

Making the Most of Double Haploid Breeding in Line Development

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This presentation outlines one breeder's experience attempting to develop a breeding strategy that exploits the potential of double haploid technology as well as genome wide selection. This presentation goes step by step through a breeding program discussing several very practical solutions regarding issues we all have to address as we bring both of these valuable breeding tools into our programs. This presentation is not about drawing a road map regarding how to utilize these technologies. Instead it is meant to generate new ideas and initiate a vigorous discussion regarding the value and interaction of these technologies in a large commercial corn breeding program.

Making the most of Doubled Haploid Breeding in line development

Dr. Tim B. Cupka

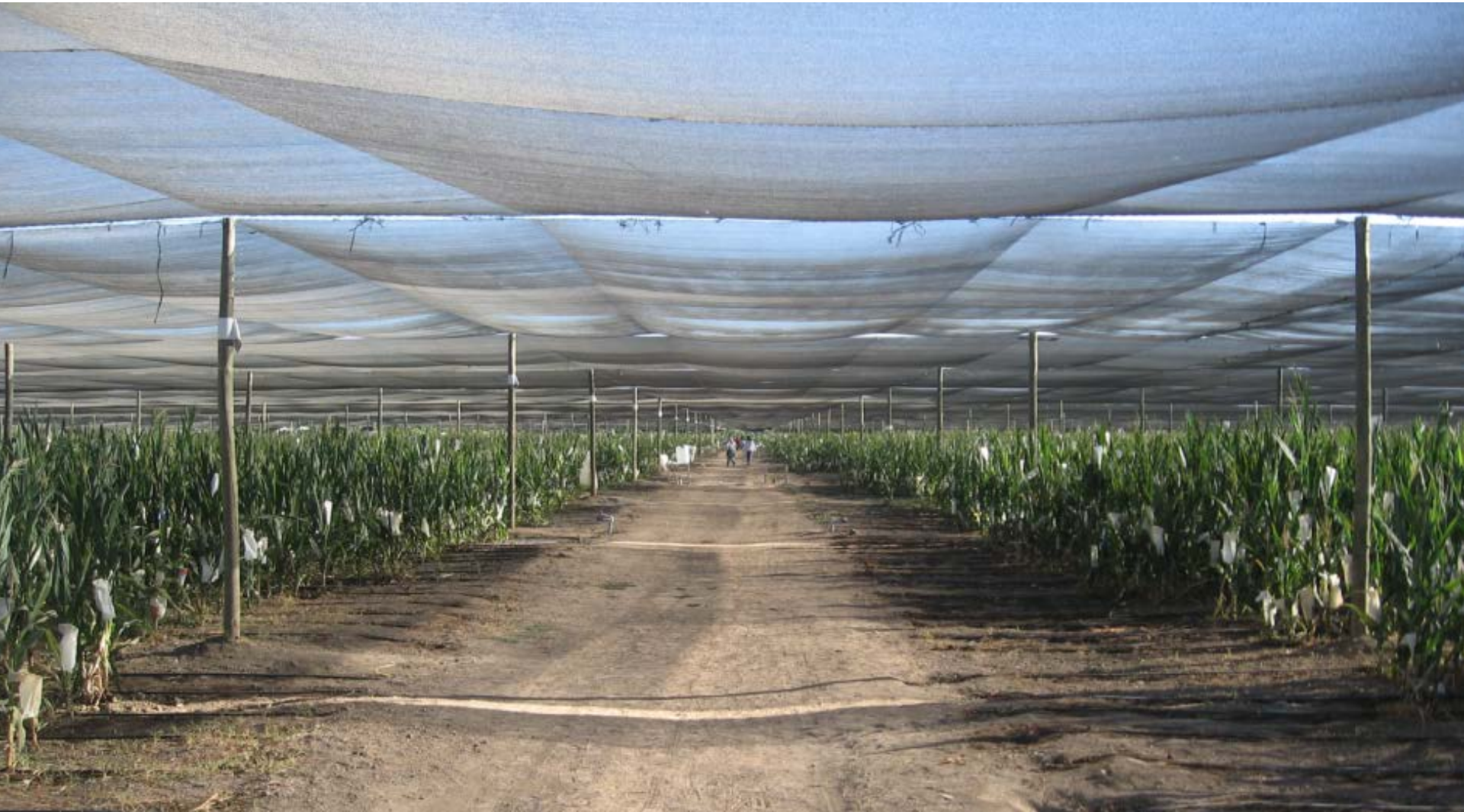
Corn Breeder

AgReliant Genetics LLC

Creating Broad-based Half Sib Composites (HSB)

1. SS HSB and NSS HSB
2. Induce in Summer to create haploid seed and Doubled in Winter to create DH Progeny
3. Each spring I receive 15,000-20,000 new DH lines, 14,000-17,000 have 4 or more kernels.

Doubling Nursery in Rancagua Chile





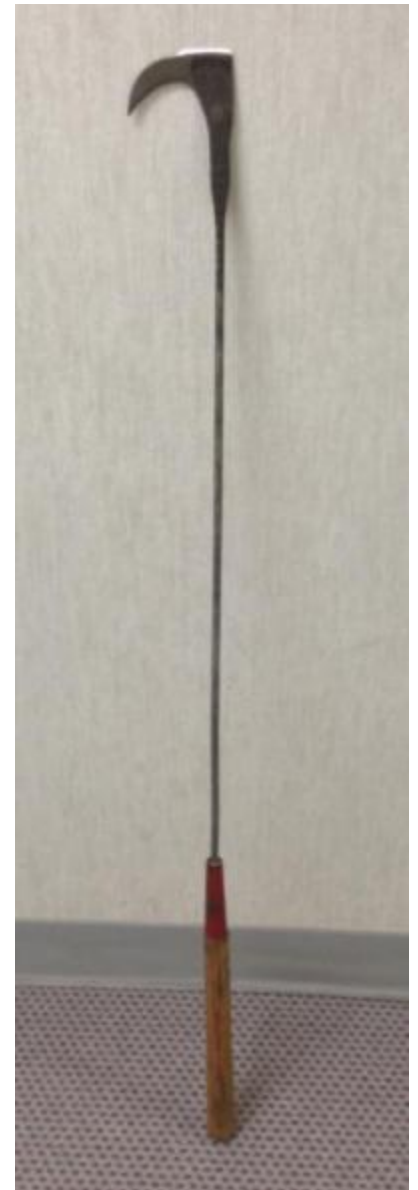


Planting DH Lines in Nursery

1. In the spring we plant approximately 15,500 DH lines, each in a single 5' long row with a minimum of 4 kernels up to 10 kernels.



The Double Haploid lines you evaluate from this point forward either phenotypically in hybrid combinations and/or utilizing Genome Wide Selection (GWS) are only as good as the selections you make on a per se basis in your initial DH increase nursery.



Beginning the Selection Process

- Beginning 3 weeks prior to pollination I begin the rogueing process by physically killing any rows that are unacceptable for utilization in a commercial seed production operation. The rogueing continues at least one week after pollinations in a nursery are complete.



Pre Anthesis - Post Anthesis Criteria for In field discarding of DH lines

1. Poor stalks and roots
2. Extremes for plant size, too big or too small
3. Poor tassel type for pollinators or poor tassel extension for seed parents
4. Excessive ear height, either too high or too low
5. Lack of stress tolerance, heat, drought or cold
6. Severe disease response
7. Poor Nick of tassel and silk, sneaky silks, silk balling, etc...
8. Excessively Late flowering Habit

Additional Reasons for significant rogueing within a row

1. Establishing proper spacing of plants to produce a maximum amount of high quality seed, maximum 6 plants per row
2. Removing 1 or 2 contaminants in an otherwise clean row
3. Eliminating weakened plants due to seed quality, pests or uneven growing conditions

Pollination Activities

1. One to three uniform healthy plants from each row are shoot bagged.
2. Two healthy uniform plants are selfed.
3. The goal is to produce a minimum of 20 kernels for all NSS lines and 80 kernels for all SS lines.





Percent of lines eliminated after harvest

- | | | |
|----|--------------------------------------|------------|
| 1. | 4.5% never germinated | 700 lines |
| 2. | 6.5% too big (outcross) or too small | 1000 lines |
| 3. | 6.5% poor stalks and roots | 1000 lines |
| 4. | 6.5% Poor Tassels type | 1000 lines |
| 5. | 3% unacceptable ear height | 500 lines |
| 6. | 3% poor stress tolerance | 500 lines |
| 7. | 3% poor nick or silk issues | 500 lines |
| 8. | 2% late flowering | 300 lines |
| 9. | 6.5% lack of high quality seed | 1000 lines |

Final Number of DH lines to Advance

1. 41.5% are eliminated, leaving 9000 DH lines available for further evaluation (TC1)
2. All 9000 DH lines are genotyped for GWS. 2500 are crossed to a tester(s) to create the training set. The remaining 6500 are placed in cold storage until the training set goes through yield trials.
3. The 2500 DH lines in the training set are either placed in paired rows (NSS) or isolations (SS) to create the experimental hybrids.

Evaluating DH lines in TC1

1. The 2500 experimental lines making up the training set are placed in Yield trials consisting of 24 entries (21 experimental lines and 3 check hybrids containing the same elite tester)
2. They are evaluated in 4 row plots at 6 locations across the cornbelt in 1 rep trials
3. Traditionally 3% - 5% of the experimental lines significantly outperform the existing commercial lines on the same tester.

Selection of GWS lines

1. Based on the performance of the training set, GWS is utilized to estimate the performance of the remaining 6500 DH lines in cold storage based on their genetic similarity to the genotypes of the individuals in the training set.
2. If 5% of the training set is identified as significantly superior to existing commercial lines, then 5% of the (GWS only) lines are also selected for advancement to the next level of a testing (fast track). An additional percentage up to a maximum of 10% are advanced at a slower pace (slow track).

TC2 Testing for the top 450 Selects

1. Each selected DH line goes to winter nursery and is crossed to up to two testers, plus a single row is selfed for increase.
2. TC2 trials are designed similar to TC1, except they are now 10 location trials. The DH Lines are observed in summer and increased as needed.
3. Traditionally 11% - 15% continue to significantly outperform important commercial or pre-commercial lines on the same testers. The top 50-60 lines are advanced to TC3 testing.

TC3 Testing for the top 50 - 60 Selects

1. Each selected DH line goes to winter nursery and is crossed to 3 testers, plus each DH line is increased as needed.
2. TC3 trials are designed similar to previous TC levels, except they are now 25 location trials. Traditionally 15% - 20% continue to significantly outperform important commercial or pre-commercial lines on the same testers. The top 6-10 lines are coded and sent to EX1 level testing

EX1 Testing for top 6-10 DH lines

1. EX1 testing is Company-Wide testing. These trials contain all newly coded DH lines in a specific maturity on several testers. Checks are best internal commercial hybrids as well as the best competitor hybrids available. These hybrids tend to be fully traited.
2. Traditionally 1 – 2 DH lines from each program are identified for advancement to our research strip trial program known as NET. These same hybrids will also be tested simultaneously in our most advanced small plot testing called EX2.
3. Coded lines are increased by the breeder to a quantity adequate for product development activities.

EX2 and NET Trials

1. EX2 trials are 2 replication, 4 row plots with 60-70 replications planted across the entire cornbelt.
2. NET trials are 4 - 8 row plots, 400' - 800' in length. 60-80 NET trials are planted in each maturity across the entire cornbelt.
3. Checks are identical between the two trials, however, EX2 trials may contain additional hybrids that did not make it into NET trials.
4. Traditionally 0 -2 hybrids get advanced to commercial status for each program after performing well in NET trials.


Commercial Release

1. Once a hybrid has successfully completed NET trials it becomes available for our Brands to determine if they need it in their product lineups.
2. During the NET trial phase all new lines are evaluated by Product Development for purity and increased to foundation level status.
3. Once a Brand has shown interest in commercializing a hybrid the new inbred is increased by the Foundation Department to commercial status and released to the Production Department for hybrid seed production.

Typical DH Time Line

1. Make Breeding Cross, induce Haploids
2. Double Haploids, evaluate DH lines per se
3. Create TC1 hybrids, evaluate Hybrids
4. Create TC2 hybrids, evaluate Hybrids
5. Create TC3 hybrids, evaluate Hybrids
6. Create EX1 hybrids, evaluate Hybrids
7. Create NET hybrids, evaluate Hybrids
8. Pilot Commercial hybrid, Sell and produce

7-8 years to create a completely new commercial hybrid!

A photograph of a cornfield at sunset. The sky is filled with vibrant orange, yellow, and pink clouds, with the sun low on the horizon. The corn plants in the foreground are dark green and silks are visible. The text is overlaid in white, bold font.

**Thank you for your time
and interest,
Are there any questions?**