USE OF CELL AND TISSUE CULTURE IN SUGARCANE PLANT IMPROVEMENT

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ABSTRACT

Sugarcane is an industrial crop and plays a considerable role in the world economy. Almost, 80% of the world sugar is produced from sugarcane and the rest is from sugar beet. In 1960's biotechnological research work start on sugarcane crop with in vitro culture worldwide. Efforts to improve sugarcane crop by molecular applications have commenced in last five years in Pakistan. As sugarcane crop have limitations such as complex genome size (2n = 120-180), narrow genetic base, poor fertility, susceptible to biotic and abiotic stresses and long duration to breed elite genotypes. It is a suitable candidate for application of biotechnology and genetic engineering tools. Biotechnological applications for sugarcane plant improvement have been applied in the areas of: (1). Cell and tissue culture for rapid propagation genetic transformation and molecular breeding (transgenic and marker assisted breeding) (2). Molecular diagnostics of sugarcane pathogens (3). Use of molecular markers for development of genetic maps (4). Variety identification and testing and (5). Molecular characterization of various traits. The purpose of this review is to highlight the recent research work done in sugarcane biotechnology in Pakistan with special focus on cell and tissue culture for rapid propagation genetic transformation for sugarcane plant improvement.
Key words: Biological approaches, Sugarcane, genetic improvement, Pakistan

INTRODUCTION

Sugarcane, belonging to the Saccharum spp., is a significant industrial crop and is among the top ten most cultivated crops globally. It contributes more than 70% of the world's sugar and is a vital raw material for sugar-producing and allied industries. Pakistan is the 5th largest leading producer of sugar, including traditional cane sugar sweeteners, khandsari, and Gur, production. The Saccharum complex comprises crucial sugarcane genotypes derived from S. officinarum, S. spontaneum, and S. robustum crosses. Even though conventional breeding has led to agronomically improved cultivars, challenges such as a narrow gene pool, complex genome, poor fertility, and a long breeding/selection cycle make further improvements challenging. Conventional breeding for incremental improvements in economic traits and increased production of sugarcane in Pakistan is hindered due to the unavailability of specific climate for flowering and lack of certain economic traits (Patade *et al.,* 2009).

Furthermore, contemporary sugarcane cultivars have a fluctuating chromosome count (2n=100-120) and infrequently blossom. As a typical glycophyte, sugarcane displays stunted growth or no growth when exposed to salinity, resulting in a yield that is 50% or less than its actual potential. To maintain sugarcane production and enhance productivity, addressing concerns such as tolerance to biotic and abiotic stresses, nutrient management, and improved sugar recovery is crucial. Both conventional and biotechnological techniques have contributed significantly to overcoming some of these challenges. Genetic transformation is necessary, but slow multiplication procedures and declining varietal vigor pose economic and biological problems. Therefore, a rapid, efficient, and callus-free in vitro method for clonal propagation is crucial. This article outlines the development of in vitro culture systems and biotechnological approaches for sugarcane improvement.

Somatic Embryogenesis an in vitro culture systems

Sugarcane has two primary methods of plant regeneration: direct and indirect morphogenesis. With direct morphogenesis, plants are regenerated directly from tissues such as immature leaf roll discs and shoot tip culture, which is the primary method for commercial propagation of sugarcane (Suprasanna et al., 2006). Indirect morphogenesis involves the initial culturing of leaf roll sections or inflorescences on an auxin-containing medium to produce an undifferentiated mass of cells, or callus. Somatic embryogenesis techniques have two primary objectives:

(i). the development of a highly efficient method for propagating a large number of uniform plants in less time and possibly at a lower cost than conventional propagation methods; and (ii). a cell culture-based regeneration system useful for genetic transformation.

Embryo genic cultures have also been applied in various areas, such as obtaining virus-resistant plants through somaclonal variation, mutagenesis and in vitro selection, and developing transgenic plants.

Efforts have been continuously made to refine in vitro morphogenesis protocols for improved efficiency. Literature studies showed that two successfully developed protocols were available for that;

a). direct somatic embryogenesis (DSEM) and

b). indirect somatic embryogenesis (ISEM)

using young leaf rolls and immature inflorescence segments from sugarcane cultivars. In different lab experiments various media combinations of coconut water (CW), kinetin, zeatin, and TDZ were compared to optimize callus growth and regeneration. CW and zeatin were found to be more effective than other growth regulators for callus induction, while CW alone was effective for plant regeneration (Ali and Iqbal, 2012)

Somatic embryo differentiation through partial desiccation

Mutation breeding has made significant contributions, resulting in the development of several mutant varieties. In our work, we aim to improve sugarcane using in vitro culture in combination with radiation-induced mutagenesis (Suprasanna *et al.,* 2007). The combination of soma-clonal variation and in vitro mutagenesis can be advantageous in the rapid isolation of salinity and drought tolerant lines through in vitro selection. Previous studies have used radiation-induced mutagenesis and in vitro techniques to develop salt-tolerant mutants in sugarcane (Ali *et al.,* 2010).

Various steps of a mutation-breeding program can utilize in vitro techniques. Meristematic cells or tissues and mitotically active cells can be propagated under tissue culture conditions to obtain a sufficient amount of material for mutagenic treatments. Intrasomatic competition, which can discriminate against mutagen-affected cells and result in a loss of their cell progenies, can be controlled by modifying in vitro conditions to enhance the competitiveness of mutant cells (Desai *et al.,* 2007). In sugarcane, we have successfully demonstrated the use of partial desiccation for 4-6 hours to stimulate and improve the somatic embryo differentiation and regeneration response of gamma-irradiated embryogenic callus cultures. This method has also been successfully extended to other sugarcane cultivars. Partial desiccation induces water deficit, which stimulates ethylene evolution and may influence morphogenetic response in vitro. Therefore, partial desiccation treatment can be a simple and innovative approach to enhance the regeneration response of higher-dose gamma-irradiated cultures.

Induction of Stress tolerance through priming

Priming techniques have been utilized to hasten the synchronized germination of seeds, improve seedling establishment, stimulate vegetative growth, and enhance crop yield in several field crops, particularly under sub-optimal conditions such as salinity stress (Bruce etal., 2007). Primed plants are believed to exhibit enhanced stress tolerance owing to the activation of cellular defense responses, improved osmotic adjustment, and a better antioxidant system upon exposure to stress(Suprasanna et al., 2008). The molecular mechanisms responsible for priming effects are thought to involve the accumulation of signaling proteins or transcription factors, as well as

chromatin remodeling that potentially facilitates faster and stronger responses to subsequent stress exposure. We examined the impact of halopriming on germination and subsequent growth in four sugarcane cultivars with varying salt tolerance. Priming during germination led to an improvement in both the percentage and rate of germination. Two-month-old sugarcane plants subjected to 15 days of isosmotic (-0.7 MPa) NaCl (150mM) or polyethylene glycol (PEG 8000; 20% w/v) stress showed improved growth performance in terms of shoot length, shoot and root fresh weight (Patade *et al.,* 2009). The primed plants also exhibited less salt- and dehydration-induced leaf senescence. Improved osmotic adjustment was found to be more crucial than antioxidant capacity in facilitating growth under stress conditions. Expression analysis of stress-responsive genes revealed up-regulation of NHX and down-regulation of SUT1, P5CS, and PDH. Our findings through review suggest that halo-priming can be an effective approach for enhancing abiotic stress tolerance in sugarcane (Patade *et al.,* 2010).

Transgenic Sugarcane

The potential applications of gene transfer, leading to the creation of transgenics, are rapidly expanding in sugarcane (Suprasanna *et al.,* 2007). These applications include insect and herbicide resistance, alteration of sucrose content via down-regulation of pyrophosphate-dependent hosphofructokinase and soluble acid invertase gene, and the production of high-value compounds such as pharmaceutically important proteins, functional foods and nutraceuticals, biopolymers, precursors, enzymes, and bio-pigments. Sugarcane can serve as a bio-factory for these products in the near future. The availability of efficient transformation systems provides the opportunity to improve commercially important traits in elite germplasm, ultimately leading to the development of an ideal plant type of sugarcane.

CONCLUSION

The cellular and molecular toolbox available for sugarcane research has created numerous opportunities. Ongoing studies focus on creating novel in vitro culture techniques for quick propagation and developing germplasm with desirable traits. In the near future, the progress in sugarcane biotechnology has the potential to revolutionize plant productivity and commercial outcomes.

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