Heterosis in Flue-Cured Tobacco and Its Utility in Predicting Transgressive Segregation in Derived Populations of Inbred Lines

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Breeding for Yield Increase

- Yield increases are a major objective for plant breeders
  - Low heritability trait, generally selected for in later generations
- Aim to find populations with high numbers of transgressive segregates: progeny with a phenotype more extreme than the parentals
  - Higher than the high parent or lower than the low parent
- Hybrid cultivars already used in tobacco to use disease resistance alleles in heterozygous state and for variety protection
  - Heterotic effects for yield may be underappreciated and could allow for significant yield increases in tobacco hybrids

Heterosis

- Heterosis: Improved performance of hybrid progeny over the parents for a particular trait
  - Mid-parent heterosis: $\frac{F1-MP}{MP} x\ 100$
  - Better-parent heterosis: $\frac{F1-BP}{BP} x\ 100$
- Described in 1876, defined in 1914, widely exploited in many crops
  - 15%-50% yield increase due to heterosis, depending on the crop
  - As of 2002, most corn and 50% of rice was produced using hybrids

Heterosis Observations

- Generally thought to occur at higher rates in cross-pollinated species, with minimal levels observed in self-pollinated species
  - Observed heterosis levels cross specific and differ for each parental combination

- Highest levels observed in the F1 generation, decreases in population during inbreeding
  - in process called inbreeding depression

- Single loci associated with heterotic effects identified in tomato, Arabidopsis, and maize, but largely considered to be due to accumulated effects at numerous loci

Mechanisms of Heterosis

- Several genetic mechanisms, thought to be a combination
  - Generally three theories:
    - Dominance: heterosis due to deleterious recessive alleles being masked by superior alleles
    - Over-dominance: superior phenotype due to heterozygous state
    - Epistasis: heterosis due to interactions
- Typically greater genetic diversity between parents is associated with higher heterosis levels
  - Seen in wide crosses or through the use of exotic material
  - Molecular measures of diversity not always a good predictor of heterosis level
- Role of gene complementation of superior alleles seen
  - Huang et al. 2014 demonstrated dominance complementation in rice

Implications of Heterosis

- Some of these genetic mechanisms can be fixed during the inbreeding process
  - Heterotic effects due to dominance can be fixed in the derived lines

- Heterosis measurements could be indicative of superior gene combinations between parents

- Suggests crosses with higher heterosis levels have the potential to produce greater numbers of superior derived lines

Past Tobacco Heterosis Observations

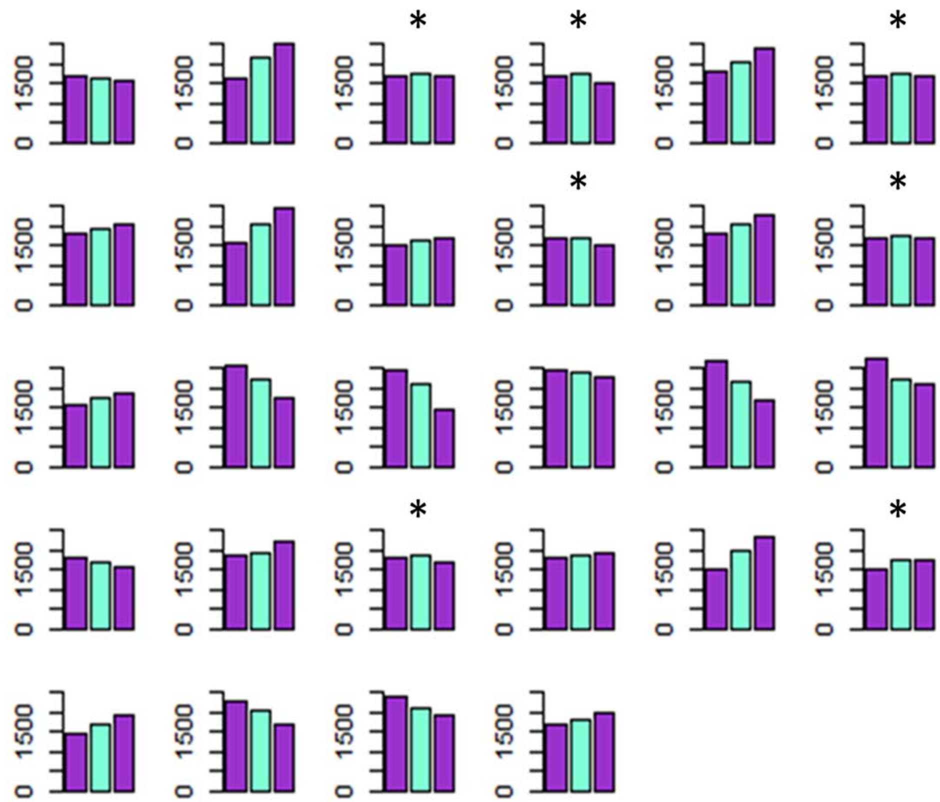
- Early studies by East (1936) to demonstrate heterosis in plants found crosses between *Nicotiana tabacum* and *N. rustica* had some of the largest biomass increases observed

- Previously observed average mid-parent yield heterosis levels in tobacco:
    - 5% - 9.8% in burley
    - 21% in oriental
    - 3% in flue-cured
    - 10% average in inter-type crosses
    - 15% in Turkish varieties
    - 12% in oriental x flue-cured crosses

Past Observations in Flue-Cured Tobacco

- 8 parent half-diallel
  - 7 of 28 hybrids outperformed the better parent
- Better-parent heterosis:

  -20% - 5%
- What about heterosis levels in present day cultivars?



Data from Matzinger and Mann, 1962

Study Objectives

- 1) to examine the potential of exploiting heterosis for yield increases in flue-cured tobacco

- 2) to allow for the prediction of more successful breeding crosses by looking for associations between yield heterosis level in the F1 and number of transgressive segregates among derived lines

Evaluation of F1 Lines

- Selected 14 flue-cured parents for half-diallel, making 91 hybrid combinations
  - Parental lines selected as diverse representation of flue-cured germplasm
  - Tested in 2016 at 3 locations in an alpha-lattice design with 4 replications

	Speight G-28	McNair 944	Speight G-70	NC82	K149	NC606	Speight 220	NC925	L09-1305-1	NC61 SE Line D	Speight 168 SE Line A	NC8640	OX2047 Wz/Wz	Line #17
Speight G-28		X	X	X	X	X	X	X	X	X	X	X	X	X
McNair 944			X	X	X	X	X	X	X	X	X	X	X	X
Speight G-70				X	X	X	X	X	X	X	X	X	X	X
NC82					X	X	X	X	X	X	X	X	X	X
K149						X	X	X	X	X	X	X	X	X
NC606							X	X	X	X	X	X	X	X
Speight 220								X	X	X	X	X	X	X
NC925									X	X	X	X	X	X
L09-1305-1										X	X	X	X	X
NC61 SE Line D											X	X	X	X
Speight 168 SE Line A												X	X	X
NC8640													X	X
OX2047 Wz/Wz														X
Line #17														

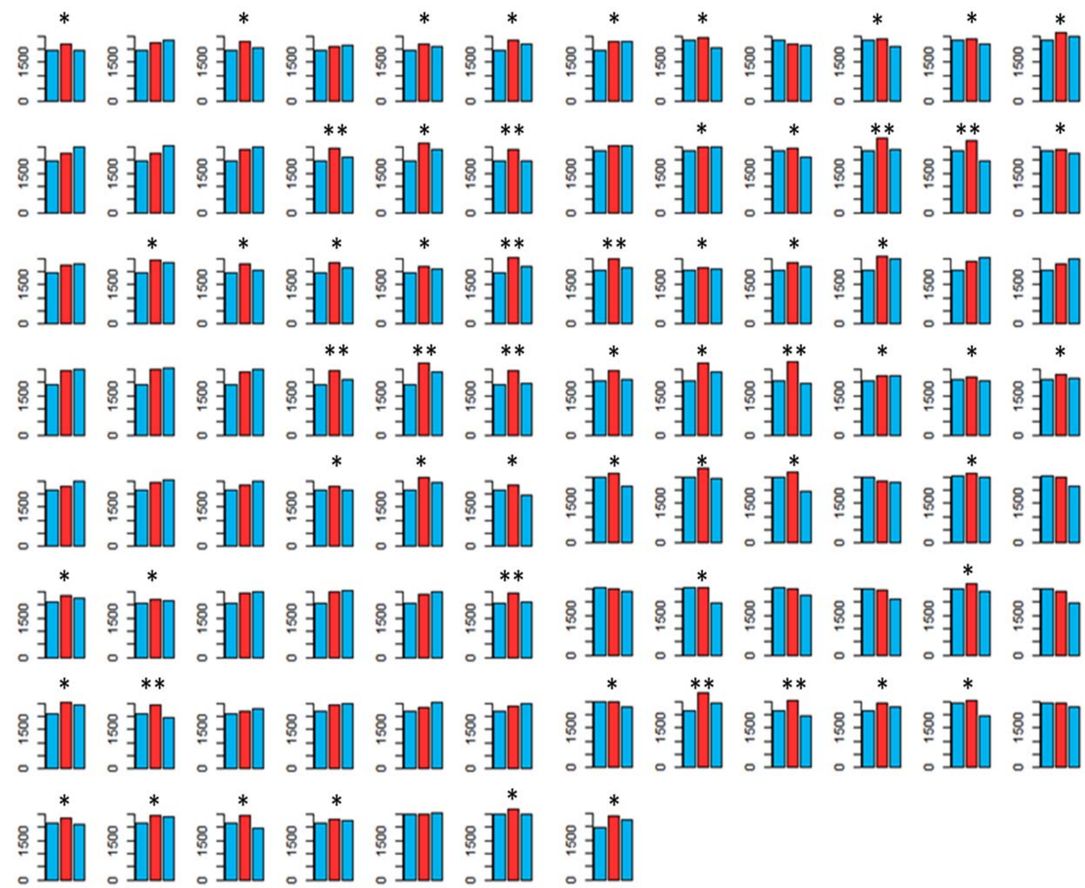
Observed Yield Heterosis

- 60 hybrids yielded more than the better parent
    - 14 significantly better

- Better-parent heterosis:

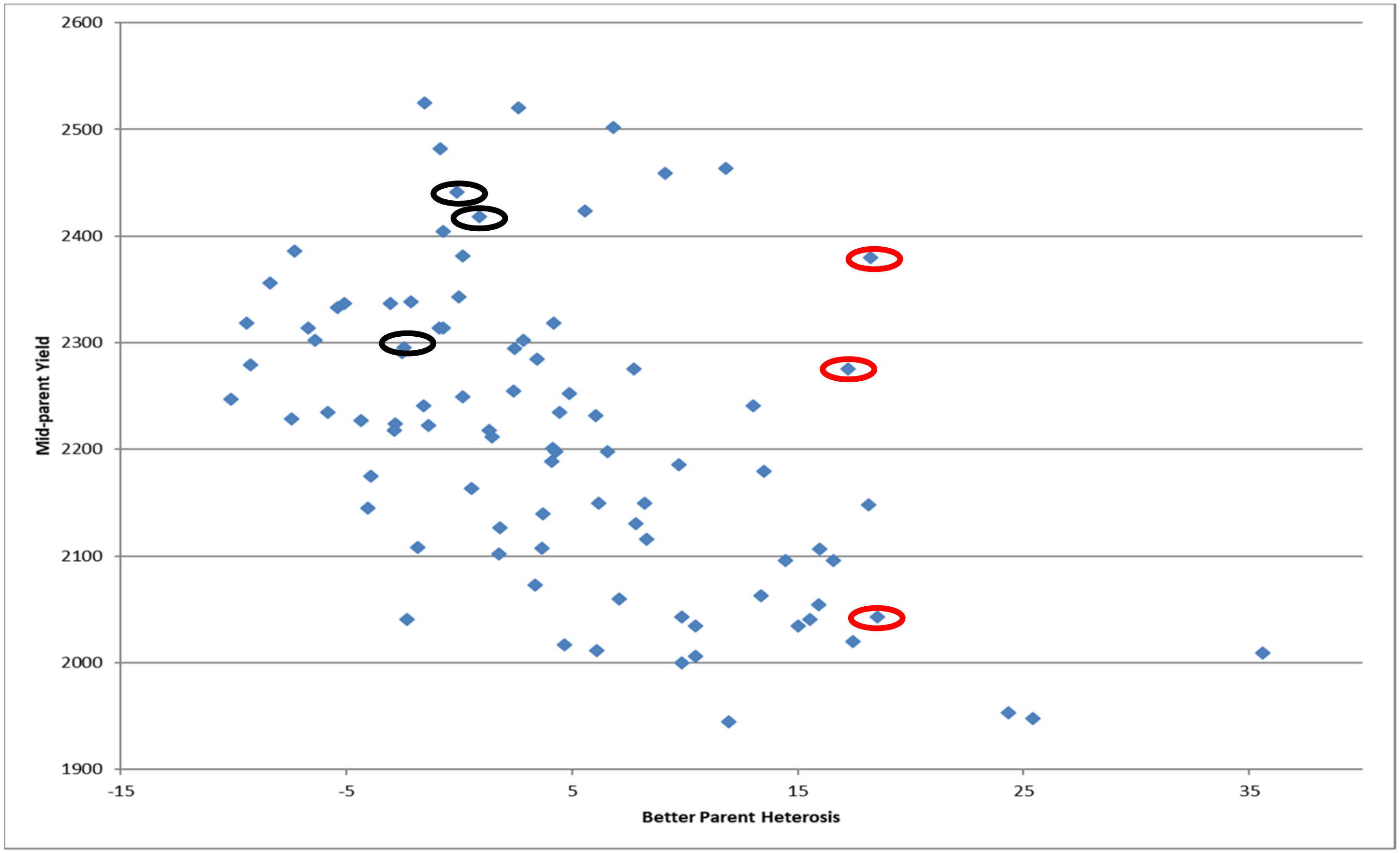
  -10% – 35%

- Wanted to select high and low heterosis populations for comparisons



Hybrid Selection

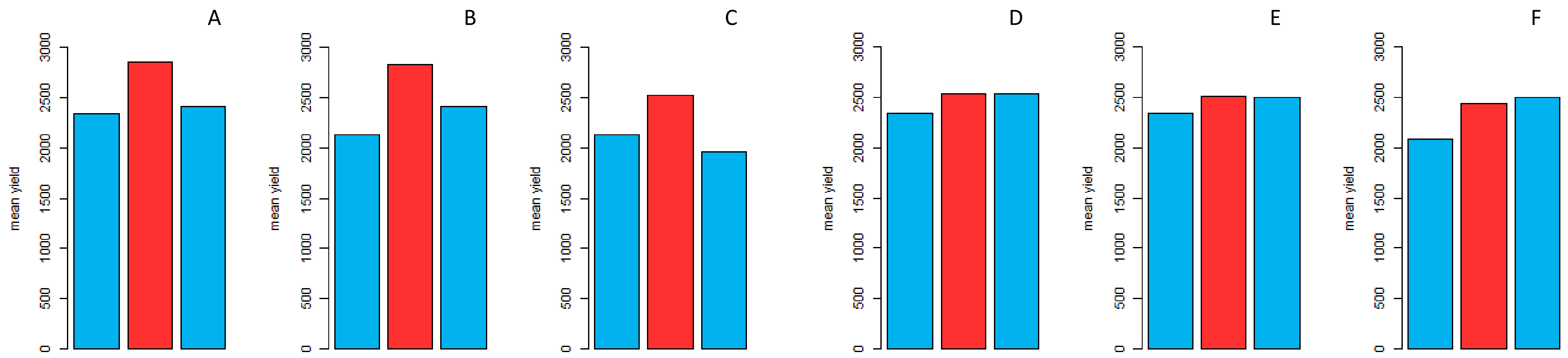
- Selected for high mean families of differing heterosis levels
  - Selection constraints due to seed production



Selected Populations

- 3 high heterosis populations
  - 17 - 18% better-parent
- 3 low heterosis populations
  - -2 - 0% better-parent

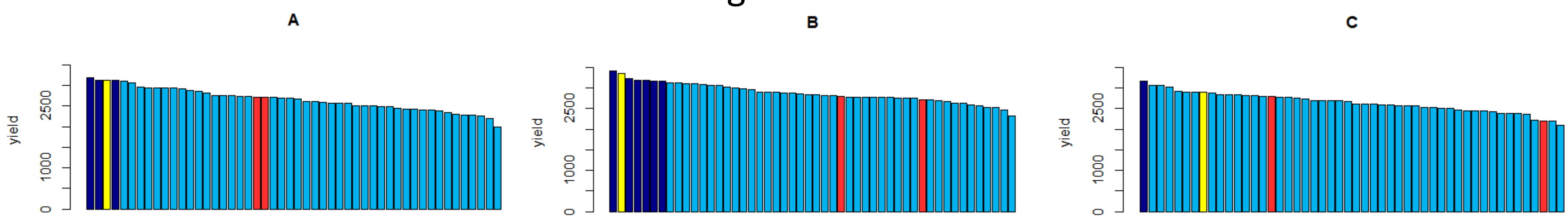
- Populations advanced to the F3:4 generation via SSD



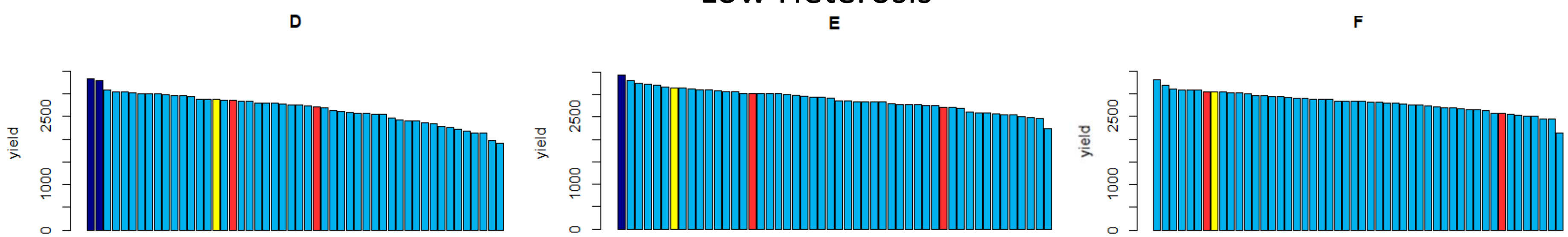
Yield Performance of Derived Lines

- Tested 6 populations of 47 F3:4 derived lines, F1, and parentals
  - 3 locations in 2017 in alpha-lattice design with 2 replications

High Heterosis



Low Heterosis



Comparisons Between Derived Populations

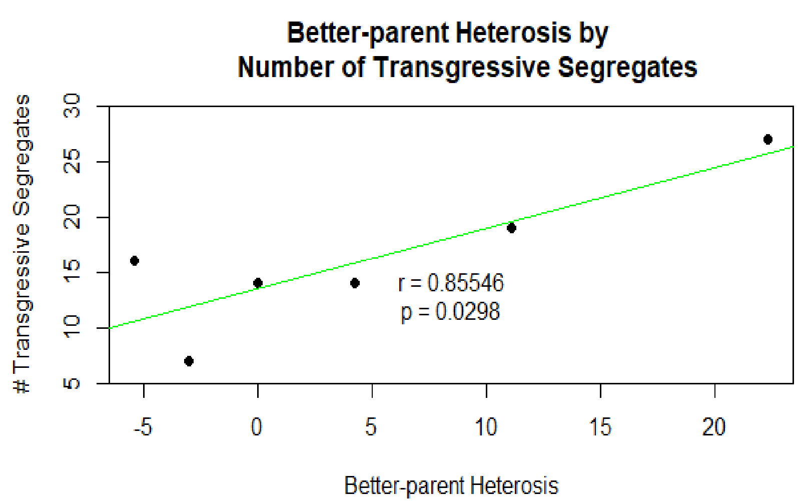
- Based on 2017 yield data, calculated better-parent heterosis levels and number of transgressive segregates

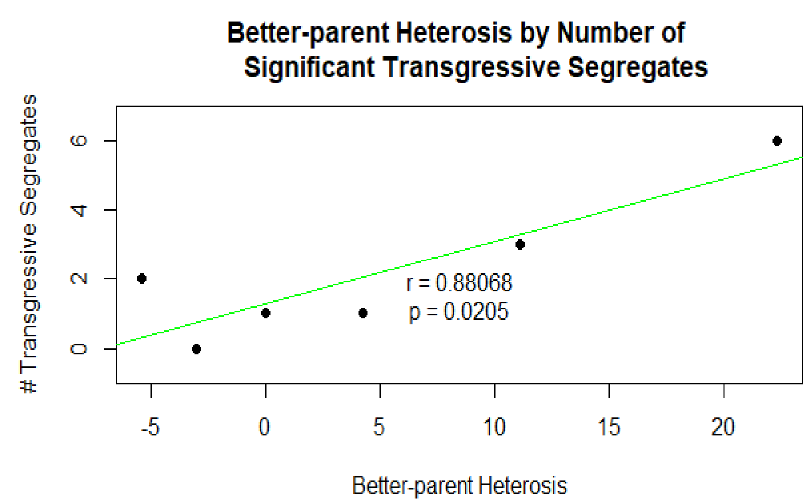
- Better-parent heterosis levels differed from 2016 to 2017
  - 17-18% -> 0-22%   -2-0% -> -5-4%

	Selected for high heterosis			Selected for low heterosis		
	A	B	C	D	E	F
ParentA mean yield	2821.335	2702.892	2702.892	2821.335	2821.335	2494.028
ParentB mean yield	2723.097	2723.097	2068.845	2884.423	2988.535	3056.175
F1 mean yield	3134.63	3331.305	2703.283	2729.325	3114.93	2964.303
Better-parent heterosis	11.10449	22.33517	0.014491	-5.3771	4.22933	-3.0061
Derived lines mean yield	2659.762	2874.453	2658.852	2646.4	2879.334	2818.403
Numerically Better	19	27	14	16	14	7
Significantly Better	3	6	1	2	1	0

Correlation Between Better-parent Heterosis in 2017 and Transgressive Segregates

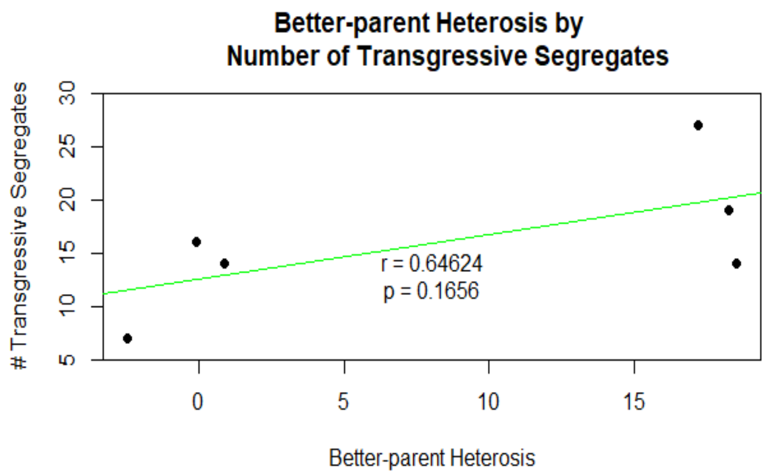
- Positive correlations were observed between better-parent heterosis and number of transgressive segregates
  - Pearson's correlation coefficient of .85 for numerically better than the better parent
  - Pearson's correlation coefficient of .88 for significantly better than the better parent

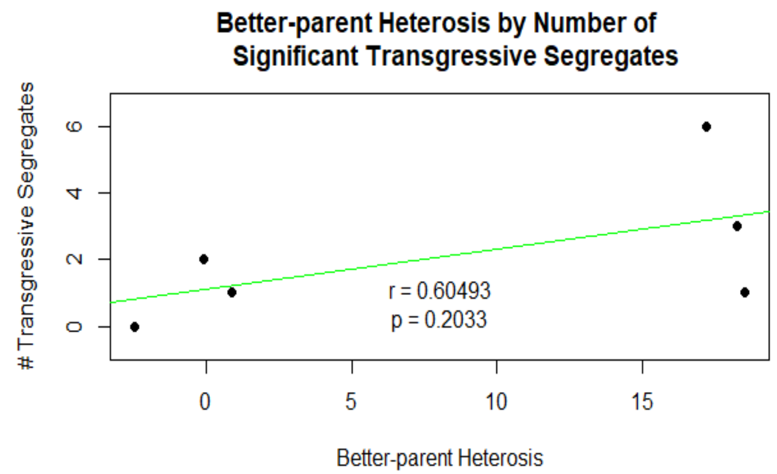




Correlation Between Better-parent Heterosis in 2016 and Transgressive Segregates

- Non-significant positive correlations observed between better-parent heterosis levels from 2016 yield data and transgressive segregates from derived populations in 2017
  - Pearson's correlation coefficient of .64 for numerically better than the better parent
  - Pearson's correlation coefficient of .60 for significantly better than the better parent





Next Steps for the Project

- Relationship between heterosis for yield and black shank resistance will also be examined

- Alkaloid and quality data will also be analyzed for these populations

Implications for Breeding Programs

- Shows potential for significant heterotic effects for yield in flue-cured tobacco crosses
- Positive relationship between better-parent heterosis and number of transgressive segregates
- Suggests year to year variation could complicate using heterosis as predictive factor
- Demonstrates potential for using heterosis level as screen for tobacco crosses when breeding for increased yield

Proposed Application of Heterosis Screen in Breeding Programs

- Generate diverse series of crosses
- Grow yield trials of all hybrids and parentals to calculate heterosis while also selfing F1’s to generate F2 populations
- Based on F1 heterosis data, advance only selected populations the following year
- Selections from the high heterosis populations should yield more transgressive segregates and increase yield of developed cultivars

Acknowledgements

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