Phenotyping Common Beans for Adaptation to Drought:
Protocol for Greenhouse Evaluation

Common bean *Phaseolus vulgaris* L. is cultivated by smallholder farmers in Latin America and Eastern and Southern Africa, often under unfavorable conditions and with minimal inputs (Beebe et al., 2008). It is estimated that 60% of bean cultivation is at risk of either intermittent or terminal drought (White and Singh, 1991; Thung and Rao, 1999). The effects of drought on common bean are dependent on the intensity, type (e.g., early, intermittent, and terminal drought), and duration of the stress (White and Izquierdo, 1991; Terán and Singh, 2002a, b; Muñoz-Perea et al., 2006). In Africa as much as 300,000 tonnes of beans are lost to drought annually (Wortmann et al., 1998). Bean producing areas where drought is endemic include highland Mexico, Central America, Northeast Brazil, as well as much of Eastern and Southern Africa. Development of drought adapted common bean cultivars is an important strategy to minimize crop failure and increase food security in the face of climate change. Identification of key plant traits and mechanisms that contribute to improved drought adaptation (e.g., root length, root depth, canopy biomass, pod partitioning index, and pod harvest index) can increase the efficiency of breeding programs through the selection of superior genotypes.

The following protocol allows for the identification of phenotypic differences in drought resistance under greenhouse conditions through the quantification of root development (total length, distribution across the soil profile, diameter, volume, and biomass, and depth), vigor (shoot biomass and leaf area), plant water status (stomatal conductance), and nutrient content. Plants are grown in transparent soil tubes, allowing for the monitoring of root development.

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Set-up

Soil preparation

Soil is collected from a bean growing field site, homogenized (ground and sieved), and mixed with river sand to form a soil:sand mixture in a 2:1 proportion by weight. The presence of sand will help for inducing drought treatment faster than with soil alone.

The soil-sand mixture is fertilized with an adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo) using different sources, and mixed thoroughly.

Source	Nutrient	Content of nutrient by source %	Nutrient level (kg/ha)
Urea	N	46.0	80.0
Triple super phosphate	P	20.0	50.0
	Ca	14.0	35.0
KCl	K	52.0	100.0
Dolomitic lime	Ca	22.0	66.0
	Mg	9.8	29.4
Elemental sulfur	S	86.0	20.0
$ZnCl_2$	Zn	47.0	2.0
CuCl2 $2H_2O$	Cu	37.1	2.0
H_3BO_3	B	17.4	0.1
Na_2MoO_4 $2H_2O$	Mo	39.4	0.1

Soil tube preparation

The soil-sand mixture is poured into plastic, transparent tubes that are 80 cm in length and 7.5 cm in diameter with a small hole at the bottom for water drainage. The soil-sand mixture is added until reaching the 75 cm mark of the tube. The 5 cylinders are then weighed and the empty weight of the plastic tube is subtracted to determine the average weight of the soil-sand mixture in each tube. This weight will depend on the bulk density of the soil-sand mixture and may range from 4 to 6 kg.

Next, water is added to the same 5 tubes until it drains from the bottom. Once draining stops, the tubes are weighed and the average is taken to determine the amount of water held by the soil at field capacity.

The tubes are then placed within PVC pipes.

Seeds are sterilized by soaking them in a solution of calcium hypochlorite at 5% for 5 minutes. They are then dried under ambient conditions. After they have dried, they are placed on germination paper. After 48 hours, the seedlings should have developed small roots.

One seedling is then planted in the center of each cylinder.

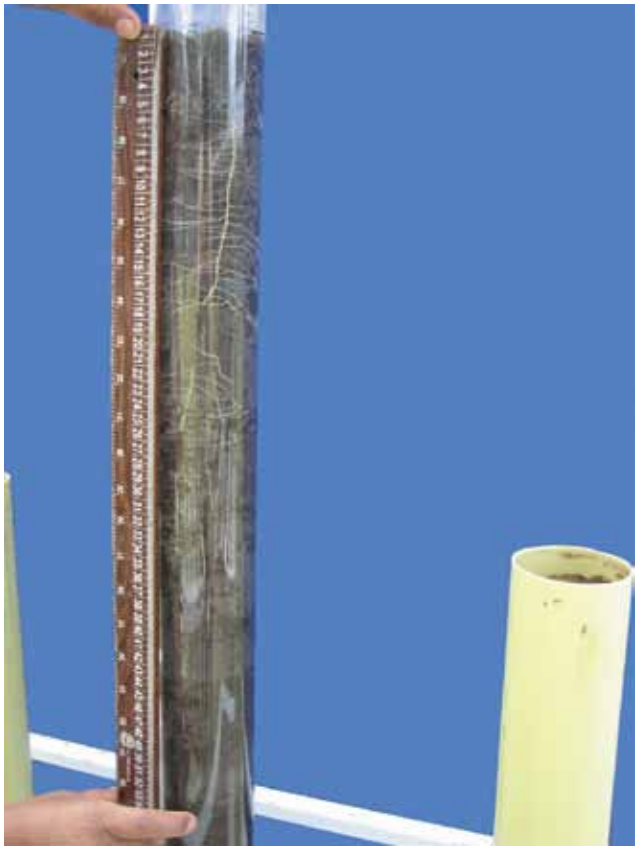
Irrigation

Trials are kept under greenhouse conditions and are planted as a randomized complete block design with two levels of water supply and three replications. For the first 10 days after planting, both plots are watered so that their soil moisture levels are maintained at 80% field capacity. After 10 days, one plot is weighed and watered every two days to maintain 80% field capacity, while the other plot receives no irrigation to simulate terminal drought conditions. Cylinders under terminal drought conditions are weighed every 2 days until harvesting to monitor the decrease in soil moisture content.

Measuring physiological characteristics during plant development

Visual rooting depth

At the same time that the cylinders are weighed for soil moisture monitoring (see above), the depth of the deepest roots should be estimated with a ruler.



SPAD Chlorophyll Meter Readings

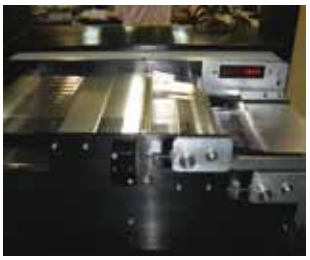
Using a non-destructive, hand-held chlorophyll meter (e.g., SPAD-502 Chlorophyll Meter), SPAD is measured once a week on a fully-expanded young leaf of a plant from each replication.

Measuring physiological characteristics 45 days after planting

Biomass and leaf area

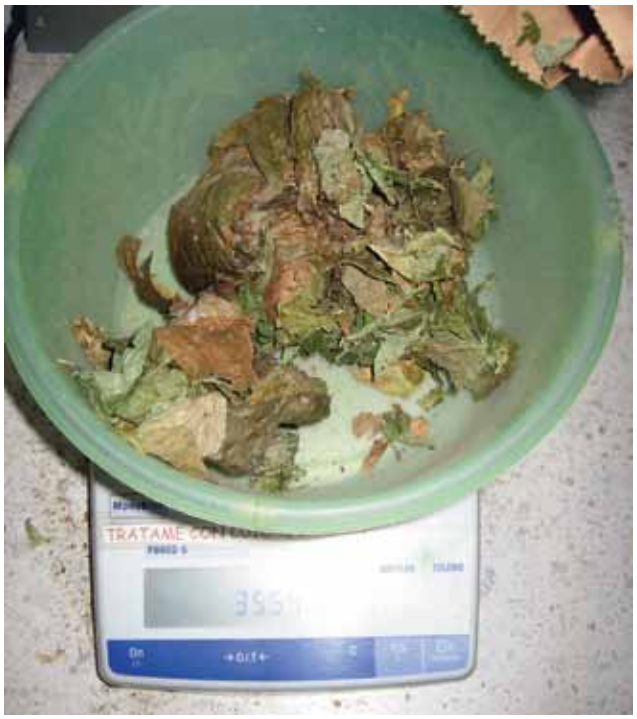
All plants are cut at the soil surface and are separated into leaves (without petiole), stems, and the remaining plant parts (pods and reproductive structures).

If a leaf area meter is available (e.g., LICOR model LI-3000), the leaf area can be determined.



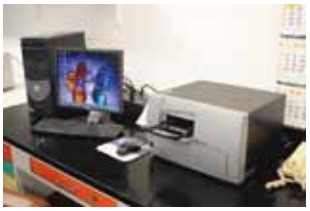
Plant parts are put in separate paper bags for oven drying at 60° C for 2 days.

Total dry matter production and dry matter distribution into different plant parts (leaf biomass, stem biomass, pods biomass, total shoot biomass) is recorded.



Shoot nutrient, ash and TNC content

To determine shoot nutrient (N, P, K, Ca and Mg), ash, and TNC (total nonstructural carbohydrates) content, dried plant samples are ground using a mill or other device. The ground samples are packed in glass tubes and sent to the laboratory for analysis. Concentration of TNC is determined using NaOH as an extraction medium and anthrone as a reagent. Absorbance of the solution is measured with a spectrophotometer at 620 nm and TNC concentration is determined by comparison with glucose standard (Adapted from Kand and Brink, 1995). Analysis of variance is calculated by using statistical analysis software (e.g., SAS/STAT Software). A probability level of 0.05 is considered statistically significant.

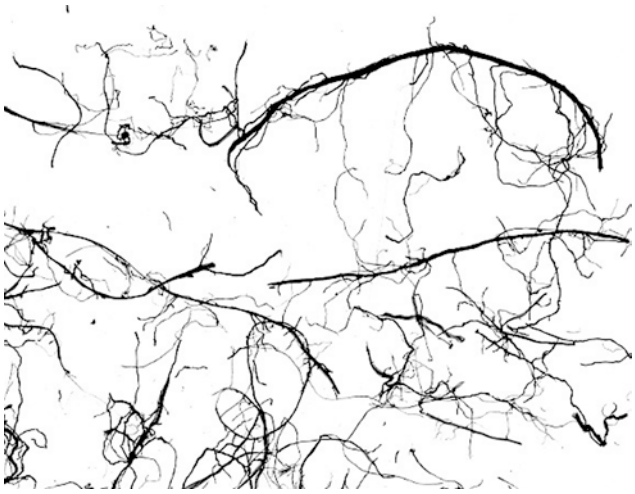


Root length, diameter and dry weight

Every soil cylinder is sliced into 6 layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75 cm).

Soil and roots are separated by hand.

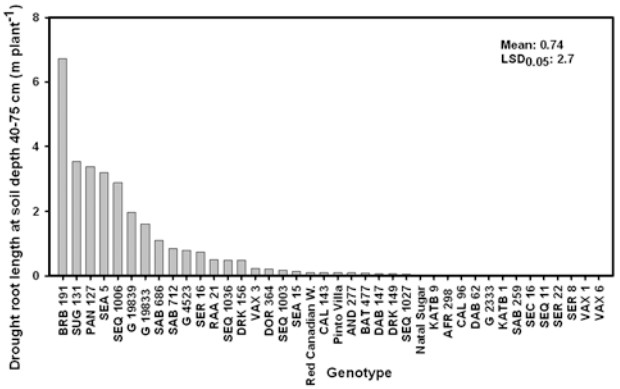
Roots are scanned. Root length and root diameter are determined by an image analysis system (WinRHIZO).



Roots are put in a paper bag for oven drying at 60° C for 2 days and their dry weight is determined.

Statistical analysis

Statistical analysis is performed on total root length, shoot biomass, SCMR, visual rooting depth, root length distribution, to determine genotypic differences in these traits. Results are then plotted on graphs to identify which genotypes exhibit better performance under drought conditions.



References

Beebe, S. E., I. M. Rao, M. W. Blair and J. A. Acosta-Gallegos. 2010. Phenotyping common beans for adaptation to drought. In: J. M. Ribaut and P. Monneveux (eds.) Drought phenotyping in crops: from theory to practice. Generation Challenge Program Special Issue on Phenotyping, pp. 311-334.

Beebe SE, Rao IM, Cajiao C, Grajales M (2008) Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Sci 48:582–592

Butare L, Rao I, Lepoivre P, Polania J, Cajiao C, Cuasquer J, Beebe S (2011) New genetic sources of resistance in the genus Phaseolus to individual and combined aluminium toxicity and progressive soil drying stresses. Euphytica 181(3):385–404

Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B, editors. 2003. Breeding rice for drought-prone environments. Los Baños (Philippines): International Rice Research Institute. 98 p.

Gale, M.R, Grigal, D.F. (1987) Vertical root distribution of northern tree species in relation to successional status. Can J. For. Res, 17:829-834.

Kang, J.H. y G.E. Bringk. 1995. White clover morphology and physiology in response to defoliation interval. Crop Sci. 35, 264-269

Muñoz-Perea, C.G., H. Terán, R.G. Allen, J.L. Wright, D.T. Westermann, and S.P. Singh. 2006. Selection for drought resistance in dry bean landraces and cultivars. Crop Sci. 46:2111–2120.

Polania J, Rao IM, Beebe S, Garcia R (2009) Desarrollo y distribución de raíces bajo estrés por sequía en frijol común (Phaseolus vulgaris L.) en un sistema de tubos con suelo. Agron Colombiana 27:25–32

Terán, H., and S.P. Singh. 2002. Selection for drought resistance in early generations of common bean populations. Can. J. Plant Sci. 82:491–497.

Thung, M., and I.M. Rao. 1999. Integrated management of abiotic stresses. p. 331–370. In S.P. Singh (ed.) Common bean improvement in the twenty-fi rst century. Kluwer Academic Publishers, Dordrecht, The Netherlands.

White, J.W., and J. Izquierdo. 1991. Physiology of yield potential and stress tolerance. p. 287–382. In A. van Schoonhoven and O. Voysest (ed.) Common beans: Research for crop improvement. CAB International, Wallingford, UK & CIAT, Cali, Colombia.

White, J.W., and S.P. Singh. 1991a. Breeding for adaptation to drought. p. 501–560. In A. van Schoonhoven and O. Voysest (ed.) Common beans: Research for crop improvement. CAB International, Wallingford, UK & CIAT, Cali, Colombia.

Wortmann, C.S., R.A. Kirkby, C.A. Eledu, and D.J. Allen. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali, Colombia.