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Contents

Project Summary

Theme : Teak Productivity Improvement

Project Title : Clonal Seed Orchard Management for higher seed productivity

Component 1 : Parentage confirmation of existing clones with molecular markers

Component 2 : Assessment of reasons for poor flowering

Duration : Two years (Phase - 1)

Investigators

Component 1 : K.K. Seethalakshmi, E. M. Muralidharan and George Thomas*

Component 2 : M. Balagopalan, C. K. Somen and K. K. Seethalakshmi

Outputs

1. Confirmation of parentage of clones in the orchard.
2. Reasons for poor productivity of orchards will be identified

Total Budget **: Rs. 12, 94,665/-**

Implementing Agency : Kerala Forest Research Institute and Rajiv Gandhi Centre for Biotechnology

Collaborating Agency : Kerala Forest Department

* Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram

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We are indebted to Dr. K. V. Sankaran, Director; Dr. J. K. Sharma and Dr. R. Gnanaharan, former Directors for their continuous support and encouragement for the implementation of the project and preparation of report.

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Clonal Seed Orchard Management for Higher Seed Productivity in Teak

Abstract

For genetic improvement of planting stock of teak, clonal seed orchards were established in Kerala during 1979- 81 by bud grafting selected plus trees from different geographic origins. Even after 15 years of growth, flowering and seed production in most of the clones were not promising. It was suspected that lack of flowering may be due to any one or more of the following reasons. 1. Growth of root stock instead of scion during the establishment of grafts. 2. Stock - scion interaction 3. Nutritional imbalances. 4. Physiological reasons like hormonal inadequacy, insufficient light, temperature, moisture, etc.

To find out the nature of flowering and seed production in existing clones, a study was undertaken in the seed orchard at Kalluvettamkuzhi, Arippa. Observations were recorded on nature of flowering, molecular characteristics of root and shoot DNA of 11 trees belonging to three plus trees, nutritional status and physiological parameters among flowered and non-flowered trees.

The study revealed that the percentage of flowering varied from 2-3 in 1999 and 2000 which increased to 5 in 2001 and 2002. The phonological phase fruiting were spread over a period of seven months i.e., from June to December. The clones differed in flower production per inflorescence. The fruit production varied from 65g/tree to 300g/tree. The average fruit production was 125g/tree.

Cluster analysis of RAPD data revealed five distinct clusters. Three plus trees stood separate from rametes and formed a distinct cluster. Flowered and non-flowered clones showed a tendency to group together into separate clusters. Two clusters each were obtained for flowered and non-flowered clones. With minor exceptions, the DNA from the scion of a clone clustered with the DNA

from its stock. The results indicated that the stock and scion of a clone were highly similar at the DNA level and the scions did not evince any relation to the plus trees from which they were derived. In addition, the flowered and non-flowered clones were genetically distinct. Since the number of samples analysed was limited a definite conclusion could not be drawn from these results. No polymorphism could be found in isozyme analysis in the material collected from seed orchards also.

The foliar nutrient contents N, P, K, Ca and Mg showed considerable variation within clones and between clones. There was a decrease in P and K contents in the leaves of trees flowered from the time of flowering till fruit maturation after which there was an initial increase, though not prominent. If a stress was given to the trees by giving more P and K at the time of flowering and very low N, Ca and Mg, to a certain extent the problem of poor flowering could be solved if all other conditions were conducive to flowering.

Eco-physiological studies in the orchards showed that parameters like light, temperature or rainfall were not a limitation for seed production at Kalluvettamkuzhy. This may be due to the wider spacing (8x8m minimum) given while planting ramets in this orchard.

The results obtained in the study points to the possibility of graft failure in the ramets and the low variability between the clones and ramets used in the study. Use of rooted cuttings of micropropagated plants and adopting a more favourable spacing in future clonal seed orchards is suggested.

Clonal Seed Orchard Management for Higher Seed Productivity in Teak

Introduction

Establishment of clonal seed orchards is considered as a promising strategy for genetic improvement of teak. Orchards are established by assembling ramets of plus trees by bud grafting. Different clones are planted in the orchard area in completely randomized design to avoid inbreeding and to promote maximum cross pollination. The orchard sites are selected away from the natural populations and plantations of teak.

Orchard establishment in Kerala was initiated in 1979. A total of 34.6 ha seed orchards were established including the 6.1 ha pilot seed orchards established by Kerala Forest Research Institute (KFRI) at Nilambur, Palappilly and Arippa and 28.5 ha production seed orchard by Kerala Forest Department (KFD) at Kalluvettamkuzhi. The KFRI orchards at Nilambur, Palappilly and Arippa consist of 17, 20 and 25 clones respectively and that at Kalluvettamkuzhy has 25 clones.

Although precocious and profuse flowering were anticipated in orchards, flowering occurred only at the normal age of phase change in teak, i.e., after 7-8 years and the percentage of flowered clones was less than 5 in all the orchards.

KFRI in collaboration with KFD has initiated different projects to address various issues related to flowering and fruit production in teak in general, and clonal orchards, in particular. Some of the completed studies show that lack of effective pollinators, self incompatibility and fungal attack are some of the parameters limiting seed set after flowering (Indira and Mohanadas, 2002; Mohanadas *et al.*, 2002). In addition to the poor seed set when flowering

occurs, teak seed orchards all over the world face the problem of poor flowering. This investigation was taken up to understand the factors that result in poor flowering in teak seed orchards.

Before planning any experiment to induce flowering in orchards, it is a pre-requisite to confirm that the existing clones are true to their parents. For genotype identification, molecular markers like isozymes and DNA finger printing have been successfully used for plant species. Initially different molecular markers can be tried to standardize the most suitable one to use regularly for identification of teak clones. In the present study, two methods viz., molecular and biochemical markers were used for parentage confirmation.

To find out the nature of flowering and seed production in existing clones, it is essential to carry out an assessment of current status of flowering and seed production. The levels of nitrogen, phosphorus, potassium and a number of micro-nutrients have been shown to influence flowering and fruiting. An assessment of the status of macro and micro nutrients in soils as well as in foliage of different clones in the existing orchards will help to check whether the poor flowering is due to nutritional imbalances.

Observations on flowering of teak have shown that single tree and trees in the border of the plantations flower profusely. This indicates the role of light in flowering of teak. To test this hypothesis, eco-physiological parameters need to be recorded from different locations in the orchards.

This investigation was taken up to check the genotype of teak clones in the existing orchards, to assess the reasons for poor flowering and thereafter standardize management practices for higher seed productivity from seed orchards.

The results of the investigation are presented in the following chapters, parentage confirmation of orchard trees, nutritional aspects related to flowering and physiological parameters in the orchard.

Parentage confirmation of clones in the teak seed orchards – DNA Fingerprinting

Introduction

It is likely that, instead of scion, the rootstock can grow and the orchard tree will exhibit the characteristics of rootstock. To verify this, DNA finger printing of bark from root and shoot of selected trees along with the bark of the parent plus tree from which the grafting was done was undertaken.

Materials and Methods

Standardization of protocol for isolation of DNA from teak wood: The method of Sharp *et al*. (1988) was found to be suitable to isolate high quality total genomic DNA from teak wood. The detailed protocol is given below.

Collection and processing of plant material: The stem and root bark along with the sapwood was cut with a sharp knife for estimation of genomic DNA. The tissue was collected in labeled polythene bags and stored on ice. The samples were brought to the laboratory as quickly as possible and stored at –70^{o}C till extraction. Just before extraction, the samples were taken out and cleaned in double distilled water. The sapwood was cut out of the bark with a knife and blotted to dry. The tissues were minced with a knife, frozen in liquid nitrogen and ground to a fine powder using pestle and mortar. The processing was done quickly and care was taken to minimize the time needed to reach the powder stage.

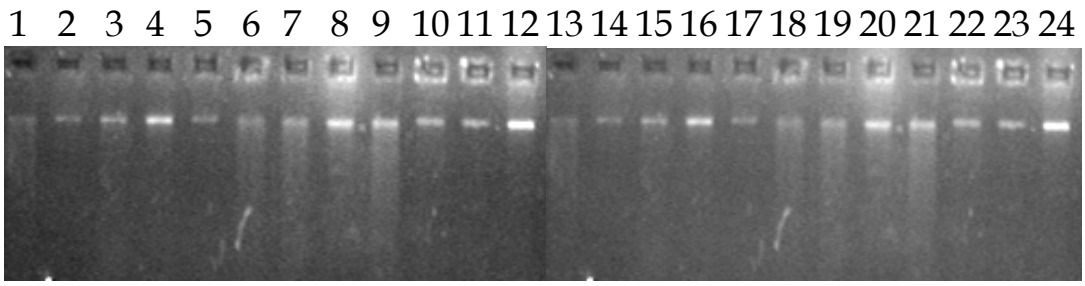
Extraction and purification of DNA: The powder was transferred into a conical flask and suspended in 5ml extraction buffer (100 mM Tris-HCl [pH 8.5], 100mM NaCl, 50mM EDTA, 2% SDS) per gram of tissue and incubated at 37^{o}C for 2.5h. An equal volume of 1:1 ratio of Tris buffered phenol : CHISAM (24 chloroform : 1 isoamyl alcohol) was added into the lysate, stirred for 10

min and centrifuged at 12,000 x g for 15 min. The supernatant was collected and DNA was precipitated with 0.6 volume of isopropanol. DNA was pelleted at 12,000 x g, washed twice in 70% ethanol, dried at room temperature (23-25^{o}C) for 10 min and dissolved in 1x TE (10 mM Tris-HCl [pH 8.0], 1mM EDTA [pH 8.0]). DNA was treated with Rnase A (50 µg/ml final concentration) followed by proteinase K (50µ g/ml final concentration) at 37^{o}C for 1h each. DNA was extracted thrice with equal volume of 1:1 ratio of Tris-buffered phenol : CHISAM and once with equal volume of CHISAM alone. DNA was reprecipitated by adding 1/10 volume of 3M sodium acetate (pH 5.2) and 2 volumes of chilled (-20^{o}C) absolute ethanol. DNA was pelleted at 12,000 x g for 10 min, washed twice with 70% ethanol, dried at room temperature (23-25^{o}C) for 10 min and dissolved in 1x TE. The DNA was of high quality as evidenced by the results of agarose gel electrophoresis (Plate 1).

Standardization of protocol for DNA fingerprinting of teak wood using Random Amplified Polymorphic DNA (RAPD) technique: The conditions to generate reproducible amplified DNA fragments were optimized by varying the temperature regimes and amplification cycles. In a final volume of 200 l, the optimized amplification reaction contained 7 ng genomic DNA, 1 U *Taq* DNA Polymerase (Bangalore Genei, India), 5 pmol 10-mer primer, 0.2 mM dNTPs, 10 mM Tris-HCl (pH 9.0), 1.5 Mm $MgCl_2$, 50 Mm KCl and 0.01% gelatin. The mixture was overlaid with two drops of mineral oil and subjected to 43 amplification cycles in a Bio-Rad PCR machine (iCycler) as follows: 1 min at 94^{o}C for denaturation, 50 sec at 36^{o}C for annealing, 2 min at 72^{o}C for primer extension. Before the first cycle, the samples were denatured for 5 min at 94^{o}C and after the final cycle, the samples were incubated for a further 10 min at 72^{o}C to ensure complete primer extension. The amplification products were separated on 1.2% agarose gels stained in EtBr and photographed using a gel documentation system (Bio-Rad).

The RAPD profiles yielded by some of the DNA samples had only few amplification products and were often not reproducible. Such DNA samples were further purified using Wizard DNA clean-up system (Promega). The purified samples generated optimum number of amplification products and were reproducible.

Plate 1. Samples of DNA extracted from different samples of teak leaves and bark



Fingerprinting of plus trees and respective clones, and interpretation of data

Materials used: Three plus trees and eleven ramets derived from these plus trees were selected for genetic analysis (Appendix 2.1). DNA was isolated from the bark tissue of plus trees and from the bark and root tissues of the clones. The major objective of the project was to examine the difference(s) between the flowered and the nonflowered clones that were developed from the same plus tree. Therefore, both flowered and nonflowered clones developed from the same plus tree were included in the analysis.

Fingerprinting and statistical analysis: Twenty random 10-mer primers (Operon Technologies, Alameda, California) (Appendix 2.2) were used to fingerprint the DNA samples following RAPD technique. The PCR amplifications were carried out as described earlier. The DNA fingerprint profiles of the plus trees and those of the root and shoot of the clones

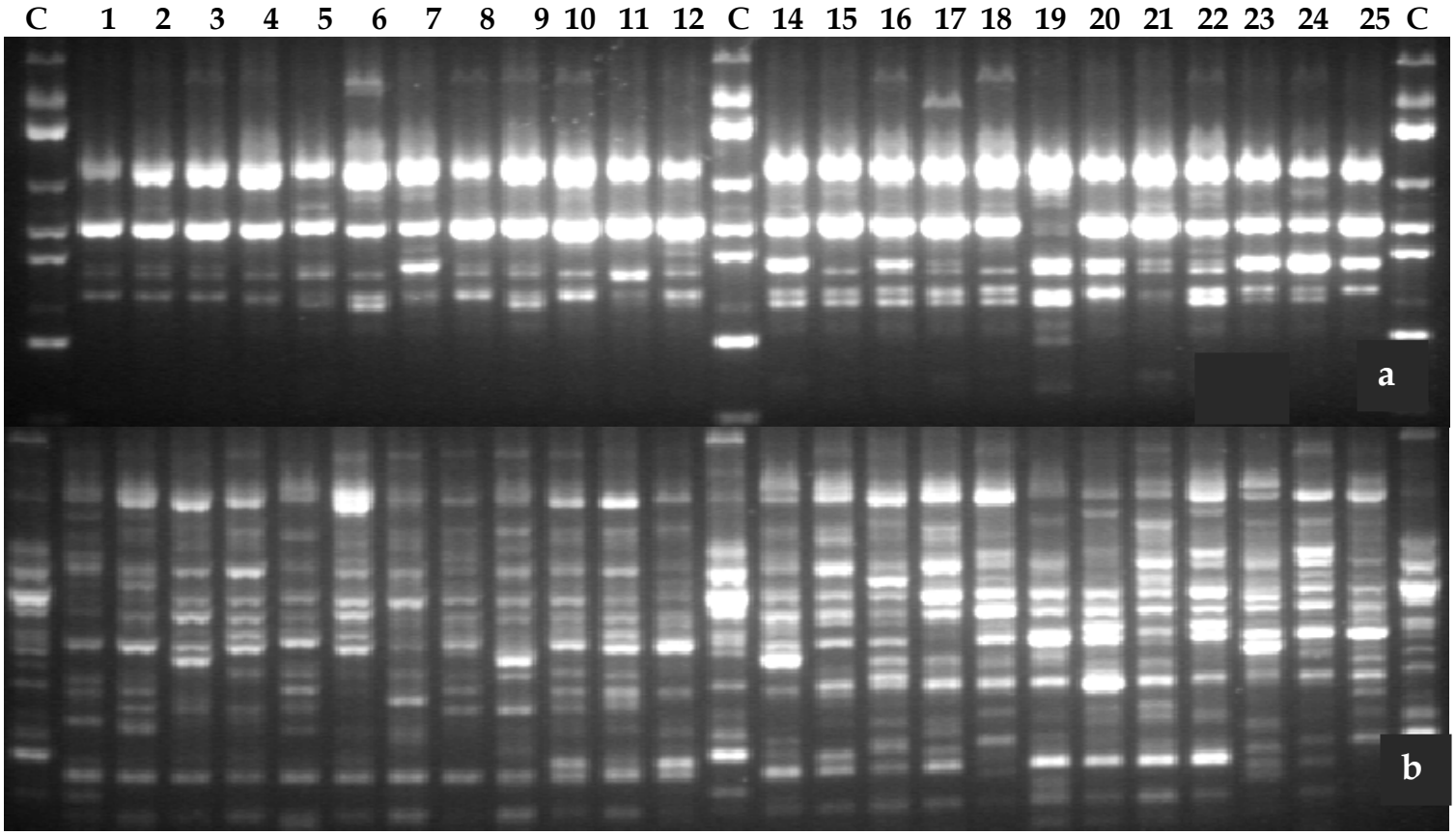
generated by the 20 random primers (Appendix 1.2 a-t) were analysed using BioNumerics software (Applied Maths, Kortrijk, Belgium) in order to examine the genetic relatedness between the DNA samples used. The resulting dendrogram is given in Appendix 3.1.

Interpretation of the data: Cluster analysis of the dice coefficient estimate, based on the RAPD data using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) revealed five distinct clusters (Appendix 3.1; 3.2). Seven DNA samples were entered in cluster I (Clusters are numbered arbitrarily). The root and shoot DNA samples of clones - 1, 2 and 3, and the root DNA of ramete - 4 were grouped in this cluster. All the 4 rametes represented in this cluster were flowered. Of the four clones two were derived from plus tree - 2, one from plus tree - 1 and one from plus tree - 12. In cluster II, root and shoot samples of all the flowered clones derived from plus tree - 12 (clones - 5 and 6) and the shoot of clone - 4 were grouped together. The shoot DNA of clone – 7, and the root and shoot DNA from clones - 8 and 9 were separated into cluster III. All these clones were nonflowered and originated from either plus tree - 1 or plus tree - 2. DNA from the root of ramete – 7 was not included in the analysis. Cluster IV consisted of DNA samples from all the three plus trees and the shoot DNA sample from one of the nonflowered clones (ramete - 10) originated from plus tree 12. The root DNA of this clone (ramete – 10), and the shoot and root DNA of the other non flowered clone (ramete-11) from the same plus tree (plus tree – 12) were entered in cluster V. The year of planting of the clones had no correlation with their clustering (Appendix 3.2).

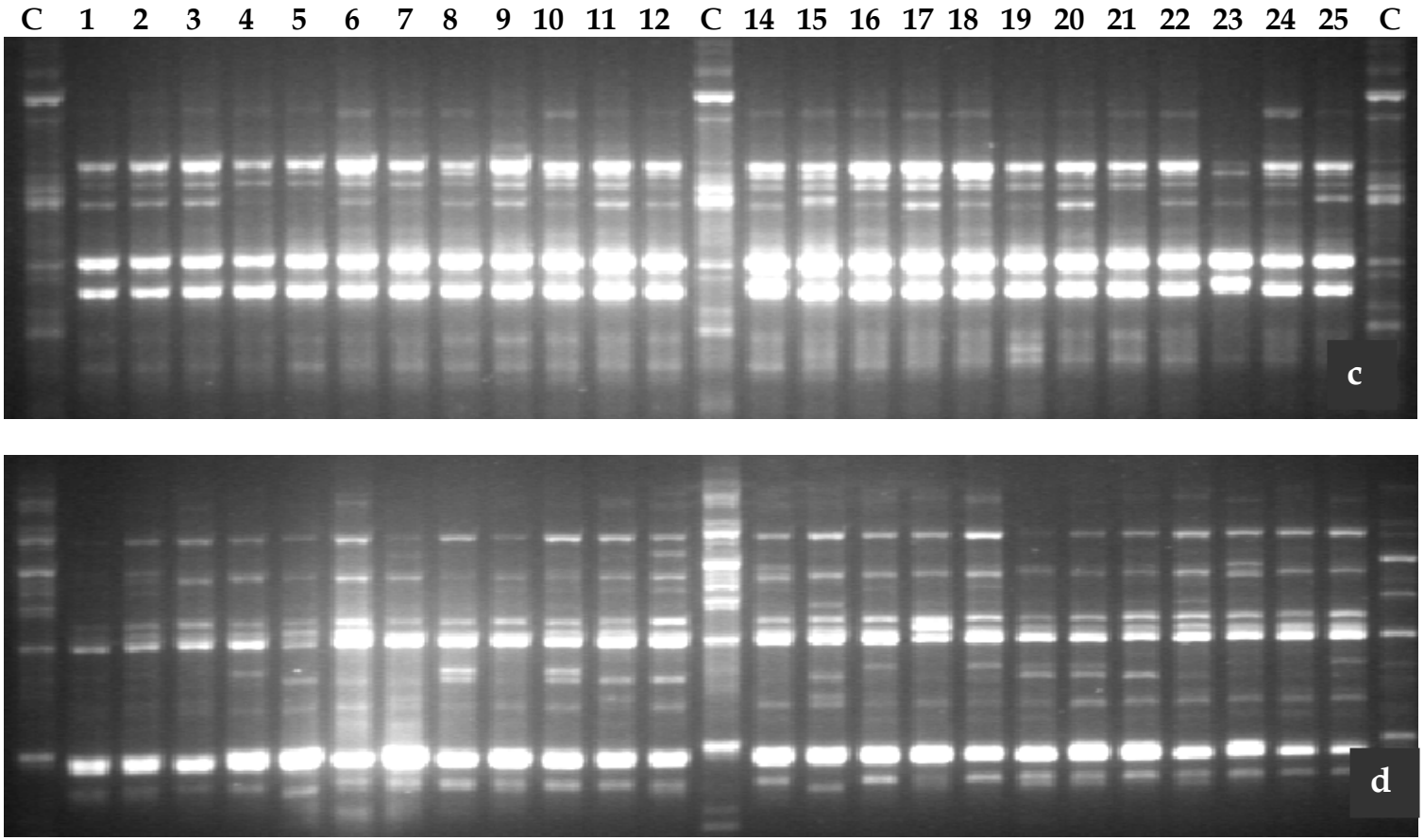
The DNA sample from the scion of a clone was expected to cluster with the DNA from the plus tree from which the scion was taken. However, with minor exceptions, the results of the cluster analysis clearly show that DNA which is closely related to the DNA of the scion of a clone is that from its own stock. The DNA isolated from the plus trees formed a distinct cluster, away

from all the other clones, indicating that the plus trees are genetically distinct and have almost similar level of relatedness to both the stock and scion of the rametes. It can be concluded that the scions of the rametes examined in this study were closely related to their own stock, rather than to the plus trees from which the scions were taken.

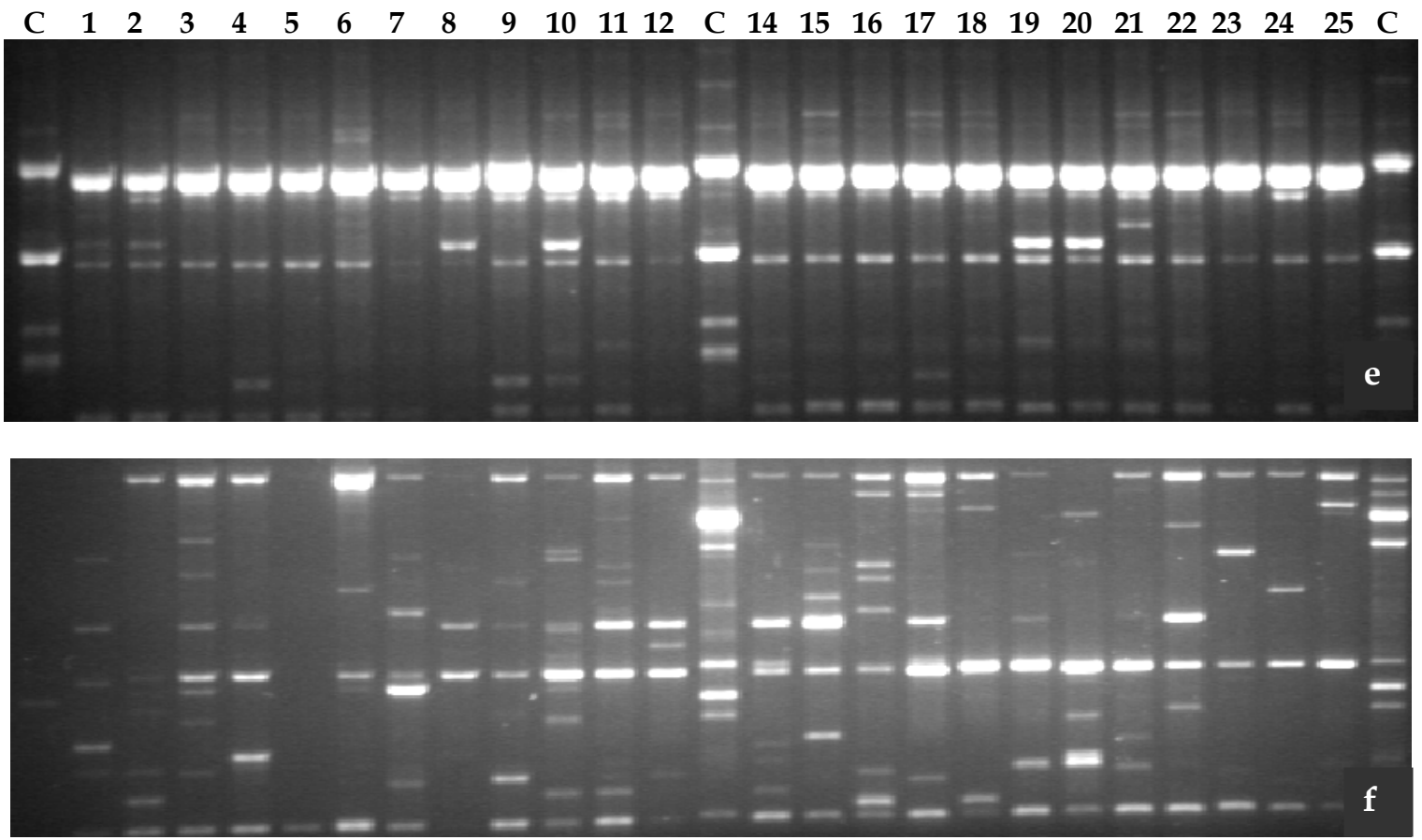
Another striking result was the tendency of the flowered and non flowered clones to form separate clusters. Flowered and nonflowered clones from plus trees 1 and 2 were separated into two distinct clusters, whereas those from plus tree 12 stood away from these two clusters, and formed two other distinct clusters. The results imply a fundamental difference between the flowered and the non clones. Since the DNA of stock and scion of rametes are segregating together, the root stocks of flowered and non-flowered rametes are distinct. It is difficult to make a definite conclusion with limited sample analysis.



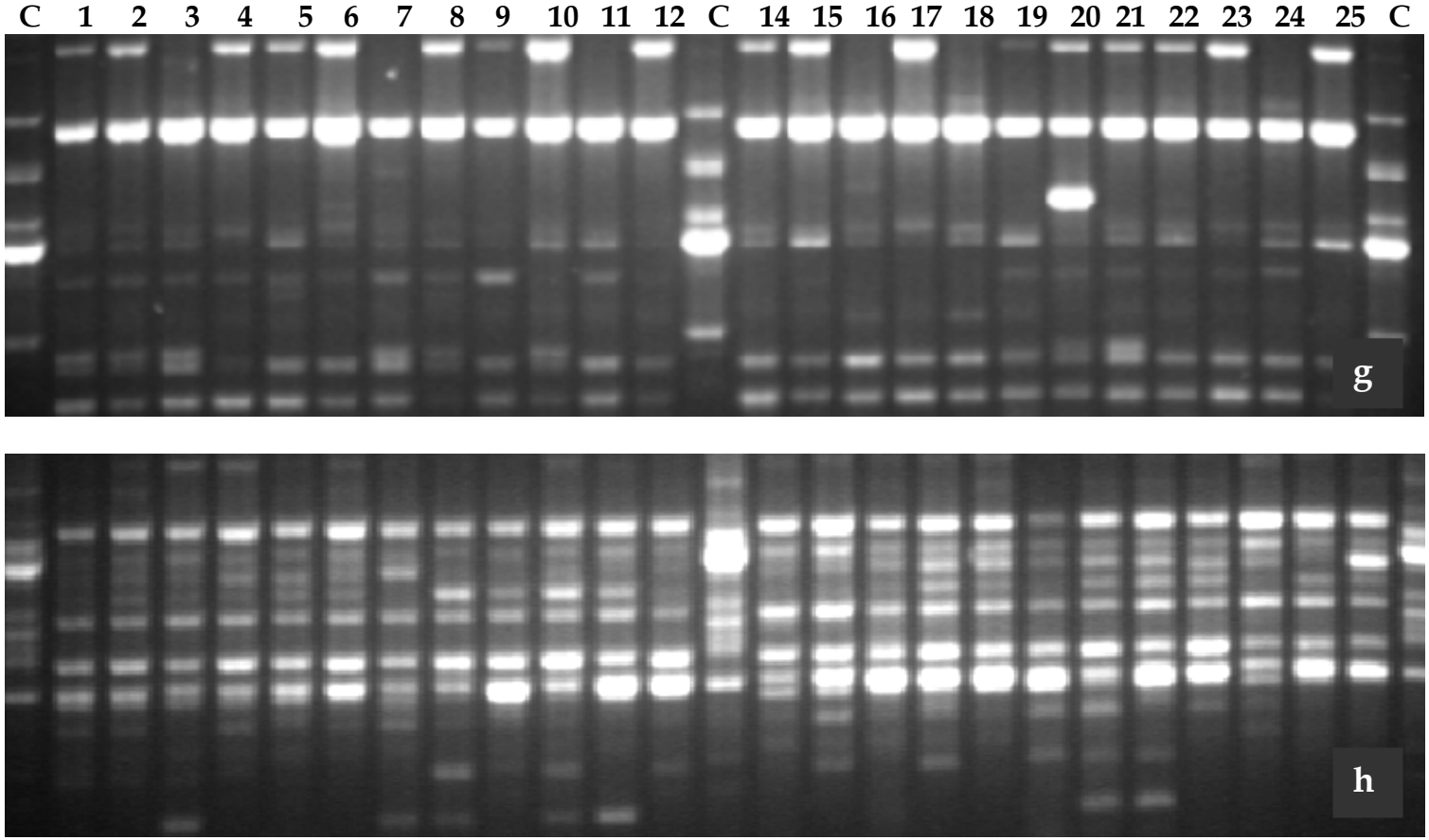
Appendix 1.2 (a-b)



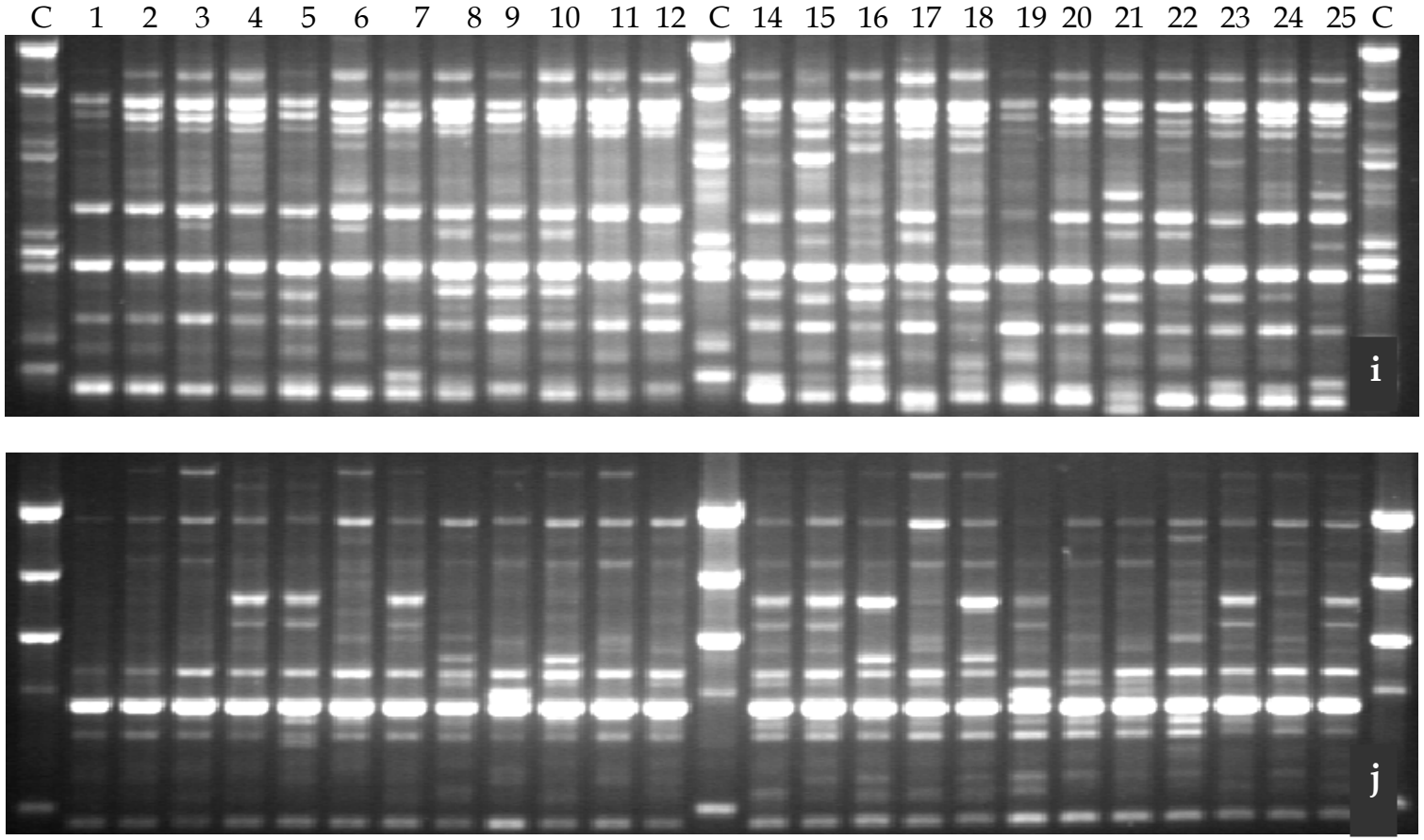
Appendix 1.2 (c-d)



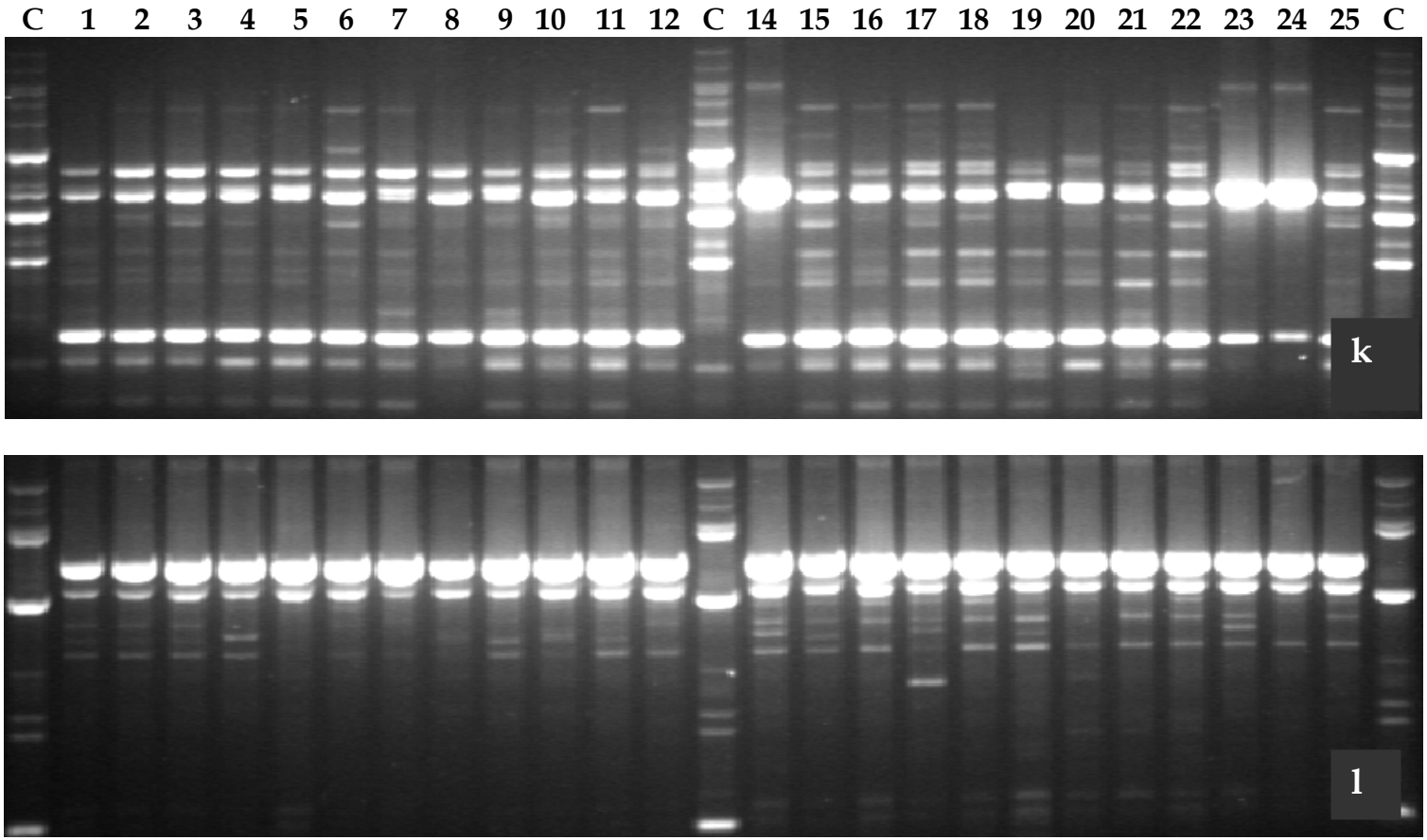
Appendix 1.2 (e-f)



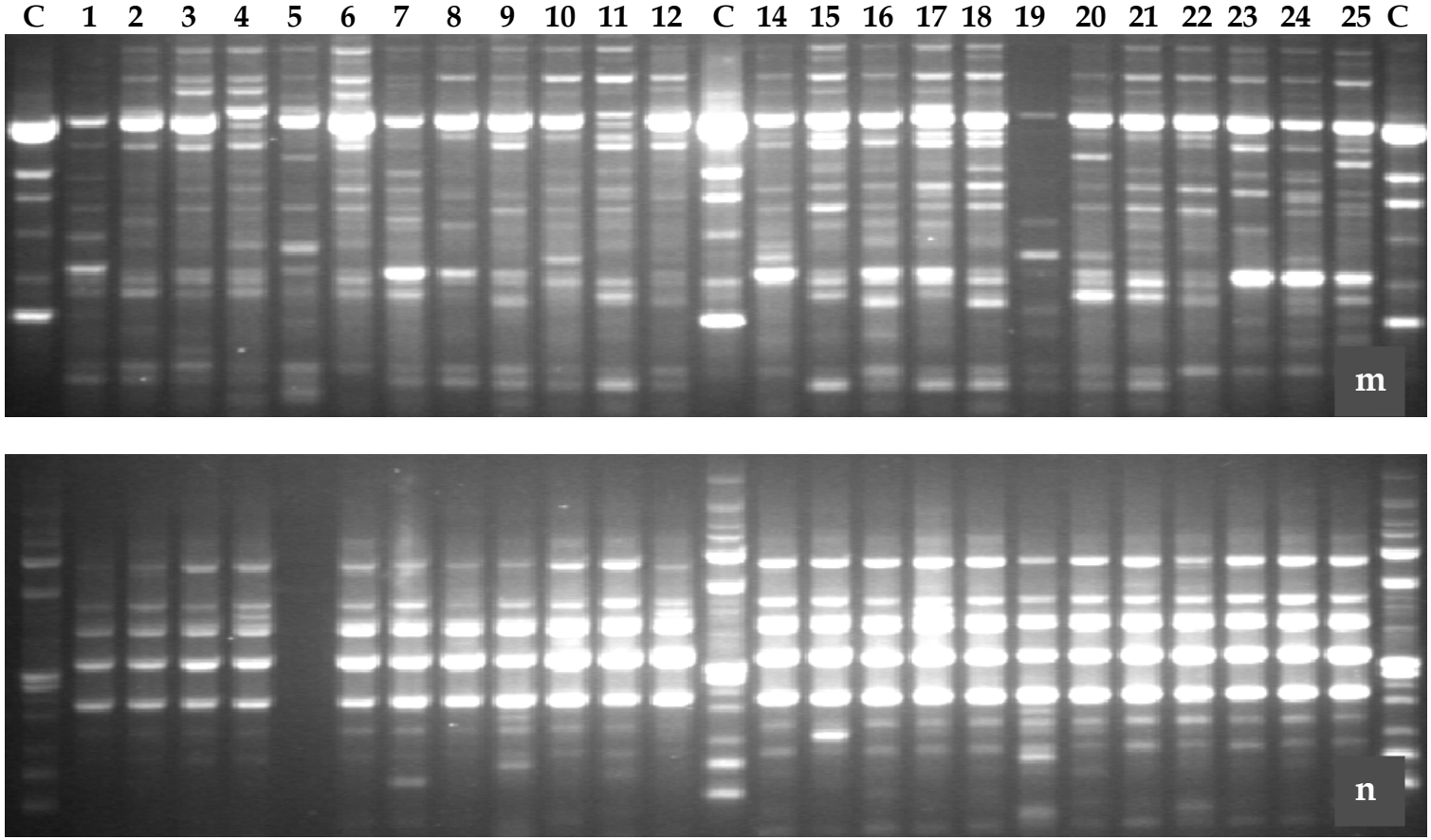
Appendix 1.2 (g-h)



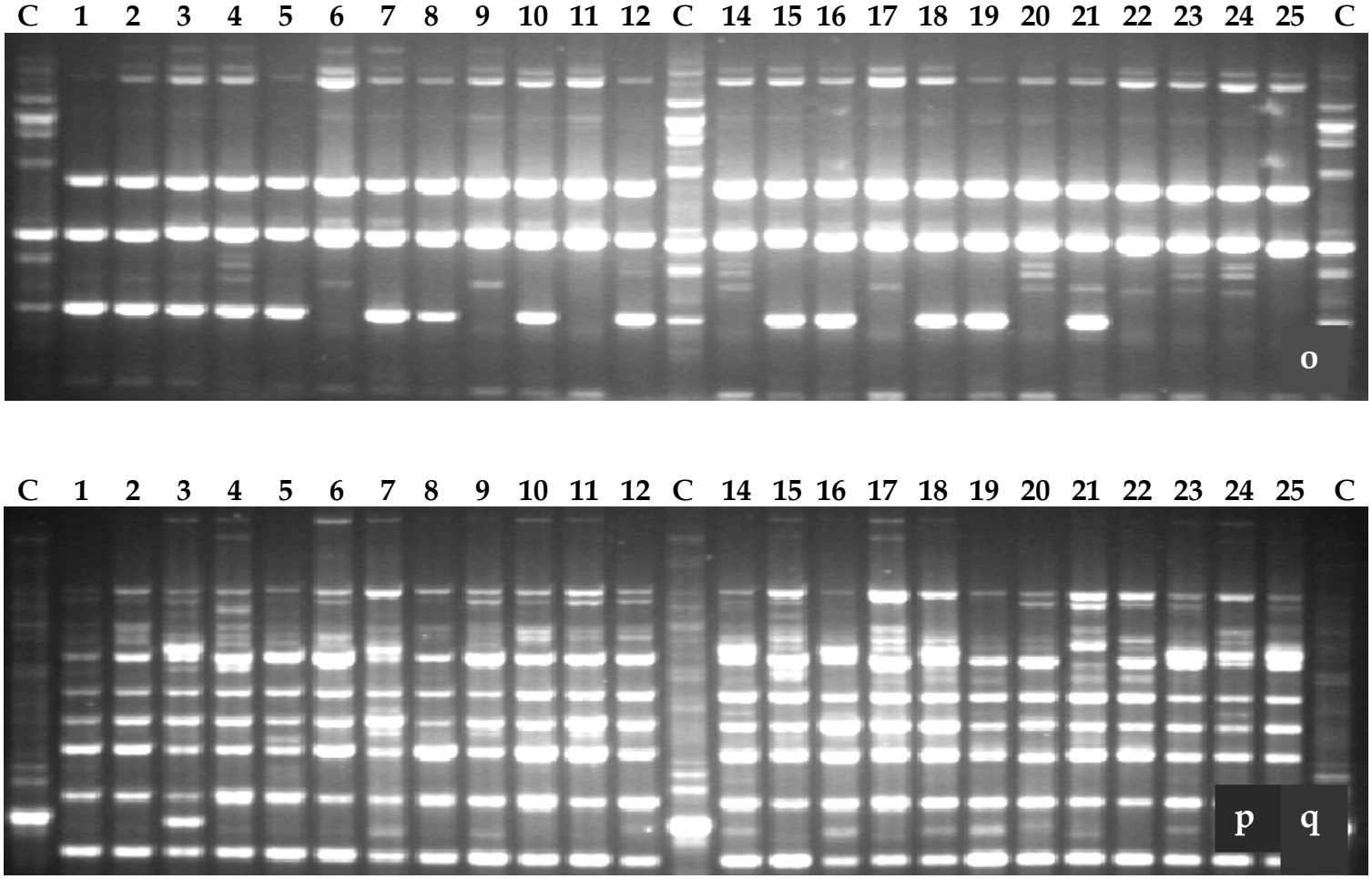
Appendix 1.2 (i-j)



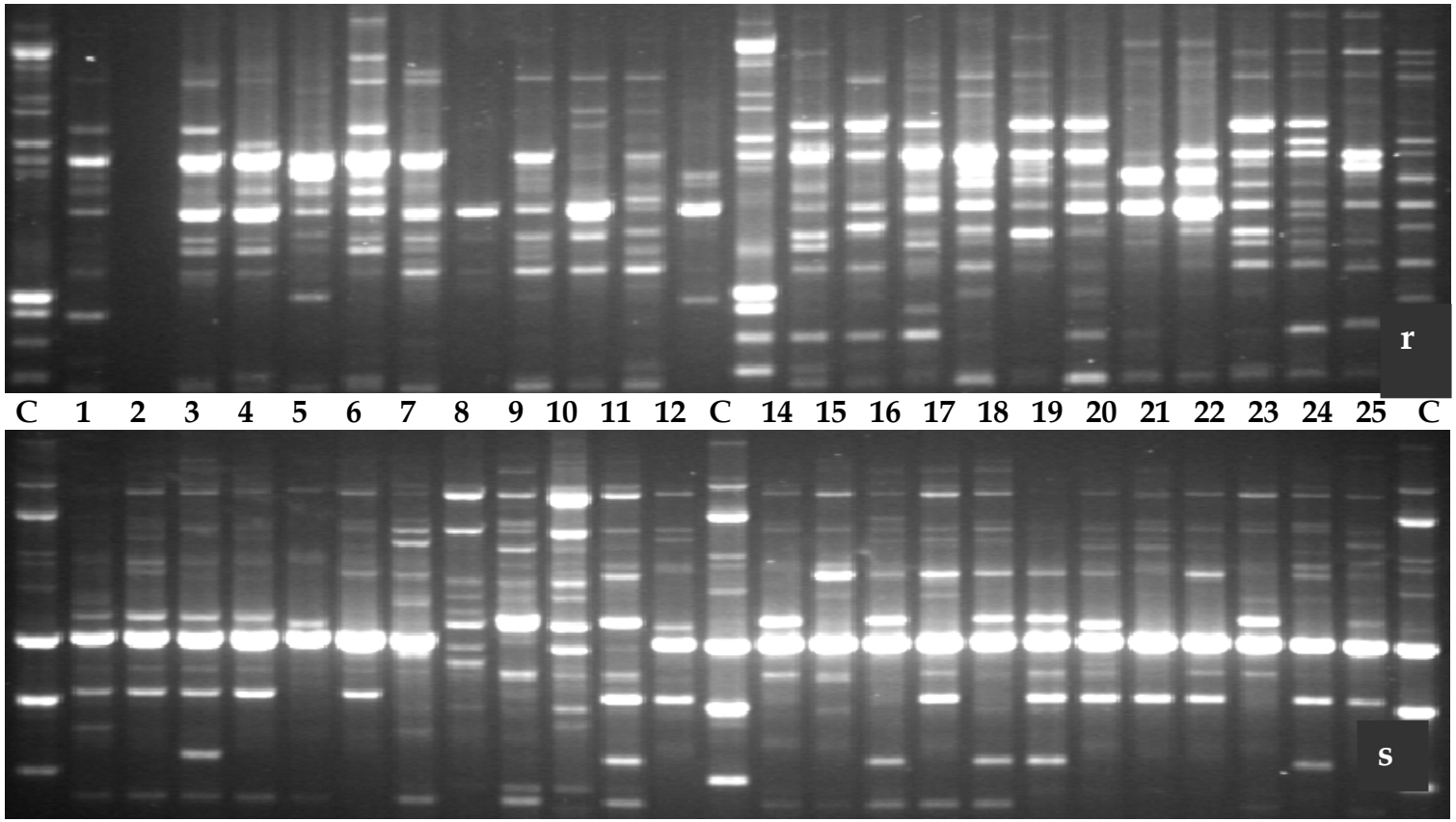
Appendix 1.2 (k-l)



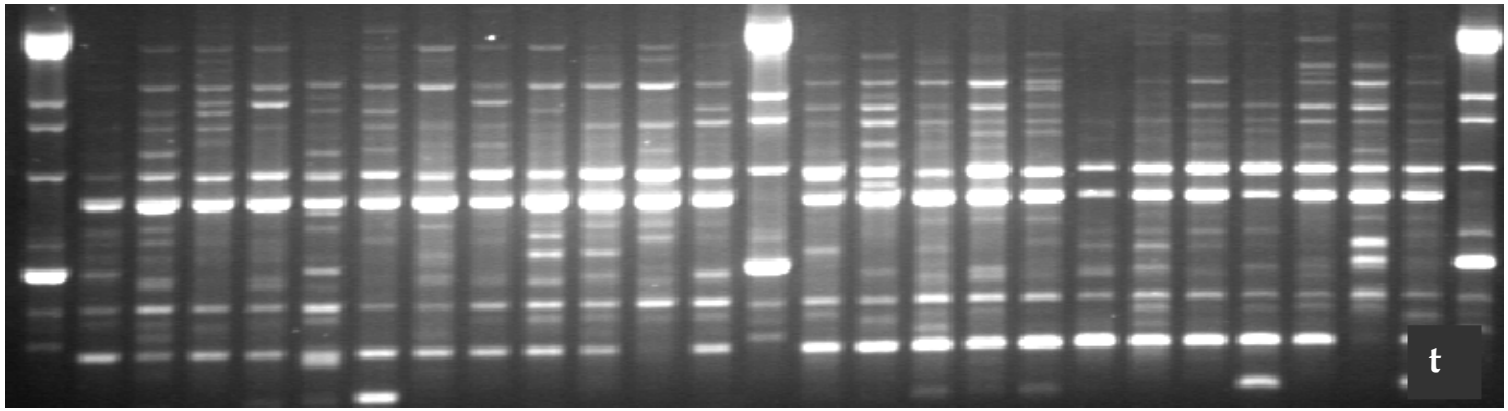
Appendix 1.2 (m-n)



Appendix 1.2 (o-p-q)



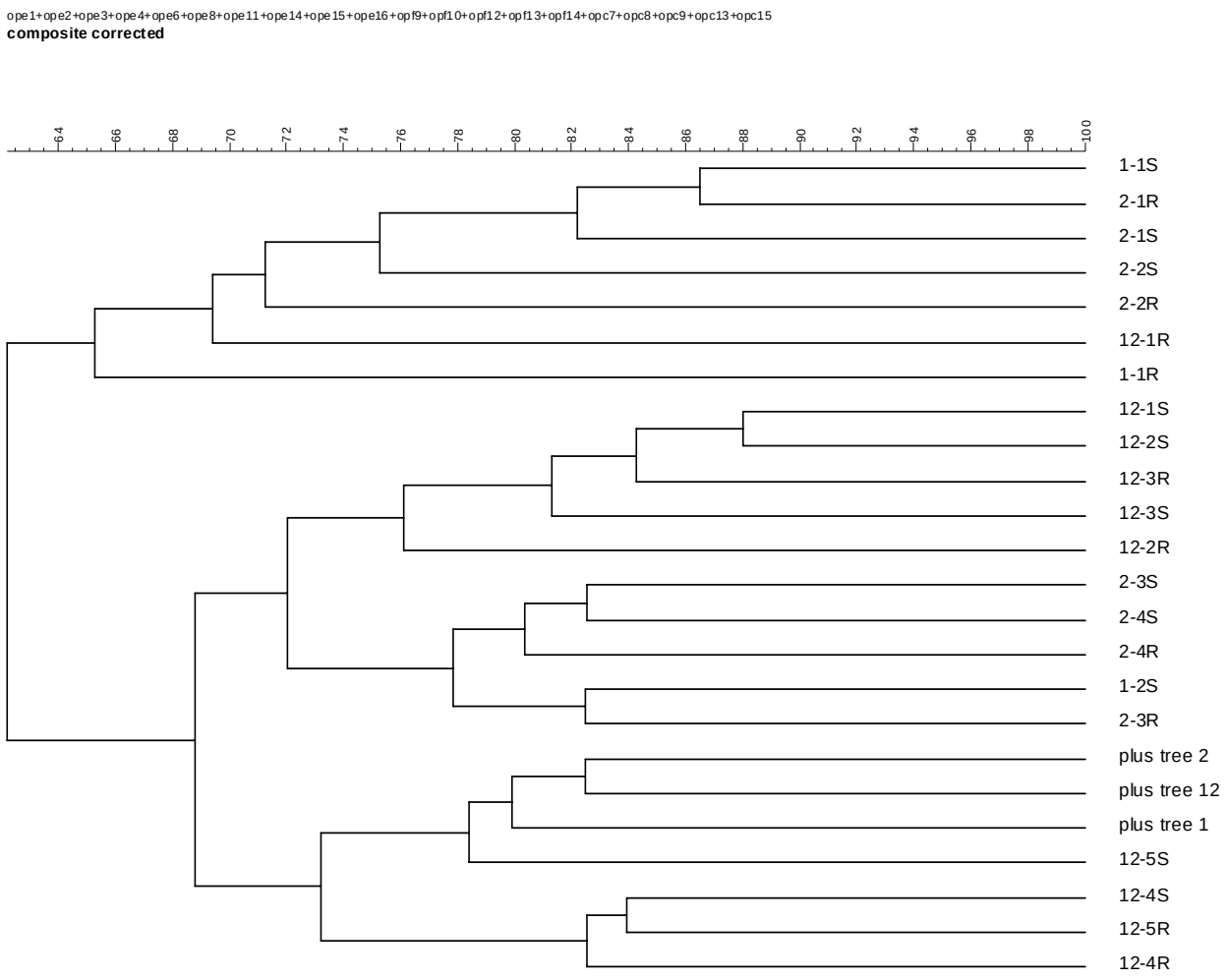
Appendix 1.2 (r-s)



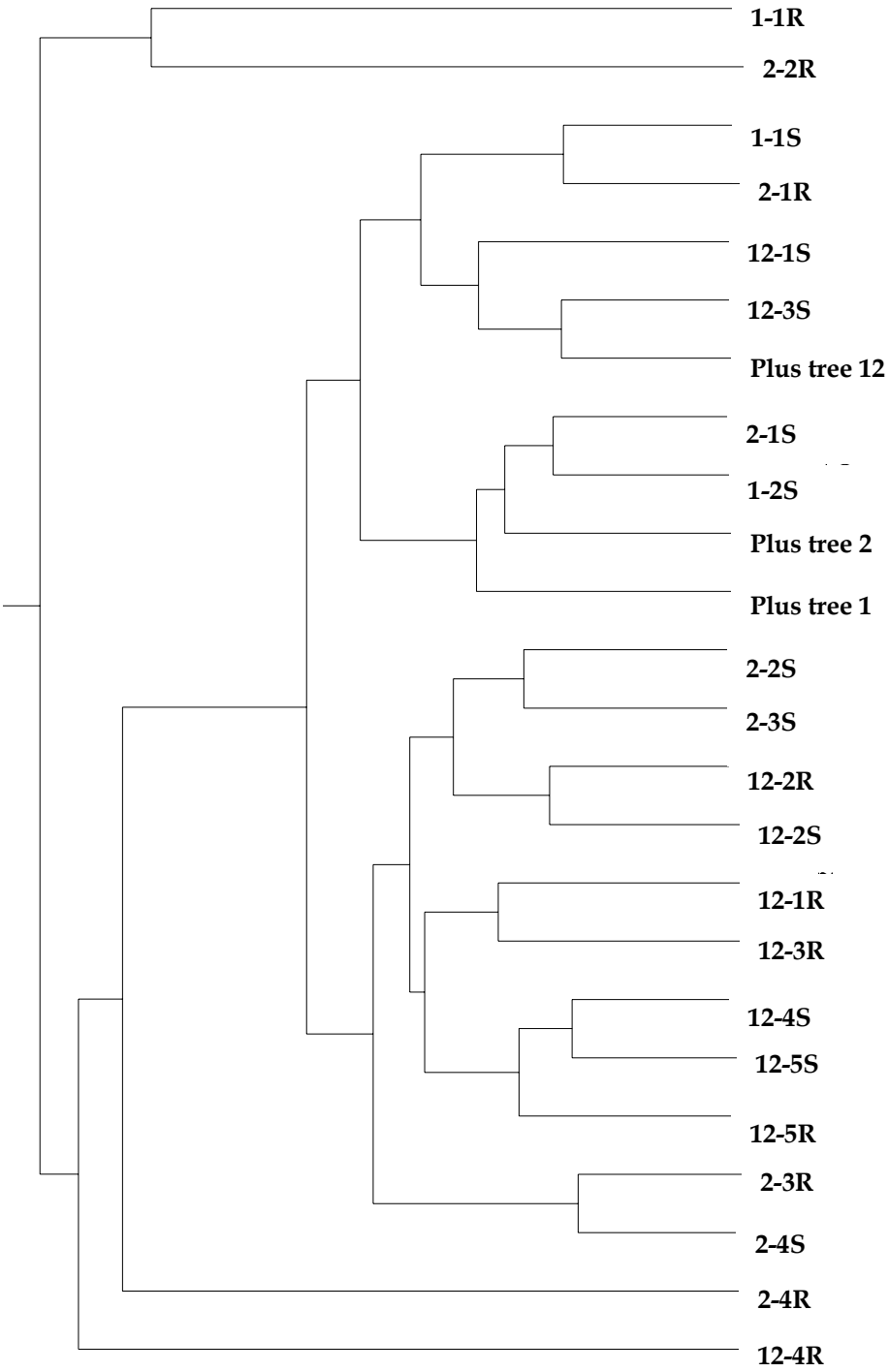
Appendix 1.2 (t)

Appendix III

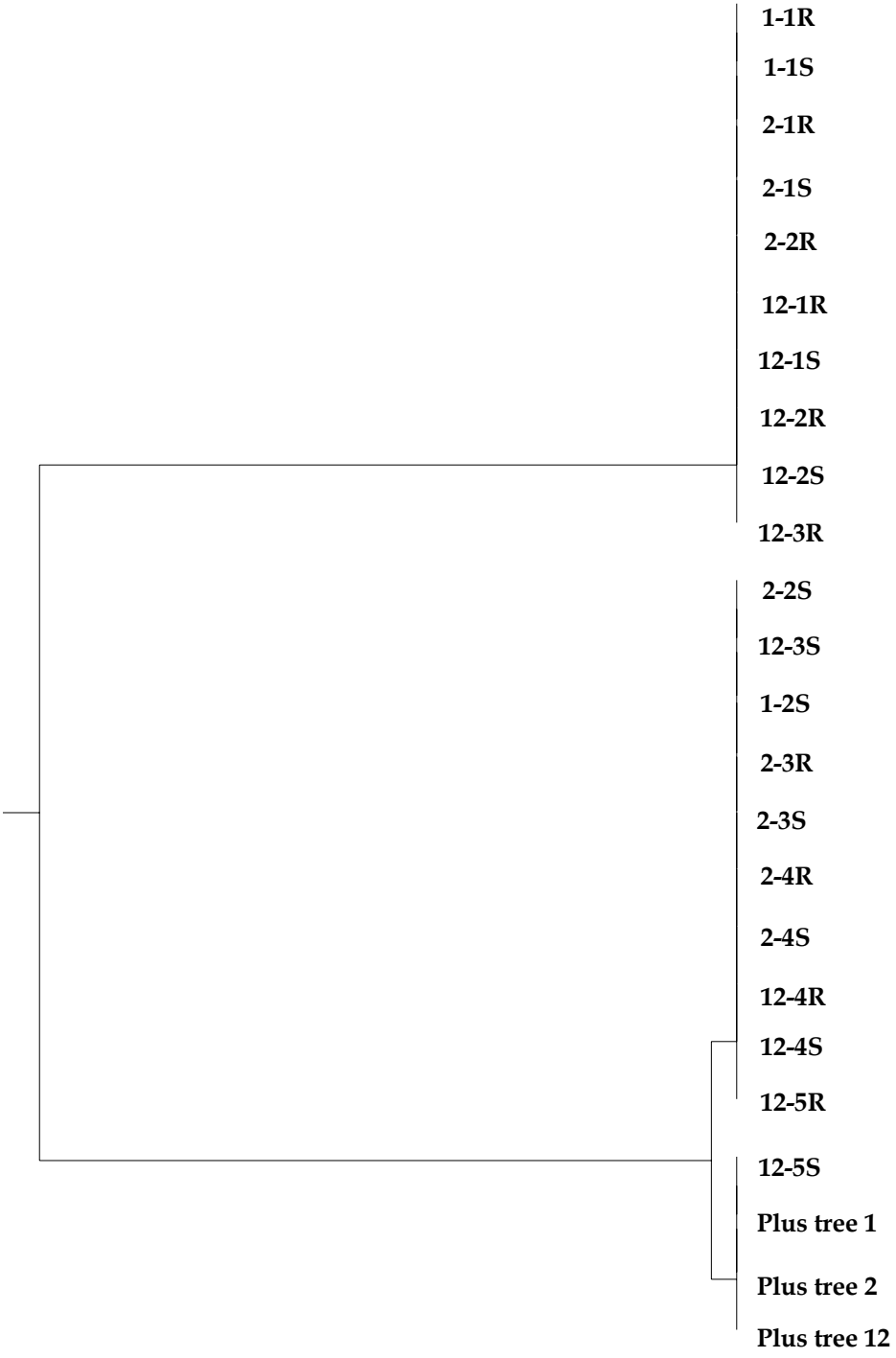
Dendrograms obtained from RAPD profiles showing similarity among clones and ramets.



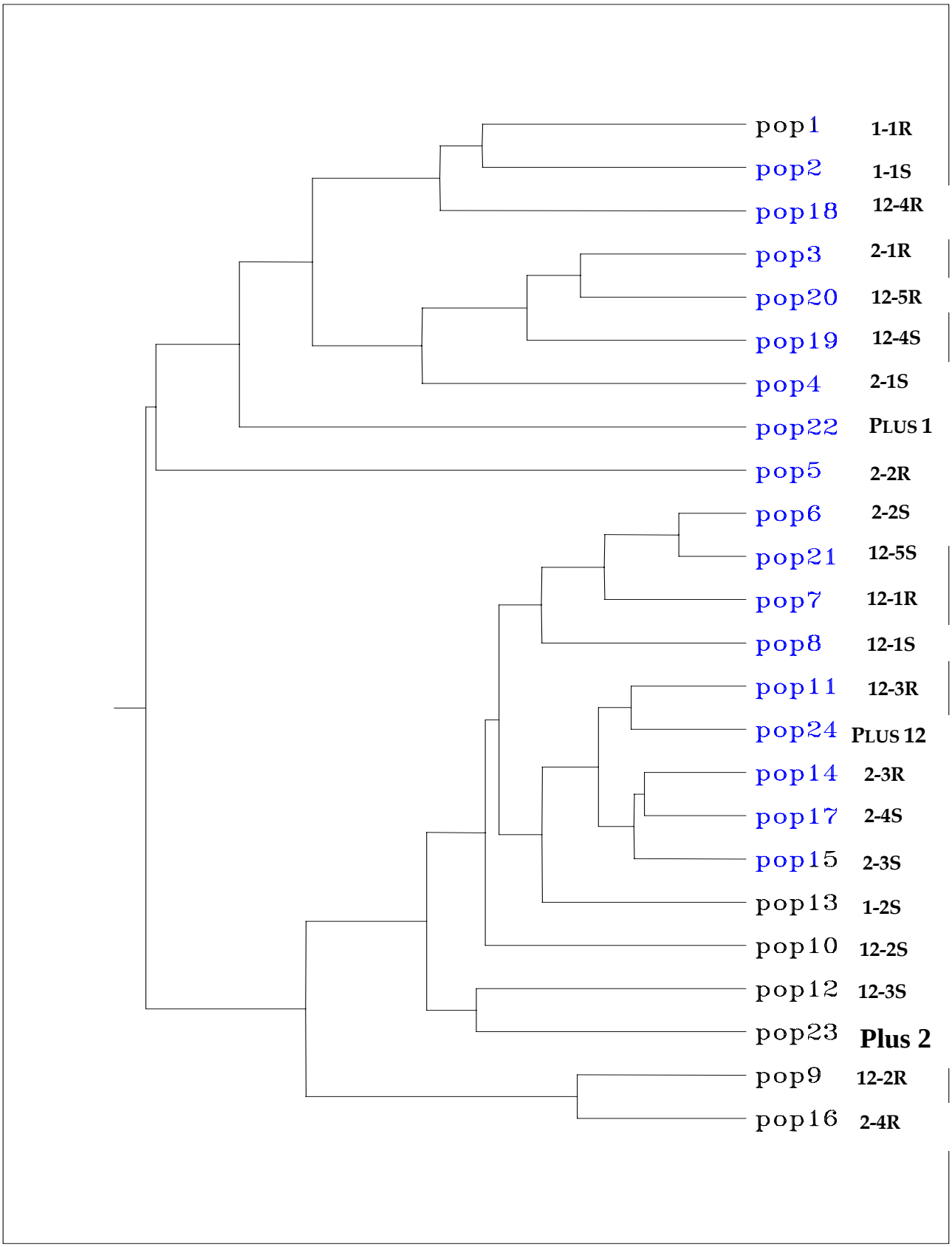
Dendrogram generated by BIONUMERICS software



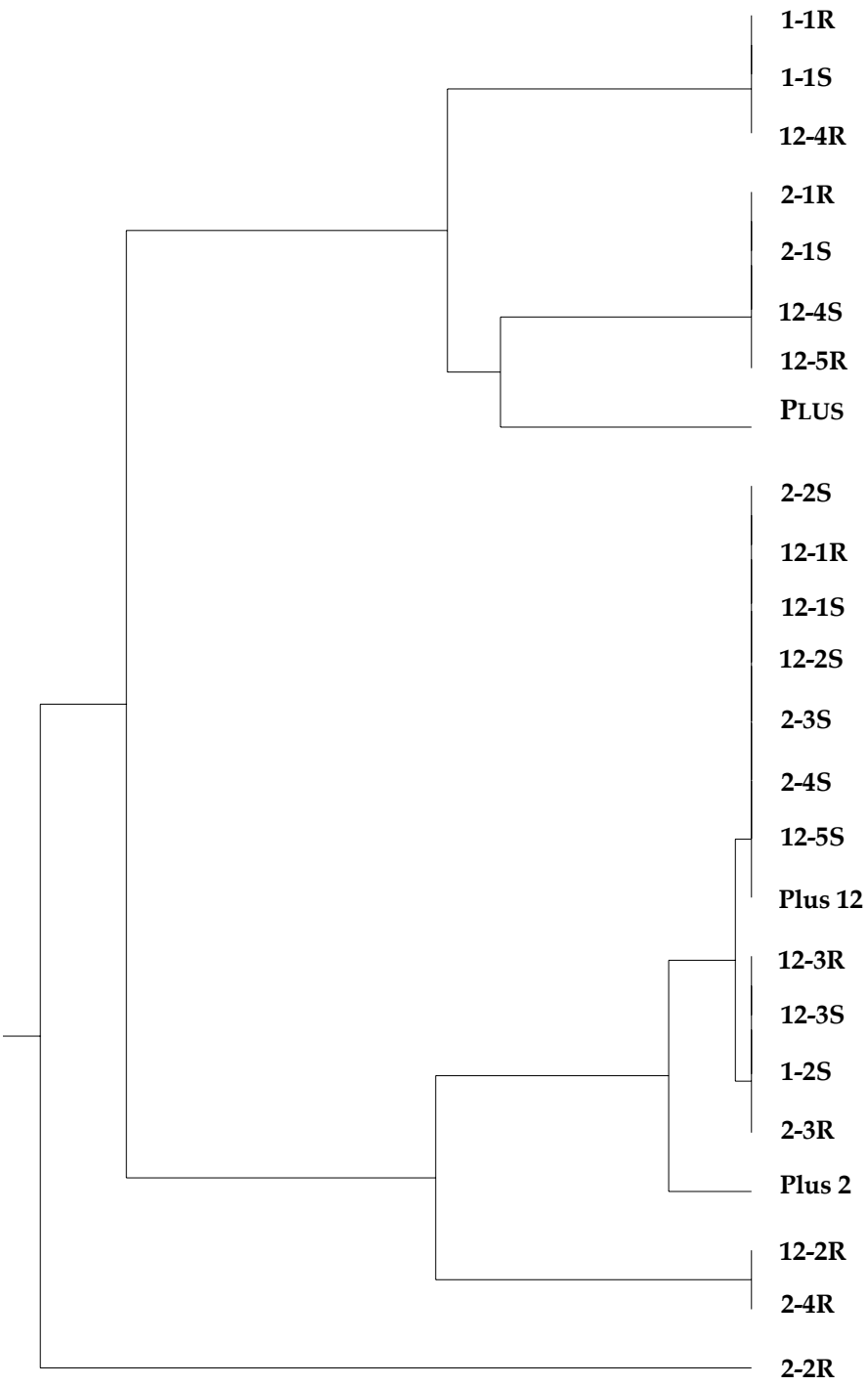
Dendrogram generated by POPGENE using the data from BIONUMERICS (using Nei's regular genetic distance)



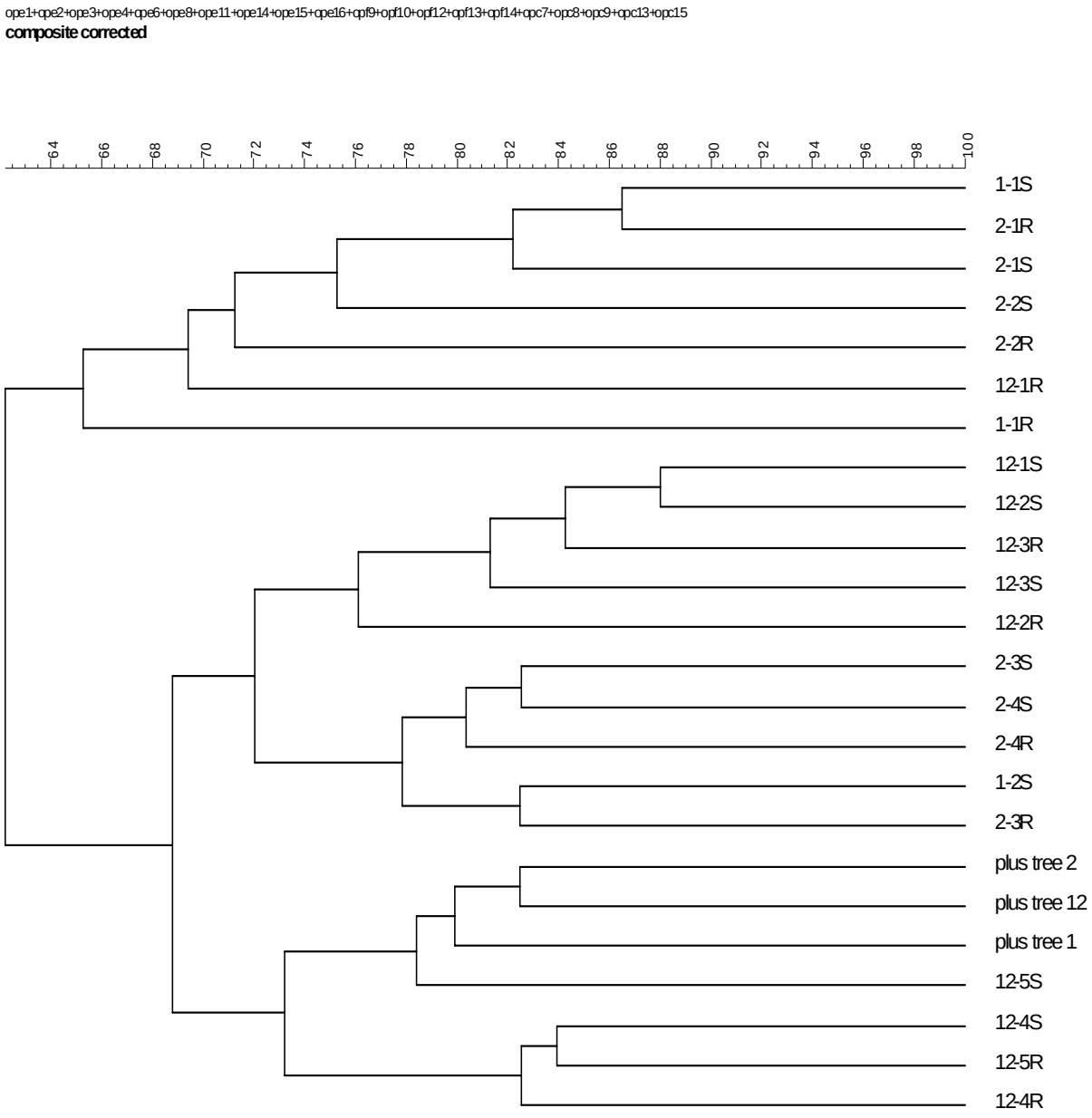
Dendrogram generated by POPGENE using the data from BIONUMERICS (using Nei's unbiased genetic distance)



Dendrogram generated by POPGENE using the data from visual analysis (using Nei's regular genetic distance)



Dendrogram generated by POPGENE using the data from visual analysis (using Nei's unbiased genetic distance)



Appendix 3.1 Dendrogram showing the relation between the teak clones generated by BioNumerics. The sample nos are as in Appendix 2.1.

Parentage confirmation of clones in the teak Seed orchards – Isozyme analysis

INTRODUCTION

To confirm or dismiss the possibility of graft failure, it would be necessary to ascertain the genotype of the aerial parts and match it with that of the roots of the graft. The aerial tissues of a clone should also match with those of the corresponding plus tree from which the scion was taken for the grafting. Conversely the shoot and root of a grafted clone should be of different genotypes. Any differences seen would indicate that the grafts had failed and the strongest possibility was that the aerial part was of root stock origin or that there was mislabelling. Since seedlings from the same seed origin are normally used as roots stock, if the same genotype is revealed when isozyme analysis of different clones is carried out then it points to the possibility of failure of grafts and proliferation of the root stock buds.

In this study isozyme analysis was used to understand the genotype of the clones in the CSOs. Isozyme analysis is suited for such a purpose and has been previously used in identification of clones (Kumaravelu, 1979). Isozymes are multiple molecular forms of enzymes that have different electrophoretic mobility by which share a common substrate. When tissue extracts are subjected to gel electrophoresis and treated with enzyme specific stains, the enzyme products can be visualised as bands. Genetic interpretation of the number and mobility of these bands can reveal single or multilocus genotypes. The relative simplicity of the technique has made isozymes popular as single gene markers.

Isozymes are however influenced by the morphological or physiological status of the tissues and may yield confusing and inconclusive results unless care is taken to use the same type of tissues for analysis. A standardisation of

the technique was first required to undertake the study. This includes the selection of suitable tissues for extraction, the appropriate buffers, and electrophoresis technique and visualisation procedure.

MATERIALS AND METHODS

The extraction and staining protocols were standardized using teak tissues collected from different parts of the trees in the CSO at Palapilly, Thrissur or directly from the epicormic shoots induced in branch cuttings taken from the plus trees. Randomly selected trees from different localities in Thrissur were used for preliminary experiments to standardise the methods. Young dormant buds, green opened leaves, reddish brown unopened leaves, inner bark of the stem and roots of trees were used for the purpose.

Methods reported by Weeden and Wendel (1989) and Sadasivam and Manickam (1992) were followed for the running of the gels and visualization of the bands.

The following 10 enzyme systems were tested for suitability as markers.

Malate dehydrogenase (MDH), Superoxide dismutase(SOD), Malic enzyme (ME), Esterase(EST), Peroxidase, Shikimic acid dehydrogenase (SAD), Alcohol dehydrogenase, Catalase , Glutamate dehydrogenase (GDH) and Glucose 6 phosphate dehydrogenase (G6PDH).

For selecting the suitable extraction buffer, three buffers with different supplements were tested by extracting young dormant shoot buds and staining for MDH.

1. Phosphate buffer 50 mM (pH 7.5)
   Sucrose 5 %
   DTT 0.1 %

2. Phosphate buffer 50 mM
   Sucrose 5 %
   DTT 0.1 %
   PVP 40. 5 %

3. Tris HCL (50 mM) pH 7.5
   Sucrose 5 %
   DTT 0.1 %

4. Tris HCL (50 mM) pH 8.0
   Sucrose 5 %
   PVP 0.1 %

5. Sodium tetra borate 50 mM (pH 8.3)
   DTT 0.1 %
   PVP 2 %
   Sucrose 0.14 M
   Sodium metabisulphate 20 mM

6. Sodium tetra borate 50 mM (pH 8.3)
   DTT 0.1 %
   PVPP 10 %
   Sucrose 0.14 M
   Sodium metabisulphate 20 mM

Extraction: 500 mg of fresh plant material was ground in 1.2 ml of the ice cold buffer using a pre-chilled mortar and pestle, centrifuged at 15000 X g for 20 min. in a refrigerated centrifuge and the supernatant was used for analysis.

Gel Electrophoresis: Electrophoresis was carried out on Polyacrylamide Gels. Preliminary experiments with starch gels were carried out so as to take the advantage of slicing each gel into 4 and simultaneous staining for different enzymes. Sigma potato starch failed to give proper gels that could be sliced and separated, at concentrations of 9- 12 % (w/v). PAGE was therefore used for this study using 10 % separating gel and a 6% stacking gel on a Bangalore Genie apparatus. The temperature was maintained at 4°C inside a refrigerator during the run. Approximately 20-35 µl of sample was loaded into each well with tracking dye and gels were subjected to electrophoresis for 1.5 hrs at 10-15mA following which the staining of the gels was carried out. Bands were either scored in diagrammatic sketches or photographed on a white light Transilluminator.

RESULTS AND DISCUSSION

Of the ten enzymes tested, visualization of bands was obtained with varying degrees of success in the ten enzyme systems tested. The best results were obtained in MDH followed by SOD, ME and EST.

Dormant buds gave better results compared to newly opened or fully developed leaves. Inner bark from the trunk and root gave poor results. Four buffers were screened for their suitability and Sodium tetra borate buffer with 10% PVPP which was found to give best results was used as standard for further experiments.

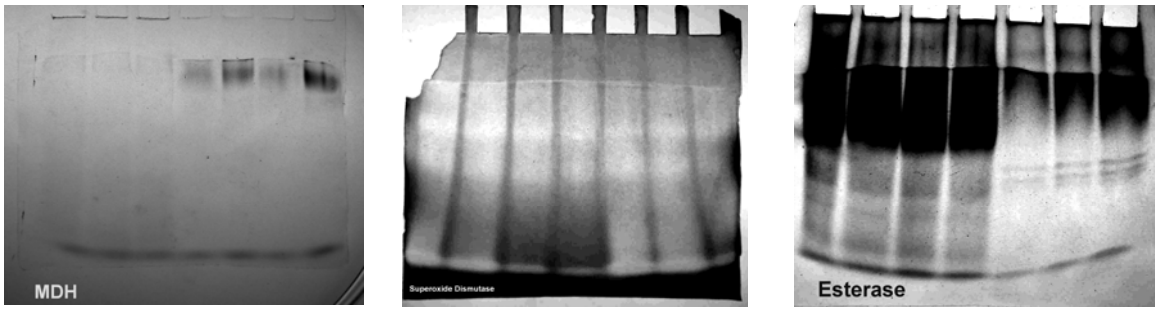
No polymorphism could be found in the material collected from five different clones in the Clonal Seed Orchard at Palapilly or from stem cuttings of plus trees at Nilambur (Fig. 1). A similar study (Indira and Muralidharan, 2005) carried out concurrently with the present work also revealed little polymorphism in isozyme analysis of 10 plus trees. In the preliminary screening, occasional polymorphism was seen in randomly selected wild trees

growing in different areas of Thrissur. Lack of sufficient genetic variation between the individuals tested may be a probable reason for the results obtained in this study. But further improvements in the techniques can result in improved enzyme activity and resolution of bands. Isozyme analysis has been used successfully for identification of clones by Kumaravelu (1979). Kertadikara and Prat (1995) had also used isozyme analysis for understanding the mating system and genetic structure of teak provenances, but worked with young seedling tissues and the populations were from different teak growing areas of the world.

Tissues from mature trees contain compounds like tannins, phenols, quinones and flavanoids which can interfere with the enzyme activity. The use of compounds like PVPP in the buffers is to bind these metabolites and improve the activity of the enzymes. Further modification of the buffers to improve the activity in teak tissues is therefore to be carried out.

The amount of enzyme protein in the sample loaded is known to influence the resolution. Therefore the proportion of tissue to buffer volume, the volume of sample loaded per well, the amount of substrate used in the staining solutions etc. can be modified to bring about a better result.

Figure 1. Banding pattern of seven clones from the seed orchard at Palapilly using three isozyme systems.



MDH **SOD** **EST**

Nutrient status in foliage and soil of flowering and non-flowering clones

To study the nutrient status in foliage and soil of flowering and non-flowering clones analysis of samples from both flowering and non-flowering trees were carried out.

MATERIALS AND METHODS

The pilot seed orchards established by KFRI at Nilambur, Palappilly which were handed over to the Kerala Forest Dept. during 1986-87 and Arippa were surveyed. The seed orchard established by KFD at Kalluvettamkuzhi near Kulathupuzha was also visited. The seed orchads at Nilambur and Palappily were not properly maintained. This resulted in growth of other species and attack by insects. The seed orchard at Arippa was also not fully maintained. The orchard trees were tall and from the physical appearance it could be possible to see that the typical bushy nature of orchards was not followed.

In order to assess the status of flowering and seed production in seed orchards and also to evaluate the nutrient status in foliage and soil of flowering and non-flowering clones, seed orchards established by Kerala Forest Department at Kalluvettamkuzhi near Kulathupuzha were selected.

Details with respect to flowered and non flowered plants during the two flowering seasons in 1999 and 2000 were gathered. Regular observations on the nature of flowering were recorded. Ten soil pits were taken from different locales of the seed orchard and soil samples collected from 0-20, 20-40 and 40-60 cm layers. In addition to this, 20 surface samples were also collected. The samples were air dried, passed through 2 mm sieve and particles > 2 mm (gravel) were found out. Analyses were carried out for particle-size separates, bulk density (BD), particle density (PD), soil pH, organic carbon (OC),

maximum water holding capacity (WHC), available N,P,K,Ca and Mg and $CaCO_3$ as per standard procedures in ASA (1965) and Jackson (1958).

As flowering started in June in 1999 and 2000, intensive survey was carried out during June to December in 2001 and 2002, when flowering was found to be at its peak. The per cent of flowering in 2001 and 2002 was assessed. As flowering was comparatively better in Nilambur clones (N7 and N28), leaf samples were collected from ten trees each. In addition to these, leaf sampling was also done from 10 each adjacent non trees in clones 7A and 28A (Aryankavu) as well as from the Nilambur clones in N7 and N28 which did not flower in 1999 and 2000. Leaf sampling was done in June, July, August and December 2001 i.e., from the time of flowering to fruit maturation. The experiment was repeated in 2002. The details with respect to the trees are given in Table 1. The leaf samples were analysed for N, P, K, Ca and Mg.

Table 1. Details with respect to the clones flowered and those selected from non flowered adjacent groups.

Sl. No	Clone No			No. of trees flowered in each clone
	Flowered	Non flowered	Adjacent	
1	7 N, 8 N	7 N, 8 N	7 A, 28 A	7 N – 30 8 N - 25

Results and Discussion

The mean values of soil properties are given in Table 2. In accordance with the name of the place, the area was lateritic. The soils were sandy loam and medium acidic (soil pH. 5.8- 6.0). The water holding capacity was poor (36-40%). The soils were highly compacted (bulk density 1.40 – 1.51). The organic

carbon content was low (0.40-1.10 %) and the general soil nutrient status was poor.

Table 2. Mean values of soil properties in different layers in the seed orchard.

Soil Properties														
Depth (cm)	G %	S%	Si%	Cl%	BD	PD	WHC%	pH	OC	N	P	K	Ca	Mg
0-20	23.13	77	12	11	1.51	2.49	40.12	6.0	1.10	75	9	78	76	56
20-40	18.47	74	13	13	1.41	2.23	38.26	5.8	0.63	54	7	45	61	41
40-60	12.15	71	15	14	0.40	2.02	36.28	5.8	0.40	37	6	36	43	32

G=Gravel; S=Sand; Si=Silt; Cl=Clay; BD=Bulk density; PD=Particle density; WHC=Water holding capacity; OC=Organic carbon;

The per cent of flowering varied from 2-3 in 1999 and 2000 which increased to 5 in 2001 and 2002. There was not much difference in per cent of flowering in 2001 and 2002. The phenological phases of fruiting were spread over a period of seven months i.e. from June to December. As the flowering started in June, early fruit development occurred in rainy season and late development of the fruits coincided with dry season.

The clones differed in flower production per inflorescence. The same clone flowered in one locale did not flower in another locale. Flowering and fruiting characteristics of the clones in the clonal seed orchard differed very much from those of a nearby local tree which flowered annually. It was also noticed that there was considerable variation among the clones for the time of fruit initiation and duration of fruit maturation. The fruit production also varied within clones from 65g/tree to 300g/tree. The average fruit production was 125g/tree. The seed size also showed great variation. Seeds from the same clones varied in size in different locales. The intra-clonal variation for number of fruits per inflorescence could not be found out. There was not continuous

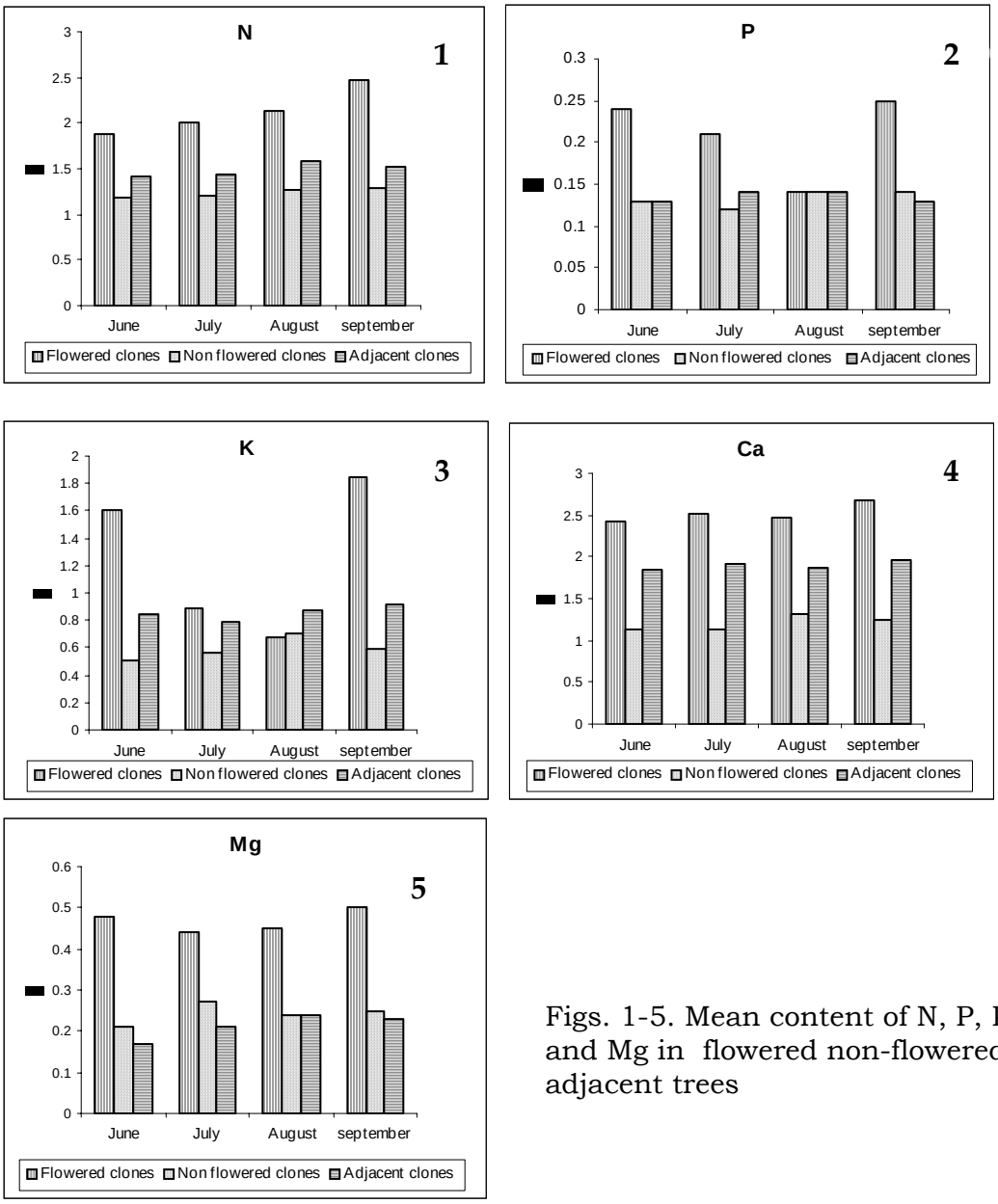
flowering for the same tree in subsequent years. Those trees which flowered in 2001 did not flower in 2002. The period of flowering to fruit maturation was 6-8 months. This has also been noted by Palupi and Owens (1996).

The mean values of leaf nutrient contents in flowered, non flowered and adjacent trees are given in Table 3.

Table 3. N, P, K, Ca and Mg contents in the leaves of flowered, non flowered and adjacent trees.

Properties	Flowered		Non-flowered		Adjacent	
	Min	Max	Min	Max	Min	Max
N	1.23	3.05	1.09	1.43	1.13	1.76
P	0.16	0.20	0.11	0.14	0.12	0.15
K	0.45	2.30	0.23	0.89	0.19	1.10
Ca	1.84	5.65	1.03	1.56	1.06	3.11
Mg	0.26	0.66	0.14	0.32	0.15	0.34

The foliar nutrient contents N, P, K, Ca and Mg showed considerable variation within clones and between clones. The trend in the mean values are shown in Figs 1-5. There was a decrease in P and K contents in the leaves of flowered trees from the time of flowering till fruit maturation after which there was an initial increase, though not prominent. No appreciable variation in foliar nutrient status was seen in non flowered and in those trees of adjacent area.



Figs. 1-5. Mean content of N, P, K, Ca and Mg in flowered non-flowered and adjacent trees

As the flowering started in June i.e., at the time of south west monsoon, the poor fruit production could be assumed to be due to peak rainy season. Heavy rains may cause a high abortion rate either because flowers are knocked off from the pedicel or because there are fewer pollinators foraging on the inflorescences. These findings agree with those of Palupi and Owens (1996) and Tangmitcharoen and Owens (1996).

The variations in foliar nutrient contents reveal that P and K were the dominant nutrients which affected flowering along with other factors. Hence,

if a stress is given for the trees by giving more P and K at the time of flowering and very low N, Ca and Mg, to certain extent the problem of poor flowering can be solved if all other conditions are conducive for flowering.

Eco-physiological characters with respect to flowering of teak clones

Introduction

Variation in growth forma was seen for same clones in different orchards. Some of the ecophysiological aspects were checked with grafted teak clones planted in one of the Orchards at Kalluvettamkuzhy where clonal trees have flowered. Except Kalluvettamkuzhy in all other places, planting was done at 4 x 4 m espacement in quinquinnial method. It was observed that in these places, the trees grew tall and did not maintain a bushy structure as expected. In many cases flowering and seed setting were correlated with biomass of trees. In Kalluvettamkuzhy, the trees were planted at wider spacing of 8 x 8 m, 10 x 10, and 12 x 12 m where all the plants exhibited comparatively low height branchy bole. Previous studies in this orchard show that yield of seeds were 3 kg/ha/yr.

Materials and methods

Experimental plots of different espacements and age group were selected for light interception studies. Photosynthetically active radiation was measured in the plots using a PAR sensor connected to a data logger. Hourly readings in the open and below the canopy were taken at 30 seconds interval and averaged hourly.

Results and discussion

The Photosynthetically Active Radiation (PAR) above the canopy varied between 502- 1334 μmol m^{-2} s^{-1} while below canopy it was 124-1218 μmol m^{-2} s^{-1}. Light interception studies showed that 71 % of PAR reached below the canopy during noon time. There is a gradual increase in light interception below the canopy ranging from 46. 5% at 9.00 am to 71% by 11.30 am and 54 % after 1.00 pm on a sunny day. This difference can be attributed to the

plantation crops growing nearby which blocks the sun during morning and evening hours. As the spacing was enough with comparatively small bushy trees, there was no shortage in light interception in any of the plots at Kalluvettamkuzhy. Due to wider spacing there is no blockage of light o n different sides of the crown. Flowering was also noticed irrespective of position and direction. This indicates that light is not a problem in Kalluvettamkuzhy orchard in the three types of espacement. Leaf area index taken in January showed an average value of 0.19 indicating a lower value due to leaf defoliation. But at Arippa, where the trees were taller, reaching a height of 12 to 15 meters, the canopy was seen closed leading to less light interception. Here, the adjacent plantation and forest trees seemed to block solar radiation from east and west. The plots seemed to be congested among a thick forest with a sloping landscape receiving less light. The triangular (quinquinnial) planting at 8m x 8 m espacement was not helpful in preventing early canopy closure. The trees grew well in the fertile soil but the trees did not show any bushy nature. At Palappilly the trees grew to a height of 8 to 10 meters. Here also the planting was quinquinnial with 8m x 8m espacement. Even though the light interception was good in this area, seed setting was poor for many clones. This may be due to poor quality of the site or clonal differences.

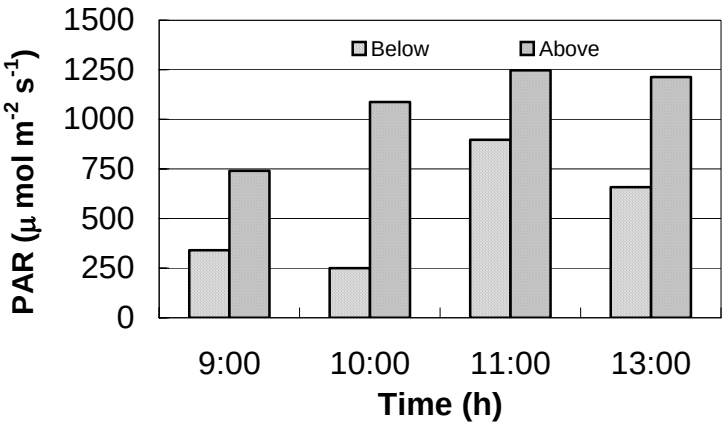


Fig. 1. Light interception above and below the canopy in the teak seed orchard

Solar radiation: Daily pattern of solar radiation was measured using a Pyranometer connected to a data logger outside the plot. Hourly solar radiation was monitored and summed up for each day and plotted for different days. (Fig. 2). The daily solar radiation varied between 8 to 23 MJ m^{-2} s^{-1} at Kalluvettamkuzhy. Total monthly solar radiation during summer months amounted to vary between 500 to 650 MJ m^{-2}. This seems to be good for teak clones. There was no hindrance to receive solar radiation from all directions as the trees were widely planted in an open plain area. But at Arippa, as the adjacent plantations were thick and tall, most of the solar radiation from the east west direction was blocked. Only during noon time, the lower canopy received maximum solar radiation. At Palappilly the solar radiation was not a limiting factor.

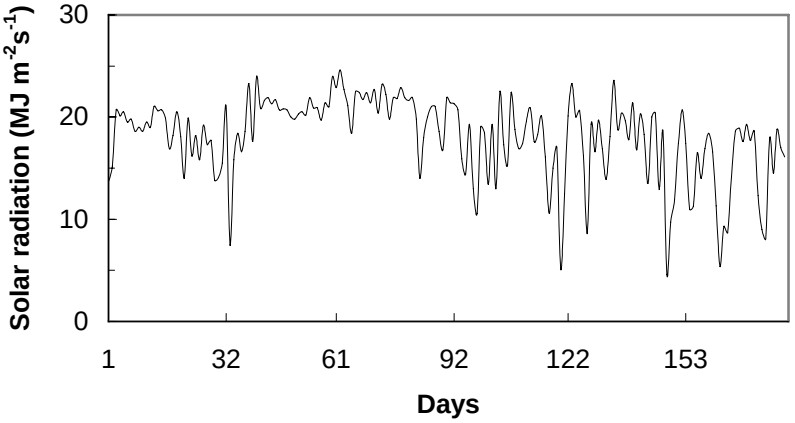


Fig.2. Daily variations in solar radiation outside the plots at Kalluvettamkuzhy

Temperature and relative humidity: Variations were measured using electronic sensors at 30 seconds interval and averaged hourly. Daily patterns of temperature and humidity were plotted. (Fig 3) The mean maximum temperature varied between 25 to 37^{o} C while mean minimum temperature varied between 20 to 25 ^{o} C at Kalluvettamkuzhy throughout the year. When the temperature for the past ten years were examined , it was understood that comparatively high temperature prevailed in this area. Even though high humidity was noticed, ranging between 30 to 100 %, it did not seem to affect

seed setting or premature fall of fruits as the day time humidity decreased due to high temperature and solar radiation in the plots. The wind speed did not exceed 1-2 m^{-2} s^{-1} but contributed to maintain a high VPD in the orchard.

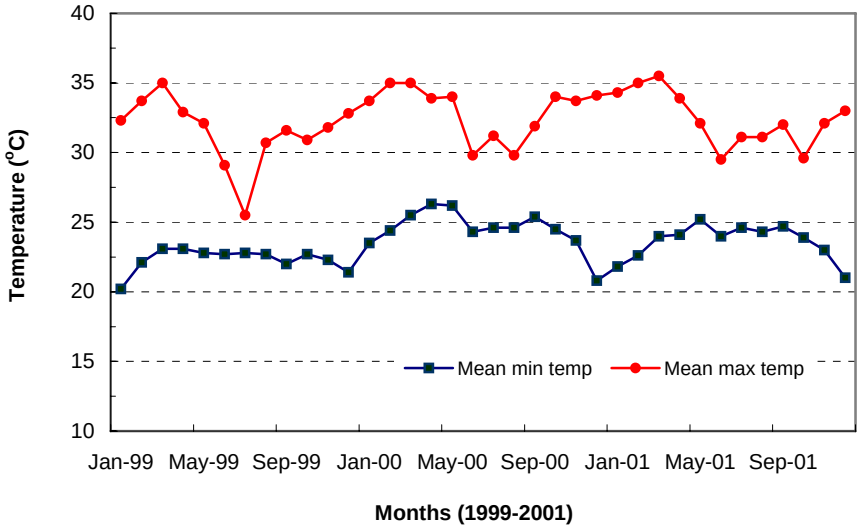


Fig. 3a. Monthly variations in temperature at Kalluvettamkuzhy

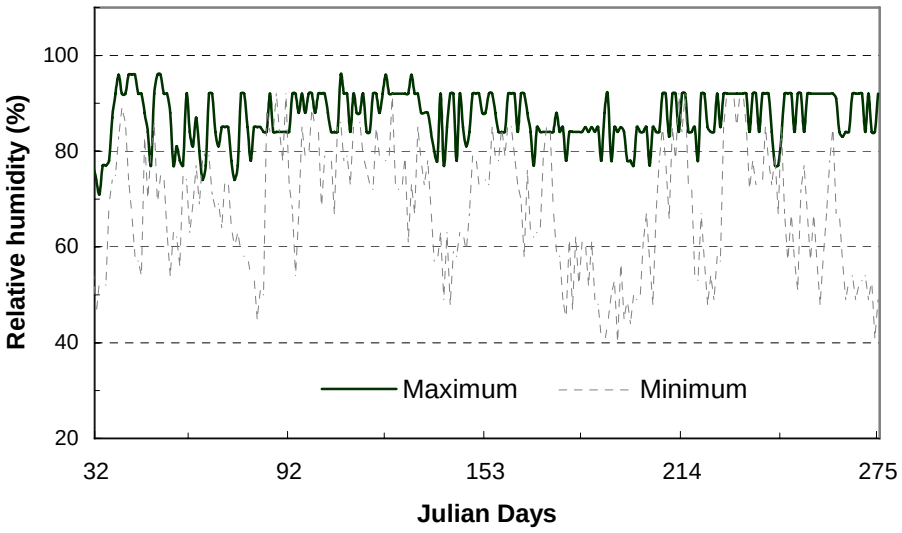


Fig. 3b. Daily variations in relative humidity at Kalluvettamkuzhy

Rainfall data in the area for the last 10 years were collected from the nearby station and analysed. It was noticed that almost all months received rain throughout the year in the plots of Kalluvettamkuzhy.

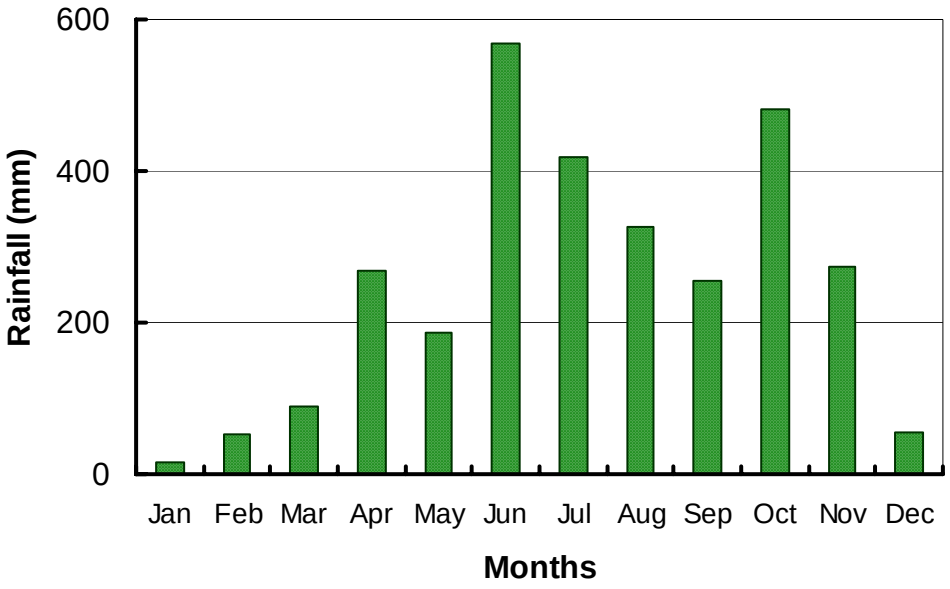


Fig 4. Average rainfall for different months for 10 years at Kalluvettamkuzhy

Average rainfall for 10 years in this area amounted to 3000 mm (Fig.4). This is comparatively very good rainfall and conducive to the growth of teak trees. Rainfall was maximum in June followed by July and October. During heavy rains, the flowering branches may break leading to poor fruit yield. Heavy rains leading to higher humidity may also lead to fungal attack on the peduncle and flowers, leading to abscission of floral parts. It is known that teak flowering starts in June and ends in September and it takes 4 to 6 months to the production of mature fruits. In Kerala, this period comes under rainy season, influenced by South West and North East monsoons. Rawat (1994) has mentioned the influence of raindrops which may wash away the pollen and breaking of very large number of buds, flowers and fruits. The teak flowers open soon after sunrise and get pollinated mainly by small insects. During rains the fate of pollinating insects is important to achieve cross pollination, where maximum number of viable seed is developed. During heavy rainy

days activity of pollination come to a minimum leading to low fruit set (Mohanadas *et al*.2002).

It is known that in teak seed orchards clones may flower at different times. The pollen grains should reach the stigma within a short time after the opening of flowers usually before noon. There are also chances of failure of endosperm development which may result from a higher incidence of self pollination (Palupi and Owens, 1997). Destruction of apical buds during various stages may delay flowering in teak also as seen in various plants. In teak also during grafting and after planting in the orchard, animals may graze the apical bud leading to suppression of flowering. In general profuse flowering and seed set is found in isolated trees of teak where light is available from all sides. In the orchards the possibility getting light like isolated trees are limited. This may lead to less branching and poor flowering.

Discussion and Conclusions

DNA fingerprinting of root and stem material of the ramets showed that there could have been unexpected mixing of planting material. The most probable reason is that the stock plant buds have sprouted and replaced the failed scion tissues due to failure of the graft. This can occur when the clonal seed orchard has not been closely monitored. Further studies with molecular markers could confirm that the DNA is similar. There is also the possibility of the scion and stock plant being very closely related to each other and therefore not being distinguished by the RAPD technique. Isozyme studies also did not reveal any polymorphism.

A possible solution to the problem is to use rooted stem cuttings or micropropagated plants for raising clonal seed orchards in future. Nutrient uptake is seen to vary between the flowered and non-flowered trees. Eco-physiological studies indicated that availability of light is not different between flowered and non-flowered trees.

Although there are chances for the development of rootstock of the grafted plants, this cannot explain the poor flowering since the phase change to flowering should have occurred at 7-10 years even in the tree growing from the root stock. One limitation could be that selection criteria for plus trees of teak give more emphasis to vegetative characters such as superior stem form and timber quality, fast growth (height and diameter), a trunk free from fluting, buttressing and epicormic branches, resistance to leaf skeletoniser, defoliator, drought and frost than its reproductive behaviour (Subramanian et al., 1994). From the limited sample size analysed for DNA and other observations made in this study and explanation for poor flowering could not be made except that the absorption of nutrients are different.

The study in the clonal seed orchard at Kalluvettamkuzhi revealed that

1. The per cent of flowering varied from 2-3 in 1999 and 2000 which increased to 5 in 2001 and 2002.

2. The fruiting pheno phases were spread over a period of seven months i.e. from June to December.

3. The clones differed in flower production per inflorescence.

4. The fruit production varied from 65g/tree to 300g/tree. The average fruit production was 125g/tree.

5. The cluster analysis of RAPD data revealed five distinct clusters. The three plus trees stood separated from the clones and formed a distinct cluster. Flowered and non-flowered clones showed a tendency to group together into separate clusters. Two clusters each were obtained for flowered and non-flowered clones. With minor exceptions, the DNA from the scion of a clone clustered with the DNA from its stock. The results demonstrate that the stock and scion of a clone are highly similar at the DNA level and the scions do not evince any relation to the plus trees from which they have been derived. In addition, the flowered and non-flowered clones are genetically distinct. It is likely that there may be differences between the stock material of flowered and non-flowered ramets.

6. No polymorphism could be found in the material collected from seed orchards using isozyme studies also.

7. The foliar nutrient contents N, P, K, Ca and Mg showed considerable variation within ramets and between ramets.

8. There was a decrease in P and K contents in the leaves of trees flowered from the time of flowering till fruit maturation after which there was an initial increase, though not prominent.

9. If a stress is given for the trees by giving more P and K at the time of flowering and very low N, Ca and Mg, to certain extent the problem of poor flowering may be reduced if all other conditions are conducive for flowering. However this needs to be tested by experiments.

10. Eco-physiological studies in the orchards showed that parameters like light, temperature or rainfall were not different in flowered and non-flowered trees.

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