**Sunflower Association Mapping (SAM)**

**Planting and Phenotyping notes modified August 13, 2010**

288 cultivar lines, 12 individuals planted per line, 2 replications per 3 locations (GA, IA, BC). At GA and IA, there are 36 rows and 16 columns for a total of 576 plots. The spacing in GA, and IA is: each plot is 10 ft long and 1 ft wide, 12 seeds were planted each. Row spacing is 60 in. The spacing in BC is slightly modified and the reps are being planted in to locations. The plants are in an alpha lattice experimental design. These designs are randomized across locations.

Please keep the naming and numbering uniform, e.g, GAP001R1 is the plot name, and each plant is IAP001aR1, IAP001bR1, IAP001cR1, IAP001dR1, etc. We are trying to keep everything very uniform since there will be thousands of data points.

Avoid phenotyping any outlier plants in a single plot that look very different from the other plants (seed contaminants) or that have obviously been damaged by insects.

We will be phenotyping the first 4 plants to flower per plot.

Note to IA and BC: The main issue is that the phenotyping is consistent throughout. When you send me your data, please ensure that it has the name of the individual, e.g, BCP001R1a, attached to the data, that way I can import it into our database. Recording in an Excel spreadsheet would be the best. I can send you an example of ours. Thanks! -JRM

Phenotyping

1. **Take a stand count of plants in each plot.**
2. **At R4/R5, collect the first fully expanded unshaded leaf ONLY (no petioles).** Notes: Leaves (when possible) should be green, healthy, free of holes, disease, etc. Leaves should be collected in the AM so they are fully hydrated and not wilted. Leaves can be kept at 4C for up to four days. Scan leaf on desktop scanner at 300DPI. Name the scan with the name of the plant, e.g., BCP009cR2. Then place leaf in a coin envelope and dry for 4 days at 60C. Weigh leaves after drying. *GA: Specific scanning instructions are given in a separate document. IA and BC: you can do this scanning/weighing however you like using your own methods and just report the ratio data to us (leaf area/dry weight).*
3. **Record dates when each of the first 4 plants flower (R5) in the plot.** Note: We are recording flowering date and making measurements when the first whorl of disk florets has opened per J. Burke.
4. **At R5, measure plant height from the soil line to the point on the stem that connects to the terminal flower head, aka, primary capitulum.**
5. **At R5, record level/intensity of anthocyanins in the disk florets and stigmas.** Notes: 1= yellow disks and no dark pigment, 5= disks with a slight hint of pigmentation, 9= darkly pigmented disks, 1=Yellow stigmas, 5= red and yellow stigmas, 9= red stigmas. *IA and BC: We are having okay luck with this system in GA; some stigmas have just the tiniest spot of red on them. You can make a note of this or call them 2/3’s if you like.*
6. **At R5, measure flower head diameter (N/S orientation and E/W orientation at 90 degrees).** Note: If flower has oblong shape, measure in the direction of the longest and shortest sides but still at 90 degrees.
7. **After measuring flower heads, bag all four heads. Self two of them daily or every other day when possible: self plants “a” and “b”.** We are bagging the heads because the seed phenotyping needs to be done on selfed seed per L. Marek and S. Knapp.

**R9 Phenotyping Changes 8/13/2010:**

**For branching, we will only phenotype 3 plants per REP. (We did 4, and we think 3 will tell the same story).**

**No specific gravity measurement in either REP.**

**Since we are not doing green volume, you can let the plants dry in the field until they start turning brown before you harvest them. This will help with drying times in the ovens and ensure seed maturity.**

**For stem diameter, we will do 3 plants from the most uniform REP and only 2 from the other REP.**

**Biomass on only 3 plants from your most uniform REP.**

**Collect 3 heads from most uniform rep and only 2 from the other. Choose the two that you manually selfed when possible. (But use judgment, if the heads are very small, get all 4).**

**You will still need quite a bit of drying space although obviously not all at once. (~1400 stem sections, ~1400 heads, 864 stalks). Let me know if you think this will be a problem.**

1. **At R9 (physiological maturity), characterize branching in 3 plants/REP (total:6).** Notes: There should be a ton of variation in branching ranging from apical, basal, full branching and everything in between. See branching power point and below\*.
2. **At R9,** **measure the stem diameter of a stem section.** **3 plants from your most uniform and 2 from the other.** Notes: At maturity, cut an ~25 cm section of stem starting at the soil line and going up the stem 25 cm. Remove all the leaves and branches. Cut the top at a ~45 degree slant so we can identify the orientation of the stem later. Transport the stems in a plastic bag on ice back to the lab. Measure stem circumference at the base (proximal end) of the stem (N/S orientation and E/W orientation at 90 degrees). The stem should then be placed into a paper bag and dried in a forced air oven at 60C for at least 1 week. Once the stem section has reached a constant weight, record the dry weight and add this to the biomass for the most uniform REP ONLY. Dried stem samples will then be shipped to NREL for MBMS analysis. *More info on NREL later. (I don’t know if they are supposed to be a certain size for NREL. I’ll try to find this out.)*
3. **At R9, measure plant biomass in most uniform REP ONLY.** Notes: After cutting the stem section, cut the head and place in a labeled paper bag for drying. Place the rest of the stalk/stem into a paper bag and dry both heads and stalks in an oven at 60C. Plants will take different times to dry because of biomass variability; usually a week gets most when collected brownish in the field. Record dry weight of the stalk. Weigh dried sunflower heads. Add the dry weight of the stalk + the dry weight of the stem section for stalk biomass. *IA and BC: We are drying our heads separately, but if it is more tractable to do it together, feel free just let me know.*
4. **Dormancy: UGA is setting up dormancy trials. We should discuss the possibility of this at other locations, but I don’t know how feasible.**
5. **Process heads to get seed out.**
6. **Measure 100 seed weight in grams.**
7. **Measure seed characteristics, length, width, shape, etc. (Any other traits that L. Marek might suggest?)**
8. **Measure chemical seed traits, TBD and to be completed at UGA. *IA and BC can send the packets of measured seed, and we will do the chemical phenotyping here at UGA.***

Flavonoids – Anthocyanins – Phenolics – HPLC

Fatty Acids – GC

Tocopherols – HPLC

Total oil content – NMR + NIR

Protein content – NIR

Branching Phenotyping:

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