Summary & Conclusions
SUMMARY AND CONCLUSIONS

*Sorghum* is one of the world’s most important grain crops, staple food for many people in Africa and Asia and a major feed crop for domesticated animals. However, number of reports describing the use of transgenic *Sorghum* for basic studies in biology is limited in comparison with other crops. The success of the transformation techniques is mainly dependent upon the availability of optimal protocols for highly efficient tissue culture techniques. The use of tissue culture as a tool in genetic improvement of most of the cereals has been hampered by the fact that plant regeneration from various cultures is of low frequency. *Sorghum* has been considered as one of the most difficult plant species to manipulate through tissue culture and transformation. Hence, in the present study thorough investigation of such procedures was attempted in six grain *Sorghum* varieties.

An attempt has been made in this study to establish a plant tissue culture protocol for efficient and rapid plant regeneration and a stable transformation system in *Sorghum*. The present study has achieved efficient callus induction (100%), enhanced regeneration from different explants of *Sorghum* by using appropriate conditions like selection of genotypes, explant size, culture medium, PGR type and concentration. The frequency of embryogenic rate, percentage of regeneration and number of regenerated plantlets per explant were studied and high frequency of embryogenic callus induction and efficient regeneration was observed in the variety IS 3040.

A simple and efficient method for plant regeneration using immature embryo, mature embryo, immature inflorescence and shoot meristems was established using the embryogenic calli derived from these four explant types of six genotypes.
In vitro conditions were optimized for callus initiation, somatic embryogenesis and regeneration. MS medium supplemented with different concentrations and combinations of plant growth regulators (PGRs) viz., 2, 4-D, IBA, IAA, BAP, NAA, GA₃, KN, and ZN used for induction of embryogenic calli. Efficiency for callus induction, somatic embryogenesis and regeneration varied with the explants and was highest in immature inflorescence explant of IS 3040 genotype.

Auxin and cytokinin combination of 2.0 mg/L 2, 4-D plus 0.5 mg/L KN was favourable for immature embryos whereas 2.0 mg/L 2,4,5-T plus 1.0 mg/L ZN for mature embryo, 1.5 mg/L 2,4-D plus 0.5 mg/L Kinetin for immature inflorescence and 2.5 mg/L 2,4-D plus 1.5 mg/L BAP for shoot meristems for efficient embryogenic callus than cytokinins or auxins alone.

Regeneration efficiency of somatic embryos exceeded that of embryogenic calli derived from the same in vitro conditions where more number of embryogenic calli were induced. The size of explant influenced the response for somatic embryogenesis and regeneration. Immature embryos of 0.5 to 2.0 mm, immature inflorescence of 1-4 cm, and shoot meristems of 3-4 mm long were more suitable compared to other younger or older stages.

In view of the importance of multiple shoots in *Sorghum* for further use in transgenic and genetic engineering experiments, the present study also attempted to produce multiple shoots from various explants and more than 106 shoots were noticed from each explant of immature inflorescence. The cytokinin and auxins concentration (BAP + TDZ + IAA) in addition to L-asparagine, L-proline and casein hydrolysate was
found more favourable for multiple shoot induction instead of taking only cytokinin (BAP/ZN), whereas for multiple root induction good response was seen with 1.0 mg/L of NAA. The level of L-asparagine, L-proline and casein hydrolysate enhanced the multiple shoot induction in different explants of *Sorghum bicolor* (L.) Moench.

Regarding direct somatic embryogenesis, a new and efficient protocol was developed by using various explants of *Sorghum bicolor*. Such a callus free development and regeneration pattern through the induction of direct somatic embryogenesis is advantageous. In the present study, shorter time required to obtain direct somatic embryogenesis might contribute to minimize somaclonal variation. The high sensitivity of explants with TDZ in combination with KN and ZN, concentration of AgNO₃, casein hydrolysate, L-proline and L-asparagine, temperature, light and other media elements have permitted the programming of direct somatic embryogenesis in diverse explants of *Sorghum bicolor* (L.) Moench.

Further, immature inflorescence derived calli were more responsive for somatic embryogenesis than immature embryo, mature embryo and shoot meristem. Scanning electron microscopy (SEM) revealed that the immature inflorescence explants followed the somatic embryogenic pathway. The results suggest that *in vitro* somatic embryogenesis from immature inflorescence is the desired pathway for successful plant regeneration. *In vitro* somatic embryogenesis was found highly suitable for successful plant regeneration.

*Agrobacterium*-mediated method of transformation is feasible in *Sorghum*, as evidenced by transient GUS expression. There was variation in the GUS expression
depending on the explants used for transformation. Transformants were obtained from the immature embryo, immature inflorescence and shoot meristem explants of the genotype IS 3040 used in this study and thus the above-developed protocol can be used for *Sorghum* transformation to introduce other agronomically desirable traits such as downy mildew resistance and/or nutritional quality improvement.

Finally, the present study has also succeeded in producing transgenic *Sorghum* plantlets from the immature inflorescence, immature embryos and shoot meristem explants of the genotype, IS 3040 out of six genotypes (IS 3040, IS 3949, IS 8191, IS 81, IS 2746 and IS 8887) by using *Agrobacterium* strain LBA 4404 with pCAMBIA 1305.1 (for GUS expression). The transformation protocol described recovered transformants within 4 to 5 months after culture initiation. Though transformation is successful, it is well established that a number of known and unknown factors reduce the efficiency of the process and therefore, further studies on the transformed and regenerated plants are essential to determine the number of integrated sequences, transgene expression, etc., before introducing the gene/s of interest into the best performing genotypes of *Sorghum*. 