

## NATIONAL TURFGRASS EVALUATION PROGRAM (NTEP)

### ANCILLARY TRIAL – KENTUCKY BLUEGRASS DNA GENOTYPING

#### Report on NTEP Kentucky Bluegrass DNA Genotyping

Rutgers University - Josh Honig Ph.D., Stacy Bonos Ph.D., and William Meyer Ph.D.

#### Background

In Fall 2011, the NTEP Policy Committee decided to fund an ancillary study that uses new DNA genotyping techniques, developed by Rutgers University researchers, to classify Kentucky bluegrass entries. Therefore, each entry sponsored and submitted to the 2011 National Kentucky Bluegrass Test was charged an additional fee to conduct this analysis by Rutgers University. The results of this analysis were completed in spring 2015 and are therefore, published for the first time here (see the following sheets). The Rutgers University team of Dr. Josh Honig, Dr. Stacy Bonos and Dr. William Meyer will prepare and publish, in a refereed scientific journal, more detailed information on the procedures conducted and data collected. The following is a summary of the results from the NTEP entries only.

#### Procedures

One hundred fifty-six (156) cultivars and accessions of Kentucky bluegrass were seeded into in the turf greenhouse facility in New Brunswick, NJ. The 156 entries included the following: all 82 entries of the 2011 NTEP Kentucky Bluegrass test, 74 additional Kentucky bluegrass cultivars/accessions, and one natural *Poa annua* population collected at Rutgers Horticultural Research Farm II (North Brunswick, NJ). These entries were field planted in spring 2012.

In June 2013, all individual entries were visually inspected for apomixis in the field nursery during the flowering period of Kentucky bluegrass. Of the 156 original Kentucky bluegrass cultivars/accessions, 13 Kentucky bluegrass cultivars/accessions (12 from the added cultivars/accessions, plus the standard cultivar America) exhibited less than 50% apomixis in the field nursery. This low level of apomixis was assumed to be contamination in the affected entries, resulting in all 13 cultivars/accessions being removed from further analysis. The loss of the standard cultivar America was compensated for by including a number of America-type cultivars in the added group of cultivars/accessions.

Due to the findings of Bushman et al. (2013), DNA from eight (8) plants of each entry was collected to represent a cultivar/accession. Thirty-five (35) microsatellite markers from Honig et al. (2010) were used to DNA genotype eight plants from each of the 144 bluegrass cultivars, experimental selections, and accessions (1152 total samples).

## **Results and Summary**

Some departures from the previously defined (Honig et al., 2012) bluegrass classification types include Julia cultivars/accessions being split into two groups in the current analysis, as well as a number of European cultivars/accessions/collections (mixed with some North American cultivars/accessions/collections) being interspersed between some of the traditional classification types.

The current microsatellite analysis of the 2011 NTEP Kentucky Bluegrass Test shows that the vast majority of all cultivars, experimental selections, and collections were uniquely identified (genetically differentiated) with the current set of microsatellite markers. Strong genetic support was found for the Midnight, Cynthia, Julia, BVMG, Limousine, Mid-Atlantic, America, Shamrock, P-105, and Compact bluegrass classification types. Additional entries or analyses may be needed to clarify the genetic relationships of the split Julia classification type and the remaining unclassified (predominantly European) entries.

## **Literature Cited**

- Bushman, B. S., et al. 2013. Molecular markers highlight variation within and among Kentucky bluegrass varieties and accessions. *Crop Sci.* 53(5): 2245-2254.
- Honig, J. A., et al. 2010. Isolation and characterization of 88 polymorphic microsatellite Markers in Kentucky bluegrass (*Poa pratensis* L.). *Hort Sci.* 45(11): 1759-1763.
- Honig, J. A., et al. 2012. Classification of Kentucky bluegrass cultivars and accessions based on microsatellite markers. *Hort Sci.* 47(9): 1356-1366.

2011 NTEP KENTUCKY BLUEGRASS TEST - DNA GENOTYPING RESULTS FROM RUTGERS UNIVERSITY

| Entry No. | Entry Name    | Classification/Type   | Entry No. | Entry Name    | Classification/Type |
|-----------|---------------|-----------------------|-----------|---------------|---------------------|
| 1         | BAR 12PP 612  | Eurasian              | 42        | Avid          | P-105               |
| 2         | BAR 8PP 504   | Eurasian              | 43        | Empire        | America             |
| 3         | America       | Unreliable seed stock | 44        | Burl 3-51     | Eurasian            |
| 4         | A05-361       | Mid-Atlantic          | 45        | Burl 06-11    | America             |
| 5         | Cabernet      | Mid-Atlantic          | 46        | PST-K4-3      | Eurasian            |
| 6         | Award         | Midnight              | 47        | PST-K9-90     | Eurasian            |
| 7         | NuChicago     | Midnight              | 48        | PST-K10-106D  | Julia               |
| 8         | Kenblue       | Eurasian              | 49        | BAR PP 119327 | Eurasian            |
| 9         | J1770         | Midnight              | 50        | Barduke       | BMVG                |
| 10        | J-1136        | Midnight              | 51        | Baron         | BMVG                |
| 11        | Rush          | Limousine             | 52        | Barvette HGT  | Eurasian            |
| 12        | Sudden Impact | Midnight              | 53        | BAR VV 112916 | Eurasian            |
| 13        | J-1853        | Midnight              | 54        | BAR PP 110358 | Eurasian            |
| 14        | Waterworks    | America               | 55        | BAR PP 119326 | Eurasian            |
| 15        | A05-329       | America               | 56        | BAR VV 118532 | Eurasian            |
| 16        | Oasis         | America               | 57        | Skye          | Midnight            |
| 17        | A98-363       | America               | 58        | Blue Coat     | Eurasian            |
| 18        | RAD-1492      | Eurasian              | 59        | AKB 2555      | Shamrock            |
| 19        | A04-38        | Shamrock              | 60        | A00-4199      | Eurasian            |
| 20        | A05-360       | Mid-Atlantic          | 61        | A06-26        | America             |
| 21        | PPH 9131      | Eurasian              | 62        | PICK TD8      | America             |
| 22        | DPPP 818      | BMVG                  | 63        | SRX 5321      | Shamrock            |
| 23        | PP 10847      | Limousine             | 64        | PICK TD9      | Eurasian            |
| 24        | PICK 033      | Mid-Atlantic          | 65        | Blackjack     | Eurasian            |
| 25        | PICK MP07     | Eurasian              | 66        | Arrowhead     | Shamrock            |
| 26        | PICK 4340     | America               | 67        | PST-K9-99     | Eurasian            |
| 27        | SRX 446       | Mid-Atlantic          | 68        | Lunar         | Eurasian            |
| 28        | SRX 2758      | Mid-Atlantic          | 69        | PST-T10-18    | Eurasian            |
| 29        | SRX 4338      | America               | 70        | Endurance     | Eurasian            |
| 30        | LTP-08-6      | America               | 71        | PST-K9-97     | Eurasian            |
| 31        | A00-2882      | Compact               | 72        | Rubix         | Eurasian            |
| 32        | Keeneland     | America               | 73        | 3733          | Eurasian            |
| 33        | A06-46        | Eurasian              | 74        | Dauntless     | Shamrock            |
| 34        | A05-TB-382    | Mid-Atlantic          | 75        | A05-999       | Eurasian            |
| 35        | H99-1653      | Eurasian              | 76        | A06-47        | Eurasian            |
| 36        | Bluenote      | America               | 77        | RAD-507       | America             |
| 37        | Legend        | America               | 78        | A04-36        | Shamrock            |
| 38        | Zinger        | Shamrock              | 79        | Shamrock      | Shamrock            |
| 39        | A03-1017      | Midnight              | 80        | A01-1106      | Compact             |
| 40        | A04-74        | America               | 81        | Midnight      | Midnight            |
| 41        | RAD-849       | Eurasian              | 82        | Thermal Blue  | Eurasian            |

SORTED BY ENTRY NUMBER

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| Entry No. | Entry Name    | Classification/Type | Entry No. | Entry Name    | Classification/Type   |
|-----------|---------------|---------------------|-----------|---------------|-----------------------|
| 14        | Waterworks    | America             | 1         | BAR 12PP 612  | Eurasian              |
| 15        | A05-329       | America             | 2         | BAR 8PP 504   | Eurasian              |
| 16        | Oasis         | America             | 8         | Kenblue       | Eurasian              |
| 17        | A98-363       | America             | 18        | RAD-1492      | Eurasian              |
| 26        | PICK 4340     | America             | 21        | PPH 9131      | Eurasian              |
| 29        | SRX 4338      | America             | 25        | PICK MP07     | Eurasian              |
| 30        | LTP-08-6      | America             | 33        | A06-46        | Eurasian              |
| 32        | Keeneland     | America             | 35        | H99-1653      | Eurasian              |
| 36        | Bluenote      | America             | 41        | RAD-849       | Eurasian              |
| 37        | Legend        | America             | 44        | Burl 3-51     | Eurasian              |
| 40        | A04-74        | America             | 46        | PST-K4-3      | Eurasian              |
| 43        | Empire        | America             | 47        | PST-K9-90     | Eurasian              |
| 45        | Burl 06-11    | America             | 49        | BAR PP 119327 | Eurasian              |
| 61        | A06-26        | America             | 52        | Barvette HGT  | Eurasian              |
| 62        | PICK TD8      | America             | 53        | BAR VV 112916 | Eurasian              |
| 77        | RAD-507       | America             | 54        | BAR PP 110358 | Eurasian              |
|           |               |                     | 55        | BAR PP 119326 | Eurasian              |
| 22        | DPPP 818      | BMVG                | 56        | BAR VV 118532 | Eurasian              |
| 50        | Barduke       | BMVG                | 58        | Blue Coat     | Eurasian              |
| 51        | Baron         | BMVG                | 60        | A00-4199      | Eurasian              |
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| 31        | A00-2882      | Compact             | 65        | Blackjack     | Eurasian              |
| 80        | A01-1106      | Compact             | 67        | PST-K9-99     | Eurasian              |
|           |               |                     | 68        | Lunar         | Eurasian              |
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| 20        | A05-360       | Mid-Atlantic        | 75        | A05-999       | Eurasian              |
| 24        | PICK 033      | Mid-Atlantic        | 76        | A06-47        | Eurasian              |
| 27        | SRX 446       | Mid-Atlantic        | 82        | Thermal Blue  | Eurasian              |
| 28        | SRX 2758      | Mid-Atlantic        |           |               |                       |
| 34        | A05-TB-382    | Mid-Atlantic        | 48        | PST-K10-106D  | Julia                 |
|           |               |                     |           |               |                       |
| 6         | Award         | Midnight            | 19        | A04-38        | Shamrock              |
| 7         | NuChicago     | Midnight            | 38        | Zinger        | Shamrock              |
| 9         | J1770         | Midnight            | 59        | AKB 2555      | Shamrock              |
| 10        | J-1136        | Midnight            | 63        | SRX 5321      | Shamrock              |
| 12        | Sudden Impact | Midnight            | 66        | Arrowhead     | Shamrock              |
| 13        | J-1853        | Midnight            | 74        | Dauntless     | Shamrock              |
| 39        | A03-1017      | Midnight            | 78        | A04-36        | Shamrock              |
| 57        | Skye          | Midnight            | 79        | Shamrock      | Shamrock              |
| 81        | Midnight      | Midnight            |           |               |                       |
|           |               |                     | 3         | America       | Unreliable seed stock |
| 42        | Avid          | P-105               |           |               |                       |

SORTED BY CLASSIFICATION/TYPE