# Review of vegetative propagation of cacao (*Theobroma cacao* L.) by rooted cuttings. 2. Environmental and technical considerations

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**ABSTRACT**

The paper reviews the unfavourable environmental factors and technical difficulties that have limited the extensive use of vegetative propagation by rooted cuttings in multiplying cacao. Important among the external factors affecting rooting of cacao cuttings are light, temperature, humidity, and the air-moisture relations of the rooting media. The most important technical factors that affect rooting of cuttings are hormonal treatment, wounding, etiolation, and exogenous supply of carbohydrates and minerals to cuttings. These factors may influence rooting success in isolation or in combination with each other because it is often impossible to separate the effect of one factor from the other. The light intensity at which rooting occurs is dependent on temperature. The relative humidity within the propagation set-up is related to the light intensity and associated temperature. The effects of various media on rooting of cuttings are usually closely influenced by the temperature and water relations. The most effective hormonal treatment for cacao is a mixture of indole butyric acid (IBA) and naphthalene acetic acid (NAA) in equal proportions at a concentration dependent on the dipping time and the size of cutting. Wounding and etiolation are treatments which have been reported to improve rooting in most tree species, but have not had wider application in cacao propagation. Exogenous application of carbohydrates may improve rooting of cuttings. The need is for an effective manipulation of the external environment and a wider exploitation of some technical factors to improve on rooting in cacao.

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**RÉSUMÉ**

AMOAH, F. M.: *Propagation végétative de cacao* (Theobroma cacao *L.*) *par les boutures prenant des racines. 2. Les condidérations environnementales et techniques.* Cet article fait une révision de facteurs environnementaux favorables et les diffecultés techniques qui ont limité l'utilisation extensive de propagation végétative pour la multiplication de cacao. Inclus dans les facteurs extérieurs importante influençcant l'enracinement de boutures de cacao saont: la lumière, la température, l'humidité et les relations d'air-humidité de substances de la prise de raines. Les facteurs techniques les plus importants qui influencent la prise de racines par les boutures sont le traitement hormonal, la coupe blessante, étiolement et la provision exogène de hydrates de carbone et de minérals. Ces facteurs pourraient influencer le succès de la prise de racines en isolement ou en combinaison l'un evec l'autre comme ce n'est pas souvent possible de séparer l'effet d'un facteur de l'autre. L'intensité de lumière à laquelle la prise de racines se produit dépend de la température. L'humidité relative à l'intérieur de l'instensité de la lumière et à la température associés. Les effets des différents substances sur le plus souvent étroitement influencés par les rapports de température et d'eau. Le traitement hormonal le plus efficace pour le cacao et une mèlange de IBA et NAA à parts égales d'une concentration dépendant de temps d'enfoncement et les dimensions des boutures. La coupe blessante et l'étiolement sont des traitements qui ont été annoncé d'améliorer la prise de racines en plusieurs espèces d'arbre mais qui n'ont pas réalisé de plus grande application de propagation de cacao. L'application exogène de hydrates de carbone pourrait améliorer la prise de racines de semis. Il y a la nécessité d'une manipulation efficace de l'environnement extérieur et une exploitation plus grande de quelques facteurs techniques pour améliorer la prise de racines de cacao.

## Introduction

Difficulties in the vegetative propagation of cacao have been the foremost limiting factors with the rapid multiplication of some selected clones. The demand for cacao clonal materials has greatly changed in recent years. The decline in cacao yields as a result of pests, diseases, old and moribund trees, and natural disasters such as drought and bushfires in some cacao-producing countries has led to the need for intensifying production of young cacao to meet the increasing demands of the industry. However, environmental and technical difficulties in vegetative propagation by rooted cuttings have limited the adoption and extensive use of this method in clonal propagation of cacao. Rooting success has sometimes been low, and results are even poorer when peculiar problems arise.

Environmental conditions around the cuttings are the most readily controlled factors. Evans (1953) observed that external factors such as light, temperature, humidity, and the air-moisture relations of the rooting media constitute the most important of such factors. These factors either influence successful rooting singly or in combination with one or more of other factors. He further stated that the influence of light and temperature must be considered together because they interact to a marked degree under practical propagation conditions. Ramadasan (1979) also stated that light and temperature, especially in enclosed propagation systems such as closed bins, cannot be separated because temperature increases with light intensity.

Of the technical factors (pre-treatments) which favour rooting of cuttings, hormone treatment, wounding, etiolation, and supply of carbohydrates and mineral nutrients have been reported extensively.

This paper aims to discuss the interaction among these environmental and technical factors and how they affect rooting in cacao.

## Light and temperature

The minimum light intensity at which rooting occurs has been observed (Evans, 1953) to depend on temperature. At light intensity of 1000 lux and 32 ºC, Evans (1953) observed that cuttings starved. At 1000 lux and 27 ºC, cuttings remained alive, but produced relatively few roots after 5 weeks when the period of illumination was 12 h. Rooting was prolific at 27 ºC when the illumination period was extended to 24 h. At 5000 lux and from 27 to 28 ºC, rooting was prolific in 3 weeks at an illumination period of 12 h. At this same light intensity, however, rooting did not improve when the period of illumination was increased from 12 to 24 h. With the normal variation in light intensity during the day, rooting is optimal when the maximum light intensity in the midday hours is 10,000 lux. As more light is allowed into the propagation unit, the temperature rises, and in closed propagation units, humidity drops unless watering is more frequent.

In normal propagation systems, the light intensity is kept below 50 per cent of full midday sunlight of between 3000 and 7000 lux (Ramadasan, 1979). However, at very low light intensities, yellowing of the leaves occurs from the base of the lamina near the veins. Carbohydrate resources also become depleted owing to inadequate photosynthesis, resulting in death. Bowman (1950) similarly noted that at very low light intensities, defoliation is rapid. However, when the light intensity is increased, rooting improves to normal levels. Although higher light intensities could be used with the open spray techniques because of the absence of the effects of excessively high temperatures, bleaching and chlorophyll decomposition were noted at intensities 75 - 100 per cent daylight (i.e., 7500 - 10000 lux) (Evans, 1953). Evans (1953) also noted that under high light intensity, carbohydrates accumulate in excess over nitrogenous fractions so that the leaf becomes pale-yellow and subject to breakdown. In practice, light of suitable intensity is provided by overhead shade. If the day is particularly hot, the temperature is kept down by frequent water spraying of cuttings during the hottest hours.

This may be automatically synchronized using electronic leaf mist units.

Ramadasan (1979) tested the effectiveness of various propagation systems under Malaysian conditions and concluded that shade regimes were optimal at 2 to 6 per cent daylight, about 200 to 600 lux, for nearly all methods used. It was critical for closed polybag propagators which were prone to developing unfavourably high temperatures with increasing light levels. With continuous mist, Evans (1953) observed that cuttings survived well under light regimes up to 100 per cent, with optimal results at light intensities ranging from 5 to 40 per cent. Evans (1951), working under Trinidad conditions, also suggested that 5 to 75 per cent light could be used to root cuttings successfully. It seems, therefore, that the percentage of light required for rooting depends on local conditions such as cloud cover, season of rooting, and effect of light-intercepting structures such as glasshouses. Evans (1951) showed that cuttings could be rooted in complete darkness when treated initially with adequate supplies of sucrose and amino acids. This shows that light-induced photosynthesis was more important to the rooting of cuttings than the photoperiodic effect of light *per se;* and that light intensity, divorced from the complicating effects of temperature and humidity, is not a crucial factor in rooting cacao, if intensity and durations are within certain limits (Evans, 1953).

### Atmospheric humidity

The relative humidity within any propagation system is related to the light intensity and temperature. Several workers have emphasized the need for a near-saturated atmosphere around the leaves of rooting cuttings. Loach (1977) stated that the ability of a propagation system to provide a near-saturated atmosphere around cuttings is a good indicator of its effectiveness. Evans (1953) also stated that cuttings rooted well in a near-saturated atmosphere. He, however, indicated that it is virtually impossible to realise 100 per cent humidity whilst allowing 10 per cent sunlight to enter the propagation chamber without occasionally spraying the cuttings with water. Nichols (1958) stated that semi-hardwood cuttings in an atmosphere near 100 per cent relative humidity at a reduced light intensity (15-205 of the tropical incident sunlight given adequate aeration at the base of the cuttings) will produce roots in the order of 70 to 90 per cent rooting efficiency. The number of water sprayings necessary to maintain the air humidity in the propagator at almost 100 per cent varies with the external relative humidity and climatic conditions. The frequency of watering may be reduced by having the rooting medium in trays over a free-water surface (Evans, 1953).

Hartman & Kester (1983) emphasized that in slow-rooting species, water loss from the leaves must be reduced to a very low rate to keep the cuttings alive until roots form. For transpiration of the leaves to be low, the vapour pressure of the water in the atmosphere surrounding the leaves should be maintained nearly equal to the water vapour pressure in the intercellular spaces within the leaf. Automatically operated devices such as mist and fogging units give a beneficial effect primarily in increasing the amount of water vapour in the air. Under mist, transpiration can be reduced to a low level while maintaining a high light intensity to promote full photosynthesis. In cuttings, a water deficit caused by a fall in the relative humidity may be made up by intake of water through the leaf surface (Evans, 1953). However, under severe water stress, localized yellowing areas may be seen on the leaf a few days afterwards. Even a slight water deficit, which may not be enough to cause any visual symptoms of distress, results in considerable delay or reduction in rooting responses. It may, therefore, be concluded that full turgidity is necessary for rapid root differentiation.

### Rooting medium, temperature, and water relations

Cuttings of many plant species root easily in several rooting media, but those more difficult to root may be greatly influenced by the kind of rooting medium used. This effect could be seen not only in the percentage of cuttings rooted, but also in the quality of root system formed (Long, 1932). The merits of different rooting media for cacao have been investigated by several workers with varying conclusions (Keeping, 1950; Evans, 1951; Garcia, 1954; Perez, 1954; McKelvie, 1957). The effects of various media on the rooting of cuttings are usually closely influenced by the temperature and water relations.

The water relations of non-rooted cuttings are critical to their survival, particularly in leafy softwood cuttings with the leaves actually transpiring without a root system (Al Barazi, 1983). Different rooting media may have different water relations, and they are able to facilitate favourable rooting of cuttings over a narrow optimal watering regime. Hartman & Kester (1983) noted that while under-watering could kill cuttings owing to excessive net transpiration, over-watering can lead to asphyxiation, rotting, and reduced rooting. The preference for any particular rooting medium may reflect its local availability and economic considerations. With optimum light and moisture supplies, rooting can be successful in a wide range of media (Bowman, 1950). Hardy (1960) also observed that the suitability of a rooting medium depends on the amount and frequency of watering. The ideal medium consists of a range of particle sizes which create a distribution of pore sizes from which water and air are readily available to the cutting during rooting.

Evans (1953) reported that the physiological factor underlying rooting responses in different rooting media is the gaseous composition of the atmosphere around the base of the cutting. This factor has a pronounced effect on the formative changes taking place at the base of the cutting. In studies on the rooting of cacao cuttings, the author observed that in moisture-saturated air, a thick pad of callus developed from the cambial tissues exposed at the cut surface. Root primordial are either not initiated or their appearance is greatly delayed. Without or with very low concentration of oxygen, the basal part of the cutting rots. With a higher but inadequate supply of oxygen, little or no development of callus occurs at the cut end, but the division of the cells of the phellogen is active, giving rise to roots of undifferentiated callus tissue which emerge from the lenticels. When the gaseous composition at the base of the cutting is optimal for rooting, Evans (1953) observed a pronounced activity of the cambium, resulting in a swelling of the base in which root primordial rapidly appear.

The temperature of the rooting medium can also be very critical for successful rooting in many plant species, especially in difficult-to-root candidates. Evans (1951) observed that a suitable temperature in the growing medium is as necessary for initiating and developing roots as the correct level of ambient temperature is important for the shoot system of a plant to grow and develop. However, careful temperature control at the upper limit may be equally essential as that at the lower limit because supra-optimal temperatures can adversely affect the rooting of some plants. This adverse effect may be mediated through direct heat damage to the region of root initiation, or as a result of excessive respiration. This depletes the carbohydrate reserves which would have been used by the cutting for the initiation, development and subsequent growth of roots.

Hess & Snyder (1957) observed that a basal temperature of 25 ºC was optimum for most ornamentals, whilst Hartman & Kester (1983) stated that a basal temperature of 21 to 25 ºC was favourable for root emergence in a wide range of subjects. These observations may hold for temperate crops, but tropical plants may require a higher temperature for optimum rooting. Evans (1951) soaked the base of cacao cuttings in water maintained between 5 and 50 ºC for either ½ or 1 h before rooting. He observed after 3 weeks that immersion of the base of cuttings at 40 ºC for 1 h produced good rooting. At lower temperatures, roots were fewer while immersion at 50 ºC for ½ h damaged a proportion of the cuttings, but rooting was observed above the damaged region. These observations suggest that cacao cuttings may respond to basal temperature during the rooting period. However, evidence of such possible response is lacking, though it could be an additional tool in speeding and improving rooting in cacao as has been observed in many other crops.

**Hormone treatment**

Lek (1925) first suggested that hormones were essential for forming adventitious roots in cuttings. The most common exogenously applied rooting hormones are indole acetic acid (IAA), NAA, and IBA either with talc or in solution. Tinley (1961) observed that substances other than those normally accepted as growth substances may also be effective in stimulating rooting. Grace & Thistle (1939) showed that ethyl mercuric phosphate and cane sugar were able to induce root formation in cuttings. Sometimes fungicides such as ferric dimethyl-diacarbonate have also been observed to stimulate rooting in cuttings.

The effect of growth substances on rooting may be indirect, mediating through the supply of soluble sugars for root initiation and development. Considerable evidence shows that insoluble higher polysaccharides such as starch are hydrolysed to soluble sugars after being treated with growth substances (Howard, 1962). The relationship between mobilizing carbohydrates and improved rooting response of cuttings treated with growth substances is supported by experiments on the inter-relationship between boron, sugar metabolism, and rooting (Gauch & Dugger, 1953). The use of growth substances in propagation has facilitated rooting in many plants, especially difficult-to-root ones (Sultan, 1974). Different workers have expressed preference for different rooting hormones. However, IBA and NAA and their mixtures are probably the best materials for general use, because they are non-toxic over a wide range of concentrations and are also less readily broken down chemically compared to the naturally occurring IAA (Al Barazi, 1983).

Evans (1951) extensively investigated the effect of synthetic growth substances on rooting in cacao. He observed that the most effective treatment was a mixture of IBA and NAA in equal proportions. The best single substance was IBA, but this was slightly less effective than the mixture. Optimal concentrations were equal for the mixture and IBA alone. These concentrations ranged from 8000 to 10000 ppm in 50 per cent alcohol for the quick-dip method, and from 800 to 1000 ppm for the 24-h dip. For single-node cuttings, the optimum treatment was by the quick-dip method, using half the concentration for stem cuttings. Contrary to the observations of Evans (1951), Ramadasan (1979) observed that the IBA and NAA mixture proved inferior to all other hormone solutions for root length and root number in rooted cuttings. This might be due to genetic differences in the cuttings used by the two workers, and probably also to the microclimate environment within in the propagation units.

Ramadasan (1970) observed that concentrations of IBA alone of 6000 to 10000 ppm were the most effective for softwood and semi-hardwood cuttings when applied as a quick dip. Lower hormone concentrations seemed to favour increased root length whilst higher concentrations of 8000 to 12000 ppm favoured root dry weight. Ramadasan (1979) attempted to apply hormones through the leaf and observed that dilute concentrations of foliar hormone sprays were ineffective in improving the propagation of cuttings rooted, but they did confer benefits in length, number and dry weight of roots. Evans (1951) also attempted to induce root initiation on cuttings before removal from the stock plants. The treatments consisted of spraying 10, 25 and 50 ppm of rooting hormone at 1, 2 and 3 weeks before removal from the tree. The result of the pre-treatments was invariably a delay in rooting as compared to the untreated controls.

### Wounding

Deliberate wounding of cuttings besides the cut base is a common means of improving the rooting of certain plants, especially the difficult-to-root ones. Hartman & Kester (1983) observed that after wounding, callus production and root development are usually heavier, especially along the margins of the wound. Wounding may be horizontal or vertical, shallow or deep, single or double. Cuttings may be wounded before or after auxin treatment. Howard (1973) found that wounding in apple cuttings before auxin treatment was more effective than after auxin treatment. This was almost certainly due to greater uptake of the applied growth regulators. Edward & Thomas (1979, 1980) observed in a range of materials, including *Ilex* and *Juniperus* species, that wounding broke the sclerenchyma ring which formed a continuous ring constituting a barrier to root formation. Thus, wounding facilitates rooting in cuttings in which sclerenchymatous cells inhibit or delay rooting.

Ylatalo (1979) observed that wounding of cuttings as a treatment before hormones are applied can improve the rooting percentage and quality. Various workers have observed that wounding reduced the time required for roots to appear in cuttings besides giving a higher percentage success (Day, 1932; Synder, 1954). Day (1932) gave increased water intake as the reason, while Snyder (1954) suggested that because plants have a band of thick-walled cells external to the point of origin of the root, the roots are unable to penetrate them and are forced to emerge from the base of the stem. Hartman & Kester (1983) reported that wounding may relieve tissue tension and can stimulate cell division which may lead to an increase in the rate of respiration. It may also stimulate ethylene production which promotes formation of adventitious roos (Krishnamoorthy, 1970).

Wells (1962), however, remarked that wounding would not cause any cutting to root that would not otherwise root if given enough time after being given other treatments such as hormone application. This suggests that wounding may be synergistic with the effect of other treatments to improve rooting, but not a substitute for their effects. Little information is available on the response of cacao cuttings to wounding treatment. Ramadasan (1979) stated that mechanical treatments such as bark removal and wounding were ineffective in improving rooting of cacao; in fact, they seem to be detrimental. This is contrary to results with other plant species (Edward & Thomas, 1980), probably because of the type and degree of wounding applied. Hassan (1983) observed that wounding favoured rooting in Durian, a tropical fruit tree.

### Etiolation

Etiolation has been reported to promote the formation of adventitious roots in plants (Sachs, Lorett & De Bie, 1964; Hackett, 1970; Howard & Schmidt, 1979). Harrison-Murray & Howard (1981) stated that etiolation treatment does not substitute for auxin treatment, but rather makes the cuttings more responsive to auxin, particularly in numbers of roots produced. The influence of etiolation on rooting may be anatomical (Priestly, 1923; Herman & Hess, 1963), physiological (Kawase, 1965), or biochemical (Herman & Hess, 1963). Anatomically, etiolation affects rooting by modifying the structure of the shoots, the derivative tissue becoming less well differentiated (Priestly, 1923; Herman & Hess, 1963). Beakbane (1961) observed that etiolation also reduces the amount of mechanical tissue which would otherwise restrict root emergence. Thompson (1951) remarked that etiolation may also delay tissue maturation (i.e., makes tissues more juvenile) because light accelerates initiation and completion of cell multiplication and elongation, resulting in the early beginning and ending of both successive phases.

In a comparative study of etiolated and non-etiolated stem cuttings of *Hibiscus* and red kidney beans, Herman & Hess (1963) observed that the etiolated stem cuttings had a reduced starch content, reduced mechanical strength of tissues, reduced cell wall thickness, diminished cell wall deposits and vascular tissues. The non-etiolated cuttings had more parenchyma cells and more tissues in a less-differentiated condition. The etiolated cuttings also reacted strongly to IBA application in root formation. This was presumed to be due to more rooting co-factors, which implied the possible interaction of anatomical and chemical factors. Physiologically, etiolation has been shown to affect rooting by slightly increasing levels of endogenous auxins (Herman & Hess, 1963; Kawase, 1965).

Little is known about the effect of etiolation on rooting in cacao. However, some tropical and subtropical crops, for example avocado, have been reported to root well after etiolation treatment (Frolich, 1961). Evidence that cacao may respond to some form of etiolation is shown by the fact that Evans (1951) observed that cuttings from plants provided some form of shading rooted better than cuttings from plants grown in full sunlight. Etiolation may involve total exclusion of light from the stock plants for a specific time before the taking of cuttings (Hackett, 1970; Harrison-Murray & Howard, 1981), or may involve the exclusion of light from the base of potential cuttings (Harrison-Murray, Howard & Mackenzie, 1980). The latter is commonly called blanching. Harrison-Murray & Howard (1981) observed that the effect of etiolation is not rapidly reversed by transfer to light conditions, and seems to involve a factor which can move within the plant at least over short distances. Harrison-Murray & Howard (1981) further stated that for some subjects which do not respond to all the available post-cutting collection treatments, growing stock plants in darkness or at a reduced light intensity for a few weeks at the start of the growing season can greatly increase their rooting ability.

**Carbohydrate and mineral supply to cuttings**

The carbohydrate status of stock plants may be so low that rooting in cuttings might be poor or delayed or both. Several workers have attempted to reinforce the carbohydrate status of cuttings by exogenous supply of nutrients, mainly sucrose (Evans, 1951), and other elements and vitamins such as boron, nitrogen and potassium. Such exogenous supply of nutrients have been reported to yield beneficial results in some plant species (Howard & Sykes, 1966; Haum & Cornell, 1951; Pearse, 1943). Cuttings placed under mist may be damaged from leaching,especially of potassium. Here, the supply of nutrients in the mist may be a primary requisite for successful rooting (Wott & Tukey, 1967). Howard & Sykes (1966) modified the carbohydrate resources in softwood hop cuttings by supplying sugars in solution during a 5-day pre-rooting dark period. They observed considerable improvement in rooting compared with cuttings which initially had low contents of carbohydrates.

Evans (1951) attempted to improve rooting in cacao by modifying the carbohydrate and mineral contents of cuttings. Excised shoots for rooting were steeped in solutions of sucrose, amino acids, coconut water, tomato juice, vitamin B, sodium glycerophosphate, vitamin C, yeast, and manganese sulphate. He observed that most nutrients, except for sucrose, were ineffective alone, but improved rooting when combined with others. Sucrose was necessary because without it, no beneficial effects were produced by the other nutrients.

The influence of an additional supply of nutrients may be particularly marked in difficult-to-root clones and leafless cuttings. Evans (1951) stated that without leaves, cuttings of cacao failed to root. However, when large amounts of sucrose were injected into leafless cuttings, some rooted in 4 to 5 weeks. With the supply of sucrose, most difficult-to-root clones were observed to root well. Evans (1951)concluded that optimum hormone treatment is in itself important, but activity is enhanced by sucrose and possibly arginine.

Several investigators have observed that carbohydrates *per se* are not critical to rooting in cuttings, because with adequate light in the propagator, leafy cuttings photosynthesise to meet their carbohydrate demands; and that the ratio of carbohydrates to nitrogen is a more critical factor to the rooting of cuttings (Harvey, 1923; Pearse, 1943; Samish & Spiegel, 1957; Kraus & Kraybill, 1918; Kim *et al*., 1977; Mokash, 1978). But Ali & Westwood (1968) found that high carbohydrate levels were negatively correlated with good rooting in three *Pyrus* species. Smith & Thorpe (1975) also could not establish any relationship between available carbohydrate and the ease of rooting in Pecan cuttings. These contradictory results suggest that different crop species, and probably the system of managing the stock plant and environmental conditions under which cuttings are rooted, may respond differently to the exogenous supply of carbohydrates and other minerals in rooting percentage and quality of roots produced. Thus, while there seems to be no generally applicable rule, an adequate supply of carbohydrates–preferably in soluble forms–seems to be needed for optimum rooting.

## Conclusion

Environmental conditions around cacao cuttings are the most readily controlled factors. Of the external factors affecting rooting of cacao, light, temperature, humidity and the air-moisture relations are the most important. The effect of light on rooting of cacao is mediated through the photosynthetic activity. Light intensity, divorced from the complicating effects of temperature and humidity, is not a crucial factor in rooting cacao if intensity and duration are within certain limits. Full turgidity in cacao cuttings is necessary for roots to differentiate rapidly. The effects of various rooting media on rooting in cacao are closely influenced by temperature and water relations. Cocoa cuttings would normally respond to hormonal treatment; the most common rooting hormones for cacao being IBA and NAA, using a concentration dependent on the size of the cutting. Wounding of cuttings besides the cut base may help improve rooting because it aids the uptake of hormones and moisture. However, this technique has not been fully exploited on cacao. Little is known about the effect of etiolation on rooting in cacao. However, some tropical and subtropical crops have been reported to root well after etiolation treatment. The exogenous supply of carbohydrates may induce leafless or difficult-to-root clones of cacao cuttings to root.

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