

PROPAGATION OF *CALLIANDRA CALOTHYRSUS* THROUGH CUTTINGS: EFFECT OF STOCKPLANT SHADING

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WOLF, J. DE & JAENICKE, H. 2000. Propagation of *Calliandra calothyrsus* through cuttings: effect of stockplant shading. Seven clones of *Calliandra calothyrsus* were raised under different irradiance and red:far-red (R:FR) treatments: open (100% sunlight, R:FR 1.05); black net (31% sunlight, R:FR 0.94) and green net (27% sunlight, R:FR 0.54). Cuttings were taken, treated with one of four auxin treatments [0, 10, 30, 60 µg indole-3-butyric acid (IBA) per cutting] and set to root in non-mist polypropagators for six weeks. Rooting success and number of roots per cutting were determined. Reduction of the light intensity to c. 30% almost doubled the rooting of *C. calothyrsus* (from 42 to 72%). Reduction of R:FR also increased the rooting by another 20–30%. The use of shade to precondition *C. calothyrsus* stockplants can therefore be recommended to farmers. The application of minimal amounts of IBA (10 µg per cuttings) is recommended as it increases the number of roots for successful hardening off and survival.

Key words: *Calliandra calothyrsus* - cuttings - light - shading - vegetative propagation

WOLF, J. DE & JAENICKE, H. 2000. Pemiakan *Calliandra calothyrsus* melalui keratan: kesan naungan tumbuhan stok. Tujuh klon *Calliandra calothyrsus* ditanam di bawah pendedahan sinaran dan rawatan merah: merah jauh: terbuka (100% cahaya matahari, R:FR 1.05); jaring hitam (31% cahaya matahari, R:FR 0.94) dan jaring hijau (27% cahaya matahari, R:FR 0.54). Keratan diambil, dirawat dengan salah satu daripada empat rawatan auksin [0, 10, 30, 60, µg asid indol-3-butirik (IBA) setiap keratan] dan ditanam dalam polipropagator tanpa-kabut selama enam minggu. Kejayaan pengakaran dan bilangan akar bagi setiap keratan ditentukan. Sinaran cahaya yang dikurangkan kepada hampir 30% menyebabkan pengakaran *C. calothyrsus* bertambah hampir dua kali ganda (daripada 42 kepada 72%). Penurunan R:FR juga meningkatkan pengakaran sebanyak 20–30%. Oleh itu, penggunaan naungan terhadap tumbuhan stok *C. calothyrsus* yang dirawat terlebih dahulu dapat disyorkan kepada peladang. Penggunaan IBA yang minimum (10 µg bagi setiap keratan) disyorkan kerana langkah ini dapat meningkatkan jumlah akar bagi memastikan pengerasan dan kemandirian.

Introduction

In recent years, increased interest has developed in using *Calliandra calothyrsus* Meissner in agroforestry systems. Its main uses are in erosion control and firewood

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production, but is also yields fodder with high protein content and nutritional value (Paterson *et al.* 1996). Although calliandra is native to Central America, it is widely used in Indonesia and has been introduced to other Asian and African countries (Chamberlain & Pottinger 1995). Limited seed (Chamberlain & Rajaselvam 1996) has led to interest in vegetative propagation of calliandra (Dick *et al.* 1996a,b). In addition, the growing interest in the species for fodder production has led to a call for the selection of superior provenances (Palmer & Ibrahim 1996). Vegetative propagation can target specific genotypes and thus increase the speed of selection and capture of superior genotypes.

Calliandra calothyrsus is a species with a relatively high rooting potential (76%) from cuttings from 5-month-old seedlings (Dick *et al.* 1996a). For a meaningful selection of superior genotypes, however, the vegetative propagation of proven, that is, older material is necessary.

The rooting of cuttings depends on many factors, most importantly, the genotype (Haissig & Riemenschneider 1988, Dick *et al.* 1996b) and the age of the cuttings (Gardner 1929, Raviv *et al.* 1987, Sanchéz *et al.* 1995). The age effect is influenced by factors such as the balance and status of nutrients, water and carbohydrates (Leakey *et al.* 1992) which in turn are influenced by stockplant management: irrigation, fertiliser application and shading.

Andersen (1986) and Moe and Andersen (1988) have reviewed literature on the effect of stockplant irradiance on non-woody species, and concluded that in most cases high irradiance has negative effects on rooting, although the effects were positive for *Begonia*, *Chrysanthemum*, *Campanula*, *Euphorbia*, *Kalanchoe* and *Phaseolus*, while some other species were unaffected by light treatment. Leakey and Storeton-West (1992) confirmed the benefits of shade for the tropical hardwood *Triplochiton scleroxylon*, when cuttings from stockplants which were grown under low irradiance ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$) rooted better and faster than at higher irradiance ($650 \mu\text{mol m}^{-2} \text{s}^{-1}$). Increased stockplant irradiance also decreases the number of roots developing on cuttings (Poulsen & Andersen 1980 for *Hedera helix*, Mesén 1993 for *Cordia alliodora*). In *T. scleroxylon*, these negative effects are less pronounced in basal internodes (Leakey & Storeton-West 1992), whereas in *H. helix*, they were found only in intermediate internodes (nodes 3–4) (Poulsen & Andersen 1980).

Very little attention has been paid to the influence of the light quality received by the stockplants on the subsequent rooting capacity of their cuttings. Heins *et al.* (1980) found for *Chrysanthemum moriflorum* that stockplant light quality did not affect rooting percentage but that far-red light (FR) reduced the number of roots per cutting, although the difference was small. *Phaseolus mungo* seedlings grown under FR did not root at all (Gupta *et al.* 1977). However, better rooting under low R:FR ratio was observed for *Eucalyptus grandis* (Hoad & Leakey 1996) and *T. scleroxylon* (Leakey & Storeton-West 1992, Newton *et al.* 1996) but rejected for *Terminalia spinosa* (Newton *et al.* 1996).

A possible explanation for the effects of irradiance, suggested for non-woody species, is an improved basipetal transport rate of auxin, photo-destruction of auxin (Moe & Andersen 1988), a change in sensitivity of the tissue to auxin, or changes in auxin cofactors or auxin inhibitors, in particular phenolic acids (Maynard & Bassuk 1988, Jarvis 1986). In trees, however, the effects of both light quality and quantity seem to be on the distribution of dry matter between shoots, the morphology of the

internode and on assimilation and carbohydrate metabolism (Leakey & Storeton-West 1992, Leakey *et al.* 1992).

The present study was conducted with the objectives of (1) assessing the effects of light quantity and quality (R:FR) received by the stockplants on rooting success of *Calliandra calothyrsus* cuttings, (2) studying the interaction of light treatments with auxin, and (3) assessing the significance of the pre-severance position of the cutting on the shoot. Various clones were used to include genetic differences into the study.

Materials and methods

Procedures

Mother plants from rooted cuttings of nine *C. calothyrsus* clones originating from Guatemala were planted in February 1996 at the Agroforestry Research Center, Maseno, Western Kenya (34°35'E, 0°N; 1560 masl; 1800 mm mean annual rainfall, 23 °C mean annual temperature). They were arranged in six plots containing nine plants (i.e. one plant for each clone). The plots were separated from each other and surrounded by a single guard row. All plants were spaced at 1.5 × 1.5 m. Each plant received 15 g of diammonium phosphate every 2 months and was allowed to grow for 10 months until all plants had started flowering. At that time, all were cut back to a height of 30 cm. Two types of shade net (black and green) and the non-shaded control were installed to provide the experimental treatments. The nets were placed on support frames at 2 m above the ground-level ensuring that neighbouring treatments did not shade each other. The plots were arranged within two blocks, each containing the three light treatments.

The light conditions were recorded with a Campbell Scientific CR-10 datalogger and were as follows: under black net: 31% photosynthetic active radiation (PAR, measured with a quantum sensor SKP 215, Skye Instruments Ltd., Powys, Wales) and red:far-red ratio (R:FR, measured with a 660:730 sensor SKR 110, Skye Instruments Ltd., Powys, Wales) of 0.94; green net: 27% PAR, R:FR of 0.54. Open sunlight at midday had a PAR of 2050 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a R:FR of 1.05 (Table 1).

The coppiced plants grew shoots up to 2 m within 10–12 weeks, when they were pruned again. The third flush of shoots obtained in this way was used for the experiment. The plants were by then 19 months old (7 October 1997). Only for seven of the nine clones enough experimental material could be obtained. The two other clones did not yield enough cutting material in any of the light treatments. Diameters of the shoots were measured to verify if shade treatments would affect the volume of the cuttings after reducing them to a standard length.

Table 1. Light characteristics of the experimental treatments

	No shade	Black shade	Green shade
PAR ($\mu\text{mol m}^{-2}\text{s}^{-1}$)*	2050 (100%)	641 (31%)	560 (27%)
R:FR (660:730 nm)**	1.05	0.94	0.54

* Photosynthetic active radiation, integrated value of 30 measurements at midday on a cloudless day,

** red: far-red ratio.

Single-node cuttings were taken from the shoots. The soft tip with undeveloped leaves was discarded and only cuttings from the apex (internodes 1–2) and the base of the shoot (internodes 5–6) were used. These classes represent different stages in chronological age that impact on lignification and perhaps other aspects of morphology which have already started in internodes 5 and 6. The leaves were trimmed leaving only two basal pinnae, resulting in an average leaf area of 10 cm² for the apical and 15 cm² for the basal cuttings. The stems were reduced to a standard length of 7 cm, and trimmed cleanly at the base. A 10 µl drop of IBA in ethanol (0.1, 0.3 or 0.6 % w/v, according to the required treatment) was applied to the base of the cuttings. The control cuttings remained untreated because in preliminary experiments the solvent did not have an effect on rooting of calliandra cuttings. To speed up the evaporation of the ethanol, cuttings were put in a cold airstream for approximately 30 seconds. The cuttings were then set into six 2 × 1 m non-mist polyethylene propagators, built according to Longman (1993), that were filled with steam-pasteurised, coarse sand (2 mm fraction). The propagators were covered with black 75% shade netting to reduce heat build-up. The average daily maximum PAR inside the propagator was 186 µmol m⁻² s⁻¹ (measured with the same quantum sensor SKP 215 mentioned earlier). The cuttings were left in the propagators for 6 weeks and sprayed with water twice a day.

The cuttings were checked for death (due to basal stem rot and leaf abscission) and rooting weekly. Dead cuttings were removed. Cuttings which showed roots or emerged root primordia were considered as rooted, put back in the rooting medium and left undisturbed for the rest of the experiment. At week 6, the number of roots longer than 1 cm was recorded for all rooted cuttings. At that time, nearly all (99.5%) surviving cuttings had rooted and all cuttings that were considered rooted earlier survived the entire experiment.

Experimental design and analysis

The rooting experiment was carried out as a 3 (shade) × 4 (IBA) × 2 (node) factorial experiment replicated twice for each of the 7 clones. Each plot contained three cuttings. In other words, we used 336 cuttings per shade treatment, 252 cuttings per auxin treatment, 504 cuttings per node position and 144 cuttings per clone. The entire experiment included 1008 cuttings. Plots were arranged in 12 incomplete blocks, resulting in 28 plots per block. In this design, no formal analysis could be carried out to compare results between clones which had been included as a second blocking factor, not a main treatment.

The analysis of percentage rooting and number of roots was done through mixed model REML (residual maximum likelihood) procedure of Genstat. This algorithm estimates effects of both fixed and random factors. The factor 'block' as well as 'clone' is considered as random, since the clones used are a random selection of a larger population. Residuals of percentage of rooting and number of roots were checked for normality and homogeneity. No transformation was needed for percentage rooting, while numbers of roots were natural logarithm (ln) transformed. The REML method is also capable of handling the incomplete structure of the design. The figures given in Tables 2 to 6 are predicted means obtained through REML.

Results

Percentage rooting

Shading and auxin application were the two main factors affecting rooting (Table 2). Shading improved the average rooting from 42 to 77% (green nets) and 72% (black nets). The difference between green and black shades is not significant. IBA has a less pronounced effect. Small doses (10 μg) seem to increase the rooting success slightly but significantly in the 'no shade' and 'green shade' treatments, whereas the response to IBA is less clear in 'black shade'.

The difference in rooting depended also on the original node position with a significant 'node'-'shade' interaction. Apical nodes rooted better than basal ones in the 'no shade' and 'black shade' treatments, whereas the reverse was true with green shading (Table 3).

A strong clonal difference was noted. Rooting success (combined over all treatments) varied between 39 and 86% (Table 4).

Table 2. Rooting success (%) of *Calliandra calothyrsus* cuttings under shade and IBA treatments (n = 84)

IBA (μg /cutting)	No shade	Black shade	Green shade
0	32.7	71.0	64.6
10	56.8	64.8	88.1
30	43.3	83.6	76.2
60	37.0	66.5	77.0
mean	42.5	71.5	76.5

Sed (standard error difference): for comparison between IBA levels: 8.4–9.2
for comparison between shade treatments: 8.6–9.5
for comparison between means: 4.5

Table 3. Rooting success (%) of *C. calothyrsus* cuttings under shade treatments and node positions (n = 168)

Node position	No shade	Black shade	Green shade
Apex	52.3	78.6	63.6
Base	32.6	64.3	89.3

Sed: 6.3

Table 4. Shoot diameter (mm), leaf retention (% after 2 weeks) and rooting success (% after 6 weeks) of cuttings of various clones of *C. calothyrsus* averaged over all other experimental treatment factors (n = 144)

Clone	Shoot diameter	Leaf retention	Rooting
C16	5.5	18.1	79.2
C26	5.5	18.8	76.6
C31	4.4	39.0	38.9
C35	5.8	17.7	54.5
C51	5.2	34.8	71.0
C58	3.3	18.3	85.7
C69	6.8	36.9	45.7
Sed	1.0	*	*

*: Because of the design no sed can be calculated for clones in the rooting experiment (see text).

In order to understand the factors influencing final rooting success, it is also important to look at their survival rate after a few weeks. Cuttings do vary in their ability to survive severance and the period that they remain without roots. Most of the cutting death occurred in the first three weeks (Table 5), when rooting had just started. Shading with black nets clearly improved survival rate of cuttings (87%) compared to the control (65%). This difference explains a major part of the difference in rooting found between these two treatments (see Table 3). The green net caused a big difference in survival between basal and apical cuttings. Nearly all basal cuttings (99%) survived the first three weeks in the propagators (Table 5).

Number of roots per rooted cutting

The number of roots formed per rooted cutting was strongly affected by the IBA treatments. Similar to the percentage of rooting, numbers of roots increased sharply with application of IBA and levelled off with increasing concentrations (Table 6).

Shading also influenced the number of roots, albeit to a lesser extent. Again, the main difference occurred between irradiance treatments, regardless of the light quality. As for rooting success, stockplants grown in the open gave the worst results. Root numbers did not vary between node positions, or among clones.

In the preliminary experiments we found that seven roots seems to be a critical number to guarantee a successful hardening-off of calliandra cuttings.

Table 5. Percentage of *C. calothyrsus* cuttings that were dead three weeks after setting under shade treatments and node positions (n = 168)

Node position	No shade	Black shade	Green shade
Apex	33.0	13.8	25.5
Base	36.2	13.2	1.2

Sed: 6.1

Table 6. Number of roots per rooted cutting of *C. calothyrsus* under shade and IBA treatments (ln)

IBA ($\mu\text{g}/\text{cutting}$)	No shade	Black shade	Green shade
0	2.1 (0.72)	2.4 (0.86)	2.4 (0.87)
10	5.1 (1.62)	8.9 (2.19)	11.9 (2.48)
30	9.9 (2.29)	17.3 (2.85)	16.4 (2.80)
60	9.4 (2.24)	20.5 (3.02)	20.7 (3.03)

Sed on the ln transformed data: 0.20

Discussion

Rooting success of *C. calothyrsus* cuttings was strongly influenced by pre-severance stockplant management. Reducing the irradiance by 70% increased rooting success and the number of roots developed. These results are in accordance with findings for a range of species (for example, Moe & Andersen 1988, Leakey & Storeton-West 1992).

Additionally, reducing R:FR from 0.94 to 0.54 only increased the rooting of low IBA treatments and basal cuttings. A similar trend has also been observed for *Triplochiton scleroxylon* (Leakey & Storeton-West 1992, Newton *et al.* 1996), *Eucalyptus grandis* (Hoad & Leakey 1996) and *Acacia tortilis* (Dick & East 1992). Newton *et al.* (1996), using *T. scleroxylon* cuttings of fixed node number, attributed increased rooting to longer internodes on plants grown under low R:FR, resulting in increased stem volume and hence a higher carbohydrate storage capacity, and to differences in pre- and post-severance photosynthetic activity. However, in experiments with *Terminalia spinosa*, using a standard cutting length, they did not find the same effect. In our study a clear increase of rooting under low R:FR could be seen with a slightly reduced cutting volume, resulting from a standard cutting length and smaller average diameter (4.9 mm) than under ambient R:FR (5.5 mm, sed: 0.54). However, the increased rooting was present only in the lowest IBA treatments, and was contrasting depending on the node position (chronological age) of the cuttings: reducing R:FR increased rooting success of basal (chronologically older = physiological more vigorous) cuttings, whereas it decreased rooting of apical (chronologically younger) cuttings. Basal cuttings, therefore, were allowed to express their potential for increased rooting as expected by their high physiological vigour only when grown under low R:FR. A possible explanation can be found in the difference in pre-rooting survival of the cuttings which was significantly different depending on shading and original node position (Table 5). It could be that the final rooting success is attributed to morphological differences due to the pre-severance treatments, such as the thickness of the tissue (Newton *et al.* 1996), that influence their abilities to withstand the post-severance stress, but have only little influence on the rooting process *sensu stricto*.

Another explanation could be low post-severance photosynthesis rates of cuttings from highly-assimilating stockplants and node positions due to end-product inhibition, because accumulation of starch produced during pre-severance leads to lower post-severance assimilation of the cutting (Leakey & Storeton-West 1992). Since starch accumulation is more pronounced in basal nodes than in apical ones, this negative feedback system might be only applicable to basal cuttings and explain the lower rooting success of cuttings from stockplants grown under high R:FR. On the other hand, stockplants grown under lower R:FR might have had a sufficiently low assimilation rate to avoid end-product inhibition. Hoad and Leakey (1994) found that plants grown under low R:FR had a reduced net photosynthetic rate and lower chlorophyll concentration.

Previous experiments testing the influence of alteration in R:FR have been carried out in controlled environment conditions with extremely low PAR and often unnatural ranges of R:FR up to 6.5 (full sunlight: *c.* 1.0) (Table 7). Also, some experiments were carried out with young seedling stockplants (Hoad & Leakey 1996, Dick *et al.* 1996a, b). The present experiment was carried out with stockplants derived from cuttings that were grown under natural conditions. Black shading was used to establish data for reduced irradiance while maintaining the R:FR around 1.0; green netting was used to change R:FR in a natural range (canopy) while maintaining a similar irradiance as under the black (neutral) shading. It is therefore challenging to interpret the data in combination.

The data show that there is contrasting behaviour of species under the different light conditions. Looking at only the results from experiments in which irradiance

Table 7. Literature overview of percentage rooting of cuttings from different species under different irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and R:FR

Species	IBA*	Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R:FR								Source
			0.4-0.5	0.6-0.7	0.9-1.1	1.2-1.3	1.6	3-3.5	4.5	6.3-6.5	
<i>Terminalia spinosa</i>	0.8%	150	77.5		-92	~87			93.7		1
<i>Triplochiton scleroxylon</i>	40 μg	150	54.1		-48			-40	31.1		1
<i>T. scleroxylon</i>	40 μg	106						-54			2
<i>T. scleroxylon</i>	40 μg	202						-47			2
<i>T. scleroxylon</i>	40 μg	246						-31			2
<i>T. scleroxylon</i>	40 μg	294						-90		~54	2
<i>Eucalyptus grandis</i>	0.3%	200	30.0	38					-10		3
<i>E. grandis</i>	0.3%	200		47		-30			-29		3
<i>Acacia tortilis</i>	0.8%	150		77	79.0				-62	~21	4
<i>Calliandra calothyrsus</i>	0 μg	560/640	64.6		71.0						5
<i>C. calothyrsus</i>	10 μg	560/640	88.1		64.8						5
<i>C. calothyrsus</i>	30 μg	560/640	76.2		83.6						5
<i>C. calothyrsus</i>	60 μg	560/640	77.0		66.5						5
<i>C. calothyrsus</i>	0 μg	2050			32.7						5
<i>C. calothyrsus</i>	10 μg	2050			56.8						5
<i>C. calothyrsus</i>	30 μg	2050			43.3						5
<i>C. calothyrsus</i>	60 μg	2050			37.0						5

*: Either quantity per cutting (μg) for individual applications or concentration in solvent (%) for dips

Sources: 1. Newton *et al.* (1996); 5 cm cuttings with 20 cm² leaf
 2. Leakey & Storeton-West (1992); single node cuttings with 50 cm² leaf
 3. Hoad & Leakey (1996); two node cuttings with half a leaf
 4. Dick & East (1992); four node cuttings
 5. Present study; 7 cm cuttings with 2 pinnae

was kept constant or near constant, the effect of changed R:FR seems negligible. *Terminalia spinosa* showed rooting reduction by about 10% between R:FR of 1.2 and 0.4, *Triplochiton scleroxylon* a slight increase of *c.* 10%, similar to *E. grandis*. *Acacia tortilis* did not show any effect when reducing R:FR from 0.9 to 0.6. Our data with *C. calothyrsus* confirm the small effect of reducing R:FR: there was either a 10% reduction in rooting success (0, 30 μg IBA) or a 20–30% increase (10, 60 μg IBA).

A significant clone effect could be shown. Dick *et al.* (1996b) have speculated that clonal variation in rooting success of *C. calothyrsus* is greater than provenance differences and that it depends on variation in plant morphology, especially leaf area and leaf retention up to day 10 of setting the cuttings. In order to minimise such effects in the present study, cuttings were trimmed to equal size and leaves were trimmed to two basal pairs of pinnae, therefore greatly reducing the leaf area and the chance of abscission. In spite of these precautions, the volume of cuttings still differed because variation in diameters and also leaf retention was different among the clones. There was, however, no evidence that clones with larger diameters and/or better leaf retention rooted better (Table 4). Our results indicate that, in contrast to Dick *et al.* (1996b), cutting morphology and leaf retention may not be responsible for genotypic variation in rooting ability.

In conclusion, in *C. calothyrsus*, a) reduction of light intensity by 70% significantly increases rooting success and number of roots; b) reduction of R:FR while maintaining low PAR increases rooting only in basal cuttings and when small amounts of IBA are applied, whereas it decreases rooting success in apical cuttings. Minimal application

of IBA increases rooting success and the number of roots so that survival during hardening-off can be guaranteed.

The practical implications of this work will relate to the purpose, resources and training of the user. Farmers, in the absence of any treatment, could expect a maximum of 33% rooting, whereas using optimal treatments they can raise this to almost 90%. The advantages of imposing such treatments need to be demonstrated to user groups. For example, alteration of the light quality, resulting in doubling the rooting success, could be achieved by growing stockplants under a light shade of small-leaved trees. Although obtaining IBA might be difficult for rural communities, the results presented here show that only minimal quantities are needed for a considerable increase of root numbers on cuttings.

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