Identification of Cold Tolerant Napiergrass Acessions Using Intergeneric Transferable Microsatellite Markers

Buxani, Namrata

Hypothesis: If I conduct a polymerase reaction using microsatellite markers, a positive amplification of DNA will suggest the presence of a cold tolerance gene in napiergrass accessions. Procedure: DNA used in this experiment was decided by past experimentation that has narrowed the research into 7 main DNA strands of napiergrass, combined with 4 primers(accessions are from USDA-ARS, Tifton Nursery): N66, N128, N147, N157,N188, N215,L7921002; Markers: FTBN5, FTBN6,FTSN1, FTSN2 Preparing mastermix: Depending on how much DNA you are testing effects the amount of master mix you will need. The seven components are: Buffer, Magnesium Chloride, dNTP, taq, and water.PCR Cycling Parameters:1)Incubate at 94°C for 5:002)Incubate at 91°C for 1:003)Incubate at 55/60°C for 1:004)Incubate at 72° for 2:005)Cycle to step 2 for 30 more times 6)Incubate at 72°C for 5:007)Incubate forever at 4°C until ready to run gel electrophoresis.8)Analyze with UV light reader Conclusion: The positive amplification of microsatellite markers was observed supporting the hypothesis. Out of the seven accessions, only six showed positive amplification.N128 did not produce a positive amplification. The amplified products were shorter than expected amplicon sizes. Further experimentation is needed to validate the clarity and specificity of the amplified products. The amplified napiergrass accessions could be potentially cold tolerant.Intergeneric markers can be used for crops with least genomic sequences,being cost effective after DNA sequencing has been finalized. Optimization of PCR is require for conclusive data that can be used in field screening and breeding programs (next step). Changing the annealing temperatures and concentration of components till optimized sharp bands are obtained is necessary.