

Molecular Marker Applications for Improving Sugar Content in Sugarcane

1 Introduction

Sugarcane is an important crop which plays a substantial role in the world economy. About 75% of the world sugar is produced from sugarcane and the rest is from sugarbeet. The mature stalks of sugarcane contain juice with sucrose content as low as 8–9% to 18–19%. Sugarcane is unique in the sense that storage of sucrose in the storage parenchymatous tissue takes place at a very high concentration, as against starch storage in most other higher plants. Sugarbeet and sweet sorghum are other important crops that store photosynthetic assimilates in the form of sucrose. Apart from the traditional use as a source of sugar, sugarcane is fast becoming a source for ethanol and biomass production as an alternative energy source. The residue after sugar extraction from the sugarcane stalk, i.e., bagasse, is used for generating electricity. Direct utilization of bagasse for bioethanol production has also been facilitated by novel fermentation technologies. Even though the concept of sugarcane as a bioenergy crop is fast picking up, the use of sugarcane as a primary source of sugar remains the top priority till now. Sucrose is considered to be the major sugar, making up to almost 60% of the dry weight of sugarcane culm, with glucose and fructose present at lower concentrations. Thus sucrose accumulation, its retention in the stalks and regulation of the process assumes great significance.

Sugarcane is a complex polyploid belonging to the subtribe Saccharinae of tribe Andropogoneae. The genera *Saccharum*, *Erianthus* (sect. *Rimpidium*), *Miscanthus* (sect. *Diantra*), *Sclerostachya* and *Narenga* constitute a closely related inbreeding group referred to as “*Saccharum complex*” (Mukherjee 1957). The genus *Saccharum* comprises of six species—*Saccharum officinarum*, *S. barberi*, *S. sinense*, *S. edule*, *S. robustum* and *S. spontaneum*—the first four are cultivated and the last two grow wild in nature (Daniels and Roach 1987). Modern sugarcane varieties are derived from the interspecific hybridization involving *S. officinarum*, *S. spontaneum* and *S. barberi* with contributions from

other species also. For the successful utilization of any crop in breeding, the interrelationship among the different members of the species/genus/related genera has to be understood. Several workers have suggested various groupings among the *Saccharum* complex and related genera with modifications from time to time (Dutt and Rao 1951; Daniels and Roach 1987).

Sugarcane improvement, from selection of existing variation in pre-historic time to the current bi/multi-parental crossing and subsequent use of non-conventional techniques, has concentrated mostly on improving the yield and sugar content. Due to the complexities associated with this crop, not many investigations were made regarding the genetics and inheritance of important traits. Since most of the economically important characters are quantitative in nature, the quantitative inheritance has been studied. Hogarth (1968) and Brown et al. (1968) have reviewed some of the problems associated with quantitative genetic studies in this crop. The general assumptions for any quantitative genetic analysis may not be met in the case of sugarcane. Even then, the developments in the field of genetics and breeding—both at classical and molecular level—has helped us to make a quantum leap in the area of sugarcane improvement.

2 Sugarcane Cytology

The cytology of the crop has been studied in great detail by earlier workers (Bremer 1961a, b, c, d; Price 1964, 1965, 1968, 1969). It has been described as a complex autopolyploid with many cytogenetic complexities like high chromosome number, variability in chromosome number, *en masse* elimination, female restitution in interspecific or even in species X cultivar, crosses etc. Mostly, the chromosome number in various species and cultivated clones were studied during the early years, with the numbers varying from 80–120. In general, normal bivalent formation, low to fairly high frequencies of univalents, and other irregularities like laggards, bridges and spindle abnormalities have been reported in different species level clones and species hybrids. The cultivated clones mostly have a range of chromosome number between 100 and 130 (Sreenivasan et al. 1987). Chromosomal studies conducted earlier were not confined to *Saccharum* species clones or cultivated hybrids. Interspecific hybrids, intergeneric hybrids, crosses with related genera like *Erianthus* and *Miscanthus*, *Sorghum*, *Zea mays* etc., were also studied in detail by earlier researchers (Jagathesan and Ramadevi 1969; Jagathesan and Sreenivasan 1967, 1971; Jalaja 1983; Janaki Ammal 1938a, b; Janaki Ammal et al. 1972). Chromosomal irregularities were reported in all the above material.

An important peculiarity in sugarcane is the $2n + n$ transmission that has been observed in interspecific crosses. Interspecific hybrids, particularly involving *S. officinarum* $\times$ *S. spontaneum* with *S. officinarum* as the female parent, have a triploid chromosome number relative to the parents (Bremer 1923, 1924, 1925, 1961a, b, 1962; Dutt and Rao 1933). This was attributed to the transmission of

double the haploid set ($2n$) of chromosomes by the female parent and haploid set (n) of chromosomes by the male parent. It has also been reported that this increase in chromosome number continues only up to the second back cross (Bremer 1961b, c, 1962). $2n+n$ transmission of chromosomes has been reported in *S. officinarum* X cultivar crosses also (Piperidis et al. 2010) indicating that this phenomenon is not confined to interspecific crosses alone. Even though several mechanisms have been postulated to explain this phenomenon the exact process has not been unambiguously established. Unreduced egg cells, endoduplication, post-meiotic fusion, endomitosis, differential survival of gametes, selective zygotic failure etc., are some of the possible reasons suggested (Sreenivasan et al. 1987; Bhatt and Gill 1985). These cytological peculiarities invariably effect the pattern of meiotic chromosomal transmission and thereby, the inheritance of important economic traits. Needless to say, these will have an effect on the efficiency of techniques like molecular marker applications.

A comparison of the initial advancement in the field of biotechnological interventions in other crops like rice, wheat, maize and in sugarcane brings out the fact that the initial progress in sugarcane was not as quick as in other crops. It is here that conventional cytological tools assume significance. If we look into the cytogenetic studies in these crops, availability of cytogenetic tools like monosomics, nullisomics, addition-substitution lines, transposons etc. have had important roles in conventional gene location and mapping studies. In rice a complete series of monosomic alien addition lines (MAALs) were produced (Jena and Khush 1989), each with a complete set of chromosomal complement from *Oryza sativa* and a single chromosome from *O. officinalis*. Such lines have been useful in transferring important traits to cultivated rice and also to study the gene/chromosomal effects. In maize, tomato and barley extensive studies were undertaken to assign linkage groups to specific chromosomes using trisomics (McClintock and Hill 1931; Rick and Barton 1954; Rick et al. 1964; Tsuchiya 1959; Riley et al. 1968). Trisomics have also been used in locating genes on specific chromosomes and for physical mapping (Tsuchiya 1991). Monosomics and nullisomics have been used in locating genes to specific chromosomes and chromosome arms in wheat. Transposon-tagging has helped in initial genetic studies in maize. Thus a lot of work was done using these conventional cytogenetic tools in chromosome mapping, physical mapping etc. This had given the workers of these crops a starting point from where, molecular tools had taken over. In the case of sugarcane, the large genome and the numerical complexities, along with the small size of individual chromosomes had been a hindrance to the development and successful utilization of such cytogenetic tools. The buffering capacity of this genome would also have been a drawback to study any gene/chromosomal effects in monosomics, nullisomics or substitution lines. Thus no such cytogenetics based gene location or chromosome mapping was carried out in this crop during the early days. Researchers employing the molecular tools did not have much initial data to rely upon, thus slowing down the pace of molecular studies in this crop at the initial stages. But with the advent of advanced molecular tools, the pace in this crop has also picked up.

3 Breeding in Sugarcane for Quality Attributes

As mentioned before, crop improvement efforts have mainly concentrated on improving the sugar yield. Final sugar yield involves many component traits like stalk length and stalk girth, sugar content and cane yield. Thus sugar yield can be improved by improving the sugar content or the cane yield. Selection criteria in sugarcane include commercial cane sugar (CCS) apart from cane yield. The sugar content in the commercial crop has reached a plateau with not much improvement being brought about by conventional practices. Thus, many a times, cane yield forms a major criteria for selection. Since this is an industrial crop, the sugar factories and mills which procure the canes from sugarcane growers, play a major role in the sugarcane development scenario. In many countries including India, the sugarcane growers are being paid on the basis of cane yield rather than by the final sugar content (even though there are incentives for growing an early maturing high sugar variety). Thus many a times, the emphasis for breeding shifts to a better yielder along with the sugar content. This might be one reason due to which the molecular interventions for increasing sugar content per se has not been put to practice to a greater extent in this crop.

A successful breeding programme necessitates proper flowering of the parental lines. Suitability of different climatic zones for sugarcane flowering varies. For example in India, Coimbatore at southern part of the country is best suited for flowering. Here also, a lot of variation exists among different clones with respect to flowering and extent of flowering. Temporal variation can also be observed for the same genotype. This, to some extent, creates difficulty in repeating specific crosses during subsequent years. The flowers are bisexual and depending on the pollen fertility the individual varieties/clones are designated as male or female. No emasculation is carried out in artificial crossing programmes and thus, unless a genotype is male sterile with zero pollen fertility, there may be chances of selfs even among progenies of controlled crosses. Some desired genotypes may not flower at all during a particular year or the flowering of desired parents may not synchronize at a given time. Thus the sugarcane breeding programmes have been more of a “hit and miss” affair. All these peculiarities in the reproductive biology of this crop determine to what extent the application of molecular marker techniques (and for that the matter, that of other biotechnological tools) are successful in this crop.

As in any other crop the conventional methods of selection, hybridization etc. have been the method of choice for breeding in sugarcane also. Collection, maintenance and proper evaluation of germplasm is an important prerequisite for success in breeding. Traditional breeding starts with the selection of parental material from the source population. For each breeding programme the selection of parental material depends on the aim of the programme. Since the commercial breeding activities invariably emphasizes on high sugar varieties, the parental material for commercial breeding programme essentially involves utilization of high sugar parents. Hybridization follows, with the crosses consisting of biparental

crosses, polycrosses or general crosses (where the pollen from many unknown parents can pollinate a single female parent). Selfing too forms a part of varietal development strategy, as enough variation can be obtained in selfed progeny also. Thus this can also serve as a source population for elite clone selection. In India as mentioned before, Coimbatore situated in the tropical region (77°E longitude and 11°N latitude) is endowed with suitable climatic conditions for flowering. So most of the hybridization programmes are carried out at the National Hybridization Garden (NHG) situated at Sugarcane Breeding Institute, Coimbatore, established under the All India Coordinated Research Project (AICRP) on Sugarcane (even though some individual research centres make crosses on a small scale at some of their own facilities. After seed set the fluff is collected, dried appropriately and is sent to the different research centres for further studies. The fluff obtained after crossing are sown in glasshouse or mist chambers. The seedlings can be transplanted to a secondary nursery or to individual polybags at about 2 months stage. Under proper conditions the seedlings are transplanted to the field. Since each clump has its own unique genetic constitution, individual clumps can be considered as separate entity. Individual seedlings are subjected to hand refractometer brix (HR Brix) observations to identify the high sugar clones. This is followed by selection of suitable clones. These are advanced further and after a series of vegetative propagation cycles the superior clones are released as elite varieties for commercial cultivation.

The involvement of high sugar parents in hybridization invariably calls for some pre-breeding strategies which forms an important component of crop improvement programmes. A pre-breeding programme for high sugar parental development (and also for other traits) exists in most of the crop improvement activities. Pre-breeding for high sugar involves crossing among high sugar elite clones and indigenous as well as exotic parents and further selection. The idea is essentially to introgress new gene combinations in the progeny that can eventually result in high sugar early maturing clones. For this a recurrent selection cycle for high sugar can be employed. The initial cycle of crossing and selection will give rise to a set of high sugar parents, which in turn can be intercrossed among themselves. Alternately, this can also be an outcome of the regular varietal development programmes. For example, elite clones which have high sugar content but do not meet the expectation of a commercial variety due to disease and pest incidence, low yield etc., can be used as parental lines after studying their reproductive behaviour. One such programme at India has yielded a large number of high sugar breeding stocks which have been included in the National Hybridization Garden facility for use by other researchers in their breeding programmes. Selection history and contribution of *S. spontaneum* to the clone's pedigree influence adaptation to sugar yield and its components as proposed by Srivastava et al. (1994). In a study involving a random sample of 64 clones from the germplasm collection of CSR, Australia, and their bi-parental progenies, (6 each, from each of the 32 full-sib families) a strong association, was recorded between the level of expression of characters including those for sugar, with the

Molecular Marker Applications for Improving Sugar
Content in Sugarcane

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2012, VII, 49 p. 5 illus., 1 illus. in color., Softcover

ISBN: 978-1-4614-2256-3