Phenotyping Common Beans for Adaptation to Drought: Protocol for Field 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 field conditions through the quantification of traits related to vigor (canopy biomass and leaf area), plant water status (canopy temperature and stomatal conductance), photosynthate mobilization (pod harvest index and pod partitioning index), shoot/seed nutrient content, and root characteristics.

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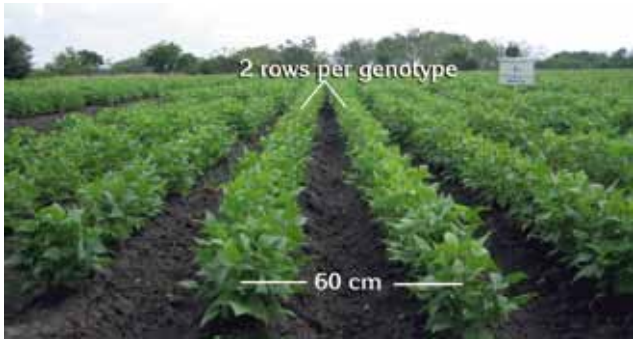
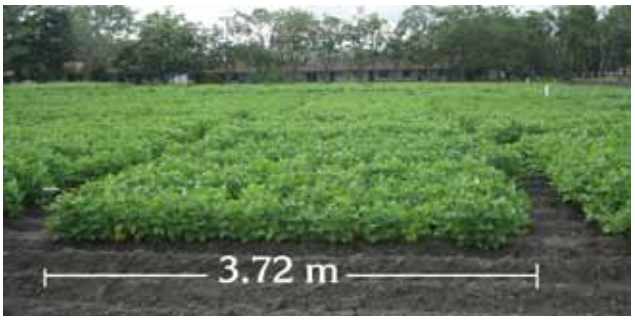
Contents

Planting Design

Field trials need to be conducted during the dry season to determine genotypic differences in drought resistance. Trials could include germplasm accessions, bred lines, and recombinant inbred lines as entries. Two levels of water supply (irrigated for no stress, and rainfed for drought stress) need to be applied to quantify the effects of drought on crop growth and seed yield.

Depending on the number of genotypes to be evaluated, a partially balanced lattice design with 3 replications could be used. The field trials can be planted in continuous rows with each genotype per replication planted in 2 side-by-side rows (or 4 rows for small trials) of 2 to 4 m in length. Rows should be spaced 60 cm apart. Seeds should be planted 7.5 cm apart (i.e. 15 seeds per m), thus yielding 10 to 15 plants per meter.



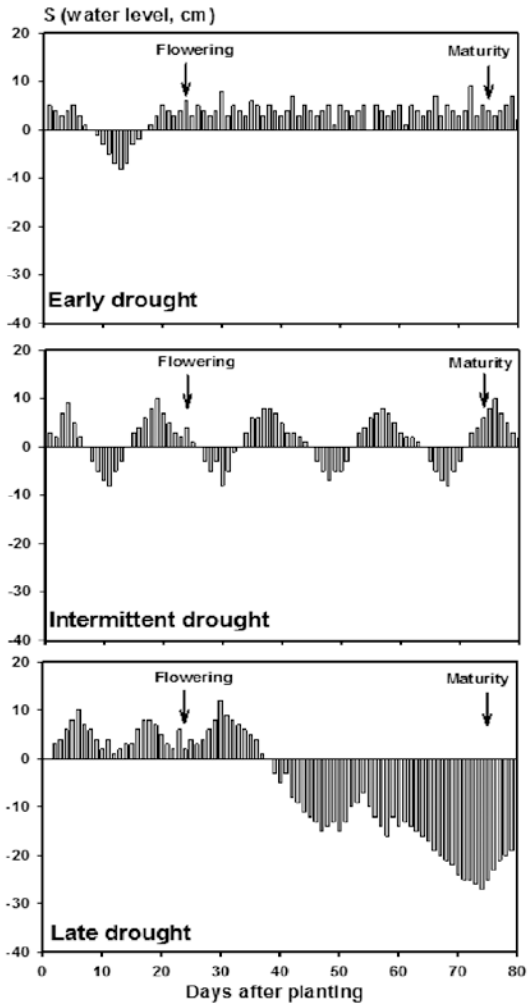


Monitoring Drought Conditions

Inducing drought stress

Depending on the rainfall, 2 to 3 gravity irrigations (approximately 35 mm each) are needed to establish the trials with control and drought treatments (one irrigation 6 days before planting and one 10 to 12 days after emergence). The control treatment may require 4 to 5 additional irrigations depending on the rainfall. The drought treatment will not receive any additional irrigation. It is important to monitor the amount of water applied during each irrigation (i.e. 35 to 50 mm).

Weather parameters (daily rainfall, minimum and maximum temperature, relative humidity, and pan evaporation) need to be recorded with an automated weather station (e.g., Davis Vantage Pro2).



Depending on rainfall distribution, trials can be conducted under early drought, intermittent drought or late drought conditions.

Soil samples for measuring drought stress

Soil samples from each replication need to be collected every 10 days, from planting to harvest. These measurements will allow for the quantification of drought stress at different growth stages.

Soil samples need to be collected with a soil corer at 6 depths (0-5, 5-10, 10-20, 20-40, 40-60 and 60-80 cm) to quantify gravimetric soil moisture content. A known volume of soil is sampled for each depth (e.g., cylinders of 5 cm height x 5 cm diameter).

Samples are immediately fresh weighted and dried in an oven at 105°C until reaching a constant weight, which is generally found between 48-72 hours.

Gravimetric soil moisture content (%) and bulk density (g cm^{-3}) are calculated.

Øm = Mw * 100 / Ms

Øm = gravimetric soil water content (%)

Mw = mass of water evaporated, g

Ms = mass of dry soil, g

Soil bulk density (g cm^{-3})

Øb = Ms / Vb

ρb = Soil bulk density (g cm^{-3})

Ms = mass of dry soil, g

Vb = volume of soil sample, cm^3

Measuring soil water with sensors

Various instruments are available to measure soil water, such as the watermark irrometer. Watermark sensors (granular matrix sensor) are an indirect, calibrated method of measuring soil water. They use an electrical resistance sensor, read by data logging equipment or a soil moisture meter which converts the electrical resistance reading to a calibrated reading of centibars (or kPa) of soil water tension.

These sensors should be installed at soil depths of 0-10, 10-20, 20-40, 40-60 and 60-80 cm at selected sites in each replication in both the control and drought stress plots (i.e., for 3 replications, a total of 30 sensors would needed). Soil water tension readings need to be recorded every day from planting to harvesting, at a defined hour (e.g., 9 am). Before these readings are taken, a measurement of soil temperature must be entered into the data logger.

Measuring evapotranspiration

Complementary to soil water tension measurements, variation in evapotranspiration (ET) needs to be monitored using an ET gage device. A ceramic evaporator at the top of the instrument and a replaceable green canvas covering it allow the instrument to mimic the ET response of plants to solar radiation and atmospheric conditions. As water is drawn from the instrument's reservoir, the water level falls in the sight tube. Every millimeter that the water level drops in the sight tube represents one millimeter of ET. Rain is prevented from entering the instrument.

Days to flowering is measured individually for each plot when 50% of the plants are in a fully flowered state.

Days to maturity is measured individually for each plot when 50% of the plants are at maturity (e.g., pods crack open easily when pinched)

Sampling at mid-pod filling

Determining "mid-pod filling"

Mid-pod filling occurs just before leaves begin to turn yellow, when the plant has no more flowers and seeds are clearly defined in pods.

SPAD Chlorophyll Meter Readings (SCMR)

SCMR is measured by using a non-destructive, hand-held chlorophyll meter (e.g., SPAD-502 Chlorophyll Meter). SPAD-502 determines the relative amount of chlorophyll present in the leaf by measuring the absorbance of the leaf in two wavelength regions. Chlorophyll has absorbance peaks in the blue (400-500 nm) and red (600-700 nm) regions, with no transmittance in the near-infrared region. SPAD-502 measures the absorbance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates a numerical SPAD (Soil Plant Analysis Development) value, ranging from 0 to 80 which is proportional to the amount of chlorophyll present in the leaf. SPAD is measured on a fully expanded young leaf of one plant for each replication.

Photosystem II Quantum Yield (QY)

Photosystem II quantum yield (QY) is measured by using a non-destructive, hand-held Qy meter (e.g., Fluorpen FP100). Fluorpen FP100 is a fluorometer that enables quick and precise measurement of chlorophyll fluorescence parameters. FP100 measures **F_T (continuous fluorescence yield in non-actinic light).** F_T is equivalent to F_0 if the leaf sample is dark-adapted) and **QY (Photosystem II quantum yield)** is equivalent to F_V/F_M in dark-adapted samples and $F_{V'}$ / $F_{M'}$ in light-adapted samples. QY is measured on a fully expanded young leaf of one plant for each replication (the same leaf used for SPAD measurement).

Stomatal conductance

Stomatal conductance to water vapor is measured with a portable leaf porometer (e.g., Deacon SC-1). This instrument measures the water vapor flux from the leaf surface to the atmosphere. A fixed diffusion path is clamped to the surface of the leaf, and the vapor flux is determined from the vapor pressure gradient in the diffusion path and the known vapor conductance through the fixed path. If the vapor flux and the conductance in the diffusion path are known, then the stomatal conductance can be easily calculated. Stomatal conductance is measured on a fully expanded young leaf of three different plants within each replication (the same leaf where SPAD is measured).

Canopy temperature

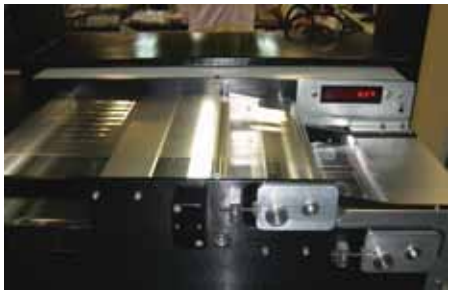
An infrared thermometer (e.g., Telatemp AG-42D) is used to record canopy temperature depression (CTD). It is held at 50 cm from the canopy surface in a 45° angle in order to measure the canopy temperature and the difference in temperature between the leaf canopy and the surrounding air temperature. Note that other models of infrared thermometer (e.g., OS562 Infrared Thermometer) require aiming at a specific spot on a bean leaf. These measurements should be recorded on a fully expanded young leaf of three different plants within each replication (the same leaf where SPAD value and QY are measured).

Biomass and leaf area

A row length of 0.5 m for each plot should be selected for destructive sampling. The plants are counted, cut to the soil surface, and put in a plastic bag, and transported for processing.

Plants are separated into leaves (without petioles), stems, and remaining plant parts (i.e. pods and reproductive structures).

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



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



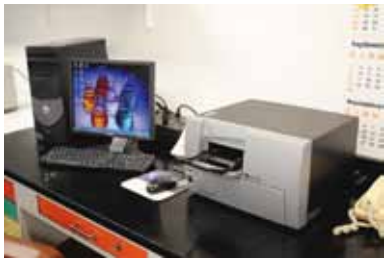

Total dry matter production and the distribution of dry matter into different plant parts (leaf biomass, stem biomass, pod biomass, total above-ground biomass) is quantified.


Shoot nutrient, ash, and TNC content

To determine shoot nutrient (N, P, K, Ca and Mg), ash, and TNC (total nonstructural carbohydrates) content, one plant of each genotype from each plot (i.e., irrigated and drought) is selected for destructive sampling. The plant is cut to the soil surface, put in a paper bag, and transported to the laboratory.

The plant is then washed with deionized water and dried in the oven at 60°C for 2 days. If analysis by plant part is desired, the plants can be separated into leaves, stems and other plant parts before drying.

After oven drying, the plant is ground using a mill. The ground samples are packed in glass tubes and sent to the laboratory for analysis. Concentration of TNC in different plant parts 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)



Root length, diameter, biomass and distribution

To determine differences in root growth and distribution across the soil profile, root samples are taken at mid-pod filling growth stage at irrigated and rainfed plots. Five soil cores are taken in each replication with a 5 cm diameter soil corer. Three cores are taken between rows and two are taken within rows. For each coring, samples are taken at 5 different depths (0-5, 5-10, 10-20, 20-40 and 40-60 cm).

Samples from the same depth and replication are pooled together in plastic bags. The 5 bags from each replication (i.e. one for each sampling depth) are then transported to the lab for analysis.

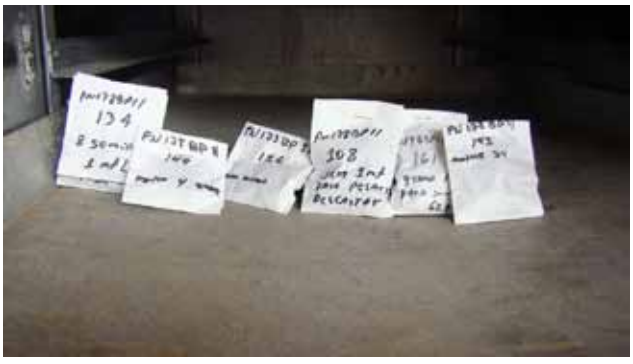
To facilitate washing, samples are first soaked for 30 minutes in 5% sodium hexametaphosphate solution.

Soil and roots are separated by hand washing and straining.

Roots are then placed in a tub of water and separated from organic matter using tweezers.

An image analysis system (e.g., WinRHIZO) is then used to determine root length and diameter.

After scanning, the roots are placed in paper bag for oven drying at 60°C for 2 days and dry weights are recoarded.



Differences in rooting among genotypes can be estimated by using a model of vertical root distribution developed by Gale and Grigal (1987).

$$Y = 1-\beta^{d}$$

Y= the proportion (a value between 0 and 1) of total root biomass or root length from the soil surface to depth d (cm)
ß= the fitted "extinction coefficient". It provides a simple numerical index of root biomass or length distribution, where high ß values (e.g., 0.98) correspond to a greater proportion of root biomass or root length deep within the soil profile and low ß values (e.g., 0.91) imply a greater proportion of root biomass or root length near the soil surface.

Sampling at harvest time

Biomass

A 0.5 m long row is selected for each genotype.

The number of plants are counted and cut to the soil surface.

The plants are put into a paper bag and transported for analysis.

Plants are separated into stems, pods and seeds. The number of pods and seeds per harvested area is counted.

Stems, pods and seeds are dried in the oven at 60°C for 2 days and dry weights are recorded.

Seed nutrient, ash and TNC content

After seeds are oven dried and dry weights are recorded, seeds are ground using a mill or other device. The ground samples is packed in glass tubes and sent to the laboratory to determine seed nutrient (N, P, K, Ca and Mg), ash, and TNC content.

Seed yield and 100 seed weight

The remaining plants of each genotype are then harvested. Seeds are separated and laid out to dry under ambient conditions. Moisture content is monitored using a seed moisture meter. Once seeds reach a moisture content of around 14%, they are weighed to determine seed yield in kg/ha.

Finally, 100 seeds are randomly selected and weighed.

Evaluating Adaptation to Drought

- **Drought intensity index (DII):**
  The DII for each growing season = 1 – Xds/Xns, where Xds and Xns are the average seed yield of all genotypes under drought stress (ds) and no stress (ns) treatments, respectively.

- **Drought susceptibility index (DSI) for seed yield:**
  The DSI for each genotype = (1 – Yds/Yns)/DII, where Yds and Yns are mean yields of a given genotype in drought stress (ds) and no stress (ns) treatments , respectively (Fisher and Maurer, 1978, Australian Journal of Agricultural Research 29:897–912).

- **Harvest index (HI) (%)**:
  The HI for each genotype = (seed biomass dry weight at harvest) / (total shoot biomass dry weight at mid-pod filling) x 100.

- **Pod harvest index (PHI) (%)**:
  The PHI for each genotype = (seed biomass dry weight at harvest) / (pod biomass dry weight at harvest) x 100.

- **Pod wall biomass proportion (%):**
  The pod wall biomass proportion for each genotype = (pod wall biomass dry weight at harvest) / (total pod biomass dry weight at harvest) x 100.

- **Pod partitioning index (%):**
  The pod partitioning index for each genotype = (pod biomass dry weight at harvest) / (total shoot biomass dry weight at mid-pod filling) x 100.
  Note that this measurement assumes that the maximum canopy biomass production is at mid-pod filling, and under intermittent drought stress this index may generate values higher than 100.

- **Stem biomass reduction (%)**:
  The stem biomass reduction for each genotype = [(stem biomass dry weight at mid-pod filling) – (stem biomass dry weight at harvest)] / (stem biomass dry weight at mid-pod filling) x 100.
  Note that this measurement may result in very low or negative values because of spatial variability and plant growth after mid-pod filling under intermittent drought.

- **Grain filling index (GFI) (%):**
  The GFI for each genotype = (100 seed dry weight under rainfed conditions) / (100 seed dry weight under irrigated conditions) x 100.

- **Yield production efficiency (g g^{-1}):**
  The yield production efficiency for each genotype = (seed biomass dry weight at harvest) / (total shoot biomass dry weight at mid-pod filling)
  Adapted from Board and Maricherla, 2008.

- **Seed production efficiency (no. g-1):**
  The seed production efficiency for each genotype = (seed number per area) / (total shoot biomass dry weight at mid-pod filling per area)
  Adapted from Board and Maricherla, 2008.

- **Pod production efficiency (no. g-1):**
  The pod production efficiency for each genotype = (pod number per area) / (total shoot biomass dry weight at mid-pod filling per area)
  Adapted from Board and Maricherla, 2008.

- **Geometric mean (GM) of seed yield, 100 seed weight, and days to maturity**:
  $GM = (ns \times ds)^{0.5}$ where ns and ds are values for drought stress and no stress treatments, respectively.

Statistical analysis: For all of the above quantifications, variance is calculated by using a statistical software system, such as SAS/STAT Software. A probability level of 0.05 is considered statistically significant.

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