EVALUATING RAPID DROUGHT TOLERANCE SCREENING ASSAYS FOR MAIZE

*M. Stalcup, S. Murray, S. Hague, and D. Hays
Department of Soil and Crop Sciences; Texas A&M University; College Station, TX

Introduction

In an attempt to reduce drought losses, many breeders primarily focus on yield stability under water stress, but little progress has occurred due to the complex nature of this trait. This has led to a search for secondary traits to improve drought tolerance. In 2009 and 2010, two screening methods evaluated sixty-two maize inbred lines and their hybrid test-cross progeny. A seedling screening method for individual plant analysis in a greenhouse measured germination, survival and recovery after a series of drought cycles. In a related field experiment, epicuticular wax was extracted from flag leaves of the same sixty-two entries at flowering under full and limited irrigation regimes.

Materials & Methods

The seedling screening experiment occurred under greenhouse conditions from January-April 2009 and 2010. A potting medium of play sand, held in Ray Leach 5 3/8" Cone-tainers™ and trays with a cotton ball, were the most effective materials for screening large amounts of germination. The inbred and hybrid tests had two planting dates. Irrigation to field capacity occurred once at planting and once to induce two drought cycles. The first drought cycle measured % germination and seedling % survival and the second measured % recovery. The first drought cycle for the inbred study began three days after planting (DAP) while that for the hybrid test began five DAP. The experiment was a completely randomized design with seventy entries per replication. Each entry contained four seeds of each line/testcross planted into individual cone-tainers™. There were six replications in 2009 and eight in 2010. Germination was calculated as the average germination of each entry across replications each year divided by the total planted. Survival and recovery measurements were on a scale of “tolerant” or “susceptible.” Wilting, curling and discoloration denoted “susceptibility.” When the majority of the seedlings were observed susceptible, irrigation induced a second drought cycle. Measurements for % recovery occurred two days later. The observation day which explained the highest percent variation for inbred and testcross entries, was used to determine drought tolerance and form an inbred-hybrid correlation.

Results

Germination for hybrid testcrosses was 5% greater than inbreds (Table 1). Hybrid testcrosses also had 1% greater overall survival by the end of the experiment. Observation days 1-4 represent the first drought cycle, while 5-9 represent the second cycle. The 3rd and 6th observation days explained the greatest % observed variation between entries for inbred lines and hybrid testcrosses, respectively (Figure 1 & 2). Four inbred lines and fourteen hybrid testcrosses were significant (P < 0.05) with above average % survival and % recovery (Figure 3 & 4). Entry 13 (G13) was significant as both an inbred and hybrid testcross. No correlation was evident between inbreds line and hybrid testcross performance estimates (R² = 0.0087) (Figure 5).

Discussion & Conclusions

• Breeders normally isolate inbred lines with desirable traits and attempt to incorporate them in hybrids.
• Of the four significant inbred lines, one performed well as a hybrid testcross. With no correlation, one could not suggest that seedling drought tolerance is inheritable between inbred lines and hybrid testcross progeny.
• Given that more hybrid genotypes were isolated as seedling drought tolerant than as inbreds, screening only hybrids may be a more conservative method but in turn may over-estimate significance.
• The results from all measurements and the fact that maize producers only grow hybrids, suggest that screening hybrid seedlings may best support selection efforts from breeders.
• This method was time efficient and cost friendly. Methods like this could reduce research and development costs incurred from selecting by yield stability.

Germplasm

Germination of sixty-two diverse maize inbred lines and two commercial checks consisted of a wide array of colors including white, yellow, red and blue grain types derived from tropical, Argentine and temperate backgrounds. In 2009, each of these sixty-two inbred lines were test-crossed in a College Station, TX field nursery to a single-cross hybrid parent of the two checks (A x B). In 2010, we evaluated testcross progeny as well as additional checks that included the best and worst performing inbred from the previous year and the hybrid parent (A x B).

Seedling Screening Method

Objectives

1) Develop an efficient and cost-friendly method to evaluate mass germplasm for seedling drought tolerance
2) Determine if seedling drought tolerance is inheritable

Materials & Methods

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Epicuticular Wax Extraction Method

Objectives

1) Modify and adapt epicuticular wax extraction methods for whole leaf samples
2) Determine if epicuticular wax on flag leaves develops in response to drought stress at flowering
3) Establish epicuticular wax heritability

Materials & Methods

The epicuticular wax study was conducted in College Station and Weslaco, Texas in 2009 and 2010. Fields were sown in a randomized complete block design with seventy entries, two blocks and two replications, and twenty-five seed per plot. Blocks were full and limited irrigation treatments. Irrigation treatments occurred as needed except at flowering for limited irrigation blocks to induce stress. Three random, non-consecutive flag leaves were sampled from each plot. Sampled plants were harvested separately from remaining plants in each plot. Additional measurements included plant and ear height, ear number and length, bulk and grain weight, germination %, flowering and silking.

• Preparation: Chloroform resistant, 20 mL vials were weighed to obtain a pre-extraction weight on a scale calibrated in grams. Extraction jars (1 oz) were calibrated to an equivalent weight.
• Sampling: Leaf samples were removed from plants with scissors, and stored in gallon size plastic bags in a cooler to slow biological processes and preserve wax integrity.
• Extraction: Each plot’s leaves were placed in an extraction jar. Leaf weights were recorded, and jars received 25 mL of HPLC grade chloroform. After covering with a Teflon coated lid, shaken for 15 seconds and removed with tweezers, leaves were photographed for leaf area analysis. The remaining solution was poured into a vial via glass funnel. Samples were evaporated under a fume hood to obtain wax weights. (Post-extraction vial weight) - (pre-extraction vial weight) = final wax weight.

Additional measurement results not provided.

Discussion & Conclusions

As block was insignificant, we conclude that epicuticular wax production does not increase due to drought stress. Hybrid testcross analysis for % wxwtf presented a much higher heritability than inbred lines; suggesting that this measurement would best determine genotypic differences from hybrid testcrosses in these environments. The heritability of % wxwta for inbred lines was the highest over all the measurements. Unfortunately, a poor correlation between inbred and hybrid wax production based on leaf area does not support heritability from inbreds to hybrids.

• Flag leaves should be sampled from entries that flower at the same time, rather than sampling from an entire block.

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