CHAPTER-VI

SUMMARY AND CONCLUSIONS

J.curcas is one of the most important plant species of the world valued for its biofuel property, belongs to the family Euphorbiaceae. The species is primarily propagated through seeds, and thus the seed yield and oil content varies significantly. Propagation can also be carried out without losing the traits by stem cutting but the limitation is generation of large scale quality planting material. Thus, conventional propagation through seeds is not reliable and vegetative cuttings are inadequate to meet the demand of large scale quality planting material. Therefore, *Jatropha* improvement programme by modern methods of agrobiotechnology are of interest worldwide. Camara Machado has reported that regeneration in *J. curcas* is highly genotype dependent. Concerted efforts are being made to optimize genotype independent regeneration and genetic transformation protocol.

Development of an efficient plant regeneration and genetic transformation protocol is a major objective of the present thesis.Therefore, this study was initiated with an objective of developing a reproducible and efficient *in vitro* regeneration method from different types of explant (Leaf, petiole, cotyledonary leaf and petiole) and source of explant (*in vitro* and *in vivo*) of toxic and non-toxic cultivar of *J. curcas*. This study also aimed to standardize *Agrobacterium* mediated transformation and molecular characterization of transformed plants.

The main findings of the entire work have been summarized as below:

In the present study shoot bud induction directly from *in vitro* and *in vivo* leaf and petiole explant of toxic and non-toxic *J.curcas* could be achieved on MS basal medium supplemented with TDZ or BAP individually and in combination with IBA. The main factors that affected regeneration in our study were the concentration and combination of PGRs, source and type of explant, orientation of petiole explant and genotype/cultivar. The percentage response of explant forming shoot bud increased with increase in the concentration of TDZ in both

toxic and non-toxic *J.curcas.* In the present study it was observed that TDZ was more effective than BAP and the response of explant forming shoot bud decreased with the addition of IBA to TDZ or BAP containing medium due to formation of callus. Regeneration efficiency was also affected by source of explant., *In vitro* explant has higher rates of regeneration efficiency and more number of shoot buds as compared to *in vivo* explants. The frequency of adventitious shoot regeneration or shoot morphogenesis differed depending on the type of explant. The regeneration efficiency and number of shoot buds were higher in cotyledonary leaf and cotyledonary petiole as compared to leaf and petiole from established shoot culture in both toxic and a non-toxic cultivar of *J.curcas*. The regeneration efficiency and number of shoot buds were higher in leaf than petiole irrespective of nature of explant source. The orientation of petiole explant in the medium also affected the regeneration efficiency. The regeneration efficiency and number of shoot buds were higher in horizontally placed petiole explant as compared to vertically placed explant. Genotype/cultivar is one of the most important factors affecting regeneration. The regeneration efficiency and number of shoot buds were higher in toxic cultivar as compared to non-toxic cultivar.

MS Medium containg 2 mg/L Kn, 1mg/L BAP and 1 mg/L NAA was found suitable for shoot proliferation and upon transfer to another medium could achive desired elongation. The maximum elongation was obtained in BAP and IAA combinations as compared to BAP and IBA, and BAP and NAA combinations. The best elongation was obtained in medium containing 0.5 mg/L BAP and 1.5 mg/L IAA. Auxins, strength of MS medium, culture type and cultivar had a significant effect on rooting. Significant differences were observed between toxic and non-toxic cultivar however, the differences were not significant with in genotypes of toxic *J.curcas*.

The present study was carried out with the objectives of optimization of cocultivation with *Agrobacterium* to achieve transformation in *J.curcas* leaf explant. For optimization of transformation protocol, a range of parameters were evaluated. These parameters included the length of the pre-culture period, bacterial growth phase, bacterial cell density, method of wounding of leaf explant,

infection time, length of co-cultivation period, pH of the co-cultivation medium and acetosyringone concentration.

Leaf explants pre-cultured for 4 days had the highest transformation efficiency. At the late-log phase, corresponding to OD_{600}= 0.6, we obtained the maximum rate of transformation efficiency. The highest transformation efficiency was scored when intact leaf explant was used as compared to injured leaf explant. Optimum results were obtained with a density of 10^9cells/ml. With increasing durations of incubation period as tested for explant infection with diluted *Agrobacterium* culture, the maximum transformation frequency was recorded with 20 min long treatment at a bacterial cell density of 10^9 cells/ml. The maximum transformation efficiency was achieved after 4 days of co-cultivation. Co-cultivation medium pH of 5.6 was found to be the best with respect to transformation efficiency. At 100 µM of acetosyringone we obtained the maximum rate of transformation. Gene integration was confirmed by PCR, Dot Blot and Southern hybridization while the expression of gene was confirmed by GUS assay. After transformation, regenerated putative transformed buds could be proliferated on M.S.Medium containg 2 mg/L Kn, 1mg/L BAP and 1 mg/L NAA and upon transfer to elongatiom medium desired shoot was obtained. About 40% rooting was observed on half strength MS medium supplemented with 2% sucrose and 3 mg/L IBA, 1 mg/L IAA, 1 mg/L NAA and 0.25 mg/L activated charcoal for rooting. After 6-8 weeks, 50-60% of plants survived.

CONCLUSION:

- An efficient, reproducible and genotype independent regeneration method has been developed from *in vitro* and *in vivo* leaf and petiole explant collected from mature plants of toxic and non-toxic cultivar of *J.curcas*.
- Reproducible and genotype independent regeneration method has been also developed using *in vitro* and *in vivo* cotylrdonary leaf and petiole explant of toxic and non-toxic cultivar and found more efficient as compared to leaf and petiole explants collected from mature plants.
- 0.5 mg/L of TDZ was found optimum for efficient regeneration in in both toxic and non-toxic cultivar of *J.curcas.*

- Regeneration efficiency was higher in toxic cultivar as compared to non-toxic cultivar at all PGRs used.
- Regeneration efficiency (93.42%) for any toxic cultivar studied and 81.07% in non toxic cultivar was achieved at 2 mg/L TDZ using *in vitro* cotyledonary leaf explant.
- Regeneration efficiency was higher in cotyledonary leaf and cotyledonary petiole explant as compared to leaf and petiole explants from established culture.
- Regeneration efficiency was higher in i*n vitro* leaf and petiole explant as compared to *in vivo* explant.
- Regeneration efficiency was higher in horizontaly placed petiole explant as compared to vertically placed petiole explant.
- Rooting percent was higher on half strength MS medium as compared to full strength MS medium.
- Rooting percentage was higher in toxic as compared to non-toxic cultivar.
- Rooting percent was higher in pulse treated as compared to shoot cultured directly on auxins conatning MS medium.
- Highest rooting percentage (51.96%) was obtained in pulse treated shoot with 3 mg/l IBA, 1 mg/L IAA and 1 mg/L NAA for 4 days in toxic cultivar.
- Highest rooting percentage (19.91%) was obtained in pulse treated shoot with 1 mg/l IBA, 1 mg/L IAA and 3 mg/L NAA for 4 days in non-toxic cultivar.
- This is the first report for genetic transformation protocol from leaf explants of *J. curcas*.

In conclusion, the present thesis entitled "**Studies on Regeneration and Genetic Transformation in *Jatropha curcas***" besides, providing an efficient, reproducible and genotype independent regeneration protocol from different types and source of explant of toxic and non-toxic cultivar of *J. curcas*, also provides optimized protocol for successful genetic transformation of *J. curcas* leaf explants which help in improvement of this important biofuel plant.