

6-15-2016

# Selective Phenotyping Traits Related to Multiple Stress and Drought Response in Dry Bean

Jennifer J. Trapp  
USDA-ARS

Carlos A. Urrea  
University of Nebraska-Lincoln, currea2@unl.edu

Jianfeng Zhou  
Washington State University, zhoujianf@missouri.edu

Lav R. Khot  
Washington State University, Lav.khot@wsu.edu

Sindhu Sankaran  
Washington State University, sindhuja.sankaran@wsu.edu

*See next page for additional authors*

Follow this and additional works at: <http://digitalcommons.unl.edu/panhandleresext>

---

Trapp, Jennifer J.; Urrea, Carlos A.; Zhou, Jianfeng; Khot, Lav R.; Sankaran, Sindhu; and Miklas, Phillip N., "Selective Phenotyping Traits Related to Multiple Stress and Drought Response in Dry Bean" (2016). *Panhandle Research and Extension Center*. 106.  
<http://digitalcommons.unl.edu/panhandleresext/106>

This Article is brought to you for free and open access by the Agricultural Research Division of IANR at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Panhandle Research and Extension Center by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Jennifer J. Trapp, Carlos A. Urrea, Jianfeng Zhou, Lav R. Khot, Sindhu Sankaran, and Phillip N. Miklas

## RESEARCH

# Selective Phenotyping Traits Related to Multiple Stress and Drought Response in Dry Bean

Jennifer J. Trapp, Carlos A. Urrea, Jianfeng Zhou, Lav R. Khot, Sindhu Sankaran, and Phillip N. Miklas\*

## ABSTRACT

Abiotic stress tolerance in dry bean (*Phaseolus vulgaris* L.) is complex. Increased population sizes are contributing to finding QTL conditioning stress response but phenotyping has not kept pace with high throughput genotyping for such studies. Our objectives were to determine effectiveness of 20 most tolerant and 20 most susceptible lines representing phenotypic extremes from a RIL population ('Buster' × 'Roza' [BR]) to facilitate examination of 19 traits for relevance to stress response and to validate existing QTL conditioning stress response. Using phenotypic extremes tested across multiple trials, eight of the 19 traits were clearly associated with drought stress. Pod wall ratio (PW), plant biomass by weight or a visual rating, and greenness index (NDVI) were most associated with seed yield (SY) under stress followed by phenology traits. The phenotypic extreme lines were also useful for validating QTL previously identified in the whole RIL population conditioning SY, seed weight (SW) and days to flower (DF), harvest maturity (HM), and seed fill (DSF). New QTL were identified for biomass, PW, and NDVI which co-segregated with major QTL for seed yield SY1.1<sup>BR</sup> and SY2.1<sup>BR</sup>. The preliminary finding of NDVI 1.1<sup>BR</sup> supports aerial imaging in larger genetic populations geared toward QTL analysis of stress response. In summary, phenotypic extremes helped sort through traits relevant to stress response in the Buster × Roza RIL population and verified the effect of two major QTL in response to terminal drought.

J.J. Trapp, Crop and Soil Sciences, Washington State Univ., Prosser, WA 99350; C.A. Urrea, Univ. of Nebraska, Panhandle Research and Extension Center, 4502 Ave. I, Scottsbluff, NE 69361; J. Zhou, L.R. Khot, and S. Sankaran, Biological Systems Engineering, Washington State Univ., PO Box 646120, Pullman, WA 99163; P.N. Miklas, USDA-ARS, Grain Legume Genetics and Physiology Research Unit, 24106 N. Bunn Rd., Prosser, WA 99350. Received 6 May 2015. Accepted 16 Mar. 2016. Assigned to Associate Editor Seth Murray. \*Corresponding author: (phil.miklas@ars.usda.gov).

**Abbreviations:** BMWT, biomass weight; BMRT, biomass rating; CIM, composite interval mapping; CT, canopy temperature; DF, days to flowering; DII, drought intensity index; DSF, days to seed fill; DSI, drought severity index; GM, geometric mean; HM, days to harvest maturity; LOD, logarithm of odds; MAS, marker assisted selection; NDVI, normalized difference vegetation index; PW, pod wall ratio; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SW, 100 seed weight; SY, seed yield; UAV, unmanned aerial vehicle.

**P**HENOTYPING is quickly becoming the limiting factor in measuring stress response as mapping populations increase in size to capitalize on increased marker density generated by low-cost and high-throughput molecular platforms. Larger populations of segregating individuals or lines provide greater resolution for defining genomic regions affecting quantitative traits of economic importance in crop plants such as drought stress in dry bean. Yet, obtaining accurate phenotypic data for response to drought stress on large populations of lines in the field is an arduous task. Moreover, the high genotype × environment interaction for drought related traits necessitate phenotyping across multiple environments (locations and years). Conversely, marker genotyping is often a single event in time and space.

Xu and Crouch (2008; in Blum, 2011) determined quality phenotyping to be “the most significant factor affecting the accuracy of genetic mapping and thus the power of the resultant MAS,

Published in *Crop Sci.* 56:1460–1472 (2016).  
doi: 10.2135/cropsci2015.05.0281

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA  
This is an open access article distributed under the CC BY-NC-ND license  
(http://creativecommons.org/licenses/by-nc-nd/4.0/).

particularly for complex traits". Accurate phenotyping of drought adaptation traits has been a key issue in breeding for drought tolerance (Serraj et al., 2003; Beebe et al., 2013; Blum, 2011; Mir et al., 2012; Tuberosa, 2012). The time of day and plant growth stage must be considered when measuring drought response traits such as osmotic adjustment, stomatal conductance, total chlorophyll content (SPAD measurement), canopy temperature, and relative water content (RWC) (Beebe et al., 2013). Given these constraints, extensively phenotyping only the extreme lines based on low and high yield performance under drought stress may help elucidate traits which condition drought tolerance in the larger population. Selective phenotyping has been reported as a tool for evaluating complex traits and mapping quantitative trait loci (QTL) in *Brassica napus* (Jestin et al., 2012) and pepper (*Capsicum annuum*) (Barchi et al., 2009).

The methods for measuring phenotypic data are evolving fast as high-throughput phenotyping platforms become more readily available. Remote sensing is based on the spectral reflectance and radiation emittance from plant surfaces (Blum, 2011) and has been utilized for measuring canopy temperature, and visible-near infrared spectral reflectance. Normalized difference vegetation index (NDVI) is related to plant health. Based on wavebands in the visible and the near infrared regions, a higher NDVI reading is associated with greater vegetation and higher chlorophyll content, and has been reported as a useful trait for breeding under water stress in turfgrass (*Poa pratensis* L and *Poa arachnifera* Torr.  $\times$  *P. pratensis* L.) (Merewitz et al., 2010), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Subash et al., 2011), and maize (*Zea mays*) (Araus et al., 2008; Lu et al., 2011). From push carts to unmanned aerial vehicles (UAVs), there are numerous approaches for obtaining phenotypic data on numerous plots within large field experiments. The instrumentation that accompanies these vehicles is also rapidly evolving for measuring plant greenness, canopy water content, plant biomass, canopy height, and phenology (White et al., 2012; Sankaran et al., 2015b). The application of UAV-enabled remote sensing can reduce the genotype-phenotype throughput gap and improve phenotyping accuracy necessary to develop drought related markers.

A recent genomic revolution in bean has provided a 5398 SNP bead chip (Song et al., 2015) and a gene annotated reference genome sequence (Schmutz et al., 2014; www.phytozome.net). These resources and technologies like GBS (genotyping by sequencing), WGS (whole genome resequencing) and RNA sequencing can be used to enhance genetic analyses and facilitate physical mapping of the targeted traits. To save on genotyping costs, DNA pools consisting of lines within a segregating population representing the phenotypic extremes have been assayed (bulk-segregant analysis [BSA]; Michelmore et al., 1991) to identify markers linked with a specific gene (Felicetti et al., 2012) or QTL (Miklas et al., 2006) in dry

bean. A related method used for mitigating costs associated with QTL mapping was selective genotyping (Darvasi and Soller, 1992). Otherwise known as trait-based marker analysis (Lebowitz et al., 1987) or distribution extreme analysis (Lander and Botstein, 1989), by selectively genotyping individuals from a population that represented the phenotypic extremes (or tails), costs could be reduced. The potential disadvantages are overestimation of QTL effects and the potential need to reformulate selected genotypes for each trait studied (Tanksley, 1993). Nonetheless, unlike selective phenotyping, selective genotyping has been utilized extensively in plant breeding over the past decade.

In the current study, a subset of lines representing the phenotypic extremes from a whole recombinant inbred line (RIL) population were selected for a more thorough assessment of drought related traits. Furthermore, we sought to conduct extensive phenotyping of the phenotypic extremes to validate and further characterize two major QTL for yield under multiple stress and drought identified by Trapp et al. (2015).

## MATERIALS AND METHODS

### Plant Material

Forty lines representing the extreme phenotypes for multiple stress and drought response as measured by yield performance were selected from an original population 'Buster'  $\times$  'Roza' consisting of 140 F<sub>7,9</sub> RILs previously tested across seven environments (Trapp et al., 2015). The extreme phenotypes, that is, the 20 most tolerant and the 20 most susceptible RILs, were selected based on high vs. low yield performance in three multiple stress and four terminal drought stress trials (Trapp et al., 2015).

### Field Conditions and Phenotyping

#### Multiple Stress Site

Experiments under multiple stress (MS) for this study were conducted in 2013 and 2014 on the 'purgatory plot' at the Washington State University, Roza Research Farm in Prosser, WA, which is located at 46°29' N, -119°73' W and has a Warden soil (coarse-silty, mixed, superactive, mesic, Xeric Haplocambid). An average rainfall of 51 mm (Hoogenboom, 2014) and mean temperature of 20.6°C during the growing season (May–August) provides exceptional conditions for drought tolerance testing. Each year, 40 Buster  $\times$  Roza RILs (20 most tolerant and 20 most drought susceptible) and parents and checks were planted in a 7  $\times$  7 lattice design with three replications. Trials were planted mid to late May. A plot consisted of one row of 3-m length in 2013. A spacing of 0.6 m between rows was used. Target seeding rate was 285,000 plants ha<sup>-1</sup>. A four-row plot with the same row spacing, length, and seeding rate was used in 2014. Due to space limitations, only the stress treatment was planted each year. Multiple stress was generated by compacted soils due to reduced tillage practices, low soil fertility (<10 mg P kg<sup>-1</sup>, and <30 kg N ha<sup>-1</sup> [available N]), and intermittent drought stress imposed by only applying approximately 25 mm of water by overhead irrigation via hand-lines every 8 to 10 d post stand establishment. This represented about 30% of the rate of evapotranspiration during the same time period (Hoogenboom, 2014).

Of the 19 traits measured, canopy temperature, SPAD, and nine root traits were not measured in 2014 due to insignificant results obtained in 2013, and therefore these 12 traits were omitted. In 2013, shoot biomass (BMWT, kg ha<sup>-1</sup>) was estimated from 2.4 m of the row at harvest. Plants were cut at soil level and weighed prior to threshing. Other traits measured included days to flower (DF), days to harvest maturity (HM), days to seed fill (DSF; DSF = HM - DF), seed weight (SW, g 100 seed<sup>-1</sup>), and seed yield (SY, kg ha<sup>-1</sup>). Pod wall ratio (PW; PW = threshed/unthreshed pods [g/g]) was obtained from 20 pods of uniform size collected from different plants within the plot at harvest.

In 2014, DF, shoot biomass estimated using a visual rating (BMRT), HM, DSF, PW, SW, and SY data were obtained. Instead of weighing the biomass for each plot in 2014, which is cumbersome for a large number of plots, we estimated the amount of biomass visually using a scale from 1 to 9 (Fig. 1). The biomass scale BMRT incorporates visual inspection of row closure and density of the canopy, where 1 = complete row closure and densest canopy with minimal porosity, 3 = 75% closed rows and dense canopy allowing 25% light penetration, 5 = 50% closed rows and 50% light penetration, 7 = 25% closed rows and 75% light penetration, and 9 = no canopy to estimate biomass at mid pod-fill. Prior to 2013 and 2014, SY was the only trait measured on the purgatory plot for the entire Buster × Roza mapping population.

### Drought Stress Sites

Terminal drought stress trials were conducted at the Washington State Univ., Research Farm in Othello, WA (in 2014) and Univ. of Nebraska, Research Station in Mitchell, NE (in 2013 and 2014) with the phenotypic extreme subsets from the Buster × Roza RIL population as above. Othello is located at 46°49' N, -119°10' W and has a Shano soil (coarse-silty, mixed, superactive, mesic, Xeric Haplocambid). The average rainfall is 64 mm and the mean temperature is 20°C during the growing season. Mitchell is located at 41°57' N, -103°42' W, has a Mitchell soil (coarse-silty, mixed, superactive, calcareous, mesic, Ustic Torriorthent), an average rainfall of 203 mm, and a mean temperature of 19°C during the growing season. The experiment consisted of 40 RILs and parents in a randomized complete block design with two replications and the non-stress (NS) and drought stress (DS) treatments planted side by side. At Othello, lines were planted in four-row plots, with 3-m length and 0.6-m row spacing. The Mitchell trials were planted in two-row plots, with 7.6-m length and 0.6-m row spacing. Planting density was 285,000 seeds ha<sup>-1</sup>.

Trials at both locations used furrow irrigation. Both treatments were watered on a regular schedule until flowering (R1 growth stage) when terminal drought was simulated by ceasing irrigation for the DS treatment. Conversely, the NS plots received four to six more irrigations after flowering. Four- and eight-row buffers were planted the length of the field between treatments to reduce the lateral movement of irrigation water between the NS and DS plots. Soil water content was measured at Othello (Neutron Probe, Campbell Pacific Nuclear 503DR, Martinez, CA) and Mitchell (Watermark probe, Spectrum Technologies, Aurora, IL) at three depths (23, 46, and 76 cm) below the soil surface, three times during the growing season: after the first irrigation to determine field capacity (R1), at mid-pod fill (R4-5), and at harvest maturity (R9). Data were obtained for DF,

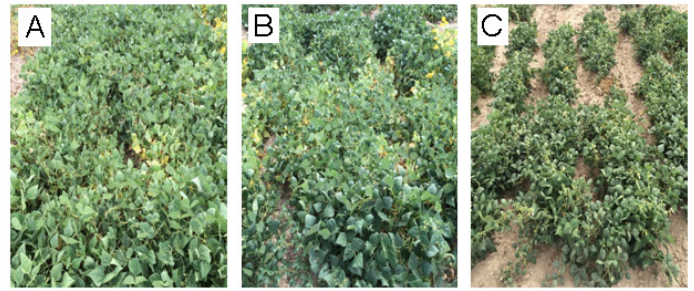


Fig. 1. Plot images depicting a visual biomass rating using a scale of 1 to 9. (a) A rating of 3 with 75% closed rows and dense canopy. (b) A rating of 5 with 50% closed rows and 50% light penetration. (c) A rating of 7 with 25% closed rows and 75% light penetration.

HM, DSF, SW, and SY at Othello and Mitchell in both years. PW was determined in 2014 only. To assess line yield response across NS and DS environments, the geometric mean (GM), the drought susceptibility index (DSI), and the drought intensity index (DII) were calculated as  $GM = \sqrt{(Y_s)(Y_i)}$  where  $Y_s$  is the mean seed yield of a line under DS and  $Y_i$  is the mean yield of the line under NS (Schneider et al., 1997),  $DSI = [1 - (Y_d / Y_p)] / DII$  where  $Y_d$  is mean yield of a line under DS and  $Y_p$  is mean yield for the same line under NS, and  $DII = 1 - (X_d / X_p)$  where  $X_d$  is mean yield averaged across lines under DS and  $X_p$  is mean yield under NS (Fischer and Maurer, 1978).

### Aerial High-Resolution Images

A UAV (HiSystems GmbH, Moormerland, Germany) with modified digital camera (XNiteCanon SX230 NDVI, LDC, Carlstadt, NJ) collected high-resolution multispectral images to estimate NDVI and canopy area at R3 and R5 growth stages. Details regarding the camera, radio controller, and UAV used in this study can be found in Sankaran et al. (2015a). Image analysis was performed to estimate the average NDVI and canopy area in the imaged study area. The feature extraction algorithms were developed using ImageJ (Schneider et al., 2012) and Matlab (v. R2009b, MathWorks, Natick, MA). Initially, the images were converted to NDVI image, using the green and near-infrared bands (Sankaran et al., 2013). Following this, the locations of the individual plots were registered and data were saved. For extracting NDVI data for individual plots, a window (39 × 27 pixels) covering the central region of the plot was selected and average NDVI was calculated. In regard to canopy area data, a threshold of 0.04 was used to remove the soil background, and number of pixels within the plot area with NDVI > 0.04 was calculated.

### Statistical Analyses

The multiple stress trials (Prosser, WA) were analyzed separately from the terminal drought trials (at Othello, WA, and Mitchell, NE) by PROC Mixed (SAS Institute, 2008). Environment, replication, and blocks were fit as random effects and genotype and treatment as fixed effects. Least square means were determined for all traits. Phenotypic correlations between pairs of traits were calculated with least square means from the combined analyses using the PROC CORR Spearman procedure of SAS. The trait means for the DS and NS treatments for the terminal drought trials are reported separately. Associations of significant SNP markers with specific traits were reconfirmed using simple and multiple regression analysis (PROC REG).

## Genotyping, Mapping, and QTL Analyses

The genotypic data and genetic linkage map reported by Trapp et al. (2015) for the Buster × Roza RIL population was used. However, only the genotypic marker data for the 40 RILs representing the phenotypic extremes were used for QTL analysis conducted using composite interval mapping (CIM) with QGene 4.0 (Joehanes and Nelson, 2008). A 1000-permutation test was used to set a significant QTL threshold at the 0.01 level of probability to determine the significant LOD level for declaration of a QTL. Estimates of the phenotypic variation ( $R^2$ ) explained by the individual QTL and the effect of substituting one allele for the other were also determined in QGene. The marker within the QTL peak with the highest  $R^2$  and  $P \leq 0.01$  was used to define the genomic position of the QTL. Identified QTL were named according to guidelines for QTL nomenclature (Miklas and Porch, 2010). Note that SNP names are truncated from the NCBI numbers which contain the prefix ss7156.

## RESULTS

### Climatic Conditions

Low precipitation across the five separate trials provided the opportunity to develop drought stress by limiting or terminating irrigation (Table 1). In 2013, and at Othello in 2014, the majority of the rainfall from May through September occurred prior to flowering which allowed, at minimum, moderate drought stress. The average soil

moisture content as measured by neutron probe across eight sites and three depths (22, 45, and 75 cm) was 55% less in the terminal drought versus non-stress treatments at Othello. For Nebraska, an accurate soil moisture reading was not obtained either year. In 2013, 71 mm of rainfall accumulated during the growing season; however, less than half of this precipitation occurred after flowering. Even so, a low drought index was achieved (0.2). In 2014, more than half of the accumulated rainfall occurred between flowering and harvest and a low to moderate drought intensity index of 0.3 was still obtained.

### Seed Yield and Seed Weight Multiple Stress

Parents and groups differed for SY both years (Table 2). Buster yielded only 25% of Roza, and the tolerant group yielded 36% more than the susceptible. There was more stress in 2014 as indicated by lower yield compared to 2013. For SW there was no significant difference between parents in 2013 or 2014. The susceptible group had a significantly lower SW ( $P < 0.01$ ) than the tolerant group in 2014.

### Terminal Drought Stress

At Mitchell in 2013, there was no significant difference for SY between the parents. There was a significant group

**Table 1. Temperature and precipitation data recorded during the growing season from May through September including maximum, minimum, and average temperatures (°C), total precipitation (mm), precipitation between planting to flowering (from DAP to DF), precipitation from flowering to harvest (from DF to HM), and the drought intensity index (DII).**

Location	Year	Temperature			Precipitation			DII
		Max	Min	Ave	Total	DAP to DF	DF to HM	
		°C			mm			
Prosser, WA	2013	37.7	3.3	19.6	91.2	76.7	14.5	NA
	2014	41.4	3.6	20.1	31	8.9	22.1	NA
Mitchell, NE	2013	31.4	6.6	21.1	71.1	38.1	33	0.2
	2014	31.3	5.7	19.4	157.5	68.6	88.9	0.3
Othello, WA	2014	38.6	1.1	19.4	23.8	15.2	8.6	0.5

**Table 2. Trait means for the phenotypic extremes, 20 most drought susceptible (S group) and 20 most drought tolerant (T group) RILs and parents from Buster × Roza RIL population, measured under multiple stress on the 'purgatory plot' at Prosser, WA, in 2013 and 2014.**

Trait	Year	'Buster'	'Roza'	Parents	S group	T group	Group
Seed yield, kg ha <sup>-1</sup>	2013	1000	3947	***	1488	2328	***
	2014	289	2337	*	770	1808	***
Seed weight, g 100 seed <sup>-1</sup>	2013	38.5	34.5	ns†	35.1	34.6	ns
	2014	33	30	ns	31	33	**
Pod wall ratio	2013	0.29	0.23	*	0.31	0.24	***
	2014	0.29	0.22	ns	0.29	0.24	***
Biomass weight, kg ha <sup>-1</sup>	2013	3577	7655	***	3942	5329	***
	2014	6.3	1.8	***	4.7	2.1	***
Biomass rating, 1-9 scale	2013	52	57	**	54	62	***
	2014	52	50	ns	51	54	***
Days to flower, d	2013	115	116	ns	116	116	ns
	2014	98	93	ns	92	100	***
Days to harvest maturity, d	2013	63	59	ns	63	53	***
	2014	46	44	ns	41	46	***

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† ns, not significant.

effect ( $P < 0.001$ ) and a group by treatment interaction ( $P < 0.05$ ) as the difference between the tolerant and susceptible group under well-watered conditions was 154 kg ha<sup>-1</sup> compared to 1836 kg ha<sup>-1</sup> under DS. At Mitchell in 2014, the DII was slightly greater than 2013 (0.3 versus 0.2) and the drought tolerant group out-yielded the susceptible group by 25%. For both years seed size was

significantly larger for the susceptible lines under non-stress and drought stress ( $P < 0.001$ ).

A higher level of drought stress (DII = 0.5) was achieved at Othello in 2014. Roza yielded 85% more than Buster under DS and only 7% more under NS. Significant differences for SY were observed between the tolerant and susceptible groups (Table 3). For SY under DS the tolerant

**Table 3. Trait means for parents and the 20 most drought susceptible (S group) and 20 most drought tolerant (T group) RILs from Buster × Roza RIL population, measured under terminal drought stress (DS) and non-stress (NS) at locations Mitchell, NE, in 2013 and 2014 and at Othello, WA, in 2014.**

Trait	Year	Loc.	Trt.	Parents			RIL population				
				'Buster'	'Roza'	Parents	S group	T group	Group	Trt.	GT†
Yield, kg ha <sup>-1</sup>	2013	NE	DS	2859	2940	ns‡	1495	3331	***	***	*
			NS	3829	3282	ns	3648	3802			
	2014	NE	DS	2438	2524	ns	1853	2471	***	*	ns
			NS	4058	3595	ns	2928	3448			
		WA	DS	442	3043	***	1100	1953	***	ns	**
			NS	3951	4261	ns	2994	3412			
Seed weight, g 100 seed <sup>-1</sup>	2013	NE	DS	37.3	26.8	**	34.6	32.9	***	ns	ns
			NS	34.8	29.6	ns	34.3	32.8			
	2014	NE	DS	40	31	ns	38	37	***	ns	ns
			NS	43	32	ns	40	37			
		WA	DS	48	36	***	42	43	ns	ns	**
			NS	53	37	***	41	39			
Days to flowering, d	2013	NE	DS	45	45	ns	44	45	***	ns	**
			NS	45	48	*	43	46			
	2014	NE	DS	46	48	*	46	46	***	ns	ns
			NS	46	49	*	45	46			
		WA	DS	44	47	*	44	48	***	ns	**
			NS	44	48	**	44	47			
Days to harvest maturity, d	2013	NE	DS	83	90	**	81	85	***	***	ns
			NS	84	93	**	83	88			
	2014	NE	DS	87	95	ns	87	91	***	ns	ns
			NS	95	94	ns	86	92			
		WA	DS	101	98	ns	103	102	***	**	***
			NS	101	98	ns	96	100			
Days to seed fill, d	2013	NE	DS	38	45	*	38	40	***	***	ns
			NS	39	45	*	40	42			
	2014	NE	DS	41	47	ns	41	44	***	ns	ns
			NS	49	46	ns	41	45			
		WA	DS	57	51	*	59	54	***	ns	**
			NS	57	50	*	52	53			
Pod wall ratio	2014	NE	DS	0.2	0.2	ns	0.23	0.20	***	ns	ns
			NS	0.2	0.2	ns	0.23	0.20			
	2014	WA	DS	0.29	0.22	**	0.31	0.23	***	ns	***
			NS	0.21	0.23	ns	0.24	0.21			
		WA	DS	5.0	1.8	***	3.7	2.6	***	ns	*
			NS	1.8	1.3	ns	2.2	1.7			
NDVI§ at R3	2014	WA	DS	0.07	0.12	*	0.11	0.1	***	ns	ns
			NS	0.15	0.17	ns	0.16	0.15			
NDVI at R5	2014	WA	DS	0.14	0.2	ns	0.19	0.17	***	ns	ns
			NS	0.2	0.18	ns	0.18	0.17			
Canopy area at R3	2014	WA	DS	3194	4063	ns	3842	3435	**	ns	**
			NS	4423	4876	ns	4316	4615			
Canopy area at R5	2014	WA	DS	4020	4640	***	4368	4237	**	ns	ns
			NS	5354	5349	ns	5304	5280			

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† GT, Group × Treatment.

‡ ns, not significant.

§ NDVI, normalized difference vegetation index.

group had 43% and susceptible group 63% less yield compared to SY under NS. The tolerant group exhibited a greater difference for SW (39 and 43 g 100 seeds<sup>-1</sup>, respectively) between NS and DS than the susceptible group.

## Remote Sensing NDVI and Canopy Area Terminal Drought Stress

During the cropping season at Othello in 2014, a multi-spectral aerial image was acquired to capture greenness (NDVI) and canopy area (reported below). Aerial images were taken with the multiband camera at mid (R3) and late pod fill (R5) growth stages. Figure 2a shows the entire drought study under low resolution, and Figure 2b is a high-resolution image of the selected lines from the Buster × Roza mapping population. The plots were segmented into rectangular regions during image processing for feature extraction (NDVI, canopy area). Buster and Roza differed significantly ( $P < 0.05$ ) for NDVI at the R3 stage under DS only. Correlations with the remote sensing data were analyzed with and without separation into the tolerant vs. susceptible groups. Ungrouped correlation coefficients for NS and DS are reported in Supplemental Tables S1 and S2, respectively. Ungrouped, more greenness (NDVI) was related to increased SY under DS (R3,  $r = 0.76$ ,  $P < 0.001$ ) and with phenology traits including later DF (R3,  $r = 0.43$ ,  $P < 0.01$ ; R5,  $r = 0.61$ ,  $P < 0.001$ ), later HM (R5,  $r = 0.62$ ,  $P < 0.001$ ), and decreased DSF (R3,  $r = -0.44$ ,  $P < 0.01$ ). Lower PW (better partitioning) was associated with more greenness ( $r = -0.31$ ,  $P < 0.05$  to  $r = -0.73$ ,  $P < 0.001$ ) under DS and NS. More biomass was correlated with higher NDVI at R3 ( $r = -0.74$ ,  $P < 0.001$  and  $r = -0.67$ ,  $P < 0.001$ ) and R5 ( $r = -0.60$ ,  $P < 0.001$  and  $r = -0.63$ ,  $P < 0.001$ ) respectively under DS and NS.

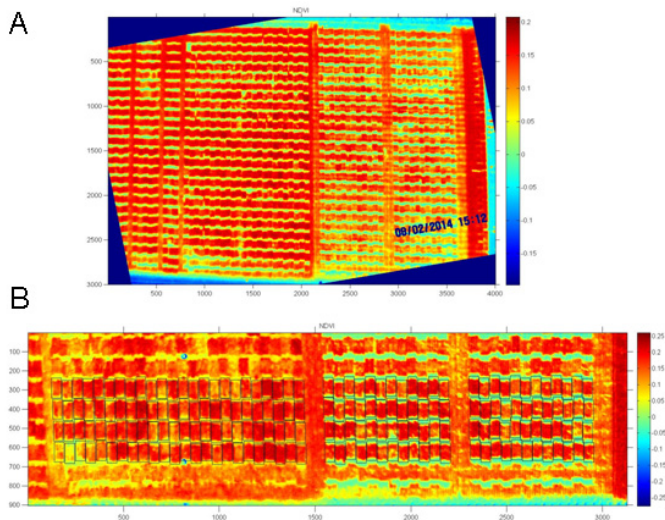


Fig. 2. Aerial image of the non-stress (left) and drought stress (right) trials at Othello, WA, in 2014, obtained at the: (a) R3 stage of development capturing two replications of the 20 most drought tolerant and 20 most susceptible RILs selected from the Buster × Roza mapping population; and (b) R5 stage of development capturing higher resolution.

By group, the tolerant group had higher NDVI values for both treatments. More greenness (NDVI, R3) within the tolerant group correlated with increased SY under DS ( $r = 0.64$ ,  $P < 0.01$ ). For the susceptible group, more greenness NDVI (R3) was related to greater SY under NS and DS (Table 4). Higher NDVI measured during the second reading (R5) was correlated with later DF and HM for the tolerant and susceptible groups under NS and DS (Fig. 3).

Canopy area estimated from aerial images at both growth stages was greater at R5 (Table 3). Increased canopy area at R3 was associated with higher seed yield ( $r = 0.31$ ,  $P < 0.05$ ) and later DF ( $r = 0.31$ ,  $P < 0.05$ ), and at R5 was associated with later DF ( $r = 0.52$ ,  $P < 0.001$ ) and HM ( $r = 0.43$ ,  $P < 0.01$ ) under DS (Supplemental Table S2). More efficient pod wall partitioning was associated with increased canopy area at R3 ( $r = -0.45$ ,  $P < 0.01$ ) and R5 ( $r = -0.42$ ,  $P < 0.01$ ) under DS. Biomass (BMRT) only correlated with canopy area at the R3 stage under NS ( $r = -0.36$ ,  $P < 0.05$ ) and under DS at R5 ( $r = -0.59$ ,  $P < 0.001$ ). The canopy area measurements at the R5 stage significantly correlated with the biomass 1 to 9 rating (BMRT) under the DS treatment within the susceptible group ( $r = -0.72$ ,  $P < 0.001$ ) (Supplemental Table S3).

## Pod Wall Ratio and Biomass Multiple Stress

Buster and Roza differed significantly for PW ( $P < 0.05$ ) in 2013 but not 2014. The differences for PW between groups were highly significant both years (Table 2). But the association of increased SY with better pod partitioning was consistent across years for both groups, except for the non-stress treatment in Mitchell, NE, for the tolerant group (Table 4). Although the methodology (harvest weight versus the 1 to 9 scale) for estimating biomass was different between years, the findings were similar with highly significant differences ( $P < 0.001$ ) in biomass observed between parents and groups. Associated with increasing yield was increased biomass based on actual weight (kg ha<sup>-1</sup>) within the tolerant group ( $r = 0.64$ ,  $P < 0.001$ ) and susceptible group ( $r = 0.85$ ,  $P < 0.001$ ) of lines (Supplemental Table S4). In 2014, more biomass (indicated by lower scores) in the tolerant group was associated with higher SY ( $r = -0.47$ ,  $P < 0.05$ ) but the correlation was not as high, perhaps due to the different method used for obtaining the data (Table 4).

## Terminal Drought Stress

PW was not measured in Nebraska in 2013. For Mitchell, NE, in 2014, both Buster and Roza had a PW of 0.20 under NS and DS and although the susceptible group averaged a higher PW than the tolerant group (0.23 versus 0.20), there was no difference between NS and DS (Table 3). Conversely, at Othello, there was a significant difference between parents ( $P < 0.01$ ) under DS only. For groups, the



**Table 4. Pearson correlation coefficients (*r*) for only those traits of 19 measured that relate to seed yield (SY) measured in the same environment in 2014: multiple stress (MS) at Prosser, WA, and non-stress (NS) and drought stress (DS) at Othello, WA, and Mitchell, NE, by group.**

Trait	Group†	Prosser, WA		Othello, WA		Mitchell, NE	
		SY in MS	SY in NS	SY in DS	SY in NS	SY in DS	
Days to harvest	T	ns‡	ns	ns	ns	ns	
	S	ns	ns	ns	ns	-0.57**	
Days to seed fill	T	ns	ns	-0.56**	0.45*	ns	
	S	ns	ns	ns	ns	-0.63**	
Pod wall ratio	T	-0.64**	-0.48*	-0.71***	ns	-0.59**	
	S	-0.61**	-0.58**	-0.69***	-0.65**	-0.71***	
Biomass rating	T	-0.47*	ns	-0.50*	ns	ns	
	S	ns	-0.59**	ns	ns	ns	
NDVI§ at R3	T	na¶	ns	0.64**	na	na	
	S	na	0.60**	0.73***	na	na	

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

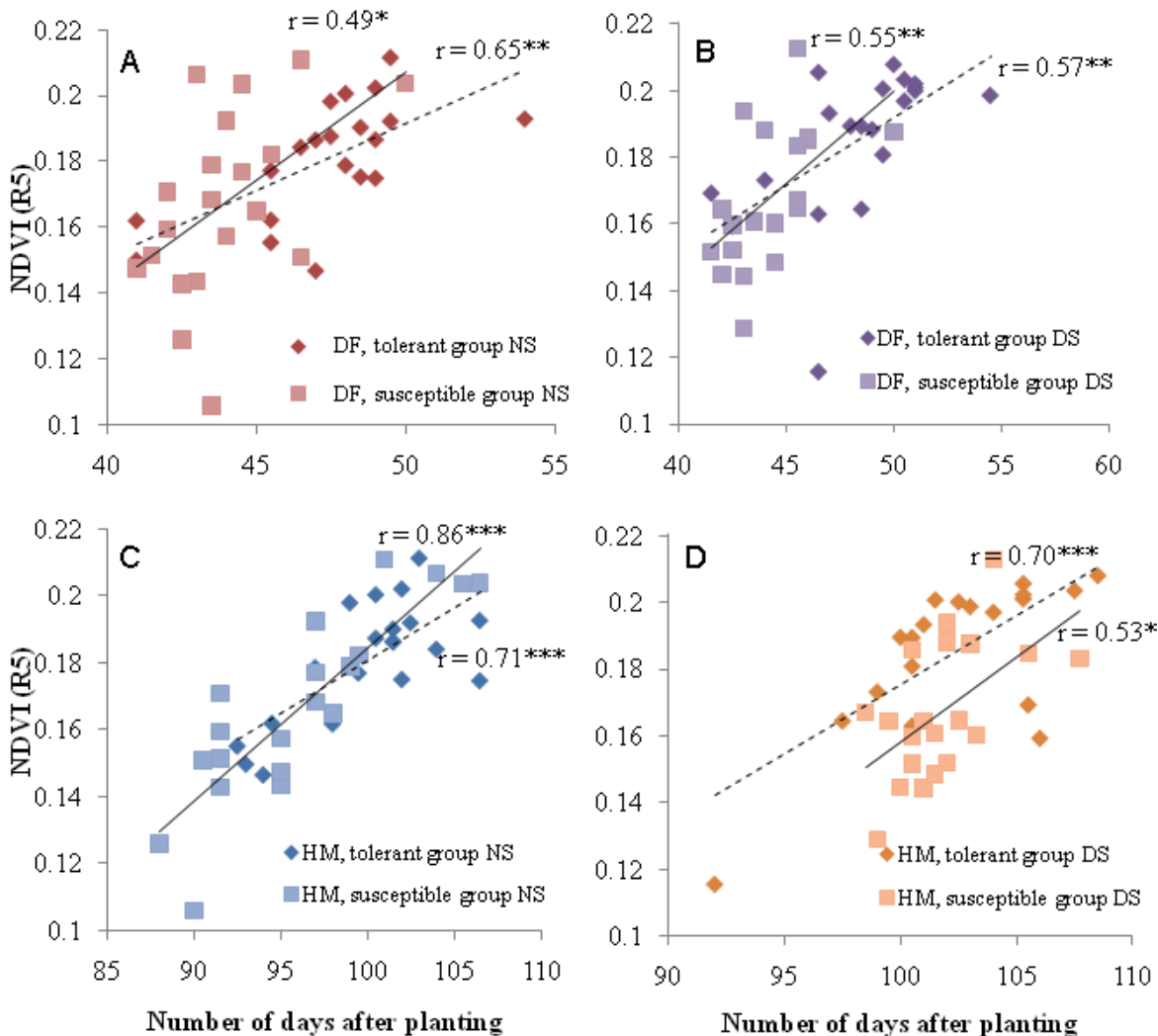
\*\*\* Significant at the 0.001 probability level.

† T, drought tolerant; S, drought susceptible.

‡ ns, not significant.

§ NDVI, normalized difference vegetation index.

¶ na, not applicable.



**Fig. 3. Correlations between normalized difference vegetation index (NDVI) measured at the R5 growth stage with days to flower (DF) under (a) non-stress (NS) and (b) terminal drought stress (DS) and harvest maturity (HM) under (c) NS and (d) DS for the 20 most drought tolerant and 20 most susceptible RILs selected from the Buster × Roza mapping population grown at Othello, WA, in 2014. The drought tolerant group is represented by a dotted line. \*, \*\*, \*\*\* indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively.**

difference for PW between treatments was larger for the susceptible group (0.07) than for the tolerant group (0.02).

Of all traits in 2014, lower PW indicative of increased partitioning from pod walls to seeds had the greatest association with increased SY across trials and groups (Table 4). The strongest association observed between lower PW and increased SY was for the tolerant group under DS at Othello in 2014 ( $r = -0.71$ ,  $P < 0.001$ ).

In 2013, plant biomass was not evaluated under terminal drought stress. There was a significant ( $P < 0.001$ ) difference between Buster and Roza and the susceptible group averaged less BMRT (3.7) than the tolerant group (2.6) in 2014 (Table 3). Increased BMRT (lower scores) was associated with higher yield for the tolerant group under DS ( $r = -0.50$ ,  $P < 0.05$ ) and the susceptible group under NS ( $r = -0.59$ ,  $P < 0.01$ ) (Table 4).

## Phenology Traits

### Multiple Stress

For 2013, DF differed significantly ( $P < 0.01$ ) between Buster and Roza as well as the tolerant and susceptible groups ( $P < 0.001$ ). No significant difference was observed for HM and DSF between the parents either year; however both traits did differ ( $P < 0.05$ ) between the tolerant and susceptible groups in 2014 (Table 2). Within the tolerant group of lines increasing SY was related to earlier HM ( $r = -0.52$ ,  $P < 0.01$ ) and shorter DSF ( $r = -0.47$ ,  $P < 0.05$ ) (Supplemental Table S4).

### Terminal Drought Stress

Overall, Buster flowered and matured earlier, and had fewer DSF than Roza in NE 2013. Likewise, the susceptible group followed suit compared to the tolerant group for all three traits (Table 3). No phenology traits measured under DS correlated with SY; however under NS, later DF ( $r = 0.57$ ,  $P < 0.05$ ), later HM ( $r = 0.85$ ,  $P < 0.01$ ), and more DSF ( $r = 0.59$ ,  $P < 0.01$ ) correlated with increased SY in the susceptible group and more DSF ( $r = 0.49$ ,  $P < 0.05$ ) was related to increased SY in the drought tolerant group (Supplemental Table S5). At Mitchell in 2014, only DF differed ( $P < 0.05$ ) between parents and there was a significant group effect ( $P < 0.001$ ) for DF, HM, and DSF. The tolerant group flowered one day later and reached harvest maturity later than the susceptible group (Table 3). PW was the most significant correlation with SY under DS and NS ( $r = -0.71$ ,  $P < 0.001$  and  $r = -0.65$ ,  $P < 0.001$ , respectively) and was only correlated for the tolerant group under DS ( $r = -0.59$ ,  $P < 0.001$ ) (Table 4).

At Othello in 2014, the parents differed ( $P < 0.05$ ) for DF and DSF and there was a genotype  $\times$  treatment interaction for the groups regarding DF, HM, and DSF ( $P < 0.01$ ) (Table 3). The greatest differences between treatments occurred in the susceptible group (HM was 7 d later and DSF 7 d greater duration under DS). On average,

the tolerant group had shorter days to seed fill than the susceptible group (Table 3).

## QTL Mapping with Extreme Phenotypes

The major QTL for SY (SY1.1, SY2.1, and SY5.1), identified by Trapp et al. (2015), were also observed for the 40 RILs representing the phenotypic extremes in the 2013 and 2014 multiple stress and terminal drought trials (Table 5). Each SY1.1 and SY2.1 had more consistent expression under terminal drought stress than was observed previously. One additional QTL for seed yield, SY3.1<sup>BR</sup>, was identified on chromosome Pv03 and was detected only under terminal drought stress ( $R^2 = 32.2\%$ ). The minor effect QTL for yield SY5.1<sup>BR</sup>, previously found in Nebraska 2011 under non stress was similarly only detected at Mitchell in 2013 under NS.

Likewise, the phenology trait QTL for DF, HM, and DSF, were found to co-segregate with SY1.1 (DF1.2 HM1.1, DSF1.1) and SY2.1 (DF2.1, HM1.2, and DSF2.1) confirming the importance of phenotypic plasticity for yield response under stress (Trapp et al., 2015).

A preliminary QTL for harvest maturity HM1.2<sup>BR</sup> was associated with DF1.1 identified in the previous study. Of the three previously identified QTL for SW (SW2.1<sup>BR</sup>, SW8.1<sup>BR</sup>, SW8.2<sup>BR</sup>) only SW2.1 was clearly identified using extreme phenotypes in 2013 and 2014.

Three traits not previously mapped using the Buster  $\times$  Roza whole mapping population, PW, BMRT, and NDVI (R3), also mapped to the major SY1.1 and SY2.1 QTL regions using the phenotypic extremes. PW1.1<sup>BR</sup> had the highest range of any drought related trait ( $R^2$  of 35–52%). An additional significant QTL (46%) for PW was located near SNP47282 (37.8 Mb) on Pv01 (PW1.2<sup>BR</sup>) but was only detected in one environment, MS in 2014. Regardless of the methodology behind biomass data collection, weight at harvest maturity in 2013 trials, or a 1 to 9 visual scale at the R5 stage in 2014 trials, both estimates mapped to the same QTL, BMWT2.1<sup>BR</sup>, accounting for 33 and 24% of the variation (Table 6). The QTL for NDVI (at R3 growth stage) mapped to Pv01 (56.9 Mb) under NS and DS ( $R^2 = 28$  and 39%, respectively) and to Pv02 near the SY2.1 QTL under DS only ( $R^2 = 27\%$ ).

## DISCUSSION

Precise phenotyping is essential to plant breeding. In this study, the 20 most tolerant and 20 most susceptible RILs from a recombinant inbred population (Buster  $\times$  Roza) were used to facilitate evaluation of 19 potential drought specific traits, of which eight of the most significant were reported, for response to multiple stress and terminal drought stress in multiple environments. An accurate evaluation of the aforementioned traits would not have been possible for the whole mapping population of 140 RILs, because many traits are time sensitive measurements

**Table 5. Validation of QTL previously reported in Trapp et al. (2015) using the 20 most drought tolerant and 20 most susceptible lines extracted from the Buster × Roza RIL population, phenotyped across environments consisting of three locations: Prosser, WA (P\_2013, P\_2014), Othello, WA (O\_2014), and Mitchell, NE (M\_2013, M\_2014) and three treatments: multiple stress (MS), drought stress (DS), and non-stress (NS).**

QTL†	Environment	Chr.	Location, Mb	Closest marker	LOD‡	LOD TH‡	R <sup>2</sup>	Add.§
SY1.1 <sup>BR</sup>	P_2013_MS	1	47.7	SNP50809¶	2.3#	2.0	23.2	-345.6
	P_2014_MS	1			3.7	2.7	35.0	-450.2
	O_2014_DS	1			3.3	3.1	31.2	-455.2
	M_2013_DS	1			2.6#	2.0	25.9	-302.2
	M_2014_NS	1			3.0	2.9	29.1	-306.0
SY2.1 <sup>BR</sup>	P_2013_MS	2	11.8	SNP40055	4.5	3.1	40.1	-458.0
	P_2014_MS	2			3.5	3.2	33.5	-445.4
	O_2014_DS	2			5.3	3.6	45.4	-553.2
	M_2014_DS	2			3.8	3.6	35.2	-354.2
SY5.1 <sup>BR</sup>	M_2013_NS	5	38.7	SNP45307	1.9#	1.9	19.2	-204.8
DF1.1 <sup>BR</sup>	P_2014_MS	1	3.3	SNP49655	3.1#	3.0	30.0	1.9
	O_2014_NS	1			2.2#	2.2	22.1	1.0
	M_2014_DS	1			3.8	4.0	35.4	1.1
	M_2014_NS	1			2.9#	2.3	28.4	0.9
DF1.2 <sup>BR</sup>	P_2013_MS	1	47.7	SNP50809	7.2	3.0	56.4	-4.5
	P_2014_MS	1			5.8	4.2	48.9	-2.7
	O_2014_NS	1			8.5	3.3	62.4	-2.4
	O_2014_DS	1			8.2	2.8	60.9	-2.7
	M_2013_NS	1			4.5	3.4	40.5	-1.5
	M_2013_DS	1			4.9	3.0	42.8	-1.3
	M_2014_NS	1			4.8	3.2	42.4	-1.2
	M_2014_DS	1			3.3	4.0	31.4	-0.9
HM1.1 <sup>BR</sup>	P_2014_MS	1	47.7	SNP50809	4.4	3.4	39.7	-5.2
	O_2014_NS	1			2.5#	2.0	25.2	-2.5
	M_2013_NS	1			6.1	2.9	50.2	-2.6
	M_2014_NS	1			2.8	2.8	27.9	-3.4
DSF1.1 <sup>BR</sup>	P_2013_MS	1	47.7	SNP50809	7.1	3.2	56.0	4.6
	P_2014_MS	1			2.1#	2.0	21.1	-2.5
	O_2014_DS	1			3.0#	2.5	29.4	2.5
SW2.1 <sup>BR</sup>	M_2013_DS	2	11.8	SNP40055	3.8	3.4	35.5	2.1
	M_2014_NS	2			2.5	2.1	25.4	2.2
	M_2014_DS	2			2.7	2.1	27.0	0.4

† Abbreviations in QTL names: SY, seed yield; DF, days to flowering; HM, days to harvest maturity; DSF, days to seed fill; SW, seed weight.

‡ LOD, logarithm of odds. LOD thresholds (LOD TH) were calculated via 1000 permutations at  $P = 0.01$ .

§ Additive value represents effect of the Buster allele on the trait.

¶ SNP names are truncated from the NCBI number which uses the prefix ss7156.

# QTL significant at  $P < 0.05$  and only reported if an additional trait was significant at the same location at  $P < 0.01$ .

confounded by slight changes in temperature, sun inclination, and cloud cover. Moreover, plant growth stage may confound measurement of these traits necessitating staggered data collection to obtain meaningful data; phenology traits, for example. Likewise, root traits are best phenotyped during flowering to best capture differences before senescence.

Root traits were not related to performance in any stress or non-stress trials in this study. Conversely, several studies (White and Castillo, 1992; Asfaw and Blair, 2012; Beebe et al., 2013) have reported on the significant role of root traits for adaptation to water stress in common bean. Although, a direct comparison with field studies using grafting and greenhouse methods is arguable, the fact that plants can manage water stress by avoiding drought by deeper rooting has been recognized in the

field for rice (Henry et al., 2011) and soybean (*Glycine max*) (Fenta et al., 2014). Similarly, tepary bean (*Phaseolus acutifolius*), one the most drought tolerant relatives of common bean, exhibits a deep rooting pattern (Beebe et al., 2013). The parents had similar root phenes in this study which makes it plausible that root traits do not represent important components of stress tolerance in the Buster × Roza population. However, deep excavation of roots was not conducted, so deep rooting measurement in the Buster × Roza RIL population may represent an important follow-up study.

Canopy temperature and SPAD readings did not have a consistent relationship with yield performance within or between groups. Conversely, other studies indicated efficacy using infrared thermometry in phenotyping for drought stress in common bean (Asfaw et al., 2012) as well

**Table 6. Preliminary QTL detected for drought related traits not previously studied in the entire Buster × Roza mapping population, using the 20 most drought tolerant and 20 most susceptible RILS evaluated across environments consisting of three locations: Prosser, WA (P\_2013, P\_2014), Othello, WA (O\_2014), and Mitchell, NE (M\_2013, NE\_2014) and three treatments: multiple stress (MS), drought stress (DS), and non-stress (NS) in 2013 and 2014.**

QTL†	Environment	Chr.	Location, Mb	Closest marker	LOD‡	LOD TH‡	R <sup>2</sup>	Add.§
SY3.1 <sup>BR</sup>	O_2014_DS	3	2.6	SNP46941¶	3.4	2.6	32.2	502.1
SW8.3 <sup>BR</sup>	O_2014_NS	8	52.2	SNP46093	4.9	2.9	43.1	3.3
	O_2014_DS	8			3.2	3.0	31.2	2.6
	M_2013_NS	8			4.2	2.9	38.2	2.1
	M_2013_DS	8			2.7	2.7	26.8	2.4
	M_2014_NS	8			4.2	2.8	38.7	2.7
	M_2014_DS	8			4.0	2.7	37.0	2.7
HM1.2 <sup>BR</sup>	M_2013_NS	1	3.3	SNP49655	2.1#	1.9	21.4	1.4
	M_2014_DS	1			2.0#	1.9	20.4	2.7
PW1.1 <sup>BR</sup>	P_2013_MS	1	47.7	SNP50809	4.5	3.6	40.6	0.03
	O_2014_NS	1			4.3	3.7	39.4	0.02
	O_2014_DS	1			6.3	3.1	51.8	0.04
	M_2014_NS	1			3.8#	3.8	35.1	0.02
	M_2014_DS	1			3.7#	3.1	34.9	0.01
PW1.2 <sup>BR</sup>	P_2014_MS	1	31.8	SNP47282	5.3	2.7	46.4	0.03
PW2.1 <sup>BR</sup>	O_2014_NS	2	11.8	SNP40055	2.7#	2.4	26.8	0.03
BMRT1.1 <sup>BR</sup>	P_2014_MS	1	47.7	SNP50809	6.3	3.0	51.7	1.3
	O_2014_NS	1			2.4#	2.4	24.2	0.4
	O_2014_DS	1			5.1	3.2	44.7	0.8
BMWT2.1 <sup>BR</sup>	P_2013_MS	2	11.8	SNP40055	3.5	3.0	32.8	-0.1
BMRT2.2 <sup>BR</sup>	P_2014_MS	2			2.4	2.1	23.9	0.9
NDVI(R3)1.1 <sup>BR</sup>	O_2014_NS	1	56.9	SNP46304	2.2#	2.2	28.1	-0.01
	O_2014_DS	1			4.3	3.0	39.3	-0.01
NDVI(R3)2.1 <sup>BR</sup>	O_2014_DS	2	11.8	SNP40055	2.7#	2.3	26.5	-0.01

† Abbreviations in QTL names: SY, seed yield; SW, seed weight; HM, days to harvest maturity; PW, pod wall ratio; BMRT, biomass rating; BMWT, biomass weight; NDVI, normalized difference vegetation index.

‡ LOD, logarithm of odds. LOD thresholds (LOD TH) were calculated via 1000 permutations at  $P = 0.01$ .

§ Additive value represents effect of the Buster allele on the trait.

¶ SNP names are truncated from the NCBI number which uses the prefix ss7156.

# QTL significant at  $P < 0.05$  and only reported if an additional trait was significant at the same location at  $P < 0.01$ .

as wheat (Olivares-Villegas et al., 2007) and rice (Takai et al., 2010). Asfaw et al. (2012) also found consistent QTL for SPAD reading in the DOR364/BAT477 RIL population, but the correlations between both measured traits and seed yield in common bean were insignificant or inconsistent. Lack of a cooler canopy or more leaf greenness for the tolerant group in this study suggests these traits do not contribute to drought response in the Buster × Roza population. It is possible that the CT and SPAD measurements were affected by other environmental factors. Canopy temperature data were obtained with a hand-held infrared thermometer, and care was taken to avoid cloudy days and exposed soil. Similarly SPAD was measured with a hand-held meter and the sample area is limited to a small area (3–4 mm) of each leaf. Perhaps aerial imaging will provide a more efficient means of measuring such time intensive and sensitive traits in the future.

Alternative to hand held devices, UAVs can carry high quality instrumentation and capture data quickly and consistently. Aerial imaging can capture data from a large number of plots within a few minutes reducing micro-environmental variability, whereas it can take

several hours or days to obtain similar data with hand-held devices. We used remote sensing to measure greenness and canopy area in the terminal drought study at Othello, WA, in 2014. With one year of preliminary remote sensing data, we were able to measure differences within and between groups, and observe correlations with yield and partitioning traits. Significant correlations were observed for NDVI with SY and PW under DS in the tolerant group. For the susceptible group, NDVI and SY were correlated under both NS and DS. Moreover, QTL were detected for NDVI (R3) using phenotypic extremes. Canopy area estimated at R5 growth stage was related to BMRT which suggests aerial imaging may have a role for estimating biomass in dry bean in the future.

The partitioning trait PW, as well as biomass (BMWT or BMRT), were consistently associated with seed yield across multiple stress and terminal drought stress trials. The association of seed yield with increased plant growth and efficient partitioning is unsurprising and has been reported in common bean for decades (Adams, 1982; Wallace and Masaya, 1988; Kelly et al., 1998). Assefa et al. (2013) also observed significant correlations between PW

and seed yield and suggested PW could be used to select drought tolerant genotypes. The important relationship between biomass accumulation and partitioning in breeding for yield under multiple and terminal drought stress is affirmed in the Buster × Roza RIL population.

Another partitioning trait, shorter DSF under stress, may be the most important factor contributing to higher seed yield in the Buster × Roza population as observed by Trapp et al. (2015) and again in this study. Selective genotyping with the extreme phenotypes further supports the important relationship of efficient photosynthate accumulation and partitioning to yield under drought stress because BMRT1.1 and PW1.1 QTL co-located with the same QTL for SY1.1. Previous studies reported QTL for pod wall partitioning (Asfaw et al., 2012; Mukeshimana et al., 2014) and biomass (harvest index; Asfaw et al., 2012) on Pv01.

Studies under multiple stresses and terminal drought are difficult to manage to obtain the same level of stress each year. The parents and groups responded differently between years on the purgatory plot because the level of multiple-stress differed and was much greater in 2014. There was little difference for phenology traits between the parents in either year but the greater stress in 2014 decreased maturity and DSF. The same trend occurred for the groups but maturity and DSF decreased more for the susceptible group in comparison to the tolerant group. For the terminal drought studies, less stress in the NE trials as indicated by lower DII likely contributed to the differential response of the parents and groups among trials. Generally, Buster compared to Roza, and the susceptible group compared to tolerant group, had decreased maturity and DSF in NE trials for DS and NS. Conversely, increased maturity and DSF was observed for the susceptible group under the 2014 (Othello, WA) environment with the higher level of terminal drought stress.

In addition to allowing a broader survey of drought related traits, selective phenotyping provided validation and further characterization of previously identified QTL and enabled detection of a few novel QTL. There is potential for using selective phenotypes as a preliminary step to identify relevant traits; however, it is important to recognize that increased false positives and inflated associations are a consequence of mapping with reduced population sizes and phenotypic extremes. Nonetheless, using the phenotypic extremes, two major yield QTL SY1.1<sup>BR</sup> and SY2.1<sup>BR</sup> previously identified using the entire Buster × Roza mapping population were similarly detected under MS, DS, and NS. The SY QTL were consistently expressed under DS confirming their role in drought tolerance. Two new traits, PW and biomass, both co segregated with these major QTL for seed yield. Given the importance of biomass accumulation in breeding for drought tolerance it is worth emphasizing that both methods (BMWT and BMRT) for estimating biomass mapped to SNP40055 under multiple stress and

had similar correlations to SY. Using a visible scale (1 to 9) to estimate biomass versus waiting until harvest to obtain actual weight is less laborious and enables earlier discernment in the growing season, providing more efficient use of time and resources. Further refinements and additional experience using the visual scale may be necessary to better predict biomass as determined by weight. In addition, there is an exciting potential for remote sensing to measure biomass, leaf greenness, and canopy temperature in the future.

In summary, by selecting a smaller set of lines representing the phenotypic extremes (tails) from a larger recombinant inbred population, more drought related traits were evaluated and the traits most relevant to the population were identified enabling a more focused study on those select traits in subsequent trials. Selective genotyping with the phenotypic extremes was useful for validating and characterizing previously identified QTL, and provided a first step toward the discovery of novel QTL. Overall, the findings support selective phenotyping as a tool for determining key traits related to drought stress paired with selective genotyping to reduce costs associated with genetic mapping and gene discovery in water limited environments.

## Supplementary Material Available

Correlations among all traits by treatment, location, and year are available in supplemental tables available with the online version of this article.

## Acknowledgments

This work was supported, in part, by funding received from the Norman Borlaug Commemorative Research Initiative between US Agency for International Development and the USDA-ARS, through Cooperative Agreement No. 58-0210-3-012 (408-5354-012) and from the Feed the Future Innovation Lab for Climate Resilient Beans Project No. AID-OAA-A-13-00077.

## References

- Adams, M.W. 1982. Plant architecture and yield breeding. *Iowa State J. Res.* 56:225–254.
- Araus, J.L., C. Sanchez, L. Cabrera-Bosquet, and J. Crossa. 2008. Maize phenotyping for drought adaptation: potential contribution of new physiological protocols. CIMMYT, Texcoco, Mexico.
- Asfaw, A., and M. Blair. 2012. Quantitative trait loci for rooting pattern traits in common beans grown under drought stress versus non-stress conditions. *Mol. Breed.* 30:681–695. doi:10.1007/s11032-011-9654-y
- Asfaw, A., M. Blair, and P. Struik. 2012. Multienvironment quantitative trait loci analysis for photosynthate acquisition accumulation and remobilization traits in common bean under drought stress. *G3: Genes Genomes Genetics* 2:579–595.
- Assefa, T., S.E. Beebe, I.M. Rao, J.B. Cuasquer, M.C. Duque, M. Rivera, A. Battisti, and M. Lucchin. 2013. Pod harvest index as a selection criterion to improve drought resistance in white pea bean. *Field Crops Res.* 148:24–33. doi:10.1016/j.fcr.2013.04.008

- Barchi, L., V. Lefebvre, A.M. Sage-Palloix, S. Lanteri, and A. Palloix. 2009. QTL analysis of plant development and fruit traits in pepper and performance of selective phenotyping. *Theor. Appl. Genet.* 118:1157–1171. doi:10.1007/s00122-009-0970-0
- Beebe, S.E., I.M. Rao, M.W. Blair, and J.A. Acosta-Gallagos. 2013. Phenotyping common beans for adaptation to drought. *Front. Physiol.* 4:35. doi:10.3389/fphys.2013.00035
- Blum, A. 2011. *Plant breeding for water-limited environments*. Springer, New York.
- Darvasi, A., and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theor. Appl. Genet.* 85:353–359.
- Felicetti, E., Q. Song, G. Jia, P. Cregan, K.E. Bett, and P.N. Miklas. 2012. Simple sequence repeats linked with slow darkening trait in pinto bean discovered by single nucleotide polymorphism assay and whole genome sequencing. *Crop Sci.* 52:1600–1608. doi:10.2135/cropsci2011.12.0655
- Fenta, B.A., S.E. Beebe, K.J. Kunert, J.D. Burrige, K.M. Barlow, J.P. Lynch, and C.H. Foyer. 2014. Field phenotyping of soybean roots for drought stress tolerance. *Agronomy* 4:418–435. doi:10.3390/agronomy4030418
- Fischer, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29:897–912. doi:10.1071/AR9780897
- Henry, A., V.R.P. Gowda, R.O. Torres, K.L. McNally, and R. Serraj. 2011. Variation in root system architecture and drought response in rice (*Oryza sativa*): Phenotyping of the OryzaSNP panel in rainfed lowland fields. *Field Crops Res.* 120:205–214. doi:10.1016/j.fcr.2010.10.003
- Hoogenboom, G. 2014. AgWeatherNet. Washington State Univ. www.weather.wsu.edu (accessed July 2014).
- Jestin, C., P. Vallee, C. Domin, M.J. Manzanares-Dauleux, and R. Delourme. 2012. Assessment of a new strategy for selective phenotyping applied to complex traits in *Brassica napus*. *Open J. Genet.* 2:190–201. doi:10.4236/ojgen.2012.24025
- Joehanes, R., and J.C. Nelson. 2008. QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics* 24:2788–2789. doi:10.1093/bioinformatics/btn523
- Kelly, J.D., J.M. Kolkman, and K. Schneider. 1998. Breeding for yield in dry bean (*Phaseolus vulgaris* L.). *Euphytica* 102:343–356. doi:10.1023/A:1018392901978
- Lander, E., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.
- Lebowitz, R.J., M. Soller, and J.S. Beckmann. 1987. Trait-based analyses for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. *Theor. Appl. Genet.* 73:556–562. doi:10.1007/BF00289194
- Lu, Y., Z. Hao, C. Xie, J. Crossa, and J.-L. Araus. 2011. Large-scale screening for maize drought resistance using multiple selection criteria evaluated under water-stressed and well-watered environments. *Field Crops Res.* 124:37–45. doi:10.1016/j.fcr.2011.06.003
- Merewitz, E., W. Meyer, and S. Bonos. 2010. Drought stress responses and recovery of Texas × Kentucky hybrids and Kentucky bluegrass genotypes in temperate climate conditions. *Agron. J.* 102:258–268. doi:10.2134/agronj2009.0328
- Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* 88:9828–9832. doi:10.1073/pnas.88.21.9828
- Miklas, P.N., J.D. Kelly, S.E. Beebe, and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica* 147:105–131. doi:10.1007/s10681-006-4600-5
- Miklas, P.N., and T. Porch. 2010. Guidelines for common bean QTL nomenclature. *Annu. Rep. Bean Improv. Coop.* 53:202–204.
- Mir, R.R., M. Zaman-Allah, N. Sreenivasulu, R. Trethowan, and R.K. Varshney. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor. Appl. Genet.* 125:625–645. doi:10.1007/s00122-012-1904-9
- Mukeshimana, G., L. Butare, P.B. Cregan, M.W. Blair, and J.D. Kelly. 2014. Quantitative trait loci associated with drought tolerance in common bean. *Crop Sci.* 54:923–938. doi:10.2135/cropsci2013.06.0427
- Olivares-Villegas, J.J., M.P. Reynolds, and G.K. McDonald. 2007. Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Funct. Plant Biol.* 34:189–203. doi:10.1071/FP06148
- Sankaran, S., L.R. Khot, and A.H. Carter. 2015a. Field-based crop phenotyping: multispectral aerial imaging for evaluation of winter wheat emergence and spring stand. *Comput. Electron. Agric.* 118:372–379. doi:10.1016/j.compag.2015.09.001
- Sankaran, S., L.R. Khot, J.M. Maja, and R. Ehsani. 2013. Comparison of two multiband cameras for use on small UAVs in Agriculture. Workshop on hyperspectral image and signal processing: Evolution in remote sensing (WHISPERS). Paper presented at the 5th Meeting of the Inst. of Electrical and Electronics Engineers. 25–28 June 2013. Gainesville, FL.
- Sankaran, S., L.R. Khot, C.E. Zúñiga, S. Jarolmasjed, V. Sathuvalli, G. Vandemark, P.N. Miklas, A. Carter, M. Pumphrey, R. Knowles, and M. Pavék. 2015b. Low-altitude, high-resolution aerial imaging systems for row and field crop phenotyping: A Review. *Eur. J. Agron.* 70:112–123. doi:10.1016/j.eja.2015.07.004
- SAS Institute. 2008. SAS version 9.3. SAS Inst. Inc., Cary, NC.
- Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9(7):671–675.
- Schneider, K.A., M.E. Brothers, and J.D. Kelly. 1997. Marker assisted selection to improve drought resistance in common bean. *Crop Sci.* 37:51–60. doi:10.2135/cropsci1997.0011183X003700010008x
- Schmutz, J., P.E. McClean, S. Mamidi, G. Albert Wu, S.B. Cannon, J. Grimwood, J. Jenkins, S. Shu, Q. Song, C. Chavarro, M. Torres-Torres, V. Geffroy, S. Mafi Moghaddam, D. Gao, B. Abernathy, K. Barry, M. Blair, M.A. Brick, M. Chovatia, P. Gepts, D.M. Goodstein, M. Gonzales, U. Hellsten, D.L. Hyten, G. Jia, J.D. Kelly, D. Kudrna, R. Lee, M.M.S. Richard, P.N. Miklas, J.M. Osorno, J. Rodrigues, V. Thareau, C.A. Urrea, M. Wang, Y. Yu, M. Zhang, R.A. Wing, P.B. Cregan, D.S. Rokhsar, and S.A. Jackson. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. *Nat. Genet.* 46:707–713. doi:10.1038/ng.3008
- Serraj, R., F.R. Bidinger, Y.S. Chauhan, N. Seetharama, S.N. Nigam, and N.P. Saxena. 2003. Management of drought in ICRISAT cereal and legume mandate crops. In: J.W. Kigne, R. Barker, and D. Molden, editors, *Water productivity in agriculture: limits and opportunities for improvement*. CAB International, Wallingford, Oxon, UK. p. 127–144.

- Song, Q., G. Jia, D.L. Hyten, J. Jenkins, E.Y. Hwang, S.G. Schroeder, J.M. Osorno, J. Schmutz, S. A. Jackson, P.E. McClean, and P.B. Cregan. 2015. SNP assay development for linkage map construction, anchoring whole genome sequence and other genetic and genomic applications in common bean. *G3: Genes Genomes Genetics* doi:10.1534/g3.115.020594
- Subash, N., H.S. Ram Mohan, and K. Banukumar. 2011. Comparing water-vegetative indices for rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) drought assessment. *Comput. Electron. Agric.* 77:175–187. doi:10.1016/j.compag.2011.05.001
- Takai, T., M. Yano, and T. Yamamoto. 2010. Canopy temperature on clear and cloudy days can be used to estimate varietal differences in stomatal conductance in rice. *Field Crops Res.* 115:165–170. doi:10.1016/j.fcr.2009.10.019
- Tanksley, S.D. 1993. Mapping polygenes. *Annu. Rev. Genet.* 27:205–233. doi:10.1146/annurev.ge.27.120193.001225
- Trapp, J., C. Urrea, P. Cregan, and P.N. Miklas. 2015. Quantitative trait loci for yield under multiple stress and drought conditions in a dry bean population. *Crop Sci.* 55:1596–1607. doi:10.2135/cropsci2014.11.0792
- Tuberosa, R. 2012. Phenotyping for drought tolerance of crops in the genomics era. *Front. Physiol.* 3:347. doi:10.3389/fphys.2012.00347
- Wallace, D.H., and P.N. Masaya. 1988. Using yield trial data to analyze the physiological genetics of yield accumulation and genotype  $\times$  environment interaction effects on yield. *Annu. Rep. Bean Improv. Coop.* 31:7–24.
- White, J.W., P. Andrade-Sanchez, M.A. Gore, K.F. Bronson, T.A. Coffelt, M.M. Conley, K.A. Feldmann, A.N. French, J.T. Heun, D.J. Hunsaker, M.A. Jenks, B.A. Kimball, R.L. Roth, R.J. Strand, K.R. Thorp, G.W. Wall, and G. Wang. 2012. Field-based phenomics for plant genetics research. *Field Crops Res.* 133:101–112. doi:10.1016/j.fcr.2012.04.003
- White, J.W., and J.A. Castillo. 1992. Evaluation of diverse shoot genotypes on selected root genotypes of common bean under soil water deficits. *Crop Sci.* 32:762–765. doi:10.2135/cropsci1992.0011183X003200030037x
- Xu, Y., and J.H. Crouch. 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Sci.* 48:391–407. doi:10.2135/cropsci2007.04.0191

Supplemental Table 1. Partial Pearson correlation coefficients (r) for the eight most significant traits from the most drought tolerant and susceptible lines extracted from the Buster x Roza RIL population measured under non-stress Othello, WA, 2014, ungrouped.

Trait	Days to flower (DF)	Days to harvest (HM)	Days to seed fill (DSF)	Seed yield (SY; kg ha <sup>-1</sup> )	Pod wall ratio (PW)	Biomass rating (BMRT)	NDVI (R3)	NDVI (R5)	Canopy area (R3)	Canopy area (R5)
Days to flower	-									
Days to harvest	0.68***	-								
Days to seed fill	0.1	0.80***	-							
Seed yield (kg ha <sup>-1</sup> )	0.09	0.06	0.01	-						
Pod wall ratio	-0.51***	-0.41**	-0.14	-0.58***	-					
Biomass rating	-0.57***	-0.55***	-0.27	-0.51***	0.54***	-				
NDVI (R3)	0.48**	0.48**	0.26	0.57***	-0.57***	-0.67***	-			
NDVI (R5)	0.58***	0.82***	0.65***	0.36*	-0.56***	-0.63***	0.70***	-		
Canopy area (R3)	0.13	0.13	0.07	0.29	-0.07	-0.36*	0.11	0.17	-	
Canopy area (R5)	0.17	0.2	0.14	0.23	-0.26	-0.09	0.53***	0.47**	0.12	-

\*, \*\*, \*\*\* represents significance at 0.05, 0.01, and 0.001 levels of probability, respectively.



Supplemental Table 2. Partial Pearson correlation coefficients (r) for the eight most significant traits from the most drought tolerant and drought susceptible lines extracted from the Buster x Roza RIL population measured under terminal drought stress Othello, WA, 2014, ungrouped.

Trait	Days to flower (DF)	Days to harvest (HM)	Days to seed fill (DSF)	Seed yield (SY; kg ha <sup>-1</sup> )	Pod wall ratio (PW)	Biomass rating (BMRT)	NDVI (R3)	NDVI (R5)	Canopy area (R3)	Canopy area (R5)
Days to flower	-									
Days to harvest	0.23	-								
Days to seed fill	-0.65***	0.59***	-							
Seed yield (kg ha <sup>-1</sup> )	0.40**	-0.23	-0.59***	-						
Pod wall ratio	-0.59***	-0.03	0.45**	-0.78***	-					
Biomass rating	-0.63***	-0.08	0.46**	-0.53***	0.74***	-				
NDVI (R3)	0.40**	-0.11	-0.44**	0.76***	-0.76***	-0.74***	-			
NDVI (R5)	0.64***	0.62***	-0.04	0.28	-0.53***	-0.60***	0.45**	-		
Canopy area (R3)	0.31*	0.06	-0.21	0.31*	-0.45**	-0.24	0.43**	0.36*	-	
Canopy area (R5)	0.52***	0.43**	-0.08	0.23	-0.42**	-0.59***	0.43**	0.68***	0.25	-

\*, \*\*, \*\*\* represents significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

Supplemental Table 3. Partial Pearson correlation coefficients (r) for five traits measured under non-stress (NS) and drought stress (DS) at Othello, WA (WA), 2014, for the susceptible group extracted from the Buster x Roza RIL population.

Trait	Trt	SY		PW		BMRT		NDVI1		NDVI2		Area1		Area2	
		NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
SY	NS	-													
	DS	0.59**	-												
PW	NS	-0.58**	-0.27	-											
	DS	-0.50*	-0.64**	0.34	-										
BMRT	NS	-0.55**	-0.28	0.53*	0.39	-									
	DS	-0.38	-0.28	0.23	0.63**	0.64**	-								
NDVI1	NS	0.60**	0.20	-0.44*	-0.36	-0.61**	-0.53*	-							
	DS	0.56**	0.73***	-0.20	-0.69***	-0.37	-0.69***	0.48*	-						
NDVI2	NS	0.42	-0.18	-0.59**	-0.10	-0.52*	-0.45*	0.72***	0.54**	-					
	DS	0.32	0.25	-0.18	-0.48*	-0.54**	-0.68***	0.18	0.61**	0.38	-				
Area1	NS	0.20	0.16	-0.16	0.12	-0.45*	-0.20	0.30	0.12	0.22	0.47*	-			
	DS	0.21	0.20	-0.15	-0.23	-0.07	0.02	0.42	0.20	-0.04	0.07	0.20	-		
Area2	NS	0.28	-0.06	-0.25	-0.10	0.02	-0.18	0.59**	0.25	0.52*	0.19	0.14	0.21	-	
	DS	0.34	0.18	-0.22	-0.26	-0.74***	-0.72***	0.64**	0.52*	0.55**	0.74***	0.49*	0.08	0.17	-

\*, \*\*, \*\*\* represents significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

Supplemental Table 4. Partial Pearson correlation coefficients table that includes the most significant correlations for 8 traits measured on the most drought tolerant and susceptible genotypes from the Buster x Roza RIL population under multiple stress, Prosser, WA, 2013. Drought susceptible group correlations are reported on the top and drought tolerant group on the bottom.

Traits	Days to flowering	Days to harvest	Biomass weight	Days to seed fill	Seed yield	Seed weight	Pod wall ratio
Days to flowering (DF)	-	0.44*	0.03	-0.68***	-0.07	-0.06	-0.15
Days to harvest (HM)	0.35	-	-0.01	0.36	-0.34	-0.21	0.3
Biomass weight (BMWT)	0.24	-0.03	-	-0.04	0.85***	0.33	-0.38
Days to seed fill (DSF)	-0.77***	0.33	-0.26	-	-0.2	-0.11	0.4
Seed yield (SY)	0.11	-0.52**	0.64**	-0.47*	-	0.60**	-0.58**
Seed weight (SW)	-0.24	-0.44*	0.1	-0.06	0.26	-	-0.1
Pod wall ratio (PW)	-0.53**	0.15	-0.3	0.64**	-0.28	0.05	-

\*, \*\*, \*\*\* represents significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

Supplemental Table 5. Partial Pearson correlation coefficients table that includes the 5 most significant correlations from the most drought tolerant and susceptible lines extracted from the Buster x Roza RIL population measured under non- and terminal drought stress, (NS and DS, respectively) Mitchell, NE, 2013. Drought susceptible group correlations are reported on the top and drought tolerant group on the bottom.

Trait		Days to flowering		Days to maturity		Seed yield		Seed weight		Days to seed fill	
		DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
Days to flowering (DF)	DS	-	0.73***	0.64**	0.71***	0.12	0.64**	-0.33	-0.40	0.02	0.26
	NS	0.80***	-	0.69***	0.66**	0.09	0.57**	-0.20	-0.33	0.31	-0.07
Days to maturity (HM)	DS	0.44*	0.53*	-	0.72***	-0.08	0.53*	0.01	-0.07	0.78***	0.30
	NS	0.68***	0.76***	0.81***	-	0.33	0.85***	-0.09	-0.06	0.36	0.70***
Seed yield (SY)	DS	-0.09	-0.10	-0.31	-0.17	-	0.44	-0.21	-0.11	-0.20	0.35
	NS	0.33	0.32	0.41	0.51*	0.09	-	-0.23	-0.23	0.17	0.59**
Seed weight (SW)	DS	-0.25	-0.23	-0.23	-0.28	-0.23	0.13	-	0.90***	0.28	0.06
	NS	-0.19	-0.19	-0.41	-0.22	0.06	0.26	0.81***	-	0.23	0.24
Days to seed fill (DSF)	DS	-0.02	0.18	0.89***	0.56**	-0.30	0.29	-0.13	-0.36	-	0.19
	NS	0.34	0.29	0.76***	0.84***	-0.16	0.49*	-0.22	-0.17	0.67***	-

\*, \*\*, \*\*\* represents significance at 0.05, 0.01, and 0.001 levels of probability, respectively.