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Comparing Genetic Variation within Red Winter Wheat Populations with and without Image-Based Optical Sorter Selection

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Abstract : **Head scab is historically a devastating disease affecting not just all classes of wheat but also barley and other small grains around the world. Fusarium head blight (FHB), or head scab, is caused most often by *Fusarium graminearum* (*Schwabe*), (sexual stage – *Gibberella zeae*) although several Fusarium spp. can cause the disease. This study was conducted to determine the effect of mass selection for FHB resistance using an image-based optical sorter. lines were derived from the C0 and C2 of two populations to compare genetic variation within populations with and without sorter selection. Our overall hypothesis is that sorting grain results in improved Fusarium head blight resistance. Both of the used wheat derived line populations have genetic variation, and population 1 has more than population 17. They are significantly different from each other for fusarium damged kernel (FDK), deoxynivalenol (DON), and other FHB traits. Although both populations are suitable to be grown for bulks, population 1 seems better since it has more genetic variation as well as lower FDK and DON, and earlier heading date. Lines within each population were significantly different and some lines in each population had significantly lower FDK and DON after selection using an optical sorter. Some lines had significant reduction in both FDK and DON, and some others had either FDK or DON reduction. Lines of population 1 that had significant reduction, were more numerous than in population 17, and FDK and DON reduction were greater.**

Keywords: Fusarium head blight (FHB), image-based optical sorter, mass selection, deoxynivalenol (DON).

I. INTRODUCTION

Wheat ((*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are critical food and feed cops around the world. In the United States, planted hectares of these two crops have declined drastically since the early 1990_s. One important factor of that reduction is a very challenging plant disease. The most devastating disease of wheat and barely is *Fusarium* head blight (FHB or scab), which is a fungal disease caused by the fungus, *Fusarium graminearum* Schwabe, resulted in the 1990_s and early 2000_s in billions of dollars of wheat and barely yield quantity and quality loss (1). That loss in the United States during the 1990s were close to $3 billion, and FHB ranked as the worst plant disease to hit the nation during the past seven decades (1,2,3). The infected grains appear discolored and shriveled, which is why they are called "tombstones". The disease also reduces test weight and lowers market grade because diseased kernels are light and shriveled (2,4). Host resistance is considered to be the most practical and effective strategy of controlling wheat scab, although breeding has been hindered by the complexity of the resistance, a lack of effective resistant genes, and disease assessment difficulty and cost (5,6). Resistance to FHB is controlled by multiple genes whose effects are greatly influenced by the environment (7,8). This complexity limits our understanding of the resistance mechanisms and has made breeding of FHB resistance very difficult and time consuming (7). The most important two types of resistance are 'Type I' (resistance to initial infection) and 'Type II'

(resistance to spread within the spike) (1,9). Yield loss and deoxynivalenol (DON) contamination are the main concerns related to FHB. Direct assessment of Fusarium damaged kernel (FDK) and DON is expensive and time consuming (6,10,11).

II. Material and Methods

Two sets of F_5 wheat populations from 2- and 3-way crosses among adapted plants were evaluated in this study in 2013: populations 1 and 17 (Table A.3.4). Seeds of 30 lines of non selected population C_0 and 30 lines after 2 selection cycles were planted in two replications in the scab nursery in 2013. The scab nursery was located on Spindletop Research Farm (38°7'37. 81'' N, 84°29'44. 85'' W; Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs]) near Lexington, KY (LEX). Recommended agricultural practices for wheat production in Kentucky were followed (12).

The grain used for this study was sorted on a USDA/ARS and National Manufacturing Seed Sorter System with color camera according to a calibration that reflected visual differences between asymptomatic grain and grain showing FHB symptoms. Calibrations for the image-based optical sorter, done each year depending on the environment and the severity of disease, was done by Dr. David Van Sanford and Dr. Anthony Clark. After calibration, the device was used for mass selection and/ or sorting. The lines used for this experiment were selected randomly in 2012 from C_0 and C_2 plots, in the form of 30 heads of each cycle from each population. These heads each was broken in half to be planted in 2 replication for each line. Seeds were planted in rows 1.2 meters long and 30 centimeters apart in the nursery. The scab nursery was irrigated with an overhead mist irrigation system that works automatically 15 times a night for 5 minutes each to provide favorable disease conditions from 8:00 pm to 8:30 am. The system operated from 8:00pm to 8:00am, beginning 22 April 2013. The irrigation system switched off from 25 April to 1 May 2013 because plants were very wet and the humidity level was high. Head rows were inoculated with scabby corn (*Zea mays L.)* (13). A number of isolates of *Fusarium graminearum* taken from scabby wheat seed collected from 2010 to 2013 in multiple locations across Kentucky were used for inoculation. Corn was spread on plastic sheets and set to imbibe water on 13 March 2013 before autoclaving. After autoclaving on 14 March 2013, PDA plugs of *F. graminerium* were mixed with 0.2 gram streptomycin in 50 ml sterile water and applied to the corn on 15 March 2013 at room temperature until corn was fully colonized by the fungus. Colonization took about three weeks. Scabby corn was stored in a freezer after being placed in mesh bags. Inoculum was spread prior to heading between the scab nursery rows of a rate of about 11.86 g m^2 on 15 April 2013.

Daily heading notes were taken in the scab nursery. Head rows that headed first were scored for disease first. Heading took place between 11 May and 27 May 2013. Disease incidence was calculated as the number of visually infected spikes divided by the total number of observed spikes per head row (20 heads per row). Disease severity assessment was done by counting the number of visually infected spikelets and dividing it by the total number of spikelets in 10 arbitrarily chosen heads from each row. Along with taking incidence and severity, rating was taken as well by visually estimating the proportion of scabby heads on a scale from 0 to 9 (9 means all heads in that head row showed FHB symptoms). The scoring was occurred 24 days after heading date. Height (inch) during the seed-filling period was recorded.

The scab nursery was harvested by sickling the 1.2-meter long rows using hand sickles and banding each row by it-self (above ground plants were cut). Threshing of the sickled head rows was done in a few days after harvest. They were threshed by a small thresher with a minimum of forced air to minimize light seed loss (tombstones), which was designed for this reason. Scab nursery yield were cleaned using a cleaning machine.

FDK (%) was visually estimated for each sample (approximately 15 g). Some samples weighted less than 15-g, but that would not affect the test since it had a proportion of FDK in the sample. The same samples were run into an air-separation machine specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate infected kernels from healthy kernels in 2012 and 2013 (10, 14, 15, 16,17,18,19). The FDK

evaluation took around 50 seconds per sample, and the net time that seeds were run into the machine by exposing them to the air was 10 seconds. Two fractions from each sample using this machine were obtained. The lighter portion of wheat (estimated as scabby kernels) was weighed, and mixed with the heavier portion of wheat (estimated as healthy kernels) and weighed again to get the total weight of each sample. Visual estimation of FDK was scored for the light portion to have an adjusted weight of the scabby portion. Visual estimation of the scabby fraction was taken to make an adjustment for the scabby weight because in some samples researchers observed some asymptomatic grains. That was not because of the differences between symptomatic and asymptomatic weight, but because of the differences between the size and the weight of different varieties, in which some asymptotic kernel varieties weighed more or less than others of the same size. Data was entered into a Microsoft Excel (2007) spreadsheet that was used to calculate FDK proportion for each sample using the following formulas:

$$\textbf{FDK (\%) = (SSW / TSW) * 100}$$

For adjusted FDK after multiplying the light portion by visual proportion the formula was:

$$\textbf{FDK (\%) = (SW / TW) * 100}$$

Where, SSW = Scabby seed weight (g), TSW = Total seed weight (g), and SW = Adjusted scabby seed weight (g)

For DON measurement, the same (15 g) samples were sent in coin envelopes to the University of Minnesota DON testing Lab for DON analysis. DON concentration (ppm) was measured by gas chromatography with mass spectrometry (GC-MS) according to (Agostinelli, 2009).

III. Statistical analysis

FHB and agronomic traits were estimated for each population on an entry mean basis using the following model:

$$\mathbf{Y_{i,j,k} = \mu + R_i + C_j + L_{k\,(j)} + \varepsilon_{i,j,k}}$$

Where: $Y_{i,j,k}$ = the observation in the *k*th line in the *j*th selection cycle in the *i*th replication for each population, μ = the overall mean, R_i = the effect of *i*th replication, C_j = the effect of *j*th selection cycle, $L_{k\,(j)}$ = the effect of *k*th line, $\varepsilon_{i,j,k}$ = the residual error.
Data were analyzed using the General Linear Model procedure (Proc GLM; SAS 2009, Proc ANOVA; SAS 2009, and Proc mean; SAS 2009).

IV. Results and Discussion

A. Among populations

The ANOVA presents significant differences between populations 1 (KY93C-0004-22-1/25R37//VA05W-517) and 17 (Truman/IL99-15867//VA03W-409). Differences between these populations were significant for all FHB traits (Table 5.1). For heading date, the mean was higher (the higher the mean the later the heading date), and mean DON was significantly ($P \leq 0.05$) lower in population number 1 than in population number 17. Population 17 is significantly shorter than population 1 (Table 1).

The ANOVA suggests no significant difference for FDK between the C_0 and the C_3 (Table 1). On the other hand, some FHB traits were significantly different between the C_0 and the C_3 including DON, but that does not mean there was significant DON reduction over the population with selection (Table 2). The performance of these two populations through selection cycles in the other experiments (other than 2013 derived line experiment) in 2012 and 2013 was as follows: In the 2013 scab nursery bulk experiment, there is evidence of a non significant FDK and DON reduction for population number 1. The 2012 Lexington plot data suggested a reduction in FDK and DON

with selection from cycle to cycle (C_0, C_1,C_2) (Figure 1). Both 2013 Lexington and Princeton plots showed fluctuating FDK (%) among selection cycles (Figures: 1, 2, 3, and 4).

B. Within population

To evaluate the selection done using an image based optical sorter, although there was slight non-significant reduction in FDK with selection, some lines in the C_3 were showed a significant FDK and DON reduction in both population (Tables 3 and 4).

Population 1 showed significant differences among lines in both C_0 (P-value = 0.0093) and C_2 (P-value = 0.0004). The same was observed in population 17 lines at (P=0.0307) in the C_0 and (P=0.0011) in the C_2 (Table 5). This indicates there is genetic variation within each population. However, population 1 seems to have more genetic variation than population 17. Out of 900 pairwise comparisons C_0 and C_2 of population 1, 426 FDK cases were lower, and out of these cases 69 cases were significant due to selection (Figure 2). Some superior lines in population 1 showed significantly lower FDK and DON reduction, (ex, line 1003-1) (Table 3). I observed 416 cases in which FDK and DON were lower after selection in population 17 (Figure 3), of which 45 were significantly lower (Table 4). Some lines had both FDK and DON reduction (e.g. 1003-1, 1003-17, and 1003-22 in population 1, and 1051-61, 1051-83, and 1051-87 in population 17) (Tables 3 and 4). Most of the lines in population 17 that had low FDK reduction had no DON reduction. On the other hand, lines that had FDK reduction in higher percentage had also DON reduction (Tables 4).

Conclusions

Both of the populations have genetic variation, and population 1 has more than population 17. They are significantly different from each other for FDK, DON, and other FHB traits. Although both populations are suitable to be grown for bulks, population 1 seems better since it has more genetic variation as well as lower FDK and DON (Tables 6, and 7), and earlier heading date. Lines within each population were significantly different and some lines in each population had significantly lower FDK and DON after selection using an optical sorter. Some lines had significant reduction in both FDK and DON, and some others had either FDK or DON reduction. Lines of population 1 that had significant reduction, were more numerous than in population 17, and FDK and DON reduction were greater.

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Table 1: ANOVA of FDK (%) assessed by air separation machine (FDK_AIR), adjusted scabby portion of FDK_AIR visually (FDK_ASJ), visual estimate (FDK_VST), deoxynivalenol (DON), heading date (HD) and plant height (HT), severity (SEV), incidence (INC), and rating (RATING) in the two derived lines populations on 2013 scab nursery.

		FDK_ADJ	FDK_AIR	FDK_VET
Source	**df**		**MS**	
Pop	1	7056.8 **	6447.3**	21206.4 **
Cycles	1	408.6	725.7 *	69.2
Pop * Cycle	1	89.2	34.5	156.8
Line (Pop * Cycle)	116	207.5 *	213.5	1011.5 **
Error	120	141.0	160.2	546.4
Total	239			
		DON	**HD**	**HT**
Source	**df**		**MS**	
Pop	1	4540.5 **	464.8 **	24.0 *
Cycles	1	391.9 **	48.6 **	66.1 **
Pop * Cycle	1	174.5 *	141**	1.0
Line (Pop * Cycle)	116	42.2 *	14.5 **	19.6 **
Error	120	29.3	1.0	5.7
Total	239			
		SEV	**INC**	**INDEX**	**RATING**
Source	**df**		**MS**	
Pop	1	87.1	0.1	16.0	1.0
Cycles	1	821.3 **	440.1	653.0 **	16.0 **
Pop * Cycle	1	5.5	30.1	7.9	0.01
Line (Pop * Cycle)	116	126.1 **	470.9 **	119.1 **	2.1 **
Error	120	68.9	188.4	57.4	1.3
Total	239				

* P-value ≤ 0.05 ** P-value ≤ 0.01

Table 2: FDK mean and standard error of each cycle within each population. Traits measured in the 2013 derived lines were: air separation machine (FDK_AIR), FDK proportion of air separation machine with visually adjusted scabby portion (FDK_ADJ), DON, incidence (INC), severity (SEV), rating, heading date (HD), plant height (HT).

	FDK_AIR		FDK_ADJ		DON		INC		SEV		Rating		HD		HT	
POP	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
1	20.0 ±2.7	22.7 ±2.7	19.2 ±2.6	20.6 ±2.6	11.2 ±0.8	15.5 ±0.8	58.8 ±2.3	62.3 ±2.3	23.0 ±1.3	26.4 ±1.3	2.6 ±0.2	3.2 ±0.2	21.7 ±0.4	21.0 ±0.4	40.0 ±0.5	38.8 ±0.5
17	29.6 ±2.7	33.8 ±2.7	28.9 ±2.6	32.7 ±2.6	21.6 ±0.8	22.5 ±0.8	59.5 ±2.3	61.5 ±2.3	23.9 ±1.3	27.9 ±1.3	2.5 ±0.2	3.0 ±0.2	17.3 ±0.4	19.8 ±0.4	39.2 ±0.5	38.3 ±0.5

Table 3: Lines from population 1 that had significant FDK and DON reduction after selection (C_2) using an image-based optical sorter in Lexington, KY, 2013.

Lines	Number of significant ($P \leq 0.05$) differences	
	FDK	DON
1003-1	10	14
1003-3	2	0
1003-4	3	0
1003-6	5	0
1003-7	2	3
1003-8	2	2
1003-12	7	3
1003-13	8	4
1003-14	2	4
1003-16	2	0
1003-17	5	11
1003-18	5	0
1003-22	6	7
1003-25	0	9
1003-26	5	0
1003-28	3	0

Table 4: Lines from population 17 that had significant FDK and DON reduction after selection (C_2) using an image-based optical sorter in Lexington, KY, 2013.

Lines	Number of significant ($P \leq 0.05$) differences	
	FDK	DON
1051-61	5	4
1051-62	2	0
1051-63	1	0
1051-64	3	0
1051-65	1	0
1051-66	1	0
1051-68	5	3
1051-69	1	0
1051-70	3	0
1051-71	1	0
1051-72	1	6
1051-73	1	0
1051-74	3	0
1051-75	1	0
1051-78	1	0
1051-79	1	0
1051-80	2	3

1051-81	1	0
1051-82	1	0
1051-83	3	8
1051-84	1	0
1051-85	0	2
1051-87	3	8
1051-89	2	0
1051-90	1	0

Table 5: ANOVA of FDK in derived lines from populations 1 and 17 evaluated in the 2013 scab nursery.

Population # 1			
		MS	
Source	**df**	**C_0**	**C_2**
Rep	1	232.6 *	0.7
Line	29	78.0 **	136.1 **
Error	29	31.8	37.6
Total	59		

Population # 17			
		MS	
Source	**df**	**C_0**	**C_2**
Rep	1	3372.8 **	4586.7 **
Line	29	231.1 *	384.8 **
Error	29	113.9	117.9
Total	59		

* P-value ≤ 0.05
** P-value ≤ 0.01

Table 6: FDK (%) mean, standard deviation, minimum, and maximum of FDK % assessed by air separation machine (FDK_AIR), FDK_AIR with visually adjusting scabby portion (FDK_ADJ), visual estimate (FDK_VST), heading date (HD), plant height (HT), deoxynivalenol (DON), severity (SEV), incidence (INC), FHB index, and rating before selection (C_0) and after selection (C_2) in population 1 (KY93C-0004-22-1/25R37//VA05W-517) in Lexington, KY, 2013.

Selection Cycle	N Obs	Variable	N	Mean	Std Dev	Minimum	Maximum
C_0	60	FDK_AIR	60	20.0	7.4	9.2	42.9
		FDK_ADJ	60	19.2	7.6	8.0	42.9
		FDK_VET	60	53.7	26.4	5.0	100.0
		HT	60	40.0	3.3	32.0	45.0
		HD	60	21.7	3.3	14.0	26.0
		DONppm	60	11.2	3.4	5.5	17.4
		SEV	60	23.0	9.7	10.0	59.3
		INC	60	58.8	18.6	20.0	90.0
		Index	60	14.4	9.2	2.5	44.4
		Rating	60	2.6	0.9	1.0	5.0
C_2	60						
		FDK_AIR	60	22.7	10.7	6.7	72.7
		FDK_ADJ	60	20.6	9.2	5.7	58.2
		FDK_VET	60	50.8	27.7	6.0	95.0
		HT	60	38.8	4.1	30.0	48.0
		HD	60	21.0	3.2	16.0	27.0
		DONppm	60	15.5	6.4	6.0	33.3
		SEV	60	26.4	8.9	10.4	47.9
		INC	60	62.3	18.6	25.0	100.0
		Index	60	17.3	9.3	2.6	38.3
		Rating	60	3.2	1.7	2.0	14.0

Table 7: FDK (%) mean, standard deviation, minimum, and maximum of FDK % assessed by air separation machine (FDK_AIR), FDK_AIR with visually adjusting scabby portion (FDK_ADJ), visual estimate (FDK_VST), heading date (HD), plant height (HT), deoxynivalenol (DON), severity (SEV), incidence (INC), FHB index, and rating before selection (C_0) and after selection (C_2) in population 17 (Truman/IL99-15867//VA03W-409) in Lexington 2013.

Selection Cycle	N Obs	Variable	N	Mean	Std Dev	Minimum	Maximum
C_0	60	FDK_AIR	60	36.1	51.0	12.8	40.7
		FDK_ADJ	60	39.2	60.3	12.8	40.7
		FDK_VET	60	70.9	27.0	7.0	100.0
		HT	60	39.2	3.5	29.0	47.0
		HD	60	17.3	1.4	14.0	20.0
		DONppm	60	21.6	6.2	8.0	33.2
		SEV	60	23.9	7.8	10.2	43.8
		INC	60	59.5	15.8	30.0	90.0
		Index	60	14.5	6.6	3.1	29.4
		Rating	60	2.5	1.2	1.0	9.0
C_2	60						
		FDK_AIR	60	33.8	18.2	11.1	100.0
		FDK_ADJ	60	32.7	18.0	7.8	95.0
		FDK_VET	60	71.3	30.1	5.0	100.0
		HT	60	38.3	3.1	33.0	46.0
		HD	60	19.8	2.7	15.0	26.0
		DONppm	60	22.5	7.2	8.0	38.1
		SEV	60	27.9	12.4	9.5	71.7
		INC	60	61.5	19.1	15.0	90.0
		Index	60	18.2	11.7	2.1	55.8
		Rating	60	3.0	1.2	1.0	7.0

Figure 1: Deoxynivalenol (DON; ppm) for three cycles of mass selection (0, 1, 2) of population 1 using an image-based optical sorter (Cycle) in Lexington, KY, 2012.

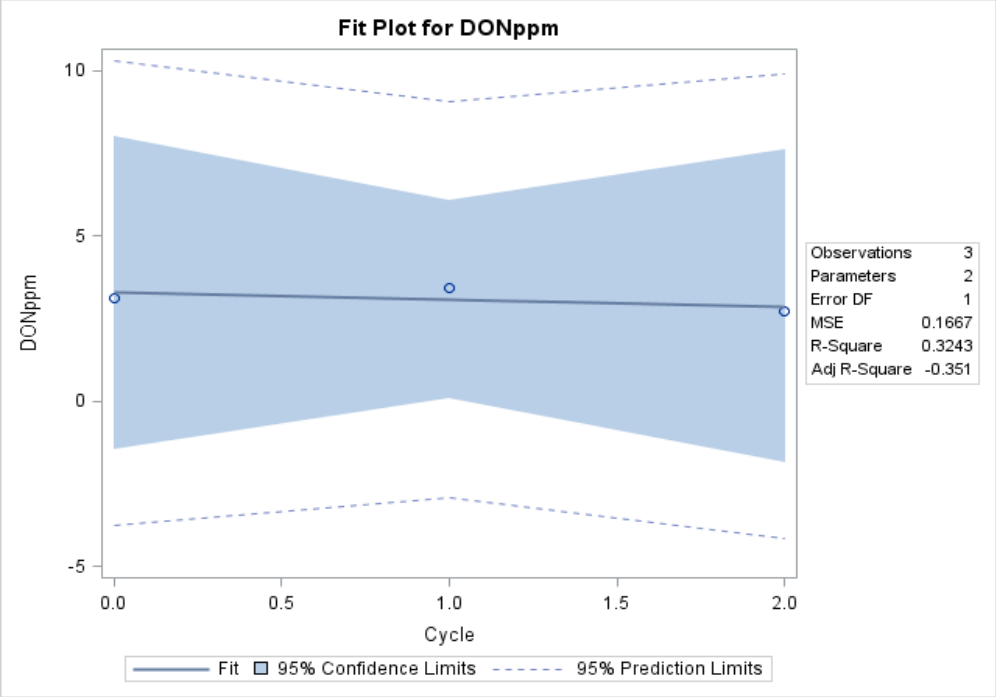


Figure 2: Combination of significant and non-significant FDK reduction due to selection derived lines of population 1 (KY93C-0004-22-1/25R37//VA05W-517) on the basis of their entry means (n=900) in Lexington, KY, 2013.

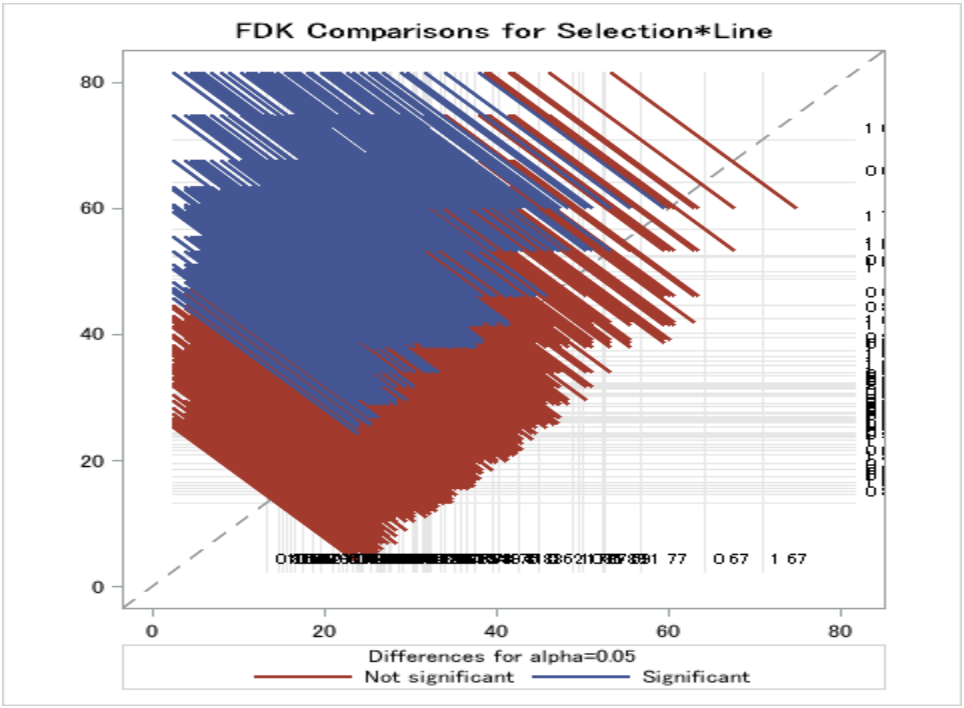
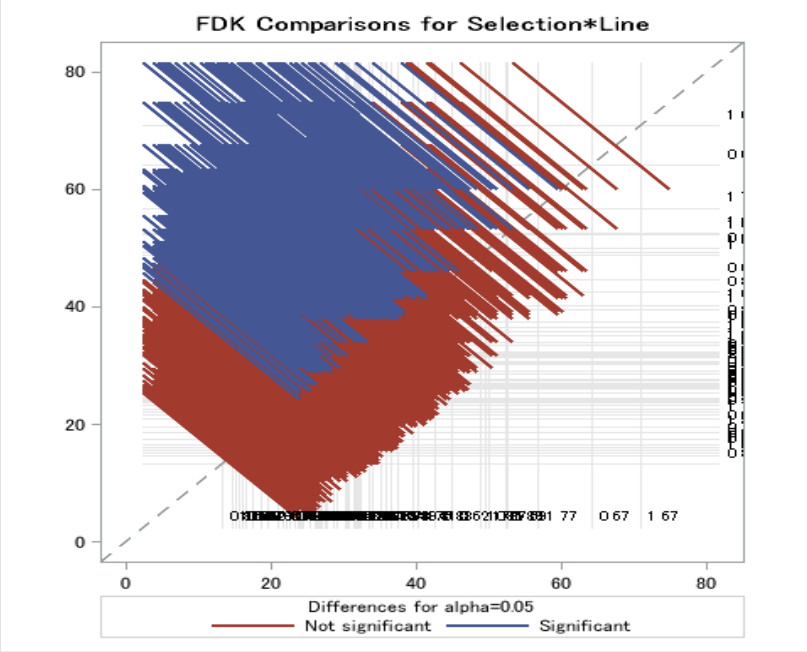


Figure 3: Combination of significant and non-significant FDK reduction due to selection derived lines of population 17 (Truman/IL99-15867//VA03W-409) on the basis of their entry means (n=900) in Lexington, KY, 2013.



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