Identification of Shoot Traits Related to Drought Tolerance in Common Bean Seedlings

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ABSTRACT. Drought is an important abiotic stress that limits common bean (Phaseolus vulgaris) productivity. The objective of this study was to determine shoot traits that are associated with drought tolerance in common bean seedlings. Ten common bean genotypes consisting mainly of cultivars and breeding lines from the Mesoamerican race of the Middle American gene pool were first evaluated in the greenhouse. Genotypes were grown in a shallow soil profile to limit root growth and assess shoot phenotypes under stress. Water stress was imposed by withholding watering for 24 days after planting. Traits evaluated included wilting, unifoliate senescence, stem greenness, and recovery from drought. Biomass and number of pods/plant produced after drought recovery were evaluated to quantify the effect of early drought stress on bean growth and reproduction. A second group of 94 common bean genotypes from the Bean Coordinated Agricultural Project (BeanCAP) were evaluated using the same protocol to determine the genetic variability for the same traits in a wider range of genotypes. In general, genotypes known to possess drought avoidance in the field conferred by deep rooting traits performed poorly in these conditions suggesting that the assay could be used to identify seedling shoot traits that contribute to drought tolerance. Genotypes from race Mesoamerica showed the greatest range in wilting. Genotypes that showed a slow rate of wilting maintained a green stem and had a higher recovery rate after watering. Importantly, these genotypes demonstrated a smaller reduction in biomass and pod number under stress compared with non-stress treatments. A few genotypes recovered completely despite expressing severe wilting, whereas the majority of genotypes with high wilting rates did not recover. Among the BeanCAP materials, genotypes bred in the rainfed midwestern United States showed overall better recovery than those bred under the irrigated production system used in the western United States. Because recovery from drought is a prerequisite to plant regrowth, biomass, and pod production after drought stress, factors that contribute to recovery were studied. Stem greenness was highly positively correlated to the recovery, whereas wilting was negatively correlated to the recovery. In a regression analysis, stem greenness and slow wilting were found to be important contributors to the variability of recovery. In addition, photosynthetic rate and stomatal conductance (g_s) explained variation in wilting and stem greenness. These results suggest that wilting and stem greenness might be useful traits to screen for drought tolerance in seedlings of common bean.

Common bean is an important food legume. Globally, 60% of common bean production is located in drought-prone areas where irrigation is not available or farmers cannot afford the cost associated with irrigation (Beebe, 2012). In addition, competition with major crops continues to push common beans into marginal lands that exhibit increased risk of drought stress. Common bean crops are subject to erratic rainfall at different growth stages resulting in substantial reduction in biomass and seed yield. Intermittent drought stress during the seedling stage affects overall plant growth, whereas terminal drought significantly reduces bean seed yield and seed size during the critical reproductive period (Singh, 2007). Seed quality is also negatively affected under prolonged periods of terminal drought. Because the incidence and duration of drought episodes are expected to increase with climate change, approaches to breeding common bean for drought tolerance were recently summarized by Beebe et al. (2013). Screening of breeding materials in controlled greenhouse and growth chambers could improve efficiency in breeding for drought tolerance. Importantly, screening for drought tolerance at the seedling stage permits efficient screening of large numbers of materials in reasonable time and space. Therefore, breeders need to determine which seedling stage traits could be used to identify drought-tolerant lines early in the selection processes. Drought episodes occurring early in plant development can also have a major negative impact.
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genotype, but the shoot genotype still played an important role
branching and climbing ability (Singh, 1982). When different
genotypes and their performance tested under drought stress,
effect of shoot genotype was small in comparison with root
tolerance in cowpea (*Vigna unguiculata*), drought during
vegetative phase reduces main stem height, stem diameter,
leaf area affecting the final yield (Rauf, 2008). Finally, drought at the seedling stage often delays
developmental events because of the inhibition of growth during
the water deficit period (Blum, 1996) and this can cause severe
yield loss if the recovery happens late in the season.

Plant shoots and roots act independently or synergistically to
enable plants to cope with drought stress. For instance, drought
tolerance in cowpea (*Vigna unguiculata*) depends on the shoot
when root volume is constrained and both root and shoot
factors mediated tolerance when root volume is unconstrained
(Watanabe et al., 1997). Common beans have four distinct
growth habits that are classified into four shoot types I to IV,
each with unique properties (Singh, 1982). These growth habits
can be determinate or indeterminate and differ in spatial layout of
branching and climbing ability (Singh, 1982). When different
common bean shoot genotypes were grafted onto different root
genotypes and their performance tested under drought stress,
effect of shoot genotype was small in comparison with root
genotype, but the shoot genotype still played an important role
in overall drought tolerance (White and Castillo, 1989). Com-
bining root traits with shoot-based drought tolerance mecha-
nisms in single genotypes should enhance the development of
drought-resistant beans.

Various shoot traits have been investigated in common bean
at the seedling stage (Beebe et al., 2013). These include
physiological processes such as photosynthetic efficiency, total chlorophyll content, gs, transpiration rate, leaf temperature, and leaf water potential (Castrillo et al., 2001; Dias and Brüggemann, 2010; Lizana et al., 2006; Ninou et al., 2013; Wentworth et al., 2006). However, these traits showed variable results in terms of association to drought tolerance and are not easily amenable to large-scale screening of breeding lines generated in most breeding programs. Fast and cost-effective methods to screen common bean seedlings are still needed. Various shoot criteria are used to evaluate drought tolerance at the seedling stage in other crops. For example, in wheat (*Triticum aestivum*), sunflower, and cotton (*Gossypium hirsutum*), seedling recovery after stress is used as a criterion to assess drought tolerance (Longenberger et al., 2006; Rauf, 2008; Tomar and Kumar, 2004). Tolerance to seedling leaf death and recovery has also been used as a screening trait in rice (DeDatta et al., 1988; Mitchell et al., 1998). In legumes, different shoots traits are used to select drought-tolerant genotypes. For instance, maintenance of green stem was shown to be an important criterion for seedling stage drought tolerance in cowpea (Muchero et al., 2008), whereas a slow wilting trait is associated with drought tolerance in soybean (Sadok et al., 2012). In cowpea, the “wooden box” seedling screening method was developed for phenotyping cowpea for drought tolerance (Singh et al., 1999). This method provides the advantage of limiting root growth to assess the shoot drought tolerance mechanisms. This technique has been adapted to screen various other crops including cotton, wheat, and watermelon (*Citrus lanatus*) (Longenberger et al., 2006; Tomar and Kumar, 2004; Zhang et al., 2011). However, one of the limitations of the wooden box method is that seedlings grown in the same box will compete with each other for limited moisture present. Evaluation of seedlings on a single-plant basis in small pots where root expansion is limited would provide the benefits of the wooden box method while eliminating competition among different seedlings.

The primary objective of this study was to develop a green-
house seedling screening test for shoot drought tolerance. This
involved 1) evaluating the response of common bean seedlings
to early drought stress when grown under root-limiting condi-
tions; 2) determining which shoot traits would be most suitable
to screen for drought tolerance under these conditions; and
3) determining the relative drought response of a larger group of
common bean genotypes previously evaluated for drought
avoidance in the field.

**Materials and Methods**

**Plant material.** In the first set of experiments, 10 common
bean genotypes were used based on previous information of
their response to drought in the field. Nine genotypes from the
Mesoamerican race of the Middle American gene pool (Singh
et al., 1991) were chosen to maintain genetic similarity among
genotypes so that drought response can be monitored easier and
not be confounded by differences in phenotype and growth habit.
These included small-seeded black bean cultivars and breeding
lines ‘Blackhawk’, ‘Jaguar’, ‘Phantom’, ‘Zorro’, L88-63, and
B98311, and red-seeded lines, TARS-SR05 and RAB 651. One
cultivar, Concepción, from the Andean gene pool was used in the
earlier experiments and was later replaced by the otebo cultivar,
Fuji, that was previously shown to be very susceptible to drought
under field conditions.

‘Zorro’ is a high-yielding, widely grown black bean cultivar
that has shown good field tolerance to drought tracing back to its
B98311 drought-tolerant grandparent (Kelly et al., 2009a).
Black bean cultivars Jaguar (Kelly et al., 2001), Phantom (Kelly
et al., 2000), and Blackhawk (Ghaderi et al., 1990) are similar to
‘Zorro’ in agronomic traits and exhibit the same upright type II
indeterminate short vine growth habit (Singh, 1982) but are not
recognized as being drought-tolerant. Michigan State University
(MSU) black bean breeding line B98311 is recognized to possess
high levels of drought tolerance conferred by a deep vigorous
taproot (Frahm et al., 2004). MSU black bean breeding line
L88-63 was developed from the cross of B98311 × TLP19 and
was selected as a drought-resistant line in field evaluations in
Honduras, Mexico, and Michigan (Frahm et al., 2004). The
TLP19 parent from the International Center for Tropical Agri-
culture (CIAT) breeding program is recognized as a shallow
rooting genotype selected to more efficiently uptake phosphorus
(Beebe et al., 2013). TARS-SR05 is a small red breeding line that
combines multiple root rot disease resistance with tolerance to
low soil fertility and is recognized as being stress-tolerant (Smith
et al., 2007). RAB 651 is a small, red-seeded, indeterminate
Mesoamerican race breeding line from CIAT that had not pre-
viously been selected for drought tolerance during its develop-
ment but was recognized to express a high level of drought

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tolerance when evaluated as an advanced line. ‘Concepción’ is a large-seeded red–purple mottled cultivar with determinate type I growth habit (Singh, 1982) that was the drought-susceptible parent in a mapping population. It was dropped from the experiment as a result of a lack of adaptation and replaced by the ‘Fuji’ otebo cultivar. ‘Fuji’ is a specialty white bean that possesses a determinate type I growth habit and is recognized as being sensitive to drought stress (Kelly et al., 2009b).

In the second set of experiments, 96 dry bean genotypes from the Bean Coordinated Agricultural Project (BeanCAP, 2011) were selected to gain greater insight in drought tolerance response in common bean. The genotypes were previously selected by a group of breeders in the United States and Puerto Rico for more extensive testing as part of the BeanCAP research projects being conducted in different production areas. The selections represent old and contemporary dry bean cultivars that differ in their response to drought in the field (BeanCAP, 2011). The study was limited to common bean genotypes from the Middle American gene pool and the experiment included 15 black, eight navy, two tan, and one criocarta genotypes from race Mesoamerica; 16 great northern (GN) and 34 pinto genotypes from race Durango; and 10 pink and eight small red from race Jalisco (Singh et al., 1991). All race Mesoamerica genotypes and contemporary race Durango and Jalisco genotypes possess the prostrate type III growth habit, whereas the older race Durango and Jalisco genotypes possess the prostrate type III indeterminate growth habit (Singh, 1982). Information on agronomic characteristics and release date of the older cultivars was previously published by Sutton and Coyne (2002). ‘Jaguar’, B98311, and ‘Fuji’ served as checks based on their reaction to drought stress in the previous study.

Greenhouse experiments. Experiments were modified from the greenhouse protocol used to study drought reaction in cowpea (Muchero et al., 2008). Four experiments were conducted in the greenhouse during Winter and Spring 2009 and 2010 on the first group of 10 genotypes. Summer seasons were avoided to limit confounding factors from excessive heat in the greenhouse and the middle bench was used to avoid microclimates associated with ventilation and cooling systems along the greenhouse perimeter. Greenhouse temperatures were kept at 22 to 25 °C and a 16-h photoperiod.

Each experiment was conducted in a completely randomized design with five replications in 1200-cm³ plastic pots. In addition, an equal number of replicates was planted and used as a control maintained under irrigation. In the first two experiments, pots were filled with 200 g of a mixture (3:1 v/v) of Baccto potting mix (Michigan Peat Co., Houston, TX) and coarse perlite. In the last two experiments, 200 g of perlite (Suremix; Michigan Grower Products, Galesburg, MI) were used as the potting medium. Small pots and the limited amount of soil were used to limit root growth so that drought-tolerant shoot phenotypes could be assessed. All pots were watered to field capacity and excess water allowed to drain for 4 h before planting. Each genotype was planted with three seeds visually selected for size and quality. A second and last water application was made after planting in drought-stressed pots, whereas the controls continued to be irrigated to field capacity on 2-d intervals. Seedlings were thinned to one plant at 7 d post-planting. Drought stress was imposed very early to avoid confounding factors associated with plant size and vigor specific to each genotype. In drought stress experiments, individual plants were scored for wilting, unifolate senescence, and maintenance of stem greenness. Wilting was scored at 18, 21, and 24 d after planting. At 24 d, watering was resumed at 2-d intervals for 14 d when genotypes were scored for the recovery. All the pots in stress and non-stress conditions were fertilized at 25 d after planting with 20 mL of 20N–8.8P–16.6K water-soluble fertilizer (Peters Professional 20-20-20; Scotts, Marysville, OH). All experiments continued to be watered to field capacity every 2 d until midpod filling where the number of pods produced/plant was counted and dry biomass determined. Additional measurements of gs and photosynthetic rates were measured at 18 d after planting in the third experiment. Stomatal conductance and photosynthetic rates were measured at ambient light intensities and a CO₂ reference concentration of 380 μmol·mol⁻¹ using a portable photosynthesis system (LI-6400XT; LI-COR Biosciences, Lincoln, NE).

Growth chamber experiments. The second set of experiments with the 96 genotypes was conducted in nine random groups as a result of the limited space in the growth chamber and the large number of genotypes. Each group consisted of 10 genotypes plus three checks (‘Jaguar’, ‘Fuji’, and B98311) except for the final group, which had only six genotypes and checks. Five replicates of each genotype were evaluated within each of the nine groups. Five blocks were created and genotypes were randomly placed in each of the five blocks. These experiments were conducted during the winter from Aug. 2011 through Apr. 2012. Growing conditions in the growth chamber were set at 26 °C, 16-h day with 8-h night. Each genotype was planted in 1200-cm³ pots in 150 g of potting soil at a seeding rate of three seeds per pot. Pots were watered to field capacity and allowed to drain at planting. After germination, plants were thinned to one seedling per pot. Drought stress was induced by discontinuing watering until data were collected ≈2 weeks after planting. No additional water was provided between planting and data collection on wilting, greenness, and unifolate senescence. After the collection of these data at ≈21 d, watering was resumed every other day for 2 weeks. At the end of the 2-week recovery period, genotypes were rated for recovery.

Variables scoring. Wilting was scored on a scale of 0 to 5 with 0 being no sign of wilting and 5 being completely wilted. Unifoliate senescing was assessed as the number of completely senescing unifoliates per pot taken when the most rapidly senescing genotypes had all the unifoliates dried. Stem greenness was scored on a scale of 0 to 5 with 0 being completely yellow and 5 being completely green. Recovery was rated on a scale of 0 to 1 (a 0 score was given to the genotype that did not recover, 0.5 if the recovery occurred at the basal node, and 1 if the recovery was from the top meristem).

Statistical analyses. All variables were analyzed using SAS (Version 9.3; SAS Institute, Cary, NC). Wilting was analyzed as repeated measurements using the generalized linear mixed model (GLIMMIX) procedure. Unifoliate senescence and recovery were analyzed as generalized linear models using the probit link function in the GLIMMIX procedure (Littell et al., 2006). Stem greenness, recovery number of pods, recovery fresh and dry biomass, gs, and photosynthetic rates were analyzed as mixed models using the MIXED procedure. Mean comparisons were performed using the least significant difference test with a significance level at α = 0.05. Phenotypic correlation and regression analyses were performed with PROC CORR and PROC REG.
Table 1. Mean scores for wilting at 18, 21, and 24 d after planting, unifoliate senescence, stem greenness, and recovery of 10 common bean genotypes evaluated under drought stress at the seedling stage.*

| Genotype     | Wilting (0 to 5 scale)** | Unifoliate senescence (0 to 2 scale)** | Stem greenness (0 to 5 scale)** | Recovery (0 to 1 scale)** |
|--------------|--------------------------|----------------------------------------|-------------------------------|--------------------------|
|              | 18 d                     | 21 d                                   | 24 d                          |                           |
| B98311       | 1.33 a*                  | 3.10 a                                 | 4.39 a                        | 0.87 a                   | 2.06 b                   | 0.60 b |
| Blackhawk    | 1.05 a                   | 2.55 a                                 | 4.20 a                        | 0.84 a                   | 2.55 b                   | 0.65 b |
| Concepción   | 0.61 ab                  | 2.61 a                                 | 4.91 a                        | 0.76 ab                  | 3.10 b                   | 0.59 b |
| Fuji         | 0.89 ab                  | 2.49 a                                 | 4.19 a                        | 0.73 ab                  | 3.50 a                   | 0.48 b |
| Jaguar       | 0.50 b                   | 0.60 b                                 | 2.65 b                        | 0.42 b                   | 3.80 a                   | 0.97 a |
| L88-63       | 1.40 a                   | 2.75 a                                 | 4.30 a                        | 0.96 a                   | 2.40 b                   | 0.60 b |
| Phantom      | 0.89 ab                  | 1.41 b                                 | 3.57 ab                       | 0.50 b                   | 3.50 a                   | 0.82 ab |
| RAB651       | 0.70 ab                  | 2.35 ab                                | 4.25 a                        | 0.87 a                   | 2.50 b                   | 0.68 b |
| TARS-SR05    | 1.25 a                   | 2.25 ab                                | 3.75 ab                       | 0.76 ab                  | 3.90 a                   | 0.95 a |
| Zorro        | 0.86 ab                  | 1.97 b                                 | 4.25 a                        | 0.73 ab                  | 2.70 b                   | 0.74 b |

*Each point represents an average of four experiments, each with five plants for each genotype.

**Wilting was scored on a scale of 0 to 5 with 0 being no sign of wilting and 5 being completely wilted.

†Unifoliate senescence was recorded as the number of senesced leaves (zero to two) when the most rapidly senescing genotype had dried unifoliates.

‡Stem greenness scored on a scale of 0 to 5 with 0 being yellow and 5 being totally green.

§Recovery scored on a scale of 0 to 1 with 0 being no recovery, 0.5 when the recovery occurred from the basal meristem, and 1 when recovery occurred from the apical meristem.

∥Means with the same letter are not significantly different.

Results

Wilting. Wilting increased over time during the stress period and average wilting scores were 1.05, 2.21, and 4.0, respectively, at Days 18, 21, and 24 after planting (Table 1). Phenotypic correlation coefficients among the measurement times were high between adjacent measurement times. Specifically, the correlation coefficients were 0.73, 0.70, and 0.87, respectively, between 18 and 21, 18 and 24, and 21 and 24 d. Significant differences in wilting existed among genotypes (P ≤ 0.0016) and measurement times (P ≤ 0.0001). There were also interactions between genotype and measurement times (P ≤ 0.03). Small-seeded genotypes ‘Jaguar’, ‘Phantom’, RAB651, ‘Zorro’, and ‘Fuji’ did not show any significant wilting at Day 18. However, all the genotypes wilted rapidly after Day 18 except ‘Jaguar’, which had a significantly lower wilting score than the other genotypes. RAB651, ‘Zorro’, and ‘Fuji’ had a low wilting score at Day 18, but the wilting rate from Days 21 to 24 was so rapid that no differences were observed with the faster wilting genotypes at Day 24 (data not shown). Wilting showed a significant positive correlation with unifoliate senescence, whereas it was negatively correlated with stem greenness, recovery, recovery number of pods, recovery fresh, and dry biomass (Table 2).

Unifoliate senescence. There was a significant difference (P ≤ 0.015) for unifoliate senescence between genotypes. ‘Jaguar’ and ‘Phantom’ had lower average score for unifoliate senescence than the rest of genotypes, whereas L88-63, B98311, and RAB651 had the highest unifoliate senescence scores (Table 1). Unifoliate senescence was negatively correlated to all variables except wilting.

Stem greenness. Significant differences (P ≤ 0.0001) for stem greenness were observed between genotypes. Genotypes TARS-SR05, ‘Jaguar’, ‘Fuji’, and ‘Phantom’ had high scores for stem greenness, whereas B98311, RAB651, and L88-63 had the lowest scores (Table 1). The stem greenness score of B98311 did not differ significantly from the score of ‘Blackhawk’, L88-63, RAB651, and ‘Zorro’. Stem greenness was negatively correlated to wilting and unifoliate senescence but was positively correlated with recovery, pod number, fresh biomass, and dry biomass (Table 2).

Recovery from drought. Significant differences (P ≤ 0.015) for recovery were observed among genotypes. Genotypes were classified into two groups (Table 1). The first group comprised genotypes ‘Jaguar’, TARS-SR05, and ‘Phantom’ with recovery rates of 0.97, 0.95, and 0.82, respectively. The rest of the genotypes were grouped into the second category with low recovery rates. Recovery was negatively correlated to wilting and unifoliate senescence, whereas it was positively correlated to stem greenness, pod number, and biomass (Table 2). Because recovery from drought stress is important for subsequent plant growth and reproduction, regression analysis of recovery on wilting, unifoliate senescence, and stem greenness

Table 2. Spearman correlation coefficients among wilting, unifoliate senescence, stem greenness, recovery, pod number, and dry biomass for 10 common bean genotypes evaluated in the greenhouse under drought stress at the seedling stage.*

|                  | Wilting      | Unifoliate senescence | Stem greenness | Recovery | Pod number |
|------------------|-------------|-----------------------|---------------|----------|------------|
| Unifoliate senescence | 0.62***     | -0.68***              | -0.52***      | 0.65***  | 0.72***    |
| Stem greenness    | -0.68***    | -0.42***              | 0.54***       | 0.79***  | 0.83***    |
| Recovery          | -0.63***    | -0.44***              | 0.57***       | 0.79***  | 0.83***    |
| Pod number        | -0.72***    | -0.57***              | 0.57***       |          |            |
| Dry biomass       |             |                       |               |          |            |

*Seedlings were grown in a shallow soil profile in small pots.

### Significant at P ≤ 0.0001.
was used to predict variables that are likely to influence seedling recovery. As results, only stem greenness and wilting fitted in the model that explained 48% of variation in recovery. Stem greenness alone accounted for 43% of the variation in recovery.

**NUMBER OF PODS.** The analysis of variance for pod number showed significant differences between stress and non-stressed treatments \((P \leq 0.0001)\) and among genotypes \((P \leq 0.0001)\). Water treatment \(\times\) genotype interaction was not significant. The stressed treatment had an average of 3.3 pods, whereas the non-stress treatment had an average 7.3 pods. Drought stress significantly reduced the number of pods for all genotypes. Percentage pod number reduction was the highest in ‘Fuji’ (99%), whereas it was lowest for ‘Jaguar’ (24%) (Tables 3 and 4). ‘Jaguar’ and ‘Phantom’ did not show significant losses in pod number as a result of drought effects.

**BIOMASS.** Significant differences \((P \leq 0.0001)\) were observed for plant biomass between stress and non-stress treatments. The average dry biomass in stressed treatments was 4.2 g, whereas the average biomass was 10.2 g in the non-stress treatment. In addition, significant differences were observed for genotype \((P \leq 0.0008)\) and genotype \(\times\) water treatment interactions \((P \leq 0.0009)\). Under non-stress conditions, all genotypes had equivalent amounts of biomass. However, under stress conditions, genotypes ‘Jaguar’, ‘Phantom’, and TARS-SR05 (18% to 36%) had lower reductions of dry biomass than other genotypes (68% to 87%) (Tables 3 and 4).

**PHOTOSYNTHESIS.** Photosynthetic rates were evaluated for Expt. 3 at Day 18. At this time, there were significant differences between water treatments \((P \leq 0.0001)\). However, there was no significant difference among genotypes within each water treatment or genotype \(\times\) water treatment interactions. At ambient light levels in the greenhouse, in the stress treatment, the average photosynthetic rate was 3.2 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\) whereas the rate was 11.5 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\) in the non-stress treatment. Although no significant differences among genotypes were observed, RAB651 and TARS-SR05 appeared to have lower photosynthetic rates under drought stress. Certain genotypes such as ‘Jaguar’, TARS-SR05, and ‘Phantom’ did not have high photosynthetic rates under drought stress. Certain genotypes such as ‘Jaguar’, TARS-SR05, and ‘Phantom’ did not have high photosynthetic rates under non-stress, but they were able to maintain relatively higher photosynthetic rates under drought conditions (Tables 4 and 5).

**STOMATAL CONDUCTANCE.** Stomatal conductance was evaluated at the same time as photosynthetic activity. Photosynthesis and \(g_s\) were highly correlated to each other \(r = 0.98** \) and 0.91** \((P \leq 0.01)\) for stress and non-stress, respectively. Although the analysis of variance did not show any difference between genotypes or a genotype \(\times\) water treatment interaction, there was a significant difference between stress and non-stress treatments \((P \leq 0.003)\). The average \(g_s\) was 0.0205 mmol-m\(^{-2}\)-s\(^{-1}\) in stress treatments, whereas it was 10-fold greater (0.225 mmol-m\(^{-2}\)-s\(^{-1}\)) in non-stress treatments. Genotypes RAB651 and B98311 had lower \(g_s\) in stress treatments. However, under non-stress, genotypes B98311, ‘Fuji’, and ‘Zorro’ had relatively high \(g_s\) compared with the other genotypes (Tables 4 and 5).

**GAS EXCHANGE IN RELATION TO WILTING, STEM GREENNESS, AND RECOVERY.** When \(g_s\) and photosynthetic rate were regressed on wilting (Fig. 1), stem greenness (Fig. 2), and recovery (Fig. 3), there was a strong negative relationship between wilting and photosynthesis and \(g_s\) \(r = –0.81**)\). Variation in stem greenness variation was positively associated with photosynthetic rate and \(g_s\) \(r = 0.94** \) and 0.91**, respectively. Similarly, recovery was

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**Table 3. Analysis of variance for number of pods, dry biomass along with percent reductions for 10 common bean genotypes evaluated in the greenhouse under drought stress at the seedling stage.**

| Genotype | Pods (no.) | Dry biomass (g) |
|----------|------------|----------------|
| B98311   | Non-stress | 7.73           | 9.68           |
|          | Stress     | 2.80           | 2.14           |
|          | Reduction (%) | 64            | 78             |
| Blackhawk| Non-stress | 7.53           | 11.34          |
|          | Stress     | 3.66           | 5.44           |
|          | Reduction (%) | 51            | 52             |
| Concepción| Non-stress | 2.78           | 12.34          |
|          | Stress     | 0.90           | 2.44           |
|          | Reduction (%) | 68            | 80             |
| Fuji     | Non-stress | 8.85           | 9.40           |
|          | Stress     | 0.05           | 1.18           |
|          | Reduction (%) | 99            | 87             |
| Jaguar   | Non-stress | 8.73           | 10.39          |
|          | Stress     | 6.66           | 7.84           |
|          | Reduction (%) | 24            | 25             |
| L88-63   | Non-stress | 6.87           | 8.93           |
|          | Stress     | 5.30           | 2.91           |
|          | Reduction (%) | 53            | 67             |
| Phantom  | Non-stress | 8.38           | 10.11          |
|          | Stress     | 5.50           | 6.50           |
|          | Reduction (%) | 35            | 36             |
| RAB651   | Non-stress | 6.93           | 9.66           |
|          | Stress     | 2.06           | 2.49           |
|          | Reduction (%) | 70            | 74             |
| TARS-SR05| Non-stress | 9.00           | 9.34           |
|          | Stress     | 5.10           | 7.63           |
|          | Reduction (%) | 43            | 18             |
| Zorro    | Non-stress | 6.20           | 9.94           |
|          | Stress     | 3.20           | 3.20           |
|          | Reduction (%) | 49            | 68             |

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**Table 4. Analysis of variance for number of pods, dry biomass along with percent reductions for 10 common bean genotypes evaluated in the greenhouse under drought stress at the seedling stage.**

| Source                  | df | Mean square  | F value  | P value |
|-------------------------|----|--------------|----------|---------|
| Genotype                | 8  | 1454.70      | 8.59     | 0.0001  |
| Water treatment         | 8  | 1945.75      | 11.12    | 0.0001  |
| Water treatment \(\times\) genotype | 8  | 14.78        | 1.59     | 0.1205  |
| Genotype                | 8  | 28.15        | 3.21     | 0.0008  |
| Water treatment         | 8  | 1454.70      | 8.59     | 0.0001  |
| Water treatment \(\times\) genotype | 8  | 24.72        | 3.64     | 0.0009  |
| Genotype                | 8  | 0.005        | 2.17     | 0.0550  |
| Water treatment         | 8  | 0.532        | 238.89   | <0.0001 |
| Water treatment \(\times\) genotype | 8  | 0.005        | 2.21     | 0.0511  |
| Genotype                | 8  | 6.174        | 1.36     | 0.2492  |
| Water treatment         | 8  | 931.6        | 205.11   | <0.0001 |
| Water treatment \(\times\) genotype | 8  | 5.824        | 1.28     | 0.2852  |

\(^a\)Stomatal conductance and photosynthesis were measured on only nine genotypes from the Middle American gene pool (‘Concepción’ was no longer in use).
Table 5. Mean values for photosynthetic rates and stomatal conductance ($g_s$) for nine common bean genotypes evaluated in the greenhouse under light conditions and drought stress and non-stress treatments at the seedling stage.

| Genotype  | Photosynthetic rate ($\mu$mol m$^{-2}$ s$^{-1}$) | $g_s$ (mmol m$^{-2}$ s$^{-1}$) |
|-----------|-----------------------------------------------|--------------------------------|
|           | Stress | Non-stress | Stress | Non-stress |
| B98311    | 2.20   | 13.42      | 0.01   | 0.27       |
| Blackhawk | 3.26   | 13.22      | 0.02   | 0.23       |
| Fuji      | 3.91   | 13.32      | 0.02   | 0.29       |
| Jaguar    | 4.27   | 10.88      | 0.03   | 0.20       |
| L88-63    | 2.66   | 9.96       | 0.02   | 0.19       |
| Phantom   | 3.59   | 11.01      | 0.03   | 0.24       |
| RAB651    | 1.94   | 8.79       | 0.01   | 0.15       |
| TARS-SR05 | 4.04   | 9.63       | 0.03   | 0.13       |
| Zorro     | 2.69   | 13.10      | 0.02   | 0.27       |

*Each point represents an average of four experiments, each with five plants for each genotype.

photosynthetically correlated with photosynthesis and $g_s$ [$r = 0.73^*$ and $0.75^* (P < 0.05)$, respectively].

**BEANCAP SCREENING.** Partial data on wilting, unifoliate senescence, stem greenness, and recovery of the 96 BEANCAP entries are presented in Tables 6–8 separately by race because initial differences in seed size may influence the rating taken in the seedling stage and invalidate comparisons among genotypes contrasting in seed size. Wilting scores of 96 entries ranged from a low of 1.6 for ‘Eclipse’ black bean to values as high as 4.4 for ‘Domino black’, ‘Matterhorn GN’, and ‘Common Red Mexican’ genotypes. The greatest range in wilting scores appeared in the Mesoamerican race with both extremes observed in black beans (Table 6). The ‘Jaguar’ control had a higher wilting value than expected based on earlier experiments, but the lowest overall values were observed in black beans. Two current commercial cultivars, Shania and Zorro, had low scores (1.8 to 2.6), whereas the older cultivars (Sutton and Coyne, 2002) had values greater than 4.0. A similar range from 1.8 to 4.4 was observed in the GN class but a smaller range in wilting was observed among pinto, pink, and small red genotypes (Tables 7 and 8). The Durango and Jalisco genotypes included both traditional type III cultivars bred under irrigation in the western United States and contemporary type II cultivars bred under rainfed conditions in the midwestern United States. Unifoliate senescence showed a similar range across all races with more striking differences in races Durango and Jalisco. This trait had an unacceptable high variability (cv = 72%). The largest contrasts in stem greenness were observed in races of Mesoamerican genotypes, but stem pigmentation may have played a confounding role in these given the contrasting seed color types between black and navy beans (Table 6). Stem greenness ranged from 2.4 to 4.2 in the Durango race and from 2.0 to 3.4 in the Jalisco race where stem pigmentation associated with seedcoat color may have influenced the ratings. Recovery ratings illustrated the ability of some lines to recover from early severe stress, whereas others completely succumbed to the stress. Those genotypes with values of 1.0 are of interest because they might have a mechanism to help sustain them through a period of severe drought. A number of well-recognized sources of drought tolerance scored very poorly in this category (i.e., B98311 (0.5), SEA 10 (0.0), and ‘Common Red Mexican’ (0.0)], suggesting that the drought tolerance observed in the field is largely dependent on the root system of these genotypes.

Discussion

Different wilting behaviors among the 10 common bean genotypes were observed during the progression of wilting under greenhouse conditions. ‘Phantom’ and ‘Jaguar’ maintained lower wilting values compared with other genotypes, whereas ‘Concepción’, B98311, and L88-63 had higher wilting values at all observation dates. Wilting response of ‘Jaguar’, ‘Phantom’, and TARS-SR05 increased at a slower rate over time compared with other genotypes (data not shown). Interestingly, TARS-SR05 increased at a slower rate over time compared with other genotypes (data not shown).

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96 BeanCAP entries. Black bean genotypes ‘Eclipse’, ‘Shania’, ‘Zorro’, and ‘Avalanche’ navy had low wilting scores and all had high recovery values (Table 6). Likewise in GN class ‘Gemini’ with a low wilting score had a recovery of 1.0 compared with ‘Beryl’ with a zero recovery and high wilting score of 4.4 (Table 7). In contrast, a few genotypes such as ‘Matterhorn’ GN and ‘Kodiak’ pinto showed high wilting scores but high recovery rates (Table 7). Interestingly, ‘Matterhorn’ has been recognized to possess moderate levels of drought resistance (Singh, 2007; Urrae et al., 2009). Among cultivars from the Jalisco race, Sedona and Merlot had intermediate wilting scores and high recovery rates (Table 8). Those genotypes with lower wilting scores may have a mechanism to slow their transpiration rate and not deplete their soil moisture reserves as quickly as genotypes that have a high wilting score as was observed in soybean (Ries et al., 2012). Because the slow wilting trait in these experiments was not associated with deep-water extraction because all genotypes were planted in shallow soil profiles, it is inferred that this trait is associated with mechanisms in the shoot.

In soybeans, slow wilting has been identified as a beneficial trait under drought. This condition involves a restricted transpiration rate under increasing vapor pressure deficit levels (Fletcher et al., 2007; Sadok et al., 2012). This phenotype was initially identified in soybean PIs (PI471938 and PI416937) (Hufstetler et al., 2007; King et al., 2009; Sloane et al., 1990). The basis for this trait is attributed to limited hydraulic conductance between the leaf xylem and the guard cells (Sinclair et al., 2008). Furthermore, studies using aquaporin inhibitors have demonstrated their involvement in this trait (Sadok and Sinclair, 2010a, 2010b). Wilting is a trait expressed by plants that have passed the leaf water potential at turgor loss point. Leaf turgor loss point has been used to quantify plant drought tolerance levels (Bartlett et al., 2012), whereas visual assessment of wilting is commonly used as a measure of leaf water potential and seedling drought survival in trees (Engelbrecht et al., 2007). Plants that wilt slowly tend to maintain gs, hydraulic conduc-
tance, photosynthesis, and growth at lower soil water potentials, which is especially important when drought occurs early during the growing season (Bartlett et al., 2012). Despite the absence of genotypic differences for photosynthetic rate and gs under stress conditions, ‘Jaguar’, TARS-SR05, and ‘Phantom’ had relatively higher values for these two parameters under stress and relatively lower values under non-stress conditions compared with the
other genotypes. These results suggest that these genotypes might have saved water, which later allowed them to maintain photosynthesis under more severe water deficits. In other grain legumes such as soybean, peanut (Arachis hypogaea), chickpea (Cicer arietinum), and cowpea, maintaining maximum transpiration rates under relatively lower vapor pressure deficits has been recognized as a soil water-saving strategy in tolerant genotypes (Belko et al., 2012; Devi et al., 2010; Sinclair et al., 2008; Zaman-Allah et al., 2011). In contrast, an early study of genotypes (Belko et al., 2012; Devi et al., 2010; Sinclair et al., 2008) showed that these two variables explained more stem greenness variability than wilting ($R^2 = 0.58* and R^2 = 0.89***$). These results suggest that bean genotypes need to maintain a viable green stem to be able to recover from drought and resume growth. However, ‘Fuji’ was an exception to this trend because it usually maintained a green stem. At the resumption of irrigation, instead of recovering, ‘Fuji’ seedlings continued to dry-down from the top and subsequently died. These results suggest that when exposed to a severe drought in the seedling stage, seedlings of ‘Fuji’ were unable to recover from drought stress relief, probably because of its determinate growth habit. Under severe drought stress of 2010 in the field in Michigan, ‘Fuji’ showed a growth pattern of producing excessive vegetative tissue instead of setting flowers and pods because small pods aborted as a result of stress. The confounding effect of stem pigmentation on the rating of stem greenness in color-contrasting seed types may limit its usefulness.
as a selectable characteristic across a broad range of bean genotypes.

Drought-tolerant genotypes B98311 and L88-63 not only had lower stem greenness and recovery rates, but also performed poorly in general in all experiments (Table 1). B98311 is a parent of L88-63 and X00822 one of the parents of ‘Zorro’. B98311 and L88-63 were selected based on root length under terminal drought (Frahm et al., 2004; Henry et al., 2010) and ‘Zorro’ performs relatively well under field drought stress. The fact that B98311 and L88-63 behaved poorly and similarly in constrained root growth conditions highlights the complexity associated with breeding for drought tolerance and strengthens the importance of knowing the target environment as well as the growth stage at which the crop is likely to encounter drought. Other genotypes such as SEA 10 and BAT 477 known as a source of drought tolerance in the field (Beebe et al., 2013) performed very poorly in these tests. Because root growth was restricted, drought tolerance in these genotypes may reside in root characteristics that were not expressed in this study. In addition, these results highlight the importance of combining root and shoot drought traits to achieve stability in drought tolerance over a range of soil conditions. The general prediction of the recovery based on high wilting scores and stem greenness in different genotypes suggests that these criteria can be used as a screening proxy for drought tolerance expressed at early stages of shoot development in common bean. An interesting trend emerged in that those genotypes such as UI-239, UI-537, ‘Montrose’, and ‘Bill Z’ developed under irrigation in the semiarid western United States tended to have lower recovery ratings than genotypes such as ‘Matterhorn’, ‘Sedona’, and ‘Merlot’ developed in programs located in the rainfed midwestern United States. The major exception was the two pinto genotypes ‘San Juan’ and ‘Fischer’ that were developed in southwestern Colorado for dryland production. They showed high recovery rates as compared with ‘Croissant’ pinto developed under irrigation for production in the rain shadow on the eastern slope of the Rocky Mountains. Interestingly, stem greenness was identified to be an important seedling trait associated with drought tolerance in cowpea, which is the closest relative of common bean (Muchero et al., 2008). Cowpea is recognized as the most drought-tolerant legume species (Hall, 2012), whereas common bean is among the least tolerant. Sharing the same trait for drought tolerance at the pod number and seedling stage suggests that this trait might be under the same genetic control in these two legume species. Common bean is generally grown in wetter environments than cowpea and rarely experiences drought conditions affecting stem greenness that occur during the bean growth season. Because wilting was negatively correlated to stem greenness and recovery \( r = -0.82^{***} \) and \(-0.81^{***}\), respectively, wilting might be a more practical trait to measure than stem greenness in common bean. For drought tolerance screening purposes, combining delayed wilting and stem greenness would provide more

Table 7. Mean scores for wilting, unifoliate senescence, stem greenness after 21 d without water, and recovery of 50 Durango common bean genotypes with specific scores for 19 representative genotypes evaluated under drought stress at the seedling stage.\(^{a}\)

| Genotype       | Seed type\(^{a}\) | Growth habit\(^{a}\) | Wilting \(^{a}\) (0 to 5 scale) | Unifoliate senescence \(^{a}\) (0 to 2 scale) | Stem greenness \(^{a}\) (0.5 scale) | Recovery \(^{a}\) (0 to 1 scale) |
|----------------|-------------------|----------------------|-------------------------------|---------------------------------|---------------------------------|-------------------------------|
| Beryl R        | GN                | III                  | 4.4                           | 2.0                             | 4.0                             | 0.0                           |
| Gemini         | GN                | III                  | 1.8                           | 0.0                             | 4.2                             | 1.0                           |
| GN9-1          | GN                | III                  | 4.6                           | 2.0                             | 3.4                             | 0.0                           |
| Marquis        | GN                | III                  | 4.8                           | 2.0                             | 3.8                             | 0.0                           |
| Matterhorn     | GN                | II                   | 4.4                           | 0.0                             | 4.0                             | 1.0                           |
| Weihing        | GN                | II                   | 4.4                           | 1.2                             | 3.8                             | 0.4                           |
| Bill Z         | Pinto             | III                  | 3.6                           | 2.0                             | 3.0                             | 0.0                           |
| Common Pinto   | Pinto             | III                  | 3.8                           | 0.4                             | 2.4                             | 1.0                           |
| Croissant      | Pinto             | II                   | 3.8                           | 2.0                             | 2.4                             | 0.0                           |
| Fisher         | Pinto             | III                  | 4.0                           | 0.0                             | 2.4                             | 1.0                           |
| Kodiak         | Pinto             | II                   | 4.0                           | 0.0                             | 4.2                             | 1.0                           |
| La Paz         | Pinto             | II                   | 2.4                           | 0.0                             | 3.4                             | 1.0                           |
| Lariet         | Pinto             | II                   | 3.6                           | 1.6                             | 3.0                             | 0.4                           |
| Montrose       | Pinto             | III                  | 3.8                           | 2.0                             | 3.0                             | 0.0                           |
| Othello        | Pinto             | III                  | 3.6                           | 1.4                             | 2.2                             | 0.7                           |
| San Juan       | Pinto             | II                   | 2.6                           | 0.0                             | 3.8                             | 1.0                           |
| Santa Fe       | Pinto             | II                   | 2.4                           | 0.4                             | 3.6                             | 0.8                           |
| Sierra         | Pinto             | II                   | 4.8                           | 0.4                             | 4.0                             | 0.8                           |
| Stampede       | Pinto             | II                   | 4.4                           | 2.0                             | 3.0                             | 0.1                           |
| Mean\(^{a}\)   |                   |                      | 3.5                           | 1.0                             | 3.3                             | 0.6                           |
| LSD\(_{0.05}\) |                   |                      | 1.2                           | 0.8                             | 0.9                             | 0.4                           |
| CV (%)\(^{a}\) |                   |                      | 26                            | 72                              | 24                              | 55                            |

\(^{a}\)Each point represents an average of four experiments, each with five plants for each genotype.

\(^{a}\)GN = Great Northern.

\(^{a}\)Growth habit types II and III described by Singh (1982).

\(^{a}\)0 = no sign of wilting, 5 = completely wilted.

\(^{a}\)Number of senesced leaves (zero to two) when the most rapidly senescing genotype had dried unifoliates.

\(^{a}\)0 = yellow, 5 = totally green.

\(^{a}\)0 = no recovery, 0.5 = recovery from the basal meristem, 1 recovery from the apical meristem.

\(^{a}\)Values derived from all 50 genotypes evaluated.
useful information about genotypic differences in terms of drought tolerance. Determining the usefulness of these two traits as predictors of drought tolerance in the field will be difficult because root traits play a major confounding role in all field studies. Biomass was significantly reduced by drought. These results suggest that severe drought in the seedling stage might have deleterious effects on yield through reduced biomass accumulation. This is important because the plants may not have sufficient time to invest in biomass production after an extended period of drought. Instead they might directly enter the reproductive period without sufficient biomass reserves for optimum yield. This could be disadvantageous for common bean genotypes with a determinate growth habit, which may not be able to initiate a second flush of pod setting when the vegetative growth period has passed.

Conclusions

Screening protocols in controlled environments are needed to select drought-tolerant bean cultivars. These screening methods need to be fast and effective to be integrated into breeding programs. Seedling drought tolerance screening methods would allow for the screening of numerous lines in a relatively short period at low cost when compared with field trials under contrasting water treatments commonly used to compare yields. This study was conducted to determine shoot traits that are associated with drought tolerance at an early development stage in common bean. The study was conducted in the greenhouse using small pots and a limited amount of soil to constrain root growth. Four shoot traits, wilting, unifoliate senescence, stem greenness, and recovery from drought, were evaluated in a wide range of common bean genotypes. Stomatal conductance and photosynthetic rate were measured to understand the cause of change in these traits in a control group. In addition, the number of pods and the biomass were evaluated to quantify the impact of seedling-stage drought stress on plant productivity. Plant growth and productivity after drought stress are dependent on the degree of recovery from stress. Wilting was negatively correlated with recovery, whereas stem greenness was highly positively correlated with recovery. Those genotypes with high recovery values that differ in wilting response could be combined because contrasting mechanisms for drought tolerance may reside in these genotypes. Certain common bean genotypes, known to have drought tolerance conferred by deep rooting capacity, performed poorly in the shallow soil profiles used in these experiments. Having an assay that allows for a separation of root and shoot traits functional in drought tolerance in common bean would provide breeders with a means to combine these mechanisms into a single cultivar that could enhance performance under a broad range of soil moisture conditions.

Table 8. Mean scores for wilting, unifoliate senescence, stem greenness after 21 d without water, and recovery of 18 Jalisco common bean genotypes with specific scores for ten representative genotypes evaluated under drought stress at the seedling stage.

| Genotype               | Seed type | Growth habit | Wilting (0 to 5 scale) | Unifoliate senescence (0 to 2 scale) | Stem greenness (0 to 5 scale) | Recovery (0 to 1 scale) |
|------------------------|-----------|--------------|------------------------|--------------------------------------|-----------------------------|------------------------|
| Gloria                 | Pink      | III          | 3.6                    | 2.0                                  | 3.4                         | 0.0                    |
| ROG 312                | Pink      | III          | 4.8                    | 1.6                                  | 2.0                         | 0.3                    |
| Roza                   | Pink      | III          | 3.6                    | 0.6                                  | 2.8                         | 1.0                    |
| Sedona                 | Pink      | II           | 2.8                    | 0.0                                  | 3.4                         | 1.0                    |
| UI-537                 | Pink      | III          | 4.6                    | 2.0                                  | 2.4                         | 0.0                    |
| Victor                 | Pink      | III          | 2.6                    | 0.2                                  | 2.4                         | 0.8                    |
| Common Red Mexican     | Small red | III          | 4.4                    | 2.0                                  | 2.2                         | 0.0                    |
| Merlot                 | Small red | II           | 3.0                    | 0.0                                  | 3.2                         | 1.0                    |
| UI-239                 | Small red | III          | 4.4                    | 2.0                                  | 2.6                         | 0.0                    |
| USRM-20                | Small red | III          | 3.8                    | 0.2                                  | 3.4                         | 0.8                    |
| Mean                   |           |              | 3.7                    | 1.2                                  | 2.7                         | 0.5                    |
| LSD0.05*               |           |              | 1.2                    | 0.8                                  | 0.9                         | 0.4                    |
| CV (%)                 |           |              | 26                     | 72                                   | 24                          | 55                     |

*Each point represents an average of four experiments, each with five plants for each genotype.
*Growth habit types II and III described by Singh (1982).
*0 = no sign of wilting; 5 = completely wilted.
*Number of senesced leaves (zero to two) when the most rapidly senescing genotype had dried unifoliates.
*0 = yellow; 5 = totally green.
*0 = no recovery; 0.5 = recovery from the basal meristem; 1 = recovery the apical meristem.
*Values derived from all 18 genotypes evaluated.

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