Field Evaluation of Burley Lines Containing Alleles Minimizing the Conversion of Nicotine to Nornicotine

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Introduction and Background

- Reduction of tobacco specific nitrosamines (TSNA), especially nitroso-nornicotine (NNN), in tobacco products has been a major objective within the tobacco industry for several years.
- The release of low conversion (LC) burley tobacco varieties, which began in 2004, reduced the conversion of nicotine to nornicotine substantially, thereby reducing TSNA levels in burley tobacco to 2-4 ppm during most growing seasons.

Introduction and Background

- More recently, researchers at North Carolina State University have identified CYP82e4, CYP82e5v2 and CYP82e10 mutant alleles that reduce nicotine to nornicotine conversion even further, resulting in TSNA levels ranging from 0.5 to 0.8 ppm.
- A current objective of the Kentucky-Tennessee Tobacco Improvement Initiative is to transfer these mutant alleles into commercial burley tobacco varieties developed by KTTII.

Materials and Methods

- BC_6S_2 parental lines were evaluated for agronomic characteristics and black shank resistance in 2013.

Materials and Methods

The parental lines and varieties that were in the 2013 field trials:

Parental Lines

- TKS 2002Z – 14
- TKF 2002Z – 3
- TKF 4024Z – 8
- TKF 4028Z – 6
- TKF 6400Z – 6
- KY 14Z – 1
- L8Z – 1

Varieties

- KT 204Z – 4
- KT 206Z – 4
- KT 209Z – 4
- KT 210Z – 4
- KT 212Z – 4
- ms KY 14 X L8Z – 1
- ms TN 90Z – 1
- ms TN 86Z – 1

Materials and Methods

- Further backcrossing was undergone with the superior performers from 2013 to be included in the 2014 field trials.

Materials and Methods

The parental lines and varieties advanced to the 2014 field trials:

Parental Lines

- TKS 2002Z – BCe10
- TKF 2002Z – D SGe10
- TKF 2002Z – A
- TKF 2002Z - C
- TKF 4024Z – SGe3
- TKF 4024Z - F
- TKF 4028Z – A BCe10
- TKF 4028e2
- TKF 4028Z - F
- TKF 6400Z - B
- ms TN86Z -
- TN 86Z - C

Varieties

- KT 204Z
- KT 209Z
- KT 210Z
- KT 212Z
- ms TN 90Z
- TN 90Z

Experimental Design

- For the agronomic evaluation of KTTII varieties, each Z iteration was compared to the original LC variety.
- For parental lines in 2013, three Z iterations were randomly selected and compared to the original LC parental line.
- For 2014 all advanced parental lines were compared to the original LC.

Experimental Design

- A randomized complete block design with 3 replications was utilized; each variety or parental line family was grouped together and analyzed independently.
- There were four locations used for the field trials: Lexington and Versailles, KY and Greeneville and Springfield, TN. However due to adverse weather conditions not all crops were utilized for analysis.

Experimental Design

- For the evaluation of black shank resistance, all Z iterations of parental lines and varieties were compared to their original counterparts.
- A nursery having high levels of both race 0 and race 1 black shank was utilized.
- All entries were replicated four times, with variety “families” grouped together in the randomization and analyzed independently.

Results

- In general, within families there were visible differences among entries for plant type, leaf angle, leaf shape, etc.

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- In general, within families there were visible differences among entries for plant type, leaf angle, leaf shape, etc.
- For some families differences were subtle, but for other families differences were quite obvious.
- There was also extreme variation among individual plants within some families.

Three phenotypes within TKF 6400e3-C

Extremely off-type plant within a TKF 2002e3-C

Results

- This variation was likely due to mutations that were not detected in FT plants during the backcrossing process.
- After making backcrosses to superior 2013 lines, the yield, physical characteristics and black shank resistance were improved, as seen in the 2014 field trials.

KT 204LC vs KT 204Z (2014)

TN 90LC vs TN 90Z (2014)

Statistical Analyses

Summary of Statistical Results for Commercial Varieties

ANOVA PR > F

2013 Lexington

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of significance						
KT 204	0.29	0.03	0.21	0.26	0.05	0.01
KT 209	0.44	0.23	0.09	0.46	0.36	0.08
KT 210	0.93	0.78	0.05	0.23	0.24	0.46
KT 212	0.19	0.17	0.01	0.40	0.66	0.14
TN 86	0.12	0.06	0.97	0.17	0.01	0.35
TN 90	0.04	0.06	0.84	0.01	0.44	0.77

Summary of Statistical Results for Commercial Varieties

ANOVA PR > F

2013 Versailles

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
KT 204	0.10	0.12	0.35	0.73	0.20	0.02
KT 209	0.02	0.01	0.06	0.57	0.42	0.04
KT 210	0.19	0.86	0.80	0.52	0.06	0.72
KT 212	0.63	0.16	0.04	0.09	0.02	0.21
TN 86	0.26	0.09	0.69	0.52	0.03	0.37
TN 90	0.93	0.62	0.01	0.83	0.25	0.36

Summary of Statistical Results for Commercial Varieties

ANOVA PR > F

2014 Versailles

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
KT 204	0.19	0.41	0.73	0.21	0.88	0.70
KT 209	0.38	0.45	0.98	0.04	0.08	0.16
KT 210	0.35	0.65	0.22	0.20	0.64	0.87
KT 212	0.86	0.88	0.91	0.83	0.62	0.46
TN 86	0.69	0.64	0.84	0.55	0.57	0.05
TN 90	0.61	0.46	0.97	0.45	0.56	0.21

Summary of Statistical Results for Commercial Varieties
ANOVA PR > F

2014 Greeneville

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
KT 204	0.72	0.37	0.29	0.94	0.06	0.14
KT 209	0.03	0.97	0.12	0.87	0.97	0.98
KT 210	0.11	0.33	0.54	0.99	0.15	0.52
KT 212	0.80	0.11	0.42	0.15	0.05	0.11
TN 86	0.85	0.97	0.60	0.36	0.27	0.55
TN 90	0.90	0.51	0.70	0.12	0.45	0.09

Summary for Statistical Results of Commercial Varieties

ANOVA PR > F

2014 Springfield

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
KT 204	0.86	0.45	0.54	0.57	0.50	**0.04**
KT 209	0.40	0.31	0.76	0.77	0.82	0.81
KT 210	0.76	0.82	0.38	0.98	0.70	0.89
KT 212	0.71	0.51	0.21	0.82	0.54	0.36
TN 86	0.85	0.47	0.80	0.95	0.80	0.78
TN 90	0.67	0.14	0.75	0.86	0.73	**0.04**

Summary of Statistical Results for Parental Lines

ANOVA PR > F

2013 Lexington

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
TKF 2002	0.50	0.88	0.16	0.33	0.11	**0.08**
TKS 2002	0.93	0.29	0.91	0.11	0.56	0.73
TKF 4024	0.39	0.13	0.40	0.44	0.28	0.13
TKF 4028	0.86	0.21	0.73	0.24	0.43	0.18
TKF 6400	0.40	0.82	**0.05**	0.40	0.15	0.58

Summary of Statistical Results for Parental Lines

ANOVA PR > F

2013 Versailles

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
TKF 2002	0.30	0.44	0.30	0.08	0.37	0.12
TKS 2002	0.36	0.97	0.01	0.01	0.09	0.02
TKF 4024	0.11	0.23	0.19	0.44	0.08	0.41
TKF 4028	0.55	0.86	0.70	0.01	0.04	0.14
TKF 6400	0.09	0.05	0.04	0.46	0.35	0.34

Summary of Statistical Results for Parental Lines
ANOVA PR > F

2014 Versailles

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
TKF 2002	0.47	0.64	0.98	0.49	0.68	**0.01**
TKS 2002	0.55	**0.02**	0.16	0.48	0.83	0.11
TKF 4024	0.52	0.50	0.83	0.66	0.52	0.36
TKF 4028	0.26	0.44	0.83	**0.01**	0.33	0.55
TKF 6400	0.92	0.61	0.36	0.96	0.96	0.66

Summary of Statistical Results for Parental Lines
ANOVA PR > F

2014 Greeneville

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
TKF 2002	0.27	**0.07**	**0.03**	0.25	0.33	0.54
TKS 2002	0.50	0.20	0.37	0.25	0.23	0.40
TKF 4024	0.44	0.26	0.53	0.37	0.21	0.63
TKF 4028	0.49	0.22	0.46	0.64	0.83	0.11
TKF 6400	0.12	0.84	0.21	0.58	0.80	0.91

Summary of Statistical Results for Parental Lines
ANOVA PR > F

2014 Springfield

Variety Family	Plant Height	Leaf Number	Leaf Internode	Leaf Width	Leaf Length	Yield
Level of Significance						
TKF 2002	**0.09**	0.58	0.43	0.33	0.67	0.96
TKS 2002	0.73	0.65	0.81	0.58	0.60	0.86
TKF 4024	0.77	0.90	0.97	0.57	0.79	0.80
TKF 4028	0.89	0.19	0.72	0.84	0.81	0.20
TKF 6400	0.97	0.73	0.86	0.24	0.76	0.76

Black Shank Resistance

It is often difficult to show significant differences for black shank resistance because the disease often stunts plants without actually killing them (often referred to as “hidden” black shank). For example, among the 14 TKS 2002Z iterations grown in 2013 there were obvious differences among lines that were not statistically significant.

Photo 3. TN 90LC: 8-27-13 Birdwell Nursery
Rep 1, 71.4%; Rep 2, 90.5%
Mean BS Survival = 81.0%

Photo 4: ms TN 90e3 8-27-13 BW
Rep 1, 40.9%; Rep 2, 14.3%
Mean BS Survival = 27.6%

Photo 5. TN 90e3 8-27-13 BW
Rep 1, 57.1%; Rep 2, 25.0%
Mean BS Survival = 41.1%

Comparison of LC vs Zyvert Parental Lines for Resistance to Race 1 Black Shank

Entry	2013 MEAN	2014 MEAN	2015 MEAN
TN 90LC	79	36	71
ms TN 90LC	84		
ms TN 90Z	38	26*	85**
TN 90Z	41	34*	76**
TKF 2002LC FS	93	87	93
TKF 2002Z	85	92*	93
TKS 2002LC FS	98	88	92
TKS 2002Z	98	90*	90
TKF 4024LC	95	87	96
TKF 4024Z FS	95	93*	97
TKF 4028LC	37	33	38
TKF 4028Z Bd Sd	0	27**	33**
TKF 6400LC	77	74	93
TKF 6400Z FS	67	74	94

*One additional backcross to LC parental line

**Two additional backcrosses to LC parental line

2014 Black Shank Yield Trials
Mean for Two Locations*

Variety	Yield Lbs/A	Black Shank Survival (%)
TN 90LC	1387	47
ms TN 90Z	1147	44
	-240	-3
KT 204LC	2511	74
KT 204Z	1874	66
	-637	-8
KT 209LC	3172	90
KT 209Z	3276	93
Difference	+104	+3
KT 210LC	2851	86
KT 210Z	2874	87
Difference	+23	+1
KT 212LC	1223	46
KT 212Z	1387	53
Difference	+164	+7

*Six reps at each location

3 reps with 1 pt/A Metalaxyl, 3 reps with 3 pts/A Metalaxyl

2014 Black Shank Yield Trials
Mean for Two Locations*

Variety	Yield Lbs/A	Black Shank Survival (%)
TN 90LC	1387	47
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KT 204LC	2511	74
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KT 209LC	3172	90
KT 209Z	3276	93
Difference	+104	+3
KT 210LC	2851	86
KT 210Z	2874	87
Difference	+23	+1
KT 212LC	1223	46
KT 212Z	1387	53
Difference	+164	+7

*Six reps at each location

3 reps with 1 pt/A Metalaxyl, 3 reps with 3 pts/A Metalaxyl

2015 Black Shank Yield Trials
Mean for Two Locations*

Variety	Yield Lbs/A	Black Shank Survival (%)
TN 90LC	2072	72
ms TN 90Z	2570	84
Difference	+498	+11
KT 204LC	3303	92
KT 204Z	3025	90
Difference	-278	-2
KT 206LC	3171	91
KT 206Z	2612	85
Difference	-558	-6

*Six reps at each location

3 reps with 1 pt/A Metalaxyl, 3 reps with 2 pts/A Metalaxyl

Effect of Additional Backcrosses on Black Shank Resistance in ms TN 90Z

Variety	Yield Lbs/A	Black Shank Survival (%)
2013 - Original ms TN 90Z		
TN 90LC	-	79
ms TN 90Z	-	38
		-41
2014 - One additional backcross to TN 90LC*		
TN 90LC	1387	47
ms TN 90Z	1147	44
	-240	-3
2015 - Two additional backcrosses to TN 90LC*		
TN 90LC	2072	72
ms TN 90Z	2570	84
Difference	+498	+11

*Six reps at each location

3 reps with 1 pt/A Metalaxyl, 3 reps with 2 pts/A Metalaxyl

Effect of Additional Backcrosses to TN 90Z on Black Shank Resistance in KT 204Z

Variety	Yield Lbs/A	Black Shank Survival (%)
2014 - Original TN 90Z as pollinator*		
KT 204LC	2511	74
KT 204Z	1874	66
	-637	-8
2015 - One additional backcross to TN 90LC*		
KT 204LC	3303	92
KT 204Z	3025	90
Difference	-278	-2
2016 - Two additional backcrosses to TN 90LC*		
KT 204LC	?	?
KT 204Z	?	?
Difference	?	?

*Six reps at each location

3 reps with 1 pt/A Metalaxyl, 3 reps with 2 pts/A Metalaxyl

Comparison of LC vs Zyvert Parental Lines for Resistance to Race 1 Black Shank

Entry	2013 MEAN	2014 MEAN	2015 MEAN
TN 90LC	79	36	71
TN 90Z	41	34*	76**
TKS 2002LC FS	98	88	92
TKS 2002Z	98	90*	90

*One additional backcross to LC parental line

**Two additional backcrosses to LC parental line

Results from Variety Evaluations

Performance of "Z" Commercial Variety Trials by Location
2014

ENTRY	Yield (Lbs/Acre)			
	TES	HRES	WC	Mean
TN 86LC	3334	2408	3499	**3080**
TN 86e	3303	2492	3209	**3001**
ms TN 86Z	3105	2582	2633	**2773**
KT 209LC	3272	2488	3080	**2947**
KT 209Z	3266	2411	2639	**2832**
KT 210LC	3499	2373	2912	**2928**
KT 210Z	3314	2343	3001	**2886**
KT 212LC	2974	2518	3109	**2867**
KT 212Z	2430	2433	2982	**2615**

Summary

- Although visible differences could be noted among individual entries within each variety or parental line family, in many cases they were inconsequential.
- Chemical analyses show that nornicotine and TSNA content is steady at lower levels in the Z varieties.

Summary

- TN 86, KT 209, KT 210, and KT 212: Differences between the best Z and LC parental lines/varieties were minor; Z versions of these varieties were released in March 2015.
- TN 90: Is expected to be released March of 2016
- KT 204 and KT 206: Additional backcrosses were needed in the Z parental lines to improve plant type and/or black shank resistance before being considered identical to the original LC varieties with respect to agronomic type and disease resistance.

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