Studies on sugarcance plant and sugarcane stereak geminivirus

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Sugarcane is considered as the most important sugar crops all over the world. As it is cultivated in over 120 countries of tropics and subtropics, and is the source of approximately 65% of the world's sugar. SSD is the most prevalent virus disease found on most sugarcane varieties grown in Upper Egypt. SSV is the causal agent of SSD. In this study, the following items were carried out:1.Detection of sugarcane streak geminivirus (SSV) using some serological and/or molecular techniques analysis.2.Establishment of the regeneration and transformation systems in a sugarcane variety(s).3- Evaluation of the transformed tissues and/or plants. The results could be summarized as follows:1.Most of the collected sugarcane leaves were exhibiting streak-virus-like symptoms.2.Using PAbs specific to SSV, the virus was detected by indirect-ELISA in sap extracted from 36 sugarcane samples of the cv. G85-37. As a number of 13 out of the 36 samples represent a ratio of 36.11% gave positive ELISA values ranged from 1.520 to 1.880.3.In case of PCR detection, 14 out of the 36 sugarcane samples were positive, as a fragment with a size of about846 by representing Rep A open reading frame (ORF) was amplified using two SSV-specific primers.4.PCR was more sensitive than ELISA, as an additional sample (No. 34) that showed a negative ELISA value (0.61 at A405 nm) was found to be positive when tested by PCR.5.The regeneration system of the sugarcane cv. F 144 was established via embryogenesis using leaf bases (apical parts of the shoot) as explants.6.A positive impact due to the use of 2,4-D for callus induction was found. As, 2,4-D at concentration of 4 mg/L (18.1 AM) was the most effective one on the frequency (68.8%) of responding explants (number of explants produced calli) and development of embryogenic calli (48.8%).7.Results showed that, when kinetin at a concentration of 2 mg/L or 9.3 iuM was combined with NAA (5 mg/L or 26.85 gM), the embryogenic calli gave 77 % response, i.e., 23 out of 30 embryogenic calli were produced shoots.8.On the level of number of shoots 483, 286, 144 and 70 with averages 23, 14.3, 9 and 7 shoots per callus were found when kinetin at concentrations of 2, 1.5, 1 and 0.0 mg/L,respectively.9.Transferring shoots on A3 medium with 0.75 mg/L IAA gave the highest number of plantlets (100%) followed by0.5 mg/L (97.7%).61Summary10.The rooted shoots were successfully acclimatized in pots containing peat-moss, clay and sand (1:1:1, v/v/v) for 2 months.11.The transformation system in sugarcane cv. F 144 was established using the plasmid pAB6 carrying the gus reporter and bar selectable marker genes, embryogenic calli derived from leaf bases of sugarcane as explants, and Biolistics Particle Delivery System using two different helium pressures, i.e., 650 and 1100 psi.12.Results showed that when 1100 psi was used for introducing the plasmid DNA in sugarcane cells, transformation frequency increased from 24% in case of 650 psi to 40% in case of 1100 psi based on the results of GUS assay.13.Leaf painting with the herbicide B ASIA a t o ne-half the recommended dose (2 g/L) was used to detect the expression of bar gene in the transgenic sugarcane plants. The transgenic plant leaves were resistant to the herbicide (stay green), while non transgenic as well as control plant leaves turned yellow and the cells died within two days.14.Molecular detection of gus and bar genes in transformed sugarcane plant materials using PCR technique confirmed the presence of gus and bar genes into their genomes, as expected band with sizes of 1800 and 540 by were amplified, respectively.