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SOYBEAN RESPONSE TO WATER: TRAIT IDENTIFICATION AND
PREDICTION

by

Shawn Jenkins

A DISSERTATION

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SOYBEAN RESPONSE TO WATER: TRAIT IDENTIFICATION AND PREDICTION

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University of Nebraska, 2020

Advisor: George L. Graef

The rising demand for soybean [*Glycine Max (L.)* Merrill] taken in consideration with current climatic trends accentuates the importance of improving soybean seed yield response per unit water (WP). To further our understanding of the quantitative WP trait, a multi-omic approach was implemented for improved trait identification and predictive modeling opportunities. Through the evaluation of two recombinant inbred line populations jointly totaling 439 lines subjected to contrasting irrigation treatments, informative agronomic, phenomic, and genomic associations were identified. Across both populations, relationships were identified between lodging at maturity ($r = -0.58$, $H = 0.86$), canopy to air temperature differential at the V5 growth stage ($r = -0.31$, $H = 0.39$), the SR680 spectral index collected at the R5 growth stage, ($r = 0.62$, $H = 0.39$), and a quantitative trait loci at approximately 30 centimorgans on chromosome 19 ($r = 0.27$) to WP. Through the integration of significant agronomic, phenomic, and genomic traits, predictive models of WP were developed across environments on an entry mean basis ($r = 0.72$, $RMSE = 0.67 \text{ kg ha}^{-1} \text{ mm}^{-1}$) and on a per plot basis ($r = 0.95$, $RMSE = 0.39 \text{ kg ha}^{-1} \text{ mm}^{-1}$) using machine learning algorithms. Our results highlight the value of integrating multiple dataset types to study and model quantitative traits. Through the application of our findings, soybean breeders can

potentially deploy multi-omic selection models in early generation screening stages to increase the rate of genetic gain in relation to soybean WP.

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LITERATURE REVIEW

Introduction

Availability of water is the primary abiotic factor influencing global food production (Matiu et al., 2017). In intensive crop production regions such as the Midwestern United States, the Chinese Corn Belt, Western Europe, and Australia, approximately two-thirds of the annual yield variability is dictated by fluctuating levels of precipitation and heat (Ray et al., 2013). Amplifying this substantial effect, crop land area limited by precipitation is projected to increase three-fold during the 21st century (Li et al., 2009). Modeling future weather trends on their impact to crop production, annual yield losses from limited precipitation are expected to increase 10.5%, 6.0%, 18.8%, and 15.6% for wheat, maize, soybean, and rice respectively during the next 80 years (Leng and Hall, 2019). As a result of the future climate and increasing populations, 20 - 60 million irrigated hectares of cropland are expected to be converted back to rainfed production during the 21st century (Elliott et al., 2014). This reversion of cropland in combination with unfavorable precipitation patterns is projected to limit the global food production during the year 2100 to levels 8 – 43% below current day totals (Elliott et al., 2014). With global food demands expected to approximately double by 2050 (Godfray et al., 2010; Tilman et al., 2011), current patterns relative to crop production and water foretell dire impending socioeconomic consequences without immediate intervention focused on crop water productivity.

A major global food crop demanding the greatest concern to the imminent limitation in production due to water is soybean [*Glycine Max (L.) Merrill*]. With estimated yield losses from limited precipitation to be roughly double that of maize,

soybean production has the highest potential to be restricted from climatic trends in the current century (Leng and Hall, 2019; Matiu et al., 2017). As the world's primary oilseed crop with over 126 million hectares projected to be planted and an estimated 360 million metric tons harvested in 2018-2019, climatic and agricultural patterns put production of the world's most important food, oil, and protein crop under danger (OECD-FAO, 2019; Singh, G., 2010).

As the world's largest producer of soybean, the United States has been identified as being especially at risk from climate changes (Elliott et al., 2014; Leng and Hall, 2019; Li et al., 2009; OECD-FAO, 2019; Zipper et al., 2016). Projected future irrigation limitations in combination with elevated risk of variable precipitation in the United States corn belt greatly increases the magnitude of future losses associated with annual rainfall amounts compared to other global soybean production environments (Elliott et al., 2014; Leng and Hall, 2019; Zipper et al., 2016). Compounding this looming unfavorable trend, soybean has experienced rapid growth in the United States over the past 100 years. Harvested hectares have increased from 181,300 in 1924 to approximately 35,751,140 hectares in 2018; even in the past decade, from 2008 to 2018, there has been an increase of approximate 60% in overall soybean production in the United States (USDA, National Agricultural Statistics Service, 2018). This swift trend is expected to continue as the 2050 projected demands will necessitate an approximate 70% increase in hectares harvested compared to year 2000 levels (Kruse, 2010). Coinciding with increased land production demands, annual soybean yield gains must increase approximately 100% from the current 1.3% rate to 2.4% annually (Hertel, 2011; Kruse, 2010; Ray et al., 2013). This demand requires an annual increase of $47.1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ from current levels of United States

soybean production (Hertel, 2011; Nelson, 2010). Continuation with current soybean yield advances is projected to result in extensive global shortages as soon as 2050 (Ray et al., 2013).

The increasing demand for soybean taken in consideration with negative climatic and irrigation trends accentuates the importance of improving soybean yield response to water. To overcome the unfavorable patterns and meet future production demands, a multidisciplinary and collaborative approach is essential to sustain soybean production. To address the approaching concerns, an increased understanding of soybean responses to water, improved characterization and deployment of soybean water response traits, and increased rates of genetic gain through breeding innovation are demanded. Only through such achievements will the great challenge of meeting future demands be realized.

Soybean Response to Water

To further our understanding of soybean response to water, a foundation of current knowledge is needed. Soybean response to water has been shown to be a highly variable trait heavily influenced by both environmental and genotypic factors (Irmak et al., 2014; Specht et al., 2001). Reports of yield to seasonal water supply, or water productivity (WP), have been reported to be approximately $13.1 \text{ kg ha}^{-1} \text{ mm}^{-1}$ in high yielding Nebraska environments with statewide averages reported as $9.9 \text{ kg ha}^{-1} \text{ mm}^{-1}$ (Irmak et al., 2014; Grassini et al., 2015). When calculated as the linear regression coefficient between limited and rainfed environments, additional water supplied through irrigation has been reported to increase soybean yield anywhere from $1.32 \text{ kg ha}^{-1} \text{ mm}^{-1}$ to $11.49 \text{ kg ha}^{-1} \text{ mm}^{-1}$ depending on environment and genotype (Irmak et al., 2014; Specht et al., 2001). A seasonal water supply of approximately 650 mm has been estimated to be

sufficient in maximizing seed yield of soybean in the U.S. Corn belt (Grassini et al., 2015). Of this 650 mm seasonal water supply, approximately 250 - 500 mm will be utilized by the soybean plant through evapotranspiration, and the remaining amount will remain in the soil profile or leach to depths unreachable by the soybean plant (Grassini et al., 2015; Irmak et al., 2014; Payero et al., 2005).

In production environments where seasonal water supplies do not exceed evapotranspiration demands, genotypic mechanisms to overcome water stress and maintain elevated WP become especially desirable (Carter, 1989; Specht et al., 2001). Historically, soybean genotypic mechanisms to manage stress imposed by limited water have been separated into two categories, drought escape and drought tolerance (Levitt, 1980). Drought escape encompasses attributes that allow the soybean plant to complete critical life cycle stages before the onset of limited water; drought tolerance pertains to traits that allow the soybean plant to maintain high water status, turgor pressure, and water use efficiency during periods of inadequate water (Manavalan et al., 2009).

In relation to drought escape traits, the timing of water stress and stage of development are indicative to the final magnitude of response in soybean. Drought stress during the pod elongation (R3-R4) and the seed filling (R4–R5) stages are estimated to have the largest impact on final seed yield (Desclaux et al., 2000; Eck et al., 1987; Kadhem et al., 1985; Korte et al., 1983; Smiciklas et al., 1992). Water stress during pod elongation has the largest influence on the number of pods, and drought stress occurring during seed filling most significantly influences seed weight and quality (Desclaux et al., 2000; Kadhem et al., 1985; Smiciklas et al., 1992). Stress during flowering stages (R1-R2) reduces pod number by increasing the frequency of aborted flowers (Korte et al.,

1983; Westgate and Peterson, 1993). Water stress during vegetative growth reduces internode length and most significantly alters final plant height (Desclaux et al., 2000; Hoogenboom et al., 1987).

In general, soybean is most sensitive to limited water during the following growth and developmental stages ranked in decreasing order of scale: (R3-R4), (R5-R6), (R1-R2), (V1-V5), (R7-R8) (Desclaux et al., 2000; Eck et al., 1987; Kadhem et al., 1985; Korte et al., 1983). Drought stress in soybean tends to hasten maturity, and reduce plant height, lodging severity, seed quantity, seed protein concentration, seed size, and harvest index, yet large differences among genotypes have been reported (Dornbos and Mullen, 1992; Kadhem et al., 1985; Korte et al., 1983; Specht et al., 1986). The large genotypic influence of soybean response to limited water can primary be attributable to the presence of drought tolerance traits if development differences are kept constant.

When considering response to water in soybean, an equation developed by Passioura in 1977 is commonly used to explain the degree of water use efficiency exhibited by an individual plant. Under water stressed environments, the grain yield (Y) is a linear function of amount of water transpired (T), water use efficiency (WUE), and harvest index (HI): $Y = T \times WUE \times HI$ (Passioura, 1977). A wide number of traits in soybean have been shown to play directly into this equation. Beneficial traits associated with WUE have been linked to variation in soybean leaf pubescence, stomatal closure intervals, ureide accumulation in petioles, leaf osmotic adjustments, abscisic acid (ABA) accumulation, maximum transpiration rate, flower abortion rates, and drought tolerant nitrogen fixation levels (Jiang and Egli, 1993; Manavalan et al., 2009; Sinclair, Thomas R. et al., 2010). Traits associated with T have been linked to variation in relative water

content, phenology, photoperiod sensitivity, development plasticity, heat tolerance, osmotic adjustment, epidermal conductance, early vigor, lateral root development, atmospheric vapor pressure deficit (VPD) responses, and tap root development (Fletcher et al., 2007; Purcell and Specht, 2004; Sadok and Sinclair, 2009). At field scale in applied agronomic research, WUE has often been estimated and referred to as water productivity (WP) (Grassini et al., 2011; Irmak et al., 2014). Due to the limitations of estimating T and HI at field scale, WP commonly calculated through the ratio of yield to unit water or effective unit water offers a quite estimation of WUE of value in comparative field experiments (Grassini et al., 2011; Irmak et al., 2014).

The large number of phenotypic traits associated with soybean response to limited water and their interaction with developmental timing illustrate the trait's complex and highly quantitative nature. Soybean can overcome limited water availability using a myriad of phenotypic traits or simply through drought avoidance. To make significant gains with such a highly quantitative trait, genotypic variation within soybean breeding populations must first be characterized. Through the construction and categorization of divergent breeding populations or population samples, researchers can better understand the genotypic variation present within soybean.

Soybean Genotypic Variation in Response to Limited Water

To effectively characterize and understand the impact of an altered water response trait, there first must be sufficient initial variation in the sample population. Considerable genetic variation in relation to T , whole-plant WUE , leaf epidermal conductance, leaf tissue relative water content (RWC), root development, and drought tolerance nitrogen fixation levels have been reported by numerous researchers (Carpentieri-Pipolo et al.,

2012; Hufstetler et al., 2007; James, A. et al., 2008; King and Purcell, 2001; Mian et al., 1998; Purcell and Specht, 2004).

First, significant variation in T and its interaction with VPD have been reported among both commercial soybean cultivars and plant introduction (PI) soybean germplasm sources (Bunce, 1981; Fletcher et al., 2007; Sadok and Sinclair, 2009). Commercial soybean cultivars have been reported to have nearly double the rate of T compared to PIs during high VPD growth conditions with measurable lower canopy temperatures ($> 2^{\circ}\text{C}$) during conditions eliciting this response (Fletcher et al., 2007; Sadok and Sinclair, 2009). Plant introduction genotypes were observed to clearly limit transpiration when VPD approached 2.0 kPa and above, yet commercial soybean cultivars exhibited no such limitation (Fletcher et al., 2007; Sadok and Sinclair, 2009). Limiting T during periods of high VPD has been theorized to be a key trait in water conservation during the growing season therefore potentially increasing both WUE and yield in certain water limiting environments (Fletcher et al., 2007). This water response trait has been modeled to improve soybean yield in approximately 70% of U.S. growing conditions over years (Sinclair et al., 2010).

In addition to transpiration rate variation, significantly different $WUEs$, leaf epidermal conductance values, leaf osmotic potential values, and RWC have been reported in soybean. Investigating commercial cultivars across water treatments, ranges of approximately 25% of maximum WUE have been reported along with a significant negative relationship between leaf epidermal conductance and WUE (Hufstetler et al., 2007). Reports of at least a two-fold range in leaf epidermal conductance values, a 2.10 MPa range in leaf osmotic potential values (52% range from maximum), and a 12-

percentage point range in RWC (20% range from maximum) during periods of drought stress have also been estimated between cultivated soybean varieties with an even greater variation among *Glycine soja* (James, A. et al., 2008). With increased leaf epidermal conductance both T and leaf RWC are anticipated to be increased along with an increased photosynthetic rate (Farquhar and Sharkey, 1982). Similar to the benefits of VPD limited T , genotypes with lower leaf epidermal conductance and lower leaf RWC likely conserve water during times of stress thereby improving WUE. In environments with extreme drought stress, these traits can be viewed as beneficial, yet in optimum environments, they would likely limit maximum yield (Blum, A., 2009; Buttery et al., 1993; Roche, 2015; Sinclair, Thomas R. et al., 2010).

Along with genotypic variation in water response traits associated with the plant canopy, variation exists among root responses. In two connected studies, variation as great as 1.3 cm day^{-1} among 105 diverse soybean lines for taproot elongation was estimated in greenhouse environments, and in the associated field study, cultivars with greater taproot elongation were able to extract water at depths over 120 cm (Kaspar et al., 1984). Greater root dry weight and total length has been found to be significantly correlated to water productivity in water-limited environments (Goldman et al., 1989; Hudak and Patterson, 1996; Read and Bartlett, 1972). Along with rooting traits, variation in nitrogen fixation during periods of drought stress have been shown to result in correlated variation in yield increase even at moderate levels of water deficit (King and Purcell, 2001; Sinclair, Thomas R. et al., 2007). Through extracting water from deeper soil depths and maintaining high levels of nitrogen fixation during water stress, growth and development remains relatively unchanged in periods of moderate stress.

With substantial genotypic variation among even commercial soybean cultivars, there is great opportunity for improving soybean response to water. Through the construction of breeding populations from parents with complementary water response traits, the likelihood of obtaining transgressive segregation and improved genetic gain is increased (Bernardo, 2002; Falconer and Mackay, 2009). Development of such soybean cultivars with improved response to water better equips producers to meet future demands despite unfavorable climatic and irrigation trends.

Breeding for Improved Response to Water in Soybean

Breeding for improved response to water has long been recognized as an area of concern in soybean breeding programs; the importance of drought on soybean's expansion into the Western United States was discussed as early as 1939 (Primmer, 1939). In 1956, the basis of drought resistance in soybean and benefits of exploiting the natural variation in limited water response traits was highlighted (Clark and Levitt, 1956). In 2010, the benefit of soybean drought traits was estimated to result in significant yield gains, up to 1000 kg ha⁻¹, in all major soybean production areas (Sinclair et al., 2010). Despite the longstanding knowledge of the benefits of drought tolerance to soybean yield and quality, most soybean breeding programs do not directly select for improved response to water (Carter, 1989; Sleper and Poehlman, 2006).

Breeding for increased levels of seed yield is often of main concern for public and private soybean breeding programs with continual focus on maximizing the amount of genetic gain per year. Releasing soybean lines with increased yield performance is commonly achieved through developing F₂ populations from elite parents and advancing these populations through single seed descent, progeny evaluation, and yield trial

evaluation over a period of six to seven years (Bernardo, 2002; Fehr, Walter R., 1987; Lynch and Walsh, 1998; Sleper and Poehlman, 2006). Yield evaluation of the progeny usually begins around the F₅ generation in testing sites representative of the intended production market (Bernardo, 2002; Fehr, Walter R., 1987). The highly quantitative nature of soybean seed yield complicates yield evaluation as both genotypic and environmental factors such as abiotic stress tolerance govern performance. Even with the great progress in soybean breeding over that past 20 years obtained through leveraging transgenic technologies, winter nurseries, marker assisted selection (MAS), data management technologies, genotyping array technologies, and genotyping by sequencing (GBS) methods, confounding environmental effects still complicate yield estimates (Blum, Abraham, 2018). The interaction that soybean seed yield exhibits with abiotic environmental factors has caused divergence in approaches for addressing these environmental limitations. Commonly, soybean breeding programs either directly address and breed for abiotic stresses such as limited water availability, or solely focus on overall yield improvement in optimum environments.

When breeding for improved drought tolerance or water productivity in limited environments in soybean, it follows that the term drought tolerance must first be clearly defined. Definitions for drought tolerance can be classified into two categories, mechanistic and empirical (Specht et al., 2001; Wilhite and Glantz, 1985). Mechanistic tolerance selection involves identifying and selecting for physiological soybean traits that are highly correlated to survival under drought stressed conditions (drought escape, dehydration avoidance, and dehydration tolerance) yet may be associated with lower seed yields in optimum environments (Jones, 1993; Kramer, 1980; Ludlow and Muchow,

1990; Nilsen and Orcutt, 1996). Empirical selection for drought tolerance involves selecting the highest-yielding genotypes in environments where drought is recurrent, or genotypes with the smallest yield decline per unit of reduced rainfall (Specht et al., 1986; Specht et al., 2001).

Determining whether to follow either a mechanistic or an empirical approach in breeding for improved water productivity in limited environments proves to be a difficult decision. Proponents of both methods have demonstrated success, yet because water productivity is a highly quantitative trait, choosing a clear superior method is problematic. Traditionally many soybean breeders have chosen to take the empirical approach, and focus on mean performance over environments even though the benefits of improved tolerance to drought are widely known (Carter, 1989). Even without a clear abiotic stress breeding objective, minor and recurrent environmental stresses at yield evaluation environments will result in continual empirical stress selection (Parlevliet, 1994). Using this viewpoint, the advantages of empirical selection for stress tolerance has been supported by the theory; with minor stresses even in optimum environments, yield performance across stress and non-stressed environments is best achieved when selection is performed in locations that maximize genetic variation (Rosielle and Hamblin, 1981). Without segregation of major drought tolerance traits, the increased genetic variation anticipated in optimum environments allows for improved selection across both optimum and stressed locations (Rosielle and Hamblin, 1981). This empirical approach has been supported with studies investigating water productivity improvement in multiple crops (Calderini and Slafer, 1998; Perez Arocho, 2017; Rizza et al., 2004; Specht et al., 2001) . When global yield increases in wheat are expressed as a percentage, no significant

advantaged arises between yield advancements in optimum and water limited regions (Calderini and Slafer, 1998). In soybean, empirical selection for drought tolerance in a diverse soybean panel has proven to effectively identify genotypes with improved performance in water-limited environments (Perez Arocho, 2017)

Irrespective of empirical drought tolerance selection successes, advances in genomic techniques over the past 20 years have increasingly challenged the empirical breeding approach. The availability of high-density genetic maps of soybean and marker assisted selection (MAS) techniques have increased the practicality of mechanistic selection. Through the application of the high-density genetic maps, 28 water response related quantitative trait loci (QTL) have been reported in soybean (Bhatnagar et al., 2005; Carpentieri-Pipolo et al., 2012; Du et al., 2009; Mian et al., 1996; Mian et al., 1998; Monteros et al., 2006; Specht et al., 2001). By characterizing soybean response to water and identifying associated molecular markers, a molecular mechanistic breeding approach is anticipated to improve accuracy, efficiency, and precision of drought tolerance progress in soybean (Pathan et al., 2007). However, the complex and highly quantitative nature of soybean seed yield limits the immediate application. With nearly half of the reported QTL explain less than 10% of the total phenotypic variation, a combination of both mechanistic and empirical selection methods or genome wide selection may prove to be most beneficial (Pathan et al., 2007).

A potential method to leverage the benefits of both empirical and mechanistic selection for drought tolerance in soybean is to focus on the response to water between two irrigation treatments (Perez Arocho, 2017; Ruff, 2016; Specht et al., 1986; Specht et al., 2001). Due to the linear response of soybean yield to varying levels of water received,

only two water treatments are needed (Specht et al., 2001). A limited and an optimum irrigation treatment will allow for yield selection under optimum conditions, mechanistic trait identification in the limited treatment, and an empirical quantification of response through the change in yield between the two extreme treatments (Grassini et al., 2015; Specht et al., 2001). Through this approach, traits correlated with significant changes in drought stress tolerance may be identified in the limited treatment and estimates of water productivity can be evaluated over treatments increasing the inference space of the experiment.

Combining such a drought tolerance experiment with high-density genetic information further improves mechanistic trait identification as QTL can be associated with yield performance in limited irrigation treatments, yield response between treatments, or water productivity over treatments. However, to fully leverage the high-dimensional genotypic data, equally high dimensional phenotypic information should be collected and paired. To effectively collect the amount of high quality phenomic information mandatory to leverage the genotypic data, high-throughput field based phenomic platforms are needed.

Field Based Phenomic Platforms

The large-scale systematic collection of high dimensional and high throughput phenotypic data has been considered imperative for advances in the genomic era (Bilder et al., 2009; Freimer and Sabatti, 2003; Houle et al., 2010; Schork, 1997). Through the union of high-dimensional phenotypic and genotypic data, rare genetic variations can be associated with phenotypic response, pleiotropy can be studied, and our knowledge of complex biological systems can be increased (Brown et al., 2014; Houle et al., 2010;

Pendergrass et al., 2011). In relationship to plant breeding, while many programs have readily adopted genomic technologies, relatively few have incorporated high-dimensional phenotypic data (Awada et al., 2018). The need to efficiently and accurately characterize plant phenotypes to keep pace with current DNA sequencing technologies has therefore piloted the development of high throughput field-based phenomic platforms (Scheben et al., 2018; White et al., 2012).

Field phenomic platforms leverage an array of visible light, thermal infrared, near infrared (NIR), ultrasonic, hyperspectral, and light detection and ranging (LIDAR) sensors to quantify crop growth and status (Andrade-Sanchez et al., 2014; Bai et al., 2016; Barker et al., 2016; Busemeyer et al., 2013; Svensgaard et al., 2014; Virlet et al., 2017; White et al., 2012). Compared to traditional phenotypic data collection processes in plant breeding programs, field phenomic platforms enable a step change in data resolution, repeatability, and dimension along with indirect quantification of phenotypes previously unfeasible to collect on a large scale (Awada et al., 2018). Through quantification of leaf reflectance parameters, biologically important yet traditionally unobtainable metrics such as chlorophyll concentration, photosynthetic rates, and canopy architecture can be rapidly collected (Chappelle et al., 1992; McKinney et al., 1989; Rainey et al., 2018). In relation to a complex and highly quantitative trait such as response to water, field phenomics provide a high-dimensional canopy reflectance dataset to identify an increased number of genes with small effect influencing the response (White et al., 2012).

Soybean Leaf Reflectance Parameters

Field phenomic platforms utilize the high correlation of many canopy reflectance parameters to biological properties of the crop. In soybean, spectral reflectance patterns offer valuable insights into both leaf structure and photosynthetic pigment concentrations. In previous studies, the amount of visible and NIR light reflected has been associated with leaf cellular structure and photosynthetic pigment concentrations (Kumar and Silva, 1973; Sinclair, TR et al., 1971; Woolley, 1971). In soybean, wavelengths relating to visible light region (400 – 700 nm) generally have lower reflectance due to the strong absorption of blue and red light by the photosynthetic pigments chlorophyll a, chlorophyll b, and carotenoids in the leaf chloroplast (Curran, 1989). As the frequency of light increases into the NIR region (700 – 1250 nm), an increase in reflectance is expected from the scattering of light through the spongy mesophyll and parenchyma cells (Gates et al., 1965; Knipling, 1970); photosynthetic pigments are also unable to use these higher energy wavelengths in the NIR region thereby increasing reflectance (Gates et al., 1965; Knipling, 1970).

The spectral reflectance curve common to plants has been used to create a multitude of reflectance indices that estimate photosynthetic pigment concentrations and activity. Through comparison of the green and red regions of the reflectance spectrum, leaf pigment concentrations can be estimated for a myriad of crop species (Rascher et al., 2011; Sims and Gamon, 2002). In soybean, reflectance differences between blue and red light wavelengths has been used to predict chlorophyll concentration and β carotene concentration with high accuracy, $R^2 = 0.93$, and 0.94 respectively (Chappelle et al., 1992). Indices related to these photosynthetic pigment concentrations have proven useful

in predicting agronomically important phenotypes in soybean such as yield and biomass. Using canopy reflectance measurements, R^2 greater than 0.80 have been reported for in season canopy reflectance parameters to yield (Babar et al., 2006; Ma, B. et al., 2001). In addition to associations with yield, recent research comparing reflectance in the visible light spectrum has shown useful for quantifying nitrogen fixation and effects of nodulation in soybean (Vollmann et al., 2011).

In conjunction with general wavelength regions of interest, the very specific Fraunhofer lines have shown promise in quantifying photosynthetic pigment concentrations (Liu, Liang-yun et al., 2006). Fraunhofer lines represent absorption wavelengths in the solar reflectance curve caused by the interaction of various elements to the sun's photosphere and the earth's atmosphere with the incoming solar radiation (Meroni et al., 2010). Due to the greatly reduced solar reflectance noise from plant canopies at Fraunhofer lines, the series of wavelengths related to chlorophyll fluorescence (656.7 nm, 686.7 nm, and 759.4 nm) has been investigated successfully for quantifying photosynthetic pigments in wheat with correlation coefficients of 0.99 to handheld chlorophyll meters (Liu, Liangyun et al., 2005; Liu, Liang-yun et al., 2006). Fraunhofer lines of 656.7 nm, 686.7 nm, and 759.4 nm have even been shown to effectively identify the presence of drought stress in the Williams 82 soybean line under controlled growing conditions with prediction accuracy of 0.96 (Mo et al., 2015).

In addition to spectral reflectance, thermal properties of the crop canopy have proven beneficial for investigating photosynthetic rates, stomatal resistance, and yield performance (Amani et al., 1996; Farquhar and Sharkey, 1982; Fischer et al., 1998; Pietragalla and Pask, 2012). Through transpiration's evaporative cooling effect on the

surface of leaf tissue, canopy temperature serves as an indirect quantification of T . As leaf transpiration increases, more liquid H_2O is converted to gas, thereby more heat energy from surrounding leaf surfaces is absorbed, and the effective temperature is thereby lowered (Farquhar and Sharkey, 1982; Roche, 2015). Using this biological rationale, soybean researchers have identified significant relationships between leaf canopy temperature to air temperature differential (CATD), water status, and yield (Harris et al., 1984; Jackson, 1982; McKinney et al., 1989; Valle et al., 1985). General trends of lower canopy temperature values relative to the environment has been shown to be positively correlated with yield, and positively correlated with leaf water potential, stomatal conductance, and transpiration rates (McKinney et al., 1989; Ries et al., 2012; Roche, 2015; Valle et al., 1985). As the primary mechanism for plants to acquire CO_2 and fix into biomass, increases in stomatal conductance and transpiration rates indirectly assessed through canopy temperature measurements offer great potential for researchers to improve yield potential, biotic stress tolerance, harvest index, and radiation use-efficiency (Roche, 2015).

Previous research has established that numerous soybean physiological and agronomic traits can be assessed from canopy reflectance parameters. Leveraging these previously identified associations, field phenomic platforms allow researchers to rapidly collect a wealth of relevant phenotypes on a per plot basis. Armed with this abundance of relevant phenotypes, researchers are better equipped to identify small variations associated with complex traits such as water stress tolerance and response (White et al., 2012).

Field Phenomics for Soybean Water Stress Categorization

Even though researchers have made progress in improving response to water in multiple crops using field phenomics, the use of field phenomics to identify water stress tolerance and response traits in soybean is lacking (Beebe et al., 2013; Masuka et al., 2012; Passioura, 2012; Spindel et al., 2018; Thompson et al., 2018). Recent research using greenhouse phenomic systems have distinguished differences in *WUE*, transpiration rate efficiency, and *T* among soybean genotypes using imagery data (Peirone et al., 2018; Pereyra-Irujo et al., 2012). Nevertheless, many of these traits identified in greenhouse conditions were shown to have little relationship to performance in field trials, suggesting a large genotype by greenhouse environmental interaction (Peirone et al., 2018). Possibly due to soybean's altered growth in greenhouse environments, few reports of phenotypic measurements collected in greenhouses have shown high correlation to field performance (Peirone et al., 2018). With the apparent limitations of greenhouse phenotyping systems, the use of field phenomics seem most adaptable to measuring water stress tolerance and response in soybean, yet few studies have been conducted.

Early work in soybean field phenomics using canopy coverage and light interception measurements from digital imagery has shown promise in evaluating soybean growth and yield in optimally irrigated experiments (Purcell, 2000). Building from this study, digital imagery of canopy development between two soybean cultivars under four irrigation treatment regimens allowed for the construction of indices with correlation coefficients of over 0.80 to yield across water treatments (Hoyos-Villegas et al., 2014). In addition to yield prediction, canopy coverage estimates have also proven useful for identifying QTL associated with yield across a diverse nested association

mapping panel in soybean (Xavier et al., 2017). Implementation of these phenomic-derived QTL has improved the accuracy of genomic selection, compared to traditional methods (Jarquín et al., 2014).

Despite these helpful early findings, to our knowledge, field phenomics has yet to be used for identifying traits in large soybean mapping populations under water treatment regimes. Although previous studies have been conducted investigating the relationship of agronomic traits between limited and optimally irrigation environments, work has yet to be done incorporating large phenomic and genomic datasets into such an experiment. Through the incorporation of agronomic, phenomic, genomic, and environmental datasets into experiments with contrasting irrigation treatments, improvements in mechanistic trait identification is expected. Our research investigates the feasibility of paring agronomic, phenomic, and genomic data to improve trait identification and prediction performance in soybean breeding programs.

RESEARCH OBJECTIVES

The objectives of this research are to 1) quantify two soybean recombinant inbred line (RIL) populations for water productivity and identify informative agronomic, phenomic, and genomic associations; and 2) integrate agronomic, phenomic, genomic, and environmental information to develop predictive models of water productivity.

MATERIALS AND METHODS

Soybean Population Development

Three maturity group (MG) III lines were selected to develop two recombinant inbred line (RIL) mapping populations based on their varying responses to water stress from an experiment conducted in Los Andes, Chile (-32.796 latitude, -70.626 longitude) during the winter of 2013-2014 (Figure 1). University of Nebraska-Lincoln (UNL) line U11-614093 was selected based on favorable yield under both optimal and water stressed irrigation treatments. University of Nebraska-Lincoln line U09-312115 was selected based on favorable yield under optimal irrigation and genetic distance from U11-614093, and University of Illinois line LD02-4485 was selected for its relatively small response to increasing amounts of water (Ruff, 2016). Breeding line U11-614093 was derived from the initial crossing of UNL line U02-242055 and Illinois line LD04-13265 (Table 1). U09-312115 was selected from the cross of UNL lines U02-242055 and U03-300134, and LD02-4485 resulted from the crossing of M90-184111 and IA3010 (Table 1) (Crochet and Hughes, 2014; Schlueter and Scofield, 2015).

The three parental lines were crossed the summer of 2013 to form two distinct RIL populations; the U11-614093 x LD02-4485 population denoted as UX3036 and the U09-312115 x U11-614093 population denoted as UX3000 at UNL's East Campus Research Farm (40.836 latitude, -96.667 longitude). Hybridity of successful crosses was assessed through DNA extracted from F₁ seed using the BioSprint 96 plant DNA extraction method and a Qiagen DNeasy Plant 96 kit (DNeasy, QIAGEN, Hilden, Germany) (Ruff, 2016). True F₁ seed was confirmed through the comparison of simple sequence repeat (SSR) markers polymorphic between parental lines (Ruff, 2016).

Confirmed F_1 hybrid seeds and parental lines were then planted in 1.0-meter length three row plots in Los Andes, Chile during the winter of 2013-2014. Bulk F_2 seed from Los Andes was then grown in 2.9-meter length four row plots at the UNL East Campus Research Farm during the summer of 2014. Seed was bulked from these plots and planted in 3.0-meter length four row plots at Los Andes during the winter of 2014-2015. Bulk F_4 seed from Los Andes was then planted in 6.0-meter length four row plots at UNL and single plants were pulled based on maturity grouping for progeny row increase. Progeny row increases of the $F_{4:5}$ recombinant inbred lines took place at Los Andes in single row 1.0-meter length plots. All seed was harvested from progeny row increases to form a total of 872 $F_{4:6}$ RILs. The total size of the UX3036 population was 403 individuals, and the total size of the UX3000 population was 469 individuals.

Preliminary Evaluation

All 872 $F_{4:6}$ RILs were then evaluated for preliminary yield and water productivity performance across four representative and uniform south eastern and central Nebraska testing environments (Table 2). An augmented incomplete block experimental design, with parental lines used as chaining mechanisms between incomplete blocks, was used to arrange RILs within each testing environment. Incomplete blocks were determined from first dividing the 876 RILs into three groups based on MG range (I, II, and III), and then randomly partitioning MGs in sub groupings of approximately 35 lines. RILs in MGs I and II were evaluated at Cotesfield and Mead whereas RILs in MGs III were grown in Clay Center and Wymore environments. Within each testing environment, RILs were planted in un-replicated 2.9 m length two-row plots

with 0.76 m row spacing and 0.91 m alley width. All testing environments were optimally irrigated to maximize seed yield.

During the 2016 preliminary evaluation, individual plots were phenotyped at the V5 and R5 stage with a multi-sensor high throughput field phenotyping platform developed at the University of Nebraska-Lincoln (Bai et al., 2016; Fehr, Walter R. et al., 1971). The field phenomic platform (FPP) is equipped with twenty independent sensors measuring an array of canopy traits including height, temperature, spectral reflectance, and digital imagery for three 0.76 m spaced two-row plots simultaneously (Table 3) (Bai et al., 2016; Yuan et al., 2018). With the large array of sensors and potential to be influenced by changing temporal and spatial factors, the starting position and movement of the FPP through each environment was randomized for each phenotyping event. Phenomic information was collected from all plots during both the V5 and R5 growth stage in 2016 apart from R5 information at Cotesfield, NE. Phenomic information was not collected during the R5 stage at Cotesfield due to concerns of damaging lodged plants from an earlier violent thunderstorm with high winds.

In addition to phenomic information collected at key developmental stages, final plant height at maturity, lodging, maturity date, seed yield, seed weight, seed quality, protein composition, and oil composition was collected for 2016 plots. Final plant height was recorded in centimeters as the average distance from the ground to tip of main stem height of mature plants in the center of the plot. Lodging was recorded at maturity according to the following 1-5 scale: 1 (almost all plants erect), 2 (all plants leaning slightly), 3 (all plants leaning, 25% - 50% down), 4 (all plants leaning, 50%-80% down), and 5 (almost all plants down) (Schlueter and Scofield, 2015). Maturity date was

recorded as the day at which 95% of pods on the main stem first reach maturity (Schlueter and Scofield, 2015). Seed yield was recorded as the plot seed yield in kg ha^{-1} adjusted to 13% moisture (Schlueter and Scofield, 2015). Seed weight was reported as the weight in grams of 100 seeds, and seed quality was assessed using an iterative 1-5 scale representative of the 1 (no blemishes, ideal seed quality) to 5 (very poor seed quality, greater than 80% of seed area blemished) range (Schlueter and Scofield, 2015). Seed composition metrics were estimated through an Infratec™ 1241 whole seed grain analyzer (Infratec™ 1241, FOSS, Hillerød, Denmark) with a transmittance scanning monochromator spectrometer. Reflectance values were transformed through SB201301 soybean bulk seed and SB201304 soybean sample transport module calibrations provided by the Iowa Grain Quality Laboratory, Iowa State University to output protein, oil and fiber compositions by weight adjusted to 13% moisture (Rippke et al., 1995). Ten subsamples were used throughout the project when analyzing plot seed samples and values were reported as the ten-subsample average.

Along with phenomic and agronomic information, estimated processed values, estimated yield WP to in season effective water (estimated evapotranspiration), and weather station information relative to testing environments was collected. Estimates for processed meal protein concentration, crude oil yield, and estimated processed value were determined from the soybean processing (SPOC) program and seed composition values (Brumm and Hurburgh, 1990). October 2013 to October 2018 average Chicago Mercantile Exchange (CME) group end of day settlement prices for soybean oil, soybean meal, and soybean hulls were used as inputs for the SPROC program (Brumm and Hurburgh, 1990). Environmental information for 2016 testing environments was

download from the High Plains Regional Climate Center (CLIMOD) webpage (<http://climod.unl.edu/>). From the online resource, daily maximum temperature in degrees Celsius, minimum temperature in degrees Celsius, and precipitation in millimeters was used to estimate accumulated growing degree days (GDD), and accumulated precipitation during the growing season. Precipitation and temperature information from CLIMOD was inputted into the soybean growth and water use crop model, SoyWater (<http://hprcc-agron0.unl.edu/soywater>), to estimate soybean growth, estimate cumulative effective water, estimate cumulative water depletion and schedule irrigation timing throughout the growing season relative to each production environment and maturity grouping (Specht et al., 2010). Water productivity (WP) was calculated by dividing total plot seed yield by the seasonal cumulative effective water estimated by SoyWater. Within SoyWater, seasonal cumulative effective water is an estimation of total transpiration influenced by weather metrics, irrigation timing and amount, soil water holding capacity, and maturity grouping (Specht et al., 2010).

Power Analysis

After the 2016 preliminary yield evaluation, a power analysis was conducted to approximate the sample size needed to estimate QTL effects and treatment differences in a water response experiment. The R package “qtlDesign” was used to estimate the sample size needed to detect QTL over critical likelihood of odds (LOD) threshold values, given estimated effect size, genotypic variance, environmental variance, and number of replications (R-Core Team, 2018; Sen et al., 2007). The PROC power procedure of SAS 9.3 (SAS Institute, 2014) was used to estimate power for yield differences between full and rainfed irrigation treatments. With large population sizes, a 50% random sample of

each RIL population was determined to provide sufficient power to detect QTL and identify treatment differences while conserving resources. With a reduced population size of 235 lines (50% of 469), replicated twice within location over two environments, the UX3000 population was estimated to have 80% power in detecting a 194 kg ha⁻¹ yield difference and identify water response QTL with LOD scores greater than 2.17. The sampling of 202 lines (50% of 403) from the UX3036 population was estimated to provide 80% power in detecting a 209 kg ha⁻¹ yield difference and identify water response QTL with LOD scores greater than 2.65. Previous QTL mapping studies involving soybean yield and drought responses have been unsuccessful in identifying QTL with LOD scores less than 2.9 (Carpentieri-Pipolo et al., 2012; Du et al., 2009; Mian et al., 1998; Specht et al., 2001). Standard errors of entry least square mean estimates across environments in the 2016 preliminary evaluation experiment averaged 146 kg ha⁻¹. Based on this information, and the variation of genetic yield potential identified in the preliminary yield analysis, power was deemed sufficient to accomplish this projects objectives.

Water Response Experiment

After determining the minimum sample size needed to accomplish objectives, the experimental design of the water response experiment was devised. Through consideration of both resource constraints and the importance of RIL estimates compared to estimates of overall irrigation effect, a split plot experimental design was determined to be most suitable. Using whole plot irrigation treatments and sub-plot RIL treatments provides an effective way to deliver and manage irrigation treatments while allowing increased power to identify RIL treatment effects (Mead et al., 2012).

Based on previous knowledge of soybean's linear response to water, only two water treatments, optimally irrigated and rainfed, were used as whole plot treatments (Specht et al., 2001). Sub-plots, representing a 2.9 m length two-row plot with 0.76 m row spacing and a 0.91 m alley width, were arranged in augmented incomplete blocks with parental lines serving as chaining mechanisms. To aid in the power of identifying RIL treatment effects, incomplete blocks were replicated within each whole plot treatment. As in the preliminary yield analysis, incomplete blocks were determined from first dividing the RILs into MG ranges. With the previous knowledge of the impact of irrigation timing and drought avoidance in soybean, four maturity groupings (2.0-2.5, 2.5-3.0, 3.0-3.5, and 3.5-4.0) were used to minimize the developmental influence of water productivity. Maturity group ranges were nested within whole-plot treatments so that irrigation could be applied independently to increase application efficiency and avoid developmental effects (Figure 2). Once RILs were partitioned into maturity group ranges, individuals were randomly assigned to sub groupings of approximately 25 lines representing the incomplete block. Incomplete blocks were then randomly assigned to a location within the whole plot irrigation treatment and environment. Randomization of RILs to incomplete blocks within maturity groups was repeated for each replication within irrigation treatment, each irrigation treatment, each environment, and each year to alleviate concerns of confounding to external effects.

Irrigation Treatment Methods

Water to optimally irrigated whole plots was delivered through 7/8" surface drip irrigation tape (Eurodrip Classic, Rivulis Irrigation Ltd., Gvat, Israel) with 15 mm thickness and 60 cm emitter spacing operating at 12 psi for 0.25 gallons per minute per

30.48 meters of tape output. The irrigation tape was placed next to the base of each plant and secured with ground staples to ensure water infiltrated directly to the roots. Water depletion for each whole plot treatment was monitored through granular matrix soil moisture sensors (200SS; Irrrometer, Co., Riverside California, USA) connected to a datalogger (Watermark® 900; Irrrometer, Co., Riverside California, USA) installed at depths of 30 cm, 60 cm, 90 cm and 120 cm below the soil surface in plots representing the mean of the maturity group range. For each maturity group range, the same entry was used across irrigation treatments, environments, and years for later comparisons; entries U16-603042, U16-604072, U16-612276, and U16-612286 with relative maturities of 2.3, 2.6, 3.2, and 3.7 respectively were targeted over years and environments. Water depletion, measured in soil centibars, was recorded every four hours from the V3 development stage onward to maturity.

Along with monitoring water depletion, irrigation timing and quantity was determined through the online irrigation scheduling and growth modeling tool SoyWater (<http://hprcc-agron0.unl.edu/soywater/>) (Specht et al., 2010). Using daily precipitation, solar radiation, temperature, humidity and wind speed information retrieved from weather stations installed directly at the testing location, daily crop water use, water depletion, and irrigation timing was calculated and validated with soil moisture sensors. A water depletion irrigation trigger of 35% from 100% field capacity based on soil type was used throughout the experiment to eliminate any potential water stress. Once the 35% trigger was reached in optimally irrigated treatments, irrigation was applied to bring the treatment back to 95% field water holding capacity.

Environmental Measurements

To aid in the comparison of RIL treatment effects, environmental information relative to soil electrical conductivity and weather station parameters were collected. Soil electrical conductivity (EC) measurements were estimated using a Dualem-1S ground conductivity instrument (Dualem, Milton, Ontario, Canada) and georeferenced with a Trimble ProXT GPS unit (Trimble, Sunnyvale, California, USA) (Abdu et al., 2008; Lv et al., 2014) in methods described in Abdu et al. (2008). Collected EC data was then corrected using normal score transformation and kriging to provide independent measurements with resolution of 3.0 m² for all testing environments. Weather station information including temperature, humidity, wind speed, solar radiation, and precipitation was collected every four hours with two comparable weather stations at both environments; a Vantage Pro (Vantage Pro2 Plus, Davis Instruments, Hayward, California, USA) at the Lincoln environment and a WatchDog (WatchDog 2900ET, Spectrum Technologies, Aurora, Illinois, USA) at the Mead environment. Based on previous knowledge of soil EC's high dependence on soil water holding capacity and soybean developmental influence from environmental parameters, environmental measurements were investigated as random covariates when estimating genotypic values to account for potential confounding environmental effects (Friedman, 2005; Setiyono et al., 2009).

Phenotypic Data Collection

As in the preliminary evaluation experiment, individual plots were phenotyped at the V5 and R5 stage with a multi-sensor high throughput field phenotyping platform developed at the University of Nebraska-Lincoln in methods described in the preliminary

yield analysis experiment. Phenomic information was collected from all plots in the water response experiment during both the V5 and R5 growth stages apart from R5 information at Lincoln, NE in 2017. Phenomic information was not collected during the R5 stage at Lincoln in 2017 due to excessive lodging. To address concerns with missing later developmental stages due to excessive lodging, additional phenotyping events were conducted at the R3 stage in 2018.

In addition to phenomic information collected at key developmental stages, final plant height at maturity, lodging, maturity date, seed yield, seed weight, seed quality, protein composition, oil composition, meal protein concentration, crude oil yield, estimated processed value and estimated WP was collected or estimated for all water response experiment plots in methods described in the preliminary evaluation experiment. Along with these agronomic variables, R1 date, R3 date, R5 date, wilting score, and yield to effective cumulative water during the reproductive period (RWP) was recorded or estimated in water response experiment plots. The R1 date was recorded as the day at which 50% of the plants in the center 1.0 m of both two rows had one emerged flower on any node on the main stem. The R3 date was recorded when 50% of the plants in the center 1.0 m of both rows have one pod on the upper four nodes at least 0.5 cm long. The R5 date was recorded when 50% of the plants in the center 1.0 m of both rows have at least one pod in the upper four nodes with one seed greater than 0.3 cm long. Wilting score was assessed using an iterative 1-5 scale representative of 1 (no wilting, no visual water stress) to 5 (permeant wilting point, plant death). Due to unseasonably high late season precipitation in both 2017 and 2018 wilting scores were only able to be collected on 2018 Mead rainfed treatment plots.

Phenomic Data Processing

After collecting in season phenomic, environmental and agronomic information for both preliminary yield evaluation and water response experiments, phenomic information was processed and correctly matched to other plot records. From easting and northing geographic Cartesian coordinate information recorded for each individual plot measurement in the FPP output, GRASS GIS version 7.2.2 was used to overlay plot range and row information enabling the correct pairing of phenomic records to unique plot record identifiers (Neteler et al., 2012). After pairing phenomic outputs to plot record identifiers, plot output files and digital images collected during each phenotyping event were renamed with the appropriate plot record identifier value to ensure accurate information flow through image processing and analysis.

Once renamed with the appropriate plot record identifier, digital images collected on a per plot basis were analyzed within various toolboxes of MATLAB version r2018b (Mathworks, 2018). First using the Color Thresholder application, a script for converting images to HSV (hue, saturation, and lightness) colorspace and filtering H, S, and V values to effectively separate plant tissue from background soil and crop residue was developed for each phenotyping event. Once an appropriate HSV thresholding script was established, the Image Batch Processor application within MATLAB was used to threshold all images collected during phenotype collection event. After thresholding, custom MATLAB scripts developed in collaboration with Wenan Yuan in the Biological Systems Engineering department at UNL were used to extract 212 variables consisting of color channel values, indices and texture metrics for each RGB image (Yuan et al., 2019)

(Appendix 1). Each variable was then correctly matched to plot records based on record identifier specified in image filenames for later analysis.

Similar to RGB image processing, handling of spectral information was facilitated through MATLAB r2018b. Through a MATLAB script developed with help from Dr. Geng Bai in the Biological Systems Engineering department at UNL, reflectance values were corrected for solar radiance and calibration target ground truths (Appendix 2). Using multiple reflectance calibration measurements for each phenotyping event collected with a MAPIR ground target (MAPIR V2, MAPIR Camera, San Diego, CA) and per plot up looking spectral reflectance information, corrected reflectance values were calculated on a per plot basis. Corrected wavelength reflectance from 500 – 950 nanometers with 0.167 nanometer resolution was then used to calculate 34 unique spectral indices (Table 3, 4). In total 2,739 variables were calculated from the spectral data set.

Genomic Data Collection

Leaf tissue samples collected from a newly emerging trifoliolate of F₄ plants at the V4 growth stage during the summer of 2015 at UNL's east campus research farm (40.836 latitude, -96.667 longitude) served as the means to acquire genotypic information relative to each RIL. Shortly after tissue collection, samples were promptly transferred to a -20°C freezer and stored until DNA was extracted in the spring of 2019. DNA was extracted through the assistance and guidance of Dr. Luis Posadas and Dr. Haichuan Wang using a modified CTAB extraction protocol adapted for a 96 well plate (Keim, 1988). DNA quantity was assessed using a QuantiFlour dsDNA system (Promega Corporation, Madison, Wisconsin, USA), and DNA quality was evaluated through electrophoresis on a

1% agarose gel. After extraction and quality checks, extracted DNA for each RIL was then diluted to a standard concentration of $12 \text{ ng } \mu\text{L}^{-1}$ for genotyping using molecular inversion probes (MIPs).

Molecular inversion probes (MIPs) were used to genotype all RIL and parental lines in the study (Hardenbol et al., 2003; Wang, Y. et al., 2012). Genotyping procedures were coordinated and conducted by Dr. Haichuan Wang. The general procedure of genotyping using MIPs involves six steps: probe phosphorylation, hybridization, extension and ligation, digestion of uncirculized DNA, amplification of DNA by PCR, and DNA sequencing. First to initiation probe phosphorylation, $84 \mu\text{l}$ of pooled MIPs were combined with $10.0 \mu\text{l}$ of 10x T4 DNA ligase bufferA, $5.0 \mu\text{l}$ of T4 polynucleotide Kinase (50U), and $1.0 \mu\text{l}$ of ATP (10 mM) for a total volume of $100 \mu\text{l}$. The mixture was then first heated to 37°C for 30 minutes, then 80°C for 20 minutes for effective phosphorylation. After probe phosphorylation, hybridization was accomplished by mixing 5 ng of the phosphorylated probes with 40 ng of standardized DNA, 10x ampligase buffer (Ampligase Thermostable DNA Ligase, Epicentre Biotechnologies, Madison, Wisconsin, USA), $0.5 \mu\text{l}$ of betaine (5M) and nuclease free water to total $6.25 \mu\text{l}$. The resulting mixture was then first heated to 95°C for 10 min, then 60°C for 24 hours. Next for extension and ligation, the hybridized solution was combined with 25 units of DNA ampligase (Ampligase Thermostable DNA Ligase, Epicentre Biotechnologies, Wisconsin, USA), 10x ampligase buffer (Ampligase Thermostable DNA Ligase, Epicentre Biotechnologies, Madison, Wisconsin, USA), 5 units of AmpliTaqDNA polymerase (Epicentre Biotechnologies, Madison, Wisconsin, USA), 0.25 mM of dNTP , and denatured water for a total volume of $7.7 \mu\text{l}$; the resulting mixture was heated at 60°C

for 24 hours for extension and ligation. Following the extension and ligation steps, uncirculized DNA was removed through adding 0.32 μl of ExoI (20U/ μl) and ExoIII (100U/ μl) exonuclease enzymes (New England Biolabs Inc., Ipswich, Massachusetts, USA) and heating at 37°C for 30 minutes then 95°C for 2 minutes. Next circulized DNA was amplified by PCR using custom primers and barcodes through 21 cycles of denaturation, annealing and extension at 95°C for 10 seconds, 60°C for 30 seconds, 72°C for 1 minute, and 72°C for 1 minute respectively using a Bio-Rad C1000 thermo cycler (C1000 Touch Thermal Cycler, Bio-Rad Laboratories, Inc., Hercules, California). Finally, amplified DNA was pooled together and diluted to a 1.5 pM standard pooled library concentration. Single-end sequencing was performed through an Illumina Next Seq 500 sequencer using custom sequence primers, 150 nucleotide single reads, a (500/550) buffer cartridge, and a NextSeq 500/550 medium output reagent cartridge (Next Seq 500, Illumina, San Diego, California, USA).

Genotypic Data Processing and QTL Mapping

Sequencing data outputted from the Illumina Next Seq 500 was first converted to the FastQ format and checked for quality using FastQC and sickle by Dr. Haichun Wang (Andrews, 2010; Joshi and Fass, 2011). Sequencing reads with phred scores greater than 30 were selected and mapped using a SNP specific reference database within Bowtie2 to produce sequence alignment map files and binary alignment map files (Langmead and Salzberg, 2012). Single nucleotide polymorphism (SNP) reads were then called using the Genome analysis tool kit (GATK version 4.1) through HaplotypeCaller and VariantFiltration commands (McKenna et al., 2010). SNPs with over eight heterozygous reads were called as heterozygous within GATK using default settings (McKenna et al.,

2010). Resulting variant call format files were then combined through the `vcf-merge` command of GATK and exported. Genotypic data in vcf file format was then converted to “A”, “H”, “B” parental base format in TASSEL (TASSEL 5 version 20190725) and processed for quality control in the “qtl” package of R (Broman et al., 2003; Glaubitz et al., 2014; R-Core Team, 2018). Lines with 10% or greater missing markers, markers unable to genotype 60% or more of the lines in the population, one line of a pair more than 98% similar, and markers with excessive segregation distortion ($p > 0.001$) from the expected 1:1 Mendelian inheritance ratio after excluding heterozygotes were omitted from the dataset (Appendix 3). The vcf hapmap file was also reduced using t-distributed stochastic neighbor embedding to examine population structure and identify possible outliers of each population (Figure 3) (Li, Wentian et al., 2017). After filtering for the previously mentioned conditions, pairwise recombination fraction versus LOD scores, and allelic frequencies were used to assess the quality of genotypic data and check for switched alleles within the “qtl” package of R (Appendix 3).

For additional quality control measures, genetic maps constructed based on marker recombination fractions versus genetic maps constructed through reference genome position interpolation were compared (Figure 4, 5). Genetic maps based on marker recombination fraction were constructed through the `orderMarkers` function within the “qtl” package of R using Haldane’s mapping function and a window size of eight. Interpolated genetic positions from the Wm82.a2.v2 reference genome were provided by Mary Happ to assign genetic position to markers (Figure 6, 7). In the UX3036 population, hilum color was also recorded and used to interpolate pubescence color. Interpolated pubescence color was then compared to the expected segregation ratio

and tested through a chi-square analysis (Figure 8). The genetic position of the T locus responsible for pubescence color was also mapped to investigate the accuracy of interpolated genetic positions (Figure 8, 9) (Palmer et al., 2004).

Interpolated genetic positions were then used to map QTL. Phenotypic values of quantitative traits, evaluated as least square mean estimates over environments, were mapped within the IciMapping integrated software for linkage analysis and genetic mapping in biparental populations (Li, Huihui et al., 2008; Li, H. et al., 2007; Meng et al., 2015). Inclusive composite interval mapping (ICIM) with additive effect mapping methods and RIL population structure were set as defaults for all QTL mapping analysis (Li, Huihui et al., 2008; Li, H. et al., 2007; Meng et al., 2015). Heterozygous markers were considered as missing through the ICIM mapping software, and logarithm of the odds (LOD) significance within the program was determined through 1,000 permutations and an $\alpha = 0.05$ significance threshold (Li, Huihui et al., 2008; Li, H. et al., 2007).

Data Analysis

After phenotypic data was collected from the experiment, the dataset was first investigated for outliers using the PROC UNIVARIATE procedure of SAS 9.3 (SAS Institute Inc, 2011). Plot observations more than three standard deviations away from the mean were investigated. If no other observation or field note seemed to be in agreement with the outlier, the observation was removed from the dataset. Along with outliers, plots with field notes describing damage from outside sources such as sprayer track damage or poor emergence were omitted from the analysis. The final 2017 – 2018 phenotypic dataset contained approximately 18,000 observations and roughly 0.1% of the original observation were omitted due to the previously mentioned conditions.

The analysis of variance to investigate overall factor significance for preliminary yield evaluation and water response experiments was performed using the PROC ANOVA procedure of SAS 9.3. With one replication per environment, environment and line effect were investigated in the 2016 preliminary yield evaluation experiment. Environment, irrigation treatment, line effect and their respective interactions along with replication within environment and irrigation treatment by replication within environment effects were examined for the 2017-2018 water response experiment. When calculating f-value ratios, replication within environment was used as an error term for the estimation of environmental effect's significance, irrigation treatment by replication within environment was used as an error term to estimate f-values for irrigation treatment and irrigation treatment by environment effect, and the residual error was used to estimate significance for all other effects.

The analysis of variance to obtain least square mean estimates of phenotypes on a RIL basis was performed using the PROC MIXED procedure of SAS 9.3. To determine the model most appropriate for estimating genotypic values, six model's Akaike information criterion (AIC) were compared using various environmental variables as random covariates for each population. The model with the lowest AIC treats environment, irrigation treatment and line as fixed effects, and individual plot maturity date within environment, replication within environment, incomplete block within replication by environment as random effects. With unequal variation expected between whole plot and subplot factors, degrees of freedom were approximated with Satterthwaite's formula, and least square mean estimations are calculated through the LSMEANS statement.

Broad sense heritability estimations on an entry mean basis were calculated through the following formula:

$$H = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{e} + \frac{\sigma_E^2}{re}}$$

Where σ_G^2 represents genetic variance, σ_{GE}^2 denotes genotypic by environmental variance, σ_E^2 represents environmental variance, e signifies the number of environments, and r is the number of replications (Fehr, Walter R., 1987). Estimates of variance and confidence intervals were calculated through a restricted maximum likelihood approach within the “lme4” package of R (Bates et al., 2015).

Predictive Modeling

Predictive models of RIL water response were built within MATLAB r2018b using scripts processed remotely at the Holland Computing Center of the University of Nebraska-Lincoln (Mathworks, 2018). To determine if parametric or nonparametric models would be most appropriate, the normality and multicollinearity of response variables in the dataset were investigated with the Lilliefors and collintest procedure within MATLAB r2018b. Due to the large portion of response variables failing to have a normal distribution and high correlation to other response variables, nonparametric multivariate analysis methods were explored. Three nonparametric statistical learning methods were used in the experiment: ensembled classification and regression trees with bootstrap aggregation and random predictor selection at each split (CART), feed forward artificial neural networks with Bayesian regulation (NET) and generalized linear regression with elastic net regulation (ENET). All three methods are especially adaptable

to non-linear datasets, datasets with excessive multicollinearity, and datasets where the number of response variables approaches or exceeds the number of observations ($p > n$) (Hastie et al., 2005; James, G. et al., 2013). Due to the suitability of the CART, NET and ENET models to datasets associated with this research, and the inability of more traditional dimension reduction techniques such as stepwise linear regression to converge, CART, NET and ENET models were solely used for predictive modeling in this study.

Classification and Regression Trees

Predictive CART models were built for datasets associated with the experiment using the `fitensemble` command of MATLAB r2018b. Ten-fold cross validation (CV), a minimum leaf size of five, 500 learning cycles, and bootstrap aggregation were used as input settings for the `fitensemble` command (Appendix 4). The CART algorithm with the above settings can be generalized as follows:

1. Randomly select one-third the number of samples using bootstrap aggregation (uniformly and with replacement) from the first nine of the ten CV datasets (Breiman, 1996).
2. Construct a regression tree with each split being chosen from the best split of a random sample of the predictors (random forest) limiting the minimum leaf size to five observations and limiting the number of ensembled learning cycles to 500 to avoid overfitting. The best split is selected based from minimizing the overall MSE of the model (Breiman, 1996; Breiman, 2001). For simplicity the split location between subsets C and D would be determined through minimizing the equation below:

$$\sum (y_i - \bar{y}_C)^2 + \sum (y_i - \bar{y}_D)^2$$

Where y_i represents response value i , and \bar{y}_C represents the sample mean of all response values in the clustered subset C and \bar{y}_D represents the sample mean of all response variables in the D subset (Sutton, 2005).

3. Aggregate the composite regression tree from nine CV fold average and apply to the predict the tenth CV fold dataset.
4. Assess performance based on the Pearson correlation coefficient between the observed and predictive response variable of interest
5. Repeat steps one through four for each of the ten CV datasets without replacement.

The performance of the final model was assessed based on the Pearson correlation coefficient between the observed and predictive response variable of interest, 95% confidence interval calculated for the Pearson correlation of each CV, mean absolute error (MAE), and the root mean square error (RMSE) between the observed and predicted response.

Artificial Feed Forward Neural Networks

Predictive NET models were built for datasets associated with the experiment using the `feedforwardnet` command of MATLAB r2018b. A 70% training, 15% validation, and 15% testing ratio was used to train the feed forward neural network with 1 hidden node and Bayesian regularization (Appendix 5). The NET algorithm with the above settings can be generalized as follows:

1. Randomly partition the dataset of interest into training, validation and testing sets using a 70/15/15 ratio respectively.
2. Train neural network using Levenberg-Marquardt optimization algorithm minimizing a linear combination of squared errors and weights (Foresee and Hagan, 1997; MacKay, 1992; Mathworks, 2018) . The neural network with the aforementioned optimization algorithm can be summarized in the following equation:

$$f(x, w) = \emptyset(x \cdot w) = \emptyset\left(\sum_{i=1}^p (x_i \cdot w_i)\right)$$

Where x and w denote the input vector and corresponding weights respectively, and \emptyset signifies the activation function (Schalkoff, 1997).

3. Validate the trained network on the validation set and calculate MSE.
4. Adjust Marquardt adjust parameter and repeat steps 1 through 3.
Terminate the algorithm if MSE increases from previous iteration or if the number of iterations exceeds 1,000.
5. Calculate performance by inputting testing dataset into final trained model.

As in the CART model, the performance of the final NET model was assessed based on the Pearson correlation coefficient between the observed and predictive response variable of interest, 95% confidence interval calculated for the Pearson correlation of each CV, mean absolute error (MAE), and the root mean square error (RMSE) between the observed and predicted response.

Generalized Linear Models with Elastic Net Regulation

Predictive ENET models were built for datasets associated with the experiment using the lasso command of MATLAB r2018b. Ten-fold CV and an alpha of 0.75 were used as input settings for the lasso command (Appendix 6). The ENET algorithm with the above settings can be simplified as follows:

1. Perform generalized linear regression from input data with a tuning parameter, λ minimizing the following problem:

$$\beta_0 \beta \left(\frac{1}{2n} \sum_{i=1}^n (y_i - \beta_0 - x_i' \beta)^2 + \lambda P_\alpha(\beta) \right)$$

Where n is the number of observations, y_i is the response at observation i , x_i is data, a vector of length p at observation i , λ is a nonnegative regularization parameter corresponding to one value of lambda and the parameters β_0 and β are a scalar and a vector of length p , respectively, and P_α is the elastic net penalty term.

2. Calculate MSE of trained regression model
3. Modify lambda and repeat steps one and two until MSE is minimized or 1,000 iterations have been performed.
4. Identify lambda value corresponding to model with MSE value one standard error above minimum MSE model and output as final model.
5. Quantity performance based on the Pearson correlation coefficient between the observed and predictive response variable of interest.

6. Repeat steps one through five for each of the ten CV datasets without replacement. Calculate final model by average variable coefficients over the ten CV folds.

RESULTS AND DISCUSSION

Agronomic Response to Irrigation

To evaluate the agronomic response to water of both the recombinant inbred line (RIL) populations and parental lines in the study, experimental units were subjected to yield evaluation trials over two locations with two irrigation treatments for two years. As expected, population means for most agronomic traits were normally distributed, with least square means close to mid-parent values (Table 5, Figure 10). The magnitude and direction of agronomic responses remained similar across both populations in the study yet remained marginal due to well-timed precipitation events reducing the stress imposed in the rainfed treatment (Table 6, Figure 11, 12, 13, 14, 15, 16, 17, 18). The timing of precipitation in relationship to developmental stage resulted in varying levels of irrigation response across environments and years. Precipitation events near the overall mean R3 reproductive stage occurred for all environments negating water stress during the most critical stage of development (Figure 12, 14, 16, 18). In both the 2017 Lincoln and 2018 Mead environments, rainfall during the R3 stage was followed by a dry period resulting in an overall significant irrigation treatment effects for yield and water response traits (Table 6). In contrast, the 2018 Lincoln and 2017 Mead environments received large rainfall events again near the R5 stage reducing the overall effect of water stress on yield (Table 6, 7, 8).

When investigating both maturity groupings and populations separately within environments, the apparent connection between precipitation timing and magnitude of water stress becomes less clear. Over populations significant irrigation treatment effects within maturity groups do not follow a clear pattern (Table 9). Within the UX3000

population, significant ($\alpha = 0.05$) yield irrigation treatment effects were observed for all maturity groupings within the 2017 Mead environment, all maturity groupings except 3.5 in the 2018 Mead environment, the 3.0 maturity group (MG) in the 2017 Lincoln environment, and no groups within the 2018 Lincoln environment (Table 10). For the UX3036 population, significant ($\alpha = 0.05$) irrigation treatment effects for yield were observed for MG 2.5 in the 2017 Mead environment, MG 3.0 in 2018 Mead and 2017 Lincoln environments, and no MGs in the 2018 Lincoln environment (Table 11).

A possible explanation for the disconnect between MG timing and irrigation yield response can be gleaned from examining mean reproductive period attributes in comparison to reproductive period ranges (Table 9, 12). Between maturity groupings assigned in the experiment, R1, R3, R5, and maturity timings differed approximately 1, 1, 1, and 4 days respectively over environments and populations (Table 9). However, ranges of reproductive stage timings differed approximately 7, 5, 5, and 13 days for R1, R3, R5, and maturity timings respectively over environments, populations and maturity groupings (Table 12, 13). Within each MG, RILs likely experienced water stress differently depending on individual rates of development in relation to precipitation timing. For example, the mean timing difference of R1 date between maturity groupings was approximately 1 day, yet the range within each maturity grouping was 7 days (Table 13). This relatively large reproductive timing range taken in consideration with marginal treatment effects supports aggregating maturity groupings together. With similar population responses and marginal treatment effects influenced by the timing of precipitation, investigating agronomic means across populations and maturity groupings

allows for more robust estimations of treatment effects within environments due to increased sample size.

When investigating analysis of variance (ANOVA) mean squares across populations and maturity groups, significant ($\alpha = 0.05$) sources of variation were observed for nearly all agronomic traits (Table 14). The only exceptions include the significance of the irrigation treatment effect on R1 timing and final plant height at maturity, the significance of the environmental by irrigation treatment effect on R1 timing, final plant height at maturity, seed quality, and lodging, and the significance of the irrigation treatment by strain effect on seed quality (Table 14). By population similar results were observed, yet a larger number of non-significant sources of variation were identified (Table 15, 16). In agreement with the trend reported earlier, investigating agronomic means across populations and maturity groupings allows for more robust estimations of treatment effects within environments due to increased sample size.

Across both populations, mean seed yield over environments and irrigation treatments ranged from 4148 kg ha⁻¹ in the 2018 Lincoln rainfed treatment to 5307 kg ha⁻¹ in the 2017 Mead rainfed treatment (Table 6). Significant ($\alpha = 0.01$) irrigation treatments in relation to seed yield were observed for two of the four environments tested (Table 6). The effect of the irrigation on seed yield ranged from 307 kg ha⁻¹ in the 2018 Mead environment within the UX3000 populations to -37.2 kg ha⁻¹ in the 2017 Mead environment within the UX3000 population (Table 13). Taken as a whole, irrigation was observed to significantly increase seed yield and weight over rainfed treatments on average approximately 126 kg ha⁻¹ and 0.3 grams per 100 seeds respectively within the

UX3000 population and 119 kg ha⁻¹ and 0.2 grams per 100 seeds respectively within the UX3036 population (Table 13).

Like seed yield and weight, the seed quality characteristics relative to seed coat quality, protein concentration, oil concentration, estimated processed value, and estimated meal product protein were all found to be significantly influenced by irrigation treatment within the experiment (Table 13). In both the UX3000 and UX3036 populations, seed coat quality and seed oil concentrations decreased with increased water availability. However, seed protein concentration, estimated processed value, and estimated meal product protein concentration were observed to be positively influenced by the irrigation treatment (Table 13). Regardless of population, optimally supplying water at key growth stages to the RIL and parental lines was observed to increase seed protein concentration by roughly 6 g kg⁻¹ and decrease seed oil concentration by 2 g kg⁻¹ resulting in slightly higher estimated processed values (+\$0.1 \$ kg⁻¹) and estimated meal product protein concentrations (+5 g kg⁻¹) (Table 13).

Unlike seed yield and quality characteristics, whole plot agronomic variables representing developmental stages and traits at maturity were not all significantly ($\alpha = 0.05$) influenced by irrigation across the experiment. Slight differences in R1, R3 and R5 date were noted for specific environments and population combinations, but overall irrigation treatment did not significantly influence flowering date or pod elongation date timing in the experiment (Table 9, 10, 11). Detectable differences in reproductive stage development timing was only evident when investigating maturity date and the length of the reproductive period (Table 9, 10, 11). Greatest differences in reproductive timing were observed when considering the length of the entire reproductive period (R1-R8) for

each plot measured in days. Over populations in the rainfed treatment, the reproductive period averaged 2 days less as a result of the rainfed treatment (Table 13). Along with reproductive length, lodging measured at maturity was found to significantly worsen with an increased effective seasonal water supply. On average lodging severity increased by 0.4 in the 1-5 scale through the application of irrigation compared to the rainfed treatment (Table 13).

Much like the sensitivity of lodging to irrigation, the ratio of seed yield to estimated seasonal effective cumulative water, or water productivity (WP), and the ratio of seed yield to estimated effective cumulative water during the reproductive period, or reproductive water productivity (RWP), were observed to have significant differences across treatments in all environments associated with the study. Environmental estimates for WP ranged from $12.4 \text{ kg ha}^{-1} \text{ mm}^{-1}$ under irrigation at the 2018 Mead site to $20.6 \text{ kg ha}^{-1} \text{ mm}^{-1}$ in the rainfed 2017 Lincoln environment (Table 6). When considering RWP, differences between environmental estimates were even larger ranging from $14.0 \text{ kg ha}^{-1} \text{ mm}^{-1}$ under irrigation at the 2018 Mead environment to $27.2 \text{ kg ha}^{-1} \text{ mm}^{-1}$ in the rainfed 2017 Lincoln location (Table 6). Population treatment effect averages indicated a decrease of approximately $3.0 \text{ kg ha}^{-1} \text{ mm}^{-1}$ and $5.5 \text{ kg ha}^{-1} \text{ mm}^{-1}$ to WP and RWP respectively, when supplied with water equal to evapotranspiration demands (Table 13).

Agronomic responses to irrigation identified in the study agree with similar research investigating soybean's response to limited water. Previous studies coincide with this experiment's outcomes in that water stress reduces seed yield, days to maturity, lodging severity, seed size, and seed protein concentration while increasing seed coat quality and seed oil concentration (Dornbos and Mullen, 1992; Korte et al., 1983; Specht

et al., 1986; Specht et al., 2001). With detectable agronomic responses to irrigation in agreement with previous research, the primary focus of the study is to investigate associations leverageable to soybean breeding programs.

Through the consideration of both yield and effective seasonal water, water productivity metrics are especially valuable in our study where irrigation treatments were marginal and influenced by development. Because both developmental timing and environmental factors are acknowledged in the calculation of effective seasonal water, water productivity metrics enable breeders to assess genotypic performance more precisely than focusing on yield between treatments in our situation. As the amount of seasonal effective water is altered through both irrigation treatments and environmental constraints, water productivity changes through yield performance, water supply, and estimated transpiration demands. The effectiveness of utilizing WP in comparison to yield between treatments is evident when comparing significance between irrigation treatments within and across environments, populations, and maturity groups within the study (Table 6). Due to this reasoning, WP is the primary performance metric of interest when identifying trait associations to water response in our study.

Agronomic Associations to Water Productivity

When determining the value of individual trait associations, the merit of correlated response in relation to direct selection of the trait of interest is of high value to plant breeding programs. The efficiency of indirect selection between two traits, trait X and Y, can be represented through the following formula:

$$\frac{CR_X}{R_X} = \frac{r_A h_Y}{h_X}$$

Where CR_X is equal to the correlated response of trait X , R_X is the direct response of trait X , h represents the accuracy of individual selection, and r_A denotes the additive genetic correlation between traits (Falconer and Mackay, 2009). Because phenotypic correlation encompasses both genetic and nongenetic factors, traits leverageable for indirect selection must exhibit significant phenotypic and genotypic correlations along with relatively high heritability. Therefore, when examining trait associations to metrics of interest, attributes with strong association useful to cultivar development institutions exhibit significant phenotypic correlation, genotypic correlation, and relatively high heritability (Fehr, 1991).

For the agronomic traits measured in the study, significant pairwise phenotypic Pearson correlation coefficients were found for nearly all trait combinations in both irrigated and rainfed treatments over populations (Table 17). In the irrigated treatment, WP was observed to be significantly correlated to agronomic traits related to days between planting and specific reproductive development stage timing with no association to traits pertaining to seed quality and oil composition (Table 17, 18, 19). In contrast, seed quality and oil composition traits displayed significant correlation to WP in the rainfed treatment with reproductive timing metrics showing slightly larger correlations to WP than in the irrigated treatment (Table 17). When further investigating the influence of reproductive stage intervals over populations, a similar trend is observed (Table 20). Increase pod elongation and seed setting intervals (R3-R5) was noted to have large positive correlations to WP, RWP, seed weight, seed protein, and estimated processed value regardless of irrigation treatment (Table 20). In contrast, longer flowering (R1-R3) or seed filling and maturity intervals (R5-R8) were observed to have negative correlations

to WP, RWP, and seed weight in both irrigation treatments (Table 20). By population, phenotypic correlations were consistent with overall trends (Table 17, 18, 19). Both populations were observed to have no significant correlation between seed quality and seed oil concentration to WP in the irrigated treatment, yet significant correlations were observed in the rainfed treatment (Table 18, 19).

With contrasting phenotypic associations observed between treatments, confusion can arise as to which treatment or combination should be emphasized to improve water productivity. When making advancement decisions, should the breeder place emphasis on performance in the irrigated treatment or the rainfed treatment? The primary objective of any cultivar development program is to deploy products with improved mean performance over a target region for specific traits of interest. Determining what environment or set of environments to use for evaluation of material therefore demands consideration of both the target production region and the effectiveness of improving mean performance over time. Ratios of genetic variance and genetic correlation between contrasting environments have been proposed as one way to gauge the effectiveness of selection between or over divergent treatments (Rosielle and Hamblin, 1981). Through the ratio of total genetic variance within the rainfed environment ($\sigma_G^2_2$) to the total genetic variance within the irrigated environment ($\sigma_G^2_1$), an approximation of the relative effectiveness in selecting between environments (K^2_G) can be estimated. Ratios larger than 1.0 indicate improved efficiency in selecting in stressed environments, whereas ratios less than 1.0 support greater selection efficiency in irrigated environments (Rosielle and Hamblin, 1981).

Across populations, K^2_G values significantly ($\alpha = 0.05$) different than 1.0 were observed for estimated processed value (EPV), meal product protein (MPP), reproductive water productivity (RWP), and R3 stage timing (Table 21). Within populations, the R3 stage timing and lodging score at maturity traits showed significant preference for selection in the irrigated treatment within the UX3000 populations, and selection for RWP was estimated to be superior within the rainfed treatment within the UX3036 population (Table 22, 23). With significantly greater RWP genetic variance calculated within the rainfed treatment, improving RWP within the UX3036 is estimated to be most efficient through evaluated performance in rainfed environments (Table 23). However, unlike RWP in the UX3036 population, estimated K^2_G for RWP in the UX3000 population was not significantly different than 1.0 (Table 22).

In agreement with trends of genetic variance across populations, genotypic by environmental interaction variance components differed significantly ($\alpha = 0.05$) between irrigation and rainfed treatments for meal product protein (MPP), reproductive water productivity (RWP), and R3 stage timing (Table 24). In contrast, unlike relationships reported when comparing genetic variance components, significant differences were identified when comparing genotypic by environmental interaction variance components for seed weight, seed quality, and water productivity (Table 24). No significant differences were reported for environmental variance component estimations in our study (Table 23).

As no clear advantage arises for WP selection in irrigated versus rainfed environments, focusing on the mean WP over contrasting treatments emerges as the most sensible evaluation method in our study. With large and positive correlations estimated

between least square means for irrigated and rainfed environments (r_{12}), and increased broad sense heritability estimates (Table 21, 22, 23, 25), selection for mean WP can be expected to result in gains in both water treatment regimes. Selection for mean WP over irrigation treatments will likely also increase stability of the trait over periods of selection in comparison to focusing on a single treatment (Hohls, 2001). In a study with marginal irrigation treatment effects influenced by the individual reproductive stage timing of lines within environments, focusing on mean performance allows for increased power in detecting true differences between RILs as a result of increased sample size. Finally, through considering the mean WP across treatments of primary importance, the inference space of the experiment is expanded to include both rainfed and irrigation production environments. In soybean production states like Nebraska, this is especially important given approximately half of soybean acres are irrigated and half are rainfed (UNL Cropwatch, 2018).

When investigating WP across treatments as primary importance, the negative correlation between reproductive stage timing traits and WP quickly becomes noticeable (Figure 19, 20, 21). Lines with earlier reproductive and maturity dates show strong association with improved WP across both populations and treatments (Figure 19) Because WP is calculated through the ratio of seed yield to the estimated seasonal effective cumulative water, RILs with similar seed yield but different growing season lengths will have dissimilar WP levels. Lines with increased maturity have an estimated higher effective cumulative water requirement as a longer period is required to maintain the water intensive tasks of growth and development. In contrast to conventional wisdom, this increased cumulative water requirement resulting from an extended growing season

does not immediately necessitate an increase in yield. Results from this study and others suggest little to no positive relationship between maturity date and final seed yield over MG 1.0 – 4.0 lines in Nebraska production environments (Table 10) (Posadas et al., 2014). With no strong relationship between yield and maturity date, removing maturity date's inherent influence on WP allows for identification of traits specifically critical to water use efficiency or ability to extract water irrespective of growing season length. Given the intrinsic implications of maturity to the definition of WP, and the importance of identifying superior yielding lines across a range of maturity groups to soybean breeding programs, the influence of maturity date was removed when estimating least square means estimates (LSMEANS) for genotypic values.

After removing the influence of maturity date on WP, a near identical relationship between WP and seed yield becomes obvious (Figure 19, 20, 21). With an overall Pearson correlation coefficient of 1.0, seed yield and WP LSMEANS largely coincided. However as discussed earlier, because both effective seasonal water and seed yield are considered in WP, water productivity metrics enable breeders to quickly approximate genotypic performance across different irrigation treatment management practices. Adjusted LSMEANS of seed yield and WP offer near identical assessments of performance potential across environments, but WP offers the advantage of performance approximation within environments given estimated seasonal effective cumulative water input. Due to this reasoning, WP LSMEANS continue to be the primary performance metrics of interest when identifying associations to water response in our study.

When investigating WP LSMEANS to other agronomic traits a strong association with lodging becomes apparent (Figure 22, 23, 24). Lodging has a strong negative

association with WP, with Pearson correlation coefficients ranging from -0.56 in the UX3036 population to -0.49 in the UX3000 population when considering LSMEANS adjusted for maturity date. Relationships between WP LSMEANS and agronomic traits studied through comparison of positive and negative transgressive segregate means within each population likewise indicated a similar relationship with lodging. Transgressive segregant groupings were determined through comparison of WP LSMEANS over irrigation treatments and environments for the 2017-2018 water response experiment to parental values. Positive transgressive segregate RILs with WP LSMEANS greater than U11-614093 in the UX3000 population had significantly reduced lodging, plant height, R1 and R3 stage timing, seed protein composition, estimated processed value, and estimation meal product protein when compared to negative transgressive segregates in the population (Table 26). Seed yield, seed weight, and RWP were significantly higher in the UX3000 population when comparing transgressive segregates (Table 26). In the UX3036 population, lodging, seed protein composition, estimated processed value, and estimation meal product protein continued to be negatively associated whereas seed yield, and RWP were significantly positively associated with increased WP (Table 26).

The strong negative association of WP and lodging is likely due to multiple factors in the study. First, significant differences occur in the susceptibility to lodging of parental lines used; the parental line with the highest WP, U11-614093, also has significantly ($\alpha = 0.01$) lower lodging severity on average when compared to the other parental lines (Table 5). In addition, lodging has been shown to reduce seed yield through the inability of mechanical harvest and through the reduction of photosynthate supply due to changes in canopy structure and light use efficiency (Johnston and Pendleton, 1968;

Johnston et al., 1969; Weber and Fehr, 1966). Because seed yield is a primary component of WP, yield reductions due to lodging likewise limit WP. With a strong association between WP and lodging detected in the experiment, the standard procedure of culling lodged genotypes in early generation evaluations likely likewise improves WP. Indirect selection efficiency of WP from lodging was estimated to be 0.59; moderate gains in overall WP may be expected through culling lodge genotypes.

Phenomic Associations to Water Productivity

Even with informative associations identified between agronomic traits and WP, much of the highly quantitative relationship between seed yield and seasonal effective water is still in question. Due to the dynamic and temporal nature of water stress, collecting phenotypic information at key growth and developmental stages offers an opportunity to uncover associations potentially undetectable at maturity. Furthermore, collecting high dimensional phenotypic data at key growth stages allows for the opportunity to discover specific associations within and across developmental periods. In hopes to better understand water productivity in soybean, a multi-sensor high throughput field phenotyping platform was used to collect phenomic information at V5 and R5 stages in 2017 and V5, R3, and R5 stages in 2018. The platform is equipped with a suite of sensors that capture growth and development phenotypes through digital images, spectrometer, light distance and ranging (LIDAR), ultrasonic, and radiometric datasets (Bai et al., 2016). These datasets can be grouped into three categories: (1) information relative to a red, green and blue channel digital image (RGB), (2) the spectral reflectance spectrum, and (3) whole canopy related phenotypic traits.

Stark differences between the phenomic categories, growth stage, and population were observed when examining relationships to WP across irrigation treatments in the experiment (Table 27, 28). In relationship to information gleaned from the RGB digital images, significant differences ($\alpha = 0.05$) were observed when comparing phenotypic correlations to WP and broad sense heritability across populations and growth stages (Table 27, 28). Positive correlations for red and green color channels were exhibited in the UX3036 population at the V5 growth stage, yet no significant correlations were detectable for other growth stages within the population (Table 27). In addition, no significant correlations were identified for either the UX3000 population or when considering all lines together in the experiment (Overall) (Table 27). Like phenotypic correlations, increased heritability estimations were calculated for earlier growth stages. Broad sense heritability was estimated to be approximately 0.25 across channels at the V5 growth stage with no significant differences between channels or populations (Table 28, Figure 25). Heritability estimations were slightly larger at the R3 growth stage when investigating individual channels although no significant differences between channels, populations, or between the V5 and R3 stages were observed (Table 28, Figure 25). Surprisingly red, green and blue color channels seem to offer no apparent value in accessing WP at the R5 growth stage as both the heritability estimation and phenotypic correlation is zero (Table 27, 28, Figure 25).

A possible explanation for the reduction in association of red, green, and blue color channels with increasing growth may deal with properties of the image in relation to the soybean canopy. At earlier growth stages such as V5 and R3, the soybean plant has yet to reach full closed canopy between rows. A digital image of the plot will therefore

assess both the color attributes of a plot in addition to the canopy cover fraction. Plots with slower development will return lower red, green, and blue pixel totals as pixels containing the soil background are removed before processing. In contrast, at the R5 growth stage plots have reached full canopy, and growth and expansion rates discernible in earlier growth stages are no longer able to be distinguished. When comparing phenomic means of color channels between parents, significant differences occur at the V5 growth stage, yet no differences are detectable at the R5 growth stage (Table 29).

To better understand the relationship of image background removal, or image thresholding, with the growth and development of the lines, whole canopy phenotypes should be examined. Through the computation of thresholded pixel area (Area), the canopy cover fraction for each plot can be quantified. When examining this metric, associations to WP become evident at the V5 growth stage. Pearson correlation coefficients range from 0.45 in the UX3036 population to 0.22 in the UX3000 with significant difference apparent between parental lines in both populations (Table 27, 29). Strengthening the association of pixel area to WP at the V5 stage, significant broad sense heritability values are calculated for both populations and when considering both populations together (Table 28). Like the associations of red, green, and blue channels to WP at later growth stages, thresholded pixel area offers little to no value once plots have reached full canopy between rows. Insignificant phenotypic correlations and heritability estimations were calculated for thresholded pixel area at both the R3 and R5 growth stage (Table 28).

In close agreement with the trends observed in the thresholded pixel area phenotype, the canopy to air temperature differential (CATD) trait supports the

relationship of growth and development to WP. Assumed to be largely influenced by the growth and expansion of plots at the V5 growth stage, CATD was shown to have a strong association to WP before the closed canopy with high broad sense heritability estimates (Table 27, 28). Expecting to result from the 14° field of view on the radiometer, positive CATD values are likely resulting from a combined temperature reading of both the soil background and plot vegetative tissue. Plots with greater canopy to soil fraction ratios at the V5 growth stage would be anticipated to cover a larger portion of the soil background, and therefore return a lower temperature reading when phenotyped. Reinforcing this association of phenotypes quantifying growth and development to WP, canopy height measured through the average output of LIDAR and ultrasonic sensors was observed to be significantly correlated to WP especially during the R5 growth stage (Table 27). In the UX3036 population, canopy height was estimated to have a correlation coefficient of 0.46 to WP at R5 (Table 27). With over 2.0 cm difference on average between parental lines in the UX3036 population at the R5 growth stage, and a heritability estimation of 0.69, canopy height appears to be a trait that is repeatable and controlled by genetics in the population (Table 28, 29).

With encouraging associations identified between phenotypes quantifying growth and development, and their likely impact on phenotypes pertaining to the digital image, the association of reflectance phenotypes to WP is still in question. To address some of confounding effects of canopy fraction, the reflectance spectrum can be investigated. Because no thresholding is performed when processing the spectrometer data, wavelength reflectance values represent the average reflectance including soil. When considering the total spectral reflectance of the plot, information gained in the visible

light range is limited. Phenotypic correlations of the summation of visible light reflectance is non-significant for every growth stage phenotyped in the experiment (Table 27). In addition, the summation of visible light reflectance is largely controlled by external environmental factors as broad sense heritability of the visible light range is marginal at all growth stages (Table 28).

In contrast to visible light reflectance, the summation of reflectance in the near infrared region (NIR) shows promising association to WP. Significant associations were observed in every growth stage when considering the summation of NIR reflectance with increased correlation and heritability during later growth stages (Table 27, 28). When considering the full spectrum, the usefulness of visible light phenotypes to NIR metrics quickly becomes evident (Figure 26, 27). Through the reflectance spectra comparison of lines with WP in the lower quantile to lines with WP in the top quantile, clear differences are only discernable in the NIR region (Figure 26, 27). Especially during the R5 growth stage, NIR reflectance wavelengths in the 750 nm to 800 nm range displays clear and significant ($\alpha = 0.05$) separation of the WP groups (Figure 27). Increasing the value of NIR reflectance over visible light reflectance, broad sense heritability estimations are relatively high especially in the 750 nm to 800 nm range (Figure 26, 27).

Increasing the utility of NIR wavelengths, reflectance ratios demonstrate increased association to WP when compared to either visible light or NIR reflectance individually. Compared to the summation of NIR reflectance, the ratio of NIR to visible light offers increased Pearson correlation coefficients for every stage and population investigated with significantly higher broad sense heritabilities in most situations (Table 26, 27). Furthering this trend, specific spectral indices comparing NIR wavelengths to

visible light wavelengths exhibit strong association to WP especially during later growth stages. Spectral indices such as the simple ratio index of 800 nm over 680 nm (SR680) were observed to have correlation coefficients of 0.39 and broad sense heritability estimations on an entry basis of 0.62 at the R5 growth stage over all lines evaluated in the experiment (Table 30). In addition to SR680, several spectral indices were observed to have broad sense heritability estimations larger than 0.5, yet no RGB indices were observed to have broad sense heritability estimations higher than 0.5 at any stage (Table 30, 31). Indices not including NIR wavelengths calculated solely from RGB channels, were also shown to have reduced association to WP when compared to spectral indices (Table 30, 31). Agreeing with the tendency established earlier, visible light phenotypes appear most associated to WP during earlier growth stages but fail to exhibit the strength of association observed when considering NIR reflectance.

In summary, phenotypes quantifying growth parameters or NIR reflectance indices display the strongest association to WP. In context of this experiment, genotypes with increased thresholded pixel area at the V5 growth stage, and increased NIR to red light reflectance ratio at the R5 growth stage tend to have a higher yield to seasonal effective cumulative water ratios. Genotypes with increased thresholded pixel area at V5 are likely intercepting more light which inherently improves the genotypes capacity for future yield and growth (Purcell, 2000). Through the interception of more light, genotypes with increased pixel area can be expected to increase photosynthetic activity thereby increasing the supply of photosynthates needed for growth and development. With a larger canopy area, the CATD would also be predicted to be reduced as evaporative cooling occurring at leaf stomates reduces the effective temperature of the

plot in comparison to the warmer soil background (Farquhar and Sharkey, 1982; Horton et al., 1984; Roche, 2015). As the uncovered soil background is expected to be warmer than the ambient air temperature, plots with increased canopy coverage likewise reduce the CATD. In addition, with root to above ground biomass ratios of approximately 0.75 in soybean at early stages, and the assumption of a positive correlation between thresholded pixel area and biomass, genotypes with improved canopy fraction at earlier stages may be developing a more extensive root system prior to flowering (Torrion et al., 2012). A more extensive root system offers genotypes the potential to be more productive through the ability to extract water deeper from the soil profile during periods of stress thereby sustaining growth and development through reproductive periods vital to seed yield and WP. Enhanced growth and development during the reproductive periods would in turn result in higher NIR to red light reflectance as the ratios have traditionally been used to quantify photosynthetic activity and biomass in a variety of crops (Sims and Gamon, 2002).

Although biological implications on the interpretation of promising phenomic associations to water productivity can be speculated, to better understand meaningful associations the incorporation of genomic data is necessary. Through the pairing of high-dimensional phenotypic and genotypic data, small genetic variations can be associated with phenotypic response, pleiotropy can be studied, and our knowledge of complex biological systems can be increased (Bilder et al., 2009; Freimer and Sabatti, 2003; Houle et al., 2010; Schork, 1997).

Genomic Associations to Water Productivity

To identify genomic regions related to water productivity and associated agronomic and phenomic traits, inclusive composite interval mapping (ICIM) was used to detect significant quantitative trait loci (QTL). In total, seven QTL for WP across treatments were detected in the study; five QTL were detected in the UX3000 population and two QTL were detected in the UX3036 population (Table 32). In the UX3000 population, the five identified QTL explained an estimated 44.4% of the total phenotypic variance observed for WP with additive QTL effects ranging from 0.56 kg ha⁻¹ mm⁻¹ to 0.98 kg ha⁻¹ mm⁻¹. Similarly, the two QTL identified in the UX3036 population had additive effects ranging from 0.80 kg ha⁻¹ mm⁻¹ to 1.48 kg ha⁻¹ mm⁻¹, yet when taken as a whole the two QTL only explained an estimated 15.8% of the total phenotypic variance for WP in the population (Table 32).

To begin to shed light on the function of each of the seven QTL identified, QTL mapping was first repeated considering WP within each irrigation treatment. Through the identification of WP QTL relative to either the irrigated or rainfed treatment, association of the previously identified QTL to productivity in stress or optimum environments can be interpreted. Eight QTL were identified after mapping WP within each water treatment; six within the UX3000 population and two within the UX3036 population. Of the eight QTL identified, six overlapped with significant genetic regions detected when considering WP across irrigation treatments as the response. Within the UX3000 population, QTL identified on chromosomes 1, 4, and 7 were found to coincide with genetic regions associated to WP in the rainfed treatment (Table 32, 33, Figure 28). The QTL identified on chromosomes 18 and 19 appear to be related to WP in irrigated

treatments (Table 33, Figure 28). Like the UX3000 population, the QTL on chromosome 19 in the UX3036 was associated with the irrigated treatment; in contrast, the UX3036 QTL on chromosome 12 appears to be most associated with WP in rainfed environments (Table 33, Figure 29).

When investigating WP QTL mapped within irrigation treatment, three additional minor QTL become significant in the UX3000 population as compared to WP QTL related to performance across irrigation treatments (Table 32, 33). New minor QTL on chromosome 8 and 19 appear related to WP in the rainfed treatment, and a new minor QTL on chromosome 3 is detectable in the irrigated treatment (Table 33). Both QTL detected in the rainfed treatment in the UX3000 population overlap with QTL identified in the study related to seed weight, whereas the QTL identified on chromosome 3 overlaps with a final plant height QTL. When studying the disappearance of QTL on chromosome 4 and 18 detected over treatments in the UX3000 population to QTL identified by irrigation treatment, power of detecting differences appears to be the main issue. The QTL on chromosome 4 was estimated to have a LOD score of 2.44 in the rainfed treatment, and the QTL on chromosome 18 was estimated to have a LOD score of 2.33 in the irrigated treatment falling below the significance threshold. Discrepancies between QTL results between the overall response and by irrigation treatment response are likely attributable to the population size, number of molecular markers, and number of environments associated with the study reducing the power needed to detect all minor QTL (Li, H. et al., 2010). Within the ICIM mapping framework, a population size greater than 200 lines with molecular markers spaced approximately every 20cM or less is recommended for unbiased estimates of QTL explaining more than 5% of the phenotypic

variance (Li, H. et al., 2010). In our study populations sizes of 144 and 171 lines were used for the UX3000 and UX3036 population respectively after filtering for similarity and poor marker call rate within lines; this population size is slightly sub-optimum. In addition, marker spacing often exceeded 20 cM in both populations (Figure 6, 7). These factors in combination reduce the power to detect minor QTL and are likely a reason for discrepancies between the overall and by irrigation treatment results (Li, H. et al., 2010).

Like the methodology used to investigate the seven WP QTL identified across environments to specific water treatments, QTL mapping was repeated for 3140 agronomic and phenomic traits to investigate association to WP. From the 3140 agronomic and phenomic traits, 2407 significant QTL were detected across both populations; 88 agronomic QTL were identified, and 2319 QTL were identified for phenomic trait by growth stage combinations (e.g. NDVI at the V5 growth stage, and NDVI and the R5 growth stage). Supporting the phenomic trend of increased heritability of traits in later growth stages the majority of phenomic QTL were derived from reproductive stage phenotyping (Figure 30). Of the 2407 agronomic and phenomic QTL, 178 were found to have estimated genetic positions within confidence intervals of the seven WP QTL discussed earlier (Table 33, 34). To simplify interpretation of the overlapping agronomic and phenomic QTL, eleven categories were constructed: canopy, index, NIR, reproductive, RGB, seed traits, blue, green, red, agronomic and yield (Appendix 7). From these groupings, the seven WP QTL were clustered based on their relative proportion of overlapping QTL to agronomic and phenomic categories. Using this category clustering approach, informative associations were discovered.

In the UX3000 population, WP QTL identified on chromosomes 1, 4, and 7 previously related to productivity in the rainfed treatment were found to be predominantly associated to seed yield (Figure 31, Table 34). Genetic regions significantly correlated to overall WP on chromosomes 1, 4, and 7 in the UX3000 population were observed to coincide with genetic regions associated with seed yield performance. In contrast WP QTL on chromosome 18 and 19 in the UX3000 population were found to be more related to agronomic, reproductive, or seed traits (Figure 31, Table 34). The WP QTL on chromosome 18 exhibited associations with genetic regions related to reproductive traits such as R1 date along with seed traits such as seed size (Table 34). In divergence, the WP QTL on chromosome 19 demonstrated associations with genetic regions controlling agronomic traits such as height and lodging along with spectral traits involving NIR to RGB ratios (Figure 31, Table 34). In the UX3036 population, the two WP QTL identified both displayed moderate association to yield performance and NIR wavelengths from approximately 900 – 950 nm. The WP QTL on chromosome 19 also demonstrated overlap with QTL identified for height, lodging, and canopy temperature traits (Figure 31, Table 34).

To further interpret the association of each of the seven WP QTL, all QTL reported on SoyBase (SoyBase.org) were investigated for overlapping position based on genetic positions from the genome assembly version Glyma.Wm82.a2.v2 (Grant et al., 2009). In total, 145 QTL from SoyBase overlapped with the genetic position confidence intervals estimated for the seven WP QTL identified in this study (Table 35). In an approach similar to the grouping and clustering of agronomic and phenomic traits, the fourteen QTL object type categories within SoyBase: other seed, whole plant, inorganic,

fungal, insect, leaf stem, miscellaneous, seed oil, seed protein, reproductive, yield, nematode, root and pod were used to identify WP QTL relationships (<https://www.soybase.org/search/qttlist.php>). Although no clear relationship pattern was identified for WP QTL on chromosome 12 and 18 to reported QTL on Soybase, reliable associations were reported for the remaining five QTL. Like trends observed when examining phenomic traits, UX3000 WP QTL on chromosome 1, 4, and 7 overlapped with reported QTL related to seed yield (Figure 32, Table 35). Quantitative trait loci reported for seed yield, seed set, seed width, seed height, node number, and pod number corresponded with WP QTL on chromosome 1, 4 and 7 (Figure 32, Table 35). In addition, the UX3036 WP QTL on chromosome 19 overlapped with reported QTL for pod number and seed set traits pertaining to yield and plant height traits like the yield and agronomic associations discovered earlier (Figure 32, Table 35).

Through coupling phenomic and genomic information, genetic associations can be related to correlated phenotypic responses. Unique patterns and potential phenotypic associations were identified for each of the seven WP QTL detected. In relationship to this experiment where water stress was minor, WP seems to be most associated to genetic factors potentially influencing maximum seed yield potential. The most significant QTL identified in the study on chromosome 7 of the UX3000 population is located within a genetic region that largely overlaps with QTL related to seed yield performance (e.g. seed yield, seed fill, seed set, seed size) (Table 34, 35). This genetic region is not unique to the UX3000 population as corresponding genetic regions have been reported when mapping QTL for seed yield and drought susceptibility across environments using multiple other biparental mapping populations (Du et al., 2009; Wang, X. et al., 2014). Bolstering the

agronomic associations of lodging to WP, QTL identified on chromosome 19 in both the UX3000 and UX3036 population demonstrated a similar genetic position and maximum expression within the irrigated treatment (Table 33, 34, Figure 28, 29). Finally, emphasizing the complex and highly quantitative nature of water productivity, QTL on chromosomes 1, 4, 12, and 18 showed relationships with multiple agronomic and phenomic traits including: yield, seed traits, reproductive stage timing, spectral indices along with canopy reflectance traits. These genetic regions, although significantly related to WP explain less than 10.0% of the phenotypic variance and may contain genes conferring pleiotropic effects. Marginal gains could be expected through focusing on only the most significant agronomic, phenomic, and genomic associations identified thus far. To more fully leverage all associations and the experimental dataset, predictive analytic modeling using machine learning algorithms can be employed.

Predictive Analytic Modeling of Water Productivity

Machine learning has been recently broadly applied for both quantitative trait prediction and supporting trait discovery in crops (Chlingaryan et al., 2018; Ma, C. et al., 2014; Ogotu et al., 2011; Singh, A. et al., 2016). When applied to heterogenous and complex biological datasets, nonparametric machine learning algorithms have relaxed or no assumptions about data distributions, heteroscedasticity, and multicollinearity (Hastie et al., 2005; James, G. et al., 2013). Especially suitable for quantitative traits such as water productivity, the creation and application of supervised machine learning algorithms allows for the full integration of agronomic, phenomic, genomic, and environmental data. This full integration is expected to in turn lead to a more holistic understanding of traits influenced partially by many factors.

To apply predictive analytic machine learning algorithms to the experiment, three algorithm methods, two separate cross validation (CV) schemes, and two distinct data input types were considered. First, least square mean estimations (LSMEANS) of agronomic and phenomic traits were paired with genetic data and modeled with generalized linear regression with elastic net regulation (ENET). Model performance was assessed using both a ten-fold cross validation on the input dataset (CV1) and through testing on datasets separate and distinct from the training set (CV2) (e.g. training on Mead environments and testing on Lincoln environments, training on the UX3000 population and testing on the UX3036 population, training on irrigated plots and training on rainfed, etc.). To address the issue of dimensionality presented through modeling many agronomic and phenomic traits, phenomic traits collected at the R3 growth stage were omitted due to lack of replication over years, and broad sense heritability estimations were used as an initial filtering process. Through only including agronomic and phenomic traits collected at the V5 or R5 growth stage with broad sense heritabilities greater than 0.25, approximately 100 to 150 agronomic and phenomic traits were modeled to WP depending on the input dataset. To reduce the number of SNP markers included in the modeling dataset, only polymorphic markers with non-significant ($\alpha > 0.001$) segregation distortion from the expected 1:1 Mendelian inheritance ratio after excluding heterozygotes were incorporated. These filters addressed the issue of dimensionality, while maintaining the most repeatable traits. Finally the ENET regulation and optimization helps address the $p \gg n$ problem through grouping highly correlated variables and only including one of the grouped variable set (Zou and Hastie, 2005). Through filtering on heritability, segregation distortion, and the variable selection

methodologies unique to ENET, approximately 4,000 agronomic, phenomic and genomic traits are reduced to approximately 25 variables in the final linear model (Figure 33, 34).

Even with the dramatic reduction of input variables, satisfactory predictive performance was exhibited when correlation of predicted to observed WP was evaluated through the CV1 scheme. Pearson correlation coefficients of predicted to observed observations ranged from 0.54 when considering the V5 growth stage observations during both the 2016 preliminary yield response experiment and 2017-2018 water response experiment, to 0.81 when focusing on only the UX3036 population with phenomic data collected at the R5 growth stage in 2017-2018 (Table 36). Root mean square error (RMSE) estimations of models ranged from 0.48 kg ha⁻¹ mm⁻¹ in the 2016-2018 UX3000 and V5 growth stage subset to 1.04 kg ha⁻¹ mm⁻¹ in the 2016-2018 V5 growth stage subset based on all RILs (Table 36). In addition, selected variables and linear model coefficient weights of the ENET largely agreed with previously identified associations in the study. Echoing the significant association of lodging at maturity to WP identified when focusing on agronomic data, lodging was observed to have the largest negative coefficient weight when looking at any population and growth stage subset (Figure 33, 34). In agreement with the importance of spectral indices to WP discovered earlier, variables such as the simple ratio index of 800 nm over 680 nm (SR680) or the simple ratio index of 800 to 705 nm (SR705) were observed to have positive coefficient weights at both the V5 and R5 growth stage in both populations (Figure 33, 34). Finally, in partial accordance with significant genetic associations previously identified through ICIM QTL mapping, four of the fourteen SNP markers with coefficient weights flanked or resided within a WP QTL region (Figure 33, 34).

To investigate the influence that each data type has on the predictability of WP, ENET models were built for each of the six data types and combinations: agronomic, phenomic, genomic, agronomic + phenomic, agronomic + genomic, and complete (agronomic + phenomic + genomic). A comparison of the Pearson correlation coefficients and 95% confidence intervals of predicted to observed responses for each data group allows for an assessment of the relative applied value of each data type. When evaluating performance through the CV1 scheme on WP LSMEANS, genomic data appears to offer the most utility as a single data type (Figure 35). Correlation coefficients were observed to be significantly ($\alpha = 0.05$) higher than models built using agronomic or phenomic data exclusively within the UX3000 population (Figure 35). Larger yet insignificant differences were also exhibited for the UX3036 populations and when merging both populations together (Figure 35). Models built using solely agronomic information displayed improved performance over phenomic models, especially when considering both populations (Figure 35). Continuing this trend, models merging agronomic and genomic data offered slight performance improvements when compared to models with agronomic and phenomic information (Figure 35). As expected, models containing all data types exhibited the best performance in most situations tested through CV1 (Figure 35).

When evaluating models through the CV2 scheme the relative importance of each data type changes. In contrast to performance evaluated through ten-fold cross validation in the CV1 scheme, models tested on datasets dissimilar of the training set displayed lower correlation coefficients and difference relative importance of data types. Examples of CV2 scheme evaluation would include building a model on the UX3000 population,

and testing on the UX3036 population, training on the 2017 WP LSMEANS and testing on 2018 WP LSMEANS or building a model on the 2018 Mead environment and testing on the 2017 Lincoln environment. Through such a cross validation scheme, the relative utility of genomic data over phenomic and agronomic data is decreased (Figure 36). In the CV2 scheme, the performance of agronomic or phenomic models is marginally improved or models built using exclusively genomic information (Figure 36). In agreement, models built with both agronomic and phenomic information offer slight, but insignificant improvements over models built with agronomic and genomic information (Figure 36). Like the importance of the complete dataset observed when evaluating through CV1, the complete dataset displays the highest performing models in most situations when evaluated through CV2 (Figure 36).

To further examine the relative importance of phenomic and genomic variables, predictive models for WP were developed using machine learning algorithms on a per plot observation basis. Modeling plot observations allows for a much larger dataset size when compared to focusing on LSMEANS of WP (18746 ~ 442 observations), and enables the incorporation of weather station data, soil electrical conductivity information, and spatial variables unique to each observation. Through ensembled classification and regression trees with bootstrap aggregation and random predictor selection at each split (CART), and feed forward artificial neural networks with Bayesian regulation (NET), the predictability and relative predictor importance of trait categories was investigated. Model performance was much higher than models built on WP LSMEANS; Pearson correlation coefficients of observed to predicted responses through the NET algorithm ranged from 0.94 when considering phenomic data at the V5 growth stage in the UX3000

population across both irrigation treatments, to 0.80 when subsetting the irrigated treatment and phenomic data collected at the R5 growth stage (Table 37). Similarly, the models built through the CART algorithm exhibited correlation coefficients ranging from 0.95 when using all plot information collected in the study to 0.80 when using only irrigation plot information and phenomic data at the R5 growth stage (Table 38).

Similar to results obtained through the CV1 scheme, when plot observations were used for modeling and performance was evaluated through the CV2 scheme, Pearson correlation coefficients were observed to be higher than models built using WP LSMEANS (Figure 36, Table 39, 40). Correlation coefficients ranged from 0.89 when considering all plot observations to 0.71 when subsetting all phenomic observations from the rainfed treatment within the UX3036 population for the CART algorithm, and coefficients ranged from 0.89 in the UX3036 using R5 phenomic variables to 0.25 using all phenomic variables at the R5 growth stage in both populations over the irrigated treatment (Table 39, 40). In addition, models built from plot observations have improved robustness to predict in untested datasets and are apparently robust to overfitting as well when compared to models developed from WP LSMEANS (Table 36, 39, 40).

In an attempt to investigate the gain in performance of per plot prediction versus WP LSMEANS prediction, relative predictor importance of each variable type was estimated through the CART algorithm using an interaction curvature test (Loh, 2002). On a per plot basis, soil electrical conductivity measurements and spatial variables such as longitude and latitude were observed to have the highest relative predictor importance across both populations (Figure 37, 38). Strengthening the evident influence of plot WP from environmental factors, weather station variables, categorized as environmental,

displayed high predictor importance regardless of the phenomic stage, and indicated increased importance when considering all phenomic observations (Figure 37, 38).

In summary, the full integration and modeling of agronomic, phenomic, genomic, and environmental data on a per plot basis through the CART and NET algorithms offers improved predictive importance when compared to modeling on a per line LSMEAN basis. As observed earlier, WP is largely controlled by environmental factors; environmental variances are much larger on average than genotypic variances (Table 24). Collecting and modeling traits that quantify environmental factors influencing WP would therefore be expected to improve the performance of the model. The predictability on a per plot basis compared to a per line basis appears to be improved partially through the incorporation of soil electrical conductivity, spatial coordinates, and weather station information. It therefore follows that to most effectively model and investigate a trait largely influenced by environmental factors, spatial and environmental data should be collected or effectively accounted for through an appropriate experimental design.

In relationship to applications in plant breeding, unbiased genotypic estimates are of primary importance. To effectively evaluate and compare genotypes, experimental design accounting for environmental factors potentially influencing the trait of interest is needed. Using experimental design factors such as blocking, replication, and augmented check lines, researchers obtain genotypic estimates adjusted for extraneous environmental effects. Therefore, in order to evaluate genotypes most effectively, plant breeders focus on adjusted genotypic means such as LSMEANS for advancement decisions. Because genotypic means are of primary importance in the line evaluation stages of plant breeding programs, modeling WP in the line evaluation stages for advancement decisions has

limited applicability. Since WP can be easily obtained through the incorporation of weather station and agronomic data normally collected in a plant breeding program, gathering estimates of water productivity over representative environments is preferable to predictive modeling. In contrast, in evaluation stages where agronomic data is not routinely generated, modeling WP shows potential for application. In earlier generation evaluation such as maturity separations, progeny row evaluations, or hill to row evaluations where yield data is not measured, collecting and modeling agronomic, phenomic, genomic, and environmental data has great potential to estimate WP. This estimation could then be used for selection purposes potentially increasing in the rate of genetic gain. The improve accuracy of multi-omic prediction models when compared to genomic selection models, may offer increase opportunity of application of rapid recurrent selection prediction success. Furthermore, the routine collection of agronomic, phenomic, genomic, and environmental data in yield evaluation generations can be used as a sort of insurance policy. With seemingly increasing volatile weather patterns and the large resource requirements needed to maintain a breeding program, regular collection of multi-omic data enables the opportunity of quantitative trait prediction should the need arise. Finally, through this routine collection of omic data in yield evaluation stages, product placement decisions in untested environments could be expected to be improved. Through training models on testing environments representative of the products growing region, predictive models using algorithms such as the CART method, could be used to predict performance in untested environments with improved confidence when compared to traditional methods.

CONCLUSION

The rising demand for soybean [*Glycine Max (L.) Merrill*] taken in consideration with current climatic trends accentuates the importance of improving soybean seed yield response per unit water, or water productivity (WP). To further our understanding of the quantitative nature of soybean WP, a multi-omic approach was implemented for improved trait identification and predictive modeling opportunities. Through the evaluation of two recombinant inbred line populations jointly totaling 439 lines subjected to contrasting irrigation treatments, informative agronomic, phenomic, and genomic associations were identified.

Population specific associations to WP were identified for ultrasonic plant height collected at the R5 growth stage in the UX3036 population ($r = 0.46$, $H = 0.69$) along with a QTL identified on chromosome 12 ($r = 0.29$). Within the UX3000 population, unique significant associations were found for QTL on chromosomes 1, 4, 7, and 18 ($r = 0.30$, 0.28 , 0.33 , 0.26). Across both populations, significant relationships were found between WP and lodging at maturity (LG) ($r = -0.58$, $H = 0.86$), the canopy to air temperature differential (CATD) at the V5 growth stage ($r = -0.31$, $H = 0.39$), the SR680 spectral index collected at the R5 growth stage, ($r = 0.62$, $H = 0.39$), and a QTL at approximately 30 cM on chromosome 19 ($r = 0.27$).

Through the coupling of field phenomic data with agronomic and genomic data routinely collected in plant breeding programs, interpretation of identified traits and predictive performance of models was increased. The shared genetic association on chromosome 19 in both populations overlapped with genetic regions indicating association to both LG and the SR680 spectral index collected at the R5 growth stage.

Through the integration of significant agronomic, phenomic, and genomic traits, predictive models of WP were developed across environments on an entry mean basis ($r = 0.72$, $RMSE = 0.67 \text{ kg ha}^{-1} \text{ mm}^{-1}$) and on a per plot basis ($r = 0.95$, $RMSE = 0.39 \text{ kg ha}^{-1} \text{ mm}^{-1}$) using machine learning algorithms.

Findings from this study shed light on both soybean response to water and the application of field phenomic data to soybean breeding programs. Our results highlight the value of integrating multiple dataset types to study and model quantitative traits. Through the application of our findings, soybean breeders can potentially deploy multi-omic selection models in early generation screening stages to increase the rate of genetic gain in relation to soybean WP.

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TABLES

Table 1. Soybean lines used as parents of the two RIL populations, UX3000 and UX3036; parental pedigree information, and metrics of genetic similarity to the common parent U11-614093 included

Strain	Parentage			Descriptors [§]						
	Female	Male	Originator	Stem Type	Flower Color	Pubescence Color	Pod Color	Seed Coat Luster	Seed Coat Color	Hilum Color
LD02-4485	M90-184111	IA 3010	Univ. of Illinois	I	P	G	Br	D	Y	Bf
U11-614093	U02-242055	LD04-13265	Univ. of Nebraska	I	P	T	Br	D	Y	Bl
U09-312115	U02-242055	U03-300134	Univ. of Nebraska	I	P	T	Br	D	Y	Bl

§ W = White, P = Purple, T = Tawny, G = Grey, Y = Yellow, Bl = Black, Bf = Buff

* Approximate coefficient of parentage to U11-614093 (Ruff, 2016)

± Genotypic similarity in terms of identity by descent to U11-614093 from 9,513 GBS SNP data (Ruff, 2016)

Table 2. Spatial characteristics, soil characteristics, growth parameters and management metrics of environment study

Environment		Spatial Characteristics			Soil Characteristics			Cumulative Plant Growth Parameters					
Location	Year	Latitude	Longitude	Elevation	Soil Type	ECS [†]	ECD [†]	Precipitation	Irrigation	Irrigated Effective Water [¶]	Rainfed Effective Water [¶]	GDD [§]	Planting Date
				m		ms m ⁻¹	ms m ⁻¹	mm	mm	mm	mm		
Clay Center	2016	40.573	-98.138	556	Crete silt loam			386	201	375		3385	5/20/2016
Cotesfield*	2016			575	Hord silt loam			293	229	360		3055	5/21/2016
Mead	2016	41.159	-96.420	350	Filbert silt loam			642	76	455		3354	6/3/2016
Wymore*	2016			401	Kennebec silt loam			508	126	344		3814	6/4/2016
Mead	2017	41.157	-96.424	350	Yutan silty clay loam	29.31	0.67	708	120	411	375	3354	5/16/2017
Lincoln	2017	40.864	-96.598	347	Kennebec silt loam	42.74	0.63	691	101	368	234	3357	5/31/2017
Mead	2018	41.159	-96.423	350	Tomek silt loam	29.58	0.82	746	125	384	324	3359	5/8/2018
Lincoln	2018	40.863	-96.595	347	Kennebec silt loam	33.17	0.69	706	114	270	222	3424	5/28/2018

[†]Soil electrical conductivity shallow signal (0 - 1 meters, ECS) and soil electrical conductivity deep signal (0 - 3 meters, ECD)

[¶] Seasonal effective cumulative water calculated through SoyWater (<http://hprcc-agron0.unl.edu/soywater>)

[§]Growing degree days (GDD)

*Latitude and longitude information not included to preserve privacy of cooperators

Table 3. Sensors modules information, field of view, and associated canopy traits measured through the fi platform

Sensor Model and Manufacturer	Field of View	Canopy Trait Measured
ToughSonic30, Senix Corporation, Hinesburg, Vermont	NA	Canopy height
SRS, Decagon Devices, Pullman, Washington	18°	Canopy NDVI
SI-131, Apogee Instruments, Inc., Logan, Utah	14°	Canopy temperature
CCS175, Thorlabs Inc., Newton, New Jersey	13°	Canopy reflectance spectra
C615, Logitech, Fremont, California	33° by 20°	Canopy RGB image
AgGPS 216, Trimble Navigation Ltd., Sunnyvale, California	NA	Plot GPS position
CS215-L, Campbell Scientific Inc., Logan, Utah	NA	Atmospheric temperature and humidity
VLP-16 Puck, Velodyne LiDAR Inc., San Jose, CA	360° vertical; 30° horizontal	Canopy height and density
LI-200, LICOR Biosciences, Lincoln, Nebraska	NA	Atmospheric solar radiation

Table 4. Spectral indices, acronyms, calculation formation, biological trait estimation and sources of spectral indices calculated from field phenomic platform

Index	Acronym	Wavelength formula	Trait estimated	Source
Anthocyanin reflectance index	ARI	1/550 - 1/700	Anthocyanin levels	Gitelson et al., 2001
Chlorophyll Red-Edge	CHLRE	$[(760:800) / (540:560)]-1$	Chlorophyll degradation	Gitelson et al., 2001
Crop Water Index	CW11	531 + 570	Water stress	Moran et al., 1994
Crop Water Index	CW12	$\sum 520 - 530 \text{ nm}$	Water stress	McDermid et al., 2008
Crop Water Index	CW13	$\sum 570 - 590 \text{ nm}$	Water stress	McDermid et al., 2008
Crop Water Index	CW14	$\sum 690 - 710 \text{ nm}$	Water stress	McDermid et al., 2008
Crop Water Index	CW15	$\sum 500 - 700$	Yield	Aparicio et al., 2000
Crop Water Index	CW16	$\sum 700 - 950$	Yield	Aparicio et al., 2000
Grain Yield	GY11	$\sum 500 - 700 \text{ nm}$	Yield	Ferrio et al., 2005
Grain Yield	GY12	$\sum 700 - 950 \text{ nm}$	Yield	Ferrio et al., 2005
Grain Yield	GY13	680 nm	Yield	Ferrio et al., 2005
Green Normalized Vegetation Index	GNDVI	$(801-550) / (800+550)$	Biomass, nitrogen use	Gitelson et al., 1996
Modified Chlorophyll Absorption Ratio Index	MCARI	$[(700-670) - 0.2 (700:550) * (700/670)]$	Chlorophyll concentration	Daughtry et al., 2000
Near infrared to green division index	NIRGreen	801 / 550		
Near infrared to red division index	NIRRed	801 / 670		
Near infrared to visible light division index	NIR_VIS	$\sum 500 - 700 / \sum 700 - 950$		
Normalized Difference Water Index	NDWI	$(800 - 680) / (800 + 680)$	Plant Water Status	Gao, 1996
Normalized Vegetation Index 680	NDVI680	$(800 - 680) / (800 + 680)$	Photosynthesis parameters	Sims and Gamon, 2002
Normalized Vegetation Index 705	NDVI705	$(750 - 705) / (750 + 705)$	Photosynthesis parameters	Sims and Gamon, 2002
Optimized Soil Adjusted Vegetation Index	OSAVI	$(1+0.16) [(800-670) / (800 + 670 + 0.16)]$	Chlorophyll concentration	Rondeaux et al., 1996, Steven, 1998
Photochemical Reflectance Index	PRI	$(531-570) / (531+ 570)$	Photosynthesis parameters	Gamon et al., 1997
Red Edge	RE	$\sum 690 - 740$	Chlorophyll concentration	Horler et al., 1983
Red Edge Division Index	RE3/RE2	$\sum(734 - 747) / \sum(715 - 726)$	Photosynthesis parameters	Vogelmann et al., 1993b
Red Edge Inflection Point	REIP1	Maximum in 680-780	Photosynthesis parameters	Vogelmann et al., 1993b
Simple Index	SI1	710 / 810	Drought Stress	Jiang, Y. and Carrow, 2007
Simple Index	SI2	710 / 760	Drought Stress	Jiang, Y. and Carrow, 2007
Simple Ratio	SR	$\sum(750 - 900) / \sum(660 - 720)$	Photosynthesis parameters	Sims and Gamon, 2002
Simple Ratio 680	SR680	800 / 680	Photosynthesis parameters	Sims and Gamon, 2002
Simple Ratio 705	SR705	730 / 705	Photosynthesis parameters	Sims and Gamon, 2002
Soil Adjusted Vegetation Index	SAVI	$(1+0.5) [(800-670) / (800 + 670 + 0.5)]$	Chlorophyll concentration	Huete et al., 1988
TCARI to OSAVI division index	TCARI_OSAVI	TACARI / OSAVI	Chlorophyll concentration	Haboudane et al., 2004
Transformed Chlorophyll Absorption Reflectance Index	TCARI	$3 * [(700-670) - 0.2 (700:550) * (700/670)]$	Chlorophyll concentration	Haboudane et al., 2004
Vogelmann Index 4	D715/705	$\sum(710 - 720) / \sum(700 - 710)$	Photosynthesis parameters	Vogelmann et al., 1993b

Table 5. RIL parent and population least square mean estimations for seed, water response, and plant characteristics based on two replications across two irrigation treatments and four environments for 2017-2018 water response

Parent	Population	n ^φ	Seed Characteristics							Water Response		Reproductive Period Attributes		
			Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP [¶]	RWP [¶]	R1 [§]	Maturity [§]	R1-R8 [§]
			kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days
LD02-4485		1	4332**	14.2**	1.7	332**	193**	5.0**	471**	14.1**	17.5**	41.3	127**	86***
	UX3036	203	4586	15.2	1.8	340	192	5.1	476	14.9	19.1	41.4	130	88
U11-614093		1	5012	16.6	1.8	351	191	5.3	485	17.0	21.4	41.9	131	89
	UX3000	235	4856	14.8	1.8	342	194	5.2	480	15.5	20.3	42.5	130	88
U09-312115		1	4643**	13.4**	1.7	337**	197**	5.2**	477**	14.9**	19.9**	42.7±	128**	85***

φLine count

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group price monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), the date at which greater than 95% of pods have reached maturity on the main stem (Maturity) between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates difference from U11-614093 significance at $\alpha = 0.10$

* indicates difference from U11-614093 significance at $\alpha = 0.05$

** indicates difference from U11-614093 significance at $\alpha = 0.01$

Table 6. Environmental agronomic means across both populations for environments used in the study including preliminary evaluation and 2017-2018 water response experiment environments

Environment			Seed Characteristics							Water Response		Reproductive Period Attributes				
Location	Year	Treatment	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶¶	R1§	R3§	R5§	Maturity§	R1-R8
			kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
Clay Center	2016		5284	15.5	1.4	328	201	5.4	473	14.1					125	
Cotesfield	2016		6285	17.5	1.4	327	198	5.5	475	15.8						
Mead	2016		4920	15.9	1.6	330	199	5.5	474	15.5					118	
Wymore	2016		4798	17.0	1.2	337	200	5.5	475	12.3					117	
Mead	2017	Irrigated	5304	16.1**	1.4	339**	196**	5.5**	479**	12.7**	16.7**	47*			135**	88**
Mead	2017	Rainfed	5307	15.6**	1.3	324**	201**	5.4**	466**	14.3**	19.9**	47*			134**	87**
Lincoln	2017	Irrigated	5033**	16.0**	1.2	350	193	5.6	485	14.5**	17.0**	38**			121**	83**
Lincoln	2017	Rainfed	4824**	15.4**	1.2	349	194	5.6	484	20.6**	26.3**	39**			118**	79**
Mead	2018	Irrigated	4538**	15.1	2.2**	337**	190**	5.5**	475**	12.4**	14.0**	41*	63	77*	137**	96**
Mead	2018	Rainfed	4281**	15.1	2.0**	332**	195**	5.4**	473**	13.2**	15.1**	40*	63	77*	135**	95**
Lincoln	2018	Irrigated	4170	15.2	2.5**	352**	187*	5.6**	483**	14.8**	19.7**	38	61	71**	126±	88±
Lincoln	2018	Rainfed	4148	15.3	2.3**	347**	188*	5.6**	480**	18.6**	27.2**	38	61	70**	125±	87±

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&mo>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φSubset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 7. Environmental agronomic means for the UX3000 population for environments used in the study preliminary evaluation and 2017-2018 water response experiment environments

Environment			Seed Characteristics							Water Response		Reproductive Period Attributes				
Location	Year	Treatment	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R
			kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
Clay Center	2016		5331	15.4	1.3	328	202	5.4	472	14.2					126.0	
Cotesfield	2016		6547	17.1	1.4	327	201	5.5	474	16.4						
Mead	2016		5233	15.9	1.6	329	203	5.5	473	16.5					119.0	
Wymore	2016		4858	16.7	1.1	336	201	5.5	474	12.5					118.0	
Mead	2017	Irrigated	5453	15.8**	1.4	340**	197**	5.6**	480**	13.0**	17.3**	47			136*	89**
Mead	2017	Rainfed	5492	15.4**	1.5	326**	200**	5.4**	469**	14.8**	20.8**	48			134*	86**
Lincoln	2017	Irrigated	5095**	16.0**	1.2**	353**	194**	5.7*	487*	14.7**	17.4**	39			121**	82**
Lincoln	2017	Rainfed	4858**	15.2**	1.2**	351**	195**	5.7*	485*	20.7**	27.1**	40			118**	78**
Mead	2018	Irrigated	4621**	15.1±	2.2	338	192	5.5	477	12.7**	14.5**	41	63	78	137**	96**
Mead	2018	Rainfed	4313**	14.9±	2.0	333	196	5.5	475	13.3**	15.4**	41	63	78	135**	94**
Lincoln	2018	Irrigated	4229	15.1	2.5**	352**	188	5.6**	483**	14.7**	19.8**	38	61	71	125.0	87±
Lincoln	2018	Rainfed	4231	15.2	2.3**	347**	190	5.6**	481**	19.0**	28.2**	39	61	70	125.0	86±

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group and monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φSubset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 8. Environmental agronomic means for the UX3036 population for environments used in the study preliminary evaluation and 2017-2018 water response experiment environments

Environment			Seed Characteristics							Water Response		Reproductive Period Attributes				
Location	Year	Treatment	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8
			kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
Clay Center	2016		5159	15.4	1.3	328	202	5.4	472	14.2					126	
Cotesfield	2016		6173	17.1	1.4	327	201	5.5	474	15.5						
Mead	2016		4787	15.9	1.6	329	203	5.5	473	16.5					119	
Wymore	2016		4643	16.7	1.1	336	201	5.5	474	12.5					118	
Mead	2017	Irrigated	5125	16.4**	1.4	338**	194**	5.5**	478**	12.3**	16.1**	47			135*	88**
Mead	2017	Rainfed	5080	15.8**	1.2	323**	200**	5.3**	464**	13.7**	19.0**	47			133*	86**
Lincoln	2017	Irrigated	4981**	16.1**	1.2**	348**	193**	5.6*	483*	14.4**	16.7**	37			120**	83**
Lincoln	2017	Rainfed	4776**	15.7**	1.2**	347**	193**	5.6*	483*	20.4**	25.5**	38			118**	80**
Mead	2018	Irrigated	4432**	15.3	2.2	336	188	5.4	473	12.1**	13.4**	40	63	77	137**	97**
Mead	2018	Rainfed	4236**	15.4	2.1	331	193	5.4	470	13.1**	14.7**	40	63	77	135**	95**
Lincoln	2018	Irrigated	4084	15.3	2.5**	352**	185	5.6**	482**	14.6**	19.2**	37	61	70	127	88±
Lincoln	2018	Rainfed	4053	15.4	2.3**	346**	187	5.5**	480**	18.2**	26.1**	37	61	70	126	87±

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-r>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φSubset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 9. Agronomic means by environment, treatment, and maturity grouping across populations for 2017-2018 water response experiment environments

Environment				Seed Characteristics					Water Response		Reproductive Period Attributes				Plant Characteristics					
Location	Year	Treatment	MG	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP [¶]	RWP [¶]	R1 [§]	R3 [§]	R5 [§]	Maturity [§]	R1-R8 [§]	Height	Lodging [‡]	n [¶]
				kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days	cm	1-5 scale	
Mead	2017	Irrigated	2.0	5476*	16.3**	1.1**	331**	201	5.5**	475**	11.3**	13.4**	44.6**			130.1	85.5	94.6**	1.1	120
Mead	2017	Rainfed	2.0	5314*	16.0**	1.3**	325**	201	5.4**	468**	14.2**	18.3**	45.3**			130.2	84.9	89.5**	1.1	120
Mead	2017	Irrigated	2.5	5505**	16.3**	1.2	338**	197**	5.6**	478**	13.5**	17.4**	46.1*			134.4**	88.3**	101.2**	1.2**	349
Mead	2017	Rainfed	2.5	5313**	15.5**	1.3	319**	203**	5.3**	461**	14.2**	18.8**	46.3*			131.9**	85.6**	93.5**	1.0**	346
Mead	2017	Irrigated	3.0	5143**	15.9**	1.6**	342**	194**	5.6**	480**	12.5**	17.1**	48.2			137.2**	89.0**	110.1**	1.7**	414
Mead	2017	Rainfed	3.0	5311**	15.5**	1.4**	328**	198**	5.4**	470**	14.2**	20.1**	48.3			135.8**	87.5**	105.6**	1.3**	414
Mead	2017	Irrigated	3.5	4874*	16.3±	1.5	341**	195*	5.6**	480**	12.2**	16.3**	47.5**			137.2±	89.7**	110.4	1.8**	54
Mead	2017	Rainfed	3.5	5196*	15.9±	1.4	329**	198*	5.4**	471**	15.9**	29.3**	48.7**			136.3±	87.6**	108.1	1.2**	54
Mead	2018	Irrigated	2.0	4887	15.8*	2.0	335**	194**	5.5**	476**	13.9**	15.1**	38.9	60.8	75.8	130.7**	91.9**	98.2**	1.6*	82
Mead	2018	Rainfed	2.0	4772	15.5*	2.0	325**	201**	5.4**	468**	15.6**	17.3**	38.8	61.7	75.5	128.4**	89.6**	102.5**	1.8*	82
Mead	2018	Irrigated	2.5	4728**	14.7**	2.1**	336**	192**	5.5**	475**	13.4**	14.6**	39.3	61.7	77.0	133.6**	94.3**	108.3	2.7**	242
Mead	2018	Rainfed	2.5	4556**	15.1**	2.0**	331**	195**	5.4**	472**	14.1**	15.7**	39.3	61.6	77.0	131.9**	92.6**	108.9	2.3**	242
Mead	2018	Irrigated	3.0	4468**	15.4**	2.3**	336	189**	5.4**	474**	11.6**	13.0**	40.8**	63.9**	77.5**	137.7**	97.0**	119.8**	2.8**	378
Mead	2018	Rainfed	3.0	4010**	15.2**	2.1**	335	193**	5.5**	475**	12.4**	14.1**	40.3**	63.1**	77.3**	136.5**	96.1**	115.0**	2.3**	378
Mead	2018	Irrigated	3.5	4311	15.2	2.1**	340**	189**	5.5**	477**	12.3**	14.6**	42.4	63.7	78.4	141.1**	98.7**	121.0	2.2	234
Mead	2018	Rainfed	3.5	4261	15.1	2.1**	330**	195**	5.4**	471**	12.7**	15.3**	42.4	64.0	78.1	138.7**	96.3**	121.7	2.3	234
Lincoln	2017	Irrigated	2.0	5099	15.2	1.5**	347**	194*	5.6*	482*	17.0**	20.2**	36.8			114.4**	77.5**	104.4	2.1*	120
Lincoln	2017	Rainfed	2.0	5118	15.3	1.1**	344**	196*	5.6*	481*	22.0**	27.6**	37.0			113.2**	76.2**	104.1	1.9*	120
Lincoln	2017	Irrigated	2.5	4950	15.6*	1.1**	344**	196**	5.6**	481**	14.8**	17.3**	37.7			118.5**	80.8**	106.6**	2.2*	349
Lincoln	2017	Rainfed	2.5	4951	15.8*	1.3**	350**	193**	5.6**	484**	21.0**	26.4**	37.9			116.8**	78.9**	112.7**	2.1*	346
Lincoln	2017	Irrigated	3.0	5102**	16.6**	1.2	356**	192**	5.7**	489**	13.9**	16.0**	38.7**			123.5**	84.8**	119.4**	2.7**	412
Lincoln	2017	Rainfed	3.0	4670**	15.3**	1.2	350**	194**	5.7**	485**	19.9**	26.0**	39.6**			119.3**	79.7**	114.2**	1.8**	408
Lincoln	2017	Irrigated	3.5	4883*	16.1**	1.1	350**	192*	5.6**	483*	13.4**	15.6**	39.5*			123.6**	84.1**	116.5	2.1	52
Lincoln	2017	Rainfed	3.5	4547*	14.7**	1.2	345**	194*	5.6**	481*	19.6**	25.8**	40.4*			118.3**	77.9**	115.9	1.9	54
Lincoln	2018	Irrigated	2.0	4411	15.5	2.4*	348**	187**	5.6**	480**	13.1**	17.1**	36.6±	49.4±	69.2±	119.4**	82.8**	99.9**	2.1**	82
Lincoln	2018	Rainfed	2.0	4342	15.6	2.2*	336**	194**	5.5**	476**	18.8**	27.0**	36.2±	48.7±	68.6±	116.9**	80.7**	94.2**	1.4**	82
Lincoln	2018	Irrigated	2.5	4241	14.4**	2.8**	349**	190**	5.6**	482**	16.6**	22.1**	36.5	50.2	69.5	121.5**	85.1**	104.8**	2.4**	242
Lincoln	2018	Rainfed	2.5	4302	15.1**	2.5**	341**	192**	5.5**	478**	19.4**	27.2**	36.5	50.4	69.3	120.7**	84.2**	101.1**	1.8**	242
Lincoln	2018	Irrigated	3.0	4176**	15.6**	2.3	353**	186	5.6**	483**	15.5**	21.0**	37.8±	51.5±	70.8*	128.0*	90.2**	111.3±	1.9**	380
Lincoln	2018	Rainfed	3.0	4097**	15.3**	2.3	351**	186	5.6**	482**	18.4**	27.4**	38.0±	51.7±	70.3*	127.8*	89.7**	110.2±	1.6**	380
Lincoln	2018	Irrigated	3.5	3987	15.4	2.6**	354**	186	5.6**	484**	12.0**	15.4**	39.3±	50.5±	71.9	130.3	91.0*	115.1	2.4**	233
Lincoln	2018	Rainfed	3.5	3985	15.5	2.2**	351**	187	5.6**	482**	17.9**	26.7**	39.6±	50.3±	71.9	130.1	90.5*	114.2	1.6**	234
Overall		Irrigated	2.0	4968±	15.7	1.8*	340**	194**	5.5**	478**	13.8**	16.4**	39.2	55.1	72.5±	123.7*	84.4**	99.3*	1.7**	404
Overall		Rainfed	2.0	4887±	15.6	1.6*	332**	198**	5.5**	473**	17.6**	22.5**	39.3	55.2	72.1±	122.2*	82.9**	97.6*	1.5**	404
Overall		Irrigated	2.5	4856**	15.3	1.8	342**	194**	5.6**	479**	14.6**	17.8**	39.9	56.0	73.2	127.0**	87.1**	105.2*	2.1**	1182
Overall		Rainfed	2.5	4780**	15.4	1.8	335**	196**	5.5**	474**	17.2**	22.0**	40.0	56.0	73.2	125.3**	85.3**	104.1*	1.8**	1176
Overall		Irrigated	3.0	4722**	15.9**	1.8**	347**	190**	5.6**	481**	13.4**	16.8**	41.4	57.7±	74.2*	131.6**	90.2**	115.2**	2.3**	1584
Overall		Rainfed	3.0	4522**	15.3**	1.7**	341**	193**	5.5**	478**	16.2**	21.9**	41.6	57.4±	73.8*	129.8**	88.3**	111.3**	1.7**	1580
Overall		Irrigated	3.5	4514.0	15.7±	1.8**	346**	191**	5.6**	481**	12.5**	15.5**	42.2±	57.1	75.2±	133.0**	90.9**	115.7	2.1**	573
Overall		Rainfed	3.5	4497.0	15.3±	1.7**	339**	193**	5.5**	476**	16.5**	24.3**	42.8±	57.1	75.0±	130.8**	88.1**	115.0	1.8**	576

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average mean over the October 2013 - October 2018 time period. (retrieved from <https://www.industry.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§ Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡ Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φ Subset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 10. Agronomic means by environment, treatment, and maturity grouping within the UX3000 population for 2017-2018 water response experiment environments

Environment				Seed Characteristics							Water Response		Reproductive Period Attributes					Plant Characteristics		
Location	Year	Treatment	MG	Yield	Weight	Quality†	Protein	Oil	EPV‡	MPP‡	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8§	Height	Lodging‡	n¶
				kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days	cm	1-5 scale	
Mead	2017	Irrigated	2.0	5655**	16.3**	1.1**	332**	203	5.5**	477**	11.7**	13.8**	44.5**			130.8	86.3**	91.8**	1.0	72
Mead	2017	Rainfed	2.0	5484**	15.9**	1.3**	327**	202	5.4**	471**	14.6**	18.9**	45.5**			130.5	85.0**	86.8**	1.0	72
Mead	2017	Irrigated	2.5	5722**	15.8**	1.3	338**	198**	5.6**	479**	14.0**	18.2**	46.2*			134.8**	88.6**	99.3**	1.0*	168
Mead	2017	Rainfed	2.5	5446**	15.0**	1.3	318**	204**	5.2**	458**	14.5**	19.4**	46.5*			132.0**	85.5**	90.8**	1.0*	166
Mead	2017	Irrigated	3.0	5237**	15.5±	1.7	343**	194**	5.6**	480**	12.7**	17.7**	48.8			137.3	88.4	109.8**	1.4**	238
Mead	2017	Rainfed	3.0	5524**	15.4±	1.6	332**	198**	5.5**	474**	14.7**	21.2**	48.8			136.9	88.1	106.5**	1.1**	238
Mead	2017	Irrigated	3.5	5154*	16.1	1.4	342**	196	5.6**	481**	12.9**	17.4**	47.8**			137.8	90.0	111.2	1.5**	24
Mead	2017	Rainfed	3.5	5522*	15.9	1.7	332**	197	5.5**	475**	16.9**	31.7**	49.1**			138.0	88.9	110.1	1.0**	24
Mead	2018	Irrigated	2.0	5189**	15.7	2.0	334**	196**	5.5**	477**	14.7**	16.1**	39.0	60.7	76.0	130.6**	91.6**	97.9**	1.3*	48
Mead	2018	Rainfed	2.0	4934**	15.5	2.0	326**	202**	5.4**	471**	16.1**	17.9**	39.0	61.5	75.8	127.5**	88.5**	102.4**	1.6*	48
Mead	2018	Irrigated	2.5	4956**	14.8±	2.0	339**	194**	5.5**	478**	14.0**	15.3**	39.5±	61.8	77.0	133.3**	93.8**	105.0	2.3**	112
Mead	2018	Rainfed	2.5	4712**	15.1±	2.0	333**	197**	5.5**	475**	14.6**	16.3**	39.8±	61.7	77.3	131.3**	91.5**	105.5	1.9**	112
Mead	2018	Irrigated	3.0	4555**	15.2**	2.3	337	191**	5.5**	476*	11.9**	13.5**	41.3**	64.2*	77.9	137.6**	96.3±	119.4**	2.7**	192
Mead	2018	Rainfed	3.0	4036**	14.7**	2.0	337	194**	5.5**	477*	12.4**	14.4**	40.8**	62.8*	77.7	136.6**	95.9±	111.0**	1.8**	190
Mead	2018	Irrigated	3.5	4273	14.9	2.2	340**	190**	5.5**	478**	12.2	14.7*	42.8	63.8	78.5	140.8**	98.0**	118.8	2.0	150
Mead	2018	Rainfed	3.5	4170	14.8	2.1	332**	196**	5.4**	474**	12.4	15.3*	42.9	64.0	78.2	138.2**	95.2**	119.2	2.1	150
Lincoln	2017	Irrigated	2.0	5226	15.2	1.4	349**	195**	5.7**	483**	17.4**	20.7**	38.1			114.0**	75.9*	102.7	1.8*	72
Lincoln	2017	Rainfed	2.0	5176	15.1	1.0	344**	197**	5.6**	481**	22.2**	28.0**	37.8			112.7**	74.9*	100.5	1.5*	72
Lincoln	2017	Irrigated	2.5	5013	15.5	1.1	346**	196**	5.6**	483**	14.9**	17.7**	38.7			118.5**	79.8**	104.7**	2.0**	168
Lincoln	2017	Rainfed	2.5	5035	15.5	1.2	352**	194**	5.7**	486**	21.3**	27.1**	38.9			116.6**	77.7**	110.1**	1.7**	168
Lincoln	2017	Irrigated	3.0	5129**	16.5**	1.2	359**	192**	5.7**	492**	13.9**	16.2**	39.7**			124.4**	84.7**	118.0**	2.6**	237
Lincoln	2017	Rainfed	3.0	4665**	15.1**	1.1	352**	194**	5.7**	486**	19.9**	26.9**	40.6**			119.7**	79.1**	111.7**	1.6**	235
Lincoln	2017	Irrigated	3.5	4936	15.6**	1.0	350	193*	5.6	484.0	13.5**	16.1**	40.8			123.5**	82.7**	114.4	2.0	23
Lincoln	2017	Rainfed	3.5	4558	14.5**	1.3	348	195*	5.6	483.0	19.6**	26.4**	41.2			117.5**	76.3**	114.6	2.0	24
Lincoln	2018	Irrigated	2.0	4604±	15.0	2.4	346**	189**	5.6**	481**	13.7**	17.9**	36.9	49.2	69.2	118.5**	81.6**	98.0**	1.8**	48
Lincoln	2018	Rainfed	2.0	4470±	15.4	2.1	336**	196**	5.5**	477**	19.4**	28.2**	36.7	48.6	68.5	116.0**	79.3**	91.9**	1.2**	48
Lincoln	2018	Irrigated	2.5	4324±	14.3**	2.9	349**	193	5.6**	483**	16.9**	22.8**	36.8	50.0	69.4	119.7	82.9	98.8**	1.8**	112
Lincoln	2018	Rainfed	2.5	4452±	15.2**	2.5	342**	193	5.6**	479**	20.0**	28.7**	37.0	50.4	69.2	119.8	82.8	95.3**	1.4**	112
Lincoln	2018	Irrigated	3.0	4244±	15.3*	2.3	352**	187	5.6**	482*	15.7**	21.7**	38.6	52.0	71.0	127.2*	88.7**	108.1	1.4**	192
Lincoln	2018	Rainfed	3.0	4168±	15.1*	2.3	350**	187	5.6**	481*	18.8**	28.4**	38.7	51.9	70.4	126.9*	88.2**	107.6	1.2**	192
Lincoln	2018	Irrigated	3.5	4019	15.1	2.5	355**	187	5.6**	484**	12.1**	15.6**	39.8	50.6	71.9	129.9	90.1±	113.2*	2.1**	149
Lincoln	2018	Rainfed	3.5	4069	15.2	2.2	351**	188	5.6**	482**	18.3**	27.4**	40.0	50.4	71.8	129.7	89.7±	111.3*	1.3**	150
Overall		Irrigated	2.0	5223**	15.2	1.6*	341**	196**	5.6**	480**	14.4**	17.2**	40.0	55.0	72.6	123.2*	83.3**	97.5**	1.5*	240
Overall		Rainfed	2.0	5079**	15.1	1.5*	334**	199**	5.5**	476**	18.2**	23.3**	40.1	55.1	72.1	121.7*	81.5**	95.0**	1.3*	240
Overall		Irrigated	2.5	5076*	14.8	1.7	343**	196**	5.6**	481**	14.9**	18.4**	40.7	55.9	73.2	126.6**	85.9**	102.0*	1.7**	560
Overall		Rainfed	2.5	4976*	14.8	1.6	336**	197**	5.5**	474**	17.7**	23.0**	41.0	56.0	73.2	124.8**	83.8**	100.5*	1.5**	558
Overall		Irrigated	3.0	4833**	15.2**	1.8**	348**	191**	5.6**	483**	13.5**	17.2**	42.3	58.1±	74.5	131.5**	89.2**	113.9**	2.0**	859
Overall		Rainfed	3.0	4653**	14.7**	1.7**	342**	194**	5.6**	480**	16.6**	22.9**	42.5	57.3±	74.0	129.9**	87.4**	109.2**	1.4**	855
Overall		Irrigated	3.5	4269.0	14.2	2.2**	347**	189**	5.6**	481**	12.3**	15.4**	41.7	57.2	75.2	134.8**	93.0**	115.6	2.0**	346
Overall		Rainfed	3.5	4246.0	14.2	2.0**	341**	192**	5.5**	478**	15.8**	22.4**	42.0	57.2	75.0	133.1**	91.1**	114.8	1.7**	348

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

‡Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

§Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average mean over the October 2013 - October 2018 time period, (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

¶Subset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 11. Agronomic means by environment, treatment, and maturity grouping within the UX3036 population for 2017-2018 water response experiment environments

Environment				Seed Characteristics						Water Response		Reproductive Period Attributes					Plant Characteristics			
Location	Year	Treatment	MG	Yield	Weight	Quality†	Protein	Oil	EPV‡	MPP‡	WP§	RWP§	R1§	R3§	R5§	Maturity§	R1-R8§	Height	Lodging †	n¶
				kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days	cm	1-5 scale	
Mead	2017	Irrigated	2.0	5208	16.3	1.1*	329**	199*	5.4**	472**	10.7**	12.8**	44.8			129.1	84.3	98.7*	1.3±	48
Mead	2017	Rainfed	2.0	5060	16.1	1.3*	322**	201*	5.3**	463**	13.5**	17.3**	45.0			129.8	84.8	93.5*	1.1±	48
Mead	2017	Irrigated	2.5	5306*	16.5**	1.2	337**	196**	5.5**	478**	13.0**	16.8**	46.1			134.3**	88.1**	103.8**	1.3**	181
Mead	2017	Rainfed	2.5	5168*	15.9**	1.2	320**	202**	5.3**	462**	13.8**	18.3**	46.3			132.1**	85.8**	96.5**	1.1**	180
Mead	2017	Irrigated	3.0	5005	16.3**	1.5**	341**	192**	5.5**	479**	12.2**	16.4**	47.7±			137.4**	89.7**	111.0**	2.1**	176
Mead	2017	Rainfed	3.0	5016	15.6**	1.2**	326**	198**	5.3**	467**	13.4**	18.9**	48.0±			134.8**	86.8**	106.2**	1.5**	176
Mead	2017	Irrigated	3.5	4649	16.4±	1.6**	341**	195*	5.6**	479**	11.6**	15.4**	47.2**			136.7**	89.5**	109.7	2.1**	30
Mead	2017	Rainfed	3.5	4935	15.9±	1.1**	327**	198*	5.4**	468**	15.1**	27.5**	48.4**			134.9**	86.5**	106.6	1.4**	30
Mead	2018	Irrigated	2.0	4460	13.9	2.0	336**	190**	5.4**	474**	12.6**	13.8**	38.7	61.0**	75.6**	130.9*	92.2±	98.7	1.9	34
Mead	2018	Rainfed	2.0	4543	13.6	2.0	323**	198**	5.3**	463**	14.9**	16.5**	38.4	62.0**	75.2**	129.5*	91.1±	102.7	2.2	34
Mead	2018	Irrigated	2.5	4508	12.6**	2.1**	335**	190**	5.4**	473**	12.7**	13.9**	39.2	61.7**	76.8**	133.9**	94.8**	111.8	3.1**	130
Mead	2018	Rainfed	2.5	4470	13.2**	2.0**	331**	193**	5.4**	471**	13.9**	15.4**	39.0	61.6**	76.7**	132.4**	93.4**	111.2	2.6**	130
Mead	2018	Irrigated	3.0	4398**	13.5	2.3**	335	188**	5.4**	472*	11.5**	12.6**	40.3**	63.5**	77.0**	137.9**	97.6**	119.4	2.9	186
Mead	2018	Rainfed	3.0	3935**	13.5	2.1**	335	192**	5.4**	474*	12.1**	13.6**	39.9**	63.2**	77.0**	136.1**	96.2**	118.8	2.7	188
Mead	2018	Irrigated	3.5	4379	13.7	2.1	339**	187**	5.4**	475**	12.5**	14.3**	41.6	63.5**	78.2**	141.5**	99.8**	124.8	2.4	84
Mead	2018	Rainfed	3.5	4424	13.5	2.1	328**	193**	5.3**	466**	13.2**	15.4**	41.6	64.0**	78.0**	139.8**	98.1**	126.0	2.7	84
Lincoln	2017	Irrigated	2.0	4910	15.2	1.5**	343	194	5.6	481.0	16.3**	19.4**	34.9*			114.9	80.0**	106.8	2.5	48
Lincoln	2017	Rainfed	2.0	5031	15.5	1.1**	343	193	5.6	481.0	21.6**	27.1**	35.8*			114.0	78.3**	109.5	2.4	48
Lincoln	2017	Irrigated	2.5	4895	15.6**	1.1**	342**	195**	5.6**	480**	14.6**	16.9**	36.9±			118.6**	81.7**	109.2**	2.5	181
Lincoln	2017	Rainfed	2.5	4893	16.1**	1.3**	349**	192**	5.6**	484**	20.7**	25.8**	37.3±			117.4**	80.0**	116.2**	2.5	178
Lincoln	2017	Irrigated	3.0	5105**	16.8**	1.2	354**	190**	5.7**	487**	13.9**	15.8**	37.6**			123.3**	85.7**	123.3**	2.9**	175
Lincoln	2017	Rainfed	3.0	4627**	15.3**	1.2	347**	193**	5.6**	482**	19.8**	24.7**	38.5**			119.0**	80.5**	117.3**	2.0**	173
Lincoln	2017	Irrigated	3.5	4841	16.4**	1.1	350**	191	5.6**	483*	13.3**	15.2**	38.5*			123.7**	85.2**	118.2	2.2	29
Lincoln	2017	Rainfed	3.5	4539	15.0**	1.1	342**	194	5.6**	480*	19.5**	25.4**	39.8*			118.9**	79.1**	116.9	1.9	30
Lincoln	2018	Irrigated	2.0	4139	15.7	2.5	349**	184**	5.6**	480**	12.3**	15.8**	36.2	49.6**	69.1**	120.7**	84.5*	102.6*	2.5**	34
Lincoln	2018	Rainfed	2.0	4162	15.7	2.4	336**	191**	5.4**	474**	18.1**	25.2**	35.5	48.9**	68.7**	118.1**	82.6*	97.4*	1.6**	34
Lincoln	2018	Irrigated	2.5	4125	14.4**	2.6	349**	187**	5.6**	481**	16.2**	21.2**	36.2	50.4**	69.5**	122.9**	86.8**	109.7**	2.9**	130
Lincoln	2018	Rainfed	2.5	4196	15.0**	2.4	340**	191**	5.5**	477**	18.9**	26.1**	36.1	50.4**	69.5**	121.5**	85.3**	106.2**	2.2**	130
Lincoln	2018	Irrigated	3.0	4114±	15.7	2.3	353*	185	5.6	482*	15.2**	20.3**	37.1**	51.1**	70.7**	128.7	91.6**	113.8*	2.3**	188
Lincoln	2018	Rainfed	3.0	4033±	15.5	2.2	351*	185	5.6	481*	18.1**	26.4**	37.4**	51.4**	70.2**	128.4	91.0**	111.9*	2.0**	188
Lincoln	2018	Irrigated	3.5	3932	15.6	2.7**	353	185	5.6	483.0	11.9**	15.2**	38.5	50.3**	72.0**	131.0	92.5*	118.4	2.8**	84
Lincoln	2018	Rainfed	3.5	3835	15.8	2.3**	352	185	5.6	482.0	17.3**	25.6**	38.8	50.1**	71.9**	130.7	91.9*	119.5	2.1**	84
Overall		Irrigated	2.0	4744.0	15.4	1.7	339**	193**	5.5**	477**	13.1**	15.6**	38.9	55.3	72.4	123.6	84.7±	101.9	2.0*	164
Overall		Rainfed	2.0	4758.0	15.3	1.6	331**	196**	5.4**	471**	17.1**	21.6**	38.9	55.4	72.0	122.7	83.8±	100.9	1.8*	164
Overall		Irrigated	2.5	4773.0	15.0*	1.7	341**	192**	5.5**	478**	14.1**	17.1**	39.9	56.1	73.2	127.3**	87.4**	108.3	2.4**	622
Overall		Rainfed	2.5	4738.0	15.2*	1.7	335**	195**	5.5**	473**	16.9**	21.5**	40.0	56.0	73.1	125.7**	85.6**	107.3	2.0**	618
Overall		Irrigated	3.0	4642**	15.5**	1.8**	346**	188**	5.5**	480**	13.2**	16.3**	40.6	57.3	73.8	131.8**	91.2**	116.9**	2.5**	725
Overall		Rainfed	3.0	4388**	15.0**	1.7**	340**	192**	5.5**	476**	15.8**	20.9**	40.9	57.3	73.6	129.7**	88.8**	113.6**	2.1**	725
Overall		Irrigated	3.5	4308.0	15.1±	2.1**	346**	188**	5.5**	480**	12.2**	14.9**	40.8	56.9	75.1	134.7*	93.9**	119.6	2.5	227
Overall		Rainfed	3.5	4290.0	14.9±	1.9**	338**	191**	5.5**	474**	15.8**	22.0**	41.2	57.0	74.9	133.0*	91.8**	119.9	2.2	228

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

‡Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

§Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average mean over the October 2013 - October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1 = all plants erect to 5 = all plants prostrate

¶Subset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 12. Ranges of reproductive period attributes by environment, population, and maturity grouping for 2017-2018 water response experiment environments; ranges of overall location represent the mean of four environments

Environment				Reproductive Period Attributes					n ^φ
Location	Year	Population	MG	R1 [§]	R3 [§]	R5 [§]	Maturity [§]	R1-R8 [§]	
				days	days	days	days	days	
Mead	2017	UX3000	2.0	5			11	12	48
Mead	2017	UX3036	2.0	6			12	14	48
Mead	2017	UX3000	2.5	9			17	18	181
Mead	2017	UX3036	2.5	9			17	18	180
Mead	2017	UX3000	3.0	7			14	15	176
Mead	2017	UX3036	3.0	8			20	18	176
Mead	2017	UX3000	3.5	6			8	10	30
Mead	2017	UX3036	3.5	7			12	16	30
Mead	2018	UX3000	2.0	5	4	5	10	11	34
Mead	2018	UX3036	2.0	5	7	7	14	12	34
Mead	2018	UX3000	2.5	5	7	6	10	11	130
Mead	2018	UX3036	2.5	7	6	7	10	10	130
Mead	2018	UX3000	3.0	9	7	6	15	16	186
Mead	2018	UX3036	3.0	9	6	6	12	16	188
Mead	2018	UX3000	3.5	7	5	5	14	14	84
Mead	2018	UX3036	3.5	7	6	8	18	18	84
Lincoln	2017	UX3000	2.0	8			12	13	48
Lincoln	2017	UX3036	2.0	9			13	14	48
Lincoln	2017	UX3000	2.5	10			15	17	181
Lincoln	2017	UX3036	2.5	12			15	18	178
Lincoln	2017	UX3000	3.0	9			14	19	175
Lincoln	2017	UX3036	3.0	11			13	22	173
Lincoln	2017	UX3000	3.5	9			10	15	29
Lincoln	2017	UX3036	3.5	9			14	16	30
Lincoln	2018	UX3000	2.0	6	4	4	10	11	34
Lincoln	2018	UX3036	2.0	8	5	4	18	15	34
Lincoln	2018	UX3000	2.5	6	5	4	13	12	130
Lincoln	2018	UX3036	2.5	6	5	4	17	14	130
Lincoln	2018	UX3000	3.0	6	5	6	10	10	188
Lincoln	2018	UX3036	3.0	6	4	5	9	10	188
Lincoln	2018	UX3000	3.5	6	5	4	11	12	84
Lincoln	2018	UX3036	3.5	5	5	8	15	13	84
Overall		UX3000	2.0	6.0	4.0	4.5	10.8	11.8	164
Overall		UX3036	2.0	7.0	6.0	5.5	14.3	13.8	164
Overall		UX3000	2.5	7.5	6.0	5.0	13.8	14.5	622
Overall		UX3036	2.5	8.5	5.5	5.5	14.8	15.0	618
Overall		UX3000	3.0	7.8	6.0	6.0	13.3	15.0	725
Overall		UX3036	3.0	8.5	5.0	5.5	13.5	16.5	725
Overall		UX3000	3.5	7.0	5.0	4.5	10.8	12.8	227
Overall		UX3036	3.5	7.0	5.5	8.0	14.8	15.8	228

§Date at which 50% of plot reach R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

φSubset count

Table 13. Agronomic irrigation treatment effects by environment and population for 2017-2018 water response environments; values represent the response to irrigation

Environment			Seed Characteristics							Water Response		Reproductive Period Attributes				
Location	Year	Population	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R8§
			kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
Mead	2017	UX3000	-37.2	0.4**	0.0	13**	(-3**)	0.2**	10**	-1.7**	-3.5**	-0.3	0.0	0.0	1.1*	1.4
Mead	2017	UX3036	49.7	0.6**	0.1	15**	(-6**)	0.2**	13**	-1.4**	-2.9**	-0.3	0.0	0.0	2.0*	2.3
Lincoln	2017	UX3000	237**	0.7±	0.1**	2**	(-1**)	0.0*	2*	-6.0**	-9.8**	-0.5	0.0	0.0	3.3**	3.8
Lincoln	2017	UX3036	202**	0.5±	0.0**	1**	0**	0.0*	1*	-5.9**	-8.9**	-0.8	0.0	0.0	2.7**	3.4
Mead	2018	UX3000	307**	0.2**	0.1	5	(-4)	0.0	2.0	-0.6**	-1.0**	0.1	0.4	0.1	1.9**	1.9
Mead	2018	UX3036	196**	-0.1**	0.1	5	(-4)	0.0	3.0	-0.9**	-1.3**	0.2	0.0	0.1	1.6**	1.4
Lincoln	2018	UX3000	-1.1	-0.2	0.2	5	(-1)	0.0	2.0	-4.2**	-8.4**	-0.1	0.0	0.4	0.4	0.3
Lincoln	2018	UX3036	30.1	-0.1	0.1**	5**	(-2)	0.0**	2**	-3.6**	-6.9**	-0.1	0.0	0.3	0.8	0.9
Overall		UX3000	126**	0.3**	0.1*	6**	(-2**)	0.1**	4**	-3.1**	-5.7**	-0.2	0.2**	0.3**	1.7**	1.9
Overall		UX3036	119*	0.2**	0.1**	6**	(-3**)	0.1**	5**	-2.9**	-5.0**	-0.2	0.0	0.2**	1.8**	2.0

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group price monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φSubset count

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 14. 2017 – 2018 water response experiment ANOVA mean squares across both UX3000 and UX3030 and parental lines and irrigation treatments

Experimental		Seed Characteristics							Water Response		Reproductive Period Attribution			
Source of Variation	df	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1	R3	R5	M
		kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	
Environment (ENV)	3	110027***	2891***	614***	16360***	3280***	85***	6090***	1450***	4044***	33891***	138427***	44587***	11
E _a	12	91	8.90	0.70	209.0	70.0	0.90	93.0	2.30	5.60	68.80	98.70	116.50	13
Irrigation Treatment (IRR_TRT)	1	6281**	115.2***	16.8*	7451***	1339**	39.8***	3497**	2465.4***	7597.8***	89.10	12.2**	44.4***	54
ENV:IRR_TRT	3	1852*	59.3**	3.10	1449**	185*	15.7***	1108**	371.2***	973.7***	50.50	11.1**	10.2**	46
E _b	12	375	5.70	1.90	158.0	44.0	1.40	138.0	0.80	0.20	158.60	1.20	1.40	63
STRAIN	440	477***	13.2***	0.5***	66***	29***	0.4***	30***	3.5***	5.4***	39.9***	7.9***	9.6***	23
ENV:STRAIN	1316	74***	1.02***	0.32***	6.2***	2.1***	0.05***	4.8***	0.78***	1.98***	3.50***	2.49***	1.44***	4
IRR_TRT:STRAIN	440	46***	0.93***	0.20	3.9***	0.9***	0.04***	3.3***	0.37***	1.30***	1.85***	1.15***	0.82***	3
ENV:IRR_TRT:STRAIN	1316	49***	0.71***	0.21*	4.8***	1.2***	0.04***	4.0***	0.37***	1.02***	1.65***	1.03***	0.74***	4
E _c	3929	37	0.51	0.19	1.5	0.4	0.01	1.1	0.25	0.58	0.89	0.60	0.36	2

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 15. 2017–2018 water response experiment ANOVA mean squares for the UX3000 RIL population across irrigation treatments

Experimental		Seed Characteristics							Water Response		Reproductive Period Attrib		
Source of Variation	df	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1	R3	R5
		kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days
Environment (ENV)	3	67824***	1525***	314**	8840***	1520***	39***	2940***	742***	313***	16300***	74209***	25093***
E _a	12	72	7.50	0.60	104.00	41.0	0.40	52.0	2.70	0.10	57.20	72.50	71.80
Irrigation Treatment (IRR_TRT)	1	3530***	86.1***	10.2*	3793***	581**	18.6**	1607**	1401.4***	54854.6***	43.20	30.7***	32.9***
ENV:IRR_TRT	3	1624**	37.3**	3.50	637*	72.0	7.2*	441.0	209.9***	7259.8***	17.30	14.9***	2.8*
E _b	12	149	5.10	1.70	173.00	35.0	1.50	154.0	0.30	0.00	113.30	0.30	0.70
STRAIN	236	377***	12.7***	0.4***	55***	19***	0.3***	22***	3.0***	5.6***	38.6***	9.0***	9.2***
ENV:STRAIN	708	64***	0.89***	0.30***	5.4***	1.8***	0.04***	4.0***	0.73***	3.56***	3.30***	2.49***	1.29***
IRR_TRT:STRAIN	236	46**	0.77***	0.21	2.9***	0.7***	0.02***	2.4***	0.36***	2.23***	1.90***	1.28***	0.89***
ENV:IRR_TRT:STRAIN	708	49***	0.70***	0.20	4.6***	1.1***	0.03***	4.0***	0.37***	1.78***	1.71***	1.14***	0.76***
E _c	2085	35	0.43	0.19	0.60	0.3	0.01	0.4	0.24	0.37	0.68	0.54	0.32

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 16. 2017–2018 water response experiment ANOVA mean squares for the UX3036 RIL population across irrigation treatments

Experimental		Seed Characteristics							Water Response		Reproductive Period Attril		
Source of Variation	df	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1	R3	R5
		kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days
Environment (ENV)	3	43692***	1371***	304***	7630***	1810***	47***	3230***	711***	1809***	17744***	64218***	19527***
E _a	12	60	8.40	0.60	137.0	33.0	0.7	6.0	0.90	1.80	26.30	31.10	49.20
Irrigation Treatment (IRR_TRT)	1	2758*	33.3***	6.7***	3652***	778***	21.2***	1903***	1066.4***	3057.0***	46.00	0.70	13.0***
ENV:IRR_TRT	3	406	28.5***	1.7*	831***	141**	8.5***	694***	164.3***	377.4***	35.70	0.80	6.7***
E _b	12	313	1.10	0.30	29.0	16.0	0.1	13.0	1.70	0.30	36.90	0.60	0.30
STRAIN	204	523***	12.9***	0.6***	74***	35***	0.4***	33***	3.6***	5.8***	26.9***	6.5***	9.2***
ENV:STRAIN	608	79***	1.16***	0.32***	6.6***	2.1***	0.06***	5.3***	0.81***	1.72***	2.99***	2.49***	1.47***
IRR_TRT:STRAIN	204	47*	1.11***	0.18	5.1***	1.0***	0.05***	4.3***	0.36***	1.07***	1.79***	0.90**	0.72***
ENV:IRR_TRT:STRAIN	608	49***	0.69**	0.22*	5.0***	1.1***	0.04***	3.8***	0.35***	0.90***	1.58***	0.89**	0.73***
E _c	1821	38	0.56	0.19	1.9	0.5	0.0	1.7	0.26	0.53	1.10	0.65	0.38

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 17. Overall Pearson correlations coefficients of agronomic means across populations and environments in the 2018 water response experiment; correlation coefficients representative of the irrigated treatment above the diagonal and correlation coefficients of rainfed treatment listed below the diagonal

	Seed Characteristics							Water Response		Reproductive Period Attributes					Plant Characteristics	
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8§	Height	Lodging
Yield		0.36**	-0.44**	-0.17**	0.43**	0.07**	0.01	0.50**	0.37**	0.31**	0.26**	0.23**	-0.01	-0.23**	-0.16**	-0.38**
Weight	0.34**		-0.37**	0.36**	0.13**	0.48**	0.42**	0.20**	0.28**	0.14**	-0.62**	-0.60**	-0.23**	-0.40**	-0.11**	-0.20**
Quality	-0.40**	-0.27**		0.08**	-0.36**	-0.12**	-0.09**	0.00	0.08**	-0.23**	-0.25**	-0.25**	0.14**	0.35**	0.10**	0.17**
Protein	-0.27**	0.24**	0.09**		-0.51**	0.82**	0.86**	0.22**	0.28**	-0.28**	-0.65**	-0.60**	-0.32**	-0.22**	0.19**	0.15**
Oil	0.40**	-0.02	-0.31**	-0.72**		0.00	-0.17**	0.01	-0.05**	0.28**	0.25**	0.22**	-0.10**	-0.32**	-0.33**	-0.27**
EPV	-0.18**	0.23**	0.01	0.91**	-0.43**		0.92**	0.26**	0.31**	-0.13**	-0.58**	-0.54**	-0.40**	-0.43**	0.00	-0.01
MPP	-0.21**	0.20**	0.06**	0.92**	-0.52**	0.97**		0.21**	0.24**	-0.16**	-0.52**	-0.47**	-0.34**	-0.33**	0.09**	0.06**
WP	0.34**	0.42**	-0.11**	0.55**	-0.25**	0.54**	0.50**		0.89**	-0.38**	-0.49**	-0.53**	-0.49**	-0.37**	-0.14**	-0.21**
RWP	0.26**	0.48**	-0.04*	0.53**	-0.30**	0.49**	0.46**	0.90**		-0.14**	-0.67**	-0.70**	-0.37**	-0.38**	-0.18**	-0.30**
R1	0.41**	0.07**	-0.29**	-0.50**	0.44**	-0.44**	-0.44**	-0.50**	-0.29**		0.68**	0.73**	0.65**	0.14**	-0.06**	-0.31**
R3	0.07**	-0.69**	-0.28**	-0.60**	0.42**	-0.50**	-0.48**	-0.80**	-0.89**	0.63**		0.94**	0.83**	0.78**	0.33**	0.19**
R5	0.04±	-0.67**	-0.29**	-0.52**	0.37**	-0.44**	-0.42**	-0.79**	-0.86**	0.69**	0.94**		0.87**	0.81**	0.36**	0.16**
Maturity	-0.03*	-0.29**	0.20**	-0.43**	0.09**	-0.46**	-0.41**	-0.76**	-0.61**	0.59**	0.78**	0.83**		0.85**	0.24**	-0.04**
R1-R8	-0.30**	-0.40**	0.44**	-0.21**	-0.17**	-0.29**	-0.22**	-0.62**	-0.57**	0.09**	0.73**	0.76**	0.86**		0.35**	0.17**
Height	-0.27**	-0.20**	0.10**	0.32**	-0.36**	0.25**	0.28**	0.04*	-0.02	-0.21**	0.34**	0.39**	0.10**	0.26**		0.39**
Lodging	-0.39**	-0.23**	0.13**	0.19**	-0.24**	0.13**	0.16**	-0.12**	-0.22**	-0.32**	0.32**	0.28**	-0.02	0.18**	0.40**	

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of month settlement price monthly average mean over the October 2013 – October 2018 time period. (retrieved from

<https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 18. Overall Pearson correlations coefficients of agronomic means across environments within the 20 response experiment for the UX3000 population; correlation coefficients representative of the irrigated treatment on the diagonal and correlation coefficients of rainfed treatment listed below the diagonal

	Seed Characteristics							Water Response		Reproductive Period Attributes					Plant Characteristics	
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8§	Height	Lodging
Yield		0.39**	-0.46**	-0.22**	0.48**	0.01	-0.03	0.49**	0.35**	0.31**	0.26**	0.19**	0.00	-0.21**	-0.21**	-0.32**
Weight	0.41**		-0.37**	0.40**	0.10**	0.50**	0.45**	0.23**	0.28**	0.09**	-0.64**	-0.62**	-0.28**	-0.42**	-0.14**	-0.16**
Quality	-0.40**	-0.24**		0.03	-0.36**	-0.16**	-0.14**	-0.02	0.08**	-0.23**	-0.28**	-0.29**	0.12**	0.31**	0.09**	0.09**
Protein	-0.33**	0.24**	0.09**		-0.54**	0.85**	0.87**	0.15**	0.19**	-0.30**	-0.65**	-0.59**	-0.38**	-0.29**	0.23**	0.24**
Oil	0.43**	-0.09**	-0.29**	-0.72**		-0.08**	-0.24**	0.04	-0.05*	0.27**	0.27**	0.21**	-0.04*	-0.24**	-0.35**	-0.22**
EPV	-0.24**	0.23**	-0.07**	0.90**	-0.44**		0.91**	0.20**	0.22**	-0.18**	-0.59**	-0.55**	-0.44**	-0.46**	0.07**	0.13**
MPP	-0.27**	0.20**	-0.02	0.91**	-0.51**	0.97**		0.13**	0.12**	-0.20**	-0.51**	-0.46**	-0.37**	-0.35**	0.18**	0.20**
WP	0.28**	0.49**	-0.19**	0.55**	-0.28**	0.50**	0.46**		0.88**	-0.39**	-0.44**	-0.52**	-0.53**	-0.44**	-0.24**	-0.26**
RWP	0.21**	0.53**	-0.08**	0.53**	-0.36**	0.45**	0.41**	0.89**		-0.13**	-0.64**	-0.69**	-0.40**	-0.44**	-0.25**	-0.35**
R1	0.46**	0.06**	-0.20**	-0.49**	0.37**	-0.44**	-0.43**	-0.49**	-0.28**		0.66**	0.72**	0.66**	0.19**	0.06**	-0.22**
R3	0.02	-0.73**	-0.28**	-0.60**	0.48**	-0.48**	-0.46**	-0.82**	-0.90**	0.62**		0.95**	0.84**	0.82**	0.44**	0.30**
R5	-0.03	-0.71**	-0.29**	-0.52**	0.40**	-0.43**	-0.41**	-0.83**	-0.88**	0.69**	0.94**		0.89**	0.85**	0.47**	0.28**
Maturity	0.00	-0.30**	0.27**	-0.46**	0.10**	-0.47**	-0.41**	-0.79**	-0.62**	0.62**	0.78**	0.84**		0.87**	0.29**	-0.02
R1-R8	-0.28**	-0.42**	0.46**	-0.29**	-0.10**	-0.32**	-0.25**	-0.70**	-0.61**	0.17**	0.75**	0.80**	0.88**		0.34**	0.12**
Height	-0.31**	-0.26**	0.09**	0.36**	-0.32**	0.33**	0.35**	0.04±	0.04±	-0.06**	0.39**	0.44**	0.15**	0.23**		0.43**
Lodging	-0.30**	-0.23**	0.13**	0.19**	-0.24**	0.13**	0.16**	-0.12**	-0.22**	-0.32**	0.32**	0.28**	-0.02	0.18**	0.40**	

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average mean over the October 2013 – October 2018 time period. (retrieved from

<https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity of the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 19. Overall Pearson correlations coefficients of agronomic means across environments within the 20 response experiment for the UX3036 population; correlation coefficients representative of the irrigated treatment are listed above the diagonal and correlation coefficients of rainfed treatment listed below the diagonal

	Seed Characteristics							Water Response		Reproductive Period Attributes					Plant Characteristics	
	Yield	Weight	Quality [†]	Protein	Oil	EPV [#]	MPP [#]	WP [¶]	RWP [¶]	R1 [§]	R3 [§]	R5 [§]	Maturity [§]	R1-R8 [§]	Height	Lodging
Yield		0.37**	-0.44**	-0.13**	0.36**	0.07**	0.02	0.52**	0.40**	0.29**	0.28**	0.25**	-0.02	-0.24**	-0.06*	-0.38**
Weight	0.33**		-0.36**	0.36**	0.20**	0.53**	0.46**	0.20**	0.30**	0.20**	-0.60**	-0.56**	-0.21**	-0.42**	-0.10**	-0.27**
Quality	-0.42**	-0.30**		0.10**	-0.40**	-0.13**	-0.07**	-0.02	0.03	-0.23**	-0.21**	-0.20**	0.19**	0.41**	0.11**	0.25**
Protein	-0.23**	0.24**	0.09**		-0.54**	0.82**	0.86**	0.25**	0.32**	-0.30**	-0.67**	-0.62**	-0.27**	-0.13**	0.20**	0.15**
Oil	0.34**	0.07**	-0.32**	-0.74**		-0.02	-0.18**	-0.03	-0.08**	0.24**	0.24**	0.22**	-0.14**	-0.37**	-0.28**	-0.22**
EPV	-0.16**	0.30**	0.04	0.91**	-0.46**		0.94**	0.27**	0.34**	-0.17**	-0.63**	-0.59**	-0.39**	-0.38**	0.05**	0.02
MPP	-0.19**	0.25**	0.07**	0.92**	-0.56**	0.97**		0.23**	0.28**	-0.18**	-0.57**	-0.52**	-0.32**	-0.28**	0.10**	0.06*
WP	0.40**	0.38**	-0.06*	0.54**	-0.26**	0.54**	0.51**		0.91**	-0.40**	-0.50**	-0.53**	-0.45**	-0.30**	0.00	-0.13**
RWP	0.32**	0.47**	-0.02	0.52**	-0.28**	0.51**	0.47**	0.91**		-0.18**	-0.70**	-0.71**	-0.34**	-0.32**	-0.07**	-0.20**
R1	0.34**	0.12**	-0.38**	-0.54**	0.46**	-0.49**	-0.50**	-0.54**	-0.35**		0.74**	0.78**	0.65**	0.12**	-0.12**	-0.33**
R3	0.12**	-0.66**	-0.27**	-0.59**	0.38**	-0.55**	-0.52**	-0.78**	-0.88**	0.69**		0.93**	0.82**	0.76**	0.24**	0.10**
R5	0.10**	-0.61**	-0.26**	-0.52**	0.32**	-0.50**	-0.47**	-0.76**	-0.84**	0.75**	0.93**		0.86**	0.80**	0.29**	0.07*
Maturity	-0.07**	-0.27**	0.16**	-0.41**	0.08**	-0.47**	-0.42**	-0.74**	-0.61**	0.57**	0.77**	0.82**		0.83**	0.17**	-0.07**
R1-R8	-0.31**	-0.41**	0.44**	-0.14**	-0.21**	-0.25**	-0.18**	-0.53**	-0.51**	0.03	0.72**	0.76**	0.84**		0.32**	0.15**
Height	-0.18**	-0.22**	0.12**	0.30**	-0.32**	0.24**	0.26**	0.13**	0.04	-0.27**	0.32**	0.39**	0.07**	0.27**		0.30**
Lodging	-0.42**	-0.23**	0.13**	0.19**	-0.24**	0.13**	0.16**	-0.12**	-0.22**	-0.32**	0.32**	0.28**	-0.02	0.18**	0.40**	

[†]Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

[#]Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average mean over the October 2013 – October 2018 time period. (retrieved from

<https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

[¶] Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

[§]Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity the main stem (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

[‡]Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 20. Overall Pearson correlations coefficients of agronomic means across populations and environments in the 2018 water response experiment for reproductive timing intervals; correlation coefficients representative of irrigated treatment above the diagonal and correlation coefficients of rainfed treatment listed below the diagonal

	Seed Characteristics							Water Response		Reproductive Period Intervals		
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1-R3§	R3-R5§	R5-R8§
Yield		0.36**	-0.44**	-0.17**	0.43**	0.07**	0.01	0.50**	0.37**	0.33**	-0.28**	-0.05**
Weight	0.34**		-0.37**	0.36**	0.13**	0.48**	0.42**	0.20**	0.28**	-0.62**	0.56**	-0.21**
Quality	-0.40**	-0.27**		0.08**	-0.36**	-0.12**	-0.09**	0.00	0.08**	-0.24**	0.21**	-0.17**
Protein	-0.27**	0.24**	0.09**		-0.51**	0.82**	0.86**	0.22**	0.28**	-0.68**	0.61**	-0.15**
Oil	0.40**	-0.02	-0.31**	-0.72**		0.00	-0.17**	0.01	-0.05**	0.26**	-0.23**	-0.18**
EPV	-0.18**	0.23**	0.01	0.91**	-0.43**		0.92**	0.26**	0.31**	-0.60**	0.54**	-0.26**
MPP	-0.21**	0.20**	0.06**	0.92**	-0.52**	0.97**		0.21**	0.24**	-0.54**	0.49**	-0.19**
WP	0.34**	0.42**	-0.11**	0.55**	-0.25**	0.54**	0.50**		0.89**	-0.37**	0.35**	-0.46**
RWP	0.26**	0.48**	-0.04*	0.53**	-0.30**	0.49**	0.46**	0.90**		-0.61**	0.53**	-0.46**
R1-R3	0.12**	-0.64**	-0.24**	-0.62**	0.44**	-0.52**	-0.50**	-0.72**	-0.87**		-0.91**	0.39**
R3-R5	-0.10**	0.59**	0.22**	0.57**	-0.41**	0.47**	0.45**	0.66**	0.76**	-0.90**		-0.45**
R5-R8	-0.21**	-0.19**	-0.14**	0.09**	-0.21**	-0.03	-0.01	-0.41**	-0.33**	0.23**	-0.30**	
Height	-0.27**	-0.20**	0.10**	0.32**	-0.36**	0.25**	0.28**	0.04*	-0.02	-0.21**	0.34**	0.39**
Lodging	-0.39**	-0.23**	0.13**	0.19**	-0.24**	0.13**	0.16**	-0.12**	-0.22**	-0.32**	0.32**	0.28**

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group of monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean> 11/28/18)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive (R1-R8) period

§Reproductive stage interval in days

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

± indicates treatment significance at $\alpha = 0.10$

* indicates treatment significance at $\alpha = 0.05$

** indicates treatment significance at $\alpha = 0.01$

Table 21. Genetic variances (σ_G^2) and least square mean Pearson correlation coefficients (r) with 95% confidence intervals for agronomic traits across populations during 2017-2018 water response experiment; treatments were denoted by following subscripts (1 = irrigated treatment, 2 = rainfed treatment, 3 = response between treatments, 4 = non-response to irrigation). Using tables in Rosielle and Hamblin (1981) K_G represents the ratio of genetic variance in stress over non-stress

	Seed Characteristics							Water Response		Reproductive Period Attributes			
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§
	kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days
σ_G^2 1	108,701 (91,268, 129,244)	0.66 (0.56, 0.76)	0.01 (0.00, 0.01)	32.39 (27.87, 37.74)	16.87 (14.71, 19.44)	0.00* (0.00, 0.00)	10.32* (8.63, 12.31)	0.98 (0.80, 1.20)	1.00* (0.72, 1.33)	2.20 (1.90, 2.55)	0.95* (0.79, 1.13)	0.94 (0.79, 1.11)	13.70 (12.02, 15.70)
σ_G^2 2	89,306 (74,822, 106,383)	0.61 (0.53, 0.70)	0.00 (0.00, 0.01)	32.18 (27.37, 37.83)	15.20 (13.12, 17.66)	0.00* (0.00, 0.01)	16.32* (13.51, 19.62)	1.16 (0.97, 1.39)	1.93* (1.53, 2.39)	2.30 (2.00, 2.67)	0.35* (0.22, 0.49)	0.98 (0.83, 1.16)	13.03 (11.42, 14.94)
σ_G^2 3	0 (0, 6,420)	0.04 (0.01, 0.07)	0.00 (0.00, 0.00)	0.00 (0.00, 0.46)	0.00 (0.00, 0.11)	0.00 (0.00, 0.00)	0.00 (0.00, 0.44)	0.01 (0.00, 0.12)	0.51 (0.20, 0.86)	0.04 (0.00, 0.11)	0.00 (0.00, 0.05)	0.00 (0.00, 0.07)	0.00 (0.00, 0.03)
σ_G^2 4	100,831 (86,443, 117,829)	0.62 (0.54, 0.72)	0.01 (0.00, 0.01)	33.33 (28.93, 38.53)	16.24 (14.19, 18.67)	0.00 (0.00, 0.00)	13.99 (11.98, 16.37)	1.10 (0.93, 1.30)	1.43 (1.15, 1.75)	2.24 (1.95, 2.58)	0.66 (0.54, 0.80)	0.96 (0.82, 1.11)	13.43 (11.81, 15.35)
r_{12}	0.83 (0.80, 0.86)	0.91 (0.90, 0.93)	0.41 (0.33, 0.48)	0.89 (0.87, 0.91)	0.95 (0.94, 0.96)	0.88 (0.86, 0.90)	0.84 (0.82, 0.87)	0.79 (0.75, 0.82)	0.74 (0.67, 0.76)	0.87 (0.84, 0.89)	0.59 (0.53, 0.65)	0.73 (0.68, 0.77)	0.91 (0.95, 0.96)
r_{13}	0.37 (0.29, 0.45)	0.22 (0.14, 0.32)	0.68 (0.62, 0.72)	0.28 (0.20, 0.37)	0.23 (0.15, 0.32)	0.11 (0.02, 0.21)	0.02 (-0.07, 0.12)	0.12 (0.02, 0.20)	0.16 (0.07, 0.25)	0.37 (0.29, 0.45)	0.59 (0.53, 0.65)	0.04 (-0.05, 0.14)	0.03 (-0.15, 0.04)
r_{14}	0.96 (0.95, 0.97)	0.98 (0.97, 0.98)	0.88 (0.85, 0.89)	0.97 (0.97, 0.98)	0.99 (0.98, 0.99)	0.97 (0.96, 0.97)	0.95 (0.94, 0.96)	0.94 (0.92, 0.95)	0.92 (0.90, 0.93)	0.97 (0.96, 0.97)	0.91 (0.89, 0.93)	0.91 (0.89, 0.92)	0.97 (0.99, 0.99)
r_{23}	-0.21 (-0.29, -0.11)	-0.19 (-0.28, -0.10)	-0.40 (-0.48, -0.32)	-0.18 (-0.26, -0.08)	-0.09 (-0.18, 0.00)	-0.37 (-0.44, -0.28)	-0.52 (-0.58, -0.44)	-0.51 (-0.59, -0.46)	-0.54 (-0.62, -0.50)	-0.13 (-0.22, -0.04)	-0.30 (-0.38, -0.21)	-0.65 (-0.71, -0.60)	-0.40 (-0.43, -0.26)
r_{24}	0.95 (0.95, 0.96)	0.98 (0.97, 0.98)	0.80 (0.76, 0.83)	0.97 (0.97, 0.98)	0.99 (0.98, 0.99)	0.97 (0.97, 0.98)	0.97 (0.96, 0.97)	0.95 (0.94, 0.96)	0.94 (0.93, 0.95)	0.96 (0.96, 0.97)	0.87 (0.85, 0.89)	0.95 (0.94, 0.96)	0.98 (0.99, 0.99)
r_{34}	0.09 (0.01, 0.19)	0.02 (-0.07, 0.12)	0.24 (0.14, 0.32)	0.05 (-0.03, 0.16)	0.07 (-0.02, 0.17)	-0.14 (-0.23, -0.04)	-0.28 (-0.36, -0.19)	-0.23 (-0.33, -0.16)	-0.23 (-0.34, -0.16)	0.13 (0.04, 0.22)	0.21 (0.11, 0.29)	-0.38 (-0.46, -0.30)	-0.20 (-0.30, -0.12)
K_G^2	0.82	0.93	0.55	0.99	0.90	1.40±	1.58±	1.18	1.92±	1.05	0.37±	1.05	0.95

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average for October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive period. Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

* indicates treatment significance at $\alpha = 0.05$

± indicates significant difference from 1.0 at $\alpha = 0.05$

Table 22. Genetic variances (σ_G^2) and least square mean Pearson correlation coefficients (r) with 95% confidence intervals for agronomic traits for the UX3000 population during 2017-2018 water response experiment; treatments were the following subscripts (1 = irrigated treatment, 2 = rainfed treatment, 3 = response between treatments, 4 = no response). Using tables in Rosielle and Hamblin (1981) K_G represents the ratio of genetic variance in stress environments

	Seed Characteristics							Water Response		Reproductive Period Attributes					K_G
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8§	
	kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days	
σ_{G1}^2	102,575 (80,058, 131,021)	0.59 (0.48, 0.74)	0.01 (0.00, 0.02)	28.11 (22.78, 34.87)	10.21 (8.34, 12.60)	0.00 (0.00, 0.00)	7.96 (6.14, 10.26)	1.03 (0.76, 1.36)	0.62 (0.25, 1.05)	2.29 (1.87, 2.83)	1.23* (0.97, 1.55)	0.98 (0.78, 1.24)	14.35 (11.93, 17.44)	5.93 (4.79, 7.37)	(52.00, 52.00)
σ_{G2}^2	70,737 (54,734, 90,907)	0.57 (0.47, 0.71)	0.00 (0.00, 0.01)	24.78 (19.47, 31.46)	9.84 (7.90, 12.30)	0.00 (0.00, 0.00)	10.10 (7.33, 13.56)	0.98 (0.76, 1.26)	0.76 (0.37, 1.23)	2.14 (1.75, 2.65)	0.35* (0.15, 0.57)	1.01 (0.80, 1.28)	14.47 (12.04, 17.56)	6.34 (5.17, 7.82)	(50.00, 50.00)
σ_{G3}^2	0 (0, 11,274)	0.01 (0.00, 0.06)	0.00 (0.00, 0.01)	0.00 (0.00, 0.54)	0.00 (0.00, 0.15)	0.00 (0.00, 0.00)	0.00 (0.00, 0.41)	0.01 (0.00, 0.16)	0.47 (0.02, 1.00)	0.04 (0.00, 0.14)	0.00 (0.00, 0.13)	0.02 (0.00, 0.13)	0.00 (0.00, 0.05)	0.00 (0.00, 0.10)	(0.00, 0.00)
σ_{G4}^2	87,455 (70,514, 108,927)	0.58 (0.48, 0.71)	0.00 (0.00, 0.01)	27.52 (22.54, 33.85)	10.25 (8.42, 12.57)	0.00 (0.00, 0.00)	9.95 (7.94, 12.50)	1.04 (0.82, 1.31)	0.66 (0.38, 1.00)	2.20 (1.81, 2.69)	0.80 (0.61, 1.03)	0.99 (0.80, 1.22)	14.51 (12.11, 17.55)	6.26 (5.15, 7.66)	(52.00, 52.00)
r_{12}	0.77 (0.71, 0.82)	0.92 (0.90, 0.94)	0.20 (0.08, 0.32)	0.89 (0.86, 0.92)	0.91 (0.88, 0.93)	0.87 (0.83, 0.89)	0.80 (0.75, 0.84)	0.73 (0.66, 0.78)	0.57 (0.48, 0.65)	0.83 (0.78, 0.87)	0.43 (0.32, 0.53)	0.53 (0.43, 0.61)	0.90 (0.95, 0.97)	0.96 (0.88, 0.92)	(0.00, 0.00)
r_{13}	0.53 (0.43, 0.61)	0.19 (0.06, 0.31)	0.90 (0.87, 0.92)	0.34 (0.22, 0.45)	0.25 (0.12, 0.36)	0.11 (-0.02, 0.23)	0.11 (-0.01, 0.24)	0.26 (0.14, 0.38)	0.38 (0.27, 0.49)	0.55 (0.46, 0.64)	0.64 (0.56, 0.71)	0.10 (-0.03, 0.22)	-0.27 (-0.48, -0.27)	-0.38 (-0.39, -0.15)	(-0.00, -0.00)
r_{14}	0.95 (0.94, 0.96)	0.98 (0.97, 0.98)	0.93 (0.91, 0.94)	0.97 (0.97, 0.98)	0.98 (0.97, 0.98)	0.96 (0.95, 0.97)	0.94 (0.93, 0.95)	0.92 (0.90, 0.94)	0.87 (0.84, 0.90)	0.96 (0.95, 0.97)	0.87 (0.84, 0.90)	0.80 (0.75, 0.84)	0.97 (0.99, 0.99)	0.99 (0.96, 0.98)	(0.00, 0.00)
r_{23}	-0.14 (-0.26, -0.01)	-0.21 (-0.33, -0.08)	-0.25 (-0.37, -0.13)	-0.12 (-0.24, 0.01)	-0.18 (-0.30, -0.06)	-0.40 (-0.51, -0.29)	-0.50 (-0.59, -0.40)	-0.47 (-0.57, -0.37)	-0.54 (-0.63, -0.44)	0.00 (-0.13, 0.12)	-0.42 (-0.52, -0.31)	-0.79 (-0.84, -0.74)	-0.66 (-0.69, -0.54)	-0.62 (-0.73, -0.58)	(-0.00, -0.00)
r_{24}	0.93 (0.91, 0.95)	0.98 (0.97, 0.98)	0.56 (0.46, 0.64)	0.97 (0.96, 0.98)	0.98 (0.97, 0.98)	0.97 (0.96, 0.98)	0.96 (0.94, 0.97)	0.94 (0.92, 0.95)	0.90 (0.87, 0.92)	0.95 (0.93, 0.96)	0.82 (0.77, 0.85)	0.93 (0.91, 0.95)	0.98 (0.99, 0.99)	0.99 (0.98, 0.99)	(0.00, 0.00)
r_{34}	0.23 (0.11, 0.35)	-0.01 (-0.14, 0.12)	0.67 (0.59, 0.73)	0.12 (-0.01, 0.24)	0.03 (-0.09, 0.16)	-0.16 (-0.29, -0.04)	-0.23 (-0.35, -0.10)	-0.13 (-0.25, 0.00)	-0.11 (-0.24, 0.01)	0.31 (0.19, 0.42)	0.18 (0.05, 0.30)	-0.51 (-0.60, -0.41)	-0.50 (-0.60, -0.42)	-0.52 (-0.59, -0.40)	(-0.00, -0.00)
K_G^2	0.69	0.97	0.46	0.88	0.96	1.32	1.27	0.95	1.24	0.94	0.28±	1.03	1.01	1.07	(0.00, 0.00)

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price montly average mean over October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and R8

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

* indicates treatment significance at $\alpha = 0.05$

± indicates significant difference from 1.0 at $\alpha = 0.05$

Table 23. Genetic variances (σ_G^2) and least square mean Pearson correlation coefficients (r) with 95% confidence intervals for agronomic traits for the UX3036 population during 2017-2018 water response experiment; treatments were the following subscripts (1 = irrigated treatment, 2 = rainfed treatment, 3 = response between treatments, 4 = no response). Using tables in Rosielle and Hamblin (1981) K_G represents the ratio of genetic variance in stress environments

	Seed Characteristics							Water Response		Reproductive Period Attributes				
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8§
	kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
σ_{G1}^2	99,478 (74,929, 130,968)	0.73 (0.58, 0.92)	0.00 (0.00, 0.02)	35.44 (28.17, 44.83)	21.16 (17.23, 26.24)	0.00 (0.00, 0.00)	10.19 (7.64, 13.46)	0.86 (0.60, 1.18)	1.03* (0.64, 1.52)	1.34 (1.05, 1.70)	0.53 (0.35, 0.75)	0.88 (0.67, 1.15)	13.79 (11.32, 16.98)	7.60 (6.17, 9.49)
σ_{G2}^2	102,358 (78,280, 133,251)	0.62 (0.50, 0.78)	0.01 (0.00, 0.02)	35.96 (28.27, 45.88)	19.42 (15.65, 24.28)	0.00 (0.00, 0.01)	17.26 (12.98, 22.74)	1.30 (0.99, 1.69)	2.10* (1.52, 2.84)	1.59 (1.26, 2.00)	0.35 (0.16, 0.55)	0.99 (0.77, 1.28)	11.97 (9.80, 14.79)	5.77 (4.61, 7.27)
σ_{G3}^2	0 (0, 9,345)	0.09 (0.04, 0.15)	0.00 (0.00, 0.01)	0.00 (0.00, 1.90)	0.00 (0.00, 0.37)	0.00 (0.00, 0.00)	0.00 (0.00, 2.16)	0.00 (0.00, 0.16)	0.31 (0.00, 0.78)	0.04 (0.00, 0.15)	0.00 (0.00, 0.05)	0.00 (0.00, 0.06)	0.00 (0.00, 0.17)	0.10 (0.00, 0.47)
σ_{G4}^2	103,920 (81,839, 132,379)	0.66 (0.53, 0.82)	0.01 (0.00, 0.01)	36.42 (29.41, 45.48)	20.42 (16.69, 25.23)	0.00 (0.00, 0.00)	14.03 (11.02, 17.91)	1.15 (0.88, 1.48)	1.67 (1.24, 2.22)	1.45 (1.17, 1.82)	0.47 (0.32, 0.65)	0.95 (0.75, 1.20)	12.86 (10.59, 15.81)	6.67 (5.43, 8.26)
r_{12}	0.83 (0.78, 0.87)	0.89 (0.86, 0.92)	0.47 (0.35, 0.57)	0.88 (0.85, 0.91)	0.95 (0.94, 0.96)	0.82 (0.78, 0.87)	0.80 (0.75, 0.85)	0.81 (0.74, 0.84)	0.76 (0.66, 0.79)	0.79 (0.73, 0.84)	0.54 (0.44, 0.64)	0.71 (0.63, 0.77)	0.91 (0.95, 0.97)	0.96 (0.89, 0.93)
r_{13}	0.29 (0.17, 0.42)	0.27 (0.15, 0.40)	0.52 (0.41, 0.61)	0.25 (0.14, 0.39)	0.25 (0.12, 0.38)	0.15 (0.02, 0.29)	-0.05 (-0.17, 0.11)	0.02 (-0.13, 0.14)	0.04 (-0.10, 0.17)	0.30 (0.17, 0.42)	0.65 (0.56, 0.72)	0.03 (-0.11, 0.17)	0.28 (0.10, 0.36)	0.23 (0.14, 0.40)
r_{14}	0.96 (0.94, 0.97)	0.97 (0.97, 0.98)	0.86 (0.82, 0.89)	0.97 (0.96, 0.98)	0.99 (0.98, 0.99)	0.95 (0.94, 0.96)	0.94 (0.92, 0.95)	0.94 (0.92, 0.95)	0.92 (0.88, 0.93)	0.94 (0.93, 0.96)	0.91 (0.88, 0.93)	0.90 (0.87, 0.92)	0.98 (0.99, 0.99)	0.99 (0.97, 0.98)
r_{23}	-0.29 (-0.40, -0.15)	-0.19 (-0.31, -0.04)	-0.51 (-0.61, -0.40)	-0.24 (-0.34, -0.08)	-0.05 (-0.19, 0.09)	-0.44 (-0.53, -0.31)	-0.63 (-0.70, -0.52)	-0.57 (-0.68, -0.51)	-0.62 (-0.73, -0.57)	-0.35 (-0.47, -0.23)	-0.29 (-0.41, -0.15)	-0.68 (-0.75, -0.60)	-0.14 (-0.19, 0.08)	-0.06 (-0.27, 0.00)
r_{24}	0.96 (0.94, 0.97)	0.97 (0.96, 0.98)	0.86 (0.81, 0.89)	0.97 (0.96, 0.98)	0.99 (0.98, 0.99)	0.96 (0.95, 0.97)	0.96 (0.95, 0.97)	0.96 (0.95, 0.97)	0.95 (0.93, 0.96)	0.95 (0.93, 0.96)	0.85 (0.80, 0.88)	0.95 (0.93, 0.96)	0.98 (0.99, 0.99)	0.99 (0.97, 0.98)
r_{34}	0.00 (-0.13, 0.15)	0.04 (-0.08, 0.19)	0.01 (-0.13, 0.14)	0.01 (-0.11, 0.17)	0.11 (-0.03, 0.24)	-0.17 (-0.28, -0.02)	-0.40 (-0.49, -0.25)	-0.32 (-0.47, -0.22)	-0.35 (-0.50, -0.26)	-0.03 (-0.17, 0.10)	0.27 (0.13, 0.39)	-0.41 (-0.52, -0.29)	0.07 (-0.05, 0.23)	0.09 (-0.06, 0.21)
K_G^2	1.03	0.85	1.54	1.01	0.92	1.39	1.69	1.51	2.03±	1.19	0.65	1.12	0.87	0.76

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price montly average mean over October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60, 11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive period

§ Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the interval between R1 and R8

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

* indicates treatment significance at $\alpha = 0.05$

± indicates significant difference from 1.0 at $\alpha = 0.05$

Table 24. Genetic (σ_G^2), genotypic by environment (σ_{GE}^2), and environmental variance (σ_E^2) estimations with 95% confidence intervals of agronomic traits across populations during 2017-2018 water response experiment; treatments are indicated through the following subscripts (1 = irrigated, 2 = rainfed, 3 = response between treatments, 4 = overall mean)

	Seed Characteristics							Water Response		Reproductive Period Attributes			
	Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§
	kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	\$ kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days
σ_G^2 1	108,701 (91,268, 129,244)	0.66 (0.56, 0.76)	0.01 (0.00, 0.01)	32.39 (27.87, 37.74)	16.87 (14.71, 19.44)	0.00* (0.00, 0.00)	10.32* (8.63, 12.31)	0.98 (0.80, 1.20)	1.00* (0.72, 1.33)	2.20 (1.90, 2.55)	0.95* (0.79, 1.13)	0.94 (0.79, 1.11)	13.70 (12.02, 15.70)
σ_G^2 2	89,306 (74,822, 106,383)	0.61 (0.53, 0.70)	0.00 (0.00, 0.01)	32.18 (27.37, 37.83)	15.20 (13.12, 17.66)	0.00* (0.00, 0.01)	16.32* (13.51, 19.62)	1.16 (0.97, 1.39)	1.93* (1.53, 2.39)	2.30 (2.00, 2.67)	0.35* (0.22, 0.49)	0.98 (0.83, 1.16)	13.03 (11.42, 14.94)
σ_G^2 3	0 (0, 6,420)	0.04 (0.01, 0.07)	0.00 (0.00, 0.00)	0.00 (0.00, 0.46)	0.00 (0.00, 0.11)	0.00 (0.00, 0.00)	0.00 (0.00, 0.44)	0.01 (0.00, 0.12)	0.51 (0.20, 0.86)	0.04 (0.00, 0.11)	0.00 (0.00, 0.05)	0.00 (0.00, 0.07)	0.00 (0.00, 0.03)
σ_G^2 4	100,831 (86,443, 117,829)	0.62 (0.54, 0.72)	0.01 (0.00, 0.01)	33.33 (28.93, 38.53)	16.24 (14.19, 18.67)	0.00 (0.00, 0.00)	13.99 (11.98, 16.37)	1.10 (0.93, 1.30)	1.43 (1.15, 1.75)	2.24 (1.95, 2.58)	0.66 (0.54, 0.80)	0.96 (0.82, 1.11)	13.43 (11.81, 15.35)
σ_{GE}^2 1	59,744 (47,607, 72,633)	0.22* (0.19, 0.27)	0.05* (0.03, 0.06)	13.11 (11.31, 15.05)	2.95 (2.47, 3.47)	0.00 (0.00, 0.00)	9.31* (8.17, 10.56)	1.50* (1.34, 1.68)	3.63* (3.27, 4.02)	0.50 (0.40, 0.61)	0.27* (0.18, 0.38)	0.18 (0.10, 0.26)	1.47 (1.28, 1.68)
σ_{GE}^2 2	54,854 (44,688, 65,761)	0.10* (0.07, 0.13)	0.02* (0.01, 0.03)	17.20 (14.60, 19.98)	5.06 (4.30, 5.88)	0.00 (0.00, 0.00)	15.62* (13.45, 17.97)	0.75* (0.61, 0.89)	2.69* (2.29, 3.12)	0.53 (0.44, 0.64)	0.66* (0.52, 0.81)	0.24 (0.16, 0.32)	1.73 (1.51, 1.96)
σ_{GE}^2 3	60,904 (43,862, 78,164)	0.19 (0.13, 0.26)	0.00 (0.00, 0.01)	33.81 (30.90, 36.96)	4.91 (4.15, 5.74)	0.00 (0.00, 0.00)	24.52 (22.10, 27.13)	1.08 (0.86, 1.29)	3.65 (3.09, 4.26)	0.14 (0.00, 0.30)	0.26 (0.17, 0.36)	0.06 (0.00, 0.14)	1.45 (1.18, 1.73)
σ_{GE}^2 4	43,751 (37,219, 50,774)	0.12 (0.10, 0.14)	0.03 (0.03, 0.04)	10.59 (9.37, 11.92)	3.21 (2.83, 3.62)	0.00 (0.00, 0.00)	7.73 (6.83, 8.71)	0.92 (0.82, 1.03)	2.41 (2.16, 2.67)	0.48 (0.42, 0.55)	0.40 (0.32, 0.49)	0.20 (0.15, 0.25)	1.16 (1.03, 1.30)
σ_E^2 1	441,605 (232,770, 1,372,453)	2.37 (0.94, 8.89)	0.61 (0.31, 1.97)	76.19 (33.04, 272.37)	21.11 (9.58, 73.33)	0.01 (0.00, 0.03)	30.11 (15.64, 94.71)	2.99 (1.77, 8.35)	7.94 (3.79, 26.60)	19.92 (5.94, 84.42)	75.26 (9.31, 659.31)	23.73 (3.31, 202.93)	61.28 (16.36, 269.20)
σ_E^2 2	436,822 (211,288, 1,454,822)	1.85 (0.81, 6.53)	0.47 (0.25, 1.50)	168.02 (65.12, 663.20)	34.02 (15.19, 119.55)	0.02 (0.01, 0.08)	87.46 (39.61, 304.21)	14.24 (4.83, 57.56)	37.36 (12.62, 150.88)	19.63 (5.79, 83.88)	73.49 (8.99, 632.57)	25.17 (3.39, 215.81)	67.63 (18.07, 297.23)
σ_E^2 3	331,901 (299,512, 414,270)	1.15 (1.00, 1.62)	0.43 (0.41, 0.47)	59.67 (31.43, 185.33)	16.47 (12.65, 31.50)	0.01 (0.01, 0.04)	53.61 (30.99, 153.06)	8.76 (4.40, 28.29)	21.62 (10.36, 72.25)	3.17 (2.91, 3.71)	1.67 (1.54, 2.04)	1.43 (1.31, 1.73)	5.49 (4.55, 8.84)
σ_E^2 4	351,402 (142,992, 1,301,909)	1.81 (0.62, 7.29)	0.44 (0.18, 1.62)	100.71 (32.80, 413.51)	22.18 (8.13, 87.50)	0.01 (0.00, 0.04)	41.93 (16.50, 158.24)	6.36 (2.14, 25.71)	17.09 (5.48, 70.47)	19.01 (5.15, 83.16)	73.97 (8.74, 645.05)	24.15 (3.02, 209.10)	63.01 (16.10, 280.42)

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price monthly average meal 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60, 11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water during reproductive period

§ Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the date at which greater than 95% of pods have reached maturity on the main stem (Maturity), and the date at which greater than 95% of pods have reached maturity on the main stem (Maturity)

‡Lodging is rated at maturity according to a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

* indicates treatment significance at $\alpha = 0.05$

± indicates significant difference from 1.0 at $\alpha = 0.05$

Table 25. Agronomic trait broad sense heritability (H) on an entry mean basis and 95% confidence interval for irrigated, 2 = rainfed, 4 = overall response)

Population	Subset	Seed Characteristics							Water Response		Reproductive Period Attributes			
		Yield kg ha ⁻¹	Weight g/100	Quality† 1-5 scale	Protein g kg ⁻¹	Oil g kg ⁻¹	EPV# \$ kg ⁻¹	MPP# g kg ⁻¹	WP¶ kg ha ⁻¹ mm ⁻¹	RWP¶ kg ha ⁻¹ mm ⁻¹	R1§ days	R3§ days	R5§ days	Maturity§ days
UX3000	H ₁	0.59 (0.46, 0.75)	0.65 (0.52, 0.81)	0.06 (0.00, 0.17)	0.70 (0.57, 0.87)	0.78 (0.64, 0.96)	0.65 (0.53, 0.82)	0.60 (0.46, 0.77)	0.59* (0.44, 0.78)	0.24 (0.10, 0.41)	0.49 (0.40, 0.61)	0.11 (0.09, 0.14)	0.24 (0.19, 0.30)	0.63 (0.53, 0.77)
		0.50 (0.39, 0.65)	0.70 (0.57, 0.86)	0.05 (0.00, 0.14)	0.51 (0.40, 0.65)	0.69 (0.55, 0.86)	0.49 (0.37, 0.64)	0.44 (0.32, 0.60)	0.34* (0.26, 0.44)	0.12 (0.06, 0.19)	0.48 (0.39, 0.59)	0.04 (0.02, 0.06)	0.23 (0.18, 0.29)	0.61 (0.51, 0.74)
UX3000	H ₄	0.73 (0.59, 0.91)	0.82 (0.67, 1.00)	0.18 (0.09, 0.30)	0.77 (0.63, 0.94)	0.86 (0.71, 1.00)	0.73 (0.59, 0.90)	0.70 (0.56, 0.88)	0.59 (0.47, 0.73)	0.26 (0.18, 0.35)	0.69 (0.57, 0.84)	0.22 (0.18, 0.28)	0.48 (0.40, 0.59)	0.79 (0.66, 0.96)
UX3036	H ₁	0.60 (0.45, 0.78)	0.66 (0.53, 0.84)	0.05 (0.00, 0.16)	0.73 (0.59, 0.93)	0.85 (0.70, 1.00)	0.63 (0.49, 0.81)	0.60 (0.45, 0.79)	0.53 (0.37, 0.72)	0.37 (0.24, 0.55)	0.31 (0.25, 0.40)	0.05 (0.04, 0.07)	0.23 (0.18, 0.31)	0.64 (0.53, 0.79)
UX3036	H ₂	0.62 (0.47, 0.80)	0.70 (0.56, 0.88)	0.10 (0.01, 0.21)	0.58 (0.45, 0.74)	0.77 (0.62, 0.96)	0.53 (0.41, 0.69)	0.52 (0.39, 0.69)	0.39 (0.30, 0.51)	0.30 (0.22, 0.41)	0.36 (0.28, 0.45)	0.04 (0.02, 0.06)	0.25 (0.19, 0.32)	0.59 (0.48, 0.73)
UX3036	H ₄	0.78 (0.62, 0.98)	0.82 (0.67, 1.00)	0.27 (0.16, 0.41)	0.79 (0.64, 0.99)	0.90 (0.74, 1.00)	0.73 (0.59, 0.92)	0.72 (0.57, 0.91)	0.60 (0.48, 0.77)	0.47 (0.36, 0.60)	0.54 (0.44, 0.68)	0.16 (0.13, 0.21)	0.51 (0.41, 0.63)	0.79 (0.65, 0.96)
Overall	H ₁	0.61 (0.51, 0.72)	0.65 (0.56, 0.76)	0.06 (0.00, 0.14)	0.72 (0.62, 0.84)	0.84 (0.73, 0.96)	0.68 (0.59, 0.80)	0.63 (0.53, 0.75)	0.57* (0.46, 0.69)	0.35 (0.25, 0.46)	0.45 (0.39, 0.53)	0.09 (0.08, 0.11)	0.23 (0.20, 0.28)	0.63 (0.55, 0.72)
Overall	H ₂	0.57 (0.47, 0.67)	0.70 (0.61, 0.82)	0.06 (0.00, 0.13)	0.55 (0.47, 0.65)	0.74 (0.64, 0.86)	0.56 (0.47, 0.67)	0.52 (0.43, 0.63)	0.37* (0.31, 0.44)	0.27 (0.21, 0.33)	0.47 (0.41, 0.55)	0.04 (0.02, 0.05)	0.23 (0.20, 0.28)	0.59 (0.52, 0.68)
Overall	H ₄	0.77 (0.66, 0.89)	0.82 (0.72, 0.94)	0.24 (0.16, 0.32)	0.78 (0.68, 0.90)	0.89 (0.78, 1.00)	0.77 (0.67, 0.89)	0.74 (0.63, 0.86)	0.60 (0.52, 0.70)	0.42 (0.35, 0.50)	0.67 (0.59, 0.77)	0.20 (0.17, 0.23)	0.49 (0.42, 0.57)	0.79 (0.69, 0.90)

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group end of day settlement price from October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=soybean-meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as yield per unit effective water (R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity on the main stem (Maturity) between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

* indicates treatment significance at $\alpha = 0.05$

Table 26. Least square means estimates of positive and negative water productivity transgressive segregant for the UX3000 and UX3036 populations over the 2017-2018 water response experiment across irrigation transgressive segregant groupings were determined through comparison of WP LSMEANS over irrigation environments for the 2017-2018 water response experiment to parental values

Subset	Population	Seed Characteristics							Water Response		Reproductive Period Attributes				
		Yield	Weight	Quality†	Protein	Oil	EPV#	MPP#	WP¶	RWP¶	R1§	R3§	R5§	Maturity§	R1-R8
		kg ha ⁻¹	g/100	1-5 scale	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	days	days	days	days	days
Positive	UX3000	5264**	15.3**	1.8**	341*	194	5.5**	479**	15.9**	20.8**	42.3**	56.9**	74.1	130	88
Positive	UX3036	5193**	15.5	1.8	336**	193	5.5**	474**	15.9**	20.3**	41.6	56.6	73.9	131	89
Parental	UX3000	4971	15.0	1.7	344	194	5.6	481	15.0	19.7	42.3	56.5	73.8	129	87
Parental	UX3036	4803	15.4	1.7	341	192	5.5	478	14.6	18.6	41.3	56.0	73.8	129	88
Negative	UX3000	4609**	14.5**	1.8**	344*	194	5.6**	481**	13.8**	18.6**	42.9**	57.0**	74.2	130.5	88
Negative	UX3036	4213**	15.1	1.8	341**	193	5.5**	477**	12.8**	16.4**	41.4	56.7	73.6	129.6	88

†Seed quality scored on a iterative 1 - 5 scale; 1 = seed free from blemishes and defects to 5 = greater than 75% of sample blemished

#Estimated processed value (EPV) and estimated meal product protein (MPP) calculated through the SPROC

program (Brumm & Hurburgh, 1990). Crude oil and meal prices were calculated from the Chicago Board of Trade - Chicago Mercantile Exchange group price monthly average mean over the October 2013 – October 2018 time period. (retrieved from <https://www.indexmundi.com/commodities/?commodity=meal&months=60,11/28/18>)

¶ Water productivity (WP) calculated as yield per unit effective water from planting date to maturity date; reproductive water productivity (RWP) calculated as effective water during reproductive (R1-R8) period

§Date at which 50% of plot reached R1 growth stage (R1), R3 growth stage, R5 growth stage, the date at which greater than 95% of pods have reached maturity (Maturity), and the interval between R1 and Maturity (R1-R8) expressed as days after planting

‡Lodging is rated at maturity according on a iterative 1-5 scale; 1= all plants erect to 5= all plants prostrate

φSubset count

± indicates segregant subset significance at $\alpha = 0.10$ from contrasting segregation group (eg., positive compared to negative)

* indicates segregant subset significance at $\alpha = 0.05$ from contrasting segregation group (eg., positive compared to negative)

** indicates segregant subset significance at $\alpha = 0.01$ from contrasting segregation group (eg., positive compared to negative)

Table 27. Pearson correlation coefficients and 95% confidence intervals of canopy reflectance parameters productivity collected at V5, R3, and R5 growth and reproductive stages during 2017-2018 water response irrigation treatments

Subset		Red - Green - Blue			Spectrum			Canopy Traits		
Population	Stage	R [†]	G [†]	B [†]	VIS [#]	NIR [#]	NIR/VIS [#]	Height [¶]	CATD [§]	Area [‡]
UX3000	V5	-0.07 (-0.19, 0.06)	-0.04 (-0.17, 0.08)	-0.10 (-0.23, 0.02)	-0.01 (-0.13, 0.12)	0.09 (-0.04, 0.21)	0.11 (-0.02, 0.23)	-0.09 (-0.22, 0.04)	-0.11 (-0.23, 0.02)	0.22 (0.09, 0.34)
UX3036	V5	0.19 (0.06, 0.32)	0.14 (0.01, 0.28)	0.07 (-0.07, 0.20)	-0.02 (-0.16, 0.12)	0.20 (0.07, 0.33)	0.28 (0.15, 0.40)	0.13 (-0.01, 0.26)	-0.33 (-0.45, -0.20)	0.45 (0.33, 0.55)
Overall	V5	-0.06 (-0.15, 0.03)	-0.02 (-0.11, 0.07)	-0.09 (-0.18, 0.00)	-0.07 (-0.16, 0.02)	0.19 (0.10, 0.28)	0.29 (0.20, 0.37)	0.02 (-0.07, 0.11)	-0.31 (-0.39, -0.23)	0.43 (0.35, 0.50)
UX3000	R3	0.04 (-0.09, 0.17)	0.07 (-0.06, 0.20)	0.05 (-0.08, 0.17)	-0.19 (-0.31, -0.06)	-0.10 (-0.22, 0.03)	0.32 (0.20, 0.43)	-0.07 (-0.19, 0.06)	-0.02 (-0.14, 0.11)	0.01 (-0.12, 0.13)
UX3036	R3	-0.10 (-0.23, 0.04)	-0.08 (-0.22, 0.05)	-0.09 (-0.23, 0.05)	-0.08 (-0.22, 0.06)	0.01 (-0.13, 0.15)	0.11 (-0.02, 0.25)	0.14 (0.00, 0.27)	0.09 (-0.05, 0.22)	0.14 (0.00, 0.27)
Overall	R3	-0.07 (-0.16, 0.02)	-0.04 (-0.13, 0.05)	-0.08 (-0.17, 0.01)	-0.16 (-0.25, -0.07)	-0.02 (-0.11, 0.07)	0.25 (0.17, 0.34)	-0.08 (-0.17, 0.01)	-0.02 (-0.11, 0.07)	0.16 (0.07, 0.25)
UX3000	R5	0.00 (-0.13, 0.13)	0.00 (-0.13, 0.13)	0.03 (-0.10, 0.15)	-0.05 (-0.18, 0.08)	0.17 (0.05, 0.29)	0.25 (0.13, 0.37)	0.02 (-0.11, 0.15)	-0.12 (-0.24, 0.01)	0.14 (0.02, 0.27)
UX3036	R5	0.07 (-0.06, 0.21)	0.06 (-0.08, 0.19)	0.05 (-0.08, 0.19)	-0.06 (-0.19, 0.08)	0.24 (0.11, 0.36)	0.27 (0.13, 0.39)	0.46 (0.34, 0.56)	-0.01 (-0.15, 0.13)	-0.03 (-0.17, 0.10)
Overall	R5	0.00 (-0.09, 0.09)	0.01 (-0.08, 0.10)	-0.06 (-0.14, 0.03)	-0.04 (-0.12, 0.05)	0.28 (0.20, 0.36)	0.34 (0.25, 0.41)	0.30 (0.21, 0.38)	0.08 (-0.01, 0.17)	0.15 (0.07, 0.24)

†Red (R), green (G), and blue (B) represented as pixel count in color channel

#Visible (VIS) and Near infrared (NIR) represented as spectral reflectance

¶ Canopy height (Height) calculated from mean of LiDar and ultrasonic sensors measurements in centimeters

§Canopy to air temperature differential (CATD) calculated from radiometric and ambient temperature sensors on plot basis

‡Thresholded pixel area (Area) and thresholded perimeter (Perimeter) expressed as pixel count after color thresholding

Table 28. Broad sense heritability on an entry-mean basis estimations and 95% confidence intervals of canopy parameters at V5, R3, and R5 growth and reproductive stages during 2017-2018 water response experiment treatments

Subset		Red - Green - Blue			Spectrum			Canopy Traits			
Population	Stage	R [†]	G [†]	B [†]	VIS [#]	NIR [#]	NIR/VIS [#]	Height [¶]	CATD [§]	Area [‡]	Perimeter
Overall	V5	0.25 (0.21, 0.31)	0.24 (0.19, 0.30)	0.29 (0.24, 0.35)	0.04 (0.00, 0.09)	0.10 (0.04, 0.16)	0.30 (0.24, 0.38)	0.20 (0.16, 0.26)	0.40 (0.33, 0.48)	0.11 (0.08, 0.15)	0.05 (0.02, 0.08)
UX3000	V5	0.22 (0.16, 0.29)	0.21 (0.14, 0.29)	0.27 (0.20, 0.36)	0.00 (0.00, 0.05)	0.12 (0.05, 0.20)	0.20 (0.12, 0.30)	0.15 (0.10, 0.23)	0.40 (0.30, 0.51)	0.06 (0.03, 0.10)	0.05 (0.02, 0.08)
UX3036	V5	0.24 (0.17, 0.33)	0.24 (0.16, 0.34)	0.29 (0.21, 0.40)	0.06 (0.00, 0.15)	0.09 (0.00, 0.20)	0.34 (0.24, 0.48)	0.25 (0.18, 0.35)	0.39 (0.29, 0.53)	0.12 (0.07, 0.17)	0.04 (0.00, 0.08)
Overall	R3	0.32 (0.23, 0.42)	0.31 (0.22, 0.41)	0.28 (0.21, 0.37)	0.15 (0.11, 0.19)	0.18 (0.13, 0.24)	0.27 (0.21, 0.33)	0.53 (0.44, 0.63)	0.31 (0.20, 0.44)	NA (0.00, 0.00)	NA (0.00, 0.00)
UX3000	R3	0.34 (0.21, 0.50)	0.28 (0.15, 0.45)	0.27 (0.15, 0.41)	0.15 (0.09, 0.22)	0.20 (0.13, 0.29)	0.20 (0.12, 0.29)	0.31 (0.20, 0.44)	0.30 (0.15, 0.48)	NA (0.00, 0.00)	NA (0.00, 0.00)
UX3036	R3	0.34 (0.21, 0.50)	0.36 (0.23, 0.53)	0.32 (0.21, 0.47)	0.14 (0.09, 0.21)	0.17 (0.10, 0.26)	0.30 (0.22, 0.40)	0.50 (0.38, 0.66)	0.27 (0.10, 0.49)	NA (0.00, 0.00)	NA (0.00, 0.00)
Overall	R5	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.20 (0.13, 0.29)	0.22 (0.15, 0.30)	0.53 (0.41, 0.66)	0.63 (0.52, 0.76)	0.25 (0.16, 0.37)	NA (0.00, 0.00)	NA (0.00, 0.00)
UX3000	R5	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.00 (0.00, 0.02)	0.14 (0.04, 0.27)	0.16 (0.06, 0.28)	0.38 (0.21, 0.58)	0.69 (0.54, 0.88)	0.22 (0.10, 0.38)	NA (0.00, 0.00)	NA (0.00, 0.00)
UX3036	R5	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.23 (0.12, 0.37)	0.21 (0.10, 0.34)	0.45 (0.28, 0.66)	0.55 (0.38, 0.75)	0.20 (0.05, 0.38)	NA (0.00, 0.00)	NA (0.00, 0.00)

†Red (R), green (G), and blue (B) represented as pixel count in color channel

#Visible (VIS) and Near infrared (NIR) represented as spectral reflectance

¶ Canopy height (Height) calculated from mean of LiDar and ultrasonic sensors measurements in centimeters

§Canopy to air temperature differential (CATD) calculated from radiometric and ambient temperature sensors on plot basis

‡Thresholded pixel area (Area) and thresholded perimeter (Perimeter) expressed as pixel count after color thresholding

Table 29. RIL parent and population least square mean canopy reflectance parameter estimations across environments for 2017-2018 water response experiment

Subset			Red - Green - Blue			Spectrum			Canopy Traits			
Parent	Population	Stage	R [†]	G [†]	B [†]	VIS [#]	NIR [#]	NIR/VIS [#]	Height [¶]	CATD [§]	Area [‡]	Perimeter [‡]
			pixels	pixels	pixels	ref	ref		cm	°C	pixels	pixels
LD02-4485		V5	83.1	108.4*	77.3	0.27*	0.88*	0.31*	32.0*	5.19*	501607*	17486
	UX3036	V5	83.8	109.6	76.1	0.31	1.03	0.30	34.4	4.57	556643	16789
U11-614093		V5	83.2	109.5	76.1	0.29	1.03	0.28	34.0	4.43	565707	17303
	UX3000	V5	81.3	108.0	74.6	0.29	1.06	0.28	33.8	4.32	582587	17209
U09-312115		V5	82.0*	108.2*	74.7*	0.28*	1.09*	0.26*	33.3	4.15*	593586*	17243
LD02-4485		R5	98.4	101.6	89.9	0.10	1.48*	15.5*	105.7*	(-0.90*)	1191001	4921
	UX3036	R5	99.8	103.0	91.3	0.10	1.54	15.6	107.0	(-0.88)	1192439	4929
U11-614093		R5	99.3	102.6	91.1	0.10	1.59	16.9	107.9	(-0.80)	1192994	4824
	UX3000	R5	99.3	102.5	89.9	0.10	1.64	16.7	108.2	(-0.59)	1197414	4801
U09-312115		R5	98.6	102.0	88.7	0.10	1.64*	17.3*	108.4*	(-0.81*)	1198144	4819

†Red (R), green (G), and blue (B) represented as pixel count in color channel

#Visible (VIS) and near infrared (NIR) represented as spectral reflectance

¶ Canopy height (Height) calculated from mean of LiDar and ultrasonic sensors measurements in centimeters

§ Canopy to air temperature differential (CATD) calculated from radiometric and ambient temperature sensors on plot basis

‡ Thresholded pixel area (Area) and thresholded perimeter (Perimeter) expressed as pixel count after color thresholding

* indicates difference from U11-614093 significance at $\alpha = 0.05$

Table 30. Broad sense heritability (H) on an entry-mean basis and phenotypic Pearson correlations coefficients (r_p) with 95% confidence intervals by growth stage of RGB reflectance indices during the 2017-2018 water response experiment across irrigation treatments and environments; H estimates larger than 0.50 highlighted in grey

Index	Growth Stage					
	V5		R3		R5	
	H	r_p	H	r_p	H	r_p
ARI	0.02 (0.00, 0.05)	-0.20 (-0.28, -0.11)	0.00 (0.00, 0.06)	-0.10 (-0.19, -0.01)	0.09 (0.00, 0.20)	-0.04 (-0.13, 0.05)
CWSI	0.02 (0.00, 0.08)	-0.03 (-0.12, 0.06)	0.16 (0.12, 0.20)	-0.14 (-0.23, -0.05)	0.19 (0.12, 0.27)	-0.01 (-0.10, 0.08)
D715	0.17 (0.12, 0.22)	0.35 (0.27, 0.43)	0.29 (0.19, 0.40)	0.19 (0.10, 0.27)	0.30 (0.22, 0.40)	0.26 (0.17, 0.34)
DSI1	0.01 (0.00, 0.04)	-0.04 (-0.13, 0.05)	0.16 (0.12, 0.20)	-0.15 (-0.23, -0.06)	0.17 (0.10, 0.24)	-0.01 (-0.10, 0.08)
DSI2	0.02 (0.00, 0.04)	-0.04 (-0.13, 0.05)	0.15 (0.11, 0.20)	-0.16 (-0.24, -0.07)	0.21 (0.14, 0.30)	-0.04 (-0.13, 0.04)
DSI3	0.01 (0.00, 0.04)	-0.03 (-0.12, 0.06)	0.13 (0.08, 0.19)	-0.14 (-0.23, -0.05)	0.20 (0.12, 0.30)	0.04 (-0.05, 0.13)
GNDVI	0.17 (0.13, 0.21)	0.30 (0.21, 0.38)	0.31 (0.23, 0.41)	0.18 (0.09, 0.26)	0.24 (0.15, 0.35)	0.27 (0.18, 0.35)
GYI1	0.02 (0.00, 0.04)	-0.05 (-0.14, 0.04)	0.15 (0.11, 0.19)	-0.16 (-0.25, -0.07)	0.20 (0.13, 0.29)	-0.03 (-0.12, 0.06)
GYI2	0.01 (0.00, 0.05)	0.18 (0.09, 0.27)	0.16 (0.11, 0.21)	-0.04 (-0.12, 0.05)	0.22 (0.15, 0.30)	0.27 (0.19, 0.36)
GYI3	0.13 (0.04, 0.23)	-0.13 (-0.22, -0.04)	0.14 (0.09, 0.19)	-0.16 (-0.25, -0.07)	0.19 (0.10, 0.29)	-0.03 (-0.12, 0.06)
LCI	0.24 (0.19, 0.31)	0.34 (0.26, 0.42)	0.16 (0.09, 0.24)	0.20 (0.12, 0.29)	0.44 (0.34, 0.57)	0.31 (0.22, 0.39)
MCARI	0.12 (0.09, 0.16)	0.15 (0.06, 0.24)	0.15 (0.04, 0.29)	0.02 (-0.07, 0.11)	0.27 (0.14, 0.41)	0.14 (0.05, 0.23)
NDRE	0.27 (0.20, 0.35)	0.34 (0.25, 0.41)	0.15 (0.07, 0.25)	0.19 (0.10, 0.27)	0.26 (0.14, 0.40)	0.20 (0.11, 0.28)
NDVI	0.14 (0.11, 0.18)	0.33 (0.24, 0.40)	0.17 (0.09, 0.27)	0.23 (0.14, 0.31)	0.64 (0.52, 0.78)	0.40 (0.32, 0.47)
NDVI680	0.13 (0.10, 0.17)	0.32 (0.24, 0.40)	0.00 (0.00, 0.10)	0.20 (0.11, 0.28)	0.57 (0.45, 0.71)	0.39 (0.32, 0.47)
NDVI705	0.30 (0.23, 0.38)	0.35 (0.27, 0.43)	0.21 (0.11, 0.33)	0.18 (0.09, 0.26)	0.38 (0.27, 0.52)	0.22 (0.13, 0.31)
NDWI	0.13 (0.10, 0.17)	0.32 (0.24, 0.40)	0.00 (0.00, 0.10)	0.20 (0.11, 0.28)	0.57 (0.45, 0.71)	0.39 (0.32, 0.47)
NIR / Green	0.20 (0.16, 0.25)	0.31 (0.23, 0.39)	0.41 (0.33, 0.50)	0.20 (0.11, 0.28)	0.33 (0.23, 0.46)	0.26 (0.17, 0.34)
NIR / Red	0.33 (0.26, 0.42)	0.32 (0.24, 0.40)	0.36 (0.28, 0.45)	0.29 (0.20, 0.37)	0.66 (0.54, 0.80)	0.40 (0.32, 0.47)
OSAVI	0.18 (0.14, 0.22)	0.31 (0.22, 0.39)	0.08 (0.00, 0.18)	0.06 (-0.03, 0.15)	0.24 (0.17, 0.32)	0.33 (0.25, 0.41)
PRI	0.11 (0.08, 0.15)	0.15 (0.06, 0.23)	0.08 (0.00, 0.20)	0.05 (-0.04, 0.14)	0.14 (0.04, 0.27)	0.17 (0.08, 0.25)
RE	0.23 (0.18, 0.30)	0.10 (0.01, 0.19)	0.40 (0.28, 0.53)	-0.08 (-0.17, 0.01)	0.56 (0.44, 0.70)	0.20 (0.11, 0.28)
RE3RE2	0.18 (0.13, 0.23)	0.32 (0.23, 0.39)	0.26 (0.16, 0.37)	0.16 (0.08, 0.25)	0.30 (0.20, 0.42)	0.17 (0.08, 0.26)
REDGE	0.24 (0.18, 0.30)	0.27 (0.18, 0.35)	0.38 (0.26, 0.51)	0.11 (0.02, 0.20)	0.56 (0.44, 0.70)	0.26 (0.18, 0.34)
REIP	0.05 (0.00, 0.14)	0.11 (0.02, 0.20)	0.11 (0.07, 0.16)	-0.02 (-0.11, 0.07)	0.25 (0.15, 0.37)	0.23 (0.15, 0.32)
RENDVI	0.17 (0.13, 0.22)	0.36 (0.28, 0.44)	0.21 (0.11, 0.33)	0.18 (0.09, 0.26)	0.38 (0.27, 0.52)	0.22 (0.13, 0.31)
reNDVI2	0.18 (0.14, 0.23)	0.36 (0.28, 0.44)	0.24 (0.15, 0.35)	0.18 (0.09, 0.27)	0.37 (0.26, 0.51)	0.21 (0.12, 0.29)
SAVI	0.02 (0.01, 0.04)	0.24 (0.16, 0.33)	0.05 (0.00, 0.11)	-0.01 (-0.10, 0.08)	0.18 (0.11, 0.26)	0.28 (0.20, 0.36)
SR1	0.28 (0.22, 0.35)	0.35 (0.26, 0.42)	0.16 (0.09, 0.24)	0.22 (0.13, 0.30)	0.54 (0.42, 0.67)	0.33 (0.25, 0.41)
SR680	0.33 (0.26, 0.41)	0.33 (0.25, 0.41)	0.34 (0.26, 0.43)	0.28 (0.20, 0.36)	0.62 (0.50, 0.75)	0.39 (0.31, 0.46)
SR705	0.17 (0.13, 0.21)	0.33 (0.24, 0.40)	0.32 (0.22, 0.44)	0.19 (0.10, 0.27)	0.58 (0.46, 0.72)	0.28 (0.20, 0.36)
SRWBI	0.01 (0.00, 0.03)	-0.07 (-0.16, 0.02)	0.08 (0.00, 0.23)	0.02 (-0.07, 0.11)	0.12 (0.05, 0.19)	-0.15 (-0.24, -0.07)
STI1	0.17 (0.12, 0.23)	-0.36 (-0.43, -0.28)	0.12 (0.05, 0.20)	-0.19 (-0.27, -0.10)	0.20 (0.10, 0.31)	-0.19 (-0.27, -0.10)
STI2	0.16 (0.09, 0.24)	-0.24 (-0.32, -0.15)	0.19 (0.11, 0.28)	-0.13 (-0.22, -0.04)	0.14 (0.06, 0.22)	-0.26 (-0.34, -0.17)
TCARI	0.21 (0.15, 0.28)	0.15 (0.06, 0.23)	0.11 (0.00, 0.24)	0.01 (-0.08, 0.10)	0.26 (0.14, 0.40)	0.13 (0.05, 0.22)
TCARI / OSAVI	0.16 (0.10, 0.24)	0.09 (0.00, 0.18)	0.12 (0.00, 0.26)	0.00 (-0.09, 0.09)	0.23 (0.11, 0.37)	0.09 (0.01, 0.18)
Average	0.15 (0.11, 0.20)	0.16 (0.08, 0.25)	0.18 (0.11, 0.27)	0.06 (-0.03, 0.14)	0.33 (0.23, 0.44)	0.17 (0.08, 0.25)

Table 31. Broad sense heritability (H) on an entry-mean basis and phenotypic Pearson correlations coefficients (r_p) with 95% confidence intervals by growth stage of spectral reflectance indices during the 2017-2018 water response experiment across irrigation treatments and environments

Index	Growth Stage					
	V5		R3		R5	
	H	r_p	H	r_p	H	r_p
CIVE	0.03 (0.02, 0.04)	-0.05 (-0.14, 0.03)	0.28 (0.21, 0.37)	-0.08 (-0.17, 0.01)	0.01 (0.00, 0.02)	-0.06 (-0.15, 0.03)
COM1	0.02 (0.01, 0.03)	0.12 (0.03, 0.21)	0.08 (0.02, 0.15)	-0.12 (-0.20, -0.03)	0.02 (0.00, 0.03)	0.09 (0.00, 0.18)
COM2	0.02 (0.01, 0.02)	0.03 (-0.06, 0.12)	0.35 (0.23, 0.50)	-0.14 (-0.23, -0.05)	0.12 (0.03, 0.23)	0.07 (-0.02, 0.16)
ExG	0.54 (0.46, 0.64)	0.04 (-0.05, 0.12)	0.44 (0.31, 0.58)	0.21 (0.13, 0.30)	0.14 (0.11, 0.19)	0.22 (0.13, 0.30)
ExGR	0.06 (0.04, 0.10)	0.17 (0.08, 0.25)	0.00 (0.00, 0.14)	-0.03 (-0.12, 0.06)	0.00 (0.00, 0.02)	-0.11 (-0.19, -0.02)
ExR	0.26 (0.22, 0.31)	-0.04 (-0.13, 0.05)	0.29 (0.24, 0.35)	-0.10 (-0.19, -0.01)	0.00 (0.00, 0.02)	-0.07 (-0.16, 0.02)
GRI	0.04 (0.01, 0.08)	0.12 (0.03, 0.21)	0.10 (0.04, 0.17)	0.00 (-0.09, 0.09)	0.00 (0.00, 0.01)	-0.08 (-0.17, 0.01)
MExG	0.27 (0.22, 0.33)	0.07 (-0.02, 0.16)	0.21 (0.10, 0.34)	0.14 (0.05, 0.22)	0.23 (0.18, 0.30)	0.05 (-0.04, 0.14)
NDI	0.05 (0.02, 0.09)	0.13 (0.04, 0.22)	0.13 (0.07, 0.19)	-0.02 (-0.11, 0.07)	0.00 (0.00, 0.01)	-0.09 (-0.17, 0.00)
NGBDI	0.03 (0.02, 0.05)	0.14 (0.05, 0.23)	0.18 (0.13, 0.25)	-0.04 (-0.13, 0.05)	0.05 (0.03, 0.06)	0.15 (0.06, 0.23)
NGRDI	0.05 (0.02, 0.09)	0.13 (0.04, 0.22)	0.13 (0.07, 0.19)	-0.02 (-0.11, 0.07)	0.00 (0.00, 0.01)	-0.09 (-0.17, 0.00)
VARI	0.00 (0.00, 0.01)	-0.04 (-0.13, 0.05)	0.21 (0.12, 0.30)	-0.04 (-0.13, 0.05)	0.10 (0.07, 0.14)	-0.15 (-0.24, -0.07)
VDVI	0.06 (0.04, 0.08)	0.19 (0.10, 0.28)	0.15 (0.09, 0.22)	-0.02 (-0.11, 0.07)	0.02 (0.01, 0.03)	0.12 (0.03, 0.21)
VEG	0.03 (0.00, 0.06)	0.12 (0.03, 0.21)	0.13 (0.08, 0.20)	-0.03 (-0.12, 0.06)	0.01 (0.00, 0.01)	0.10 (0.01, 0.18)
Average	0.10 (0.08, 0.14)	0.08 (-0.01, 0.17)	0.19 (0.12, 0.28)	-0.02 (-0.11, 0.07)	0.05 (0.03, 0.08)	0.01 (-0.08, 0.10)

Table 32. Water productivity QTL identified by inclusive composite interval mapping (ICIM) by population treatments and environments in 2017-2018 water response experiment

Population	Chromosome	Position [†]	Left Marker [#]	Left Marker Alleles ^ρ	Right Marker [#]	Right Marker Alleles ^ρ	LOD [¶]	PVE(%) [§]	Add. Effect [±]	Left CI ^φ	Right CI ^τ
		cM							kg ha ⁻¹ mm ⁻¹		cM
UX3000	1	48.8	SGM01.4042298	G/A	SGM01.39140734	G/A	3.77	9.2	0.66	41.3	108.0
UX3000	4	111.6	SGM04.47740685	C/T	SGM04.48222393	A/G	4.64	8.0	-0.64	108.0	108.0
UX3000	7	56.4	SGM07.5798679	T/C	SGM07.10241187	A/G	7.12	11.1	0.98	50.9	50.9
UX3000	18	117.4	SGM18.48271736	T/C	SGM18.53740575	G/A	3.86	6.8	0.56	100.0	100.0
UX3000	19	21.0	SGM19.2418392	C/T	SGM19.6458355	G/A	5.73	9.2	-0.68	15.3	15.3
UX3036	12	66.4	SGM12.34063256	G/A	SGM12.35086789	A/G	2.62	3.6	-0.80	59.9	59.9
UX3036	19	36.4	SGM19.2418392	T/C	SGM19.42257278	C/T	2.72	12.2	1.48	15.3	15.3

[†]Estimated QTL position in centimorgan units

[#] Downstream flanking SNP marker (LeftMarker) and upstream flanking SNP marker (RightMarker) of estimated QTL position

^ρ Segregating alleles of marker. Listed as female allele / male allele parental line source

[¶] Logarithm of the odds (LOD) score of estimated QTL position

[§] Phenotypic variation explained by QTL at estimated position

[±] Estimated additive genetic effect of female derived QTL at estimated position (eg. UX3000 = U09-312115 x U11-614093, effect of QTL derived from U11-614093 x LD02-4485).

^φ Lower (Left CI) and upper (Right CI) confidence interval calculated by one-LOD drop from estimated QTL position

^τ Parental line containing estimated favorable effect QTL

Table 33. Water productivity QTL identified by inclusive composite interval mapping (ICIM) by population treatment across environments in 2017-2018 water response experiment

Population	Treatment	Chromosome	Position [†]	Left Marker [#]	Left Marker Alleles ^ρ	Right Marker [#]	Right Marker Alleles ^ρ	LOD [¶]	PVE(%) [§]	Add. Effect [‡]	LD02-4485 ^τ
			cM							kg ha ⁻¹ mm ⁻¹	
UX3000	Rainfed	1	54.8	SGM01.4042298	G/A	SGM01.39140734	G/A	3.11	6.7	0.48	
UX3000	Irrigated	3	127.6	SGM03.39594385	C/A	SGM03.45039348	C/T	3.05	5.3	0.53	
UX3000	Rainfed	7	57.4	SGM07.10241187	A/G	SGM07.13784462	G/T	4.75	10.8	0.83	
UX3000	Rainfed	8	127.9	SGM08.36466450	A/C	SGM08.40695313	G/A	4.32	8.3	-0.54	
UX3000	Irrigated	19	19.0	SGM19.2418392	C/T	SGM19.6458355	G/A	3.37	7.2	-0.59	
UX3000	Rainfed	19	34.0	SGM19.32353405	C/T	SGM19.34851394	G/A	3.39	6.5	-0.49	
UX3036	Rainfed	12	69.4	SGM12.34063256	G/A	SGM12.35086789	A/G	2.96	8.7	-0.89	
UX3036	Irrigated	19	30.4	SGM19.2418392	T/C	SGM19.42257278	C/T	3.54	8.7	1.31	

[†]Estimated QTL position in centimorgan units

[#] Downstream flanking SNP marker (LeftMarker) and upstream flanking SNP marker (RightMarker) of estimated QTL position

^ρ Segregating alleles of marker. Listed as female allele / male allele parental line source

[¶] Logarithm of the odds (LOD) score of estimated QTL position

[§] Phenotypic variation explained by QTL at estimated position

[‡] Estimated additive genetic effect of female derived QTL at estimated position (eg. UX3000 = U09-312115 x U11-614093, effect of QTL derived from U09-312115 LD02-4485).

^φ Lower (Left CI) and upper (Right CI) confidence interval calculated by one-LOD drop from estimated QTL position

^τ Parental line containing estimated favorable effect QTL

Table 34. Water productivity QTL identified by inclusive composite interval mapping (ICIM) by population across irrigation treatments and environments in 2017-2018 water response experiment; QTL Trait Identifier lists trait names of QTL with overlapping genetic confidence intervals identified by population and irrigation treatment across environments in 2017-2018 water response experiment

Population	Chromosome	Position [†]	QTL Trait Identifier [#]
		cM	
UX3000	1	48.8	UX3000_17YIELD, UX3000_17WP, UX3000_YIELD_2, UX3000_WP_2, UX3000_V5ExR, UX3000_V5ExR.Homogeneity, UX3000_V5H.Homogeneity, UX3000_R5SR705, UX3000_1618YIELD, UX3036_R5
UX3000	4	111.6	UX3000_YIELD, UX3000_RWP, UX3000_17WP, UX3000_YIELD_2, UX3000_WP_2, UX3000_R5r.g.Energy, UX3000_1618YIELD
UX3000	7	56.4	UX3000_YIELD, UX3000_RWP, UX3000_17WP, UX3000_YIELD_1, UX3000_YIELD_2, UX3000_WP_2, UX3000_YIELD_1618
UX3000	18	117.4	UX3000_SW, UX3000_18WP, UX3000_WP_1, UX3000_R1, UX3000_R5H, UX3000_R5S, UX3000_R5r.g.Homogeneity, UX3000_R5NGBDL.Energy, UX3000_R5VDVI.Homogeneity
UX3000	19	21.0	UX3000_YIELD, UX3000_17WP, UX3000_WP_1, UX3000_RWP_1, UX3000_V5g, UX3000_V5g.b, UX3000_V5a., UX3000_V5ExGR.Contrast, UX3000_V5ExGR.Energy, UX3000_V5ExGR.Homogeneity, UX3000_R5SR680, UX3000_R5NIRRed, UX3000_1618YIELD, UX3000_1618R8
UX3036	12	66.4	UX3036_18WP, UX3036_YIELD, UX3036_YIELD_2, UX3036_WP_2, UX3036_R5Cr.Correlation
UX3036	19	36.4	UX3036_18YIELD, UX3036_18WP, UX3036_YIELD, UX3036_HT, UX3036_YIELD_1, UX3036_LG, UX3036_WP_1, UX3036_RWP_1, UX3036_R3g.Contrast, UX3036_R3g.Homogeneity, UX3036_R3ExG.Homogeneity, UX3036_R3R935_7, UX3036_R3R936, UX3036_R3R936_3, UX3036_R3R937_8, UX3036_R3R939, UX3036_R3R939_3, UX3036_R3R940_2, UX3036_R3R940_5, UX3036_R3R940_7, UX3036_R3R941, UX3036_R3R941_3, UX3036_R3R942_5, UX3036_R3R944, UX3036_R3R945_2, UX3036_R3R945_7, UX3036_R3R946_3, UX3036_R3R947_2, UX3036_R3R947_8, UX3036_R3R948_7, UX3036_R3R949_3, UX3036_R3NIRGreen

[†]Estimated QTL position in centimorgan units

[#] List QTL traits identified in 2017-2018 water response experiment with estimated genetic position within confidence interval estimated for corresponding water productivity QTL. Trait identifier in the following general format POPULATION_TRAIT_TREATMENT; traits without treatment listed represent response across treatments

Table 35. Water productivity QTL identified by inclusive composite interval mapping (ICIM) by population across irrigation treatment and environments in 2017-2018 water response experiment; SoyBase QTL Trait Identifier list unique object types of reported QTL with estimated positions within confidence interval of corresponding water productivity QTL

Population	Chromosome	Position [†]	Soybase QTL Trait Identifier [#]
		cM	
UX3000	1	48.8	Bean pyralid, First flower, Lodging, Node number, Plant height, Pod wall weight, Reproductive to vegetative period ratio, Seed oil, Seed set, Seed weight, shoot weight
UX3000	4	111.6	Phosphorus use efficiency, Plant height, Plant dry weight, Pod number, Root weight, SDS disease incidence, SDS disease index, Seed coat cracking, Seed daidzein, Seed height, Seed isoflavone, Seed length, Seed protein, Seed weight, Seed width
UX3000	7	56.4	Canopy height, Common cutworm, Corn earworm, First flower, Leaflet area, Leaflet chlorophyll, Pod maturity, Pubescence density, Root nodule weight, dry, Root volume, Row spacing response, Seed fill, Seed genistein, Seed oil, Seed oleic, Seed protein, Seed set, Seed thicknes, Seed width, Seed yield
UX3000	18	117.4	SCN
UX3000	19	21.0	Pod dehiscence, Pod number, Root length, Seed oil, Seed protein, Shoot weight
UX3036	12	66.4	Fe effic, Hypocotyl weight, Pod borer, Seed isoflavone, Seed linolenic, Seed oil
UX3036	19	36.4	Al tolerance, Flood tolerance, Plant height, Pod number, Root density, Row spacing response, Seed genistein, Seed isoflavone, Seed length to width ratio, Seed linolenic, Seed oil, Seed protein, Seed set, Seed sucrose, Seed total isoflavone, Seed weight, Shoot weight

[†]Estimated QTL position in centimorgan units

[#] List unique SoyBase QTL object types with estimated genetic position within confidence interval estimated for corresponding water productivity QTL. Retrived from <https://soybase.org/dlpages/>

Table 36. Summary of water productivity LSMEANS ENET algorithm models over irrigation treatments with observed to predicted Pearson correlation coefficients (r) estimated through CV1 scheme; root mean square error (RMSE), mean absolute error (MAE) and data subset size (n) reported

Population†	Growth Stage#	Year Subset*	r	RMSE	MAE	n
				kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	
Overall	V5	2017-2018	0.67 (0.57, 0.77)	0.67	0.51	442
UX3000	V5	2017-2018	0.75 (0.69, 0.81)	0.54	0.41	239
UX3036	V5	2017-2018	0.63 (0.54, 0.72)	0.82	0.63	203
Overall	R5	2017-2018	0.72 (0.66, 0.77)	0.66	0.51	442
UX3000	R5	2017-2018	0.67 (0.60, 0.75)	0.58	0.45	239
UX3036	R5	2017-2018	0.81 (0.74, 0.88)	0.65	0.50	203
Overall	V5 + R5	2017-2018	0.72 (0.67, 0.77)	0.67	0.52	442
UX3000	V5 + R5	2017-2018	0.79 (0.74, 0.84)	0.52	0.40	239
UX3036	V5 + R5	2017-2018	0.73 (0.67, 0.79)	0.66	0.52	203
Overall	V5	2016 - 2018	0.54 (0.50, 0.58)	1.04	0.81	845
UX3000	V5	2016 - 2018	0.79 (0.74, 0.85)	0.48	0.37	239
UX3036	V5	2016 - 2018	0.64 (0.57, 0.72)	0.77	0.59	203
Overall	R5	2016 - 2018	0.65 (0.62, 0.68)	0.93	0.74	845
UX3000	R5	2016 - 2018	0.73 (0.64, 0.82)	0.54	0.43	239
UX3036	R5	2016 - 2018	0.75 (0.69, 0.81)	0.69	0.53	203
Overall	V5 + R5	2016 - 2018	0.55 (0.52, 0.59)	1.03	0.83	845
UX3000	V5 + R5	2016 - 2018	0.76 (0.70, 0.83)	0.48	0.37	239
UX3036	V5 + R5	2016 - 2018	0.77 (0.72, 0.82)	0.67	0.52	203

† Population subset; Overall denotes observations from both populations considered together

Growth stages of phenomic data (e.g. V5 + R5 indicates phenomic data from both V5 and R5 data collection events were included)

*Year subset used (2016-2018 indicates observations from both the 2016 preliminary evaluation experiment and the 2017-2018 water response experiment, 2017-2018 indicates observations from the water response experiment)

Table 37. Summary of per-plot water productivity NET algorithm models using 2017-2018 water response experiment observations; observed to predicted Pearson correlation coefficients (r) estimated through CV1 scheme, and root mean square error (RMSE), mean absolute error (MAE) and data subset size (n) reported

Population†	Growth Stage#	Treatment*	r	RMSE	MAE	n
				kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	
Overall	R5	Irrigated	0.86 (0.85, 0.88)	0.43	0.34	2956
Overall	R5	Overall	0.90 (0.90, 0.91)	0.47	0.37	5921
Overall	R5	Rainfed	0.91 (0.90, 0.92)	0.47	0.37	2965
Overall	V5	Irrigated	0.87 (0.86, 0.88)	0.45	0.35	4406
Overall	V5	Overall	0.93 (0.92, 0.93)	0.49	0.38	8804
Overall	V5	Rainfed	0.93 (0.93, 0.93)	0.50	0.39	4398
Overall	V5 + R5	Irrigated	0.90 (0.88, 0.91)	0.42	0.33	2012
Overall	V5 + R5	Overall	0.91 (0.91, 0.91)	0.50	0.39	18746
Overall	V5 + R5	Rainfed	0.92 (0.92, 0.93)	0.48	0.37	2009
UX3000	R5	Overall	0.92 (0.91, 0.92)	0.44	0.33	2936
UX3000	V5	Overall	0.94 (0.94, 0.94)	0.44	0.34	4362
UX3000	V5 + R5	Irrigated	0.89 (0.88, 0.89)	0.41	0.32	4665
UX3000	V5 + R5	Overall	0.92 (0.92, 0.93)	0.47	0.36	9326
UX3000	V5 + R5	Rainfed	0.94 (0.94, 0.94)	0.45	0.35	4661
UX3036	R5	Overall	0.89 (0.88, 0.90)	0.48	0.38	2493
UX3036	V5	Overall	0.93 (0.92, 0.93)	0.49	0.39	3708
UX3036	V5 + R5	Irrigated	0.87 (0.87, 0.88)	0.45	0.35	3933
UX3036	V5 + R5	Overall	0.91 (0.91, 0.91)	0.51	0.40	7874
UX3036	V5 + R5	Rainfed	0.92 (0.92, 0.93)	0.51	0.40	3941

† Population subset; Overall denotes observations from both populations considered together

Growth stages of phenomic data (e.g. V5 + R5 indicates phenomic data from both V5 and R5 data collection events were included)

*Irrigation treatment subset used (Overall indicates observations from both treatments used, rainfed denotes observations from only rainfed treatment observed, irrigated indicates observations from only the irrigated treatment used)

Table 38. Summary of per-plot water productivity CART algorithm models using 2017-2018 water response experiment observations; observed to predicted Pearson correlation coefficients (r) estimated through CV1 scheme, and root mean square error (RMSE), mean absolute error (MAE) and data subset size (n) reported

Population†	Growth Stage#	Treatment*	r	RMSE	MAE	n
				kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	
Overall	R5	Irrigated	0.80 (0.79, 0.81)	0.52	0.41	2956
Overall	R5	Overall	0.86 (0.86, 0.87)	0.55	0.42	5921
Overall	R5	Rainfed	0.86 (0.85, 0.88)	0.58	0.44	2965
Overall	V5	Irrigated	0.83 (0.83, 0.83)	0.50	0.39	4406
Overall	V5	Overall	0.91 (0.91, 0.92)	0.53	0.40	8804
Overall	V5	Rainfed	0.91 (0.90, 0.92)	0.56	0.43	4398
Overall	V5 + R5	Irrigated	0.82 (0.81, 0.83)	0.55	0.43	2012
Overall	V5 + R5	Overall	0.95 (0.95, 0.95)	0.39	0.29	18746
Overall	V5 + R5	Rainfed	0.87 (0.86, 0.88)	0.63	0.49	2009
UX3000	R5	Overall	0.89 (0.88, 0.90)	0.51	0.39	2936
UX3000	V5	Overall	0.92 (0.92, 0.93)	0.49	0.37	4362
UX3000	V5 + R5	Irrigated	0.91 (0.91, 0.92)	0.36	0.27	4665
UX3000	V5 + R5	Overall	0.95 (0.95, 0.96)	0.37	0.28	9326
UX3000	V5 + R5	Rainfed	0.96 (0.95, 0.96)	0.38	0.28	4661
UX3036	R5	Overall	0.84 (0.83, 0.86)	0.58	0.45	2493
UX3036	V5	Overall	0.90 (0.90, 0.91)	0.57	0.44	3708
UX3036	V5 + R5	Irrigated	0.91 (0.90, 0.91)	0.39	0.29	3933
UX3036	V5 + R5	Overall	0.95 (0.94, 0.95)	0.41	0.30	7874
UX3036	V5 + R5	Rainfed	0.95 (0.95, 0.95)	0.42	0.31	3941

† Population subset; Overall denotes observations from both populations considered together

Growth stages of phenomic data (e.g. V5 + R5 indicates phenomic data from both V5 and R5 data collection events were included)

*Irrigation treatment subset used (Overall indicates observations from both treatments used, rainfed denotes observations from only rainfed treatment observed, irrigated indicates observations from only the irrigated treatment used)

Table 39. Summary of water productivity on a per-plot basis CART algorithm model using 2017-2018 water response experiment observations; observed to predicted Pearson correlation coefficients (r) estimated through CV2 scheme, and root mean square error (RMSE), mean absolute error (MAE) and data subset size (n) reported

Population†	Growth Stage#	Treatment‡	r	RMSE	MAE	n
				kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	
Overall	R5	Irrigated	0.73 (0.71, 0.75)	2.38	1.81	2956
Overall	R5	Overall	0.82 (0.77, 0.86)	1.68	1.30	5921
Overall	R5	Rainfed	0.76 (0.67, 0.84)	1.90	1.47	2965
Overall	V5	Irrigated	0.77 (0.75, 0.79)	2.13	1.63	4406
Overall	V5	Overall	0.84 (0.80, 0.88)	1.52	1.18	8804
Overall	V5	Rainfed	0.76 (0.67, 0.84)	2.03	1.60	4398
Overall	V5 + R5	Irrigated	0.82 (0.80, 0.84)	1.92	1.46	2012
Overall*	V5 + R5	Overall				18746
Overall	V5 + R5	Rainfed	0.73 (0.63, 0.83)	2.15	1.64	2009
UX3000	R5	Overall	0.74 (0.64, 0.83)	1.98	1.55	2936
UX3000	V5	Overall	0.78 (0.68, 0.87)	2.03	1.60	4362
UX3000	V5 + R5	Irrigated	0.71 (0.67, 0.74)	2.23	1.75	4665
UX3000	V5 + R5	Overall	0.79 (0.72, 0.86)	1.90	1.50	9326
UX3000	V5 + R5	Rainfed	0.70 (0.49, 0.90)	2.35	1.88	4661
UX3036	R5	Overall	0.78 (0.64, 0.91)	1.93	1.53	2493
UX3036	V5	Overall	0.79 (0.64, 0.93)	1.73	1.38	3708
UX3036	V5 + R5	Irrigated	0.72 (0.64, 0.79)	2.55	2.03	3933
UX3036	V5 + R5	Overall	0.79 (0.65, 0.94)	1.88	1.50	7874
UX3036	V5 + R5	Rainfed	0.71 (0.49, 0.93)	2.00	1.55	3941

† Population subset; Overall denotes observations from both populations considered together

Growth stages of phenomic data (e.g. V5 + R5 indicates phenomic data from both V5 and R5 data collection events were included)

‡ Irrigation treatment subset used (Overall indicates observations from both treatments used, rainfed denotes observations from only rainfed treatment observed, irrigated indicates observations from only the irrigated treatment used)

* No untested subsets to evaluated overall model performance using V5 + R5 stages through CV2 method

Table 40. Summary of per-plot water productivity NET algorithm models using 2017-2018 water response experiment observations; observed to predicted Pearson correlation coefficients (r) estimated through CV2 scheme, and root mean square error (RMSE), mean absolute error (MAE) and data subset size (n) reported

Population [†]	Growth Stage [#]	Treatment [±]	r	RMSE	MAE	n
				kg ha ⁻¹ mm ⁻¹	kg ha ⁻¹ mm ⁻¹	
Overall	R5	Irrigated	0.25 (0.15, 0.35)	5.54	4.67	2956
Overall	R5	Overall	0.54 (0.45, 0.64)	6.75	5.92	5921
Overall	R5	Rainfed	0.34 (0.18, 0.49)	4.35	3.42	2965
Overall	V5	Irrigated	0.37 (0.20, 0.55)	4.59	3.52	4406
Overall	V5	Overall	0.80 (0.76, 0.84)	1.95	1.56	8804
Overall	V5	Rainfed	0.58 (0.50, 0.67)	5.57	4.65	4398
Overall	V5 + R5	Irrigated	0.40 (0.28, 0.52)	4.29	3.45	2012
Overall*	V5 + R5	Overall				18746
Overall	V5 + R5	Rainfed	0.75 (0.72, 0.78)	2.05	1.61	2009
UX3000	R5	Overall	0.80 (0.75, 0.86)	1.92	1.49	2936
UX3000	V5	Overall	0.36 (0.22, 0.50)	4.68	3.61	4362
UX3000	V5 + R5	Irrigated	0.56 (0.46, 0.65)	4.79	4.11	4665
UX3000	V5 + R5	Overall	0.77 (0.73, 0.81)	2.62	2.10	9326
UX3000	V5 + R5	Rainfed	0.79 (0.72, 0.86)	2.17	1.74	4661
UX3036	R5	Overall	0.89 (0.88, 0.90)	1.74	1.44	2493
UX3036	V5	Overall	0.54 (0.46, 0.63)	6.27	5.27	3708
UX3036	V5 + R5	Irrigated	0.64 (0.55, 0.73)	5.46	4.55	3933
UX3036	V5 + R5	Overall	0.74 (0.71, 0.78)	2.26	1.80	7874
UX3036	V5 + R5	Rainfed	0.74 (0.70, 0.77)	2.34	1.86	3941

[†] Population subset; Overall denotes observations from both populations considered together

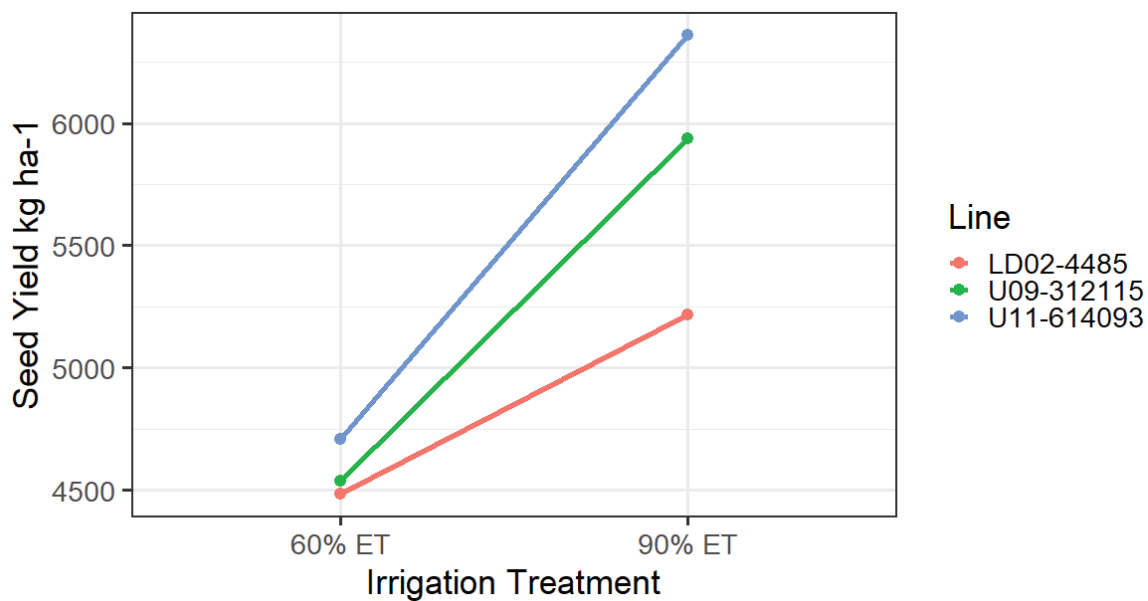
[#] Growth stages of phenomic data (e.g. V5 + R5 indicates phenomic data from both V5 and R5 data collection events were included)

[±] Irrigation treatment subset used (Overall indicates observations from both treatments used, rainfed denotes observations from only rainfed treatment observed, irrigated indicates observations from only the irrigated treatment used)

*No untested subsets to evaluated overall model performance using V5 + R5 stages through CV2 method

FIGURES

Figure 1. Least square mean estimations of parental lines for the study under limited and full irrigation treatments from 2013-2014 Chile drip and Lincoln campus irrigation evaluation



	Irrigation Treatment		
	Limited [#]	Full [#]	
Parent	Yield	Yield	n ^φ
	kg ha ⁻¹	kg ha ⁻¹	
LD02-4485	4484 ± 321	5217 ± 591	14
U11-614093	4708 ± 321	6361 ± 591	14
U09-312115	4537 ± 321	5938 ± 591	14

* indicates difference from U11-614093 significance at $\alpha = 0.05$

#Irrigation treatments for previous studies defined as limited = 60% of evapotranspiration demands replaced, and full = 90% of evapotranspiration demands replaced

φSubset count

Figure 2. Mead 2018 water response experiment field layout and experimental design. Maturity grouping and irrigation treatment denoted below each tier. Incomplete blocks outlined in black within each maturity group and irrigation treatment tier. Parental lines of population and random sampling of RILs within each population placed randomly within each incomplete block. Maturity group placement, irrigation treatment, incomplete block placement, and plot placement randomized for each year, location, treatment, and replication.

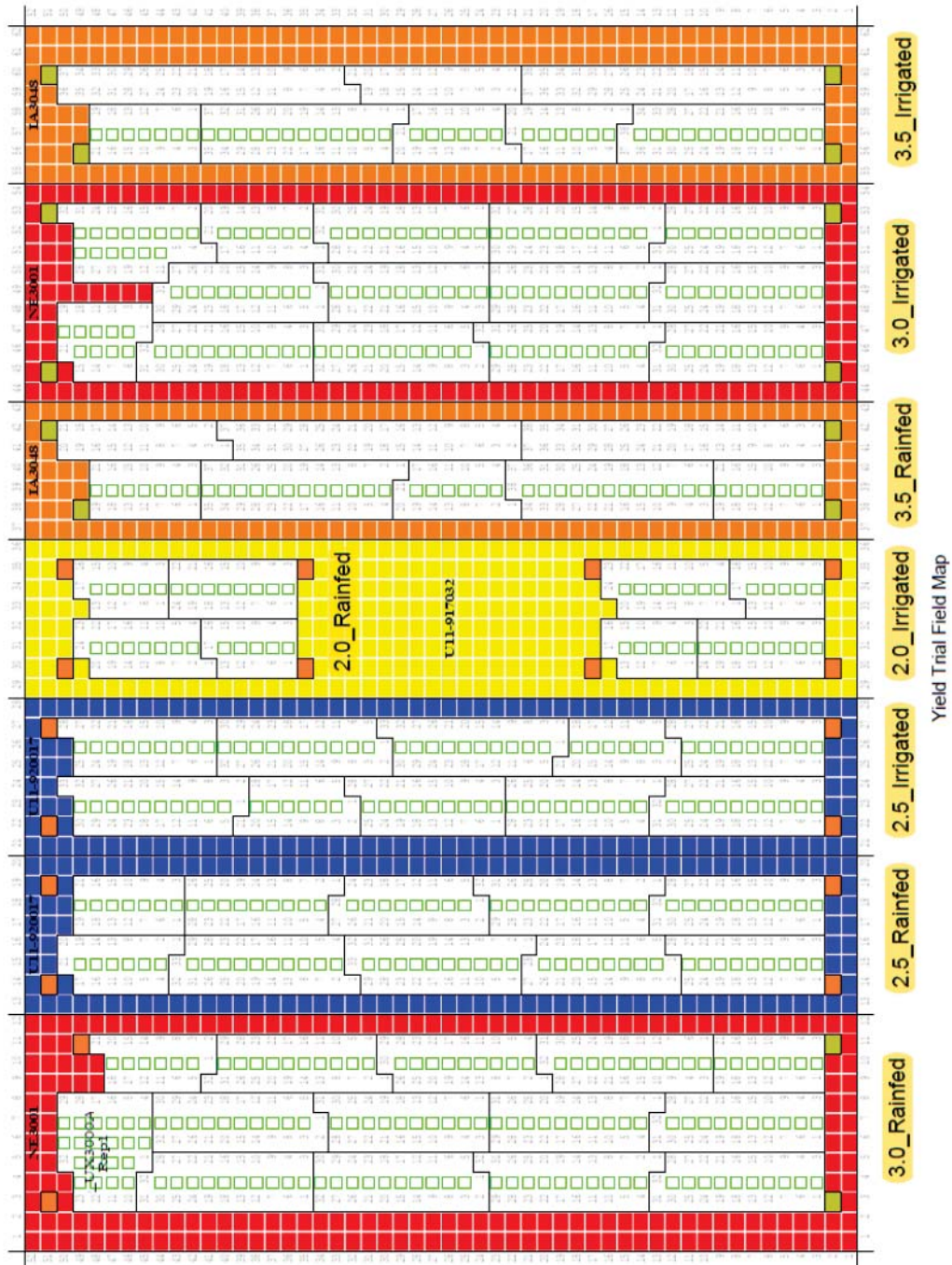


Figure 3. t-distributed stochastic neighbor embedding dimensionality reduction on SNP markers information for RILs and parental lines

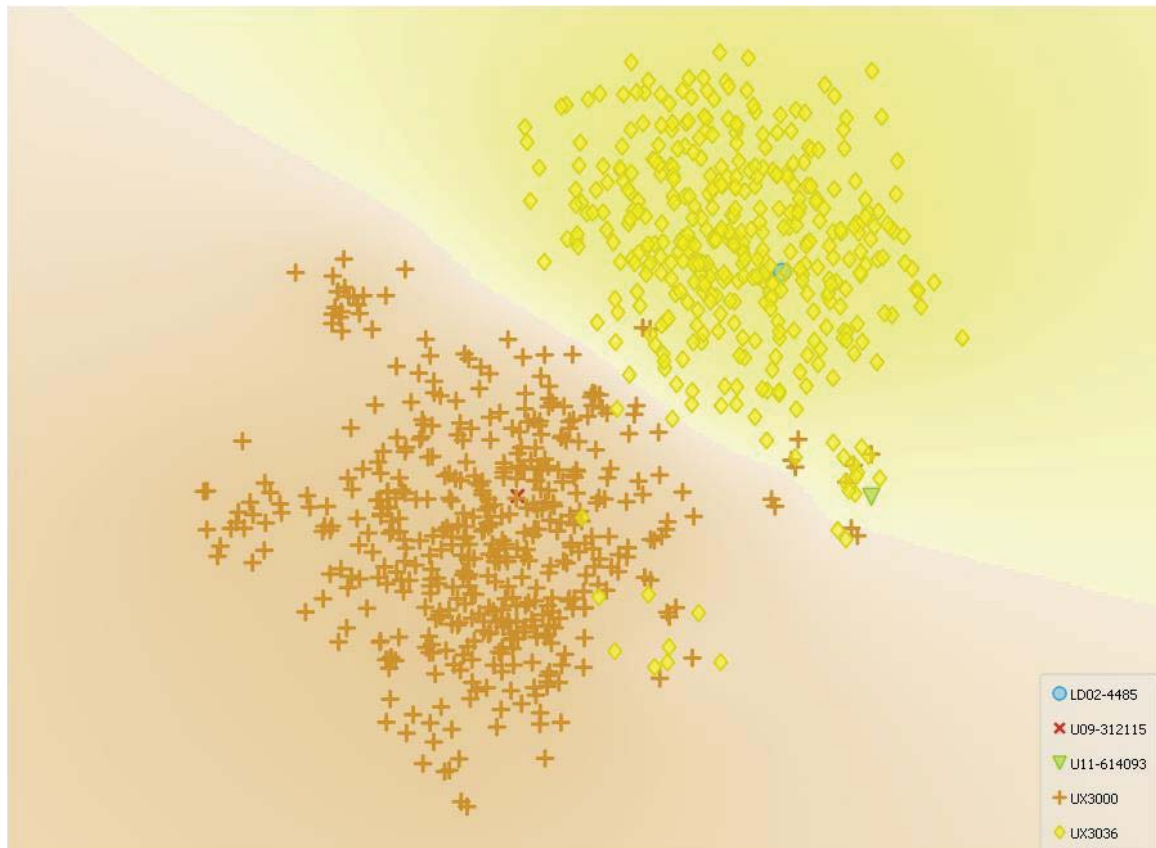


Figure 4. Comparison of genetic map created through Haldane's mapping function versus genetic map created using SNP genetic position interpolation from Wm82.a2.v2 reference genome for the UX3000 population

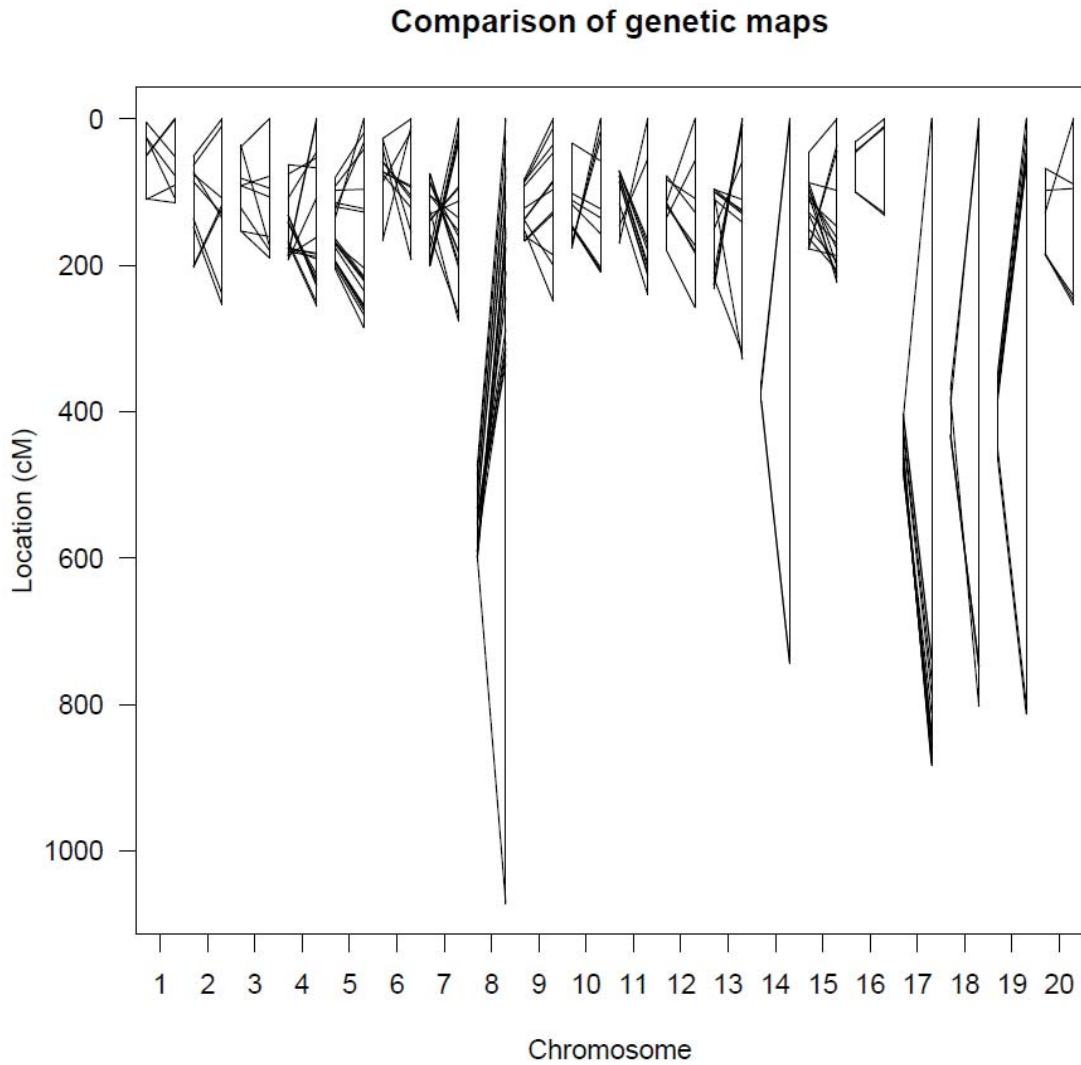


Figure 5. Comparison of genetic map created through Haldane's mapping function versus genetic map created using SNP genetic position interpolation from Wm82.a2.v2 reference genome for the UX3036 population

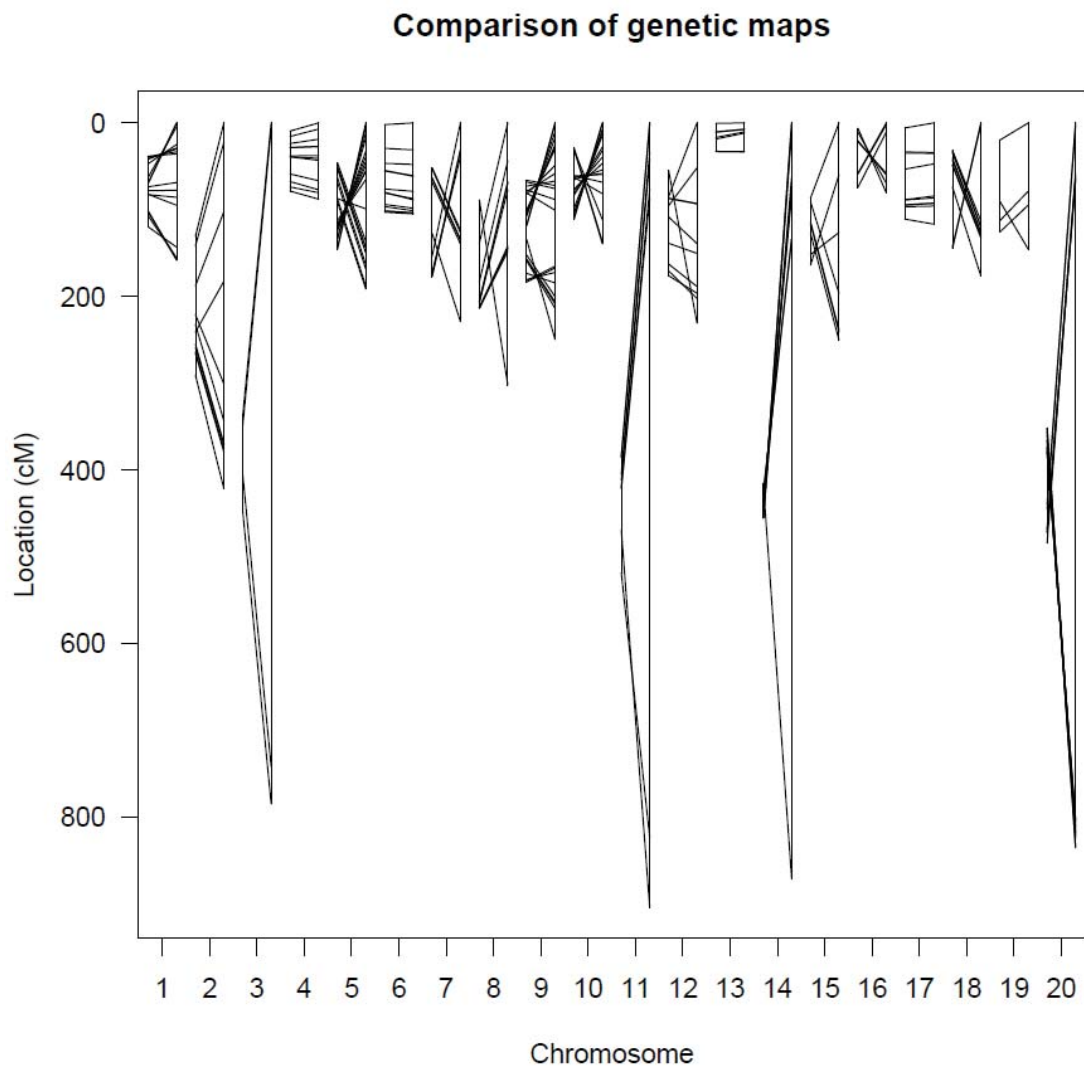


Figure 6. Genetic map of UX3000 population used for QTL mapping from Wm82.a2.v2 reference genome interpolation

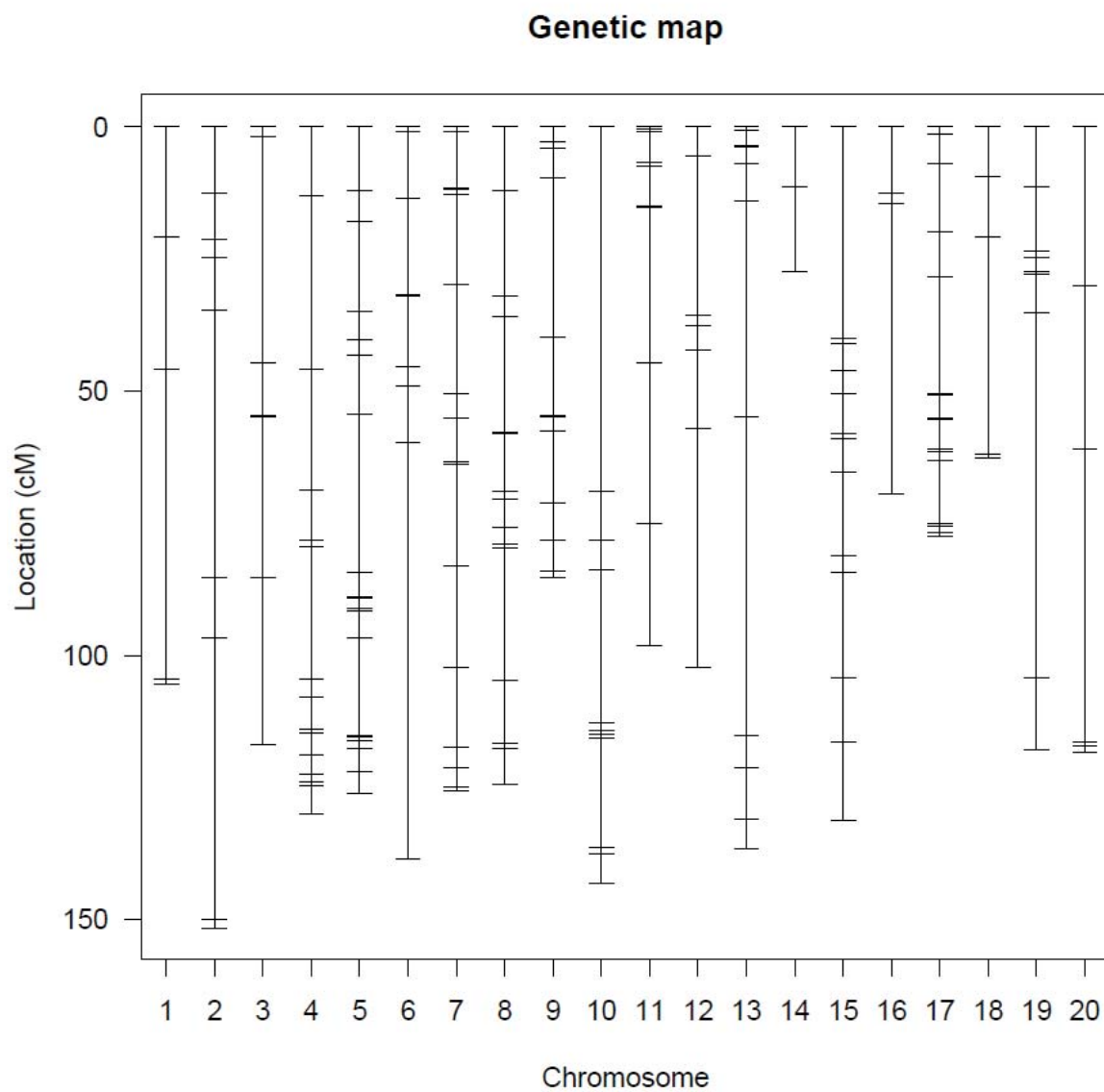


Figure 7. Genetic map of UX3036 population used for QTL mapping from Wm82.a2.v2 reference genome interpolation

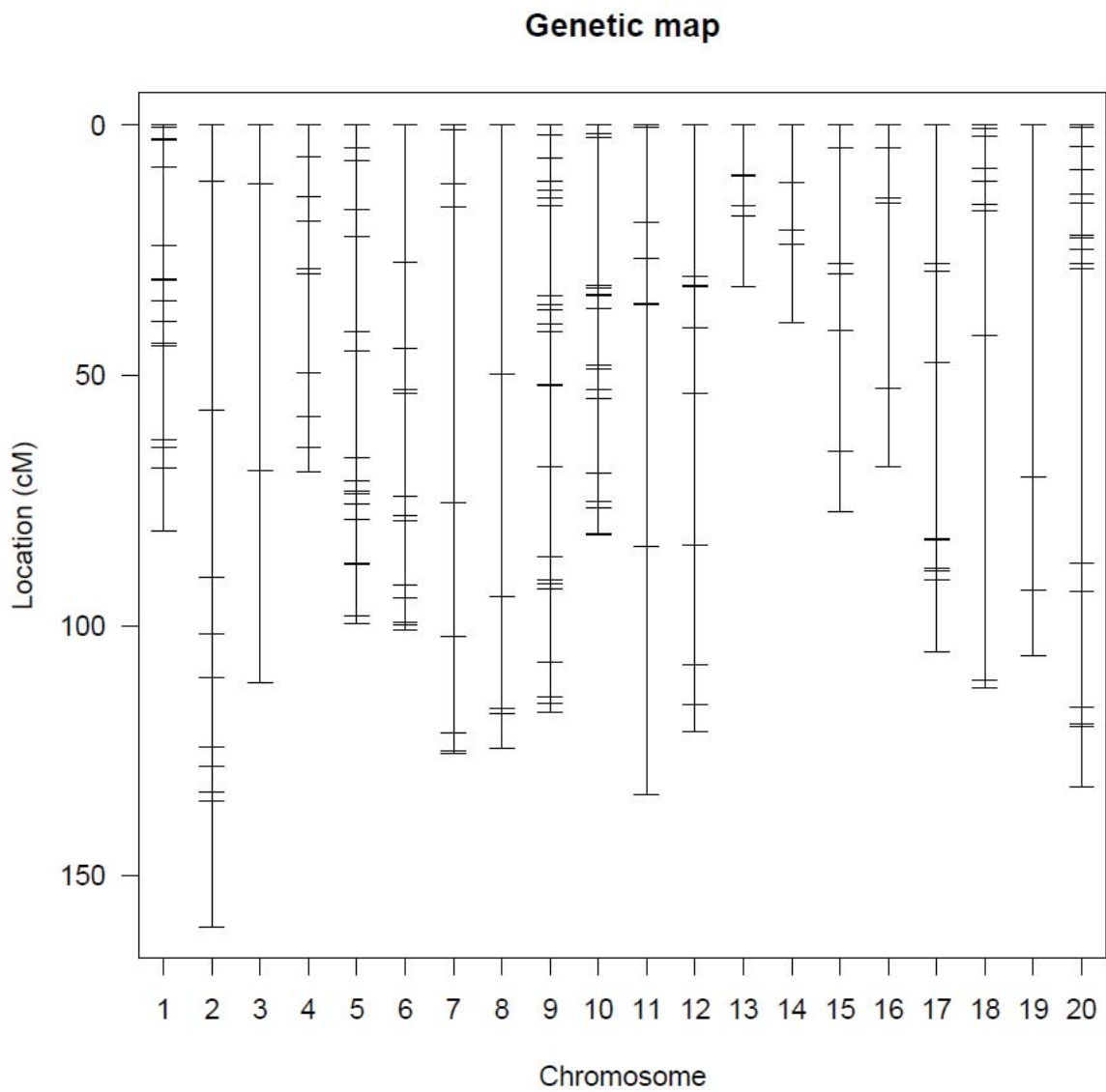


Figure 8. Pubescence color segregation ratio interpolated from hilum color in UX3036 population compared to the expected 9:7 segregation ratio through Chi-square test.

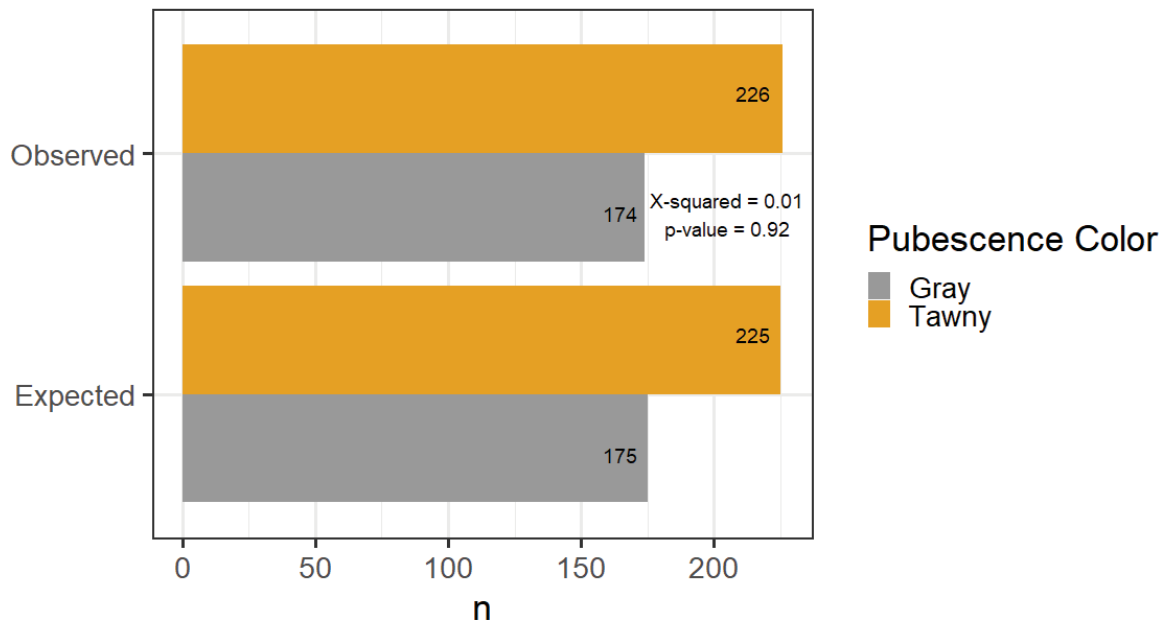


Figure 9. Estimated QTL position of pubescence color trait in UX3036 population compared to reported position of T locus on SoyBase; QTL position estimated through inclusive composite interval mapping.

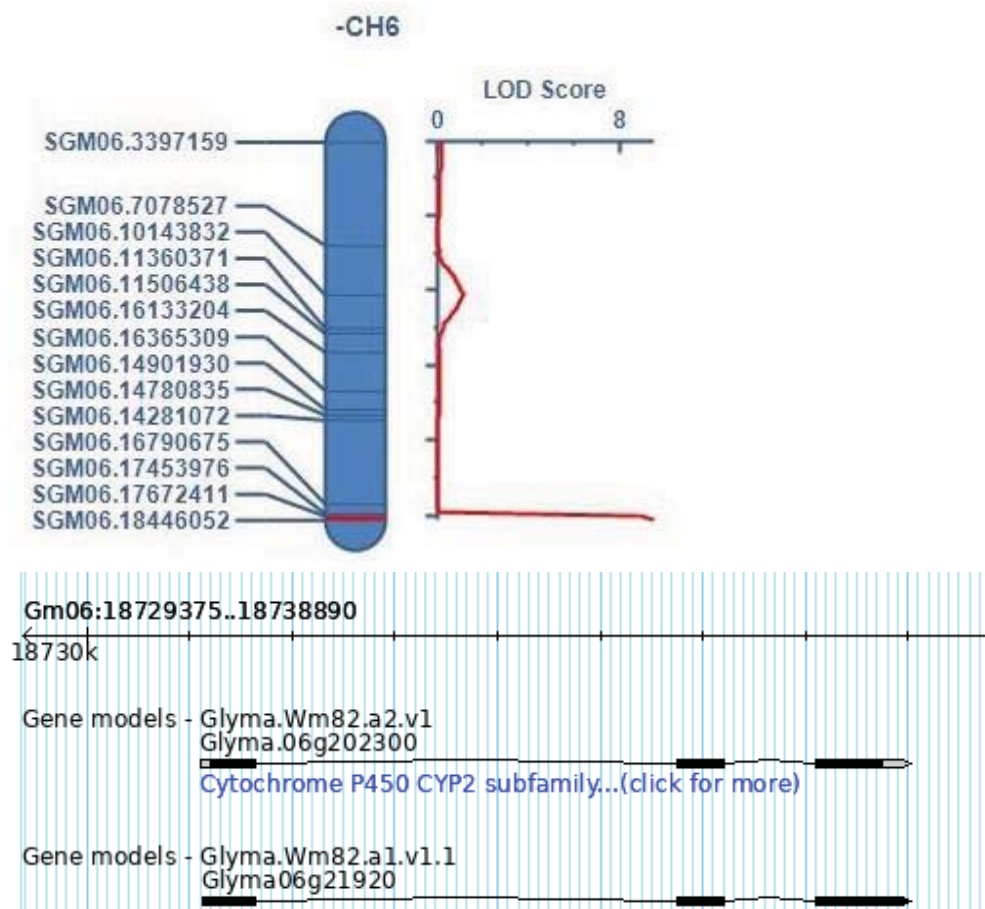


Figure 10. Violin plot of water productivity least square mean estimate (LSMEANS) distributions for RILs and parental lines in the 2017- 2018 water response experiment across environments and irrigation treatments. ANOVA p-value represents significance of population effect. Means based on two populations in each of two environments per year for two years. Number of RILs equal to 235 in the UX3000 populations and 203 in the UX3036 population.

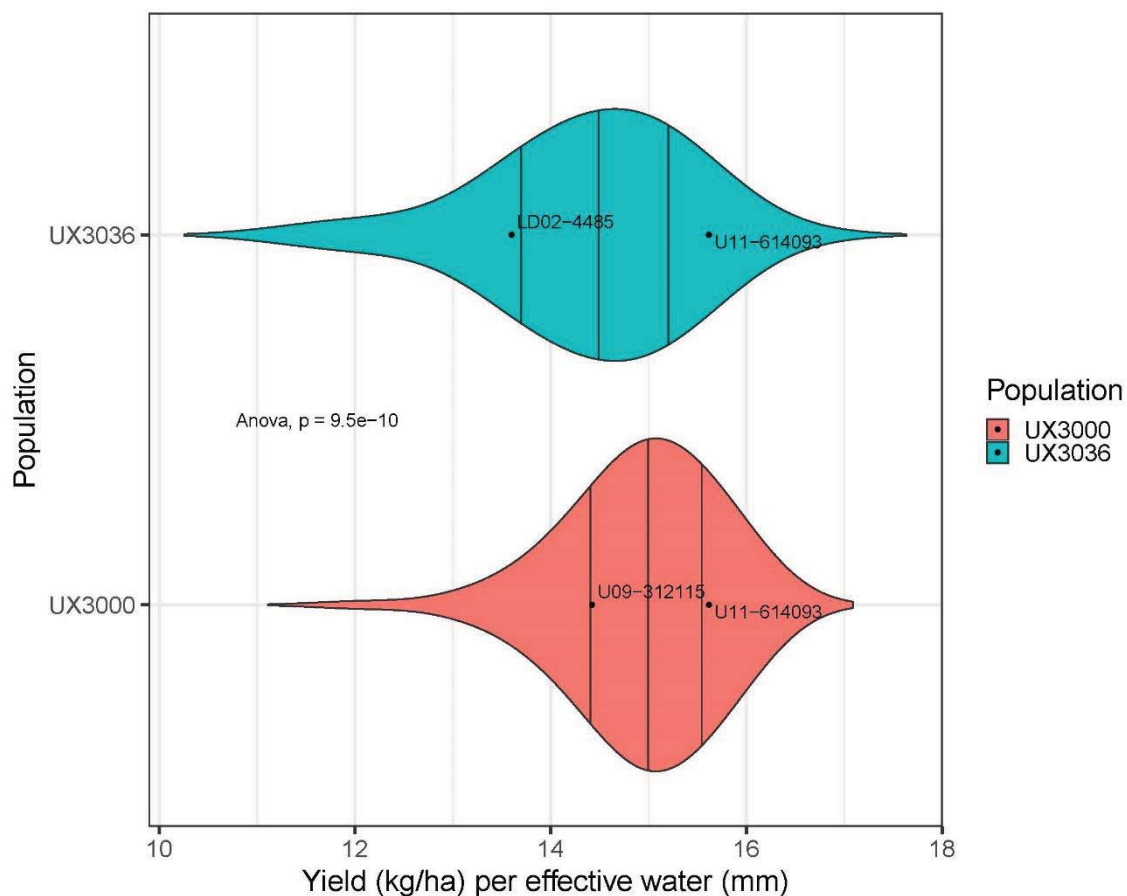


Figure 11. SoyWater water use chart representing irrigated treatments of the 3.0 maturity grouping within the 2017 Mead environment of the 2017-2018 water response experiment

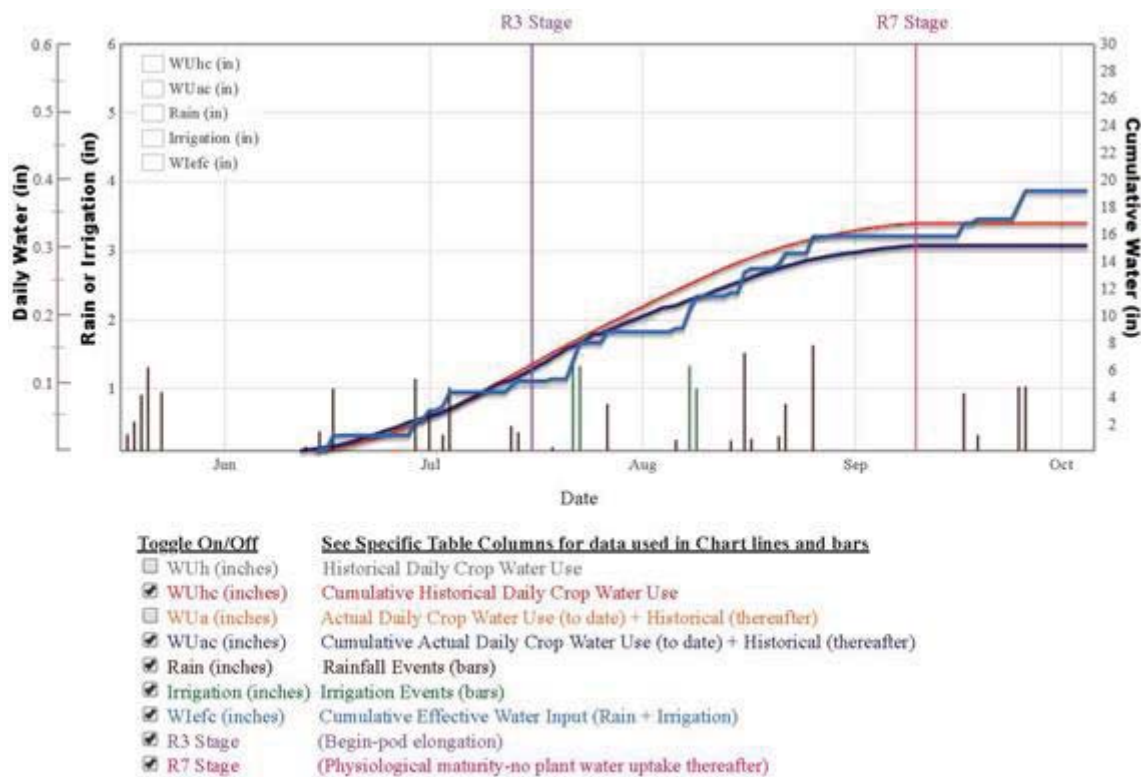
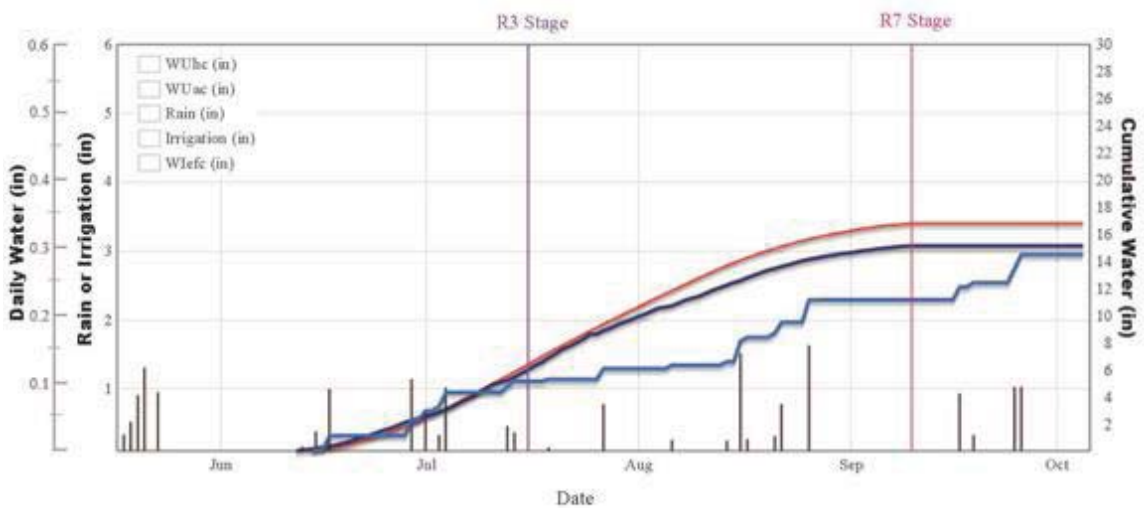
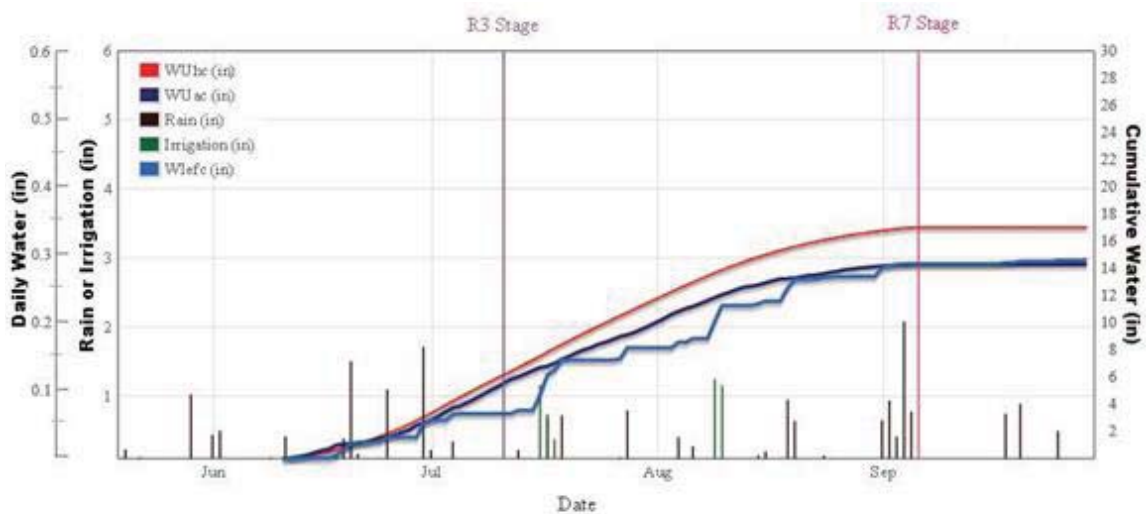


Figure 12. SoyWater water use chart representing rainfed treatments of the 3.0 maturity grouping within the 2017 Mead environment of the 2017-2018 water response experiment



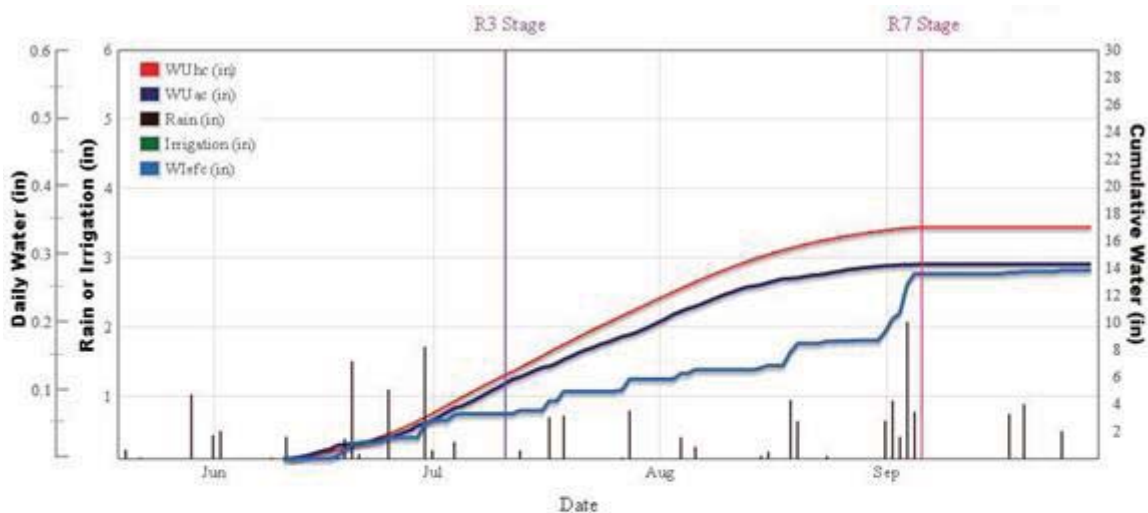
- | | |
|---|--|
| Toggle On/Off | See Specific Table Columns for data used in Chart lines and bars |
| <input type="checkbox"/> WUh (inches) | Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUhc (inches) | Cumulative Historical Daily Crop Water Use |
| <input type="checkbox"/> WUa (inches) | Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> WUac (inches) | Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> Rain (inches) | Rainfall Events (bars) |
| <input checked="" type="checkbox"/> Irrigation (inches) | Irrigation Events (bars) |
| <input checked="" type="checkbox"/> WIfec (inches) | Cumulative Effective Water Input (Rain + Irrigation) |
| <input checked="" type="checkbox"/> R3 Stage | (Begin-pod elongation) |
| <input checked="" type="checkbox"/> R7 Stage | (Physiological maturity-no plant water uptake thereafter) |

Figure 13. SoyWater water use chart representing irrigated treatments of the 3.0 maturity grouping within the 2018 Mead environment of the 2017-2018 water response experiment



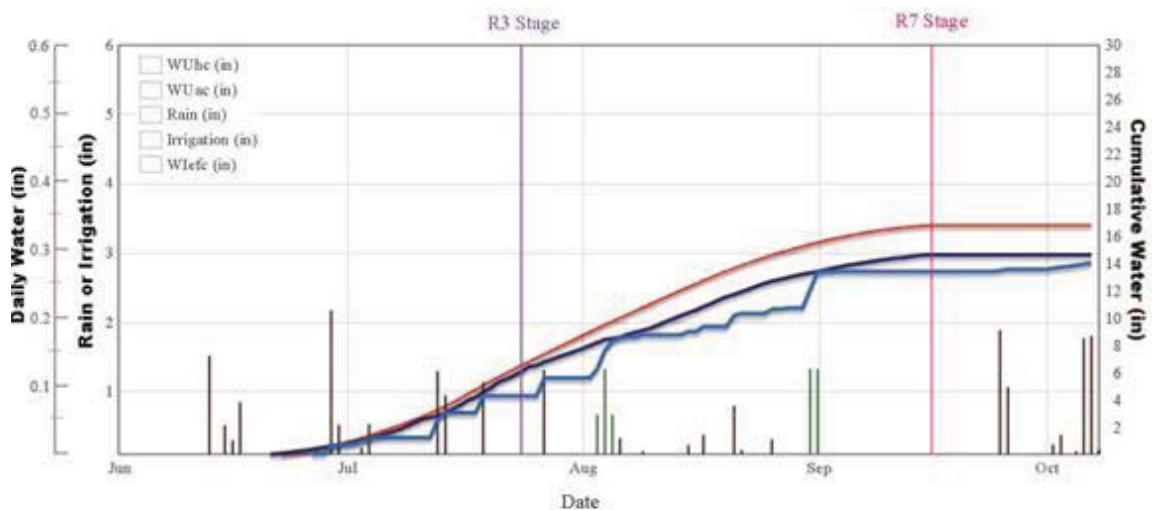
- | | |
|---|--|
| Toggle On/Off | See Specific Table Columns for data used in Chart lines and bars |
| <input type="checkbox"/> WUhc (inches) | Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUhc (inches) | Cumulative Historical Daily Crop Water Use |
| <input type="checkbox"/> WUa (inches) | Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> WUac (inches) | Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> Rain (inches) | Rainfall Events (bars) |
| <input checked="" type="checkbox"/> Irrigation (inches) | Irrigation Events (bars) |
| <input checked="" type="checkbox"/> WUfc (inches) | Cumulative Effective Water Input (Rain + Irrigation) |
| <input checked="" type="checkbox"/> R3 Stage | (Begin-pod elongation) |
| <input checked="" type="checkbox"/> R7 Stage | (Physiological maturity-no plant water uptake thereafter) |

Figure 14. SoyWater water use chart representing rainfed treatments of the 3.0 maturity grouping within the 2018 Mead environment of the 2017-2018 water response experiment



- | | |
|---|--|
| Toggle On/Off | See Specific Table Columns for data used in Chart lines and bars |
| <input type="checkbox"/> WUh (inches) | Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUhc (inches) | Cumulative Historical Daily Crop Water Use |
| <input type="checkbox"/> WUa (inches) | Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> WUac (inches) | Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> Rain (inches) | Rainfall Events (bars) |
| <input checked="" type="checkbox"/> Irrigation (inches) | Irrigation Events (bars) |
| <input checked="" type="checkbox"/> WUefc (inches) | Cumulative Effective Water Input (Rain + Irrigation) |
| <input checked="" type="checkbox"/> R3 Stage | (Begin-pod elongation) |
| <input checked="" type="checkbox"/> R7 Stage | (Physiological maturity-no plant water uptake thereafter) |

Figure 15. SoyWater water use chart representing irrigated treatments of the 3.0 maturity grouping within the 2017 Lincoln environment of the 2017-2018 water response experiment



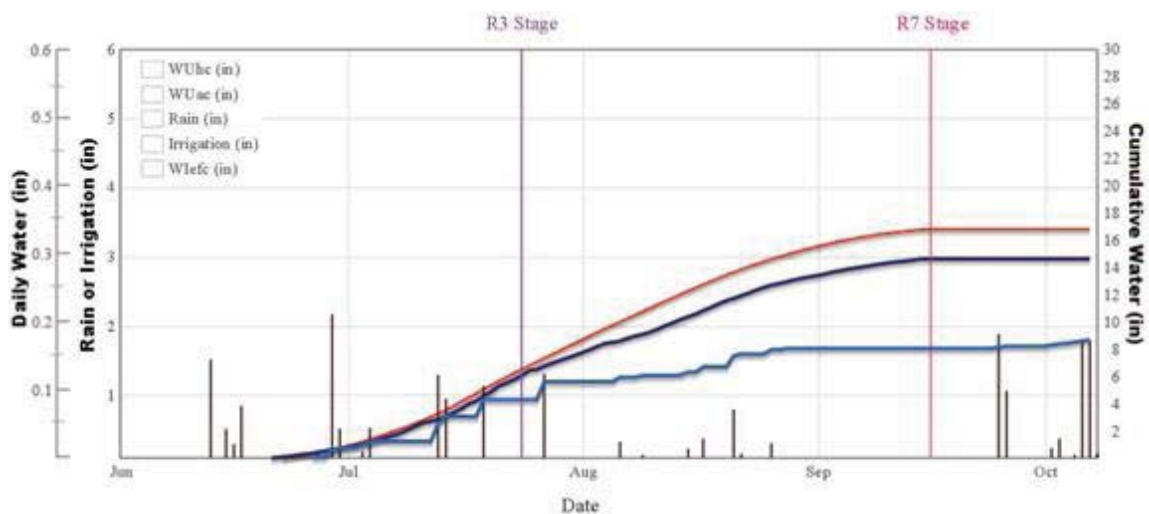
Toggle On/Off

- WUh (inches)
- WUhc (inches)
- WUa (inches)
- WUac (inches)
- Rain (inches)
- Irrigation (inches)
- WUefc (inches)
- R3 Stage
- R7 Stage

See Specific Table Columns for data used in Chart lines and bars

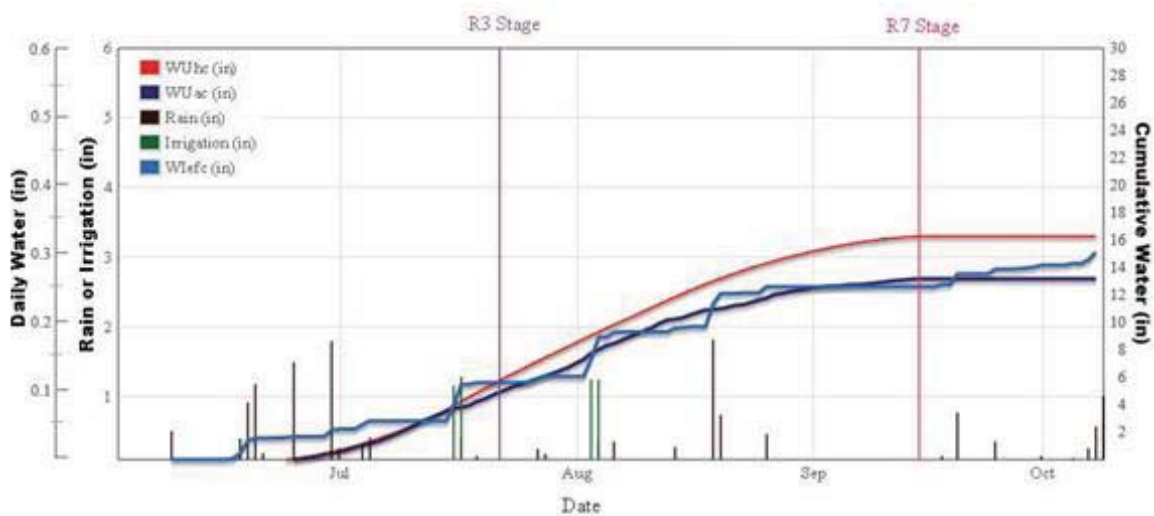
- Historical Daily Crop Water Use
- Cumulative Historical Daily Crop Water Use
- Actual Daily Crop Water Use (to date) + Historical (thereafter)
- Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter)
- Rainfall Events (bars)
- Irrigation Events (bars)
- Cumulative Effective Water Input (Rain + Irrigation)
- (Begin-pod elongation)
- (Physiological maturity-no plant water uptake thereafter)

Figure 16. SoyWater water use chart representing rainfed treatments of the 3.0 maturity grouping within the 2017 Lincoln environment of the 2017-2018 water response experiment



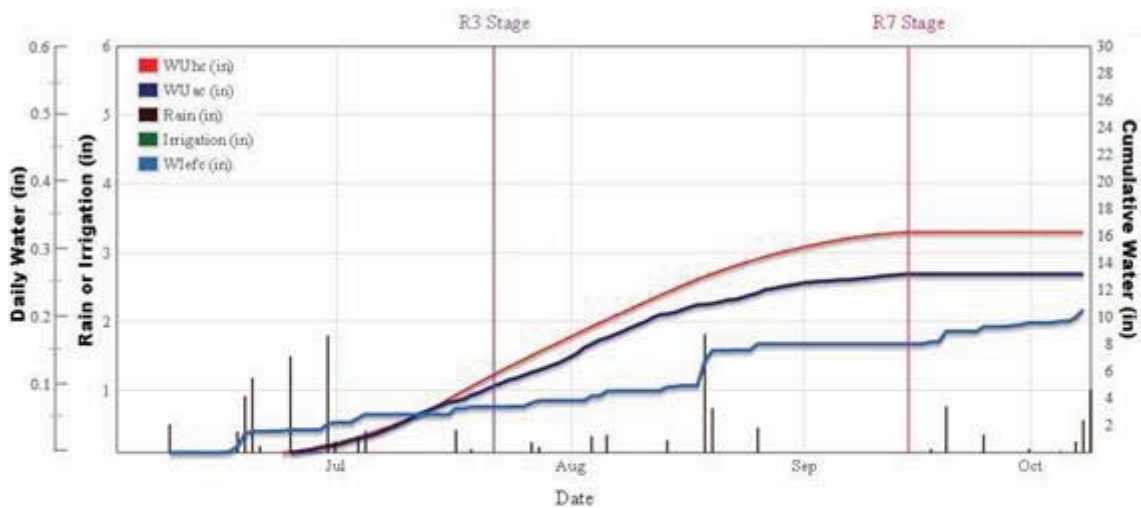
- | <u>Toggle On/Off</u> | <u>See Specific Table Columns for data used in Chart lines and bars</u> |
|---|--|
| <input type="checkbox"/> WUh (inches) | Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUhc (inches) | Cumulative Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUa (inches) | Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> WUac (inches) | Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> Rain (inches) | Rainfall Events (bars) |
| <input checked="" type="checkbox"/> Irrigation (inches) | Irrigation Events (bars) |
| <input checked="" type="checkbox"/> Wlffc (inches) | Cumulative Effective Water Input (Rain + Irrigation) |
| <input checked="" type="checkbox"/> R3 Stage | (Begin-pod elongation) |
| <input checked="" type="checkbox"/> R7 Stage | (Physiological maturity-no plant water uptake thereafter) |

Figure 17. SoyWater water use chart representing irrigated treatments of the 3.0 maturity grouping within the 2018 Lincoln environment of the 2017-2018 water response experiment



<u>Toggle On/Off</u>	<u>See Specific Table Columns for data used in Chart lines and bars</u>
<input type="checkbox"/> WUh (inches)	Historical Daily Crop Water Use
<input checked="" type="checkbox"/> WUhc (inches)	Cumulative Historical Daily Crop Water Use
<input type="checkbox"/> WUa (inches)	Actual Daily Crop Water Use (to date) + Historical (thereafter)
<input checked="" type="checkbox"/> WUac (inches)	Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter)
<input checked="" type="checkbox"/> Rain (inches)	Rainfall Events (bars)
<input checked="" type="checkbox"/> Irrigation (inches)	Irrigation Events (bars)
<input checked="" type="checkbox"/> WUefc (inches)	Cumulative Effective Water Input (Rain + Irrigation)
<input checked="" type="checkbox"/> R3 Stage	(Begin-pod elongation)
<input checked="" type="checkbox"/> R7 Stage	(Physiological maturity-no plant water uptake thereafter)

Figure 18. SoyWater water use chart representing rainfed treatments of the 3.0 maturity grouping within the 2018 Lincoln environment of the 2017-2018 water response experiment



- | | |
|---|--|
| Toggle On/Off | See Specific Table Columns for data used in Chart lines and bars |
| <input type="checkbox"/> WUhc (inches) | Historical Daily Crop Water Use |
| <input checked="" type="checkbox"/> WUhc (inches) | Cumulative Historical Daily Crop Water Use |
| <input type="checkbox"/> WUac (inches) | Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> WUac (inches) | Cumulative Actual Daily Crop Water Use (to date) + Historical (thereafter) |
| <input checked="" type="checkbox"/> Rain (inches) | Rainfall Events (bars) |
| <input checked="" type="checkbox"/> Irrigation (inches) | Irrigation Events (bars) |
| <input checked="" type="checkbox"/> WUefc (inches) | Cumulative Effective Water Input (Rain + Irrigation) |
| <input checked="" type="checkbox"/> R3 Stage | (Begin-pod elongation) |
| <input checked="" type="checkbox"/> R7 Stage | (Physiological maturity-no plant water uptake thereafter) |

Figure 19. Correlogram of agronomic means of 2017-2018 water response experiment across populations, environments and irrigation treatments. Number values represent Pearson correlation coefficients. Values graphically represented through color shading. Non-significant ($\alpha > 0.05$) pairwise relationships crossed-out.

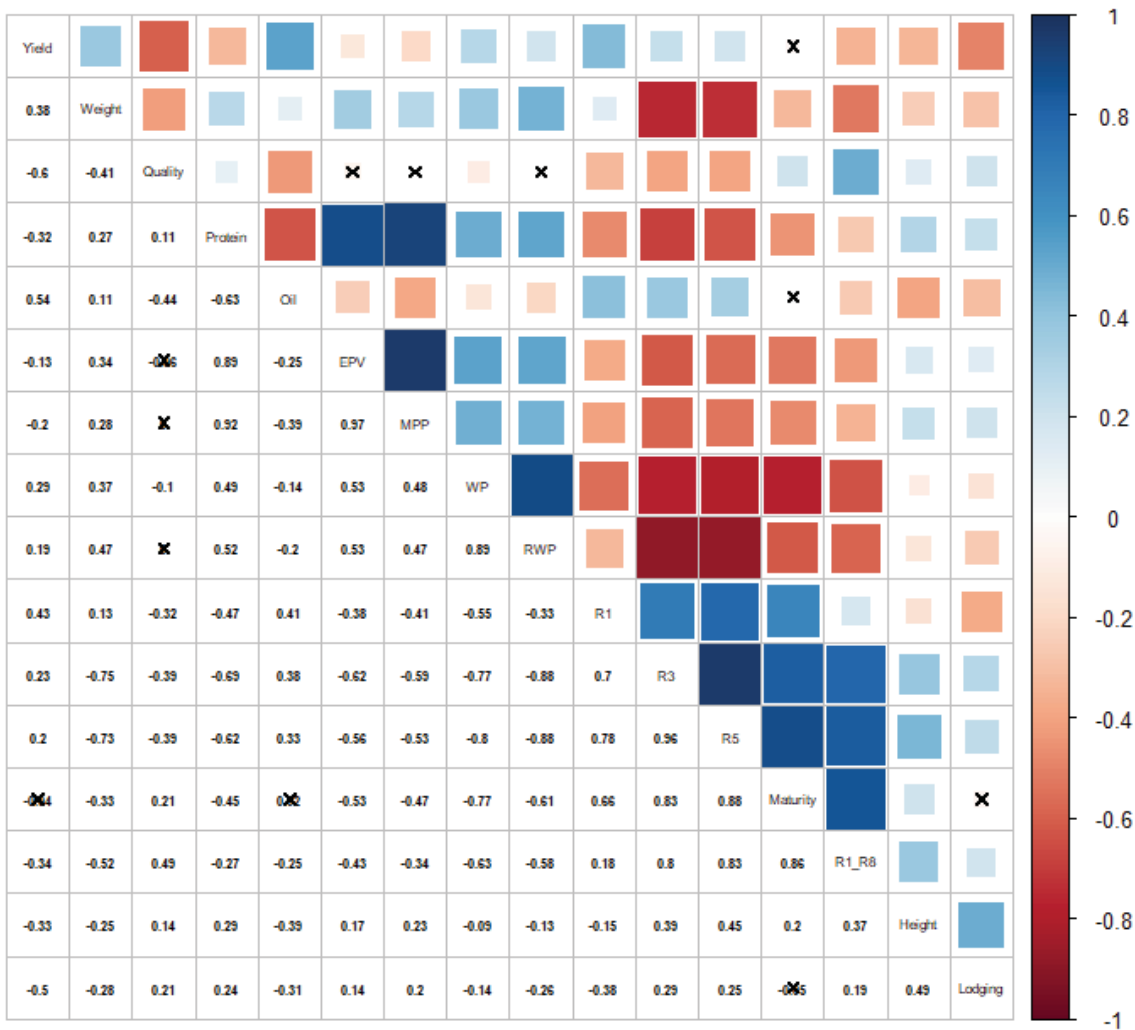


Figure 20. Correlogram of agronomic means of 2017-2018 water response experiment within the UX3000 population across environments and irrigation treatments. Pearson correlation coefficients graphically represented through color shading. Non-significant ($\alpha > 0.05$) pairwise relationships crossed-out.

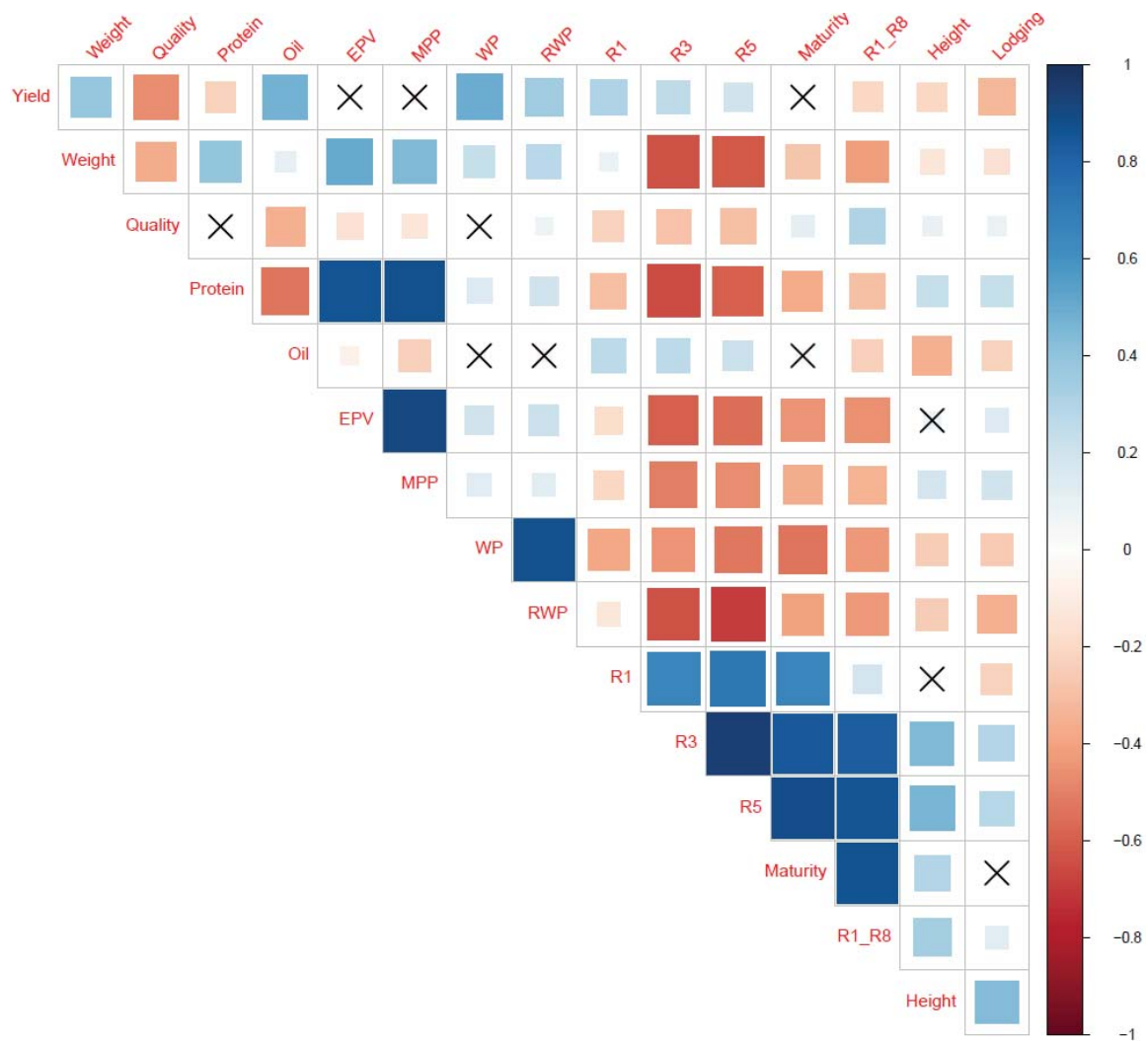


Figure 25. Red green and blue digital image channels relationship with growth stage at time of phenotyping and broad sense heritability and 95% confidence intervals on an entry mean basis during 2017-2018 water response experiment across populations, environments, and irrigation treatments

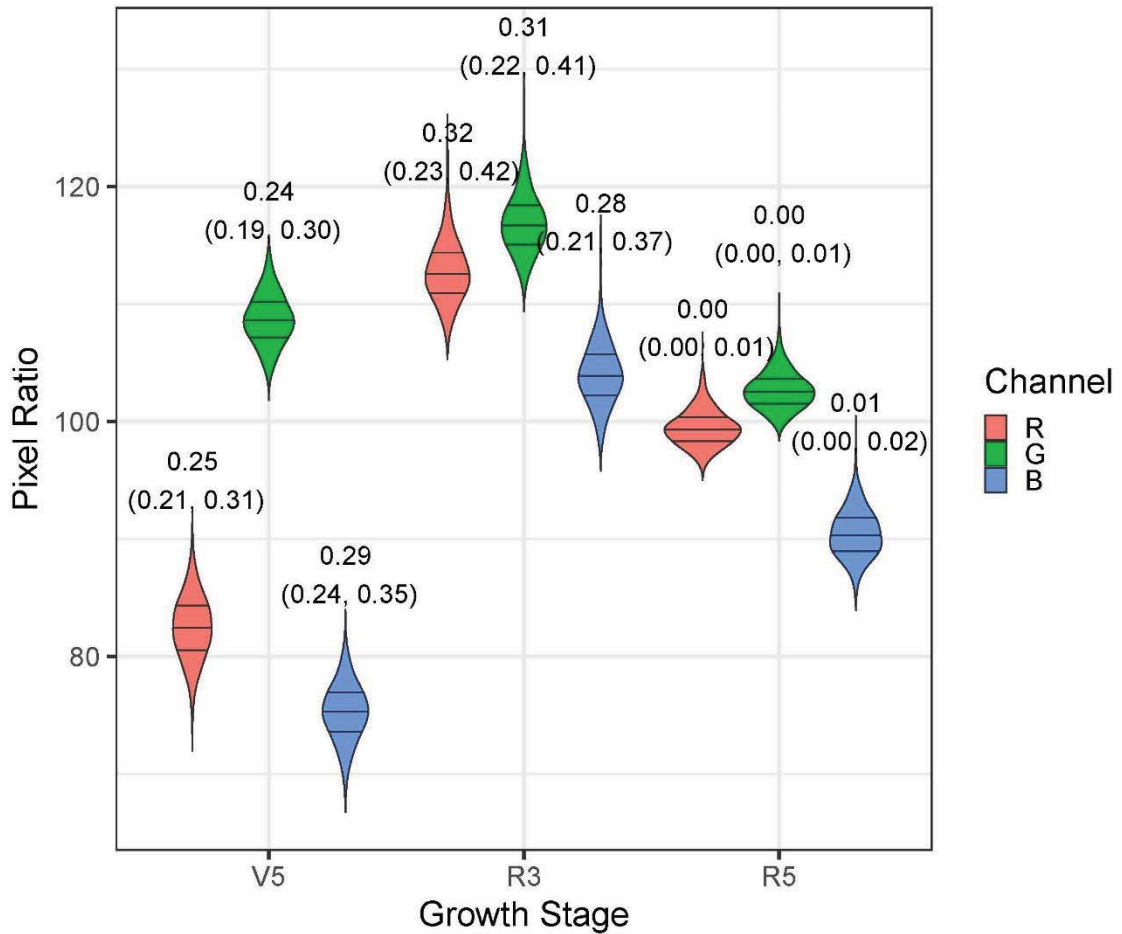


Figure 26. Water productivity least square means quartile group average and 95% confidence interval of spectral wavelength least square means across environments, populations, and irrigation treatments during the 2017-2018 water response experiment collected at the V5 growth stage; broad sense heritability on an entry mean basis of spectral wavelengths indicated through dark grey bars

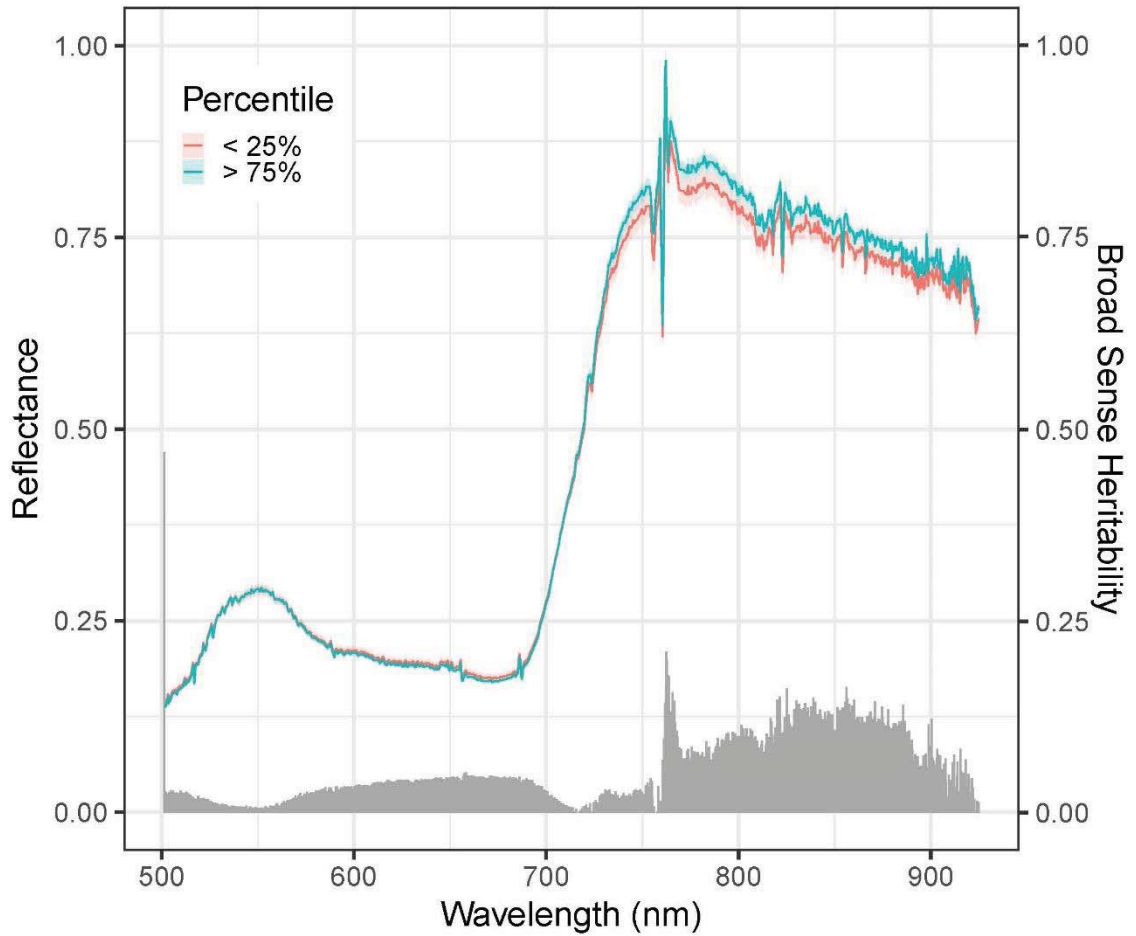


Figure 27. Water productivity least square means quartile group average and 95% confidence interval of spectral wavelength least square means across environments, populations, and irrigation treatments during the 2017-2018 water response experiment collected at the R5 growth stage; broad sense heritability on an entry basis of spectral wavelengths indicated through dark grey bars

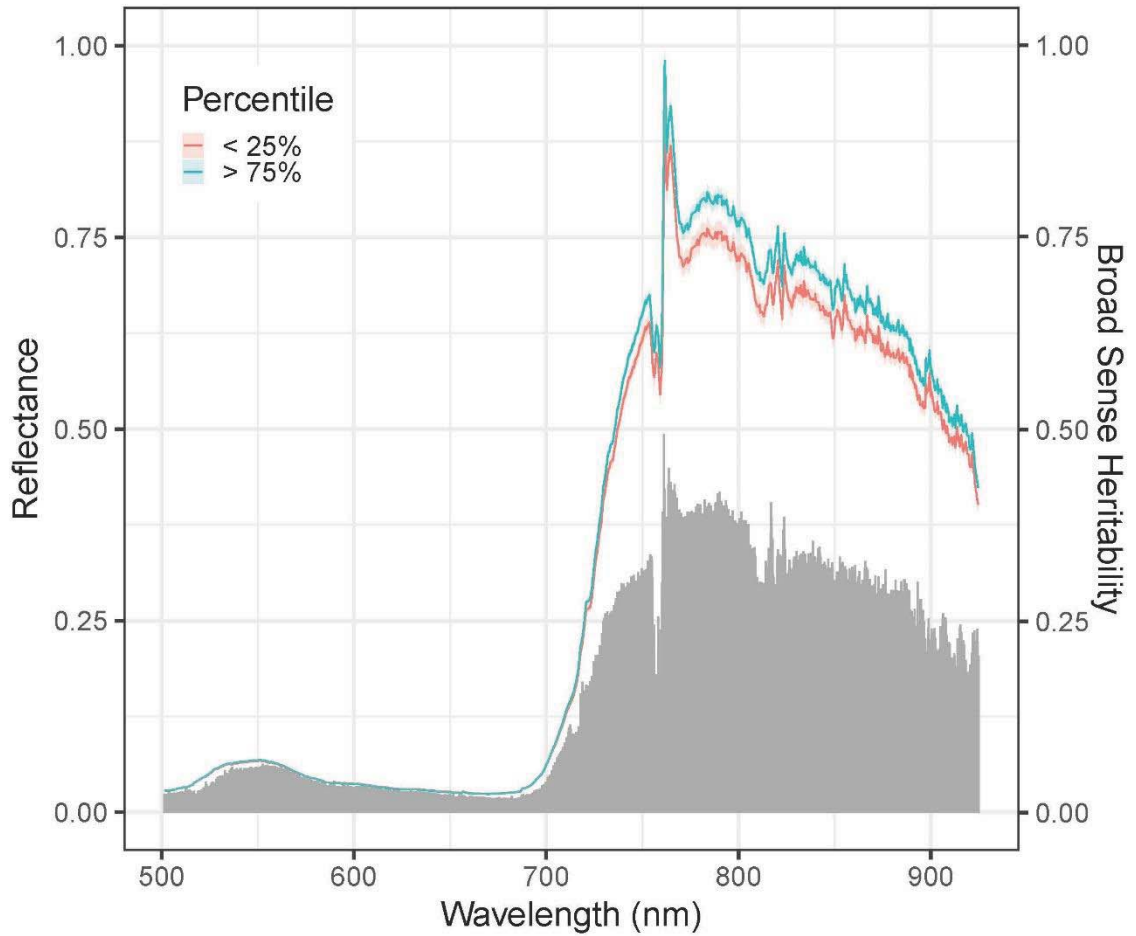


Figure 28. Manhattan plot for UX3000 population considering least square means of water productivity over environments and irrigation treatments (Overall), over environments within the irrigated treatment (Irrigated), and over environments within the rainfed treatment (Rainfed) during the 2017-2018 water response experiment

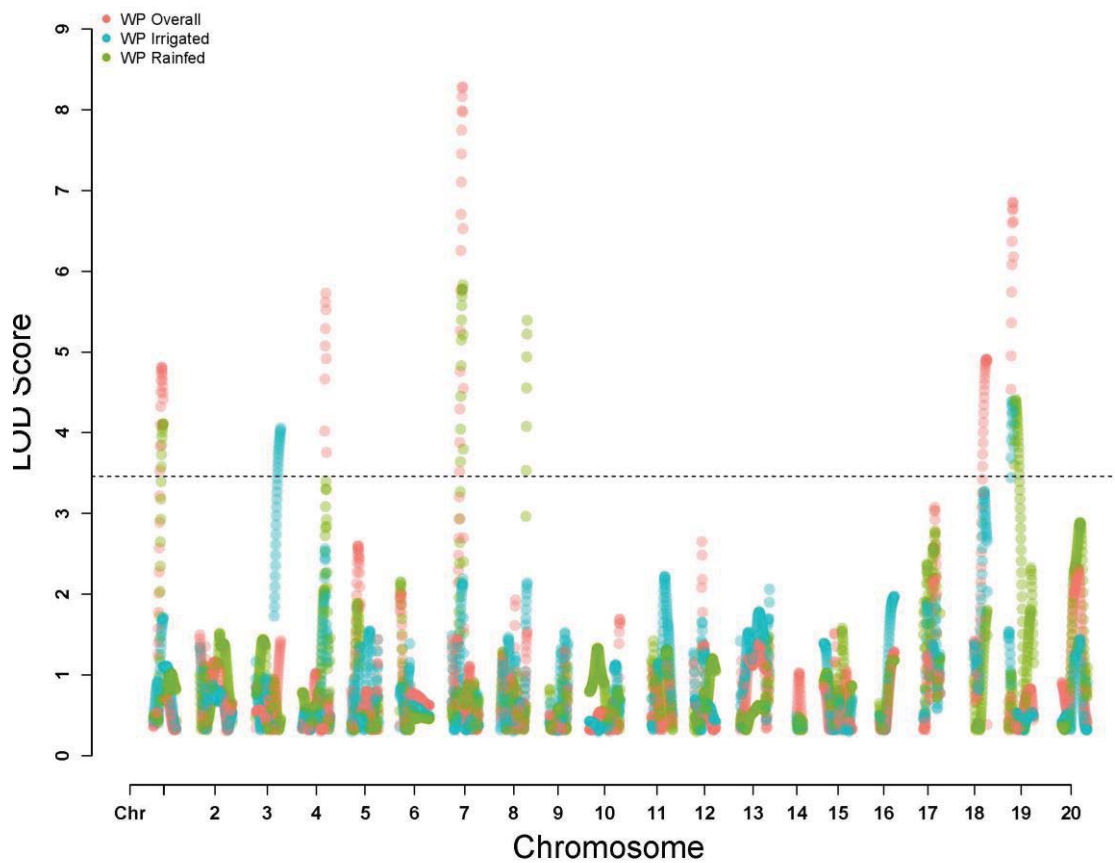


Figure 29. Manhattan plot for UX3036 population considering least square means of water productivity over environments and irrigation treatments (Overall), over environments within the irrigated treatment (Irrigated), and over environments within the rainfed treatment (Rainfed) during the 2017-2018 water response experiment

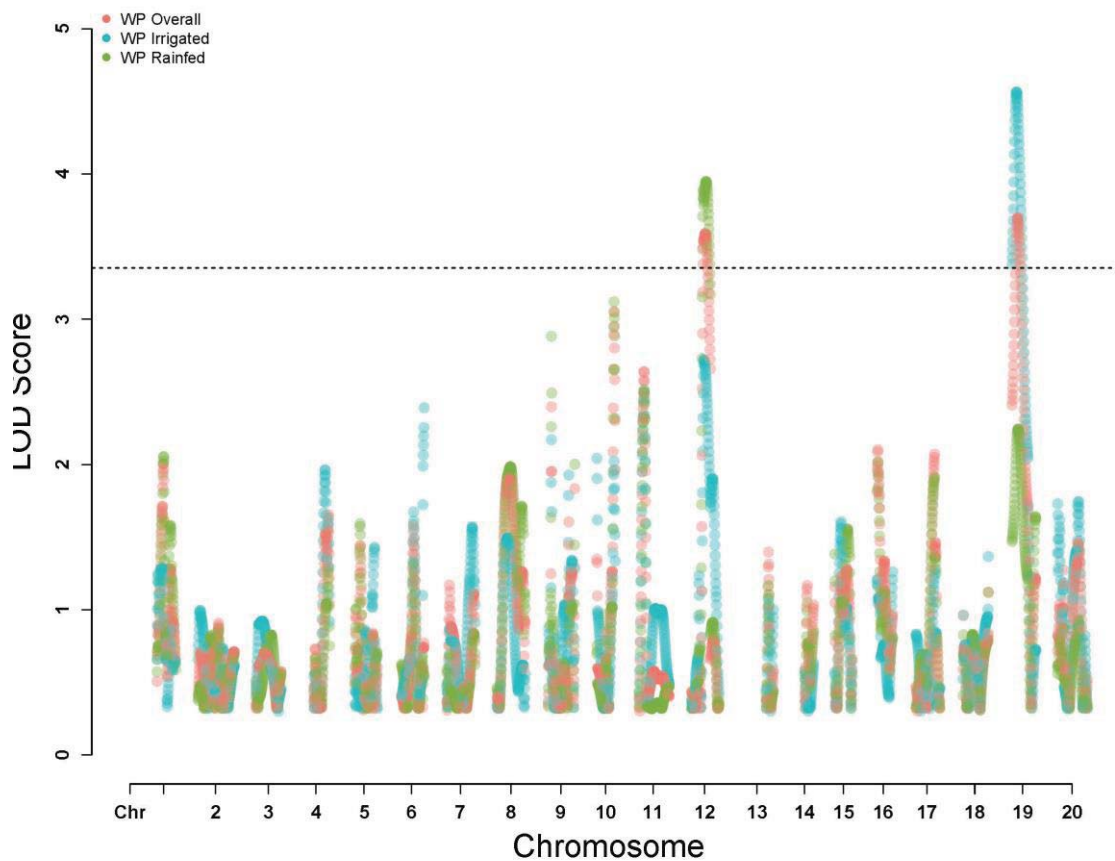


Figure 30. Summary of 2,319 unique phenomic trait QTL by growth stage and population across irrigation treatments and environments during the 2017-2018 water response experiment

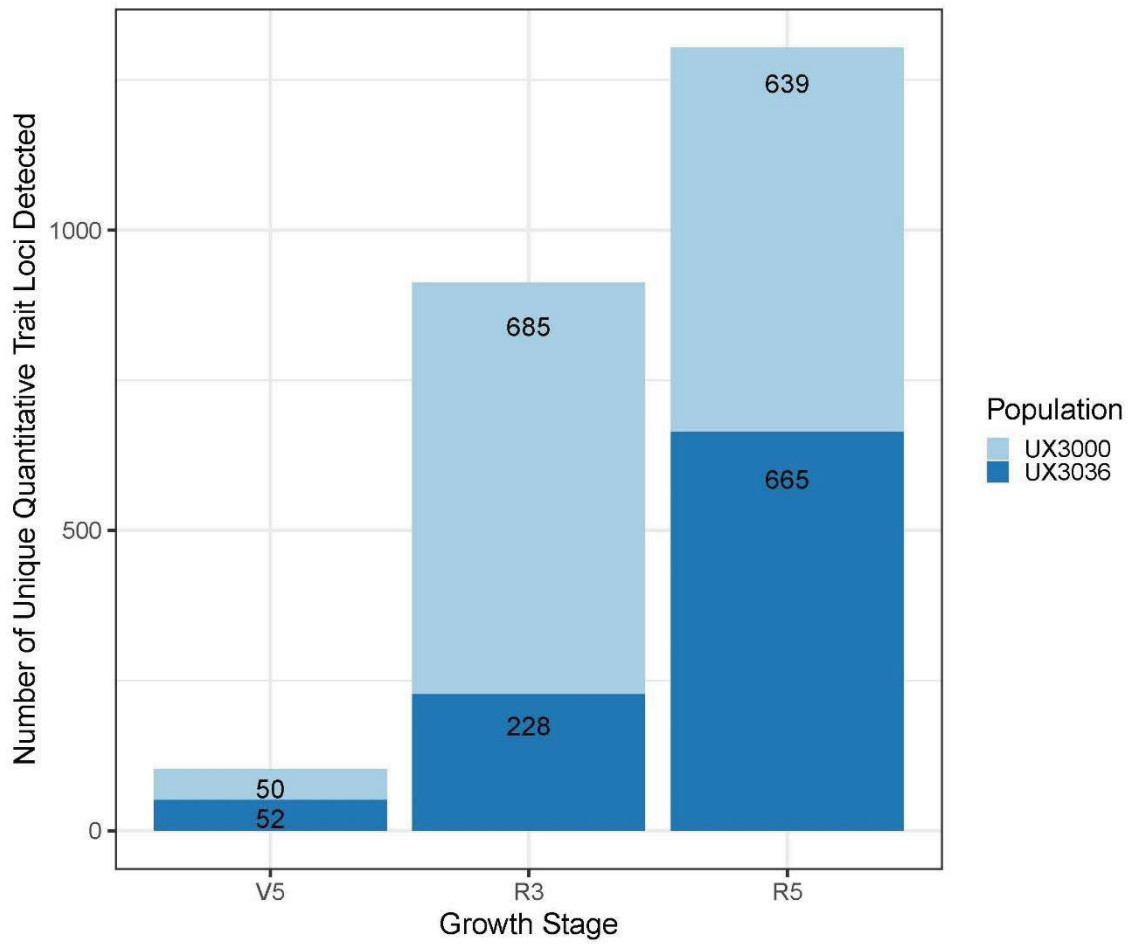


Figure 32. Identified water productivity QTL across irrigation treatments and environments during the 2017-2018 water response experiment heatmap and hierarchical clustering dendrogram to reported QTL object type categories on Soybase (soybase.org). WP QTL specified in the following format: POPUALATION_CHROMOSOME. Count data of reported QTL's object type with overlapping genetic position confidence intervals was normalized and used for construction of both heatmap and dendrogram. Values represent relative portion of object type overlapping (e.g. 1.00 represents all overlapping QTL fall one specified object type). Dendrogram on left of figure represents relative similarity of QTL in relationship to ratios of overlapping Soybase QTL object types available at soybase.org

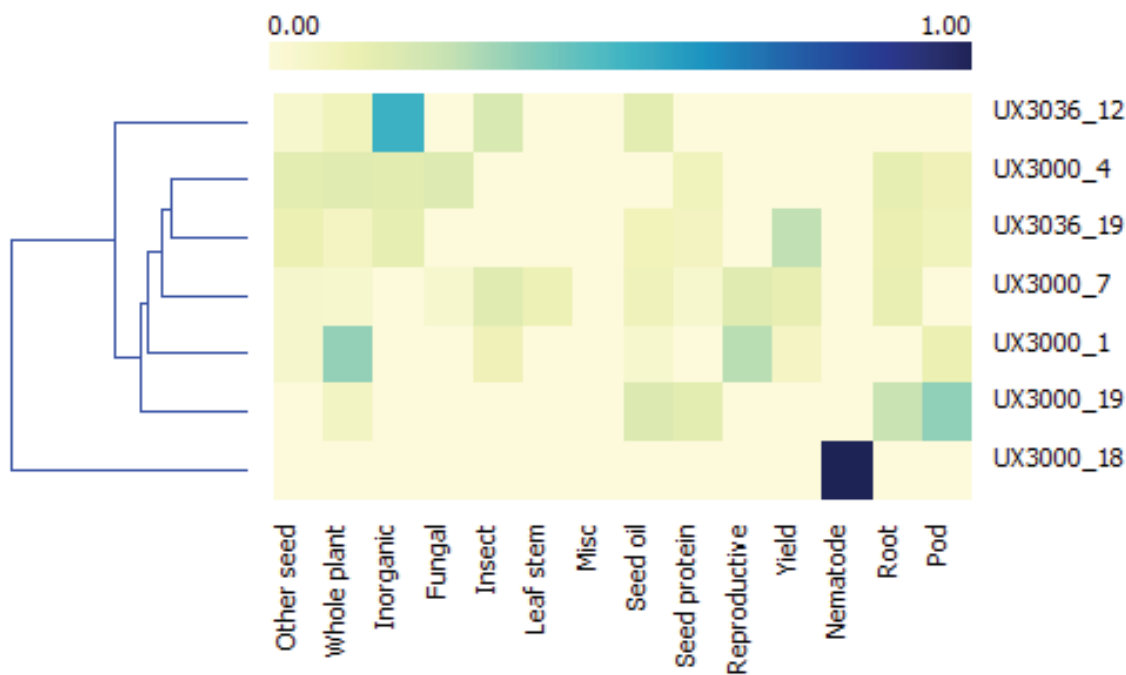


Figure 33. Regression coefficients of generalized linear regression with elastic net regulation (ENET) model variables by population using least square means (LSMEANS) of water productivity across environments and irrigation treatments during 2017-2018 water response experiment in combination with genomic data and phenomic data collected at the V5 growth stage; flanking SNP markers of detected WP QTL indicated with orange dot

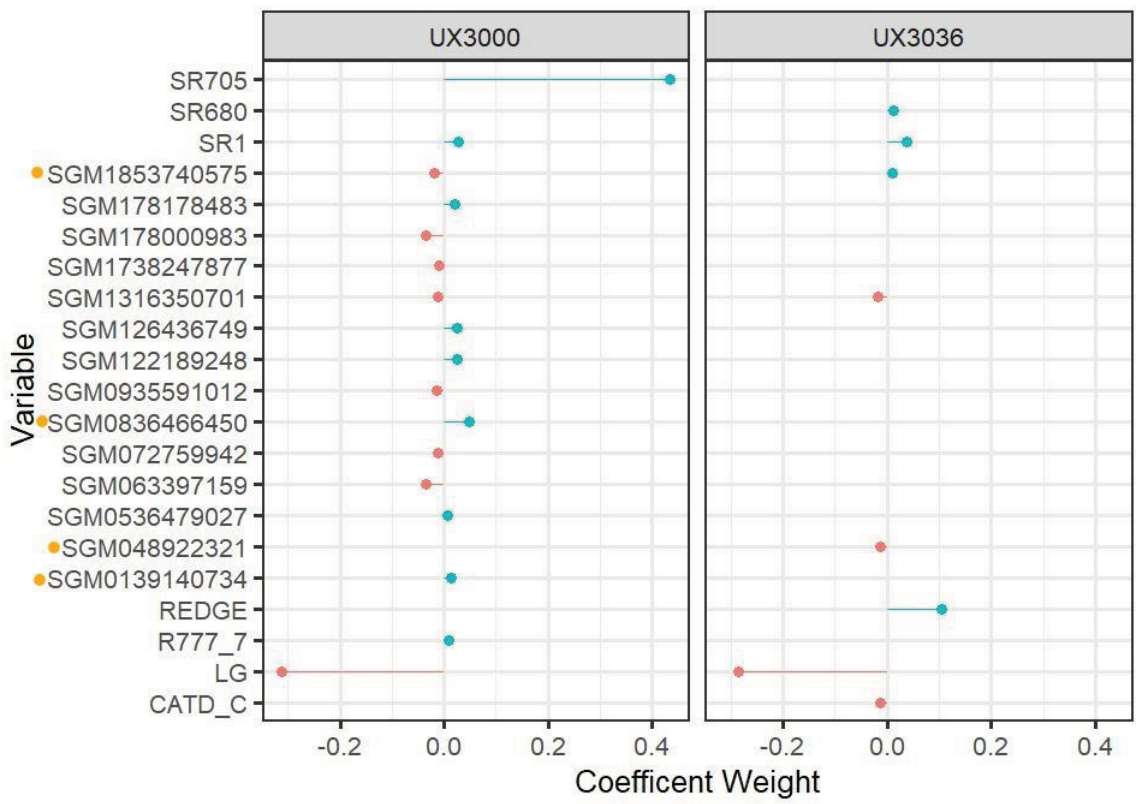


Figure 34. Regression coefficients of generalized linear regression with elastic net regulation (ENET) model variables by population using least square means (LSMEANS) of water productivity across environments and irrigation treatments during 2017-2018 water response experiment in combination with genomic data and phenomic data collected at the R5 growth stage; flanking SNP markers of detected WP QTL indicated with orange dot

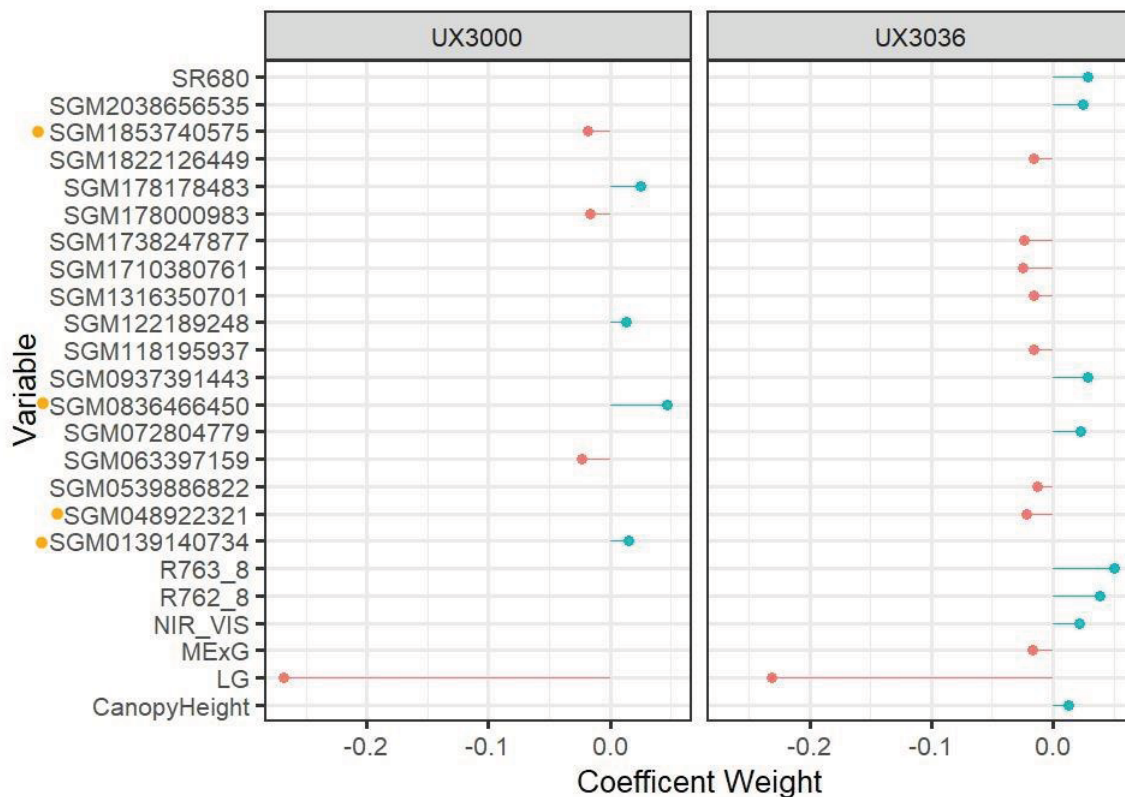


Figure 35. Predicted to observed Pearson correlation coefficient estimations and 95% confidence intervals of generalized linear regression with elastic net regulation (ENET) model over phenomic data collection growth stages and data subsets across environments and irrigation treatments in the 2017-2018 water response experiment using CV1 scheme. Date set type indicated on upper margin and growth stage relative to phenomic data collection on right hand margin.

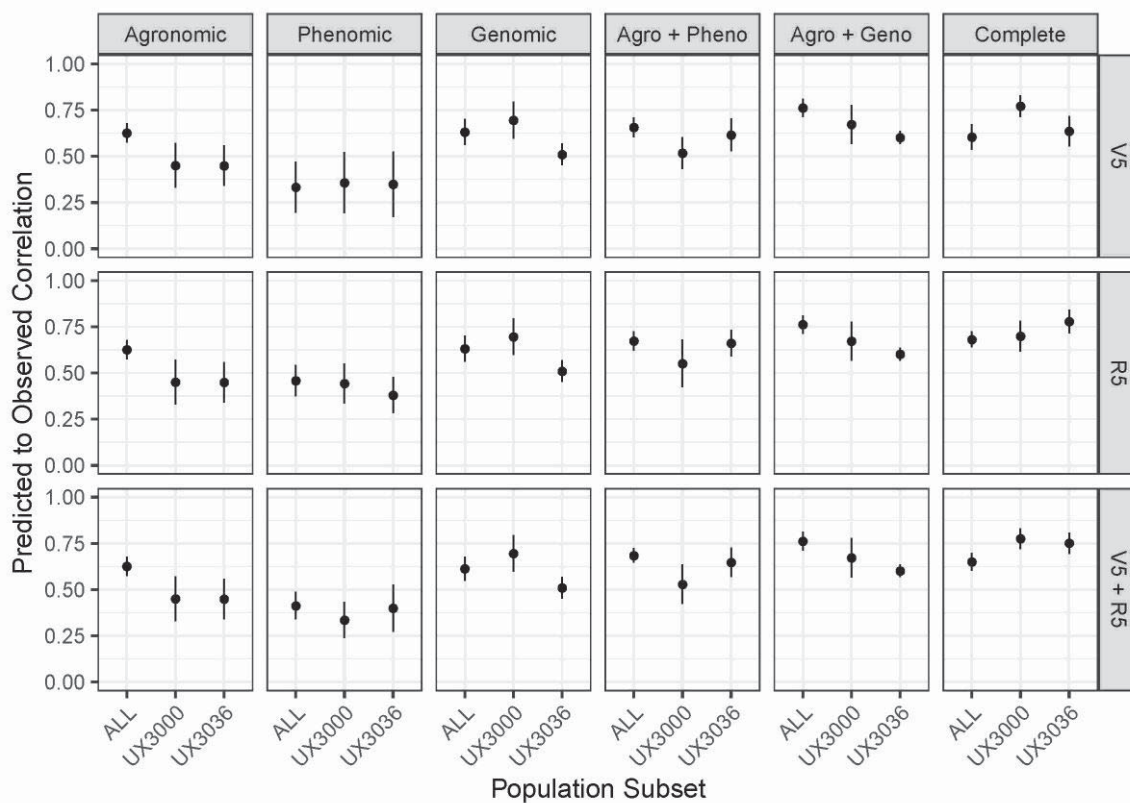


Figure 36. Predicted to observed Pearson correlation coefficient estimations and 95% confidence intervals of generalized linear regression with elastic net regulation (ENET) model over phenomic data collection growth stages, populations, environments and irrigation treatments in the 2017-2018 water response experiment using CV2 scheme.

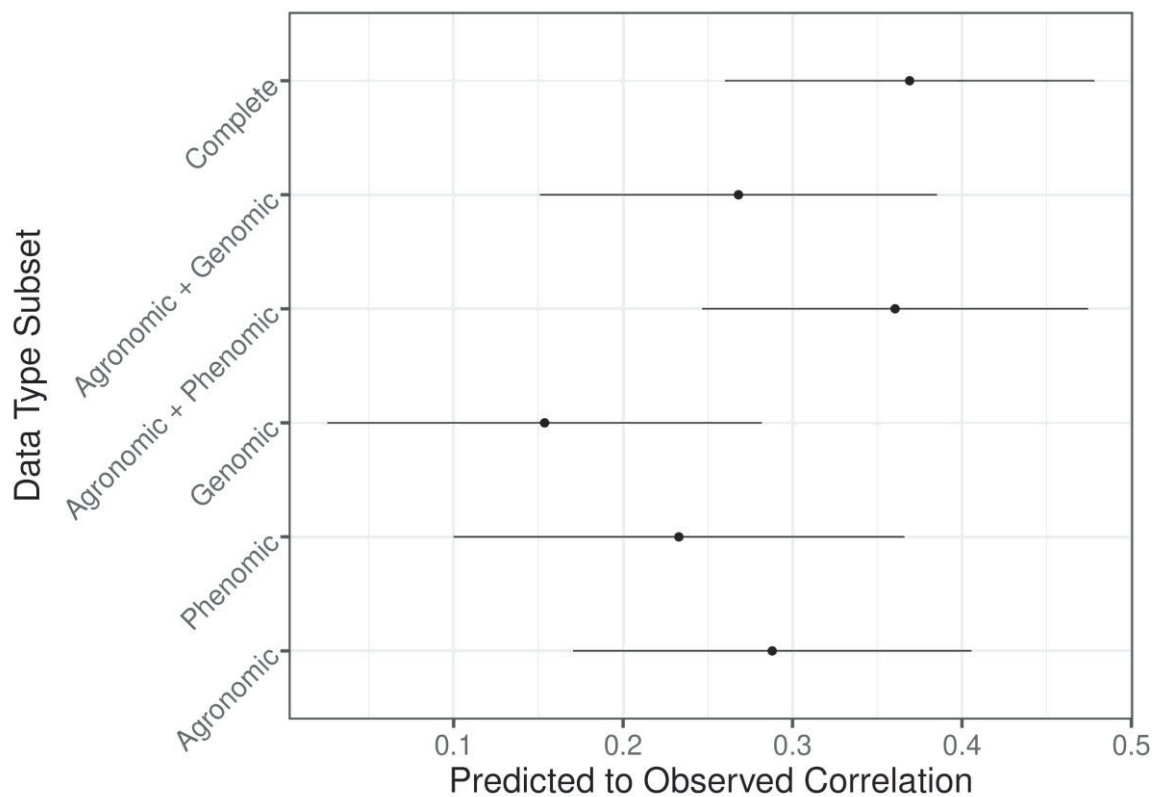


Figure 37. Relative predictor importance estimations and 95% confidence intervals estimated from interaction curvature method and CART algorithm from plot data of 2017-2018 water response experiment within the UX3000 population. Growth stage relative to phenomic data collection variables indicated in upper plot margin. Categories of individual traits listed in appendix 7.

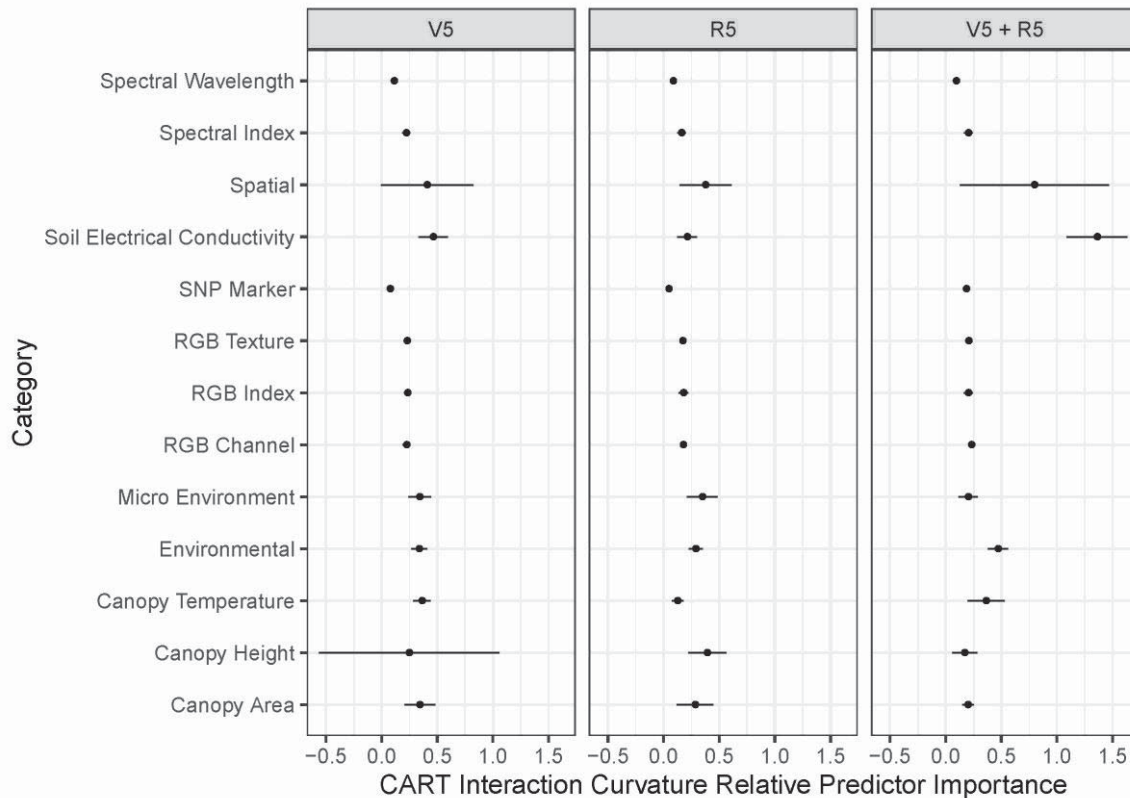
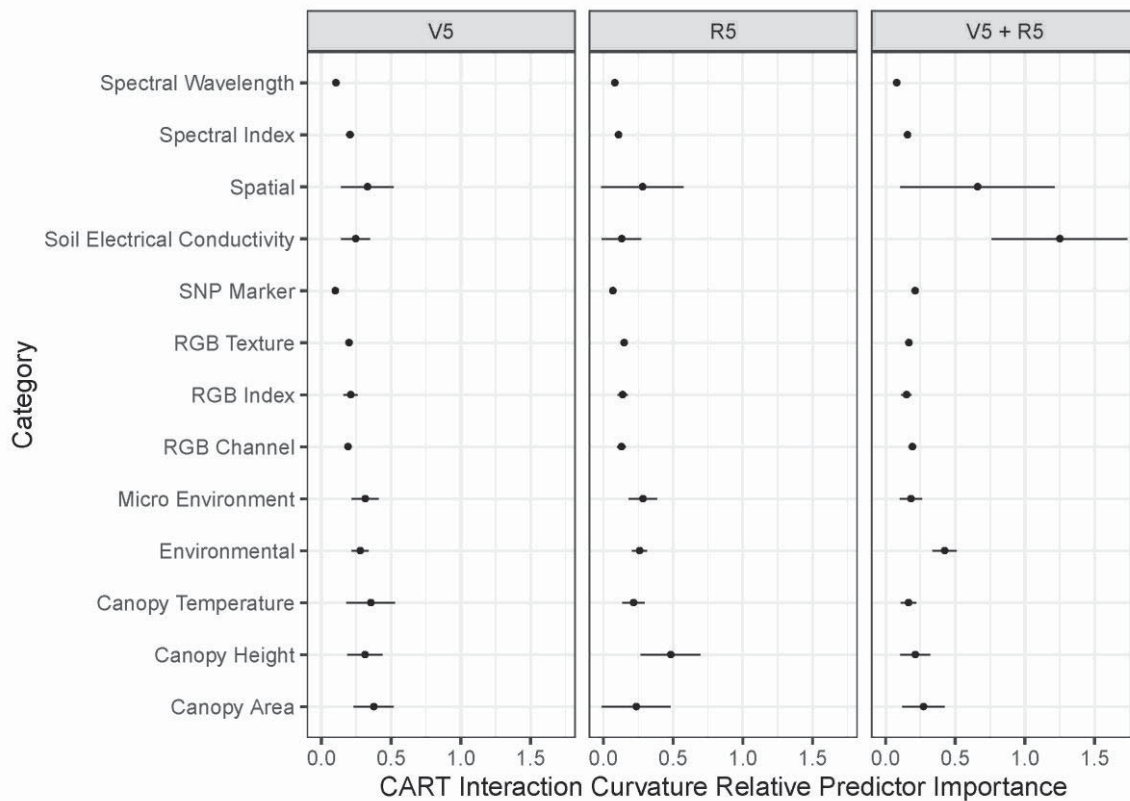


Figure 38. Relative predictor importance estimations and 95% confidence intervals estimated from interaction curvature method and CART algorithm from plot data of 2017-2018 water response experiment within the UX3036 population. Growth stage relative to phenomic data collection variables indicated in upper plot margin. Categories of individual traits listed in appendix 7.



APPENDIX

1. Image Processing Script

```

TotalnoImage = height(allresults);
srepath = 'D:\Box Sync\Dissertation\Drought Project Phenotyping
Data\2018\SC R5\SC R5 3.0\';

IndicesAllFile = ["Area", "AreaVar", "Perimeter", "PF",
"MinoxAxisLength", "ConvexArea", "EquivDiameter", "Solidity",
"Extent", "FilledArea", "Orientation", "R", "G", "B", "r", "g", "b", "r-g", "g-
b", "(g-b)/(r-
g)", "INT", "GRRRI", "NDI", "NGRDI", "NGBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG",
"MExG", "ExG", "ExR", "ExGR", "COM1", "COM2", "X", "Y", "Z", "L*", "a*", "b*", "b*
/a*", "H", "S", "V", "RF", "YF", "GF", "CF", "BF", "MF", "RGF", "YPrime", "Cb", "Cr", ];
IndicesTextureFile = ["R", "G", "B", "r", "g", "b", "r-g", "g-b", "(g-b)/(r-
g)", "INT", "GRRRI", "NDI", "NGRDI", "NGBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG",
"MExG", "ExG", "ExR", "ExGR", "COM1", "COM2", "X", "Y", "Z", "L*", "a*", "b*", "b*
/a*", "H", "S", "V", "YPrime", "Cb", "Cr", ];
for t = 1:38
    ContrastIndexName = strcat(string(IndicesTextureFile(t)), '
Contrast');
    CorrelationIndexName = strcat(string(IndicesTextureFile(t)), '
Correlation');
    EnergyIndexName = strcat(string(IndicesTextureFile(t)), ' Energy');
    HomogeneityIndexName = strcat(string(IndicesTextureFile(t)), '
Homogeneity');
    IndicesAllFile(1,53+4*t) = ContrastIndexName;
    IndicesAllFile(1,54+4*t) = CorrelationIndexName;
    IndicesAllFile(1,55+4*t) = EnergyIndexName;
    IndicesAllFile(1,56+4*t) = HomogeneityIndexName;
end
IndicesAllFile(1,209) = 'Gray Contrast';
IndicesAllFile(1,210) = 'Gray Correlation';
IndicesAllFile(1,211) = 'Gray Energy';
IndicesAllFile(1,212) = 'Gray Homogeneity';
IndicesColorWorkspace =
["R", "G", "B", "r", "g", "b", "rg", "gb", "gbrg", "INT", "GRRRI", "NDI", "NGRDI", "N
GBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG", "MExG", "ExG", "ExR", "ExGR", "COM1",
"COM2", "X", "Y", "Z", "Lasterisk", "aasterisk", "basterisk", "basteriskaaste
risk", "H", "S", "V", "RF", "YF", "GF", "CF", "BF", "MF", "RGF", "YPrime", "Cb", "Cr
", ];
IndicesTextureWorkspace =
["R", "G", "B", "r", "g", "b", "rg", "gb", "gbrg", "INT", "GRRRI", "NDI", "NGRDI", "N
GBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG", "MExG", "ExG", "ExR", "ExGR", "COM1",
"COM2", "X", "Y", "Z", "Lasterisk", "aasterisk", "basterisk", "basteriskaaste
risk", "H", "S", "V", "YPrime", "Cb", "Cr", ];

for i = 1:TotalnoImage

BW_out = allresults.output{i,1};
BW_out = bwpropfilt(BW_out, 'Area', [2500 + eps(2500), Inf]);
I = imread(allresults.fileName{i,1});

maskedImage = I;
maskedImage(repmat(~BW_out, [1 1 3])) = 0;

```

```

% Get properties for thresholded image.
properties = regionprops(BW_out, {'Area', 'ConvexArea',
'Perimeter', 'MinorAxisLength', 'EquivDiameter', 'Solidity', 'Extent',
'FilledArea', 'Orientation'});

properties = struct2table(properties);

Area = sum(properties.Area);
AreaVar = var(properties.Area);
ConvexArea = sum(properties.ConvexArea);
AreaFilled = sum(properties.FilledArea);
Perimeter = sum(properties.Perimeter);
MinorAxisLength = max(properties.MinorAxisLength);
EquivDiameter = mean(properties.EquivDiameter);
Solidity = mean(properties.Solidity);
Extent = mean(properties.Extent);
[row, column] = size(BW_out);
PF = Area/row/column;
Orientation = mean(properties.Orientation);

% R,G,B
R = maskedImage(:, :, 1);
G = maskedImage(:, :, 2);
B = maskedImage(:, :, 3);

% r,g,b,r-g,g-b,(g-b)/(r-g)
RN = R/255;
GN = G/255;
BN = B/255;
r = RN./(RN+GN+BN);
g = GN./(RN+GN+BN);
b = BN./(RN+GN+BN);
rg = r-g;
gb = g-b;
gbrg = (g-b)./(r-g);

%
INT, GRRRI, NDI, NGRDI, NGBDI, VARI, VDVI, CIVE, TGI, VEG, MExG, ExG, ExR, ExGR, COM1,
COM2,

INT = (R+G+B)/3;
GRRRI = G./R;
NDI = 128*((G-R)./(G+R)+1);
NGRDI = (G-R)./(G+R);
NGBDI = (G-B)./(G+B);
VARI = (G-R)./(G+R-B);
VDVI = (2*G-B-R)./(2*G+B+R);
CIVE = 0.441*R-0.811*G+0.385*B+18.78745;
TGI = -0.5*((670-480)*(R-G)-(670-550)*(R-B));
VEG =
uint8(double(G)./((double(R).^0.667).*double(B).^0.333));
MExG = 1.262*G-0.884*R-0.311*B;
ExG = 2*g-r-b;
ExR = 1.3*R-G;
ExGR = ExG-ExR;
COM1 = ExG+CIVE+ExGR+VEG;
COM2 = 0.36*ExG+0.47*CIVE+0.17*VEG;

```

```

maskedImage = uint8(maskedImage);

% X,Y,Z
maskedImageXYZ = rgb2xyz(maskedImage);
X = maskedImageXYZ(:,:,1);
Y = maskedImageXYZ(:,:,2);
Z = maskedImageXYZ(:,:,3);

% L*,a*,b*,b*/a*
maskedImageLab = rgb2lab(maskedImage);
Lasterisk = maskedImageLab(:,:,1);
aasterisk = maskedImageLab(:,:,2);
basterisk = maskedImageLab(:,:,3);
basteriskaasterisk = basterisk./aasterisk;

%
H,S,V,RedFraction,YellowFraction,GreenFraction,CyanFraction,BlueFraction,
MagentaFraction,RelativeGreenFraction
maskedImageHSV = rgb2hsv(maskedImage);
H = maskedImageHSV(:,:,1);
S = maskedImageHSV(:,:,2);
V = maskedImageHSV(:,:,3);
RF = (H<1/6);
YF = (H>=1/6) & (H<1/3);
GF = (H>=1/3) & (H<0.5);
CF = (H>=0.5) & (H<2/3);
BF = (H>=2/3) & (H<5/6);
MF = (H>=5/6);
RGF = (H>=1/6) & (H<=0.5);

% Y',Cb,Cr
maskedImageYCbCr = double(rgb2ycbcr(maskedImage));
YPrime = maskedImageYCbCr(:,:,1);
Cb = maskedImageYCbCr(:,:,2);
Cr = maskedImageYCbCr(:,:,3);

% Save the first indices
IndicesAllFile(i,1) = Area;
IndicesAllFile(i,2) = AreaVar;
IndicesAllFile(i,3) = Perimeter;
IndicesAllFile(i,4) = PF;
IndicesAllFile(i,5) = MinorAxisLength;
IndicesAllFile(i,6) = ConvexArea;
IndicesAllFile(i,7) = EquivDiameter;
IndicesAllFile(i,8) = Solidity;
IndicesAllFile(i,9) = Extent;
IndicesAllFile(i,10) = AreaFilled;
IndicesAllFile(i,11) = Orientation;

% Compute the average value of each color index for each
masked image
BW = double(BW_out);
for c = 1:45
    ColorIndexMatrix =
double(eval(IndicesColorWorkspace(c)));
    % Set NaN and Inf to 0
    ColorIndexMatrix(isnan(ColorIndexMatrix)) = 0;

```

```

        ColorIndexMatrix(isinf(ColorIndexMatrix)) = 0;
        % Set matrix values of soil to 0
        ColorIndexMatrix = ColorIndexMatrix.*BW;
        ColorIndex = sum(ColorIndexMatrix(:))/Area;
        IndicesAllFile(i,11+c) = ColorIndex;
    end

    % Compute 4 texture indices of most color indice matrices
    plus gray image for each masked image
    % Prepare a BW mask to convert matrix values of soil to NaN
    so that they will be ignored in GLCM
    BWCroppedTexture = double(BW_out);
    BWCroppedTexture(BWCroppedTexture==0) = NaN;

    for t = 1:38
        TextureIndexMatrix =
double(eval(IndicesTextureWorkspace(t)));
        % Remove soil effect
        TextureIndexMatrix =
TextureIndexMatrix.*BWCroppedTexture;
        % GrayLimits are set as the limits of the original
matrix; NumLevels is set as the number of unique number of the original
matrix; Offset is set as scanning vertically upward by 1 pixel
distance;
        GLCM =
graycomatrix(TextureIndexMatrix, 'GrayLimits', [], 'NumLevels', 256, 'Offset
', [-1 0]);
        TextureIndices = graycoprops(GLCM);
        IndicesAllFile(i,53+4*t) = TextureIndices.Contrast;
        % Replace NaN with 0 when there is no correlation
        if isnan(TextureIndices.Correlation) == 1
            IndicesAllFile(i,54+4*t) = 0;
        else IndicesAllFile(i,54+4*t) =
TextureIndices.Correlation;
        end
        IndicesAllFile(i,55+4*t) = TextureIndices.Energy;
        IndicesAllFile(i,56+4*t) = TextureIndices.Homogeneity;
    end

    % Gray Image
    maskedImageGray = double(rgb2gray(maskedImage));
    TextureIndexMatrix = maskedImageGray.*BWCroppedTexture;
    GLCM =
graycomatrix(TextureIndexMatrix, 'GrayLimits', [], 'NumLevels', 256, 'Offset
', [-1 0]);
    TextureIndices = graycoprops(GLCM);
    IndicesAllFile(i,209) = TextureIndices.Contrast;
    if isnan(TextureIndices.Correlation) == 1
        IndicesAllFile(i,210) = 0;
    else IndicesAllFile(i,210) = TextureIndices.Correlation;
    end
    IndicesAllFile(i,211) = TextureIndices.Energy;
    IndicesAllFile(i,212) = TextureIndices.Homogeneity;

    % Write images
    BWfilename = strcat(srepath, 'BW\ ', num2str(i), '.bmp');

```

```

        Maskedfilename = strcat(srepath, 'Mask\ ', num2str(i), '.bmp'
    );
        imwrite(BW_out, BWfilename);
        imwrite(maskedImage, Maskedfilename);

    end

    % Export Results to Exel File.
    export = IndicesAllFile
IndicesAllFile = ["FileName", "Area", "AreaVar", "Perimeter", "PF",
"MinoxAxisLength", "ConvexArea", "EquivDiameter", "Solidity",
"Extent", "FilledArea", "Orientation", "R", "G", "B", "r", "g", "b", "r-g", "g-
b", "(g-b)/(r-
g)", "INT", "GRR1", "NDI", "NGRDI", "NGBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG"
, "MExG", "ExG", "ExR", "ExGR", "COM1", "COM2", "X", "Y", "Z", "L*", "a*", "b*", "b*
/a*", "H", "S", "V", "RF", "YF", "GF", "CF", "BF", "MF", "RGF", "Y'", "Cb", "Cr", ];
IndicesTextureFile = ["R", "G", "B", "r", "g", "b", "r-g", "g-b", "(g-b)/(r-
g)", "INT", "GRR1", "NDI", "NGRDI", "NGBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG"
, "MExG", "ExG", "ExR", "ExGR", "COM1", "COM2", "X", "Y", "Z", "L*", "a*", "b*", "b*
/a*", "H", "S", "V", "Y'", "Cb", "Cr", ];
for t = 1:38
    ContrastIndexName = strcat(string(IndicesTextureFile(t)), '
Contrast');
    CorrelationIndexName = strcat(string(IndicesTextureFile(t)), '
Correlation');
    EnergyIndexName = strcat(string(IndicesTextureFile(t)), ' Energy');
    HomogeneityIndexName = strcat(string(IndicesTextureFile(t)), '
Homogeneity');
    IndicesAllFile(1,54+4*t) = ContrastIndexName;
    IndicesAllFile(1,55+4*t) = CorrelationIndexName;
    IndicesAllFile(1,56+4*t) = EnergyIndexName;
    IndicesAllFile(1,57+4*t) = HomogeneityIndexName;
end
IndicesAllFile(1,210) = 'Gray Contrast';
IndicesAllFile(1,211) = 'Gray Correlation';
IndicesAllFile(1,212) = 'Gray Energy';
IndicesAllFile(1,213) = 'Gray Homogeneity';
IndicesColorWorkspace =
["R", "G", "B", "r", "g", "b", "rg", "gb", "gbrg", "INT", "GRR1", "NDI", "NGRDI", "N
GBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG", "MExG", "ExG", "ExR", "ExGR", "COM1"
, "COM2", "X", "Y", "Z", "Lasterisk", "aasterisk", "basterisk", "basteriskaaste
risk", "H", "S", "V", "RF", "YF", "GF", "CF", "BF", "MF", "RGF", "YPrime", "Cb", "Cr
", ];
IndicesTextureWorkspace =
["R", "G", "B", "r", "g", "b", "rg", "gb", "gbrg", "INT", "GRR1", "NDI", "NGRDI", "N
GBDI", "VARI", "VDVI", "CIVE", "TGI", "VEG", "MExG", "ExG", "ExR", "ExGR", "COM1"
, "COM2", "X", "Y", "Z", "Lasterisk", "aasterisk", "basterisk", "basteriskaaste
risk", "H", "S", "V", "YPrime", "Cb", "Cr", ];
    Column_Headers = IndicesAllFile;
    xlFilename = strcat(srepath, 'ImageAnalysis.xlsx');
    xlRange = 'A1';
    xlswrite(xlFilename, Column_Headers, 'ImageAnalysis', xlRange);

    Names = allresults{:,2};
    xlswrite(xlFilename, Names, 'ImageAnalysis', 'A2');

    xlswrite(xlFilename, export, 'ImageAnalysis', 'B2');

```

2. Spectrum Processing Script

```

clear;
clc;
%-----
%WORKING DIRECTORY SETUP
%These values need to be adjusted until the following break

%Set Working Directories
srepath_all = 'D:\Box Sync\Dissertation\Drought Project Phenotyping
Data\2017\Mead\Mead Late Season\Mead-2017.8.12-Late Season\';
%srepath_all = 'D:\Box Sync\Dissertation\Drought Project Phenotyping
Data\2017\Stevens Creek\Stevens Creek-2017.7.1-Early Season-
Organized\';

%List Folders within Directory
files= dir(srepath_all);
directories = [files.isdir];
sub_folder = files(directories);

sub_folders = {'1.1';'1.2';'2.1';'2.2';'3.1';'3.2';'4.1';'4.2'};

%-----
%Code is automated from this point forward based on values denoted
above
%-----

%REFLECTANCE ADJUSTMENTS

for i = 1:(length(sub_folders))

%Import Plot Raw Values and Calculate Reflectance
%Set source directory
srepath = strcat(srepath_all, sub_folders{i}, "\");

%Create filenames
fileU = strcat(srepath, 'Spectrum-U.xls.csv');
fileL = strcat(srepath, 'Spectrum-L.xls.csv');
fileM = strcat(srepath, 'Spectrum-M.xls.csv');
fileR = strcat(srepath, 'Spectrum-R.xls.csv');
fileINT = strcat(srepath, 'Integration Time.xls.csv');
fileLog = strcat(srepath, 'Measurements.xls.csv');

%Import Reference Files
Uraw= csvread(fileU,1);
Lraw = csvread(fileL,1);
Mraw = csvread(fileM,1);
Rraw = csvread(fileR,1);
Int = csvread(fileINT);
log = readtable(fileLog);

Llog = table2array(log(strcmp(log.LMR, 'L'),1));
Mlog = table2array(log(strcmp(log.LMR, 'M'),1));
Rlog = table2array(log(strcmp(log.LMR, 'R'),1));

```



```

%Calculate Reflectance
    %Need to match up Up looking and Down looking Values in situations
where
    %sensor bars where turned off to speed up system

    %Up looking Spectrometer Calculation
    %No Black Pixel Collected in 2017 so need to adjust by scaling to
    %lowest value in each array
    Utemp = (Uraw.*Int(:,1));

%Create Table for Loop Outputs
zedsdead_U = zeros(size(Utemp,1),1);
for q = 1:size(Utemp,1)
zedsdead_U(q,1) = min(Utemp(q,:));
end

%Calculate Reflectance
U = (Utemp - zedsdead_U)./ Int(:,1);

%Left Spectrometer

    %Index for Uplooking Adjustment Values
    U_L_index = (Llog(:,1)+2)/3;
    %No Black Pixel Collected in 2017 so need to adjust by scaling
to
    %lowest value in each array
    Ltemp = (Lraw.*Int((U_L_index),2));
    U_L = U(U_L_index,:);

%Create Table for Loop Outputs
zedsdead_L = zeros(size(Ltemp,1),1);
for w = 1:size(Ltemp,1)
zedsdead_L(w,1) = min(Ltemp(w,:));
end

%Downlooking Caluclation
D_L = (Ltemp - zedsdead_L)./ Int((U_L_index),2);

%Total Reflectance
L_1 = D_L ./ U_L;

    %Row Labels
    L = [Llog L_1];

%Middle Spectrometer
    %Index for Uplooking Adjustment Values
    U_M_index = (Mlog(:,1)+1)/3;
    U_M = U(U_M_index,:);
    %No Black Pixel Collected in 2017 so need to adjust by scaling
to
    %lowest value in each array
    Mtemp = (Mraw.*Int((U_M_index),2));
    U_M = U(U_M_index,:);

%Create Table for Loop Outputs
zedsdead_M = zeros(size(Mtemp,1),1);

```

```

for e = 1:size(Mtemp,1)
zedsdead_M(e,1) = min(Mtemp(e,:));
end

%Downlooking Caluclation
D_M = (Mtemp - zedsdead_M)./ Int((U_M_index),2);

%Total Reflectance
M_1 = D_M ./ U_M;

%Row Labels
M = [Mlog M_1];

%Right Spectrometer
%Index for Uplooking Adjustment Values
U_R_index = (Rlog(:,1)+0)/3;
U_R = U(U_R_index,:);
%No Black Pixel Collected in 2017 so need to adjust by scaling
to
%lowest value in each array
Rtemp = (Rraw.*Int((U_R_index),2));
U_R = U(U_R_index,:);

%Create Table for Loop Outputs
zedsdead_R = zeros(size(Rtemp,1),1);
for r = 1:size(Rtemp,1)
zedsdead_R(r,1) = min(Rtemp(r,:));
end

%Downlooking Caluclation
D_R = (Rtemp - zedsdead_R)./ Int((U_R_index),2);

%Total Reflectance
R_1 = D_R ./ U_R;

%Row Labels
R= [Rlog R_1];

%Calculate Average Spectrometer Readings for Variable Names Output
%This is required so reflectance indices may be calculated and compared
between plots
%Import Label Files
labs = readtable('D:\Box Sync\Dissertation\Drought Project
Phenotyping Data\2017\Mead\Mead Late Season\Speclabels.xlsx');

%Create Column Names for Merged Table
columnnames = labs{1,:};

%Merge Spectrometer Files
spec = vertcat(L, M, R);
spec = array2table(spec, 'VariableNames', columnnames);

%-----
%-----
%VEGETATION INDEX CALCULATIONS

%Photochemical Reflectance Index (PRI) calculation

```

```

%PRI=(R531-R570)/(R531+R570) by SRS sensor manual (Should we use other
proven parameter?)
    spec.PRI=(spec.R531 - spec.R570_1) ./ ((spec.R531 +
spec.R570_1));%Calculate PRI index

%Normalized difference vegetation index (NDVI) calculation
%NDVI=(R800-R630)/(R800+R630)
    spec.NDVI=(spec.R799_9 - spec.R630_1)./(spec.R799_9 +
spec.R630_1);%Calculate NDVI index

%Red Edge NDVI vegetation index (RENDVI) calculation
%Red edge NDVI=(R750-R705)/(R750+R705)
    spec.RENDVI=(spec.R750_1 - spec.R705_1)./(spec.R750_1 +
spec.R705_1);%calculate RENDVI index

%Green Normalized Vegetation Index(GNDVI)calculation
%GNDVI=(801-550)/(800+550)
    spec.GNDVI=(spec.R801_1 - spec.R549_9)./(spec.R799_9 +
spec.R549_9);%calculate GNDVI index

%Anthocyanin reflectance index (ARI) calculation
%ARI = (1/550) - (1/700)
    spec.ARI=(1./spec.R549_9)-(1./spec.R700_1);%calculate ARI index

%Chlorophyll Red-Edge
%ChlRdEg = [(760:800)/(540:560)]-1
    spec.ChlRdEg2=(sum(spec{:},1168:1293},2) ./ sum(spec{:},524:581},2))-
1;

%Crop Water Status
%CWSI = 531+570
    spec.CWSI = spec.R529_9 + spec.R570_1;

%Drought Stress Index # 1
%DSI1 = 520:530
    spec.DSI1=sum(spec{:},468:496},2);

%Drought Stress Index # 2
%DSI2 = 570:590
    spec.DSI2=sum(spec{:},609:665},2);

%Drought Stress Index #3
%DSI3 = 690:710
    spec.DSI3=sum(spec{:},957:1017},2);

%Grain Yield Index #1
%GYI1 = 500:700
    spec.GYI1=sum(spec{:},413:987},2);

%Grain Yield Index #2
%GYI2 = 700:950
    spec.GYI2 = sum(spec{:},987:1783},2);

%Grain Yield Index #3
%GYI1 = 680
    spec.GYI3 = sum(spec{:},926:928},2);

```

```

%Grain Yield Index #4
%GYI4 = 950:1000
    spec.GYI4 = sum(spec{:,1783:1957},2);

%Leaf Chlorophyll Index
%LCI = (850-710)/(850+680)
    spec.LCI = (spec.R850 - spec.R710_1)./(spec.R850 + spec.R679_9);

%Stress Index #1
%STI1= 710/810
    spec.STI1 = spec.R710_1./spec.R810_1;

%Stress Index #2
%STI2= 710/760
    spec.STI2 = spec.R710_1./spec.R760_2;

%Red Edge Index
%RE= 690-740
    spec.RE = spec.R690./spec.R740_2;

%Normalized Water Index
%NDWI= (800-680)/(800+680)
    spec.NDWI = (spec.R799_9 - spec.R679_9)./(spec.R799_9 +
spec.R679_9);

%NDWI2 = sum(950 - 970)
    spec.NDWI2 = sum(spec{:,1783:1851},2);

%RED Edge Division Index
%RE3RE2= (734:747)/(715:726)
    spec.RE3RE2 =
(sum(spec{:,1089:1129},2))./(sum(spec{:,1032:1065},2));

%Red Edge Inflection Point
%REIP= Maximum 680-780
    REIPrange = spec{:,927:1230};
    [val,loc] = max(REIPrange');
    %Maximum value in range
    spec.REIP = val';
    %Wavelength of inflection point
    spec.REIPnm = (loc' * 0.33) + 680;

%Spectral Reflectance Index 1
%SR1 = (750:900)/(660:720);
    spec.SR1 = (sum(spec{:,1138:1614},2))./(sum(spec{:,868:1047},2));

%Spectral Reflectance Index 680
%SR680= 800/680
    spec.SR680 = spec.R799_9 ./ spec.R679_9;

%Spectral Reflectance Index 705
%SR705= 730/705
    spec.SR705 = spec.R730 ./ spec.R705_1;

%Normalized difference vegetation index 680 (NDVI680) calculation
%NDVI680=(R800-R680)/(R800+R680)

```

```

spec.NDVI680 = (spec.R799_9 - spec.R679_9) ./ (spec.R799_9 +
spec.R679_9);

%Normalized difference vegetation index 705 (NDVI705) calculation
%NDVI705=(750-705)/(750+705)
spec.NDVI705 = (spec.R750_1 - spec.R705_1) ./ (spec.R750_1 +
spec.R705_1);

%Difference index 715 calculation
%D715=(710:720)/(700:710)
spec.D715 = (sum(spec{:,1017:1047},2)) ./ (sum(spec{:,987:1017},2));

%NIRRed index
%NIRRed = 801/670
spec.NIRRed = spec.R801_1./spec.R670_1;

%NIRGreen
%NIRGreen = 801/550
spec.NIRGreen = spec.R801_1./spec.R549_9;

%MCARI
%MCARI = (700-670)-0.2(700-550)*700/670
%MCARI2 = (850-730)-0.2(850-570)/730
spec.MCARI = ((spec.R700_1 - spec.R670_1) - 0.2 * (spec.R700_1 -
spec.R549_9) .* spec.R700_1) ./spec.R670_1;
spec.MCARI2 = ((spec.R850 - spec.R730) - 0.2*(spec.R850 -
spec.R570_1)) ./ spec.R730;

%SAVI
%SAVI = (1 + 0.5) * (R801 - R670) / (R801 + R670 + 0.5)
spec.SAVI= (1 + 0.5) .* (spec.R801_1 - spec.R670_1) ./ (spec.R801_1
+ spec.R670_1 + 0.5);

%OSAVI
%OSAVI = (1+ 0.16) *(R801 - R670)/(R801 + R670 + 0.16)
spec.OSAVI= (1 + 0.16) .* (spec.R801_1 - spec.R670_1) ./
(spec.R801_1 + spec.R670_1 + 0.16);

%Simple Ratio Water Band Index
%SRWBI = R950 / R900
spec.SRWBI = spec.R950_1 ./ spec.R899_9;

%Water Balance Index
%WBI1 = 970/900
spec.WBI1 = spec.R969_9 ./ spec.R899_9;
%WBI2 = 905/980
spec.WBI2 = spec.R905 ./ spec.R980;
%WBI3 = 970/902
spec.WBI3 = spec.R969_9 ./ spec.R902;

%Normalized reflectance curve area (Estimation of Canopy Water
Content
%by Means of Hyperspectral Indices Based on Drought Stress Gradient
Experiments of Maize in the North Plain China)
%NRCA =1015:1020
spec.NRCA = sum(spec{:,2009:2029},2);

```

```
%Transformed Chlorophyll Absorption in Reflectance Index
%TCARI = 3 * (700 - 670) -0.2(700-550) *700/670)
spec.TCARI = 3 .* ( (spec.R700_1 - spec.R670_1) - 0.2 *
(spec.R700_1 - spec.R549_9) .*spec.R700_1) ./spec.R670_1);

%TCARI to OSAVI Ration
spec.TCARI_OSAVI = spec.TCARI ./ spec.OSAVI;

%Write Final Output
filename_out = strcat(srepath, 'AnalysisSpec.csv');
writetable(spec, filename_out);

%Print Folder is Complete for Watching Loop
fprintf(sub_folders{i})

end
```

3. Example RQTL genomic data quality control script

```

#Load Library's
library(qtl)
library(tidyverse)

#Set Working Directory
setwd("D:/Box Sync/Dissertation/Drought Project/Analysis/ICIM/QC")

#UX3036
file_UX3036=read.cross(format=c("csvr"), file="UX3036_ABH_PHENO2.csv",
na.strings="NA",
                        genotypes=c("A","H","B"), alleles=c("A","B"))
object_UX3036=convert2riself(file_UX3036)
summary(object_UX3036)

#Save pdf
#pdf("UX3036 Quality Check Figures.pdf")

#Investigate pattern of missing data
plotMissing(object_UX3036) #several individuals and markers with
missing data

#Plot genotyped markers for each individual
par(mfrow=c(1,2), las=1)
plot(ntyped(object_UX3036), ylab="No. typed markers", main="No.
genotypes by individual")
plot(ntyped(object_UX3036, "mar"), ylab="No. typed individuals",
main="No. genotypes by marker")

#Drop individuals with relative low number of markers
object_UX3036 <- subset(object_UX3036, ind=(ntyped(object_UX3036)>800))

#Drop markers with poor call rate
nt.bymar <- ntyped(object_UX3036, "mar")
todrop <- names(nt.bymar[nt.bymar < 145])
object_UX3036 <- drop.markers(object_UX3036, todrop)

#Identify duplicate individuals

```

```

cg <- comparegeno(object_UX3036)
hist(cg[lower.tri(cg)], breaks=seq(0, 1, len=101), xlab="No. matching
genotypes")
rug(cg[lower.tri(cg)])

#Identify individuals with over 95% matching genotypes
wh <- which(cg > 0.98, arr=TRUE)
wh <- wh[wh[,1] < wh[,2],]
g <- pull.geno(object_UX3036)

#Remove one paired individual with over 95% matching genotypes and
checked for duplicated markers
object_UX3036 <- subset(object_UX3036, ind=-wh[,2])
print(dup <- findDupMarkers(object_UX3036, exact.only=FALSE))
summary(object_UX3036)

#Investigate distored segregation patterns
gt <- geno.table(object_UX3036)
gt[gt$P.value < 0.001/totmar(object_UX3036),]

#Drop distored markers
todrop <- rownames(gt[gt$P.value < 1e-8,])
object_UX3036 <- drop.markers(object_UX3036, todrop)
summary(object_UX3036)

#Investigate allele frequencies
g <- pull.geno(object_UX3036)
gfreq <- apply(g, 1, function(a) table(factor(a, levels=1:3)))
gfreq <- t(t(gfreq) / colSums(gfreq))
par(mfrow=c(1,2), las=1)

for(i in 1:2){
  plot(gfreq[i,], ylab="Genotype frequency", main=c("A", "B")[i],
ylim=c(0,1))
}

#
object_UX3036 <- est.rf(object_UX3036)

```



```
checkAlleles(object_UX3036, threshold=5)

#Plot LOD score against the estimated recombination fractions for all
marker pairs
rf <- pull.rf(object_UX3036)
lod <- pull.rf(object_UX3036, what="lod")
plot(as.numeric(rf), as.numeric(lod), xlab="Recombination fraction",
ylab="LOD score")

#Investigate recombination fraction
par(mfrow=c(1,1), las=1)
plotRF(object_UX3036, alternate.chrid=FALSE)

#Investigate genetic map for coverage
plotMap(object_UX3036)

#Reorder genetic map based on recombination fraction
object_UX3036_rec = orderMarkers(object_UX3036, use.ripple = TRUE,
window = 8, map.function = "haldane")

#Investigate switched alleles
plotRF(object_UX3036_rec, alternate.chrid=TRUE)

#Look for problem individuals
plot(countXO(object_UX3036_rec), ylab="Number of crossovers")
plotMap(object_UX3036, object_UX3036_rec)

#Output cross object
chr = c("1", "2", "3", "4", "5", "6", "7", "8", "9", "10",
        "11", "12", "13", "14", "15", "16", "17", "18", "19", "20")
write.cross(object_UX3036, format="csv", filestem="UX3036_QC_2", chr,
digits=NULL, descr)
write.cross(object_UX3036_rec, format="csv", filestem="UX3036_QC_2_rec",
chr, digits=NULL, descr)
summary(object_UX3036)

dev.off()
```

4. Example CART Script

```

...
i=str2num(getenv('SLURM_TASKS_PER_NODE'));
p=parpool(i);
p.IdleTimeout = inf
...

%Load Dataset and Rename
load Workspace_Complete.mat

% Partition variables for loop output
ALL = MasterDatasetComplete;
UX3000 = MasterDatasetComplete(MasterDatasetComplete.TEST=='UX3000',:);
UX3036 = MasterDatasetComplete(MasterDatasetComplete.TEST=='UX3036',:);

UX3000_V5 = UX3000(UX3000.STAGE=='V5',:);
UX3000_R5 = UX3000(UX3000.STAGE=='R5',:);

UX3036_V5 = UX3036(UX3036.STAGE=='V5',:);
UX3036_R5 = UX3036(UX3036.STAGE=='R5',:);

V5 = ALL(ALL.STAGE=='V5',:);
R5 = ALL(ALL.STAGE=='R5',:);
R3 = ALL(ALL.STAGE=='R3',:);

IRR_TREAT1 =
MasterDatasetComplete(MasterDatasetComplete.IRR_TREAT==1,:);
IRR_TREAT2 =
MasterDatasetComplete(MasterDatasetComplete.IRR_TREAT==2,:);

UX3000_1 = UX3000(UX3000.IRR_TREAT==1,:);
UX3000_2 = UX3000(UX3000.IRR_TREAT==2,:);

UX3036_1 = UX3036(UX3036.IRR_TREAT==1,:);
UX3036_2 = UX3036(UX3036.IRR_TREAT==2',:);

V5_1 = V5(V5.IRR_TREAT==1,:);
V5_2 = V5(V5.IRR_TREAT==2',:);

R5_1 = R5(R5.IRR_TREAT==1,:);
R5_2 = R5(R5.IRR_TREAT==2',:);

R3_1 = R3(R3.IRR_TREAT==1,:);
R3_2 = R3(R3.IRR_TREAT==2',:);

```

```

% Store datasets in list
loop.names = {"UX3000", "UX3000_V5", "UX3000_R5", "UX3036",
"UX3036_V5", "UX3036_R5", "ALL", "V5", "R5",...
"IRR_TREAT1", "IRR_TREAT2", "UX3000_1", "UX3000_2", "UX3036_1",
"UX3036_2", "V5_1", "V5_2", "R5_1", "R5_2", "R3", "R3_1", "R3_2"};

water{1} = UX3000;
water{2} = UX3000_V5;
water{3} = UX3000_R5;
water{4} = UX3036;
water{5} = UX3036_V5;
water{6} = UX3036_R5;
water{7} = ALL;
water{8} = V5;
water{9} = R5;
water{10} = IRR_TREAT1;
water{11} = IRR_TREAT2;
water{12} = UX3000_1;
water{13} = UX3000_2;
water{14} = UX3036_1;
water{15} = UX3036_2;
water{16} = V5_1;
water{17} = V5_2;
water{18} = R5_1;
water{19} = R5_2;
water{20} = R3;
water{21} = R3_1;
water{22} = R3_2;

for i = 1:length(water)

% Assign inputtable dataset
%Testing Data
dataset = water{i};

%Create CV partitions for testing model
CV = cvpartition(height(dataset), 'Kfold', 10);

%Create Empty Table for Correlation Ouputs
cor = array2table(zeros(1,10));

for k = 1:10

% Create trainingg and testing datasets
cv.idx = CV.test(k);
train = dataset(~cv.idx,:);
test = dataset(cv.idx,:);

% Extract predictors and response
% This code processes the data into the right shape for training the
model.
predictorNames = {'IRR_TREAT', 'STAGE', 'LMR', 'TEST', 'ECD', 'ECDV',
'ECS', 'ECS_D', 'ECS_DV', 'ECSV', 'Time', 'Latitude', 'Longitude',
'Easting', 'Northing', 'AirTemperature_C', 'RelativeHumidity',
'SVP_Pa', 'VPD_kPa', 'ShortwaveRadiation_Wm2', 'SensorHeight_cm',

```

'CanopyHeight', 'UltrasonicCanopyHeight', 'CATD_C', 'CATD_VPD',
 'CanopyTemperature_C', 'Volume', 'Area', 'Perimeter', 'Area_Perimeter',
 'PF', 'R', 'G', 'B', 'r', 'g', 'b', 'rg', 'gb', 'Xgbrg', 'INT', 'GRR1',
 'NDI', 'NGRDI', 'NGBDI', 'VARI', 'VDVI', 'CIVE', 'VEG', 'MExG', 'ExG',
 'ExR', 'ExGR', 'COM1', 'COM2', 'X', 'Y', 'Z', 'L', 'a', 'b1', 'ba',
 'H', 'S', 'V', 'RF', 'YF', 'GF', 'CF', 'RGF', 'Y1', 'Cb', 'Cr',
 'RContrast', 'RCorrelation', 'REnergy', 'RHomogeneity',...
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 'bHomogeneity', 'rgContrast', 'rgCorrelation', 'rgEnergy',
 'rgHomogeneity', 'gbContrast', 'gbCorrelation', 'gbEnergy',
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'R35_Wlfc', 'R35_Dep', 'R5_TotW', 'R5_Wlfc', 'R5_Dep',
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    false];

% Train a regression model
% This code specifies all the model options and trains the model.
% Retrieve optimized number of learning cycles and minimum leaf size
from
template = templateTree('MinLeafSize', 5);
regressionEnsemble = fitensemble(predictors, response, 'Method',
'Bag', 'NumLearningCycles', 500, 'Learners', template);

% Create the result struct with predict function
predictorExtractionFcn = @(t) t(:, predictorNames);
ensemblePredictFcn = @(x) predict(regressionEnsemble, x);
trainedModel.predictFcn = @(x)
ensemblePredictFcn(predictorExtractionFcn(x));

% Add additional fields to the result struct
trainedModel.RequiredVariables = {'IRR_TREAT', 'STAGE', 'LMR', 'TEST',
'ECD', 'ECDV', 'ECS', 'ECSd', 'ECSdV', 'ECSV', 'Time', 'Latitude',
'Longitude', 'Easting', 'Northing', 'AirTemperature_C',
'RelativeHumidity', 'SVP_Pa', 'VPD_kPa', 'ShortwaveRadiation_Wm2',
'SensorHeight_cm', 'CanopyHeight', 'UltrasonicCanopyHeight', 'CATD_C',
'CATD_VPD', 'CanopyTemperature_C', 'Volume', 'Area', 'Perimeter',
'Area_Perimeter', 'PF', 'R', 'G', 'B', 'r', 'g', 'b', 'rg', 'gb',
'Xgbrg', 'INT', 'GRRI', 'NDI', 'NGRDI', 'NGBDI', 'VARI', 'VDVI',
'CIVE', 'VEG', 'MExG', 'ExG', 'ExR', 'ExGR', 'COM1', 'COM2', 'X', 'Y',
'Z', 'L', 'a', 'bl', 'ba', 'H', 'S', 'V', 'RF', 'YF', 'GF', 'CF',
'RGF', 'Yl', 'Cb', 'Cr', 'RContrast', 'RCorrelation', 'REnergy',
'RHomogeneity', ...
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'BContrast', 'BCorrelation', 'BEnergy', 'BHomogeneity', 'rContrast',
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'ExGEnergy', 'ExGHomogeneity', 'ExRContrast', 'ExRCorrelation',

```

'ExREnergy', 'ExRHomogeneity', 'ExGRContrast', 'ExGRCorrelation',
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'R8_TotW', 'R8_Wlfc', 'S_TotW', 'S_Wlfc', 'S_Dep', 'R7_TotW',
'R7_Wlfc', 'R7_Dep', 'R1_TotW', 'R1_Wlfc', 'R1_Dep', 'R35_TotW',
'R35_Wlfc', 'R35_Dep', 'R5_TotW', 'R5_Wlfc', 'R5_Dep',
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'Avg_Dep_R35_R7', 'Avg_Dep_R1_R7', 'TotW_R1_R35', 'TotW_R35_R5',
'TotW_R5_R7', 'Wlfc_R1_R35', 'Wlfc_R35_R5', 'Wlfc_R5_R7'};
trainedModel.RegressionEnsemble = regressionEnsemble;
response=test.YIELD_R8_Wlfc;

%Output testing predictions and observed values
yfit = trainedModel.predictFcn(test);
predtemp{k} = table(yfit, response, test.RecID, test.STAGE);

correlation= corrcoef(yfit, response);
cor{1,k}= correlation(1,2);

end

% Merge model predictions
preds = vertcat(predtemp{:});

% Write predictions file
pname = strcat(loop.names{i}, '_', 'predictions.csv');
corrname = strcat(loop.names{i}, '_', 'correlations.csv');
writetable(preds, pname);
writetable(cor, corrname);

end

```

5. Example NET Script

```

...
i=str2num(getenv('SLURM_TASKS_PER_NODE'));
p=parpool(i);
p.IdleTimeout = inf
...

%Load Dataset and Rename
load workspace_genol718.mat

% Partition variables for loop output
ALL = MasterDatasetComplete;
UX3000 = MasterDatasetComplete(MasterDatasetComplete.TEST=='UX3000',:);
UX3036 = MasterDatasetComplete(MasterDatasetComplete.TEST=='UX3036',:);

UX3000_V5 = UX3000(UX3000.STAGE=='V5',:);
UX3000_R5 = UX3000(UX3000.STAGE=='R5',:);

UX3036_V5 = UX3036(UX3036.STAGE=='V5',:);
UX3036_R5 = UX3036(UX3036.STAGE=='R5',:);

V5 = ALL(ALL.STAGE=='V5',:);
R5 = ALL(ALL.STAGE=='R5',:);
R3 = ALL(ALL.STAGE=='R3',:);

IRR_TREAT1 =
MasterDatasetComplete(MasterDatasetComplete.IRR_TREAT==1,:);
IRR_TREAT2 =
MasterDatasetComplete(MasterDatasetComplete.IRR_TREAT==2,:);

UX3000_1 = UX3000(UX3000.IRR_TREAT==1,:);
UX3000_2 = UX3000(UX3000.IRR_TREAT==2,:);

UX3036_1 = UX3036(UX3036.IRR_TREAT==1,:);
UX3036_2 = UX3036(UX3036.IRR_TREAT==2',:);

V5_1 = V5(V5.IRR_TREAT==1,:);
V5_2 = V5(V5.IRR_TREAT==2',:);

R5_1 = R5(R5.IRR_TREAT==1,:);
R5_2 = R5(R5.IRR_TREAT==2',:);

R3_1 = R3(R3.IRR_TREAT==1,:);
R3_2 = R3(R3.IRR_TREAT==2',:);

% Store datasets in list
loop.names = {"UX3000", "UX3000_V5", "UX3000_R5", "UX3036",
"UX3036_V5", "UX3036_R5", "ALL", "V5", "R5",...
"IRR_TREAT1", "IRR_TREAT2", "UX3000_1", "UX3000_2", "UX3036_1",
"UX3036_2", "V5_1", "V5_2", "R5_1", "R5_2", "R3", "R3_1", "R3_2"};

water{1} = UX3000;
water{2} = UX3000_V5;
water{3} = UX3000_R5;
water{4} = UX3036;
water{5} = UX3036_V5;

```

```

water{6} = UX3036_R5;
water{7} = ALL;
water{8} = V5;
water{9} = R5;
water{10} = IRR_TREAT1;
water{11} = IRR_TREAT2;
water{12} = UX3000_1;
water{13} = UX3000_2;
water{14} = UX3036_1;
water{15} = UX3036_2;
water{16} = V5_1;
water{17} = V5_2;
water{18} = R5_1;
water{19} = R5_2;
water{20} = R3;
water{21} = R3_1;
water{22} = R3_2;

for i = 1:length(water)

% Assign inputtable dataset
%Testing Data
dataset = water{i};

% Extract predictors and response
% This code processes the data into the right shape for training the
model.
phenoNames = {'IRR_TREAT', 'STAGE', 'LMR', 'TEST', 'Time', 'Latitude',
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'SGM19_7358532','SGM19_860428','SGM20_1042491','SGM20_1129790','SGM20_1445929','SGM20_1608482','SGM20_20469910','SGM20_2054806','SGM20_2237894','SGM20_2426117','SGM20_24674575','SGM20_294010','SGM20_3071936','SGM20_34041437','SGM20_34140804','SGM20_34188658','SGM20_34239213','SGM20_342632','SGM20_34910001','SGM20_35379699','SGM20_35766549','SGM20_36235283','SGM20_36720824','SGM20_37097315','SGM20_37573710','SGM20_38030407','SGM20_38656535','SGM20_38750487','SGM20_39691634','SGM20_39729724','SGM20_40636288','SGM20_40704783','SGM20_40820776','SGM20_41288533','SGM20_42075128','SGM20_42244655','SGM20_42885207','SGM20_42952890','SGM20_44105030','SGM20_44505799','SGM20_45714180','SGM20_45857761','SGM20_46958141',...
```

```
'SGM20_47024906','SGM20_47241278','SGM20_47447552','SGM20_690237','SGM20_824049'};
```

```
% Format Categorical Variables for LASSO
```

```
% Format Categorical Variables
```

```
dataset.IRR_TREAT = grp2idx(dataset.IRR_TREAT);
```

```
dataset.STAGE = grp2idx(dataset.STAGE);
```

```
dataset.LMR = grp2idx(dataset.LMR);
```

```
dataset.TEST = grp2idx(dataset.TEST);
```

```
dataset = convertvars(dataset, genoNames, 'single');
```

```
predictorNames = horzcat(phenoNames, envNames, genoNames);
```

```
predictors = table2array(dataset(:, predictorNames));
```

```
response = dataset.YIELD_R8_Wlefc;
```

```
input = predictors';
```

```
target = response';
```

```
% Create a Fitting Network
```

```
net = feedforwardnet(1, 'trainbr'); %One hidden layer and bayesian regularization
```

```
% Set up Division of Data for Training, Validation, Testing
```

```
net.divideParam.trainRatio = 70/100;
```

```
net.divideParam.valRatio = 15/100;
```

```
net.divideParam.testRatio = 15/100;
```

```
% Train the Network
```

```

[net,tr] = train(net,input,target);

% Test the Network
yfit = net(input);
errors = gsubtract(yfit,target);
mse = perform(net,target,yfit);

%Output testing predictions and observed values
preds = table(yfit', response, dataset.RecID, dataset.STAGE);

% Plot performance
figurename1 = strcat("FitFigures/",loop.names{i},"_PERF.fig");
figure
plotperform(tr);
savefig(figurename1);

% Plot training state status
figurename2 = strcat("FitFigures/",loop.names{i},"_TS.fig");
figure
plottrainstate(tr);
savefig(figurename2);

% Plot Error Histogram
figurename3 = strcat("FitFigures/",loop.names{i},"_ER.fig");
figure
ploterrhist(errors);
savefig(figurename3);

% Plot Regression Figures
figurename4 = strcat("FitFigures/",loop.names{i},"_REG.fig");
figure
plotregression(target,yfit,'All' );
savefig(figurename4);

% Cross Validation on other datasets
sets=1:length(water);

for k=sets(sets~=i)

valnames = loop.names;

%Create testing dataset
test = water{k};

% Extract predictors and response
% This code processes the data into the right shape for training the
model.
phenoNames = {'IRR_TREAT', 'STAGE', 'LMR', 'TEST', 'Time', 'Latitude',
'Longitude', 'Easting', 'Northing', 'AirTemperature_C',
'RelativeHumidity', 'SVP_Pa', 'VPD_kPa', 'ShortwaveRadiation_Wm2',
'SensorHeight_cm', 'CanopyHeight', 'UltrasonicCanopyHeight', 'CATD_C',
'CATD_VPD', 'CanopyTemperature_C', 'Volume', 'Area', 'Perimeter',
'Area_Perimeter', 'PF', 'R', 'G', 'B', 'r', 'g', 'b', 'rg', 'gb',
'Xgbrg', 'INT', 'GRRI', 'NDI', 'NGRDI', 'NGBDI', 'VARI', 'VDVI',
'CIVE', 'VEG', 'MExG', 'ExG', 'ExR', 'ExGR', 'COM1', 'COM2', 'X', 'Y',
'Z', 'L', 'a', 'bl', 'ba', 'H', 'S', 'V', 'RF', 'YF', 'GF', 'CF',

```

```

'RGF', 'Y1', 'Cb', 'Cr', 'RContrast', 'RCorrelation', 'REnergy',
'RHomogeneity',...
  'GContrast', 'GCorrelation', 'GEnergy', 'GHomogeneity',
'BContrast', 'BCorrelation', 'BEnergy', 'BHomogeneity', 'rContrast',
'rCorrelation', 'rEnergy', 'rHomogeneity', 'gContrast', 'gCorrelation',
'gEnergy', 'gHomogeneity', 'bContrast', 'bCorrelation', 'bEnergy',
'bHomogeneity', 'rgContrast', 'rgCorrelation', 'rgEnergy',
'rgHomogeneity', 'gbContrast', 'gbCorrelation', 'gbEnergy',
'gbHomogeneity', 'XgbrgContrast', 'XgbrgCorrelation', 'XgbrgEnergy',
'XgbrgHomogeneity', 'INTContrast', 'INTCorrelation', 'INTEnergy',
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'NGBDIEnergy',...
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'YHomogeneity', 'ZContrast', 'ZCorrelation', 'ZEnergy', 'ZHomogeneity',
'LContrast', 'LCorrelation', 'LEnergy', 'LHomogeneity',...
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'bContrast1', 'bCorrelation1', 'bEnergy1', 'bHomogeneity1',
'baContrast', 'baCorrelation', 'baEnergy', 'baHomogeneity',
'HContrast', 'HCorrelation', 'HEnergy', 'HHomogeneity', 'SContrast',
'SCorrelation', 'SEnergy', 'SHomogeneity', 'VContrast', 'VCorrelation',
'VEnergy', 'VHomogeneity', 'YContrast1', 'YCorrelation1', 'YEnergy1',
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'CrHomogeneity', 'GrayContrast', 'GrayCorrelation', 'GrayEnergy',
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'NIR', 'CWSI', 'DSI1', 'DSI2', 'DSI3', 'GYI1', 'GYI2',...
'GYI3', 'LCI', 'STI1', 'STI2', 'REDGE', 'NDWI', 'RE3RE2', 'REIP',
'REIPnm', 'SR1', 'SR680', 'SR705', 'NDVI680', 'NDVI705', 'D715',
'NIRRed', 'NIRGreen', 'MCARI', 'SAVI', 'OSAVI', 'SRWBI', 'RE', 'TCARI',
'TCARI_OSAVI'};

```

```

envNames = {'ECD', 'ECDV', 'ECS', 'ECS_D', 'ECS_DV', 'ECSV', 'GDD',
'GDD_C', 'Precip_C', 'PrecipDepart_C', 'IRR_C', 'WUa', 'WUac',
'R8_GDD', 'R7_GDD', 'R5_GDD', 'R35_GDD', 'R1_GDD', 'R8_TotW',
'R8_Wlefc', 'S_TotW', 'S_Wlefc', 'S_Dep', 'R7_TotW', 'R7_Wlefc',
'R7_Dep', 'R1_TotW', 'R1_Wlefc', 'R1_Dep', 'R35_TotW', 'R35_Wlefc',
'R35_Dep', 'R5_TotW', 'R5_Wlefc', 'R5_Dep', 'Avg_Dep_R1_R35',
'Avg_Dep_R35_R5', 'Avg_Dep_R5_R7', 'Avg_Dep_R1_R5', 'Avg_Dep_R35_R7',

```

```
'Avg_Dep_R1_R7', 'TotW_R1_R35', 'TotW_R35_R5', 'TotW_R5_R7',
'Wlefc_R1_R35', 'Wlefc_R35_R5', 'Wlefc_R5_R7'};
```

```
genoNames =
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GM20_40636288','SGM20_40704783','SGM20_40820776','SGM20_41288533','SGM2
0_42075128','SGM20_42244655','SGM20_42885207','SGM20_42952890','SGM20_4
4105030','SGM20_44505799','SGM20_45714180','SGM20_45857761','SGM20_4695
8141',...
'SGM20_47024906','SGM20_47241278','SGM20_47447552','SGM20_690237','SGM2
0_824049'};

% Format Categorical Variables
test.IRR_TREAT = grp2idx(test.IRR_TREAT);
test.STAGE = grp2idx(test.STAGE);
test.LMR = grp2idx(test.LMR);
test.TEST = grp2idx(test.TEST);
test = convertvars(test, genoNames, 'single');

predictorNames = horzcat(phenoNames, envNames, genoNames);

    predictors = table2array(test(:, predictorNames));
    response = test.YIELD_R8_Wlefc;

    input = predictors';

    % Test the Network
    yfit2 = net(input);
    %Output testing predictions and observed values
    testname = repmat(valnames{k},length(yfit2),1);
    predtemp{k} = table(yfit2', response, testname, test.RecID,
test.STAGE);
end
% Merge model predictions
preds2 = vertcat(predtemp{:});
% Write predictions file
pname = strcat(loop.names{i}, '_', 'predictions.csv');
writetable(preds, pname);
pname2 = strcat(loop.names{i}, '_', 'predcomp.csv');
writetable(preds2, pname2);
end

```

6. Example ENET Script

```

...
i=str2num(getenv('SLURM_TASKS_PER_NODE'));
p=parpool(i);
p.IdleTimeout = inf
...

% Load dataset
load("BLUPS.mat")

HeritabilityEstimationsR5.Variable(18) = "gb";
HeritabilityEstimationsR5.Variable(17) = "rg";
HeritabilityEstimationsR5.Variable(19) = "Xgbrg";
HeritabilityEstimationsR5.Variable(41) = "ba";
HeritabilityEstimationsR5.Variable(38) = "L";
HeritabilityEstimationsR5.Variable(39) = "a";
HeritabilityEstimationsR5.Variable(40) = "b";
HeritabilityEstimationsR5.Variable(50) = "Y";

% Subset only heritable predictors variables
Hvars = HeritabilityEstimationsR5(HeritabilityEstimationsR5.Hprog >
0.25, 1);
Hvarnames = convertStringsToChars(Hvars.Variable');
Hvarnames(:,120:121)=[];

% 2017- 2018
ALL_1718 = BLUPS1718ALL(:,2:22);
V5_1718 = BLUPS1718V5(:,2:22);
R5_1718 = BLUPS1718R5(:,2:22);

% Partion variables for loop output
ALL_ALL_1718 = ALL_1718(ALL_1718.TEST~="DTHY",:);
UX3000_ALL_1718 = ALL_1718(ALL_1718.TEST=="UX3000",:);
UX3036_ALL_1718 = ALL_1718(ALL_1718.TEST=="UX3036",:);

ALL_V5_1718 = V5_1718(V5_1718.TEST~="DTHY",:);
UX3000_V5_1718 = V5_1718(V5_1718.TEST=="UX3000",:);
UX3036_V5_1718 = V5_1718(V5_1718.TEST=="UX3036",:);

ALL_R5_1718 = R5_1718(R5_1718.TEST~="DTHY",:);
UX3000_R5_1718 = R5_1718(R5_1718.TEST=="UX3000",:);
UX3036_R5_1718 = R5_1718(R5_1718.TEST=="UX3036",:);

% 2016 - 2018
ALL_1618 = BLUPS1618ALL(:,2:10);
V5_1618 = BLUPS1618V5(:,2:10);
R5_1618 = BLUPS1618R5(:,2:10);

% Partion variables for loop output
ALL_ALL_1618 = ALL_1618(ALL_1618.TEST~="DTHY",:);
UX3000_ALL_1618 = ALL_1618(ALL_1618.TEST=="UX3000",:);
UX3036_ALL_1618 = ALL_1618(ALL_1618.TEST=="UX3036",:);
NT_UX3000_ALL_1618 = ALL_1618(ALL_1618.TEST=="NT_UX3000",:);
NT_UX3036_ALL_1618 = ALL_1618(ALL_1618.TEST=="NT_UX3036",:);
NT_ALL = [NT_UX3000_ALL_1618; NT_UX3036_ALL_1618];

```



```

ALL_V5_1618 = V5_1618(V5_1618.TEST~="DTHY",:);
UX3000_V5_1618 = V5_1618(V5_1618.TEST=="UX3000",:);
UX3036_V5_1618 = V5_1618(V5_1618.TEST=="UX3036",:);
NT_UX3000_V5_1618 = V5_1618(V5_1618.TEST=="NT_UX3000",:);
NT_UX3036_V5_1618 = V5_1618(V5_1618.TEST=="NT_UX3036",:);
NT_V5 = [NT_UX3000_V5_1618; NT_UX3036_V5_1618];

ALL_R5_1618 = R5_1618(R5_1618.TEST~="DTHY",:);
UX3000_R5_1618 = R5_1618(R5_1618.TEST=="UX3000",:);
UX3036_R5_1618 = R5_1618(R5_1618.TEST=="UX3036",:);
NT_UX3000_R5_1618 = R5_1618(R5_1618.TEST=="NT_UX3000",:);
NT_UX3036_R5_1618 = R5_1618(R5_1618.TEST=="NT_UX3036",:);
NT_R5 = [NT_UX3000_R5_1618; NT_UX3036_R5_1618];
Hapmapall = Hapmap;

Hapmapnames = Commonhapmap.Properties.VariableNames;
Hapmap = convertvars(Commonhapmap, Hapmapnames(2:end), 'single');

% Store datasets in list
wtrnames = [ "ALL_1718", "ALL_ALL_1718", "ALL_R5_1718",
"ALL_V5_1718",...
"UX3000_ALL_1718", "UX3000_R5_1718", "UX3000_V5_1718",...
"UX3036_ALL_1718", "UX3036_R5_1718", "UX3036_V5_1718",...
"ALL_1618", "ALL_ALL_1618", "ALL_R5_1618", "ALL_V5_1618",...
"UX3000_ALL_1618", "UX3000_R5_1618", "UX3000_V5_1618",...
"UX3036_ALL_1618", "UX3036_R5_1618", "UX3036_V5_1618",...
"NT_ALL", "NT_R5", "NT_V5", "NT_UX3000_ALL", "NT_UX3036_ALL",...
"NT_UX3000_R5", "NT_UX3036_R5", "NT_UX3000_V5", "NT_UX3036_V5"];

wtr{1} = ALL_1718;
wtr{2} = ALL_ALL_1718;
wtr{3} = ALL_R5_1718;
wtr{4} = ALL_V5_1718;

wtr{5} = UX3000_ALL_1718;
wtr{6} = UX3000_R5_1718;
wtr{7} = UX3000_V5_1718;

wtr{8} = UX3036_ALL_1718;
wtr{9} = UX3036_R5_1718;
wtr{10} = UX3036_V5_1718;

wtr{11} = ALL_1618;
wtr{12} = ALL_ALL_1618;
wtr{13} = ALL_R5_1618;
wtr{14} = ALL_V5_1618;

wtr{15} = UX3000_ALL_1618;
wtr{16} = UX3000_R5_1618;
wtr{17} = UX3000_V5_1618;

wtr{18} = UX3036_ALL_1618;
wtr{19} = UX3036_R5_1618;
wtr{20} = UX3036_V5_1618;

wtr{21} = NT_ALL;
wtr{22} = NT_R5;

```

```

wtr{23} = NT_V5;

wtr{24} = NT_UX3000_ALL_1618;
wtr{25} = NT_UX3036_ALL_1618;
wtr{26} = NT_UX3000_R5_1618;
wtr{27} = NT_UX3036_R5_1618;
wtr{28} = NT_UX3000_V5_1618;
wtr{29} = NT_UX3036_V5_1618;

% Calculate model for R WP
for i = 1:10
% Assign dataset
dat = wtr{i};
dat = rmmissing(dat, 'DataVariables', "STRAIN");
dat = innerjoin(dat, Hapmap);

% Format data
dat.response = dat.YIELD_R_Wlefc;
tbl = dat(:,8:end);
table = tbl(:,1:end-1);
X = table2array(table);
y= tbl.response;

% Lasso regularization regression
[B, FitInfo] = lasso(X,y, 'Alpha', 0.75, 'CV',10);
idxLambda1SE = FitInfo.Index1SE;
coef = B(:,idxLambda1SE);
coef0 = FitInfo.Intercept(idxLambda1SE);
yhat = X*coef + coef0;

%Output predictions
predslasso = array2table([yhat, dat.response, dat.STRAIN]);
coefstable = cell2table(transpose(table.Properties.VariableNames));
coefstble = [coefstable,array2table(coef)];

% Write files
lname =
strcat("R/",wtrnames{i},'_',"YIELD_R_Wlefc","_", 'lassopred.csv');
writetable(predslasso,lname);

cname = strcat("R/",wtrnames{i},'_',"YIELD_R_Wlefc","_", 'coefs.csv');
writetable(coefstble,cname);

% Test model
for k = 1:10

% Assign dataset
wtrdat = wtr{k};
wtrdat = rmmissing(wtrdat, 'DataVariables', "STRAIN");
wtrdat = innerjoin(wtrdat, Hapmap);

% Assign response variable
wtrdat.response = wtrdat.YIELD_R_Wlefc;
wtrtbl = wtrdat(:,8:end);
wtrtable = wtrtbl(:,1:end-1);

X2= table2array(wtrtable);

```

```

%Output predictions
yhat2 = X2*coef + coef0;
testname = repmat(wtrnames{k},height(wtrdat),1);
complasso{k} = array2table([yhat2, wtrdat.response, wtrdat.STRAIN,
testname]);

end

% Write files
compname =
strcat("R/",wtrnames{i},'_',"YIELD_R_Wlefc","_",'comppred.csv');
comparisonlass = vertcat(complasso{:});
writetable(comparisonlass,compname);

end

% Yield and WP

% Calculate model
for i = 1:length(wtr)
% Assign dataset
dat = wtr{i};
dat = dat(:,ALL_1618.Properties.VariableNames);
dat = rmmissing(dat, 'DataVariables', "STRAIN");
dat = innerjoin(dat, Hapmap);

% Assign Response Variable
respvarnames = ["YIELD", "YIELD_R8_Wlefc"];

for j = 1:2

% Format data
dat.response = table2array(dat(:,respvarnames{j}));
tbl = dat(:,8:end);
table = tbl(:,1:end-1);
X = table2array(table);
y= tbl.response;

% Lasso regularization regression
[B, FitInfo] = lasso(X,y,'Alpha', 0.75, 'CV',10);
idxLambda1SE = FitInfo.Index1SE;
coef = B(:,idxLambda1SE);
coef0 = FitInfo.Intercept(idxLambda1SE);
yhat = X*coef + coef0;

%Output predictions
predslasso = array2table([yhat, dat.response, dat.STRAIN]);
coefstable = cell2table(transpose(table.Properties.VariableNames));
coefstable = [coefstable,array2table(coef)];

% Write files
lname = strcat(respvarnames{j}, "/",
wtrnames{i},'_',respvarnames{j},'_',"lassopred.csv');
writetable(predslasso,lname);

```

```

cname = strcat(respvarnames{j}, "/",
wtrnames{i}, '_', respvarnames{j}, "_", 'coefs.csv');
writetable(coefstble, cname);

% Test model
for k = 1:length(wtr)

% Assign dataset
wtrdat = wtr{k};
wtrdat = wtrdat(:, ALL_1618.Properties.VariableNames);
wtrdat = rmmissing(wtrdat, 'DataVariables', "STRAIN");
wtrdat = innerjoin(wtrdat, Hapmap);

% Assign Response Variable
wtrdat.response = table2array(wtrdat(:, respvarnames{j}));

wtrtbl = wtrdat(:, 8:end);
wtrtable = wtrtbl(:, 1:end-1);
X2= table2array(wtrtable);

%Output predictions
yhat2 = X2*coef + coef0;
testname = repmat(wtrnames{k}, height(wtrdat), 1);
complasso{k} = array2table([yhat2, wtrdat.response, wtrdat.STRAIN,
testname]);

end

% Write files
compname = strcat(respvarnames{j},
"/", wtrnames{i}, '_', respvarnames{j}, "_", 'comppred.csv');
comparisonlass = vertcat(complasso{:});
writetable(comparisonlass, compname);

end
end

```

7. Hyperlink to variable categories spreadsheet for individual traits collected during the 2017-2018 water response experiment for QTL and predictive analytic summary figures

<https://unl.box.com/s/r62z2s84rcxlq1vgfaj8jihunthxi9s>