) in the 2011 field trial, this number was reduced to 15 for 2012 and 2013 to permit four complete replications in the space available under the irrigation equipment. To facilitate combined presentation of the data across years we report here on only the 15 varieties utilized in all three years of the trials.

Naturally occurring waters tress was quite mild in 2011. Averaged across varieties, the irrigation treatment increased yield by only 1.2 bu / acre, which was not statistically significant. (In an adjacent experiment in 2011, irrigation increased yield by 23.5%. That trial had an earlier planting date, causing the crop to be more strongly affected by an early season dry period that occurred in that year). In 2012, the driest year of the study, irrigation increased yield by almost 10 bu / acre, and in 2013, the increase was 2.1 bu / acre (Table 2). Averaged across all three years, the yield benefit from irrigation was 4.3 bu / acre (6.9%).

In the multi-year analysis, there was a treatment x variety interaction for yield ($p < 0.1$), indicating that the varieties differed for the amount of yield they lost due to soil water deficits under rainfed conditions. Looking at the individual components of yield (Table 2), variety differences became even more apparent. In 2012, 2013 and in the multi-year analysis, there was a significant treatment x variety interaction for pod number (pods produced per square meter of ground area). This indicates that water stress reduced pod numbers in some varieties more than others. In 2012 and the multi-year analysis there was also an interaction for the number of seeds per pod, even though there was no treatment main effect for this yield component. This indicates that some varieties increased the number of seeds per pod under water stress, and others decreased it. That is, soil water deficits caused some varieties to increase the ratio of 2-seeded pods to 3-seeded pods, while other varieties had the opposite response. The final yield component, 100-seed weight, was significantly affected by the treatments only in 2012. Interestingly, irrigation **decreased** the 100-seed weight. Under irrigation there were many more pods set, but individual seeds were slightly smaller.

The effect of water stress on yield and its components is further explored in Figure 2. In this figure, the percent difference in yield between the treatments (that is, the percent of yield lost due to water stress) is compared to the percentage difference in each of the yield components. Looking at the multi-year analysis (bottom panels of the figure), it is clear that pod number is the yield component that most consistently determines a variety's relative susceptibility to yield loss due to water stress. In general, there also seems to be a negative relationship between percent yield loss and percent difference in seeds per pod. In other words, varieties that lost less yield under water stress also has a larger shift towards more 2-seeded pods under water stress. Apparently, they sacrificed pod size for increased pod number.

Irrigation significantly increased total crop biomass in every year of the study ($p < 0.1$ in 2011; $P < 0.05$ in 2012, 2013 and the multi-year analysis) (Table 3). In 2013, there was a significant treatment x variety interaction for biomass, indicating that some varieties responded more strongly to irrigation than did others. By contrast, harvest index (the proportion of total biomass allocated to the seed) was not significantly affected by irrigation (Table 3). Figure 3 further illustrates the strong relationship between the effect of water stress on yield, and its effect on biomass. Varieties that were able to use the irrigation water to produce more yield did so by producing more total biomass, not by allocating a larger fraction of that biomass to the seed.

We never found a significant correlation between WUE measured in the greenhouse and yield under rainfed conditions in the field. However, in one year (2011), we found that high-WUE varieties had higher yields under **irrigated** conditions (Figure 4A). In the driest year (2012), we found that high-WUE lines were **more** susceptible to yield loss due to water stress (Figure 5A). This was not because they achieve higher yields under irrigation (Figure 4B) as in 2011, but rather because they had lower yields than low-WUE lines under rainfed conditions (Figure 5B). They also had lower biomass production than low-WUE lines under rainfed conditions in 2012 (Figure 5C).

Overall, these results suggest that, among the 15 Ontario-adapted commercial soybean varieties, high WUE is associated with high water use and high biomass production. Under water-replete conditions, this can sometimes be a beneficial trait (e.g., 2011 trial under irrigation), but under fairly severe water stress (e.g., 2012, rainfed) it is detrimental.

Public Benefit to Date

Public benefit from this research will accrue over the long term, once the information has been used to successfully breed soybean varieties with enhanced tolerance to the types of soil water deficits that occur in the Ontario growing environment. This process has already begun. After sharing the results of our 2011 greenhouse and field trials with a commercial soybean breeding company, they contracted our research group to screen 120 of their soybean lines for WUE in the greenhouse. That project went very well, and we are now screening an additional 120 lines for them. They will use this phenotypic data for their own efforts to breed more drought tolerant soybean lines.

A unique feature of our work is that we are studying varietal differences in drought tolerance, defining drought as the type (timing and intensity) of soil water deficits that actually occur under typical Ontario production conditions. Thus, we have the highest likelihood of uncovering specific traits that are of value in the target environment. We are in continuous contact with public and private soybean breeding programs in Ontario, and share our results with all of them regularly.

4. Knowledge Translation and Transfer

The primary audience for this work is Ontario's public- and private-sector soybean breeders. All of the province's breeders are aware that the work is ongoing and, as mentioned above, at least one commercial breeder is already endeavoring to make use of the results.

Results of the work have so far been presented publically at two scientific meetings:

- Visser, B. and H.J. Earl 2012. Field Performance of Soybean Cultivars Varying for Water Use Efficiency. ASA/CSSA/SSSA Annual Meetings, October 22 2012, Cincinnati OH.

- Visser, B.A. and H. J. Earl. 2013. Field performance of commercial Ontario soybean cultivars differing in water use efficiency. ASA/CSSA/SSSA Annual Meetings, November 3-6, Tampa FL.

Additional scientific communications will include i) publication of scientific manuscripts and a graduate student thesis (M.Sc. work of Bridget Visser), and ii) additional presentations at scientific conferences, including the annual GFO / OSACC research meeting.

A secondary audience is the soybean growers of Ontario, who should be made aware that soybean varieties may already differ for tolerance of water stress, and that there are ongoing efforts to further improve Ontario varieties in this regard. Appropriate outlets in this case include the farm press in general (we have done past interviews for *The Furrow, and Ontario Farmer*, among others) and the GFO magazine in particular. In early 2013 we were interviewed by RealAgriculture.com regarding this work. The two videos can be viewed here:

http://www.realagriculture.com/2013/01/soybean-school-how-much-yield-is-lost-to-drought-stress/

http://www.realagriculture.com/2013/01/soybean-school-how-important-are-drought-resistant-traits-to-soybean-yields/

We have also been asked to provide a summary of the work for publication by Manitoba Pulse Growers (in preparation).

6. Final Comments

This project was completed exactly as planned in all aspects.

While the yield losses to water stress were somewhat smaller than we observed in a previous GFO-funded series of field trials, overall the project confirmed that in Ontario, significant soybean yield is lost to soil water deficits in almost every growing season. This validates drought tolerance as a legitimate target for genetic improvement of soybean varieties adapted to this region; however, it is important that such efforts remain focused on the types of soil water deficits that actually occur in the region (i.e., mild, typically late-season stresses, rather than severe, acute stresses that actually threaten crop survival).

We investigated WUE as a trait that might impart enhanced tolerance of these types of soil water deficits. Consistent with our predictions, we found that current, commercial soybean varieties adapted to the region varied significantly for WUE. Unfortunately, in a field environment we did not find that high WUE was positively associated with yield under water stress. In fact, in one year we found the opposite. While disappointing, this is still an important contribution towards critically evaluating the various possible avenues for improving drought tolerance. Even more could be gleaned by further studying this set of varieties, to understand the precise physiological basis of the observed variation in WUE.

Encouragingly, we did uncover significant variation among these varieties for their yield responses to soil water deficits, and illuminated some details about how that variation occurs at the level of individual yield components. This provides additional valuable insight into possible avenues for genetic improvement – again, in the specific context of maximizing yields under typical, real-world drought stress.

The field data we collected on these varieties under rainfed and irrigated conditions will also be useful as we continue to explore other traits that may improve drought tolerance. GFO through the Growing Forward II program has agreed to fund an investigation into root-related traits in soybean. Once we advance to the genotype screening portion of that work we can use these same 15 varieties to see if the traits we quantify in controlled environment experiments can help explain the differences in their responses to water stress already observed in the 2011-2013 field trials described here.

Table 1. Water use efficiency (WUE) measured in the greenhouse for 23 Ontario adapted soybean varieties. For 20 of the varieties, yield (mean across two watering treatments) and days to maturity (water-replete treatment only) are shown, as measured in a field experiment. The five entries marked with an 'x' were excluded from subsequent years of the field trial.

Entry	Variety	WUE	Yield	DTM	
		g / L	kg / ha	days	
1	OAC Drayton	4.27	5059	116	
2	DH420	4.13	4464	110	
3	HDC 2701	4.42	4752	111	
4	Dares	4.22	5183	115	
5	PRO 275	3.94	4715	117	x
6	OAC Champion	4.17	4729	112	
7	Saska	3.99	4909	113	
8	Bruce	4.06	4677	114	
9	RCAT Corbett	4.03	4533	120	x
10	OAC Lakeview	4.47	4934	110	
11	OAC Wallace	4.12	4836	117	x
12	OAC Purdy	4.11	5112	115	
13	Wildfire	3.80	4616	115	
14	RR2 Cobalt	3.91	4581	115	x
15	5A090RR2	3.94	4635	116	
16	PRO 2715R	4.07	4635	115	
17	RCAT MatRix	4.27	4890	121	x
18	Absolute RR	4.27	4901	115	
19	S08-C3	4.23	4379	114	
20	Blade RR	3.79	4044	114	
21	Ceryx RR	4.30			
22	Karlo RR	4.03			
23	PRO 2625R	4.21			
	P-value	<0.0001	<0.0001	<0.0001	
	LSD 5%	0.19	274	1.7	
	N	6	3	3	

Table 2 Yield, in both imperial and metric units, and its components, pods/m², number of seeds/pod, and 100-seed weight, for two treatments, rain-fed and water-replete, and the p-value for the treatment by variety interaction (trt*variety) for 2011-2013 and all years combined.

		Yield		**pods/m²**	**seeds/pod**	**100-seed wt**
		bu/acre	kg/ha			g
2011	Rain-fed	63.9	4291	967	2.31	17.4
	Water-replete	65.1	4367	985	2.26	17.5
	P-value	*0.26*	*0.26*	*0.48*	*0.26*	*0.73*
	*Trt*variety P-value*	*0.46*	*0.46*	*0.48*	*0.35*	*0.53*
2012	Rain-fed	60.6	4064	1049	2.28	17.4
	Water-replete	70.2	4714	1283	2.32	16.3
	P-value	***<0.0001***	***<0.0001***	***<0.0001***	*0.16*	***0.0013***
	*Trt*variety P-value*	*0.69*	*0.69*	***0.0005***	***0.019***	*0.076*
2013	Rain-fed	62.6	4200	1001	2.29	17.3
	Water-replete	64.7	4343	1009	2.32	17.6
	P-value	***0.0135***	***0.0135***	*0.65*	*0.39*	*0.24*
	*Trt*variety P-value*	*0.30*	*0.30*	***0.011***	*0.50*	*0.70*
Multi-Year	Rain-fed	62.4	4185	1006	2.29	17.4
	Water-replete	66.7	4475	1092	2.30	17.1
	P-value	***<0.0001***	***<0.0001***	***0.0001***	*0.73*	*0.12*
	*Trt*variety P-value*	*0.057*	*0.057*	***<0.0001***	***0.019***	*0.28*

Table 3 The total biomass and harvest index (HI), for two treatments, rain-fed and water-replete, and the p-value for the treatment by variety interaction (trt*variety) for 2011-2013 and all years combined.

		Total Biomass g	HI
2011	Rain-fed	705	0.58
	Water-replete	734	0.56
	P-value	*0.052*	*0.14*
	*Trt*variety P-value*	*0.51*	*0.76*
2012	Rain-fed	668	0.57
	Water-replete	789	0.57
	P-value	***<0.0001***	*0.65*
	*Trt*variety P-value*	*0.81*	*0.26*
2013	Rain-fed	670	0.58
	Water-replete	690	0.58
	P-value	***0.011***	*0.97*
	*Trt*variety P-value*	***0.030***	*0.15*
Multi-Year	Rain-fed	681	0.58
	Water-replete	738	0.57
	P-value	***<0.0001***	*0.25*
	*Trt*variety P-value*	*0.11*	*0.23*

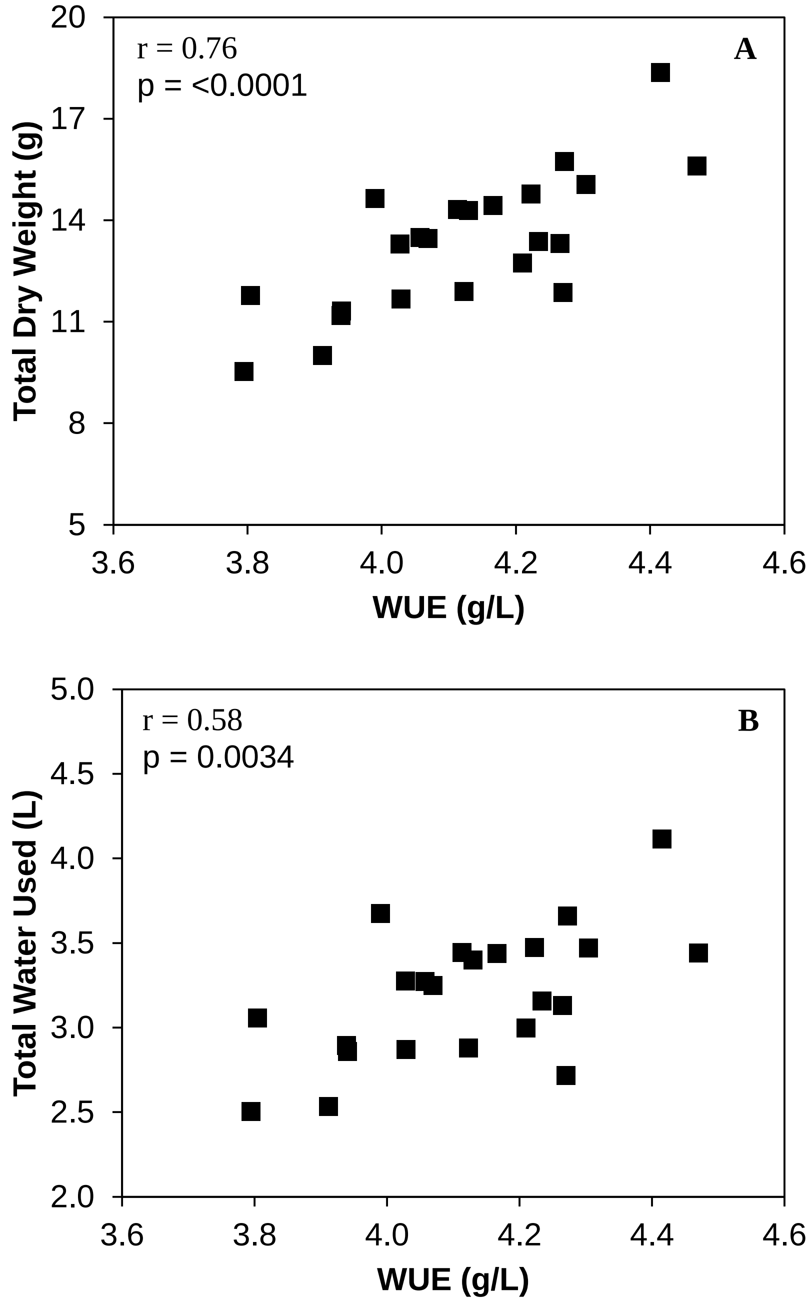

Figure 1 The (A) total dried biomass and (B) water used in comparison to the corresponding water use efficiency (WUE) for 23 varieties.

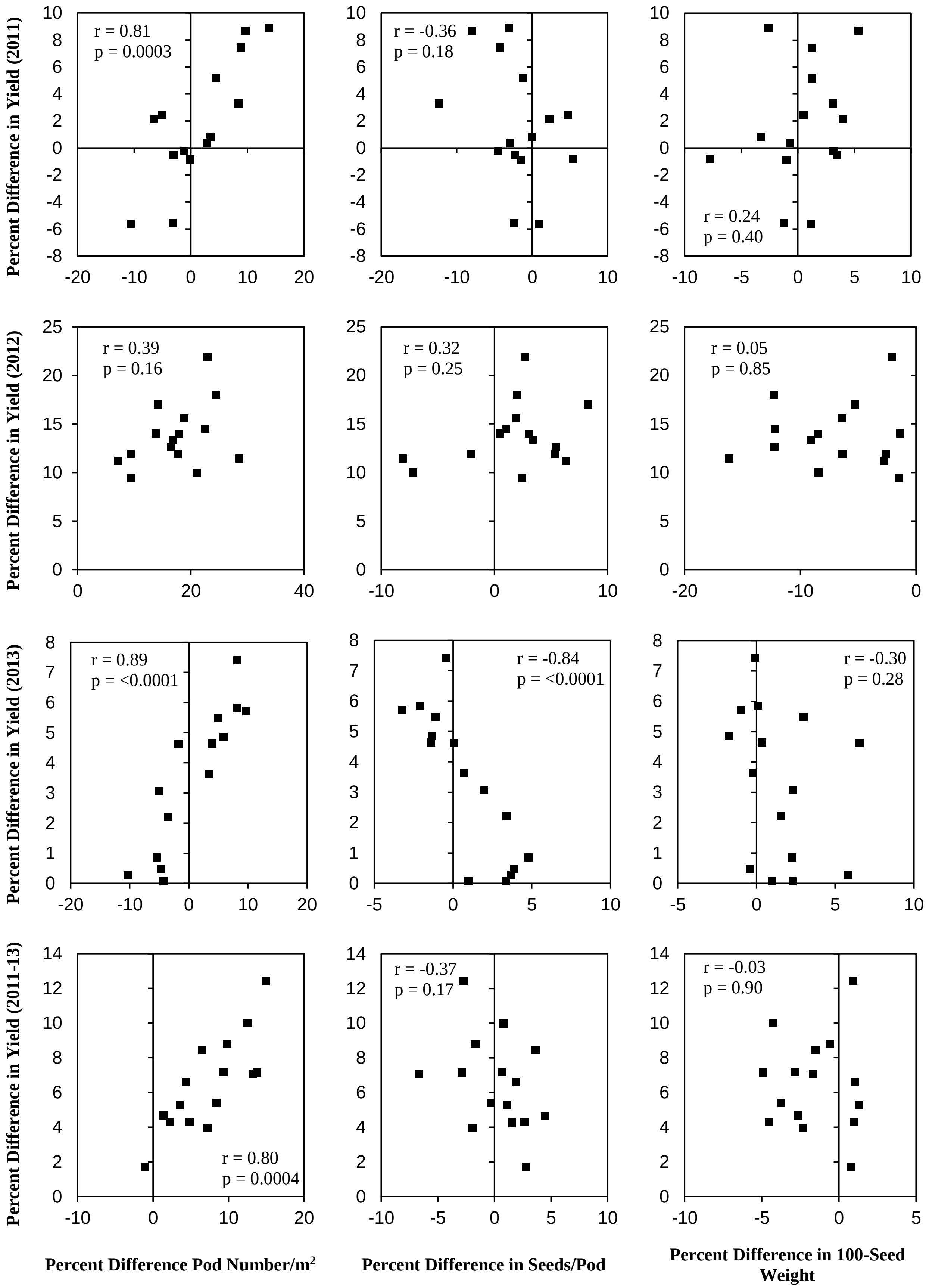

Figure 2. The percent difference in yield and percent difference (water-replete vs. rain-fed) in pod number/m^2, seeds/pod, and 100-seed weight for 15 soybean varieties in 2011 (top), 2012 (second row), 2013 (third row), and all years combined (bottom).

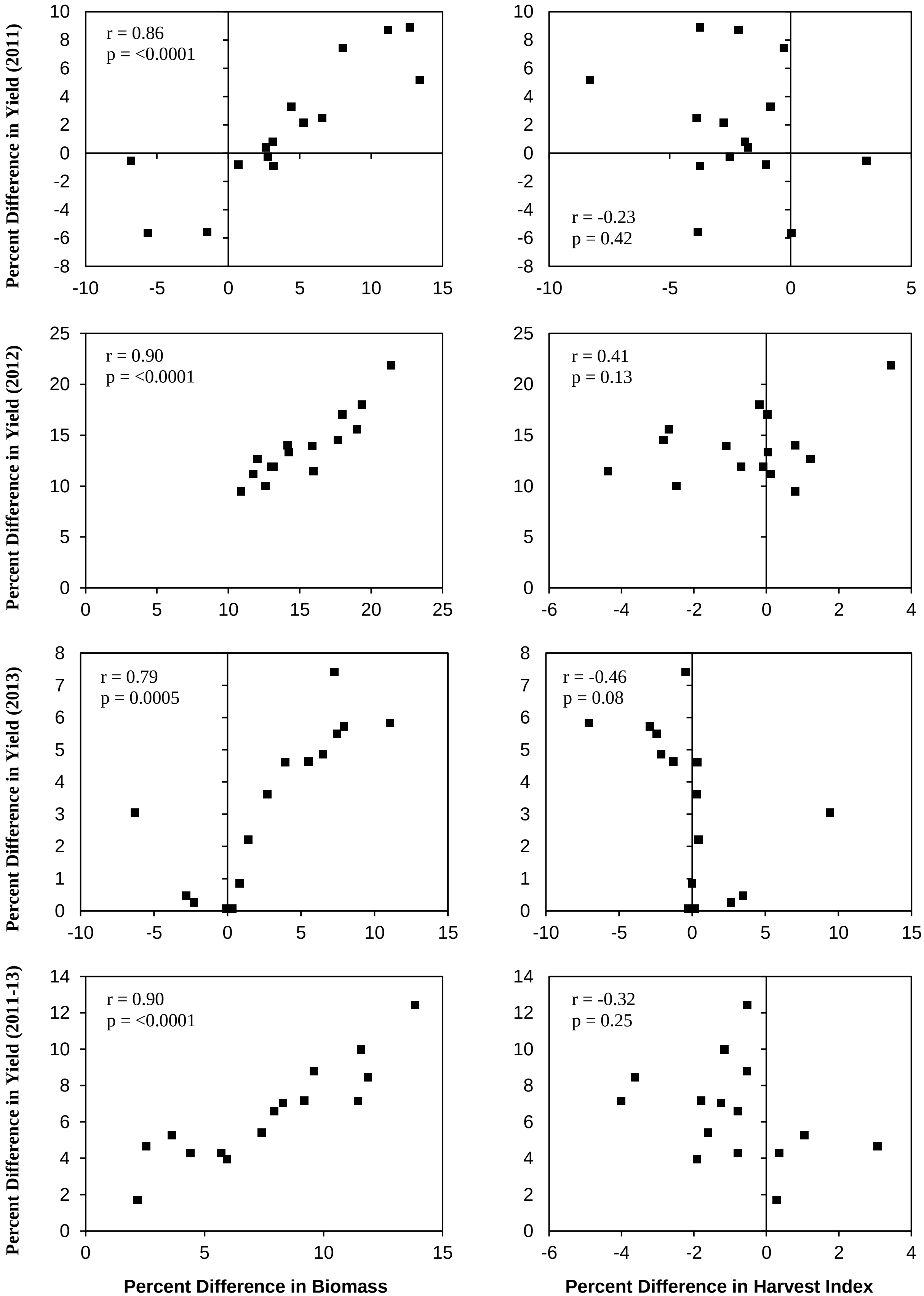

Figure 3. The percent difference in yield and percent difference (water-replete vs. rain-fed) in biomass and harvest index, for 15 soybean varieties in 2011 (top), 2012 (second row), 2013 (third row), and all years combined (bottom).

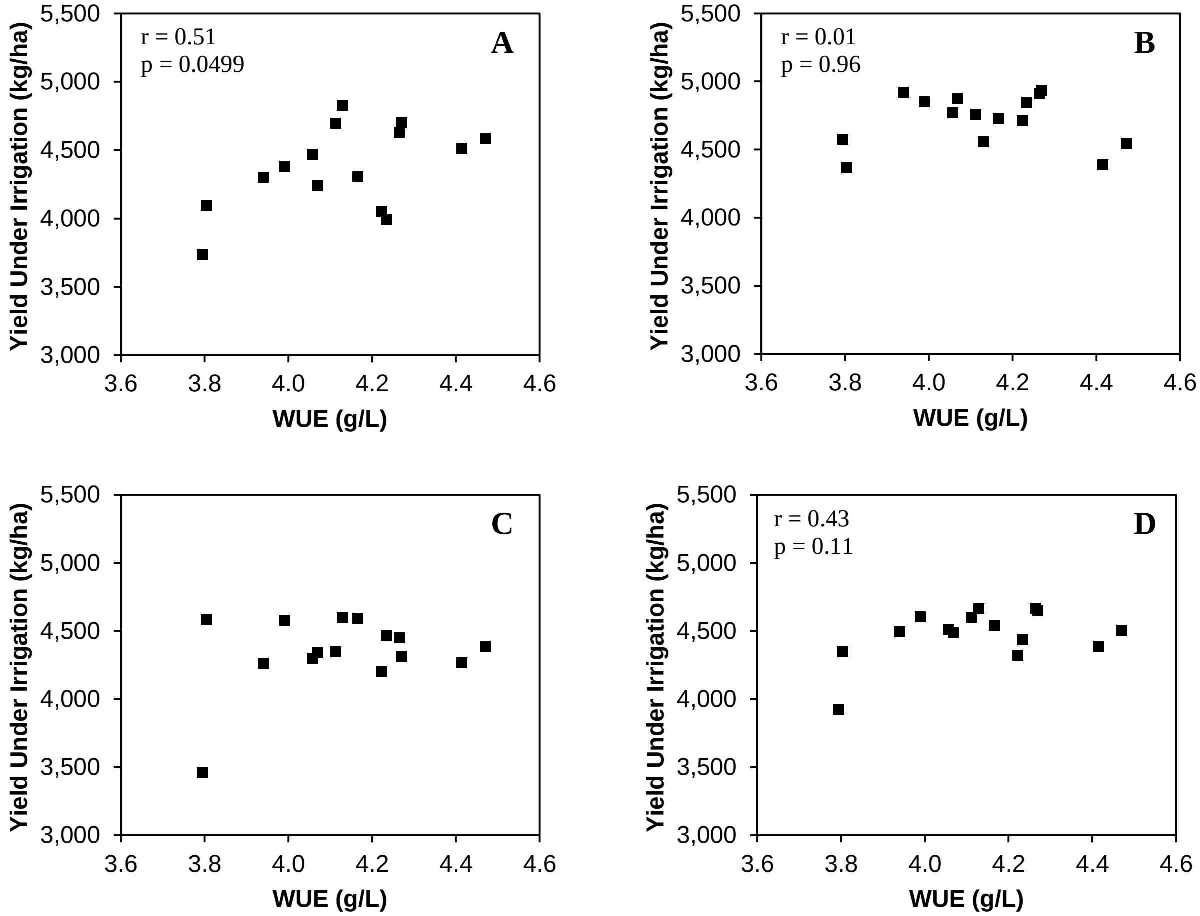

Figure 4. The water use efficiency (WUE) in the greenhouse and yield under irrigation in the field for 15 soybean varieties in (A) 2011, (B) 2012, (C) 2013, and (D) all years combined. Each data point is the mean of six replications in the greenhouse and three (2011) or four (2012 and 2013) replications in the field.

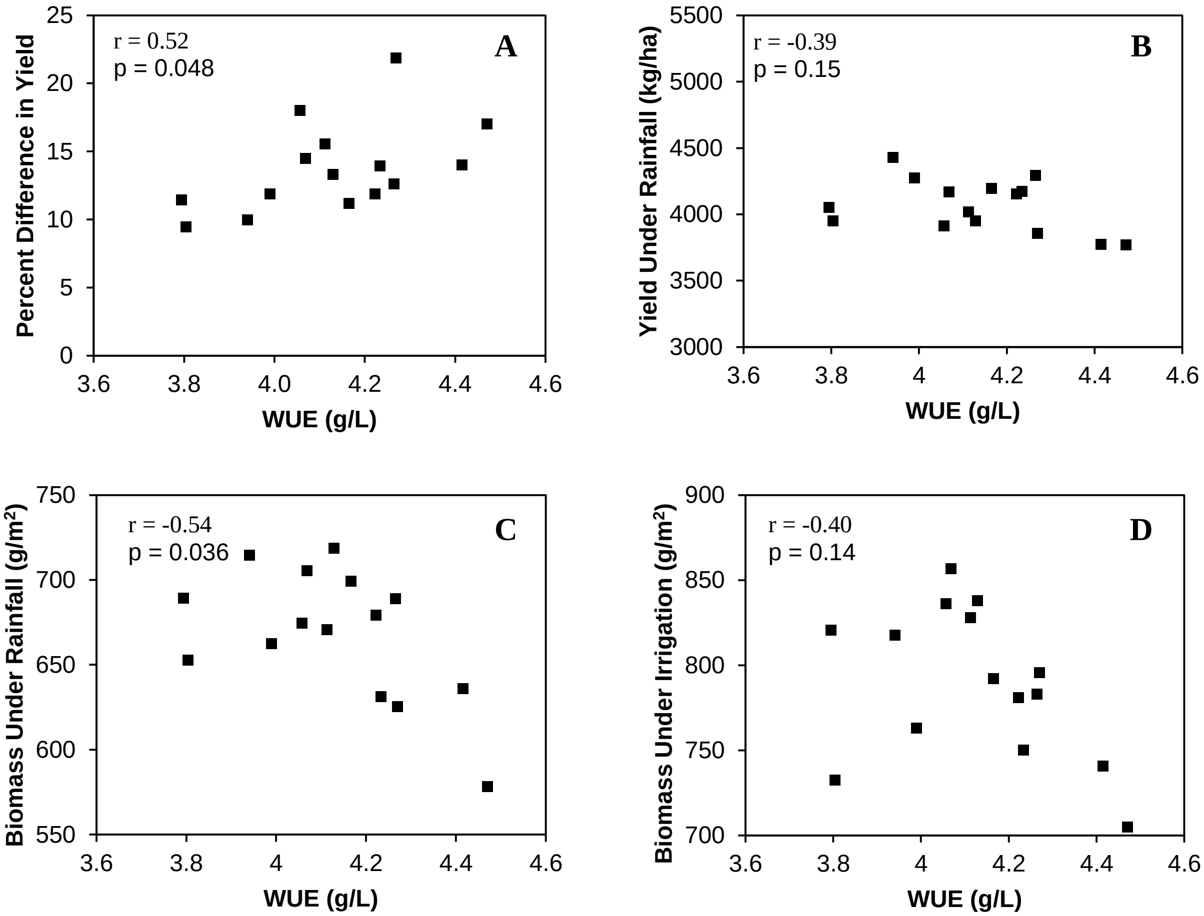

Figure 5. The water use efficiency (WUE) measured in the greenhouse and (A) percent difference in yield (water-replete vs. rain-fed), (B) yield under the rain-fed treatment (yield under the water-replete treatment can be found in Figure 4B), and total biomass under the (C) rain-fed and (D) water-replete treatments for 15 varieties in the field experiment in 2012.