

CHAPTER 5

CONCLUSION

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Plant regeneration was species dependent and the explant source was of great relevance. Nodal explants ~~were~~ responded on MT basal medium or when NAA and BAP were supplemented singly or in combinations forming shoots and/or roots for the four species. Citrumelo showed the best regeneration capacity among the species tried from nodal, internodal and leaf explants through direct shoot regeneration or ^{an} after intervening short callus stage while *C. reticulata* exhibited the highest response from root explants forming up to 15 shoots per explant on BAP enriched media. On the other hand *C. limonia* and *C. reticulata* showed the lowest response to the hormones tried. For all the species, adding cytokinin (BAP) to MT basal medium enhanced shoot regeneration and completely inhibited root formation even in the presence of NAA. The ^{sive} responded explants for citrumelo, *C. reticulata* and *C. limonia* maintained their totipotency by ^{tu} subculturing on 2 and 3 mg/l BAP every 6 to 8 weeks for more than 18 months.

It was found that regenerated shoots from different explants and species formed roots on MT enriched with 1 to 3 mg/l NAA and the plantlets obtained successfully established in soil, after acclimatisation. Hardening for a period of 4 weeks enables the plantlets to develop good leaf system to support autotrophic growth. Exposing the plantlets to desiccant in loosely closed jar for 7 to 10 days prior to transfer to pots increased the survival rate percentages. Leaf isoenzyme

analysis for citrumelo, *C. limonia* and *C. reticulata* showed there was no somaclonal variation among the regenerated plants.

Citrumelo embryo callus protoplasts were isolated by using a combination of maceration enzymes of cellulase R-10 at 3.0% (w/v) and macerozyme R-10 at 0.3% (w/v). The isolated protoplasts divided when cultured on liquid or solid MT medium with or without hormone supplementation. However, the protoplasts did not sustain regeneration due ^{to} contamination problems. Mesophyll protoplasts of citrumelo, *C. suhuiensis*, and *C. limonia* were also isolated and cultured on MT basal medium but, no division was observed.

Some physiological parameters measured on the plantlets after hardening period of 4 weeks viz. photosynthetic rate, light response curve, light compensation point, quantum efficiency and water use efficiency, showed that they behave very much like seed-grown plant. It confirms that a hardening period of 4 weeks is sufficient and effective in transplanting the regenerated plantlets into competent seedling. The quest for mass propagation is thus achieved.