In Vivo Generation of DiHaploids in Tall Fescue

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Abstract

Within the Lolium-Festuca genome complex there is a need for novel breeding approaches that can facilitate the rapid development of improved germplasm, cultivars or material for molecular studies. Two annual ryegrass lines, designated as IL1 and IL2, when hybridized by hexaploid, *L. arundinaceum* (Schreb.) Darbysh.) (syn.*Festuca arundinacea* Schreb. to generate an F1, exhibit a genome loss behavior that results in tall fescue dihaploid (DH) generation. DH are generated either through seed or chimera sectoring. The generation of homozygous, DH lines of tall fescue provides new opportunities for enhanced breeding methods and molecular research in this species. The generation of DH's is structured around a mitotic, genome loss behavior that is conferred by the novel inducer lines. The identification of DH offspring is enhanced through the use of a capillary electrophoresis fragment analyzer and flow cytometric analysis.

Introduction

DH selection methods can result in more rapid and efficient gains than other forms of selection (Niroula and Bimb, 2009). Briefly, when utilizing a DH approach, in a diploid organism, only two types of genotypes occur for a pair of alleles, A and a, with the frequency of ½ AA and ½ aa. When utilizing a selfing or backcross approach, three genotypes occur with the frequency of ¼ AA, ½ Aa, ¼ aa. Therefore, if AA represents a desirable genotype, the probability of obtaining the desirable AA genotype is higher when utilizing a DH approach. Also, if 'n' advantageous loci are segregating in a population, the probability of obtaining that desirable genotype is (1/2)n when employing a DH approach, and (1/4)n when utilizing selfing or backcrossing methods. As a consequence, the efficiency of a DH selection approach will be higher when the number of genes governing a trait are quantitative in their inheritance and expression (Kotch et al., 1992). When applied to a polyploid species such as tall fescue, the gain in efficiency will be exponential. An approach to generate DH tall fescue lines has been suggested (Kindiger & Singh, 2011, Kindiger, 2012) and a rapid approach to that identifies DH's would provide value within a breeding program.

Materials and Methods

DNA was extracted from leaf tissue by standard methods and run across a set of EST-SSR markers (Studer et al., 2006) and a series of chloroplast (Cp) derived SSR markers (Provan et al., 2004). The reaction mixtures were resolved on an ABI 3730KL capillary electrophoresis system. Fifteen nuclear genomic EST-SSR and 20 chloroplast (Cp) derived SSR markers were evaluated across the presumptive DH individuals.

Results and Conclusions

EST-SSR analysis of DH lines exhibited a single peak, which suggested homozygosity at all the evaluated loci. The Cp SSR analysis (Kiang et al., 1994) suggested all the recovered DH lines shared the identical Cp DNA fingerprint as the IL maternal parent.

The results of the nuclear genomic analysis indicated that homozygosity was achieved in IL x tall fescue hybrids that were retained in a pollen isolated environment (Figure 1). Heterozygotes were identified in an environment where the F1 were subjected to open-pollination. Homozygosity was apparently derived through rapid Lolium genome and chromosome loss with retention of the tall fescue genome. When DH were derived through seed, spontaneous doubling of the remaining genome in the unfertilized embryos yielded balanced, 42 chromosome tall fescue DH recoveries.

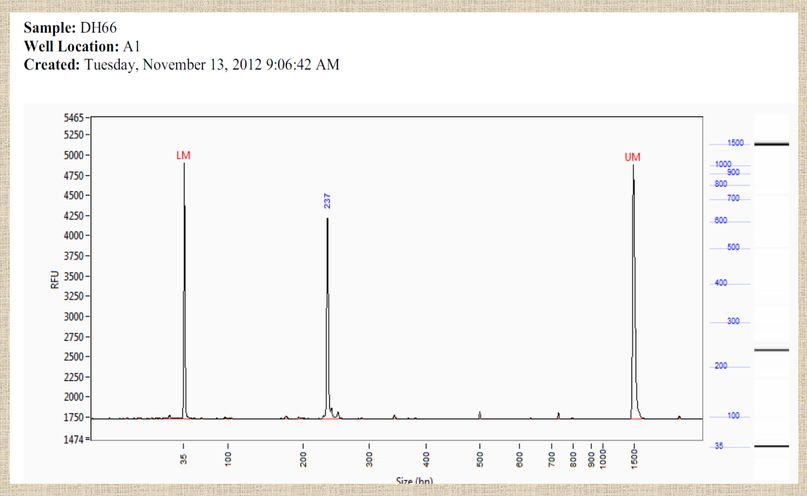

Figure 1. An example of an EST-SSR marker amplification (NFA092) with a known DH line exhibiting a single peak, suggesting homozygosity at that particular locus.

Results and Conclusions

DH were also generated via chimera generation, whereby the mitotic loss of the ryegrass genome generated sectoring plants that contained both F1 and tall fescue growing points (Figure 2).

Figure 2. Image of a F1 plant exhibiting a tall fescue chimera on the right.

The generation of DH tall fescue creates an opportunity for a robust approach to molecular analysis of this species and the implementation of gamete selection approach for quantitative trait improvement during a traditional breeding or selection program.

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