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Effects of cutting position and leaf area retention on rooting and growth of vegetatively propagated medicinal *Cannabis sativa*

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Abstract

The absence of standardized propagation procedures remains a significant barrier to the cultivation of high-quality medicinal cannabis, which is extensively cultivated in rural regions of South Africa. Addressing this issue is crucial for the commercialization of cannabis for therapeutic purposes and product development. This study looked into the impact of cutting position and leaf area retention on the adventitious rooting and growth of medicinal cannabis (*Cannabis sativa*) propagules. The trial was carried out at the Dohne Agricultural Development Institute in Eastern Cape, utilizing a Randomized Complete Block Design (RCBD with three replications. The treatments were: i) herbaceous shoot cuttings with 50% leaf area clipped (HS50%LAT), ii) herbaceous shoot cuttings with 100% leaf area (HS100%LA), and iii) basal stem cuttings. Sixty 15 cm cuttings per treatment were excised at a 45° angle below the node from actively growing, vegetatively branched wild plants in Lusikisiki. The cutting bases were treated with Indole-3-butyric acid (IBA), planted into a commercial peat-based rooting medium, and housed in 60-celled propagation incubators. These were maintained in propagation chambers at 15–20°C under 24-hour T8 fluorescent illumination. Moisture levels were adjusted daily using distilled water. Data was collected every two weeks until six weeks after planting (WAP). The parameters measured were rooting percentage, time to 50% rooting (T50), height, number of leaves, fresh and dry weights (shoot, root, and total), length of shoots and roots, and the ratio of roots to shoots. The findings indicated that HS100%LA, succeeded by HS50%LAT, excelled in all rooting and growth metrics. In contrast, the basal stem cutting produced unfavorable results for all measured parameters. This study shows that using herbaceous shoot cuttings with 100% leaf area retention improves vegetative propagation and successful establishment of medicinal *C. sativa*.

Key words: *Cannabis sativa*, herbaceous shoot cutting, basal stem cutting, propagules, rooting success.

Introduction

Cannabis is an herbaceous plant in the Cannabinaceae family with a vast historical use in human tradition as a medicinal herb (Poniatowska *et al*., 2019). It has currently gained global attention due to its multiple uses as a medicinal plant (Mejia-Londono *et al.,* 2023). Cannabis produces bioactive compounds called phytocannabinoids (Busta *et al*., 2022). Cannabinoids are primarily found in the glandular trichomes of unfertilized female flowers (Potter, 2014). Its derivatives have been reported to be used in the treatment of a variety of ailments such as asthma, epilepsy, fatigue, glaucoma, pain and multiple sclerosis (Chandra *et al*., 2020). This plant is predominantly dioecious (bearing male and female inflorescence on separate plants), and its reproductive nature is cross-pollination (Small, 2017). When cultivated from seed, the plant predominantly produces male flowers that have no commercial use except for the seed production (Monthony *et al*., 2021). Therefore, male plants are not desirable within the field as fertilized plants negatively affect the quality and concentration of cannabinoids (Trancoso *et al*., 2022; Chandra *et al*., 2017). Thus, they are removed from the cultivation area as soon as they show up to circumvent cross-pollination (Mendoza *et al.,* 2009). The female inflorescence, on the other hand, is preferred for maximum cannabinoids production and to sustain uniformity in biomass (Chandra *et al*., 2020).

Generally, plant propagation is performed to multiply, reproduce, and preserve species' genetic material (Cabahug *et al.,*2018). Numerous literature sources indicate that industrial cannabis (hemp) can be propagated sexually by seed and asexually by vegetative cuttings or micro-propagation using the in-vitro technique (Caplan, 2018; Potter, 2014; Lata *et al*., 2011). Sexual propagation method involves the production of new plants from seeds (Hartmann and Kester, 2011). Sexually produced offspring show high variation due to genetic recombination, making it difficult to maintain homogeneity in plants (Porras-García *et al*., 2023; Zarei *et al*., 2022; Lata *et al*.,2016). Asexual propagation, also known as vegetative propagation, is the plant reproductive technique that involves the use of vegetative plant parts such as shoot and stem cuttings/cloning (Cabahug *et al*., 2018).

Rooted cuttings are widely used in the reproduction of several

horticultural crops, including industrial cannabis (hemp) (Caplan, 2018). However, the rooting success of the industrial cannabis cuttings may be affected by several factors such as the age of the mother plant, number of the leaves in the cutting, cutting practices (such as retention of expanded leaves in the cutting or reduction of leaf length by 50%) and the cutting position (shoot on stem) (Campbell *et al*., 2021). It is documented that keeping the expanded leaves in the cutting encourages transpiration and increases the area for pathogens, leading to cutting loss due to stress (Mejia-Londono *et al*., 2023). Nevertheless, removing the part of the leaf length reduces the leaf area, transpiration and prevents disease appearance due to controlled density and biomass in the propagation chamber, thus leading to a successful rooting of the propagules (Porras-García *et al*., 2023).

Although numerous online grower guides exist, peer-reviewed information on medicinal cannabis propagation in South Africa remains scarce, despite rising demand for high-quality material to support the country's medicinal cannabis sector. This study therefore aimed to develop an asexual propagation technique that preserves the genetic profile of elite mother plants while reliably meeting the seedling demand of the industry. In Eastern Cape rural communities, producers increasingly use stem cuttings to obtain more female plants and enhance cannabinoid yield, yet scientifically validated protocols for producing propagules through this method are limited. Standardization of such production technology is essential not only for commercial yield but also for the conservation and domestication of specialized plant species (Samad *et al*., 2022). As seen in other underutilized crops, identifying the ideal combination of cutting size and growth regulator concentration is the most effective strategy for large-scale propagation (Hoque *et al*., 2025). Accordingly, the study compared establishment success of herbaceous shoot cuttings with full leaf area (HS100%LA), 50% trimmed leaf area (HS50%LAT), and leafless basal stem cuttings (BS) to identify the most effective protocol for generating high-quality seedlings for high-value cannabis products.

Materials and methods

Location of the experiment: The experiment, to determine the effect of cutting (propagation) technique on the success of cannabis seedling establishment was conducted during the winter season 2024 at Döhne Agricultural Development Institute (DADI), Stutterheim, Eastern Cape, South Africa. The study was conducted in a controlled environment in the propagation chamber at 15-20 °C, with 80 % humidity and low light intensity (100-200 µmol/m²/s *PPFD*).

Plant material: Plant material for cuttings was sourced from developed branches of two-month-old actively growing *Cannabis sativa* landrace plants, locally known as the "Transkei gold". This variety is predominantly found growing in the wild in Lusikisiki, Ingquza Hill Local Municipality, Eastern Cape, South Africa. The 15 cm cuttings were excised from the lateral branches of the vegetatively growing plants to ensure in situ preservation of the species as per Hartmann and Kester (2011).

Selection and screening of the "mother plant" for cuttings:

In the wild in Lusikisiki where cuttings were taken, the tagged plants of *Cannabis sativa* cultivar as identified by Dumani *et al*. (2024) were selected and used as mother plants for the propagation experiment. Vegetatively branched with vigorously representative cannabis plants were selected as "mother plants". Plants were screened for possible pests, diseases damage and those infested were considered unhealthy for propagation. On each selected mother plant, cuttings were excised from lateral developed branches of the cannabis plant above the 10th node, where the meristematic tissues are active (Hartmann and Kester, 2011).

Experimental design: The experiment was laid out in a Randomized Complete Design (RCD) and used three propagation techniques (cutting with different leaf area management) as treatment namely, i) herbaceous shoot cutting with 50% of leaf area trimmed [expanded leaflet cut into half (HS50%LAT)], ii) herbaceous shoot cutting with 100% leaf area [expanded leaflet not trimmed (HS100%LA)] and iii) basal stem cutting [lateral branch stem cutting without leaves (BS)] and were replicated three times. Each treatment (propagation technique) consisted of twenty (20) cuttings per replicate, totaling sixty (60) cuttings per treatment and a total of one hundred and eighty (180) propagules for the whole experiment.

Experimental procedure: In the propagation room, the wooden constructed propagation shelves and the propagation incubators [sixty (60) celled (57 cm x 37 cm x H:20 cm) Root-It-Lid propagation kit (Hydroponic.co.za, Cape Town, South Africa)] were disinfected using a spore-kill (didecyldimethyl ammonium chloride) and allowed to dry prior to the experimental setup. The secateurs used to excise cuttings from mother plants were also cleaned and disinfected with 70% ethanol to prevent disease or pathogen transmission. Vigorously, vegetatively branched plants were used as the mother plants where cuttings were excised. A commercial sphagnum peat-based rooting medium (Jiffy 7C pellets 40 x 45 mm) (Jiffy SA.co.za, Johannesburg, South Africa) was moistened with distilled water and inserted into the 60-celled propagation incubators before the planting of cuttings. The abovementioned different cutting techniques were taken in the lateral branches above the 10th node of the mother plant. The cuttings were excised at a length of 15 cm with a cut angle of 45° below the node, and the wounds on the mother plant and at the top of the stem cuttings were sealed with Tebuconazole 10 g/L with acrylic resin (Gold Reef Specialist Chemicals, Durban, South Africa) as per propagation standards by Hartmann and Kester (2011). On the herbaceous shoot treatments (HS100%LA and HS50%LAT), four (4) existing leaves per cutting were retained following Takoutsing *et al*. (2017). The basal parts of the cuttings

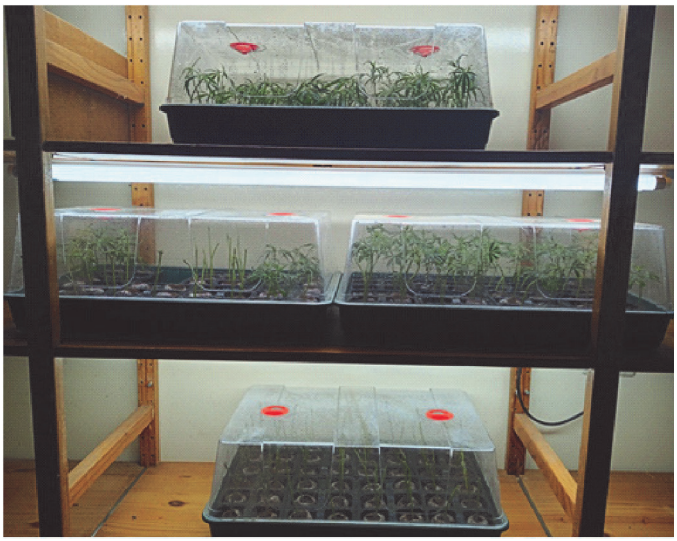

Plate. 1. Experimental set-up in the propagation room at Dohne ADI.

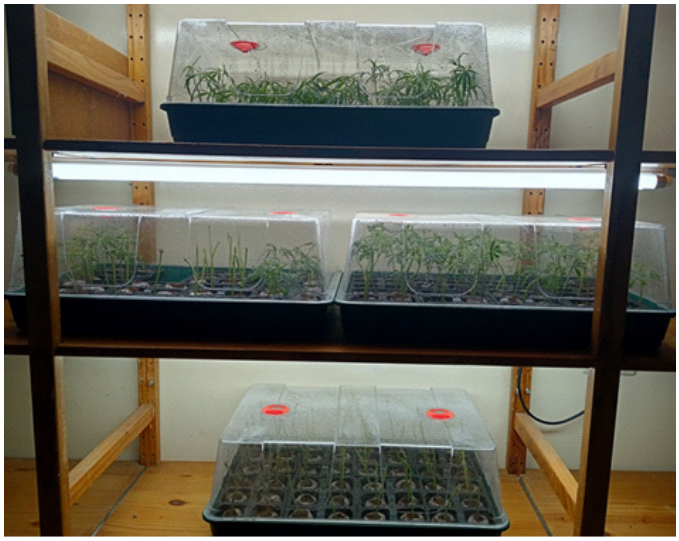

Plate. 1. Experimental set-up in the propagation room at Dohne ADI

were dipped into the rooting hormone (500 mg/l indole-3-butyric acid) [Clean-safe labs, Cape Town, South Africa] before planting into the prepared rooting medium. After planting, the incubators were kept in the propagation chambers at 15 - 20 ℃ under T8 fluorescent tube illumination for 24 hours on wooden shelves. The level of mist in the incubator was checked and adjusted accordingly with distilled water using a 2-litre misting sprayer daily as per (Takoutsing *et al*., 2017). The propagules were monitored for sprouting and rooting for six (6) weeks (Plate 1) following Chandra *et al*. (2020).

Data collection: Data was collected at two (2) week intervals after planting (WAP) until six WAP when the experiment was terminated. Cuttings were assessed following the methodology as described by Takoutsing *et al*. (2017). Thus, each cutting was lifted from the rooting medium, and the rooting status was noted ("1" for rooted or "0" for unrooted). Dead cuttings were recorded ("1" for dead cutting or "0" for alive). Live, but unrooted cuttings were reinstalled into the media for subsequent observations. The following parameters were measured for data collection:

The number of rooted and unrooted propagules was counted. The cutting was classified to be rooted when it sticks into the medium when gently pulled.

Time to 50% (T_{50}) rooting cuttings was determined following Ahmad *et al.* (2020):

$$T50 = \frac{ti\left(\frac{N}{2} - ni\right)(tj - ti)}{(nj - ni)}$$

Where N is the final number of rooted cuttings, n_j *and* n_i are the cumulative number of rooted cuttings by adjacent counts at times t_j and t_i, respectively, when $n_i<N/2<n_j$.

Cuttings rooting success percentage % was calculated following the equation described by Ekamawanti *et al*. (2023)

Cutting rooting success %= [(NRC/TNCP)x 100]

Where NRC = number of rooted cuttings, TNPC = total number of cuttings planted.

At six (6) weeks after planting when the experiment was terminated five (5) propagules per treatment per replicate were randomly sampled destructively and rinsed with clean water to remove rooting medium and the following vegetative data was taken: a) propagule height was measured using a centimeter ruler (cm), b) number of new leaves per propagule were counted, c) fresh propagule, shoot and root weight (g) were measured using *Adam ACBplus-600g scale.* ***d)*** Roots were then removed from each propagule using a razor blade, and the root length was measured using a centimeter ruler (cm). ***e)*** The samples were oven dried at 65°C for 48 hours, and the dry shoot and root weights were determined using a digital scale. ***f)*** The root: shoot ratio (RS) of the propagules was determined using the formula by De Andrade *et al*. (2023):

Root: Shoot ratio = RDW/ SDW RDW = root dry weight, SDW = shoot dry Weight.

Data analysis: Collected propagation data was subjected to R-Studio statistical software version 4.2.2, US, for analysis of variance (ANOVA). The comparison of means was done using Fisher's Least Significant Difference (LSD) (0.05), and the values were calculated at the P= 0.05 confidence level.

Results

Effect of propagation techniques on the number of propagules rooted: The study revealed that the propagation techniques significantly influenced the number of rooted propagules (Fig. 1). The highest number of rooted propagules was recorded on the herbaceous shoot cuttings with 100% not trimmed leaf area (HS100%LA) (16.8) followed by herbaceous shoot cutting with 50% of the leaf area trimmed (HS50%LAT) (13.9) the least was recorded on basal stem cutting (BS) with (0) rooted propagules and were all significantly different to each other (plate 2). Similarly, the propagation techniques significantly influenced the number of unrooted propagules only at BS cuttings, whilst the HS50%LAT (5.7) and HS100%LA (3.2) were the same.

Effect of propagation techniques on the number of weeks to the median (T50) number of rooted propagules: Propagation techniques were significantly different to each other, on the number of weeks to median (50%) number of rooted propagules (Fig. 2). Cannabis cuttings subjected to HS100%LA propagation technique demonstrated a rapid, high rooting potential, with 50% number of rooted propagules recorded on the second WAP and were significantly different to the others. The pick-up trend was observed on the fourth WAP, where HS50%LAT propagation technique recorded a 50% number of rooted propagules for the first time and was statistically at par with HS100%LA, whilst differing significantly with the BS that exhibited no rooting throughout the experiment. A similar trend was observed until

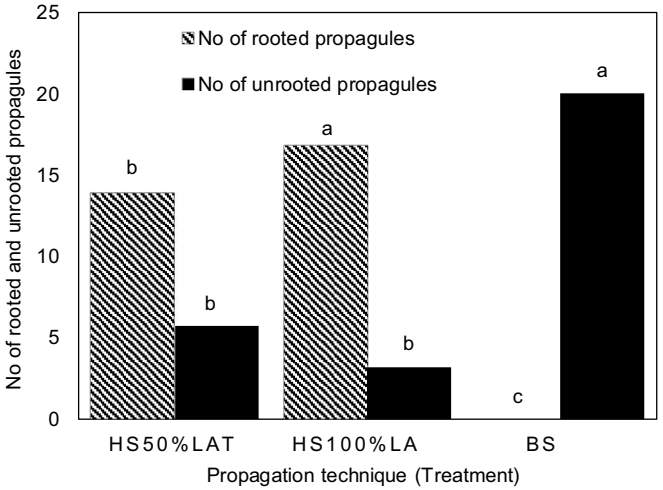

Fig. 1. Effect of propagation technique on the number of rooted and non-rooted cuttings of *Cannabis sativa*. HS50%LAT = herbaceous shoots with 50% leaf area trimmed; HS100%LA = herbaceous shoots with 100% leaf area retained; BS = basal stem cuttings. Bars with the same letter along a line are not significantly different (P= 0.05).

Plate. 2. Illustration of the response of *Cannabis sativa* propagules to different propagation (cutting) techniques. Photo: A. Dumani (2024), Dohne Agricultural Research Institute. HS50%LAT = herbaceous shoots with 50% apical leaf area trimmed; HS100%LA = herbaceous shoots with full apical leaf area retained; BS = basal stem (lateral branch) cuttings.

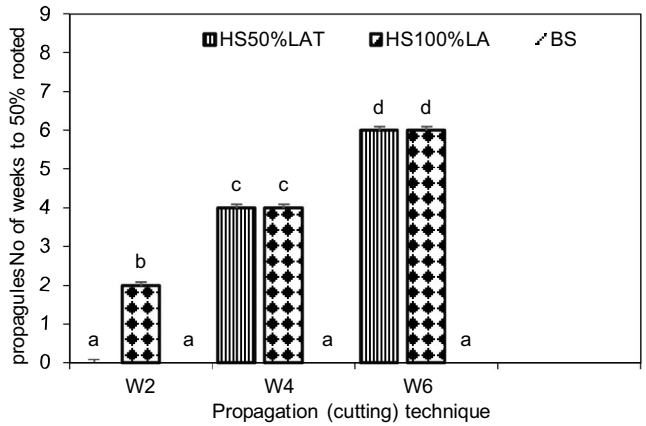

Fig. 2. Effect of propagation techniques on the number of weeks to median (T50) rooting. HS50%LAT = herbaceous shoots with 50% leaf area trimmed; HS100%LA = herbaceous shoots with full leaf area retained; BS = basal stem cuttings. Bars with the same letter are not significantly different (P= 0.05).

the sixth WAP when the experiment was terminated.

Effect of propagation techniques on the rooting success percentage (%): The study revealed that the propagation techniques significantly influenced the rooting success percentage (RS%) of *Cannabis sativa* cuttings (Fig. 3). Treatment HS100%LA (60%) had the highest RS% in week 2, followed by the HS50%LAT (38.3%), and the least was BS (0%), and were all significantly different from each other. At four (4) weeks WAP, the performance of HS100%LA and HS50%LAT was the same and were both significantly different to BS, which has no roots. A similar trend was observed in week six (6). Overall, the propagation techniques suggested that the HS100%LA demonstrated the highest rooting success % potential when propagating the *Cannabis sativa* through herbaceous cuttings, followed closely by HS50%LAT.

Effect of propagation techniques on growth and development of propagules: Table 1 showed that the propagation techniques significantly affected the growth and development of *Cannabis sativa* propagules in all the measured parameters, namely, propagule height, number of new leaves per propagule, shoot, and root length. The longest propagules were obtained in HS100%LA (25.6 cm), followed by HS50%LAT (22.9 cm) propagules and the shortest was observed in the BS (15 cm), which were significantly different to each other. HS100%LA (5.9) recorded the highest and significant number of new leaves, followed by HS50%LAT (3.2) and BS (2.5), which were not different to each other. A significant

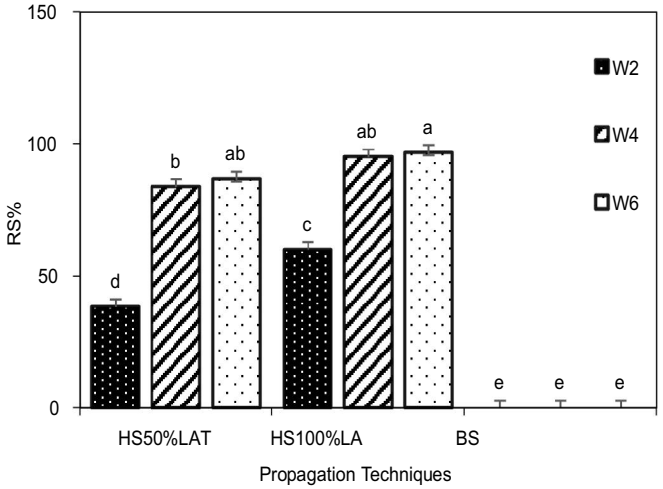

Fig. 3. Effect of Propagation techniques on the rooting success % of *Cannabis sativa* propagules. HS50%LAT = herbaceous shoot with 50% leaf area trimmed, HS100%LA = herbaceous shoot with 100% leaf area retained/ not trimmed, BS = basal stem cuttings. RS% = rooting success percentage. Bars bearing the same letters indicate no significant differences (P= 0.05).

difference was observed in shoot and root length, with the highest attributes attained in HS100%LA, followed by HS50%LAT, while BS has no rooted propagules. Overall, *Cannabis sativa,* when propagated through the HS100%LA technique, exhibited vigorous growth and development ability in all parameters compared to others, followed by HS50LAT and the BS propagation technique demonstrated the worst performance throughout the experiment (Plate 3).

Table. 1. Effect of propagation techniques on growth and development of *Cannabis sativa* propagules

Treatment	Propagule height (cm)	No. of new leaves/ propagule	Shoot length	Root length (cm)
HS50%LAT	22.9^{a}	3.2^{b}	16.4b	6.1b
HS100%LA	25.6^{b}	5.9^{a}	19.6^{a}	12.96^{a}
BS	15.0^{c}	2.5^{b}	15.0^{c}	0.00^{c}
Mean	16.8	3.8	17.2	6.35
Cv%	7.07	2.02	12.8	6.8
P-Value	0.001	0.05	0.001	0.001

HS50%T = herbaceous shoots with 50% leaf area trimmed; HS100%LA = herbaceous shoots with 100% leaf area retained; BS = basal stem cuttings. Values within a column followed by different letters are significantly different at $P \leq 0.05$. P-value: probability value; CV (%): coefficient of variation.

Effect of propagation techniques on the biological yield: Table 2 revealed that the propagation techniques significantly influenced the biological yield parameters measured. The highest and significant fresh propagule weight was found in HS100%LA,

Table. 2. Effect of propagation techniques on the biological yield of *Cannabis sativa* propagules

Treatment	Fresh propagule weight (g)	Fresh shoot weight (g)	Fresh root weight (g)	Dry propagule weight (g)	Dry shoot weight (g)	Dry root weight (g)	Root: Shoot Ratio
HS50%LAT	1.61^{b}	1.01^{ab}	0.31^{b}	0.23^{ab}	0.26^{ab}	0.07^{a}	0.26^{ab}
HS100%LA	2.40^{a}	1.39^{a}	1.09^{a}	0.36^{a}	0.30^{a}	0.11^{a}	0.46^{a}
B S	0.84^{c}	0.83^{b}	0.00^{b}	0.18^{b}	0.19^{b}	0.00^{b}	0.00^{b}
Mean	1.64	1.07	0.47	0.26	0.25	0.06	0.24
Cv%	4.7	5.34	9.5	7	4.6	15.4	15.5
P-Value	0.001	0.05	0.001	0.05	0.05	0.01	0.01

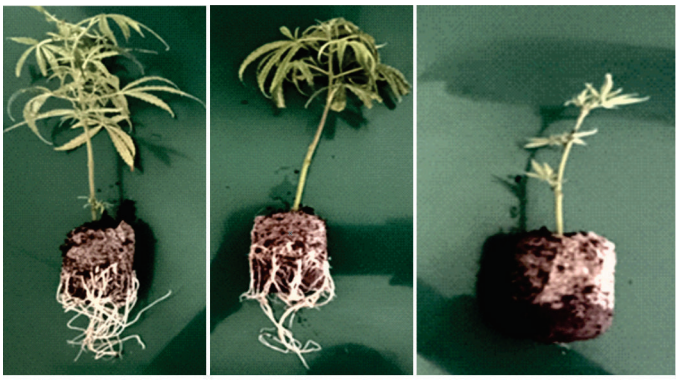

Plate. 3. Illustration of rooted propagules produced from different cutting techniques. Photo: A. Dumani (2024), Dohne ADI. HS50%LAT = herbaceous shoots with 50% apical leaf area trimmed; HS100%LA = herbaceous shoots with full apical leaf area retained; BS = lateral branch

followed by HS50%LAT, and BS recorded the least. Regarding the fresh shoot weight, the highest was obtained in HS100%LA and was significantly different to BS, whilst BS was slightly the same as HS50%LAT. A similar trend was observed in fresh root weight. Regarding dry propagule weight, HS100%LA had the highest and a significantly different weight from BS; however, HS50%LAT was slightly the same as HS100%LA and BS. A similar trend was dry root weight and root: shoot ratio.

Discussion

The results showed that the rooting of rooted cuttings/shoots was significantly influenced by propagation methods. Cuttings from herbaceous shoots with 100% leaf area (HS100%LA) gave the highest number of rooted propagules and was related to the highest percentage of rooting success (RS%) values, followed by HS50%LAT. Shoot cuttings rooted and BS cuttings failed to root. It is most likely due to a high amount of active meristematic tissues in the tops of plants (Wrobel *et al*., 2018). Source-sink relationship is also crucial for rooting success. For this partnership, leaf is the source of carbon hydrates, which the rooting zone depends (Barthwal *et al*., 2025).

Hormonal control also plays a significant role as auxins, which are required for the beginning of root growth, are predominantly produced in apical meristems and young leaves (Liu *et al*., 2025). As a result, tissues at the apex are advisable for elite clonal regeneration to achieve high rooting ability and genetic uniformity (Vassilevska-Ivanova, 2019). These results agree with those reported from Begonia rex (Latifah *et al*., 2024) and disagree with the results obtained by Mejía-Londoño *et al*. (2023), who achieved better success in trimmed cuttings of cannabis. This discrepancy might result from their use of 1-naphthaleneacetic acid and well-controlled environments in which transpiration was minimized (Mejía-Londoño *et al*., 2023).

HS100%LA achieved 50% rooting (T50) by week 2, which is in line with the usual cannabis T50 time of 2–3 weeks (Chandra *et al*., 2020). Theoretically, lack of success of BS cuttings points to metabolic starvation (Morkunas *et al*., 2012). The lack of active photosynthetic capacity led these cuttings to use their available stem reserves (Banjara, 2017), and heavy leaf removal probably caused fatal physiological damage. Rooting is closely associated with leaf processes such as photosynthesis and transpiration (Takoutsing *et al*., 2017; Kouakou *et al*., 2016).

The height, number of leaves and root length were all significantly influenced by the propagation methods, with HS100%LA performing the highest. Although BS cuttings did not produce roots, they developed buds and leaves that were probably induced by some unknown (light, temperature), stimuli and the application of exogenous auxin activities diverting the metabolites toward shoot formation (Singh *et al*., 2025). Nevertheless, intense root development is needed to deliver water and minerals for continuous shoot formation (Ramadani and Setiono, 2021). A specific critical leaf area is needed for the optimum balance of photosynthesis and transpiration to survive (Chandra *et al*., 2020).

Trimming decreases root quality and operations for carbohydrate reserves (Mejía-Londoño *et al*., 2023; Kouakou *et al*., 2016). As a result, HS100%LA cuttings showed higher biological yield (fresh and dry weights) compared to that of BS. This is consistent with previous findings where whole-leaf cuts of hemp exhibited a greater biomass response than did trimmed ones (Mejía-Londoño *et al*., 2023; Chandra *et al*., 2020; Cockson *et al*., 2019; Caplan. 2018) Finally, the higher root/shoot ratio of HS100%LA is a key indicator of seedling quality (Sheridan *et al*., 2021). This morphological feature is essential for water harvesting and endurance when the plant receives at last environmental growth conditions with high control of evapotranspirational demand (Atkinson *et al*., 2019, corresponding to Krak *et al*., 2025).

The results of this study reveal the significance of cutting position and leaf area retention during vegetative propagation of medicinal *C. sativa*. The results demonstrate that the preferable ones for high rooting percentage and vigorous growth are herbaceous shoot cuttings with 100% leaf area (HS100%LA) followed by those without half of the leaf area. In contrast, basal stem cuttings were not a suitable propagation method in such circumstances, due to the lack of success in establishment. These insights contribute to the development of a robust protocol for regional phenotypic propagation of these cultivars, increasing the quality between regional availability and high-quality commercial production. The HS100%LA process may significantly improve the productivity of medicinal cannabis production and ease its adoption in pharmaceutical development.

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