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Maize inbred line purity assessment with multiplexe microsatellite markers

Multiplexed sets of highly polymorphic microsatellite markers have been used in our laboratory to solve questions of maize inbred line identity and genetic purity in support of advanced hybrid maize breeding programs. Samples of 20 seedlings per line were analyzed for genetic purity prior to a backcross assisted line conversion program. Samples GMX1 to GMX10 were from tropical lines while GMX11 and GMX12 were from two temperate lines. Allegedly GMX1 through GMX3 were genetic seed samples and GMX4 a basic seed sample from the same line; GMX5 through 7 were genetic seed samples and GMX8 a basic seed sample from the same line; GMX9 was a genetic seed sample and GMX10 a basic seed from the same line. A bulked leaf sample consisting of 10 mg each of 20 seedlings was extracted and the DNA analyzed. Additionally between 4 and 8 individual seedlings per line were typed to discriminate between the hypotheses of line mixture versus residual heterozygosity. Nine unlinked microsatellites were used, two markers mapping to the distal ends of chromosome 1 and the other 7 to separate chromosomes. Markers phi015, phi056, phi093, phi064, phi083, phi032 and phi072 are tetranucleotide repeats while phi085 and phi053 are penta and trinucleotide repeats respectively. These markers were recommended for varietal protection due to their high discrimination power and typing robustness (Matsuoka et al. 2002 TAG 104:436). Loci were amplified in two PCR reactions and simultaneously analyzed in a ABI377XL sequencer. Virtual allelic ladders were constructed using the ABI Genotyper software macros to allow precise allelic declaration in independent studies. Samples GMX1, GMX2, GMX3, GMX5, GMX7 were found to be fully homozygous at the sampled loci indicating a high degree of line purity, expected for genetic seed lots. Sample GMX6 showed two loci with more than one allele indicating the occurrence of line mixture or residual heterozygosity. Basic seed samples GMX4, GMX8 and GMX10 showed several loci in a heterozygous state. Samples GMX4 and GMX8, basic seed samples, were highly heterozygous with at least one heterozygous seedling in six out of nine loci. Samples GMX9 and GMX10 both displayed heterozygous individual plants and additional alleles in the bulks not present in individual plants clearly indicating the simultaneous occurrence of line mixture and residual heterozygosity. Temperate line samples GMX11 and GMX12 were found to be homozygous at all loci with the exception of locus phi085 for GMX12 which is suggestive of a duplicated locus occurrence. In conclusion, line purity was generally found in the genetic seed samples but not in the basic seed samples where segregation was seen. However the allele sharing observed between the basic seed sample and the genetic seed sample in the comparison clearly indicated that one derived from the other, with the exception of comparison GMX9-GMX10 where different alleles were found. This marker assisted purity appraisal allowed the breeder to precisely select the purest genetic seed lots to guarantee the success of the backcross program. ■