CHAPTER -VI

SUMMARY
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Investigations were carried out during 1993-1995 to standardize the in vitro hardening of micropropagated plantlets of Khasi Mandarin and other three important rootstocks. In vitro acclimatalization with varying levels of sucrose, paclobutrazol, reduced humidity and antitranspirants were standarded. Attempts were made to combine ex vitro rooting and acclimatization in the same environment for successful transfer of plants to field. Physiological and biochemical changes during acclimatization were also studied. Ex vitro rooting was tried in different carriers and best carriers for rooting and acclimalization were selected. The data were subjected to analysis and summary of findings are presented here under.

1. Preconditioning and rooting of microcuttings with different concentration of sucrose had significant influence on the quality of plants that have been established in vivo. Sucrose 30gl⁻¹ was found best for growth of microcuttings in vitro.

2. Influence of sucrose concentration on ex vitro growth showed that shoot as well as root growth was better in sucrose 30 gl⁻¹ which produced more number of new leaves and retained maximum number of persistant leaves. The PC scores indicated that among different species CV recorded more loading in PC I and shoot length ,leaf area, and root length are important contributors to PC I contributed 66per cent to the variabilitity.

3. The inclusion of paclobutrazol in the growth medium inhibited the growth of in vitro microshootes, specially in relation to their height. There was a decrease in plant height for all the species,
progressive increase in the concentration of this compound. The effect of paclobutrozol is pronounced even at its lowest concentration (0.25 mg/l) tested. Paclobutrazol did not adversely influence other growth parameters, with the exception of the highest concentration tested (4mg/l) which significantly reduced leaf number and leaf weight.

4. Influence of paclobutrazol on *ex vitro* growth reveal that it had significant effect on all the parameter studied. Of the range of paclobutrazol studied, 1mg l⁻¹ proved to be optimal which produced plants with highest number of persistant leaves and new leaves with moderate leaf area. The PC loading for *Citrus* species indicated that CV recorded higher loading in PC I which is a persistant leaf number and leaf area factor. Stomatal index is an important factor in orthogonal axis recorded higher score indicating the influence of stomatal index in CLM.

5. Silica gel significantly reduced shoot growth and RWC content for all the *Citrus* species studied. Alar and 8HQ at 2mg/l as antitranspirants proved better in controlling transpirational water loss from the plant surfaces which resulted in the higher RWC values. The interactions of *Citrus* species and antitranspirants indicated that in KIN and CV stomatal index was minimum at Alar 1mg/l while at same concentration, stomatal index was maximum in KM and CLM.

6. Soilrite topped over FYM was best carrier for growth and rooting of microshoots while FYM alone as a carrier was found not suitable. Soilrite topped over FYM, Garden soil or sand give better plant growth. But FYM topped over other carriers was found not suitable for growth of microshoots due to rotting of microshoots in these carriers. The interaction between *Citrus* species and carriers showed significant variations for all the parameters studied. The PC loading for different carriers showed that SR + FYM recorded highest loading in PC I where plant height, shoot length and leaf numbers are some important
character which contributed 58 per cent towards total variance.

7. Though basal media had little effect on biochemical constituents of *Citrus* microshoots but different sucrose concentration had significant effect on biochemical constituents. Starch content increased linearly with the increase in sucrose concentration. Chlorophyll content also increased in higher concentration of sucrose. Among different *Citrus* species, KM recorded highest OD phenol, starch and protein content.

8. Inclusion of paclobutrazol in the rooting medium progressively increased starch, epicuticular wax, total phenol and chlorophyll content in the cultured plantlets.

9. *Ex vitro* survival per cent for different sucrose concentration showed that sucrose 30 g l⁻¹ is the optimum concentration as compared to higher or lower concentration, as it recorded 92.1 per cent *ex vitro* survival of Khasi Mandarin and 97.3 per cent in CV. *Ex vitro* survival was lower beyond this sucrose concentration.

10. Of the range of paclobutrazol studied, paclobutrazol 1 mg l⁻¹ proved to be the best in respect of *ex vitro* survival.

11. Silica gel recorded very low *ex vitro* survival of plants. 8HQ (2 mg l⁻¹) recorded higher *ex vitro* survival among antitranspirants followed by alar (2 mg l⁻¹).

12. Soilrite + FYM proved to be the best carrier for *ex vitro* survival coupled with vigourous growth of plants. FYM is not suitable as a carrier.
13. Scanning electron microscopic study of leaves showed that the leaf anatomy of *Citrus* plants at different stages of acclimatization showed similar organisation. However, leaves from greenhouse plants showed more number of normal stomata in the leaf surface compared to leaves from *in vitro* treatments.