SUMMARY AND CONCLUSION

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Identifying an alternative fuel source has become imperative. *Jatropha curcas* has all the properties to enable it to be considered as a promising alternative fuel source. Systematic scientific studies will help to understand the plant better. This will also help to device ways to increase its innate potential. Seed oil content of the plant ranges between 22-44%. This large variation in the oil content, unavailability of quality planting material and absence of seed banks jeopardize its commercial success. These issues could be addressed in multiple ways. One of the methods of choice of doing so is transgenic studies. In this study, a holistic approach has been attempted at increasing the oil yield from the plant. For this, tissue culture and somatic embryogeneis protocols have been developed, over-expression of DGAT has been attempted and RAPD profiling of oil content has been reported in this thesis. Micropropagation is looked as an answer for raising such plants which have been touted as economically beneficial but have not been cultivated or whose germplasm is not maintained. During the time of undertaking this study, *Jatropha curcas* was not a cultivated variety, its germplasm was not known, agronomical practices were not known and there was a lot of discrepancy with regards to its oil content. At such juncture plant tissue culture holds a lot of promise. Moreover, a standardized tissue culture protocol is a pre-condition for undertaking transgenic studies. Hence, micropropagation of *J. curcas* was studied. Direct and indirect organogenesis was studied. Direct plant regeneration without an intervening callus phase is a more reliable method for multiplication or clonal propagation. Direct organogenesis was attempted through nodal explants. Apical and axillary buds, tender, thin, green twigs cut below 2nd and 3rd nodes were taken as explants. The culture medium comprised of MS medium supplemented with 2.2 μM BAP and 4.9 μM IBA. Nodal explants produced single shoots within 10-12 days. Explants were sub cultured on MS medium supplemented with 2.22 μM BAP and 4.92 μM IBA. Multiple shoot formation was observed within a period of 30-40 days. Multiplication of shoots was obtained in the same combination. The shoots were then kept on MS basal medium for rooting. Roots were observed after nearly three weeks in MS basal medium. For indirect organogenesis a range of combination of BAP (4.5 μM - 27.0 μM) and IBA (3.0 μM -7.5 μM) were used. Curling of enlarged leaf discs was

first observed followed by callus appearance at the cut margins. Callus morphology and relative response of callusing was studied. Buds in clusters (3-5) started emerging from various locations of the callus, mostly underneath the explants. First bud initiation was observed in MS medium supplemented with 27.0 µM BAP + 3.0 µM IBA. It was also the most effective combination for shoot bud initiation and proliferation. In the present study, tweaking cytokinin levels did not have an effect on organogenesis while low IBA levels did influence organogenesis. Low levels of IBA play a key role in callus formation, bud initiation and multiple shoot formation. Rooting was observed after three weeks of transfer in the rooting medium (MS Basal medium). It could be concluded that auxins play a greater role than cytokinins in organogenesis of *J.curcas.*

Somatic embryogenesis forms other integral part of micropropagation studies. Direct, indirect somatic embryogenesis through solid media studies and liquid suspension culture studies was undertaken in this investigation. Somatic embryogenesis acts as a powerful tool for genetic improvement of any plant species because of its single cell origin. The suspension liquid culture system allows the study of different physiological and biochemical characteristics such as growth parameters, plant growth regulators effect, nutrient uptake and maturation capacity, somatic embryo morphology etc. In the current study the roles of a few factors that have an influence on the performance of explants in culture conditions have been discussed. Age of seeds is known to be an important parameter influencing the outcome of cells in culture. Here, immature and mature seeds were used to obtain first cotyledonary leaves which then served as explants. It was observed that mature seeds could give rise to cotyledonary leaves upon initiation in callus inducing media, whereas immature seeds failed to do the same. Seed viability was the next parameter found to have a bearing on the outcome of the experiments. It was observed that seeds stored at room temperature did not germinate and their cotyledons were degenerated. Unviable seeds were treated with GA10 ppm to break seed dormancy however; it did not help. Hence for all experiments seeds after collection were stored at -20°C until use. Seasonal cues like environmental conditions also have an effect on the performance of explants in culture conditions. In this study, embryogenic calli formation was found to be higher in the months of January to April. It could be explained by the fact that season dependent metabolism is retained

in tissue culture conditions and consequently, it is hard to break down the dormant state. The role of auxins in morphogenesis is evident from the fact that the cells which respond to auxin revert to a dedifferentiated state and begin to divide. For liquid suspension culture studies, the cotyledonary leaves were initiated on MS supplemented with varied concentration of 2, 4-D (0.4, 0.8, 1.2 mg/l) and NAA (5mg/l, 10 mg/l, 15 mg/l) for a period of 30 days followed by their transfer to no auxin media. Early embryogenic structures like globular, heart shaped and torpedo shaped cells were seen. Once in suspension culture, it was observed that the cell number was initially low for first 4-5 days, the cell number increased within 10-12 days and then a gradual decrease was observed after 20 days. For solid media studies short time auxin pulse treatment was used wherein the cotyledonary leaves were initiated on MS +2, 4-D (0.4, 0.8, 1.2 mg/l + 2% coconut water and 0.4, 0.8 and 1.2 mg/l +10% coconut water and 2.4, 3.6 and 4.0 mg/l) and MS+ NAA for a period of seven days or fourteen days followed by their transfer to no auxin medium. Various embryogenic structures were observed. There are atleast two stages in somatic embryogenesis, stages requiring and inhibited by auxins. Hence, auxin levels were checked on day 4, day 7 and day 14. On day 4 pre-embryogenic masses were not observed whereas on day 7 pool of embryogenic and non-embyrogenic structures were observed. On day 14 most of the calli had turned embryogenic. Hence, day 4 calli was considered as non-embryogenic calli (NE) and day 7 and day 14 had both embryogenic (E) and non-embryogenic characteristics. Total auxin levels were estimated and it was found that a significant increase is seen in total auxin in embryogenic(E) calli(7 days and 14 days) as compared to non-embryogenic calli (NE)(4 days) in various media combinations studied. It could be concluded from the present study that 2, 4-D alone at higher concentration (4.0 mg/l) is a preferred medium to induce embryogenesis in *J. curcas* as compared to lower 2, 4-D levels with coconut water. This could be due to the fact that coconut water is an undefined medium and hence may interfere with reproducibility of procedure. This is the first report of liquid suspension culture studies of *Jatropha curcas* and direct as well as indirect somatic embryogenesis.

Jatropha seeds contain 22-44% oil in its seeds. Main constituent of oil is TAG (Triacylglycerol). DGAT (Diacyl glycerol acyltransferase) is the enzyme that catalyzes the committed step of TAG

synthesis from DAG (Kennedy pathway). A correlation between DGAT transcript level and oil accumulation has been reported in common oilseeds as well as in plant species that accumulate unusual fatty acids. Binary vector pE172 contains DGAT2 gene under a seed specific promoter and binary vector pE309 contains DGAT2 gene under a constitutive promoter. Transformation of gene of interest was carried out through triparental mating. It was confirmed by restriction digestion. In species where low transformation efficiencies are expected, the study of the effect of several factors by comparing the percentage of recovered transformed plants may prove unsuccessful because limited numbers of transformants are produced. Therefore, it becomes important to evaluate the influence of diverse factors on the efficiency of T-DNA transfer. *Agrobacterium* infection time was studied. Fifteen and twenty minutes of immersion time of explants in *Agrobacterium* culture was studied and it was found that twenty minutes is more effective time period for immersion as compared to fifteen minutes. Evaluation of selective and bactericidal antibiotics was done and it was found that 20mg/L kanamycin is effective as a selective agent. Preculturing explants prior to inoculation and co-cultivation with *Agrobacterium* has been shown to improve genetic transformation frequencies in many plants. Hence, for both leaf explants and somatic embryos seven days of pre-culture was preferred. More so in case of somatic embryos as seven days pre-culture would enable to generate a few somatic embryos which would then be susceptible for *Agrobacterium* infection during co-cutivation. In the present study, co-cultivation for 4 days yielded maximum transformation efficiency.

Genetic diversity of six different *J.curcas* genotypes was studied using RAPD. Pre-requisite for RAPD is to obtain a good quality DNA. Hence, a protocol for successful DNA isolation from *J.curcas* was standardized and a modified protocol using CTAB was devised. Out of 37 primers used to study the genetic diversity of 6 *Jatropha curcas* accessions, 7 primers could generate reproducible amplification products. These primers yielded 95 amplified bands/fragments. Eleven unique alleles were detected with a total of five primers in five genotypes. The putatively similar bands originating for RAPDs in different individuals may not necessarily be homologous, although they may share the same size in base pairs. Accessions from MSU and TNAU clustered together as per Jaccard's similarity coefficient.

In conclusion direct and indirect organogenesis from different explants has been achieved. For the first time liquid suspension culture studies for *J.curcas* has been reported in this study. Solid media studies were also undertaken for somatic embryo formation and maturation. Successful *Agrobacterium* mediated transformation of leaf discs and somatic embryos of *J.curcas* have been achieved. Existing genetic variation in six different *J.curcas* genotypes has been studied through RAPD.For the same a new modified protocol for DNA isolation has been developed. Genotype from The M.S.University of Baroda and Mettupalayam fell in the same cluster.

The studies reported here can be used to cultivate *J. curcas* with greater economic returns.