

# Research of the Most Suitable Conditions for the Replication of *Lawsonia inermis* L. in Togo

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**Abstract:** Propagation of *Lawsonia inermis* L. (henna), dye, medicinal and horticultural species was investigated in Togo. The main mode of multiplication of the multi-purposes species is sowing, but this propagation faces problems, particularly low germination rates, which hardly reach 20%. Henna cuttings is possible, but this technique commonly used for low-cost vegetative propagation of plants is not well known. This study proposes a method of propagation by cutting stem segments of henna both in greenhouses and in vitro. In greenhouse, the cuttings concerned segments of identical size, i.e. 20cm, but of different positions on the branch: proximal, median and distal cuttings. A batch of cuttings was pre-treated by soaking their base in water to initiate rhizogenesis before transfer to the substratum. In vitro, single-mode segments were cultured. The results indicate that the best recovery percentages were recorded for cuttings that had been in water, and more specifically for basal cuttings (100% recovery), which were also the most efficient in terms of development: number of knots, leaves and lengthening of shoots formed. In vitro, microblocks showed high take-up and rooting percentages of nearly 70%, as well as homogeneous, although slower, growth.

**Keywords:** *Lawsonia inermis* L., stem cutting, micropropagation, type of cutting, rooting ability

## 1. Introduction

Current global concerns about sustainable development and the growing request for healthy products are generating a renewed interest in natural products in the fields of agriculture, food, health and cosmetics, etc. [1, 2]. Plants have always been widely used by humans, first as food sources, but also as materials, ornamental items and for their harmful or beneficial effects on human well-being. Their use for the extraction of dyes and tinctures is a heritage of knowledge found in all civilizations [3, 4].

Very sober and plastic, *Lawsonia inermis* L. is one of the species tolerant to several types of soil, poor, stony, sandy and with conditions combining low air humidity and drought [5]. This multi-purpose dye plant [6, 7] has a plasticity that allows it to develop in various agro-climatic zones around the world [8, 9].

Cultivated and exported mainly for its dried and powdered leaves, its colouring principle is a pigment called lawsone or naphthoquinone [10] whose maximum content is obtained when the plant is grown in tropical and subtropical regions where temperatures vary between 25-35° [11–13]. This pigment is detected in dry leaves at a percentage of 0.5-1.5% [14, 15]. The worldwide trade of Henna is largely controlled by India, which exported large quantities of about three tons between 2002 and 2003, mainly to countries in the Middle East and the United States. The demand for henna in the domestic markets of many Islamic countries in the Middle East and North Africa is so high that the only alternative is to import it [16]. Due to its very low toxicity and deep-rooted traditions, henna is one of the few natural dyes in such high demand [17, 18].

In Africa, henna is widespread in most Sahelian areas and more rarely in Sudanese areas where its cultivation provides significant income to the population [19, 13]. Henna is used in industry for its dyeing, cosmetic and ornamental properties [6, 7]. The species is used by many ethnic groups

to colour hands, feet, face during various ceremonies and also to dye hair [20, 8]. It is used in horticulture as an ornamental or hedge plant, appreciated for the powerful and pleasant fragrance of its flowers [21, 22], but also in traditional and modern medicine because of the richness of its leaves in natural biochemical components including flavonoids, terpenes and coumarins with antibacterial, antifungal, anti-inflammatory, antioxidant properties [23–25].

The use of this species in Togo is based mainly on its ornamental, cosmetic and dyeing properties [22, 26]. Its culture, not very widespread, is based on seedling propagation. This is the only known and used method of plant production by the population, although regeneration of the plant is also possible by cutting. This situation is justified, *a priori*, by the lack of available information on henna propagation methods. In view of its socio-economic importance and the added value that an expansion of its culture would bring to indigenous populations, this work has therefore been carried out in order to propose a practical, effective and inexpensive method of henna multiplication. The results of this study will not only contribute to the sustainable enhancement and conservation of the species, but also to the enhancement of knowledge on the biology of henna.

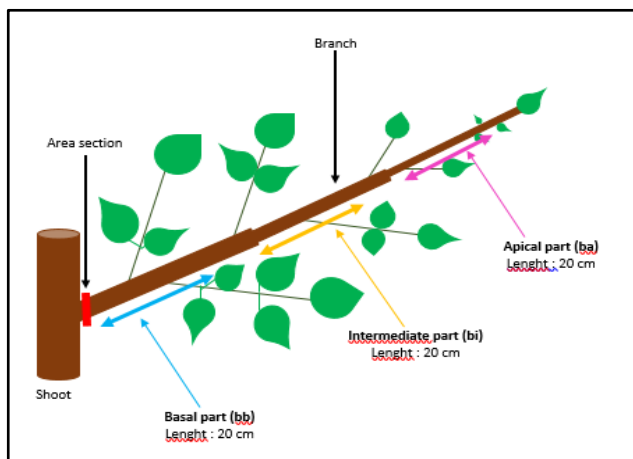
## 2. Materials and Methods

### 2.1. Greenhouse cuttings

Cuttings of the stems were carried out on shoots from wild genotypes located in a suburban district (Agoè-Zongo) of Lomé city.

On a branch of *Lawsonia inermis*, three types of cutting were identified according to their position (Figure 1): a) proximal or basal cuttings taken from the lower part of the branch, b) median or intermediate cuttings taken from between the lower and apical part of the branch, c) distal or

apical cuttings taken from the apical part of the branch. Each cutting length is 20 cm.



**Figure 1:** Position of cuttings types used in the experimentation as a function of their position on the branch. ba = apical part of the branch that after section will give the apical or distal cutting; bi = intermediate part of the branch that after section will give the intermediate or median cutting; bb = basal part of the branch that after section will give the basal or proximal cutting.

Cuttings were then briefly disinfected by soaking them in Domestos solution (4.8 g sodium hypochlorite per 100g; Unilever® France) at 10% for 30 seconds, followed by three successive rinsings with water. Then two sets are formed:

- Reference batch whose cuttings have been planted directly in culture pots, in particular polyethylene plastic bags containing the substratum that is a mixture of clean sea sand\*, garden soil and topsoil in the ratio of 50/25/25 (v/v/v). \* The sand was washed with 1: 8 hydrochloric acid solution and rinsed seven times with water.
- Treated batch: the base of the cuttings was soaked in tap water contained in glass pots before repotting.

For each type of cutting, 10 stems fragments with two repetitions were used, i.e. 20 cuttings per type and 60 cuttings for all three types. No auxinic treatments, fertilizers and pesticides were applied.

For 90 days, the following parameters were assessed: 1. rooting time, i.e. the time elapsing between soaking the base of the cutting in water and the emission of the first root, 2. recovery percentage, i. e. the ratio between the number of cuttings whose buds have broken through and the total number of cuttings planted; 3. cutting or recovery capacity [27] 4. number and length of roots before repotting, 5. number and average size of new shoots, and 6. number of knots and leaves made up.

## 2.2. In vitro cutting

Cuttings were carried out on isolated uninodal segments of 22-week-old plants, derived from *in vitro* germination on Murashige and Skoog medium [28] or MS medium with added sucrose 30g/l and solidified with agar 8g/l (pH =5.7-5.8). These explants were obtained by young stem section. Microcuts were cultured in test tubes (10 mm X 150 mm)

each containing 5 ml of MS. The tubes containing the explants are then placed in the culture room at  $25 \pm 2^\circ\text{C}$ , at a photoperiod of 16 hours of light and a light intensity of  $120\mu\text{E.m}^{-2}.\text{s}^{-1}$ , followed by 8 hours of darkness. A total of 60 microcuts were monitored over a five-week period. Percentage of budburst (regrowth) and rooting, number and average size of new shoots, number of knots and leaves per explant were recorded.

## 2.3. Processing and data analysis

Once a week, analysis of variance tests were performed using the Xlstat software (version 2008.6.03). The Student-Newman and Keuls test for comparing and classifying averages into homogeneous groups is performed when significant effects have been observed on the data obtained, at the 5% threshold [29, 30].

## 3. Results

### 3.1. Greenhouse cuttings

#### 3.1.1. Resumption of cuttings growth

The analysis of the results of the recovery percentages of the three types of cuttings indicates that, in general, all cuttings are reactive with average percentages above 60%. The highest % were recorded with cuttings that had previously been in water: 96.67% recovery of all types compared to 86.67% recovery for control cuttings (Table 1).

Table 1: Recovery percentage of *Lawsonia inermis*' cuttings after 3 months of growth as a function of their position on the branch and the treatment applied.

| Type of cutting              | Apical cuttings | Intermediate cuttings | Basal cuttings |
|------------------------------|-----------------|-----------------------|----------------|
| Reference batch              | 70%             | 90%                   | 100%           |
| Treated batch                | 90%             | 100%                  | 100%           |
| Average recovery percentages | 80%             | 95%                   | 100%           |

Considering the type of cuttings, significant differences were observed between the cuttings in batch 1 and 2. They range from 70 to 90% for apical cuttings, from 90 to 100% for intermediate cuttings and 100% for basal cuttings (Table 1).

#### 3.1.2. Root development of cuttings that have previously been in water

During the study, rooting time for these cuttings was spread over three weeks with a maximum rooting percentage of 100% recorded at the end of this time. This time is shorter in the proximal parts and longer in the distal parts (Table 2).

**Table 2:** Rooting time of *Lawsonia inermis*' cuttings that have previously been in water before repotting.

| Type of cutting | Apical cuttings      | Intermediate cuttings | Basal cuttings       |
|-----------------|----------------------|-----------------------|----------------------|
| First cutting   | 9 <sup>th</sup> day  | 8 <sup>th</sup> day   | 5 <sup>th</sup> day  |
| Last cutting    | 18 <sup>th</sup> day | 13 <sup>th</sup> day  | 11 <sup>th</sup> day |

Basal cuttings were the first and fastest to emit roots. The first roots were issued on day 5 for the first cutting and on day 11 for the last. Basal cuttings were followed by intermediate cuttings for which the time of root development is on days 8 for the first and 13 for the last. Apical cuttings

took longer to take root: day 9 for the first cut and day 18 for the last.

**Table 3:** Average number and size of roots emitted by each type of *Lawsonia inermis* 'cuttings before potting on solid substrates

| Type of cutting         | Apical cuttings | Intermediate cuttings | Basal cuttings |
|-------------------------|-----------------|-----------------------|----------------|
| Average number of roots | 3,8a ± 1,54     | 5,5ab ± 2,12          | 7,5a ± 2,79    |
| Average root size       | 2,15a ± 0,9     | 2,32a ± 0,87          | 3,04a ± 1,18   |

*Student-Newman-Keuls test: the values followed by the same letter are not significantly different at the 5% threshold.*

Comparison of the number of roots emitted by each type of cutting revealed significant differences: basal cuttings have produced the highest number of roots compared to apical and intermediate cuttings for the same length of time. In contrast, root lengths did not vary significantly from one group to the next (Table 3). This is due to the fact that repotting in the plant substrate was carried out as soon as the first of the roots emitted reached 3 cm long, a threshold

length after which repotting becomes extremely tricky because of the fragility of the roots.

### 3.1.3. Influence of cutting type and water soaking on cuttings growth

Three months after planting, analysis of the growth parameters of the cuttings reveals that the quantity of foliage shoots resulting from the development of broken buds depends neither on the type of cutting nor on the treatment applied: the average number of shoots developed at the end of the monitoring period is similar for all cuttings and is  $5.3 \pm 1.16$  shoots.

Basal cuttings were the most productive in terms of: shoot length growth ( $11.655 \pm 6.05$  cm); number of knots ( $24.35 \pm 13.19$ ) and number of leaves ( $48.52 \pm 26.23$ ). They are followed by intermediate cuttings: average shoot length of  $5.54 \pm 2.7$  cm with  $21.93 \pm 12.3$  knots and  $43.88 \pm 24.59$  leaves). Apical cuttings give the shortest shoots ( $3.005 \pm 1.9$  cm) with  $11.87 \pm 8.64$  knots and  $23.74 \pm 17.2$  leaves (Table 4).

**Table 4:** Growth parameters of *Lawsonia inermis* 'cuttings after three months of planting

| Growth parameters | Apical cuttings |                 | Intermediate cuttings |                | Basal cuttings  |                |
|-------------------|-----------------|-----------------|-----------------------|----------------|-----------------|----------------|
|                   | Reference batch | Treated batch   | Reference batch       | Treated batch  | Reference batch | Treated batch  |
| Number of shoots  | 3,78a ± 2,58    | 4,9ab ± 2,22    | 5,58ab ± 2,25         | 7,33b ± 2,03   | 4,94ab ± 2,23   | 5,3ab ± 1,65   |
| Shoot length (cm) | 2,45a ± 2,10    | 3,56a ± 1,79    | 5,5ab ± 2,83          | 5,59ab ± 2,57  | 9,77bc ± 5,77   | 13,54c ± 6,33  |
| Number of knots   | 10,55a ± 8,53   | 13,19ab ± 8,78  | 19,72bc ± 11,77       | 24,15c ± 12,83 | 21,89bc ± 13,61 | 26,82c ± 12,77 |
| Number of leaves  | 21,11a ± 17,02  | 26,38ab ± 17,57 | 39,45bc ± 23,54       | 48,31c ± 25,65 | 43,45cb ± 26,95 | 53,6c ± 25,51  |

*Student-Newman-Keuls test: the values followed by the same letter are not significantly different at the 5% threshold.*

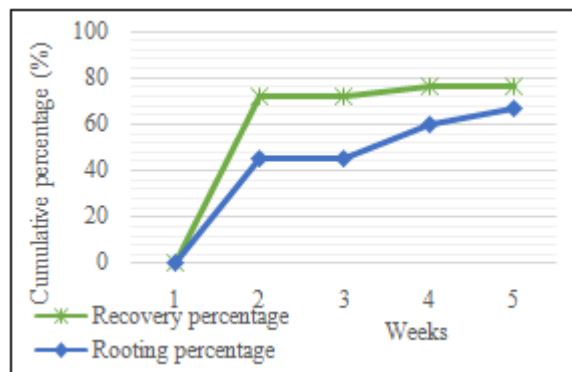
In addition, soaking the cuttings in water had no significant influence on the growth of the treated cuttings. Indeed, the growth values for each type of cutting are equivalent (Table 4).

Statistical analyses revealed that the type of cutting, particularly from the apical and basal parts, had an influence on the parameters measured ( $p < 0.001$ ), except for the average number of shoots. They also demonstrated that intermediate cuttings had growth equivalent to that of apical cuttings ( $p > 0.05$ ).

## 3.2. In vitro microbouturage

### 3.2.1. Resumption of development and rooting of microcuttings

From the first week of cultivation, the single-node segments grown on the MS medium showed positive signs of reactivity: bud break of axillary buds and root initiation. By week 2, bud break exceeded 70% while rooting reached 45%. From the second to the fifth week, these rates increased to a maximum of 76.6% for bud break and 66.7% for rooting (Figure 2).



**Figure 2:** Recovery and rooting percentages of *Lawsonia inermis* 'cuttings grown in vitro for 5 weeks.

However, the shoots emitted are developed very slowly, giving on average at the end of the experiment, 1 shoot per explant with an average length increasing from 0.1 cm (week 2) to 1.6 cm (week 5).

### 3.2.2. Growth of microcuttings

The resumption of bud activity of microcuttings was detectable at the end of the first week of cultivation and growth, measurable from the second week with an average of 1 knot, 2 leaves and 3 roots for the explants. At week 3, the results were: 2 nodes, 4 leaves and 3 roots on average and at week 4, the values recorded are: 4 nodes, 10 leaves and 7 roots (Figure 3).

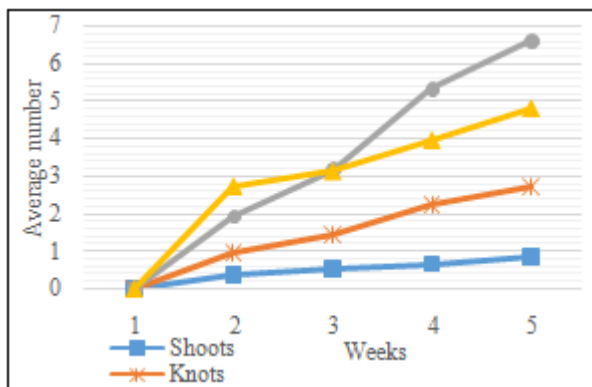


Figure 3: *In vitro*'s growth of *Lawsonia inermis*' microcuttings in culture for 5 weeks

#### 4. Discussion

A cutting is a piece of a plant that is separated, rootless from the plant of which it was part, and stored under appropriate conditions to its nature, first produces roots, then buds, and in turn produces a plant [31]. Adventitious rooting is a complex process that occurs in three successive interdependent physiological phases, and responds to different initiation conditions, namely induction, activation and expression [32, 33].

In this study, tests were carried out on the cutting of stem segments from the apical, intermediate and basal parts of a henna shoot under greenhouse. In this study, tests were carried out on the cutting of stem segments from the apical, intermediate and basal parts of a henna shoot under greenhouse conditions. The effect of soaking in water on segment development was also studied.

*Lawsonia inermis* showed significant reactivity to vegetative propagation by stem segments and the main results of this study indicated a good cutting capacity of the species. The percentages of resumption of cuttings are greater than 60% and reach 100% growth when considering the position of the stem segment on the branch. However, the species' ability to recover decreases as it moves further away from the stem. Indeed, the resumption of cutting is due to a revival of activity at the level of one or more vegetative buds that will later give shoots [27].

This decreasing gradient in the percentage of "recovery from the base of the shoot" to the tip would be explained by the juvenile nature of the axillary buds in dormancy in the proximal parts compared to those in the distal part of the shoot which are older. And as soon as the cuttings bearing these buds are cultured, they develop by passing fairly quickly through the normal stages of development, including bud break, cell multiplication and growth [12, 34]. Also, the apical dominance (induced by endogenous  $\beta$ -indolylacetic acid or AIA) which is exerted by the terminal bud on the lateral buds being uplifted, the hormonal balance (auxins-cytokinins) which mainly conditions the budburst of these buds, would be optimal in the basal cuttings [35–37, 27]. Thus, the fairly high values recorded for the growth parameters of basal-type cuttings (in particular the average shoot length, number of nodes and number of leaves) would be the positive consequence of the action of budburst-

development of vegetative buds combined with that of rapid rooting of these cuttings: having rooted fairly quickly, they could start growing earlier than others and therefore produced more biomass. Basal cuttings are therefore more effective for the recovery of organogenesis in greenhouses than intermediate and apical cuttings. They are therefore an effective vegetative propagation material.

The achievement of vegetative propagation, and more accurately of cuttings, depends on the initiation and development of rooted adventitious plants [38, 39, 34]. The success of this rooting, which is essential for successful cuttings, is influenced by a number of variable parameters: exogenous factors such as temperature, hormone use, light or, endogenous factors such as the plant's physiological age and condition [34].

Chouard's [40] work on the practical use of "Hormones" in cuttings showed that the neof ormation of a root blank is always at the expense of the deeper tissues such as the pericycle, cambium, phellogenetic base and the parenchyma that is in contact with the leaf ribs that most enjoy this capability. According to the same author [41],  $\beta$ -indolylacetic acid (AIA), a natural auxin of plants, is well known to trigger cambium divisions and promote the emergence of adventitious roots. Indeed, due to the polarized transport of auxin, its accumulation at the base of the cut organs (stem segments in this study) right in the deep tissues has triggered the proliferation of tissues with the necessary characteristics to evolve into roots. It therefore appears as a necessary factor for the initiation of rhizogenesis [42–46].

In addition, the analysis of the results obtained after the pre-soaking of the cuttings' base in water, concluded that this method accelerated the onset of henna roots and this, by spontaneous organogenesis. This rooting capability was also reported by Carrière [30] who used the same technique in germination studies of oleanders (*Nerium oleander*), Tamarix and Saule. Indeed, water makes it possible to maintain plant life [47] for a more or less long time and it is sufficient in the process of initiating rhizogenesis in cuttings of some species, which is essential to absorb the necessary principles for plant nutrition and therefore for the resumption of the development process.

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For the micropropagation tests, the MS environment was chosen as the base and propagation environment for the species. Henna is a woody shrub, which grows slowly *in vitro* after germination [48, 49]. Subculture tests established the reactivity of henna microcuts grown *in vitro*. Indeed,

after five weeks of follow-up, interesting values of growth parameters such as average numbers of knots, leaves and roots are obtained. The microcuts therefore break up and root quite easily on MS environment solidified with agar 8g/l and without use of phytohormones. However, the interknot is not sufficiently dislocated, which results in a small average size (nearly 10 mm). This behaviour is explained by the effect of subculture, which favours a better adaptation of seedlings to *in vitro* conditions and MS environment [50–52]. This remains an important determination of the reactivity of henna *in vitro*, which augurs well for a fairly quick adaptation to growing conditions (*in vitro*) and therefore for an improvement in seed production by these technologies. These results of growth and micropropagation, although weak, are better than those obtained by Chaudhury & Kajla [52], on MS environment which have neither obtained budburst nor rooting of the explants being cultured.

## 5. Conclusion and Future Scope

All the outcomes of this study would enable to establish routes for the production of cuttings from explants taken from the lower part of the shoot; the basal cuttings. To do so, it is important to soak in water beforehand in order to initiate fast rhizogenesis and improve the resumption of the development. This recovery capability appears to be higher in basal cuttings that produce the highest number of roots compared to apical and intermediate cuttings. Basal cuttings are also the most effective for regenerating individuals when considering development parameters such as shoot length, number of nodes and number of leaves on the cutting. In addition, micropropagation or *in vitro* culture makes it possible to obtain regularly growing *in vitro* plants, but tests still need to be carried out in order to accelerate and improve the development of these *in vitro* plants by taking into consideration parameters such as growth regulators, in particular phytohormones.

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