Sweetcorn Seed Testing Services

SAND EMERGENCE TESTING

SoDak uses the "top of creped cellulose with sand" method (TCS) to classify normal seedlings at 77°F or ideal emergence conditions (Figure 1). This method is an Association of Official Seed Analyst (AOSA) approved method for standard germination of sweetcorn. SoDak has found testing on the "dry side" or using less water in the sand germination is suitable for sweetcorn, along with classification normal seedlings as strong or slow at the completion of the seven day test. Sugar leakage is also a characteristic which is rated by the presence of saprophytic fungal mycelium growth on top of the sand. *Rhizopus* or *Mucor* species seem to be the prominent saprophyte and we use a "trace", one or two rating to rank the level of sugar leakage (Figure 2). In summary, we supply four responses to help you rank the seed quality: 1) total normal seedling%, 2) strong normal %, 3) slow normal% and 4) level of saprophytes/i.e sugar leakage level. SoDak provides the "raw data view" on the completed test report for clients to further understand the seed quality of the respective seed lots.

COLD IMBIBITION AND OXYGEN REGIME TESTING

Imbibition of cold water has long been a stress used for *Zea* species to rank seed vigor or field emergence. SoDak offers three levels of imbibitional chilling stress: 1) 40°F, 2) 50°F and 3) 60°F in an aerobic oxygen sand regime (Figure 3). Seedlings are classified at 120 GDDs as either strong, slow or abnormal and reported. SoDak also offers a 50°F anaerobic regime cold test called the "50°F Saturated Cold" which utilizes both stresses (chilling and oxygen depredation) and is often too stressful for sweetcorn and would be considered the most stressful regime to evaluate seed vigor (Figure 4). Seedlings are planted embryo down into a saturated sandy loam soil for a seven days 50°F cold and oxygen depredation stress followed by a growout until 100 GDDs and evaluation. Total, strong, slow and abnormal seedlings are reported along with dead seeds.

TESTING FOR ADVENTITIOUS PRESENCE (AP) OF GMO EVENTS

To test for the absence of biotech herbicide and insecticide traits in conventional or non-GMO seed, SoDak Labs, Inc. offers three AP tests; AP-Seed, AP-Grain, and PCR

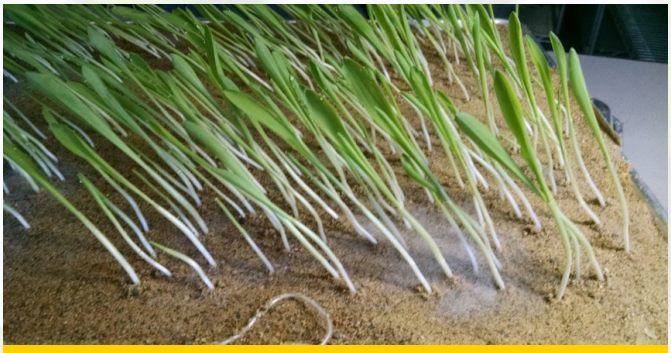

FIGURE 1. Sand emergence of sweetcorn seedlings after seven days. Sand regime allows uniform imbibition and seedling growth uniformity can be rated.

FIGURE 2. Sand germination test showing *Rhizopus spp.* mycelium growth in response to sweetcorn seeds leaking sugar into germination media.

FIGURE 3. Seedling evaluation of a 50F cold test following seven days @ 50F and 120 Growing Degree Days (GDDs).

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FIGURE 4. Sweetcorn seed response to the 50F Saturated Cold test at 100 GDD.

FIGURE 5. Herbicide bioassay showing 2 trait positive seedlings in a 1200 seed test.

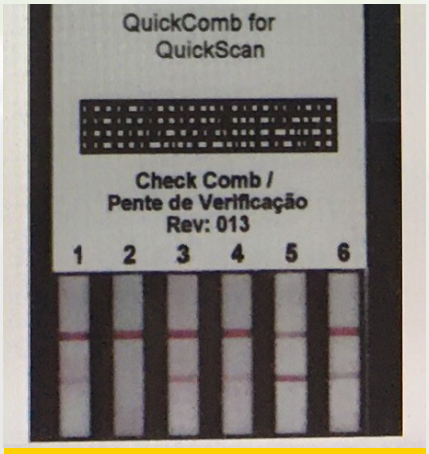

FIGURE 6. ELISA Lateral Flow strip comb showing positive and negative reactions for specific traits.

FIGURE 7. Seeds vigorously washed in 10% bleach.

GMO Screen testing. For AP-Seed, 1200 or 2400 seed is screened using herbicide bioassays (Figure 5). Insect traits can be screened using an ELISA based lateral flow strip comb. For AP Grain, 800 kernels are ground and tested with an ELISA comb (Figure 6) for both insecticide and herbicide proteins. For PCR GMO Screen Testing, seed is grouped into "pools" no larger than 1,000 seeds with typically 3000 to 10,000 seeds in total analyzed. The standard process for sweet corn begins with a vigorous 10% bleach wash (Figure 7), then seed is dried and divided into the requested pool size before processing. Samples are ground to a flour consistency then DNA is extracted and tested for the presence of CaMV P-35S and T-NOS, which are transgenic insertions commonly used in biotech traits. Sample results are determined by semi-quantitative analysis using SeedCalc8 and the qualitative results of the sample's subsamples.

ISOZYME PURITY

Isozymes are different versions of the same enzyme, resulting from distinct genes, yet performing the same metabolic function. These variations can be observed in differences such as amino acid composition, net charges, molecular sizes, and shapes. At SoDak Labs, we use specific activity stains to detect polymorphic isozymes after electrophoresis. Five stains, examining 16 loci, are applied, and detection relies on the precipitation of indicator dyes in areas of enzymatic activity. The total number of bands on a gel depends on factors like the number of coding genes, the organism's allelic state, protein product structures, and subcellular compartmentalization. Analyzing inbred parent patterns on the gel helps determine hybridity, enabling the identification of allele segregation, female and male selfs, 2 loci and 3 loci offtypes, and variants.

**Specific to sweet corn*

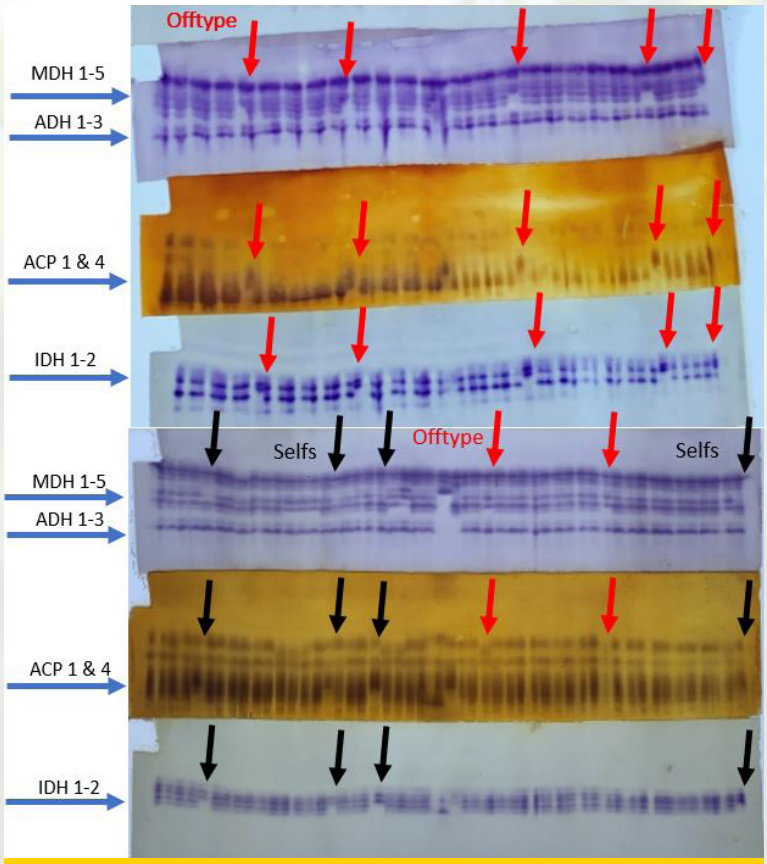

FIGURE 8. Isozyme staining patterns used for sweetcorn hybrid purity determination. GLU 1-2 and PGM 1-2 not pictured.