PP-215

Cryopreservation of *Saccharum* spp. Germplasm Using Droplet-vitrification Technique and Confirmation of Genetic Stability After Cryopreservation Using ISSR Markers

Ergun KAYA
Molecular Biology and Genetics Department, Faculty of Science, Mugla Sitki Kocman University, TURKEY
ergunkaya@mu.edu.tr

Aim of the study:*Saccharum* spp. germplasm is preserved as *ex situ* collections. On the orher hand, it is too expensive to maintain of big collections and they can also be negative effected from pests and disasters in natural conditions. In this study, our first aim was to develop an efficient protocol for cryopreservation of three lines sugarcane germplasm (Halaii, H83-6179 and NG 57-024) via PVS2-based one step freezing technique. The second aim of this study was to confirm the genetic stability of cryopreserved lines of *Saccharum* spp. using ISSR marker system.

Material and Methods: MS supplemented with $20gl^{-1}$ sucrose, 4.44µM 6-Benzylaminopurine, $3gl^{-1}$ gelrite and $3gl^{-1}$ charcoal for cultures. Shoot tips of sugarcane were precultured for 24h on MS medium containing 0.625M sucrose and they were then placed in 4-5µl PVS2 on sterile aluminum foil strips for 30, 45, 60 min. Following treatment with PVS2, the strips including shoot tips in the PVS2 droplets were directly immersed into liquid nitrogen. Rewarming was done after 24 h LN by rapidly removing the foil strips from the cryovials and immediately immersing them into room temperature washing solution (MS containing 1M sucrose) for 15 minutes prior to the transferring the shoot tips onto MS medium. ISSR-PCR were carried out using ten ISRR primers and PCR reactions were performed in a 20µl reaction mixture, containing PCR Buffer, 2,5mM $MgCl_2$, 0,4mM of each dNTP, 0,4mM primer, 40ng DNA and 1 unit Taq DNA polymerase. Amplification conditions were as follows: pre-denaturation for 3min at 95°C followed by 35 cycles of 15sec denaturation at 95°C, 30sec annealing at 55°C, 3min extension at 72°C and a final extension for 10min at this temperature. After seperation on 1,5% agarose gel, the PCR products monitored under UV light, and documented using a gel image analysis system.

Results: Results from the three sugarcane clones used in an initial evaluation of droplet vitrification indicated that this method is effective for the cryopreservation of *Saccharum* spp shoot tips. 45 minutes of PVS2 exposure yielded the highest regeneration rate for lines Halaii (70.9%) and NG 57-024 (63.3%). However, the best regeneration rate was obtained with 30 minutes of PVS2 treatment for line H 83-6179 (76.3%). Genetic stability was tested using ISSR primers and 100 % genetic stability was detected from lines of Halaii and H 83-6179 and 98,5% genetic stability was detected from lines of NG 57-024 by the ten ISSR primers. The total of 211 (Halaii), 198 (H83-6179) and 201 (NG 57-024) reproducible bands, ranging from 125 to 5500bp, were scored with this technique. However, only three bands (2000, 720 and 625bp) obtained from ISSR VII and ISSR VIII primers were polymorphic for line NG 57-024 between samples of untreated, sucrose pre-cultured, PVS2 treated shoots and cryopreservated shoots. Although there were a few differences of ISSR band profiles, these profiles indicate that three lines of Sugarcane had high rate of genetic stability after cryopreservation.

Acknowledgements: This work was supported by Mugla Sitki Kocman University, Scientific Research Projects Coordination Unit (Mugla, Turkey, MSKU-BAP 16/021).

Keywords: Cryopreservation, Droplet-vitrification, ISSR, Sugarcane.