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Effect of serial bud grafting and etiolation on rejuvenation and rooting of mature trees of Tectona grandis Linn.f.

Effect of Serial Bud Grafting and Etiolation on Rejuvenation and Rooting Cuttings of Mature Trees of *Tectona grandis* Linn. f.

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Summary

An experiment was conducted to study the effect of serial bud grafting and etiolation on rooting stem cuttings. Auxiliary buds taken from two clones of mature teak trees and were grafted on root stocks of 2-year-old seedlings in 1998 and again serially grafted on 2-year-old root stocks in 1999. The grafts were maintained under complete darkness to induce etiolation. The etiolated shoot emerging from the grafted buds were made into mono-nodal, leafy cuttings, which were cultured under mist for rooting. Serial grafting and etiolation did not affect rooting, but treatment with IBA in combination with serial grafting and etiolation promoted root formation on the cuttings. All the control cuttings taken from lateral shoots after first or second grafting failed to root whereas, IBA-treated cuttings rooted very poorly after first grafting. However, rooting response in IBA-treated cuttings improved significantly after second grafting. Thus, serial grafting as well as etiolation caused only partial rejuvenation of mature clones. The two clones differed in their response to serial grafting and etiolation for induction of juvenility. Clone FG1 exhibited stronger rejuvenation, as indicated by more profuse rooting of the cuttings after second serial grafting. Clone FG11 exhibited comparatively weaker rejuvenation, which was indicated by less profuse rooting of the cuttings under similar conditions.

Key words: Tectona grandis, IBA, etiolation, rooting, serial bud grafting, clone.

Introduction

Tectona grandis (Linn.f.) has gained worldwide reputation for high-quality timber due to aesthetic appeal and wood durability. Recently, rapid destruction of natural teak forests have taken place due to over-exploitation to meet demand. There is an urgent need to establish teak plantations in order to reduce pressure on naturally occurring stands. During the recent years clonal forestry program has emerged as a boon for improving yield and production of high quality timber in shorter rotations. In such program, it is essential to start by selecting superior clones / trees, from which the cuttings are taken for vegetative propagation (cloning). However, most trees reach maturity by the time their growth superiority can be conclusively determined. In teak like in many other wood plant species, the branch cuttings tend to lose the rooting ability with the maturity, necessitating rejuvenation of elite mature clones for their rapid, mass clonal propagation (NANDA, 1970; PAL, 1993; HARTMANN et al.,1997). Several rejuvenation techniques have been tried (PAL, 1993; HUSEN, 2002), and serial grafting is the most commonly used technique for propagation (MONTEUUIS, 1986; PLIEGO-ALFARO and MURASHIGE, 1987). Stem cuttings derived from etiolated stock plants usually root better than those from non-etiolated ones (HANSEN and ERIKSEN, 1974; HUSEN and PAL, 2001). Correspondingly, the specific objective of this experiment was to investigate the effect of serial bud grafting and etiolation on rejuvenation and rooting cuttings of teak clones in order to eventually improve the efficiency of clonal propagation of this species.

Materials and Methods

Selection of donor plant and grafting

FG1 and FG11 teak clones, each of which about 95-years-old, were selected to provide mature scions for bud grafting onto 2-year-old rootstock. Bud grafting was performed in May 1998, following the method as described by HUSEN (2002). The grafts were watered daily and kept either under normal light conditions (*Fig. 1*)/or under complete darkness. After grafting, all other sprouts on the root stock, except those growing from scion buds, were removed immediately as they emerged.

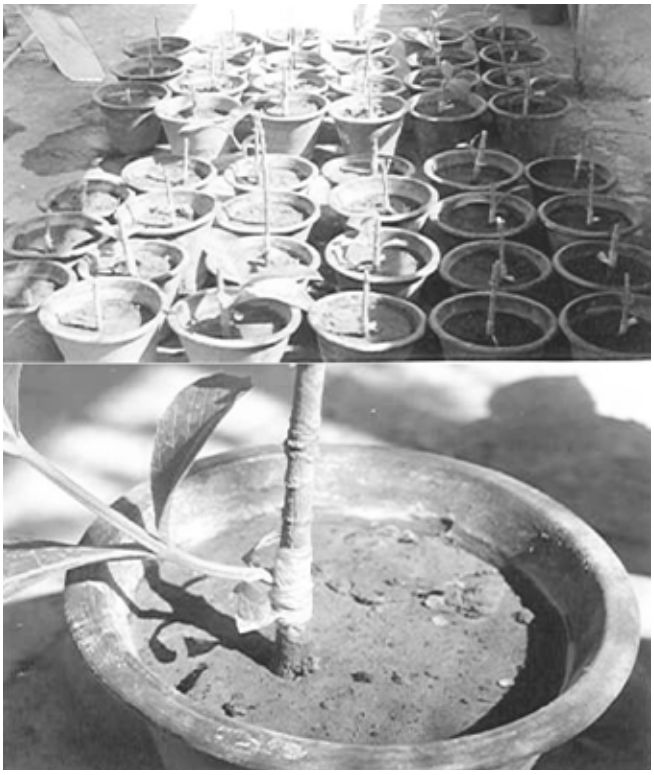

Figure 1. – Bud grafting of FG1 and FG11 clones.

Regrafting

In May 1999, visible dormant auxiliary buds were obtained from 1-year-old grafted shoot scions, which were maintained under normal light conditions, without artificial, supplemental lighting. These buds were grafted again on 2-year-old rootstocks following the above method. After grafting, the grafted clones were maintained under complete darkness to cause shoot etiolation.

Collection and preparation of cuttings

After 25 days, the etiolated shoots emerging from scion buds were harvested and made into mono-nodal leafy, softwood cut-

tings. Twenty cuttings were used per replicate and 5 replicates were maintained per treatment. Further, details of experimental deign and statistical analyses are given under forthcoming paragraphs of statistical analysis. Total leaf area per cutting was approximately 10 cm^2, and total length of cutting was approximately 4.0 cm, which comprised of 1.0 cm internodal portion above the node and 3.0 cm below it.

Treatments

The cuttings were subjected to the following treatments: (a) serial grafting: it was performed by repeated grafting of buds of mature teak clones on juvenile, 2-year-old root stocks. Details about the selection of donor plants and grafting, and regrafting of auxiliary buds are already given in the preceding paragraphs; (b) clone: as previously mentioned auxiliary buds were taken from two clones of teak i.e., FG1 and FG11. The branches emerging from these grafted buds were made into cuttings and used for the experimentation; (c) dipping the basal ends into indole-3-butyric acid (IBA). IBA treatment included application of 1000 ppm and 0 ppm (untreated control) concentrations by basal dip method. The IBA was applied in its powder formulation, which also contained 0.05 percent Bavistin. The control cuttings were treated similarly with talcum powder containing Bavastin only.

Planting

After treatment the cuttings were planted in plastic trays, which were field with sterilized vermiculite (pH 7.0). The vermiculite was presoaked in tap water for 24 hrs. before filling the trays to allow it to absorb the water. The cuttings were planted immediately after application of a treatment and kept inside a mist chamber where the relative humidity was maintained at 85 ± 2 percent with a maximum and minimum day-night temperature at 32 ± 10 °C to 26 ± 10 °C, respectively.

Observations on rooting response

After 45 days, the cuttings were carefully removed from the rooting medium and observations were recorded on callus formation, sprouting, rooting, number of roots per cutting and the mean length of roots per cutting (cm).

Statistical analysis

A factorial completely randomized design (CRD) was used for the statistical analysis of the data. Because the percentage data are based upon a binomial response and some mean percentage lie outside the stable variance range of 30 to 70 percent, all percentage data were transformed to arcsine √ p following the method of ANDERSON and MCLEAN (1974). All other analyses were performed on untransformed data. Analysis of variance (ANOVA) procedures were used to test for significant treatment effects of the treatments on each variable measured (*Table 1*). In the ANOVA, the value for each replication was estimated based on all available cuttings and subjected to the following model:

$$Y_{ijk} = \mu + g_i + t_j + c_k + (gt)_{ij} + (gt)_{ij} + (ct)_{kj} + (gtc)_{ijk} + e_{ijk}$$

Where, i = 1...2, j = 1...2 and k = 1...2

μ = overall mean

Y_{ijk} = value of serial grafting i, IBA treatment j and clone k

g_i = effect of serial grafting i

t_j = effect of IBA treatment j

c_k = effect of clone k

$(gt)_{ij}$ = interaction effect of serial grafting i and IBA treatment j

$(gt)_{ij}$ = interaction effect of serial grafting i and clone k

$(ct)_{kj}$ = interaction effect clone k and IBA treatment j

$(gtc)_{ijk}$ = interaction effect clone k and IBA treatment j

e_{ijk} = random error related to Y_{ijk}

Results

Effect of serial grafting

Serial grafting showed significant effect (P<0.05 level) on percent rooting, sprouting, mean number of roots, and root length per cutting, while variation in percent callus formation was insignificant at P<0.05 level (*Table 1*). Serial grafting caused rooting on 20.00 percent of the cuttings, while cuttings from shoots of first grafting exhibited very poor (1.67%) rooting. Percent sprouting values were higher (31.67%) in the cut-

Table 1. – Analysis of variance for the effect of serial grafting, clone, treatment of IBA and their interaction on callusing, rooting and sprouting parameters in etiolated grafted cuttings of *Tectona grandis*.

Source of variation	df	Mean sum of square				
		Percent callusing	Percent rooting	Percent sprouting	Mean number of roots per cutting	Mean length of root per cutting
Serial grafting (SG)	1	65.10	2896.59*	5058.43*	40.40*	0.03*
Clone	1	9.48	202.34	643.69	15.06*	0.65*
Treatment of IBA	1	65.10	4220.64*	2384.34*	70.76*	4.17*
SG x Clone	1	202.34	9.48	9.48	3.33	0.03
SG x Treat.	1	2271.85*	2896.59*	704.20	40.40	0.60
Clone x Treat.	1	202.34	202.34	9.47	15.06*	0.02*
SG x clone x Treat.	1	9.48	9.47	9.47	3.33*	0.02*
Error	32	206.04	94.38	278.54	1.46	0.59
		Critical differences at P<0.05 level				
Serial grafting (SG)		-	6.08	10.45	0.76	0.48
Clone		-	-	-	0.76	0.48
Treatment of IBA		-	6.08	10.45	0.76	0.48
SG x Clone		-	-	-	-	-
SG x Treat.		12.71	8.60	-	-	-
Clone x Treat.		-	-	-	1.07	0.68
SG x clone x Treat.		-	-	-	1.51	0.96

* Significant at 5 percent levels.

tings taken from scion shoots emerging after second grafting than those emerged after first grafting (6.67%) (*Fig. 2a*). The mean number of roots per cutting was higher (1.50) on cuttings of scion shoots, which emerged after second grafting than on cuttings of scion shoots which emerged after the first grafting (0.10) (*Fig. 2b*). Cuttings from the second grafting had higher mean length of root per cutting (2.34 cm) than those from the scion shoots of first grafting (0.33 cm) (*Fig. 2c*).

Effect of clones

Significant clonal variation was observed for the mean number of roots and their length per cutting at P<0.05 level (*Table 1*). More roots were produced on cuttings from the FG1 clone (1.05) than those taken from the FG11 clone (0.55) (*Fig. 3a*). Cuttings of FG1 clone produced longer roots (1.94 cm), than those of FG11 clone (0.72 cm) (*Fig. 3b*).

Effect of IBA treatment

Except for percent callusing, all other rooting parameters showed significant (P <0.05 level) variation (*Table 1*). Control cuttings did not root, but 21.67 percent of cuttings treated with IBA rooted. More cuttings sprouted with the IBA treatment

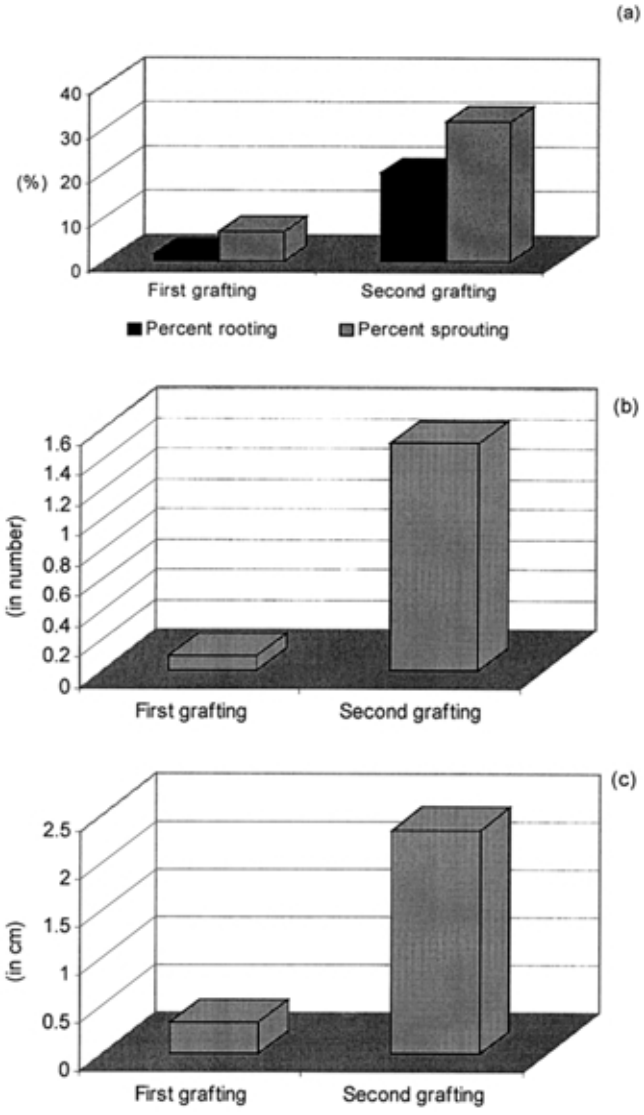

Figure 2. – Effect of serial grafting on (a) rooting response of cutting, (b) mean number of roots and (c) their length per cutting.

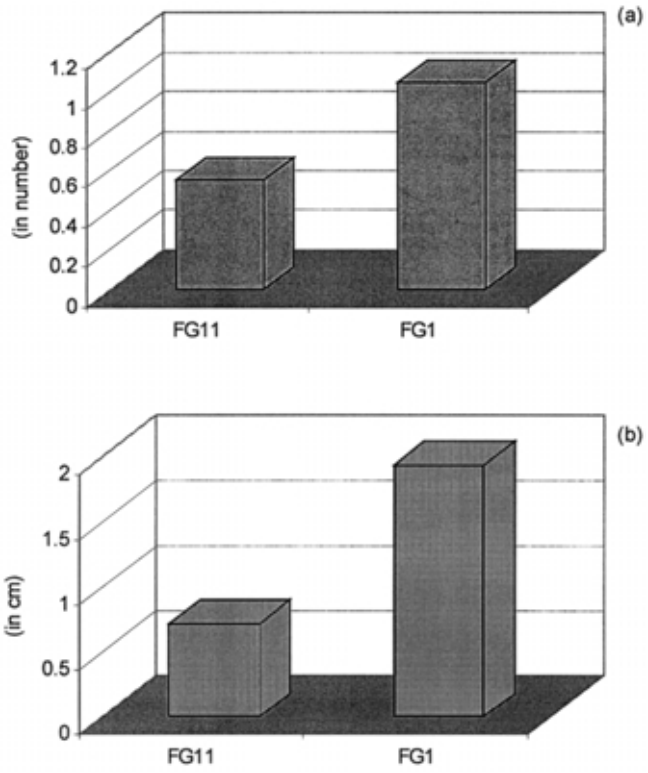

Figure 3. – Clonal variation in (a) mean number of roots and (b) their length per cutting.

(28.33%), than without any auxin treatment (10.00%) (*Fig. 4a*). The mean number of roots per cutting was higher (1.60) on the IBA treated cuttings, than on the control cuttings (0.00) (*Fig. 4b*). The mean length of root per cutting was more (2.66 cm) on IBA-treated cuttings than on control cuttings (0.00) (*Fig. 4c*).

Interactive effect of serial grafting, clones and IBA treatment

The combined effect of serial grafting and IBA treatment was significant at P<0.05 level for percent callusing and rooting (*Table 1*). Control cuttings from first grafting did not form callus, while the IBA treatment caused callusing. These trends were reverse by serial grafting where callus was formed on control cuttings, and IBA-treated cuttings did not form callus. Forty percent (40%) of the cuttings rooted, however, when taken from shoots that emerged from the second graft and were treated with IBA. In contrast, control cuttings completely failed to root irrespective of their being taken from shoots emerging after first or second grafting. Only 3.33 percent IBA treated cuttings rooted if the cuttings were taken from scion shoots of the first graft (*Fig. 5*).

The interactive effects of IBA treatment X clone were significant at P<0.05 level for mean number of roots and mean length of the roots per cutting (*Table 1*). The mean number and length of roots per cutting were higher in IBA-treated cuttings of the FG1 clone than in any other combination of IBA X clone. The three factor interaction, i.e., interaction between IBA treatment, serial grafting and clones, also produced a statistically significant effect (P<0.05 level) on the mean number of roots per cutting (*Table 1*). The combination of treatments which produced maximum roots was IBA, second grafting, and FG1 clone. Further, the interactive effect of three factors, IBA treatment X serial grafting X clone on mean length of root per cutting, was also significant at P<0.05 level (*Table 1*). The longest roots (mean length of root per cutting = 6.67 cm) were produced

on IBA treated cuttings of scion shoots of second grafting in FG1 clone.

Discussion

The results demonstrate that serial grafting coupled with etiolation may cause rejuvenation in teak as is evident from the improved rooting response of cuttings of etiolated shoots emerging from the serially grafted buds. The IBA treatment further enhanced rooting, when the cuttings were made from etiolated shoots emerging from second serial grafts. In comparison, the IBA treatment could cause rooting only in a small percentage of the cuttings prepared from etiolated shoots that emerged from the first serial grafts.

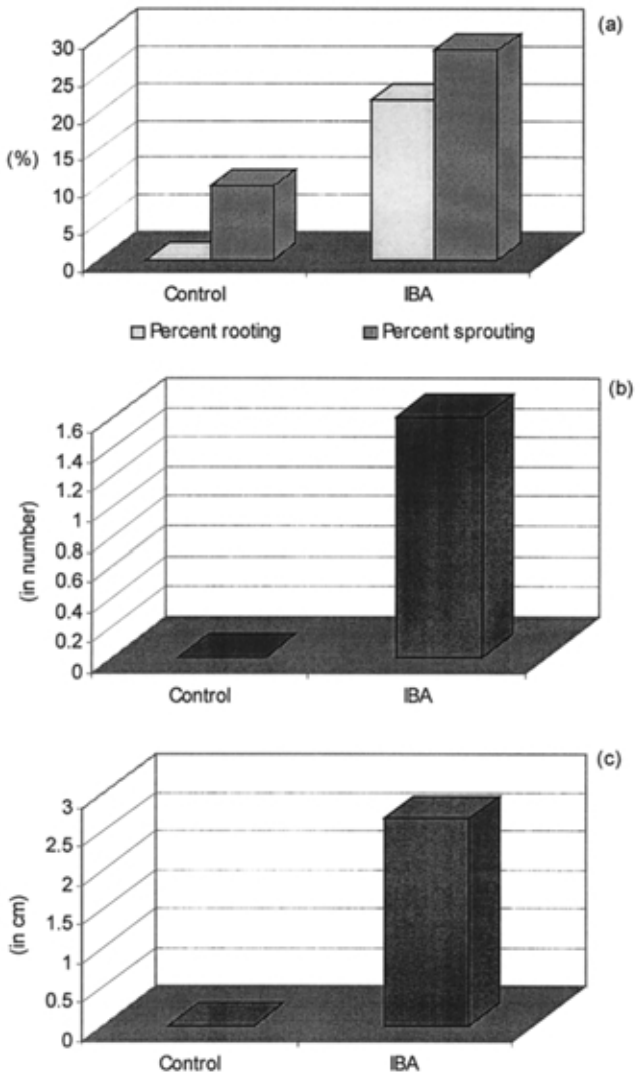

Figure 4. – Effect of IBA treatment on (a) rooting response of cutting, (b) mean number of roots and (c) their length per cutting.

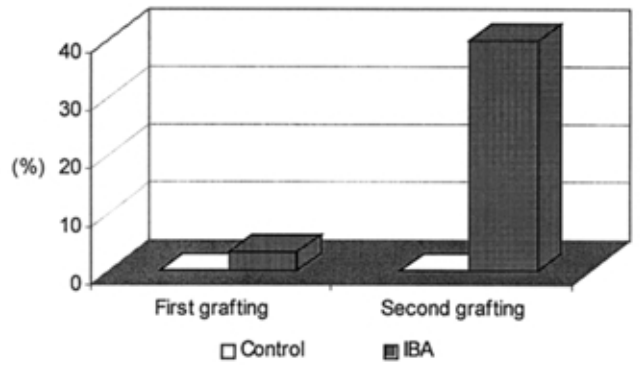

Figure 5. – Interactive effect of serial grafting and IBA treatment on percent rooting.

Induction of juvenility by serial grafting of mature scions on to juvenile root stocks has already been shown in *Hedera helix* L. (Doorenbos, 1954), *H. canariensis* Willd. (Stoutemyer et al., 1961), *Hevea brasiliensis* Willd. (Muzik and Cruzado, 1958), *Persea americana* Mill. (Pliego-Alfaro and Murashige, 1987), *Sequoiadendron giganteum* Lindley (Monteuuis, 1986), *Thuja plicata* Donn ex D. Don (Mission and Giot-Wirgat, 1985) and *Sequoia sempervirens* D. Don (Huang et al., 1991). In the present investigation, however, only partial rejuvenation could be obtained in teak by serially grafting as less than 50 percent cuttings taken from shoots emerging from serial grafted scions and subjected to etiolation and IBA treatment actually rooted. The partial rejuvenation could be related to the age of the donors (approximately 95-years-old) and that the serial grafting was carried only to the second stage. Perhaps, juvenility of higher order may be induced in teak if serial grafting in continued further and younger donors were used.

The results further showed that clones FG1 and FG11 differed in their response to serial grafting and etiolation for induction of juvenility. Clone FG1 exhibited stronger rejuvenation, as indicated by more profuse rooting of the cuttings after second serial grafting. In comparison, clone FG11 exhibited weaker rejuvenation, as shown by the less profused rooting of the cuttings under similar conditions. Clonal variation in rooting behaviour and the response to rejuvenation treatment has been reported in *Picea abies* L. by Foster et al. (1989) and in *Larix deciduas* Mill. by Ewald and Kretzschmar (1996). Hence, selection of clones that are more responsive to serial grafting for rejuvenation should be considered in teak improvement programs.

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Buchbesprechungen

Techniques in Molecular Systematics and Evolution. Edited by R. DESALLE, G. GIRIBET and W. WHEELER. 2001. Birkhäuser Verlag, Basel – Boston – Berlin, ISBN 3-7643-6256-1, 407 pages.

This book is a comprehensive guide for molecular studies in systematics and evolution. It describes the most important techniques for the generation and analysis of data and provides the reader with a great number of sources for further information. It is helpful for readers with a basic knowledge about phylogeny and molecular techniques and offers strategic help for planning such studies. The book is divided into an analytical and a laboratory part.

The analytical part includes 10 chapters describing the construction of phylogenetic trees and population genetic analyses. The reader is led from simple phylogenetic problems to complex analysis and is provided with enough knowledge to choose the right strategy for his problem.

The laboratory part includes 7 chapters and covers all aspects of molecular studies such as sampling, storage, extraction and analysis with various methods. A large amount of laboratory protocols and internet links are provided.

The book addresses a broad readership in biology and is highly recommendable for readers starting with molecular phylogenetic studies as well as for more experienced readers.

SASCHA LIEPELT

Molecular Systematics and Evolution: Theory and Practice. Edited by R. DESALLE, G. GIRIBET and W. WHEELER. 2002. Birkhäuser Verlag, Basel – Boston – Berlin, ISBN 3-7643 6544-7, 309 pages

This book provides theoretical background knowledge for systematics and evolutionary biology. It is focused on new techniques and the large amount of data that modern molecular methods generate. The book consists of three parts with five to seven chapters each. The chapters are written in the form of scientific papers, allowing for each chapter to be read separately.

The first part of the book deals with evolutionary analysis using examples to explain the special problems connected with the different taxonomic levels. It offers advice and strategies for setting up molecular systematic and evolutionary studies.

The second part deals with current problems in molecular systematics. Different approaches on how to handle the enormous sets of data, which are generated by modern studies, are discussed. Several controversial topics like the species concept or homology are covered and offer starting points for further discussion.

The third part describes new approaches to molecular evolution which are now emerging due to new techniques and new data sets. The evolution of gene families and proteins, coevolution, comparative approaches and horizontal transfer are the topics covered in this part.

This book addresses a broad scientific readership and is suited for researchers as well as students interested in evolutionary biology.

SASCHA LIEPELT

Plant Biotechnology and Transgenic Plants. Edited by KIRSI-MARJA OKSMAN-CALDENTEY and WOLFGANG H. BARZ. 2002. Marcel Dekker Inc., New York-Basel, Basel Switzerland. ISBN:0-8247-0794-X. 719 pages.

The book covers several fields of modern plant biotechnology looking from more practical points of view, but not exclusively. This enables the reader to become familiar with research strategies based on the recent progress of biotechnology in fields like plant breeding, drug discovery, increased tolerance against different agents, food and nutritional quality etc. On the other hand sufficient information is provided concerning the practical feasibility by different techniques like cell cultures, the use of bioreactors, the use of hairy roots and finally the use of transgenic plants. Nevertheless investigations on basic principles and conclusions from these results are not only the main part (e. g. signal transduction, plant cell wall, lignin genetic engineering), but included in most of the contributions. The broad range of items in 27 chapters reviewing significant results from the production and modification of metabolites (primary as well as secondary) to an application of bio- and genetechnology for nutrition, medicine, plant production and phytoremediation allow the reader an estimation of the present state of the art. Important topics are illustrated and more detailed information can be found with the references. The book is recommendable for all researchers, teachers and students who are interested in recent advances in plant biotechnology.

D. EWALD

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