

# Application of SSRs Developed and Characterized for Buffalograss to Assess Sequence Variation

Carol A. Caha, Teresa Donze-Reiner\* and Keenan L. Amundsen

Department of Agronomy & Horticulture  
University of Nebraska – Lincoln

## Introduction

Buffalograss [*Buchloë dactyloides* (Nutt.)Engel. syn *Bouteloua dactyloides* (Nutt.) Columbus] is a low input, sustainable turfgrass species. For turf aesthetic purposes, cultural practices, and increased seed yield, female plants of this dioecious species are preferred over male types.

Seed production fields are generally seeded to a segregating population of seed derived from a synthetic hybrid. These fields yield approximately 500 lbs of seed per acre annually. The production fields naturally segregate 1:1 for gender and thus half of the field consists of male plants. This is more males than necessary for pollination and reduces the number of females available for seed production.

In 2010, experimental breeder fields were vegetatively established to alternating two male rows to nine female rows. In 2011 and 2012 an increased number of male contaminants in the female rows was observed.

Figure 1. A typical buffalograss seed production field.



Figure 2. Experimental Buffalograss production field planted to alternating rows of males and females. Male floral structures are color-enhanced in red to show location of male types.



## Objectives

- 1) Identify simple sequence repeat (SSR) markers derived from buffalograss transcriptome data that can differentiate the female parents from the male parent used in a production field.
- 2) Determine if SSR data could elucidate the origin of the invasive male types in the female rows as being the result of:
  - a) aggressive males sending stolons into the female rows
  - b) seed from hybrid crosses that germinated in female rows or
  - c) reversion to monoecious status resulting in male structures on plants that were previously female.

## Materials and Methods

**Marker Development:** Next generation sequencing (NGS) technologies were used to develop SSR markers at reduced cost and effort of traditional methods. The transcriptomes of buffalograss cultivars Prestige and 378 were sequenced and assembled *de novo*.

The Prestige transcriptome was mined for SSR-containing sequences by the software program RepeatMasker. An *in silico* approach was used to identify SSRs that differed by copy number between 378 and Prestige. A total of 96 SSR primer pairs were designed from the 1,586 found SSRs.

**SSR Application:** A set of 24 SSR primer pairs was screened and two primer pairs (B4 and E7) were selected for their ability to differentiate the one male parent (2996) from the three female parents (2995, 3000, and 3005) used to establish the production field (Figure 2).

The 5' 6-FAM modified SSRs were used to amplify DNA from parents and 100 invasive male types collected in the female rows. SSRs were run on an ABI3730 at the University of Nebraska Medical Center and fragment analysis was performed using GeneMapper software.

## Results

Figure 3. B4 and E7 SSR peak overlays for the one male and three female parents used in a production field.

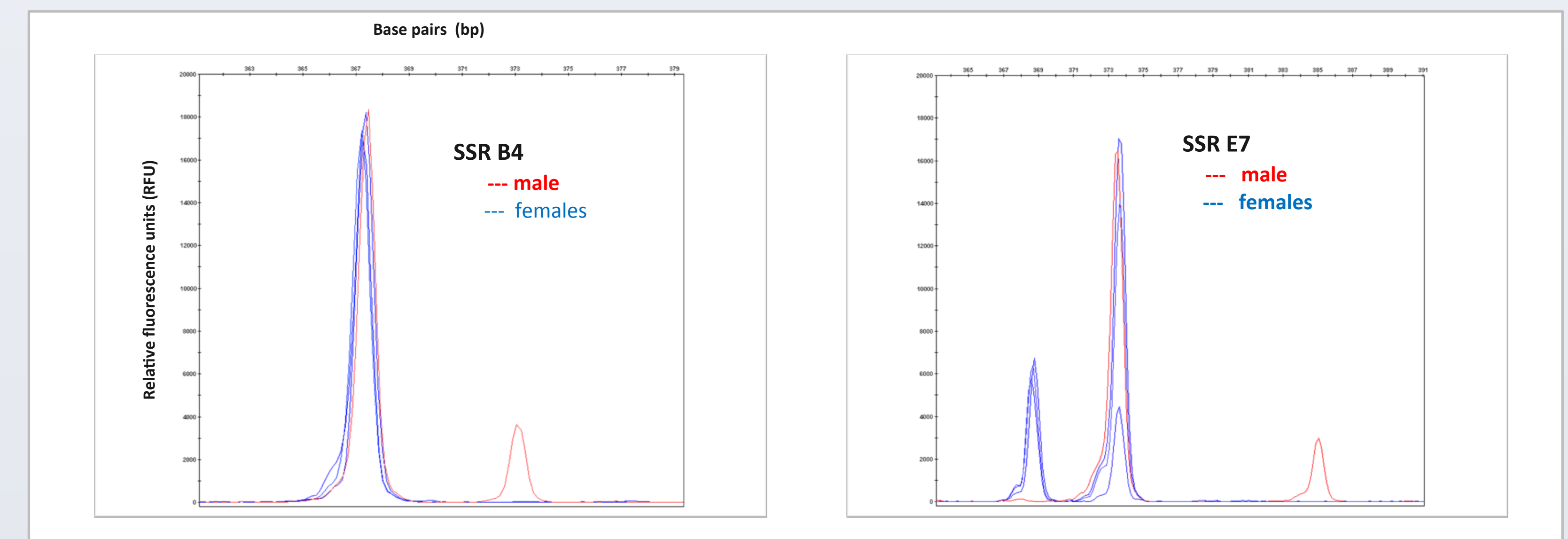


Table 1. Classification and percentage of the total number of 'male' types collected within production field female rows

SSR – B4	%
Female type [343 bp]	68
Male type [343 and 349 bp]	21
Other type A [343 and 355 bp]	8
Other type B [343, 349 and 355 bp]	3

SSR – E7	%
Female type [343 and 449 bps]	81
Male type [343, 349 and 362 bps]	3
Other type A [349 bp]	7
Other type B [349 and 362 bps]	7
Other type C [332, 343 and 349 bps]	2

## Conclusions

- The majority of invasive male types had the same SSR profile as the female parents indicating that they were likely the result of monoecy that can result in male structures on plants that were previously female.
- Some male types had the male parent SSR profile that suggests aggressive stolon growth into the female rows.
- Additionally, a small percentage of male types had 'alleles' in common with both male and female parents indicating hybrid crosses.
- The appearance of 'alleles' not found in either the male or female parents suggests pollen contribution from outside the production field.

## Acknowledgements

We would like to thank the United States Golf Association and the Native Turfgrass Group for financially supporting this research.

2011

2012