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ASSESSMENT OF FIELD PERFORMANCE OF MICROPROPAGATED TEAK AND EUCALYPT

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ABSTRACT

A field trial was conducted at the KFRI Field Research Centre, Veluppadam, Thrissur, for assessing the performance of micropropagated plantlets of *Tectona grandis* (Teak) and *Eucalyptus tereticornis* with respect to their vigour and field hardiness as compared to the conventional planting material used in forestry plantations. Plantlets of both the species were obtained from a reputed laboratory operating at commercial scale, maintained in the nursery and their quality visually assessed. Root coiling and other defects in plant form were observed in the plantlets. The defects were attributed to improper nursery practices.

The field experiments were laid out in a randomised complete block design. with four replicates. Mcropropagated plantlets of teak and eucalypt were 'planted and stumps of teak and rooted cuttings of Eucalyptus were used as controls. Observations on mean survival, height and girth at breast height (gbh) were recorded at two monthly intervals. Since the initial height of both types of propagules was different at the time of planting (stump and seedlings) height increment was taken into consideration for statistical verification of the results.

In teak, survival of both the propagules was above 90% during the initial months. The survival rate gradually showed a declining trend and in seven months, it was 76% in micropropagated teak plants and 83% in conventional stump plants. This declining trend continued in the micropropagated plants throughout the course of 21 months of field growth. However, the control plants (stumps), maintained a steady rate of high survival and were uniformly above 80% throughout the study period. There was no significant differences between controls and micropropagated plantlets with regard to height increment and also girth increment.

In eucalypt the rooted cuttings recorded a steady trend of high survival rate (above 80%) where as the micropropagated seedlings showed low survival (40%) throughout the course of 21 months. Height and girth increments were lower in micropropagated plantlets when compared with the controls.

It was concluded that better nursery management practices need to be followed if the potential of micropropagated plantlets is to be realised. The use of larger plastic bags or root trainers for raising the propagules and care during *ex vitro* rooting of microshoots is recommended. The potential of producing stumps from micropropagated teak plantlets also needs to be examined to take advantage of its unique field hardiness.

1. INTRODUCTION

Micropropagation, the application of *in vitro* culture techniques for mass clonal propagation, offers several unique advantages over the conventional methods of cloning plants and has found wide application for propagation of horticultural/ornamental plant species at a commercial scale. The advantages of the technology in the propagation of tree species are particularly important since conventional methods have limitations.

The number of tree species in which tissue culture studies have been carried out all over the world is large and the list is expanding every year. In fact, tree species were among the first plants to cultured *in vitro*. When the application of cloning in forestry is considered it is apparent immediately that the majority of species are of long life cycles and typically have a distinct juvenile and mature phases of development. Selection of superior genotypes is practically possible only when the trees are well into their mature phase of growth. However the number of species in which plant regeneration from mature tree tissues has been achieved was until recently very limited. The earliest reports of successful cloning of mature trees were in the early 80's (Gupta *et al.,* 1980, 1981). The list has grown since then and trees representing every taxonomic group have been successfully micropropagated and also include several of the commercially important tree species. In many of the gymnosperms, the technology for cloning has advanced to a stage where some amount of rnechanisation is also possible. It is now feasible to regenerate plantlets through somatic embryos produced in liquid suspension cultures grown in bioreactors. It is also possible in several tree species to produce artificial or synthetic seeds by encapsulating somatic embryos in a suitable matrix. In spite of such developments. the status of the tissue culture technology as far its application to plantation forestry is concerned has not been ven satisfactory. It is also of significance that the practising forest managers including the Forest Departments are sceptical of the benefits of micropropagation in forestry.

1.1. LIMITATIONS OF MICROPROPAGATION TECHNOLOGY

The objective of micropropagation is mass clonal propagation. The much higher multiplication rate over the conventional clonal propagation methods is a distinct advantage. However the technique is not without limitations and disadvantages.

The higher cost of plant production is the most notable of the disadvantages of micropropagation over cloning methods involving rooted cuttings. While this is true of the technique regardless of the plant species involved, there are innumerable situations where conventional vegetative methods are not practically feasible. This is particularly true for propagation of mature trees and hence micropropagation is economically justifiable. In plants where simple conventional clonal propagation is possible, the cost of plant production is always lower than that of micropropagation. Only if other unique advantages exist, can micropropagation become economically viable.

The need for a sophisticated and expensive tissue culture facility and trained technicians is another major disadvantage of micropropagation since conventional clonal propagation can often be done with inexpensive equipment and simple techniques that can be Iearnt without great effort. Tissue culture is a sterile technique and hence requires special equipment and stringent control over the environment. It is also energy intensive because of the need to control the temperature and provide artificial lighting to the cultures. Although there is tremendous scope for adopting cost reduction measures this aspect has not received sufficient attention from the researchers (Muralidharan. 1995).

Clonal propagation methods are expected to generate plants with the same genetic complement as that of the mother plant. While this is always the case with all the conventional clonal propagation methods, it is well known that *in uitro* culture carries with it a risk of somaclonal variation. The factors responsible for induction of variability are not very well understood and so are the means to avoid it in vitro cultures. In general long- term cultures and particularly those which have a callus phase are particularly prone to somaclonal variation. Hence the options available are to minimize the culture period to the bare minimum and to initiate fresh cultures as

many times as is practical and economically feasible. The use of callus cultures is necessitated only if other methods are not available or found feasible. Adequate testing of the micropropagated plants for clonal fidelity is therefore necessary before the technology can be applied on a large scale, particularly in the case of forestry crops where the growth period is substantially longer than most other crops.

Tissue culture raised plants require hardening before they are ready for planting in the field. Maintenance of high humidity and protection from microbial infections is important at the early stages of the hardening procedure. The transition from heterotrophy to autotrophy takes place at this stage and the leaves and root systems have to become fully functional. At the end of the hardening procedure the plantlet has to be hardy enough to survive in the field. While the genotype of the micropropagated plants will be the same as that of the mother plant selected, their phenotypic quality is still dependent on the proper development of plant form. In forest trees the field hardiness of micropropagated plants is of particular importance since management practices are minimal unlike agricultural crops. Planting stock of superior genotype can perform poorly if the plant quality is not satisfactory in structure and function. The potential for use of in *vitro* propagation in improving productivity of plantations is tremendous, particularly when the benefit of genetic engineering with desirable genes becomes a reality. Genetic transformation in Eucalypt has already been achieved (Mullins *et al.*. 1997).

The assessment of field performance of micropropagated forest tree species is thus an important aspect in the current scenario of forest biotechnology.

1.2. FIELD ASSESSMENT

Although the number of reports of successful plant regeneration in forest tree species is now large, the results of field assessment of micropropagated plantlets reported in literature are limited. Gupta *et al.* (1991) reviewed the reports of field performance of micropropagated plants of ten forestry species belonging to angiosperms as well as gymnosperms. They found that micropropagated plants showed superior performance over seedlings of the same trees. Growth was uniform and no morphological variation was observed in the plants derived from tissue

culture. In teak and tamarind early flowering was observed. In Douglas fir and pines, plantlets derived from adventitious buds tend to show early maturation. Teak and eucalypt are two of the most important of forest plantation tree species in India. These are also among the first tree species in which mature trees have been micropropagated (Gupta *et al.,* 1980: 1981). Field trials have been carried out with tissue cultured plants and seedling controls in both the species (Khuspe *et al.,* 1987: Gupta *et al.,* 1991; Gavinlertvatana, 1995 and Monteuuis *et al.,* 1998). Cavinlertvatana (1995) reported that over 500,000 micropropagated teak plants were planted in field and faster growth, more uniformity and less branching were observed in comparison with seedlings.

1.3. SCOPE OF THIS STUDY

Even after some two decades since the successful demonstration of micropropagation in teak and eucalypt, the technology has not found application in forestry. Part of the hurdle is perhaps the doubt among forest managers regarding the feasibility of deploying tissue cultured plants under the conventional silvicultural practice in the field. Therefore to demonstrate the feasibility of raising plantations using micropropagated plants of the two species, under typical field conditions, this study was envisaged.

It is to be stressed here that the objectives of this study were limited in scope. **A** stringently carried out field performance assessment would require comparison of micropropagated plantlets with seedlings of the mother tree. Several such field trials are being carried out in India. But it is to be noted that seedlings are not the conventional propagule in teak and rooted cuttings are being increasingly used in commercial *Eucalypt* plantations instead of Seedlings. With the time and financial resources available for this study it was only possible to examine some of the aspects of transplanting and field establishment of the micropropagated plants. Plants for this study were obtained from a reputed laboratory carrying out micropropagation on a large scale and hence represented the standard of the industry. The exact origin of the plant material used for micropropagation and the full details of the protocol followed for culture were not available and not very relevant to the study.

In this study emphasis was given on assessing the quality and field hardiness of micropropagated plants since the primary doubt that foresters had related to these aspects. This kind of study, although not comprehensive in nature, was expected to generate information to help devise better management practices in the nursery and also to serve as a demonstration plot for foresters and forest managers.

2. MATERIALS AND METHODS

2.1. TISSUE CULTURED PLANTS

Plantlets of teak and *Eucalyptus tereticornis* of mature tree origin were obtained from the DBT Tissue Culture Pilot Plant, National Chemical Laboratory, Pune (courtesy Dr. R.S. Nadgauda, Head of the Division). Plantlets were supplied as hardened plantlets grown in nursery in polythene bags of 5 cm x 10 cm (flat size) .

2.2. CONTROL PLANTS

2.2.1. Teak

Teak stumps were prepared at the KFRI Sub-centre Nursery at Nilambur from one year old seedlings grown in raised beds. Open pollinated seeds from local trees which formed the standard planting material were used for raising the seedlings. Selected good quality stumps were transported a few days before planting and kept wrapped in moist gunny bags until required.

2.2.2. Eucalypt

Rooted cuttings of *E. tereticornis* raised at the clonal propagation facility of the Institute at Kottapara were used as control plants. The cuttings were available as hardened plants in root trainers containing vermiculite as the medium. These were transferred to polybags of 15 cm x 23 cm containing soil and maintained in the nursery for approximately 2 months until planting.

2.3. EVALUATXON OF QUALITY OF MICROPROPAGATED PLANTS

The plantlets of teak and eucalypt were closely examined for the quality of the shoot and root system as soon as they were transported and transferred to the KFRI nursery. Since a preliminary and superficial examination revealed that defects were present in the shoot and root architecture, it was decided to transfer the plants to new bags so as to

enable a closer examination and take any remedial measures wherever found necessary. The polythene bags were removed and the root ball gently loosened under running water to expose the main root system without damaging the finer roots. The defects were recorded and photographed.

2.4. REMEDLAL MEASURES

Micropropagated plants were transferred into larger polythene bags of 15 cm x 23 cm (flat size), 250 microns thick, containing a potting mixture of 3 parts cleaned and sieved forest top soil, one part sand and one part finely powdered farm yard manure. While potting, care was taken to see that the roots of the plantlets were placed in the polythene bags properly stretched without disturbing the general architecture of the root system. In order to (accomplish this the plant was placed in the bag with roots positioned properly and potting mix-ture added very slowly and gradually filling the bag with utmost care. The polypotted seedlings were kept in shade and until their establishment, watering was done twice daily. Watering was further reduced to once a day and was gradually stopped. Watering was done only as and when required to avoid wilting of the established plants.

2.5. FIELD TRIALS

The field trial plots were laid out in the Field Research Center of KFRI at Veluppadam in Trichur District. The area was a clear felled teak plantation area with an undulating terrain typical of the teak plantations in Kerala. Just prior to the pre-monsoon showers, in April the area to be planted was demarcated and weeded. Aligning and staking followed during May and pits of 30 cm x 30 cm x *30* cm were dug at a spacing of 2 m x 2 m.. Field planting of the seedlings and crow bar planting of teak stumps were completed by the beginning of June with the onset of South-West monsoon in Kerala. A soil drench with termiticide (Chlorpyrifos) was given as a prophylactic measure against termites and grubs.

The experiment was laid out in randomised complete block design with two treatments - treatment (micropropagated plants) and control (stumps in case of teak and clonally propagated plants in Eucalypt) replicated four times. The plot size was 100 sq.m. with a spacing of 2 m x 2 m. Thus, in each plot, there were 25 seedlings. The orientation of the blocks was

across the slope to avoid variation within a block. Both the species were planted in four blocks of 0.04 ha at the Field Research Centre campus. Veluppadam during June 1997.

The area was fenced with bamboo to prevent browsing animals. Weeding of the plots was undertaken once in every 4 months. The area around each plant was cleared of all growth. Observations were taken once in every two months The damage and mortality if any, and growth measurements such as height, girth at breast height (gbh) and survival percentage were recorded for growth assessment and comparison.

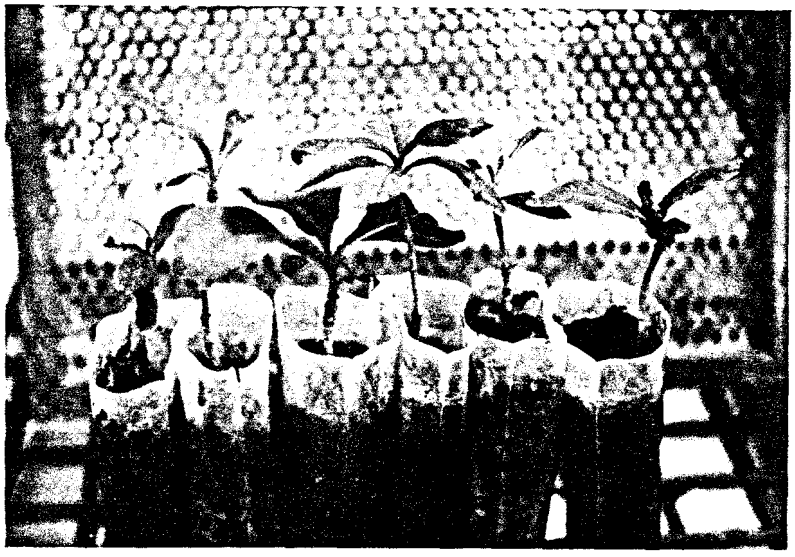
Since the initial height of both type of propagules in teak was different at the time of planting (stump and seedlings) height increment was taken into consideration for statistical verification of the results.

The increment in height and gbh over their initial measurements for different periods was worked out and statistically analysed using Analysis of Variance (ANOVA) for Split plot design (Gomez and Gomez. 1984). The data were analysed after applying appropriate transformations.

3. RESULTS AND DISCUSSION

3.1. PLANTLET QUALITY

It was apparent from the general appearance of the teak and eucalypt plantlets when received from the laboratory that they were not comparable to the normal quality of plants raised in forest nurseries using standard procedure. On examining the root system after removal of the soil. it was apparent that the use of small polythene bags for maintenance of the plantlets in the nursery for a long period had caused root coiling and other abnormalities (Fig. 1). In the procedure followed for micropropagation. rooting of microshoots was carried out ex citro in trays. followed by



Fi,g. 1. Micropropagaged plantlets of teak in small polythene bags as rcecived from the laboratory of origin.

transfer of the rooted plant to individual polythenc bags. From the shape of the roots (Figs. 2 a & b.) it appears as if the abnormalities were caused when the plantlets were transferred to the polythene bag rather than at an earlier stage. In both teak and eucalypt the root formation is adventitious

Fig.2 a. Abnormalities in the root system of teak plantlets

Fig.2 b. Abnormal root system of Eucalypt plantlets

in the micropropagated plants as well as in the control plants and several roots develop unlike the seedlings where tap 'roots are formed. It is the usual practice to press down the potting medium around the base of the stem of the plantlet after transfer to the new container. If care is not taken, the excess pressure can result in the bending of the roots which then continues to grow retaining the shape. Root coiling is not unexpected in plants retained in the poly-thene bags for long time.

The remedy for the above defects is to transfer the plantlets to bigger bags after a few months of growth. Although the larger size and weight of the containers are a disadvantage from the point of view of maintenance, transport and handling, the plantlet quality will be improved and plantlets can be kept in the nursery longer. The better alternative is to grow the plantlets in root trainers right from the beginning. The root trainers which are available in different sizes and designs will prevent root coiling and the excessive growth of the root system. Since a root plug is formed, further damage at the time of planting is avoided with root-trainer raised plantlets. Although more expensive than the polythene bags, root-trainers are reusable and make transport of plants easier because of its compact nature and the use of lighter growth medium like perlite. vermiculite or mixtures of these with soil, compost etc.

Since a non-intensive silvicultural practice comparable to the conventional method was being adopted for the field trials the remedial measures to correct the defects in the micropropagated plantlets were adopted only to ensure better survivai and growth. The transfer to fresh potting medium and maintenance in the nursery before planting allowed the plantlets to recuperate. The effect of these measures was not evaluated in the study since it is undesirable to have it adopted as standard practice in silviculture.

3.2. GROWTH IN FIELD

3.2.1. Teak

3.2.1.1. Survival

The survival percentage and establishment rate of both micropropagated teak seedlings and stumps were quite high and was above 90% during the initial months, soon after planting (Fig. 3). The survival rate gradually showed a declining trend and in seven months, it was 76% in micro-propagated teak plants and 83% in conventional stump plants (Fig. 4).

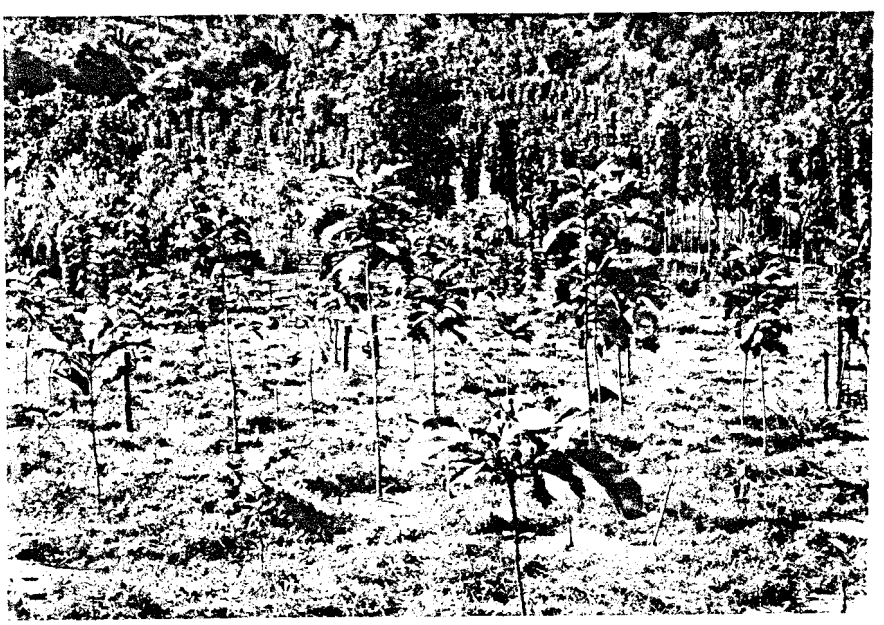


Fig. 3. Aview of the field trials of teak at Palappilly

This declining trend continued in the micro-propagated plants throughout the course of 21 months of field growth. However, the control plants (stumps), maintained a steady rate of high survival and were uniformly above 80% through out the study period.

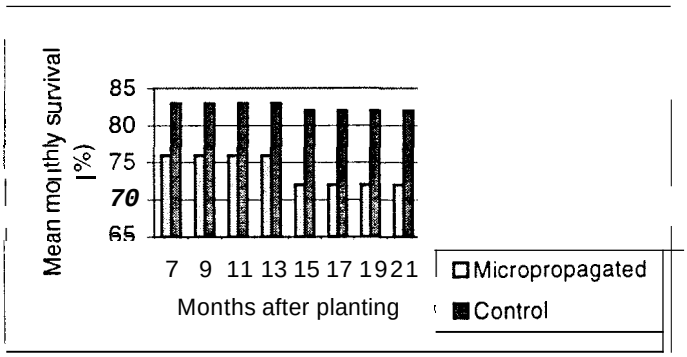


Fig. 4. Survival percentage of micropropagated teak plantlets and stump raised plants.

Stump planting has been the accepted way of planting teak in plantations in India for a long time. The advantages of stumps are that nurseries can be located away from the plantation sites and planting stock in the form of stumps can be transported and stored for several days without damage until planting. Stumps are hardy and tolerate the transplanting shock even though they are planted in crowbar holes. Stumps are prepared from one year old seedlings and consequently have some storage material available in the tissues. Teak plants developing from seedlings and stumps show not much variation difference in growth in the later stages but stumps ensure better survival in the field during the initial stages of establishment.

Tissue culture plants used in this study, on the other hand can be compared to the seedlings of teak. The stem is lower in diameter than that of the plants derived from stumps. Since in the conventional practice, micropropagated plants are grown in polythene bags and maintained in the nursery, it is also worthwhile to examine this possibility of converting them into stumps, so as to take advantage of the ease f transportation and storage and improved survival.

3.1.1.2. Height

The teak stumps in the control plots started production of above ground vegetative shoots 48 hours after planting. Meanwhile the micropropagated

seedlings were already having a mean height of 16 cm at planting. Though the height growth in these two types of propagules was not comparable due to the difference in initial height at planting the growth trend showed close similarity (Fig. 5).

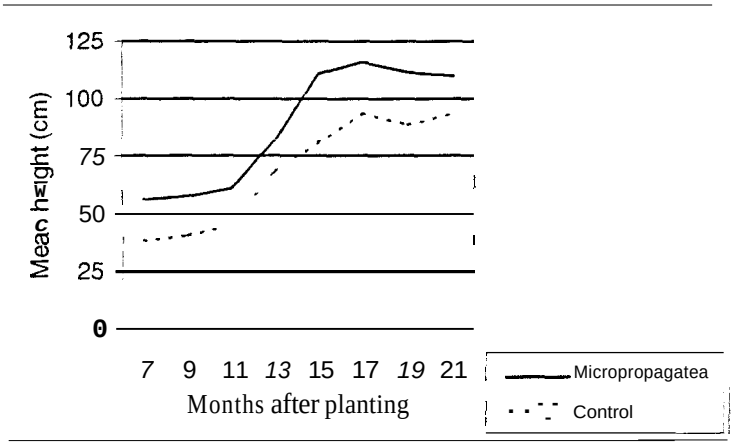


Fig. 5. Mean height of micropropagated teak plantlets and stump raised plants

However, since the initial height at the time of planting was different in both type of propagules, only the height increment was taken into consideration for statisticaI verification of the results.

The overall mean increment in height was higher in micropropagated plants than the control. ANOVA showed non-significant effect of treatment and interaction between treatment and period. However, the effect of period of observation was found to be significant.

The results suggest that micropropagated plantlets are capable of performing better than the controls in height growth if extra care were taken to maintain them during the nursery phase. Mascarenhas *et a1* (1987)had however found that although there was an initial faster growth in tissue culture raised teak plants over seedlings from the same trees, by the fifth year the difference in height increment was not significant.

3.1.1.3. Girth

The micropropagated plantlets and stumps (control) attained measurable stem girth (5.95cm) only 16 months after planting. As in the case of height

growth both type of propagules exhibited similar pattern of growth in girth. Though the stump plants revealed higher stem dimensions initially (16 months after planting) in due course the micropropagated plants picked up and showed gbh similar to the former and there was no striking difference between the two treatments (Fig. 6).

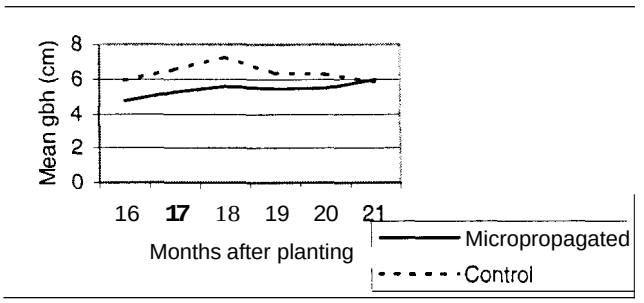


Fig. 6. Mean girth at breast height of micro-propagated teak plantlets and stump plants in the field

ANOVA showed non-significant effect of treatment, period and also the interaction between treatment and period.

As in the case of height increments, better stem diameter increments could be expected if micropropagated planting stock of better quality were used. It can therefore be inferred that the micropropagated plantlets, with adequate initial care and proper hardening in the nursery, can be considered as ideal propagules for field planting as was evident from the corresponding uniform growth trends exhibited by both type of propagules in the present study.

3.2.2. Eucalypt

3.2.2.1. Survival rate

Field establishment (Fig. 7) and growth of micropropagated seedlings of *E. tereticornis* and rooted cuttings (control) followed a different pattern when compared to that of teak. Soon after field planting there was a drastic

Fig. 7. A view of the field trials of Eucalyptus

decline in survival percentage of micropropagated plantlets. After the onset of monsoon rains the incidence of Cylindrocladium blight was observed in the micropropagated plants which resulted in damage to the young shoots and contributed to the increased mortality of the plants. The control plants recorded better survival in the field and maintained a steady trend of high survival rate (above 80%)where as the micropropagated seedlings recorded a low survival (40%)through out the course of 21 months (Fig. 8).

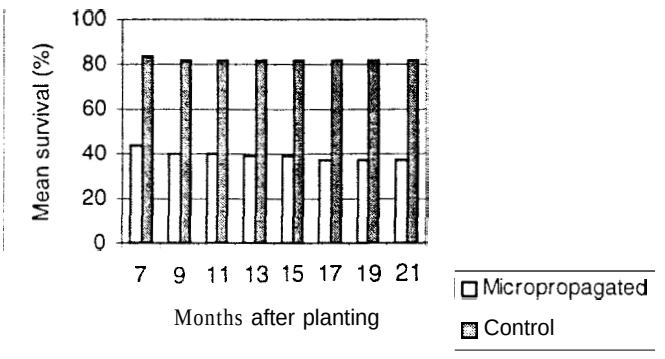


Fig.8. Mean survival of micropropagated Eucalypt plantlets and rooted cuttings (control)in field.

The high incidence of mortality due to the fungal infections in micropropagated Eucalypt plantlets can be attributed to the fact that they originated from mother trees that were not selected for disease resistance. Cylindrocladium blight of eucalypt is a severe disease during the monsoon season in Kerala but the clones used for this study as controls were selected for tolerance to the disease. The micropropagated plants were moreover selected from a different agro-climatic region. This can explain the difference in survival rates between the micropropagated plantlets and the control plants as observed in this study.

3.2.2.2. Height

Unlike the micropropagated seedlings of teak, the tissue culture seedlings of *E. tereticornis* recorded lower growth in height compared to the rooted cuttings and the trend continued through out the study period indicating probably the inferior quality of the micropropagated planting stock (Fig. 9). The micropropagated plants also failed to make up the initial set back in the nursery growth even after 21 months growth in the field. One of the reasons suggested for the poor performance may be the deleterious effect of the disease on the micropropagated plants.

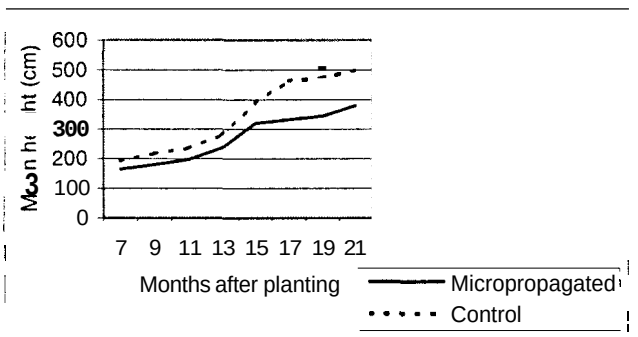


Fig. 9. Mean height of micropropagated Eucalypt plantlets and rooted cuttings (control)in field.

The overall mean increment in height was lower in treatment than the control. ANOVA showed non-significant effect of treatment and interaction between treatment and period while it showed significant effect for the period. The non-significant effect of the interaction indicates that the treatment differences remain the same over different periods.

The effect of Cylindrocladium blight, on the height growth of micropropagated plantlets cannot be ignored even in the plantlets that survived. The young leaves and shoot tip are often severely affected in infected plantlets. The poor root system can also contribute to the growth performance.

3.2.2.3. Girth

Field comparison of micropropagated plantlets and rooted cuttings showed that stem diameter increment in the former is slower and lesser than the latter (Fig. 10).

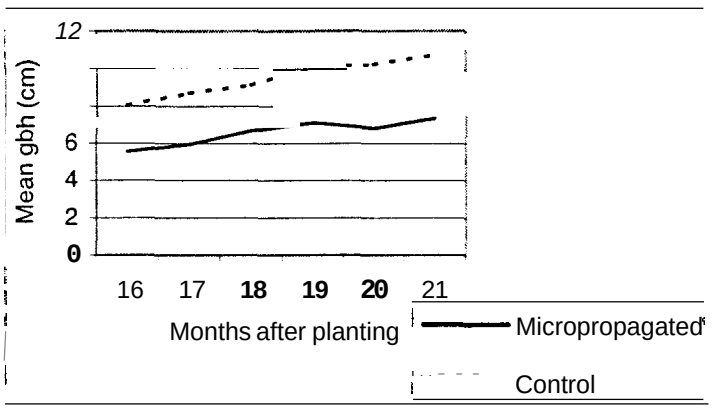


Fig. 10. Mean girth at breast height (gbh) of micro-propagated Eucalypt plantlets and rooted cuttings (control) in field.

The overall mean increment in gbh was lower in treatment than the control. ANOVA revealed non-significant effect of treatment and interaction between treatment and period while increment varied significantly over the period of measurement.

As in the case of height increment, the poor performance can be attributed to the inferior quality of the stock as well as the incidence of disease. The benefits of the selection carried out for disease resistance and growth rates are apparent in the improved performance of the rooted cuttings in this study.

4. CONCLUSIONS

Plantations are currently raised in India in both teak and eucalypt using propagules of bulked seed origin in the form of stumps in teak or seedlings. Rooted cuttings of selected clones of eucalypt have also been used in recent times particularly by the paper pulp industry. The propagules used for raising plantations are rather hardy and consequently silvicultural management is relatively non-intensive. The technology for micropropagation of mature trees has been developed for teak and Eucalypt and large scale cloning of selected elite trees is now feasible. Micropropagated plantlets produced using this technology and after conventional hardening treatments and nursery rearing appear to be less robust and hardy compared to the traditional propagules, as revealed by this study.

Proper nursery practice consisting of the right choice of container and potting media and exercising care when micropropagated plantlets are transferred to the soil so that deformation of the root and stem base is avoided, are important points to be considered to ensure production of quality planting stock.

Micropropagated teak plants have the advantage of having a stem at the time of planting but at the cost of having to be maintained with greater care at the nursery and during transportation to the plantation site. To take advantage of the field hardiness and ease of transport and storage the possibility of converting micropropagated teak plantlets to stumps should be explored.

Selection of plus trees of Eucalypt for plantations in high rainfall areas like Kerala should be based on disease tolerance in addition to other traits since poor survival of the plants during the establishment phase as observed in this study could be a obstacle to deployment of otherwise superior planting stock.

In Eucalypt, a field trial comparing the performance of micropropagated plantlets and rooted cuttings of the same age and derived from the same tree is essential for a cost benefit analysis of the two methods of clonal propagation. Micropropagation has the potential of giving very high multiplication rates but rooted cuttings can be produced using simple and cheaper facilities. Clones are also known to show differences in their response to either of the methods and to overcome this problem a large scale plantation programme will need to use both the techniques.

It is anticipated that genetic engineering of these important tree species with genes of important traits will be a reality in the near future. Regeneration of transgenics will involve tissue culture and hence it is important to standardise the micropropagation technology including proper hardening and nursery practices so as to generate quality planting stock.

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