Chapter 5 Summary and conclusions

Sugarcane is an oldest and a major crop of tropical and sub-tropical regions worldwide. This cash crop occupies about 20.4 million hectares of land (2% of total cropped area of the world) with the production of 1392.4 millions tone of the cane. India is the world’s 2nd largest producer of sugarcane. In India, it occupies about 5.15 million hectares area (over 3% of the total cultivated area) with the production of 281.7 million tons. Looking in to the future demand in year 2025 for sugar for consumption, it is double to the present production capacity. Sugarcane has also been recognized as the most efficient energy crop. The over increasing demand for power and fuel has opened up new opportunity to sugar industry and sugarcane cultivation in India in general and Maharashtra state in particular.

Sugarcane is long duration crop which undergoes different abiotic stresses like shortage of water, high temperature during summer, low temperature during winter, flooding during rainy season, nutritional stress, salinity, alkalinity and biotic stresses like diseases and pests which are responsible for reduction in sugarcane and sugar productivity. High yielding, early maturing and multiple stresses tolerant varieties are greatly needed for sustainable sugarcane cultivation.

CoC 671, a sugarcane cultivar, is an early maturing and high sugared variety, has improved the sugar recovery of Maharashtra. In Maharashtra this variety is very popular and grown on about 40% sugarcane cultivated area. But due to varietal degeneration its spread is rapidly decreasing in cultivation. Therefore there is an urgent need to improve CoC671 or replace it with better one. Conventional breeding method has resulted in failure of substantial improvement in this variety, since the single trait improvement through conventional breeding is difficult due to linkages of undesirable traits. There is also limitation to increase the sugar content of sugarcane varieties utilizing the presently available germplasm resources as the limit has been reached. Thus there is an urgent need of improvement of CoC 671 for abiotic and biotic stresses and having better potential to meet future sugar industry demand. With this background following objectives have been planned.

- Standardization of low cost, efficient embryogenic callus development and regeneration protocol for the use in mutation breeding and molecular breeding techniques.
• Induction of mutations in sugarcane variety CoC 671 by using Ethyl methane sulphonate (EMS) for agronomical traits especially early maturity, high sugar content and high yield along with resistance to smut.

• Optimization of protocol for Agrobacterium mediated NDPK2 gene transfer in sugarcane for multiple stress tolerance.

The thesis is divided into five main chapters.

• **Chapter 1:** “Introduction”, presents a general account of sugarcane and constraints in sustainable sugar production.

• **Chapter 2:** “Review of Literature” which gives information of the previous work in the respected field with the scenario of sugar industry at Global level, National Level and particularly at state level.

• **Chapter 3:** “Materials and Methods” which gives the standard methodology/laboratory and field practices adopted during the present research work.

• **Chapter 4:** “Results and Discussions” which embodies the results of the present research work and discussed in the light of relevant literature.

• **Chapter 5.** “Summary and Conclusions”, which summarizes present work with conclusions derived in.

• Literature cited in the thesis has been listed in “Bibliography”

In order to achieve the above objectives in sugarcane improvement programme significant observations have been recorded and summarized as follows:

1. An alternative low cost support matrix for the replacement of agar in sugarcane tissue culture is necessary.

2. Absorbent cotton can be used as low cost best support matrix which eliminates the adversities imposed by agar in its tissue culture. Parameters such as fresh weight (704.42, 555.86 mg), dry weight (70.16, 59.58 mg), number of shoots (20.00, 13.75) and shoot height (5.37, 4.53 cm) was significantly superior in cultures supported with absorbent cotton to that of the agar as support matrix respectively.
3. The quality absorbent cotton works as natural buffering agent in in-vitro studies and prevents drift in pH of medium and maintains the pH 5.8. It improves mineral uptake resulting in better development of explants eliminating phosphorus fixation problem generally occurs in agar based tissue culture media.

4. Furthermore, mineral studies have often been focused on growth rather than morphogenesis with very little known of the relationships between mineral uptake and morphogenesis.

5. Absorbent cotton proves to be low cost alternative to agar in sugarcane tissue culture has great potential to reduce production cost in commercial plant tissue culture industry.

6. An efficient embryogenic callus development was achieved by use of PEG (100 mg/l) in callus induction medium.

7. The analysis of transcript profiles of stage specific embryos that could contribute to reveal molecular mechanism regulating somatic embryo maturation and regeneration.

8. Based on the wide variation of inducers, somatic embryogenesis cannot be defined as a specific response to one or more exogenously applied plant growth promoters. On the contrary, these observations indicate that stress plays a critical role as an embryogenic stimulus.

9. In vitro mutagenesis in sugarcane by using EMS finds good potential to induce mutations for improving the traits.

10. The mutants had distinct stable morphological and genetic variations. Biotic stress (smut) resistant, early maturing, high yielding high sugared early maturing mutants have been identified.

11. It has been shown that in vitro technique, induced mutations and stringent selection conditions together have resulted in selection of mutants with better agronomical traits such as early maturing, high sugared, moderately smut resistant mutants (TC 906, TC 922, TC 2813 and TC 2819 TC 2826 and 2875) over the source variety CoC 671.

12. The mutant TC 906 was smut resistant with early maturity, higher cane yield (144.11 t/ha) and TC 922 was moderately resistant to smut with superior cane yield (165.33 t/ha) to its parent CoC 671 (128.44 t/ha).
13. The mutant TC 2813 and TC 2819 showed superior CCS t/ha (21.94 and 22.56), moderately resistant to smut with higher yield (129.22 t/ha and 128.65 t/ha) over its parent cultivar CoC 671(14.55 t/ha, 94.42 t/ha) respectively.

14. The mutant TC 2826 was resistant to smut disease and showed significantly superior sucrose % (23.13) than the parent CoC 671(21.39) at 12 months maturity.

15. PCR technique is reliable and fast method has been utilized for screening the smut resistance in sugarcane.

16. TC 906, TC 2813 and TC 2819 are early maturing, high sugared, high yielding mutant and TC 922 is high yielding mutant obtained through induced mutagenesis.

17. Thus the above mutants have better potential to replace CoC 671 as better parental status in sugarcane germplasm for the sugarcane hybridization work.

18. The identification of markers linked to quantitative trait loci (QTLs) for increased sugar accumulation could improve the effectiveness of current breeding strategies in sugarcane.

19. *Agrobacterium* mediated sugarcane transformation protocol has been optimized with use of Sodium benzoate (10mg/l) and Chitosan (500mg/l) by eliminating antibiotics in *Agrobacterium* growth restriction.

20. Buffering and stabilization of pH of the medium at 5.8 and thereby higher nutrient availability is useful for improvement of transformation and regeneration efficiency of calli.

21. The antibiotics have shorter active period and therefore in later phase overgrowth of bacteria causing tissue necrosis is observed. No such observation has been recorded during the biocide use in restricting *Agrobacterium* growth. This may restrict high cost antibiotics use in *Agrobacterium* transformation as well as superbug development.

22. Putative transgenic plants have been obtained with *Agrobacterium* mediated AtNDPK2 transformation in sugarcane. The transgene showed presence in first generation (T0) but it gets eliminated in subsequent (T1) generation. The work indicated that a need of suitable tissue specific and stress inducible promoters are necessary in sugarcane transformation.
Thus from the foregoing account it can be concluded that

I. An alternative support matrix has been identified in sugarcane tissue culture.

II. The \textit{in vitro} mutagenesis has resulted in improvement of cultivar CoC 671 for disease resistance, high sugar content, early maturity and high yield.

III. The \textit{Agrobacterium} transformation protocol is optimized by use of Sodium benzoate (10mg/l) and chitosan (500mg/l) for elimination of antibiotics controlling overgrowth of the bacteria.

IV. The work has significance in development of antibacterial cotton to be used in tissue culture.

V. \textit{In vitro} mutagenesis may further lead to develop multiple stress tolerant variety in sugarcane.

VI. Further studies for biocide use in \textit{Agrobacterium} mediated transformation system may helpful eliminate the use of antibiotics for killing \textit{Agrobacterium} with giving boost to plant cells minimizing the necrosis and improving regeneration. This may help in increasing the transformation efficiency.

The process of genetic improvement of sugarcane is a continuous process as observed in other field crops, however much attention is to be given on stress breeding in relation to global warming. The present piece of work is an attempt to that mission oriented breeding program.