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Advancement of Biotechnological Research on Sugarcane in the World with Reference to Bangladesh

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INTRODUCTION

Sugarcane is a member of genus *Saccharum*, tribe Andropogoneae, family Gramineae. Saccharum species are usually highly polyploid. Interspecific variability of chromosome number and maintenance of aneuploids are characteristics of sugarcane (Price, 1963, 1965). There are five species such as *S. officinarum, S. sinense, S. barberi, S. spontaneum* and *S. robustum* within the genus *Saccharum*.

By taking advantages of the capacity of callus cells that undergo genetic changes in culture, many agriculturally useful callus-derived plants have been obtained (Larkin and Scowcroft, 1981b). In sugarcane, for instance, plants resistant to Fiji virus (Krisnamurthi, 1974) and smut fungus (*Ustilago scitaminae*) (Liu, 1981), and high-yielding, high-sucrose callus derivatives (Liu and Chen, 1978a, 1978b) have been obtained. These results provide an argument for the use of tissue and cell culture techniques as biotechnological tools for sugarcane improvement.

Sugarcane has some important advantages as a target for genetic engineering. Foremost among these is an excellent callus culture and plant regeneration system which is simple and reproducible to apply, and effective for virtually all sugarcane cultivars (Heinz *et al.*, 1977; Ho and Vasil, 1983; Chen *et al.*, 1988; Taylor *et al.*, 1992). This is important because useful genes can be transferred directly into cultivars through regenerable cells in tissue culture. Furthermore, sugarcane cultivars are clones, routinely multiplied and maintained by vegetative propagation. Thus, once an improved transgenic plant is regenerated, the route to commercial application is not complicated by issues of meiotic stability or inheritance patterns. The primary criteria, which must be met by all genes introduced into food crops are environmental safety, and in the case of food crops, safety to the consumers. It will be essential to confirm the absence of any potentially harmful impurities from any transgenic food plant. This is likely to be relatively easily confirmed for sugarcane, because refined white sugar, which is the sole food product from many improved sugarcane cultivars, is the most chemically pure food produced from agriculture (i, e., > 99.98% sucrose).

For these reasons, substantial research efforts have been directed at sugarcane molecular biology, and particularly at the development of a genetic transformation system. Recently, a series of developments with promoters, marker genes, and gene transfer apparatus have culminated in the production of the transgenic sugarcane plants in the world. Advancements of biotechnological researches on sugarcane are reviewed below.

REVIEW

Research on sugarcane tissue and cell culture was started in Hawaii in 1961 by Nickell (1964), and its history has been reviewed by Heinz *et al.* (1977) and Liu (1981).

Callus induction and subsequent shoot differentiation were reported by Heinz and Mee (1968). The Taiwan Sugar Research Institute (TSRI) has been carrying out a program for the application of tissue culture techniques in sugarcane improvement since 1970 (Liu, 1971). Considerable work, specially on selecting variants resistant to Fiji virus and eye spot fungus, has been undertaken in Fiji (Krishnamurthi, 1974) and Australia (Larkin and Scowcroft, 1981a). Other research institute in Florida (Lyrene, 1976 ; Vasil *et al.*, 1979), the Philippines (Lat and Lantin, 1976), Brazil (Evans *et al.*, 1980), and France (Sauvaire and Galzy, 1980) have also investigated sugarcane tissue and cell culture. Now-a-days almost all of sugarcane research organizations of the world are trying to use biotechnological research tools for sugarcane improvement.

The initial site of callus cells in young leaf and sub apical meristem explants was first identified by Liu *et al.* (1982). The configuration of an apical meristem surrounded by leaf primordia as a shoot differentiation pattern in callus was discovered by Liu and Chen (1974). Although Nadar and Heinz (1977) have proposed that the presence of 26.9M NAA in MS (Murashige and Skoog, 1962) medium would stimulate the formation of roots in regenerated shoots, their finding has not been confirmed by sugarcane tissue culture laboratories. Alternately, SH (Schenk and Hildebrandt, 1972) medium devoid of auxin was used to induce root formation from differentiated shoots (Liu, 1981). Nevertheless, several authors (Liu and Chen, 1974; Nadar and Heinz, 1977) agreed that shoot and root are developed independently and have different requirements in nutrient medium. Rapid multiplication of sugarcane through micropropagation using shoot tip as explant was successfully carried out (Hendre *et al.*, 1983; Ali and Afghan, 2001).

Suspension culture was initiated by Nickell and Maretzki (1969), and several nutrient and biochemical studies using suspension cultures have also been completed by the Hawaiian group (Maretzki et al., 1974). Meretzki and Nickell (1973) were the first to isolate sugarcane protoplasts and to induce the formation of cell cultures. Later, Chen and Shih (1983) were able to obtain callus from protoplasts derived from suspension cells. Isolation of sugarcane protoplasts and plant regeneration were also reported by Sugimoto *et al.* (1992), Matsuoka *et al.* (1996, 1998), Aftab and Iqbal (1999).

For physiological stress studies, Liu and Yeh (1982) were the first workers to select a 0.26M NaCl tolerant cell line by using a plating technique. Development of salt tolerant sugarcane plants through tissue culture was reported by Tanvir et al. (2001) in Pakistan. Plants resistant to amino acid analogue, hydroxy proline (HYP) may be resistant against drought stress (Ramana, *et al.*, 1997). Breeding programme for drought resistance in sugarcane using polyethylene glycol (PEG) in vitro was undertaken in Thailand. The use of tissue culture technique for improving sugarcane varieties under water stress conditions was successfully conducted (www.pakissan.com, 1995).

Vasil *et al.* (1979) was the first group to study nitrogen fixation in test tubes by estimating an association between sugarcane callus and the nitrogen-fixing bacterium Azospirillum. Chinese scientists (Chen *et al.*, 1979) have reported the regeneration of green plantlets from sugarcane anther culture.

It should be mentioned that Krishnamurthi (1974) was the first to obtain a disease resistant plant for crop improvement using cell culture methods. More importantly, Liu and Chen (1978a, 1978b) were the first among sugarcane cell culturists to attain several high-yielding and high sucrose callus-derived clones. Sugarcane cell culturists have contributed significantly to the basic and applied research in the area of biotechnology.

In the case of sugarcane, suitable protocols for transformation are available. Stably transformed callus of a hybrid sugarcane cultivar (CP 72-1210) was achieved following high velocity microprojectile bombardment of suspension culture cells, and electroporation of protoplasts (Choudhury and Vasil, 1992). Particle bombardment of axillary meristem (Gambley *et al.*, 1993), embryogenic callus (Bower *et al.*, 1996; Gallo-Meagher and Irvine, 1996) and electroporation of intact cells (Arencibia *et al.*, 1995; Arencibia *et al.*, 1997) have been used to asses new genetic strategies in transgenic sugarcane. Agrobacterium-mediated gene transformation is a promising method for introducing agronomic genes into crop plant. The transformation of sugarcane using Agrobacterium has recently been reported (Arencibia *et al.*, 1998; Enriquez-Oberegon *et al.*, 1998; Matsuoka *et al.*, 2001). Agrobacterium-mediated technique assisted by the visual selection of transgenic cells using green fluorescent protein (GFP) was reported by Elliot *et al.* (1998). Transgenic sugarcane plants resistant to stem borer attack were produced using a commercial sugarcane variety Ja 60-5 in Cuba (Arencibia *et al.*, 1997). Herbicide resistance in Brazilian commercial sugarcane cultivar (SP 80-180) was transformed using trasngenic technique (Falco *et al.*, 2000). Additional traits that have been obtained through genetic transformation include resistance to various sugarcane pathogens and pests such as mosaic virus (Joyce *et al.*, 1998; Smith *et al.*, 1995), stem borer (Arencibia *et al.*, 1997), cane grub (Smith *et al.*, 1999) and leaf scald disease (Birch, 1996, 1997). Several genes for resistance to insect pests, disease or herbicides have already been isolated and shown to be effective in field trials with trasngenic plants of other crop species. Some of them could be readily adapted for testing in local sugarcane varieties. Table 1 summarizes the most important results of biotechnological research of the world.

Table 1. Current successes in sugarcane cell culture research.

Success	Explants	Reference
Callus induction and shoot differentiation	Young internode, subapical meristem, young leaf, unemerged inflorescence	Heinz & Mee, 1968; Liu,1971; Lat and Lantin, 1976; Sauvaire & Galzy, 1980
Root regeneration	Neoformed shoots	Nadar & Heinz, 1977; Liu, 1981
Regenerated plants	Subapical meristem, young leaf	Liu & Chen, 1976a
Morphological characters		Lat and Lantin,1976
Histological examination of callus origin	Subapical meristem, young leaf,	Liu *et al.*1982
Histological studies on organogenesis	Unemerged inflorescence, young leaf,	Liu & Chen, 1974; Nadar *et al.*1978
Genetic variability	Subapical meristem, young leaf	Liu *et al.* 1982
Suspension cells		
Gamma- ray	Subapical meristem, young leaf	Heinz, 1973.
Chromosome number	Subapical meristem, young leaf	Liu & Chen, 1976.
Isoenzyme patterns	Subapical meristem, young leaf	Liu & Chen, 1978a; Vasil *et al.*1979
Disease resistance Fiji disease	Subapical meristem, young leaf	Krishnamurthi, 1974.; Krishnamurthi & Tlaskal, 1974
Downy mildew	Subapical meristem, young leaf	Krishnamurthi, 1974; Chen *et al.* 1979
Eyespot disease	Subapical meristem, young leaf	Heinz et al. 1977; Larkin & Scowcroft, 1981

Table 1. Continued.

Success	Explants	Reference
Leaf scald disease	Young leaf	Birch 1996, 1997
Mosaic virus	Meristemetic tissue	Joyce *et al.* 1998
Stem borer	Young leaf	Arencibia *et al.* 1997
Canegrub	Young leaf	Smith *et al.* 1999
High yielding	Subapical meristem, young leaf	Liu & Chen,1978a, 1978b,1979 ; Liu,1981
High sucrose	Subapical meristem, young leaf	Liu & Chen, 1978b, 1980
Rapid multiplication	Shoot tip	Hendre *et al.* 1983; Ali and Afghan, 2001
Suspension culture	Young internode, subapical meristem, young leaf,	Nickel & Maretzki, 1969; Maretzki *et al.* 1974.
Protoplast isolation, culture and plant regeneration	Young leaf, Suspension cells	Krishnamurthi, 1976; Evans *et al.* 1980; Maretzki & Nickel 1973 ; Chen & Shih, 1983 ; Sugimoto, *et al.* 1992 ; Matsuoka *et al.* 1996, 1998 ; Aftab and Iqbal, 1999;
Anther culture	Anther	Moore & Maretzki, 1977 ; Chen *et al.* 1979 ; Liu *et al.* 1980;
Salt-tolerant cell line	Subapical meristem, young leaf	Liu & Yeh, 1982; Fitch & More, 1981, Tanvir, *et al.* 2001;
Drought tolerant study	Young leaf	www.pakissan.com, 1995.
Nitrogen fixation in Callus	Sugarcane callus	Vasil, 1979; Chen *et al.* 1979;
Transgenic expression and Transgenic plants	Meristem, Callus, Cells	Chowdhury & Vasil, 1992; Gambley *et al.* 1993; Bower *et al.* 1996; Gallo-Megher and Irvine, 1996; Arencibia *et al.* 1995, 1997, 1998; Elliot *et al.* 1998; Enriquez-Obregon *et al.* 1998; Falco *et al.* 2000; Matsuoka *et al.* 2001.

In Bangladesh research on sugarcane biotechnology is limited to tissue culture level. First attempt was made by Islam *et al.* (1982). They got good callus induction and shoot differentiation. However, good root formation was not possible. Induction of callus and regeneration of plants in sugarcane was reported by Hossain *et al.* (1993). They got complete plants with good root formation. Regeneration of plants and effects of mutagen and water-logging stress on regenerated plants were reported else where (Begum *et al.*, 1995a, 1995b; Hossain *et al.*, 1995). Somaclonal variation in regenerated plants from non-irradiated and irradiated calli were studied by Samad and Begum (2000). In vitro plant regeneration and propagation of sugarcane were also reported by several authors (Mannan and Amin, 1999a,b,c; Karim *et al.*, 2002). Table 2 summarizes the important tissue culture research of Bangladesh.

Table 2. Sugarcane tissue culture in Bangladesh.

Success	Explants	Reference
Shoot formation	Spindle leaf	Islam *et al.*1982
Callus induction and plant regeneration	Spindle leaf base	Hossain *et al.*1993
Callus induction	Leaf base	Begum *et al.*1995a
Mutagenic effect in the regenerated plants	Spindle leaf base	Begum *et al.*1995b
Plant regeneration and water logging test	Spindle leaf base	Hossain *et al.*1995
Callus and shoot formation	Leaf sheath	Mannan & Amin,1999a
Rooting and plantlet growth	Leaf sheath	Mannan & Amin,1999b
Plantlet production	Meristem	Mannan & Amin,1999c
Somaclonal variation	Growing point of leaf sheath	Samad & Begum, 2000
Clonal propagation	Leaf sheath	Karim *et al.* 2002

PROSPECTS

In plant breeding perspective, the production of agriculturally useful variants has already had some impact on the improvement of sugarcane. The purpose is to take advantage of the capacity of callus cells to undergo genetic change in culture particularly when only a few agronomic traits are altered. However, these genetic changes are undirected. Therefore, difficulties confronting the tissue culturist in screening a cane variety to be improve for one or two characters while still holding its high-yielding ability are the same as those faced by conventional plant breeders. To raise the frequency of success in selection, the callus-derived plant populations must be large enough and more efficient techniques must be developed to detect the changes that alter the agronomic characters.

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