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Current Status of Sugarcane Transgenic: an Overview

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Abstract

Sugarcane (*Saccharum sps*. Hybrids) is one of the most important industrial crops for sugar and biofuel production of the world. Substantial resources are being invested worldwide for sugarcane improvement through conventional and molecular breeding. The recombinant DNA technology has potential, via genetic engineering, to incorporate a specific gene which controls a particular trait, without co-transfer of undesirable genes from donor species as occurs in conventional breeding. A remarkable development is creation of sugarcane transgenic plants with improved yield characteristic and high value products. Now a days, transgenic traits has an integral part of maize, soya been, rice, tomato, cotton and oilseed breeding. Production of sugarcane transgenic events is routine in various laboratories of the world. In recent year, the potential of transformation technology in sugarcane improvement has been demonstrated with commercialization of commercial cultivars with multiple resistances. The globally cultivated area of transgenic crops has increased more than 81 million hectares. However, there are several technical limitations that still remain for multi gene transformation and creation of economically viable transgenic events. The demerits of many cultivars are not easily removed by transformation, and tools and technologies for précised integration and controlled expression of transgene have yet to be demonstrated in sugarcane. Present article exploring the possibility of sugarcane transgenic development through different methodologies and discuss the opportunities and challenges of developing and evolving commercial transgenic sugarcane based on the information available globally.

Keywords: *Saccharum* hybrids; Genetic transformation; phosphotransferase [hpt]; Biolistic device and crop improvement

Abbreviations: SiC: Silicon Carbide; PEG: Polyethylene Glycol; SCMV: Sugarcane Mosaic Potyvirus; SE: Somatic Embryogenesis; GFP: Green Fluorescent Protein; ICP: Insecticidal Crystal Protein; Ubi: Ubiquitin Promoter; nptII: Neomycin Phosphotransferase; Ti: Tumor Inducing; PAT: Phosphinothricin Acetyl Transferase; Bt: *Bacillus thuringiensis*; GUS: Beta-glucuronidase

Introduction

Plant biotechnology has made significant strides in the past two decades or so, encompassing within its folds the spectacular developments in the plant genetic engineering. Now a day, genetically engineered crops appear as the most recent technological advances to help boost food production, mainly by addressing the production constraints with minimum costs and environmental pollution. Transgenic crops offer significant production advantages such as decreased and easier herbicide use and reduced pesticide use [1]. This has a double advantage; first, it reduces the cost of production and second, it escapes environmental pollution due to the indiscriminate use of pesticides and herbicides.

Sugarcane (*Saccharum officinarum* L.) belongs to family Poaceae, is a major agricultural crop. It is a major industrial cash crop, having potential to be a key crop in biofactory evolution as it produces high yield of valuable products like sugar, biofibres, waxes, bioplastic and biofuel [2-5]. Sugarcane is highly heterogeneous and generally multiplies vegetatively by stem cutting. Large genome size, high polyaneuploidy, low fertility, complex environmental interactions, slow breeding advances and nobilization hinder the breeding for this crop. Consequently lack of suitable multiplication procedure has long been serious problem in sugarcane breeding programme [6]. Some of the most vexing problems faced in sugarcane cultivation are attributed to low cane and sugar yields, which involve development of cultivars endowed with resistance/tolerance to drought; salinity, insect-pests, fungal diseases and herbicides as major constraints [7].

Sugarcane is an important food and energy crop, there are so many reasons that make this crop an appropriate candidate for improvement via genetic engineering. To comprehend these problems, biotechnological approaches such as genetic transformation by using *Agrobacterium* and biolistic device (particle delivery system) have been applied in sugarcane with varying degrees of success. As genetic manipulation of the crops has emerged as a new tool for the crop improvement, therefore, genetic transformation of the desired genes in sugarcane may be helpful to cope with the sugarcane problems [8]. However, establishment of tissue culture base line ensure successful regeneration of plants, it's a privilege for genetic alteration. Research on sugarcane tissue and cell culture was first started by Nickell [9] in Hawaii. The first successful plant regeneration system in sugarcane was established about 40 years ago [10], however, a persuasive evidence through somatic embryogenesis was reported by Ahloowalia and Maretzki [11]. Moreover, successful somatic embryogenesis and regeneration was further studied in sugarcane using different explants and medium composition [12-15]. Development of somatic embryogenesis was a milestone in transgenesis in sugarcane [2,16]. Somatic embryogenesis is an important aspect of plant tissue culture where somatic embryos do arise in culture usually from single cells and ontogeny of somatic embryogenesis is comparable with zygotic embryogenesis. Somatic embryos are uniparental and hence the plants regenerated from somatic embryos are true to type. Thus, somatic embryogenesis is being looked upon as an attractive alternative for mass

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cloning of plants and as an important tool for genetic transformation [17]. Moreover, developments of direct somatic embryogenesis system reinforce sugarcane biotechnology because embryogenic calli is the most suitable target tissue for genetic transformation [18]. The improvement of sugarcane through different biotechnological approaches has been suggested by Suprasanna [19]. Genetic engineering of sugarcane cultivars that can produce economically important compounds such as medicinal proteins, sweetener, nutraceuticals, biopolymers, biopigments, precursors and various enzymes is concrete ways to launch sugarcane as a biofactory in coming years. Present piece of information describes the current developments in the fields of genetic engineering that are evolving in the recent years as novel strategies for use in sugarcane improvement.

Methods

Plant genetic transformation

In the last two decades plant breeders are using genetic engineering techniques for transmission of noble gene into crops to improve plant characteristics. Genetic engineering deals with introduction of foreign genes into plant genome through cells, protoplasts or tissues for the production of transgenic plants that exhibit normal physiological and biological functions [20].

During the past years major advances have been made in the plant molecular biology and genetic engineering of crops. With the advent of recombinant DNA technology, it is now possible to clone, modify, mobilize and stably integrate the gene(s) of interest irrespective of their source of origin [21]. These technologies coupled with efficient plant regeneration system have successfully been employed for the accelerated genetic improvement of crop plants.

In case of sugarcane, around 50% losses by borers have been employed. In that Insecticidal crystal protein (ICP) genes encoded from *Bacillus thuringiensis* proved highly toxic to the larvae of sugarcane borers. As of now, more than thirty species of crop plants have been transformed with the Bt (Cry genes) [22]. The commercialization of Bt crops started in 1996 with the introduction of bollworm resistant ('Boll gourd') cotton in USA. Subsequently, Bt potato and maize were also commercialized [23]. Bt crops have led to significant reduction in chemical pesticide use by which has reduced ecological susceptibility [24]. According to Krattiger [23], the Bt genes have the potential to substitute almost one third of 8100 million US dollars necessary to chemically control the insect pest. Subsequently, several vector and vector less methods have been developed for the genetic transformation of crop plants [25]. However, *Agrobacterium* and particle gun mediated genetic transformation have been more frequently used in crop plants.

Electroporation mediated transformation

The utilization of electrofusion and electroporation has a major impact on genetic manipulation of organism [26]. In this method, the foreign DNA (gene) migrates through high voltage induced pores in the plasma membrane and integrates into the plant genome. An effective method was illustrated by Arencibia et al. [27] for genetic alteration of commercial sugarcane varieties PoJ 2878 and Ja 605 based on electroporation from embryogenic calli. The transgenic plants were regenerated from electroporated tissue & confirmation was accomplished by histochemical GUS assay and Southern Blotting. Though, in most cereals regeneration of fertile plant by using protoplast is difficult to establish, they need regular maintenance & lose regenerative competence rapidly. Over the time, many significant approaches have been made to overcome these difficulties.

Electroporation also has the advantage that all the cells are in the same physiological state after transformation. In electroporation studies, concentration of carrier DNA has been found to increase the gene transfer efficiency and is influenced by the electric field intensity, pulse duration, number of pulses applied as well as by the composition of electroporation medium [28]. The utilization of a protoplast system via electroporation as a feasible alternative for sugarcane breeding requires the development of an efficient protocol for plant regeneration from protoplasts. Several authors have reported the use of electroporation for sugarcane transformation [27,29-32]. It finds a practical limitation in that there is a need for an efficient plant regeneration protocol from protoplasts [33]. Two earlier reports describe the regeneration of sugarcane plants from protoplasts [34,35], however difficulty in repeating these results has limited progress in this research area.

PEG mediated transformation

Plant protoplast can be transformed with naked DNA by treatment with PEG (polyethylene glycol) in the presence of divalent cations (Ca^{++}). The PEG and divalent cations destabilize the plasma membrane of the plant protoplast and render it permeable to naked DNA. DNA are also susceptible to degradation while the transformation in this method. Though this technique have some limitation inspite of these this methods have several advantages that protoplast can be isolated and transformed in high number in different plant species. Polyethylene Glycol also used in electroporation methods for the DNA transformation in plants. Moreover, transformation of protoplast by electroporation based on synergistic interaction of Magnesium Chloride and Polyethylene Glycol was recorded extremely efficient [36,37]. PEG-mediated hybridization for the crop improvement was studied by Aftab and Iqbal [38].

Silicon carbide fibers for transformation

It's simple technique using silicon carbide fibers without any specialized equipment. Plant materials (e.g. cells in suspension culture, embryo and embryo derived Calli etc.) are introduced into a buffer containing DNA and silicon carbide fibres which is vortexes vigorously. The fibres which are 0.3 to 0.6 micrometer in diameter and 10 to 100 micrometer long penetrate the cell wall and plasma membrane, which help DNA in to penetrate the plasma membrane and enter to the cells. Silicon carbide (SiC) whiskers have been extensively studied for high tech applications because of several advantages such as high tensile strength, high elastic modulus, excellent shock and degradation resistance [39]. SiC whiskers are fairly rigid rod nanotube that fractures readily, resulting in sharp cutting edge [40]. Silicon carbide and other whiskers from different sources have been utilized in the transformation of monocot and dicot plant species embryo and cell suspension cultures. The mechanism by which whiskers mediated transformation occurs is based on different mechanism. Kaeppler et al. [41] reported that Scanning electron microscopy of whiskers–treated BMS cells clearly shows that the fine fibres are capable of cell wall penetration of a maize cell. Both the surface of SiC whiskers and DNA molecules are negatively charged. In neutral pH medium, this negative charge possibly results in being little affinity between DNA and whiskers. Uses and application of silicon fiber for the transformation in sugarcane has been reviewed already [42,43].

Particle gun mediated transformation

Particle bombardment method is a useful tool for biotechnologists allowing direct gene transfer to a broad range of cells and tissues over the past several years. The term biolistic was coined to describe the nature of the delivery of the foreign DNA into living cells or

tissue through bombardment with a biolistic device (particle delivery system). Particle gun has shown the possibilities to transform many important crop species, which have been difficult to transform by other technique, and is more valuable for improving species which are heterozygous, vegetatively propagated and has a long generation time [44] e.g. sugarcane. It also depends on efficient and reproducible culture techniques. Use of biolistic process for transient assay of gene construct in specialized plant cells or tissue is a valuable application. Any developmentally regulated tissue specific promoter can be fused to a reporter gene and delivered into specialized cells and tissues. Upon visualization or quantification of expression of the introduced genes, one can study developmentally regulated gene functions in specific plant cells or tissues [45].

Direct gene transfer in to sugarcane through particle bombardment is routine in cultivars amenable to embryonic callus culture. Bombardment directly in to meristem or other excised tissues, followed by shoot proliferation or regeneration via a callus stage has been inefficient or unsuccessful for production of non-chimeric transgenic plants [46]. By comparison with more recalcitrant related species such as sweet sorghum [47], the surface layer of sugarcane embryogenic callus evidently has a higher proportion of cells that are able to proliferate and regenerate under conditions that permit the selection of transformed plantlets. Callus formation and regeneration vary with sugarcane genotype, explants type, culture conditions of these factors [46,48].

In sugarcane, first transgenic plants were produced by Bower and Birch [49] at University of Queens Land, Australia. They successfully recovered transgenic sugarcane plant from embryonic callus with high velocity DNA coated microprojectile by bombardment. It was observed that embryogenic callus required high bombardment velocities than suspension culture cells. Transformation of a gene npt-II was done into sugarcane into under the control of a strong monocot promoter Emu. Transformation was confirmed by ELISA and Southern hybridization.

Transformation in callus regenerated tissue by using particle bombardment method have been used by Franks and Birch [50] to achieved stable uid A (GUS) gene transfer into intact cells of commercial sugarcane cv. Q63. For the intact cell Arencibia et al. [51] developed transgenic sugarcane plant (*Saccharum officinarum* l) using a truncated Cry 1A (b) gene encoding the active region of *Bacillus thuringiensis* ð-endotoxin under the control of CaMV 35S promoter. However, Choudhary and Vasil [30] stably transformed callus of a hybrid sugarcane cultivar. The effect of tissue type and strength of promoter on the transient expressin of the GUS reporter gene was reported by Gallo-Meagher and Irvine [52] in the sugarcane cultivar NCo 310. The effectiveness of pBARGUS and pAHC25, plasmid DNA constructs for transforming cell suspensions of *Saccharum officinarum, Triticum aestivum, Zea mays, Pennisetum glaucum, Pennisetum purpureum,* and *Pannicum maximum* by microprojectile bombardment checked by Tayler et al. [53]. Gambley et al. [54] uses the sugarcane meristematic tissues for the microprojectile bombardment. Gallo-Meagher and Irvine [55] obtained herbicide resistant transgenic plants of *Saccharum* spp. hybrids variety NCo 310. Joersbo and Okkels [56] presented a novel concept for selection of the transgenic plant, also known as positive selection. This concept supports the regeneration and growth of transgenic cells, at the same time non transgenic cells are not killed but starved. The positive selection system opposed to the traditional system, where the transgenic cells become insensitive to the selective agent on selection media, while the non-transgenic cells are killed (negative selection).

By using microprojectile bombardment system sugarcane plants were co-transformed by Joyce et al. [57,58] with sugarcane mosaic viral coat protein gene (CP) for pathogen resistance and as a selectable marker npt II gene under the control of either a synthetic promoter Emu or a plant ubiquitin promoter (Ubi). Snyman et al. [59] and Subramonian et al. [60] inserted a herbicide Buster (glufosinate ammonium) resistant transgene into embryogenic callus of sugarcane variety NCo310.

Modified versions of the green fluorescent protein (GFP) gene can serve as an early reporter of plant gene expression, that can be use to establish and optimize the plant transformation protocol [61]. Identification of GFP-positive sugarcane (*Sachharum* spp. Hybrid Q117) cells has enabled the visual invivo screening for transgenic cells. Ingelbrecht et al. [62] have reported that virus resistance in transgenic sugarcane (*Saccharum* spp. Hybrid) is based on post-transcriptional gene silencing. Nutt et al. [63] produced transgenic sugarcane plants expressing either the potato proteinase inhibitor II or the snowdrop lectin gene that showed increased resistance to cane grubs. For the leaf scald (*Xanthomonas albilineans*) transgenic sugarcane produced by using alb D (albicidin detoxification) gene through microprojectile bombardment [64]. Falco et al. [65] transformed embryogenic calli of Brazilian sugarcane (*Saccharum officinarum*) genotype SP 80-180 with two plasmids containing genes coding for neomycin phosphotransferase (nptII) and phosphinothricin acetyl transferase (bar) by using the same technique. Leibbrandt and Snyman [66] genetically modified a sugarcane cultivar Nco310 with pat gene, conferring resistance to herbicide Buster, via microprjectile bombardment. Abdel-Tawab et al. [67] optimized biolistic gun transformation parameters for the Egyptian sugarcane cultivar G54/9. They bombarded calli of G54/9 with plasmid pAB6 containing bar and uid A genes and achieved transformation with the following conditions: one and two shots, 40 bar vacuum and 14 cm distance between microcarriers and target calli.

Braga et al. [68] documented that Cry 1Ab gene would express efficiently and confer resistance against the borer to the sugarcane plants in the field over an extended growing period without altering material traits of the plants. Transgenic plants obtained from two Brazilian sugarcane cultivars (SP80-3280 and SP80-1842) were found to be resistant to *Diatraea saccharalis* (sugarcane borer) by bombarding their embryogenic calli with tungsten particles coated. In that three plasmids: pCIB4421 (Cry 1Ab gene with a maize phosphenolpyruvate carboxylase promoter), pCIB4426 (Cry 1Ab gene with a pith promoter) and pHA9 (neo gene, conferring resistance to antibiotics). Co-transformation efficiency was high and 14 transgenic plants of the two cultivars were recovered. Bombarded calli were selected in vitro for geneticin resistance. Recovered plants were rooted and tested for kanamycin resistance in greenhouse. Plants were then tested through PCR analysis, bioassays and ELISA, which confirmed the integration of transgenes into sugarcane cultivars. The plants were also screened in the field for insect infestation and evaluated for phenotypic and quantitative traits.

Transgenic sugarcane plants expressing transgene against borer mainly Cry1Ab [69], aprotinin gene [69,70] and Cry 1Aa3 [71] evaluated and expression is checked through serological and molecular techniques. A number of transgenic sugarcane lines have been developed with gene expressing Cry protein, proteinase inhibitor or lectin resistance to borers, sucking insects or grubs [72]. Basnayake et al. [48] reported the controlled tissue culture condition for effectual genetic transformation via particle bombardment using a different set of Australian sugarcane cultivars. The chitinase could be detrimental to

fungi. It is a glycosyl hydrolase that catalyzes the hydrolysis of β-1,4-glycosiidic bonds in chitin, which is a major component of the fungal cell wall. The continual expression of chitinase in plants could prevent insect and fungal disease damage [73]. Creating sugarcane varieties resistant to fungal diseases by genetic transformation with a chitinase gene is an alternative means to prevent fungal damage. Khamrit et al. [74] reports on the improvement of sugarcane cultivar Phil 66-07, which is the commercially important crop of Thailand with high sensitivity to fungi, by transforming it with a chitinase gene via particle bombardment.

It was generally possible to obtain several independent transgenic plants per bombardment, with time in callus culture limited to 10-15 weeks. A caution with which this efficient transformation system is able to separate shoots arose from different primary transformed cells in more than half of the tested calli after selection for generating resistance. The result across this diverse cultivar set are likely to be a useful guide to key variables for rapid optimization of tissue culture conditions for efficient genetic transformation of other sugarcane cultivars. Amenability to tissue culture stages required for gene transfer, selection and plant regeneration are the main determinants of genetic transformation efficiency via particle bombardment in to sugarcane. The technique is moving from the experimental phase, where it is sufficient to work in a few amenable genotypes, to practical application in a diverse and changing set of elite cultivars.

Chloroplast transformation in sugarcane

Chloroplast transformation was initiated in order to overcome the problem associated with nuclear transformation, where site specific integration and high level of expression can be obtained. Through leaf discs as example has been used for the chloroplast transformation in variety CoC 671 and two antibiotics (streptomycin & genticin) were used to check the sensitivity. Transformation of organogenic and emryogenic calus of CoC 671 with the chloroplast transformation vectors pZE 27 (aadA) (Streptomycin) and pZE29 (nptII) (Geneticin) were carried out and transformed calli were shifted to selection media. Around 60% lethality of the leaf discs were observed at 25 mg/L (Geneticin) and 250 mg/L (Streptomycin) concentrations. Most of the regenerating plants growing on streptomycin gradually turned into albino plants. Four plants out of 12 tested by PCR exhibited positive for aadA gene. Out of 11 plants tested, none of them were positive for nptII gene (http://www.vsisugar.com/india/agriculture_divisions/molecular_biology_genetic_engineering/research-achievements.pdf).

Agrobacterium mediated genetic transformation

A. tumefaciens is a soil dwelling, gram negative bacterium, which has a natural ability to mobilize and integrate a part of its large tumor inducing (Ti) plasmid called transfer DNA (T-DNA) into the nucleus of infected plant cells [75]. As soon as this fact was discovered, scientists started using modified (disarmed) *Agrobacterium* strains developed by Fraley [76] lacking tumor-forming genes, to transfer useful genes into plants. This system had a number of advantages like higher transformation efficiency, integration of defined DNA pieces frequently as a single copy [77], Mendelian transmission to the next generations [78] and lower cost of equipment than biolistic. Genetic transformation by using *A. tumefaciens* system has significant advantages over Biolistic technology. *Agrobacterium* system has a stable expression and higher transformation efficiency. Moreover, fewer transgene integration results in lower frequency of transgene silencing [79].

A. tumefaciens mediated genetic transformation is a routine method of gene transfer in dicotyledonous plants. Monocots as a group were earlier considered outside the host range of *Agrobacterium*. This is because monocots, particularly the grasses, secrete little or no phenolic compounds [80], lack receptor sites on their cells for *A. tumefaciens* attachment, lack tumor formation or reduced activity of T-DNA promoters in monocots [81]. But now monocots are also being transformed using *Agrobacterium*. Key factors involved in *A. tumefaciens* mediated transformation of monocotyledons include use of meristematic tissues for transformation [82,83], addition of signal molecules, use of monocotyledon promoters [84] and use of supervirulent *Agrobacterium* strains [85]. This methodology presents several advantages over other approaches including the ability to transfer large segments of DNA with minimal rearrangement of DNA, fewer copy gene insertion, higher efficiency, minimal cost and also reducing the occurrence of transgene silencing [79]. *Agrobacterium* mediated genetic transformation system is also illustrated as described below in flow diagram (Figure 1).

Agrobacterium mediated transformation of sugarcane callus tissue had been firstly attempted successfully and recovered morphologically normal transgenic sugarcane plants with transformation frequencies 9.4×10^{-3} and 1.15×10^{-2} [86]. Viable protocols for transformation using *A. tumefaciens* mediated transformation were assessed by Enriquez et al. [87]. An herbicide BASTA resistant sugarcane plant was generated. Before using as explant, meristematic zone of sugarcane was treated with antinecrotic compound to reduce the oxidative bursts. A high regeneration rate and transformation frequencies 10-35% were recorded. Wang et al. [88] constructed a series of improved vectors useful for monocotyledonous genetic transformation by *A. tumefaciens* system; having fruitful features like selectable marker genes: hpt gene for hygromycin resistance & bar gene for phosphinothricin resistance, a polylinker sequence for transgene insertion and a number of origin of replication. Sugarcane cultivars Ja60-5 and B4362 were transformed [87] with *A. tumefaciens* harbouring the binary plasmid pGT GUSBAR (glufosinate resistance). Matsuoka et al. [89] transformed callus and cell suspension cultures of sugarcane cv. NiF4 with *A. tumefaciens* strains EHA 101 and LBA 4404 carrying a binary vector pMLH7133-GUS, which contained nptII (neomycin phosphotransferase), hpt (hygromycin phosphotransferase) and uid A genes. They reported that co-cultivation of tissue and *A. tumefaciens* in liquid medium gave greater GUS expression, and cell suspension cultures were superior to calli for *A. tumefaciens* transformation in sugarcane.

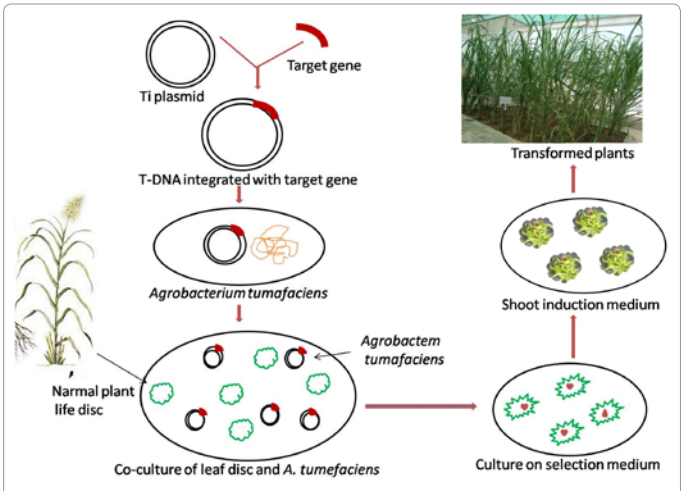

Figure 1: Flow diagram of Agrobacterium mediated transformation in sugarcane.

Mulleegadoo and Saumtally [90] transformed sugarcane cultivar M292/70 with *Agrobacterium tumifaciens* strain AGLO harboring the plasmid pTO134 which contained the phosphinothricin acetyl transferase (bar) and the green fluorescent protein (gfp) genes. Wang et al. [91] introduced trehalose synthase (maltose alpha-D-glucosyltransferase) gene from *Grifola frondosa* into calli of a sugarcane hybrid using *A. tumefaciens* EHA105 strain, which also contained bar gene. The presence of trehalose gene in phosphinothricin resistant plants regenerated from selected calli was confirmed by PCR and dot-blot analysis. Manickavasagam et al. [92] developed herbicide resistant sugarcane plants using axillary buds of sugarcane cultivars Co 92061 and Co 671 with *Agrobacterium* strains LBA 4404 and EHA 105.

In recent past, Zhangsun et al. [93] reported the most useful nptII gene (selectable marker) for selection of sugarcane callus transformation by *A. tumefaciens* system. During 1994, U.S. Environmental Protection Agency declared that nptII is safe to use in commercial transgenic crops i.e. cotton, tomato and rapeseed. The NPTII protein has no deleterious effects on human beings and it is easily degradable in gastrointestinal tract [94]. There is a lack of information regarding various factors for the optimization of *Agrobacterium* mediated transformation process in sugarcane. An effective protocol for transformation in sugarcane requires to be established. Embryonic callus of Australian sugarcane variety Q117 has been transformed availing an easy and reproducible protocol by *A. tumefaciens* system using nptII gene as selectable marker.

Conclusion

Sugarcane (*Saccharum officinarum* L.) is raised as an important industrial cash crop worldwide. It is cultivated in tropical & subtropical regions of the world in a range of climates from hot dry environment near sea level to cool & moist environment at higher elevations for the production of sugar and bioethanol. The success of discoveries & scientific research in Genetic engineering over the last decade has opened the door to improve the agricultural crops. This genetic manipulation in agricultural crops results in insect-pest resistance and abiotic stress tolerance, by which nutritional value of crops can be increased. The genetic engineering is powerful tool and has a great potential in upgrading the genetic potential of crops. *Agrobacterium tumefaciens* has become the most used gene delivery system resulting successfully integration of transgene in plant genome & regeneration of transgenic plant. Several other methods like electroporation, poly ethylene glycol, silicon carbide fiber, particle bombardment were also utilized to insert the gene of interest for the desire traits. Further researches on environment-friendly *Agrobacterium* mediated genetic transformation will be open a new window in the sugarcane biotechnology which will be useful for the crop improvement and crop protection programme.

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