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Establishment of plant regeneration and genetic transformation in hemp (Cannabis Sativa L.) using Agrobacterium-mediated

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Hemp (Cannabis sativa L.) is an annual and typically dioecious crop and is cultivated in many parts of the world for its fiber, oil, seed and in the therapeutic potential for human diseases, phytocannabinoids as a medical therapy are getting more attention recently. The species is also widely utilized as a source of fiber (such as fabrics, ropes, and paper), food, oil, and medicines plus it has a reputation as being used in religious ceremonies and/or for recreational purposes [1]. The development of new hemp cultivars with improved traits such as resistance to biotic and abiotic stresses, better nutritional and processing qualities, and with increased yields among others [2,3]. However, before the implementation of this technique in C. sativa species, it is imperative to develop an efficient plant regeneration and transformation protocol that allows the regeneration of transgenic plants. Micro propagation can facilitate high throughput propagation in many species and forms the basis of disease-free plants for certified clean plant programs. Thus, developing an optimized in vitro method for propagating clean plants is a crucial strategy to produce large-scale genetically identical plants, retain genetic integrity and maintain the long-term sustainability of the economically valuable crop [4,5,6]. The purpose of this study was to establish a protocol for Agrobacterium-mediated transformation for foreign gene introduction in Hemp. Several factors influence transformation efficiency including the effect of explant type, age of leaf explants, the concentration of silver nitrate and or calcium chloride, bacteria concentration, infection time, acetosyringone concentration, wounding, and different co-culture periods of bacteria were evaluated to optimize the transformation efficiency for Hemp. The Agrobacterium-mediated transformation was optimized using the binary vector pCAMBIA1304 (1) encoding the modified Green Fluorescent Protein (GFP) and a β-glucuronidase (GUS) as reporter genes which is driven by the Cauliflower Mosaic Virus (CaMV) 35S promoter for early detection of transgene expression and containing hygromycin phosphotransferase (HPT) gene as a selectable marker. Results obtained were based on the average number of blue spots and percentage of transformed GUS expressing leaf tissue which was observed 3 days post-transformation. The results indicated that the 14 and 21 days old fully expanded leaf and transient transformation efficiency increased with a GUS blue spot and GFP expression and decreased at 7 days. Enhanced transient GUS expression with an average frequency of 79% was noticed at 30 minutes of the co-cultivation period while 10 minutes of the co-cultivation period resulted in 20% transient GUS expression. The addition of 150 μM acetosyringone, 60 μM of silver nitrate, and 0.5 μM of calcium chloride in the co-cultivation medium increased the transient transformation frequency and the number of blue spots by 77% (150), 80% (159), and 76% (133) compared to control treatment in the leaf tissues. The bacterial inoculum concentration of OD of 0.4 at 600 nm showed 90% transient GUS expression in the histochemical GUS assay. The highest transient gene expression of 74% was obtained from a leaf incubated compared to other explants such as roots, cotyledons, petiole, and or leaf-derived callus. The results indicate that the GUS gene is superior to the GFP gene in following transgene expression in transiently transformed materials in different explants. The maximum transformation frequency was obtained by using the following parameters: 21 days of fully expanded leaf explants of commercial Joey cultivar that were left for 3 days of post-transformation incubation, co-cultivated infected with Agrobacterium for 30 min at a concentration of OD600 of 0.4, and wounding the tissue with the screen. These optimized conditions have been used to obtain stably transformed explants for subsequent regeneration.

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Biography

Sarwan Dhir is a tenured Professor of Plant Biotechnology and Director of the Center for Biotechnology at Fort Valley State University, Fort Valley, GA. He received post-doctoral training at the University of Illinois at Urbana-Champaign and Monsanto Agricultural Company in St. Louis (Bayer) in the area of genetic engineering and plant biotechnology. Since 2001, as a faculty member, he played a leadership role in the establishment of the Center for Biotechnology (Supported by NSF since 2001), developed the Plant Science-Biotechnology major (by restricting and developing 6 new courses), and founding director for Undergraduate Research Program. Besides full-time teaching appointments, as the PI, we received more than 15 million dollars from various funding agencies such as NSF (from 2001 to 2025), NIFA, and DoE. He has successfully directed NSF-funded "National Role Model" programs such as REU-Site in Biotechnology, HBCU-UP, and S-STEM scholarship program since 2001. He has served as a faculty mentor to place more than 485 students in summer internship programs at major institutions, federal labs, and industries and mentored more than 625 undergraduates to prepare their abstracts and present their research at national and international scientific meetings, winning more than 105 awards. He has mentored, 212 Pre-bridge program students, and out of those, 198 enrolled at FVSU and 152 chose STEM majors. Every year, he provides financial support to students enrolled in the Plant Science-Biotech program to 35-40 students in scholarships amounting to $4,200 per academic year for 4-years. He has served as a research mentor for more than 55 middle and high school students and helped them to develop award-winning science fair presentations at national and international science fair competitions. He is a winner of the 2005 White House-Presidential Awards for Excellence in Science, Mathematics, and Engineering Mentoring (PAESMEM) from President George Bush in STEM, a two-time Outstanding Mentoring Excellence Award from NSF HBCU-UP program, and the John W. Davidson Award for Outstanding Teaching and Outstanding Million Dollar Grantsmanship Award given by the FVSU Foundation.

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