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Seed germination and vegetative propagation of *Helichrysum odoratissimum*

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ABSTRACT

Helichrysum odoratissimum is a popular indigenous herb of South Africa, well known for its aromaticity. It offers potential as a source of extracts for the development of cosmeceutical products as it has been reported to be a natural antibiotic, and has antifungal, antimicrobial, antioxidant and antiviral properties. In order to be commercialized, a sustainable production system is required to ensure a consistent supply of good quality material. Therefore, the production of *H. odoratissimum* by seed and stem cutting was evaluated. The interactive effect of different seed priming methods and temperature levels were investigated on germination indices of *H. odoratissimum* seeds. A randomized complete block design with three replicates was used to evaluate the effect of season, cutting position, rooting hormone and growth media on survival percentage, number of buds, number of leaves, rooting percentage, number of roots per cutting and root length of *H. odoratissimum* stem cuttings. Data for both aspects was analysed using ANOVA and means with significant differences were separated with a *t*-test at 5% level of significance. Smoke treated seeds incubated at 20 °C had a significantly higher germination percentage (91,7%), mean germination rate (0.59) and coefficient velocity of germination (59.5) compared to most other treatments. Almost all factors tested in vegetative propagation significantly affected parameters measured. Apical cuttings taken during autumn resulted in significantly higher survival (78.5) and rooting percentages (71.1%) compared to other seasons. Cuttings planted in a perlite, vermiculite and sand mixture during autumn significantly outperformed all other season/media combinations with regards to rooting percentage (75%). PGR application improved the number of roots obtained significantly in autumn and winter, but not in summer and spring, compared to the control. It was therefore concluded that, for higher seed germination *H. odoratissimum* seeds must be primed with smoke water and incubated at 15–20 °C and cuttings may be taken autumn for higher rooting and survival rates as well as improved root length. A PGR application can be beneficial depending on the type of medium used.

1. Introduction

Helichrysum is a member of the sunflower family (Asteraceae), consisting of about 500–600 species (Van Vuuren et al., 2014; Malolo et al., 2015; Akinyede et al., 2021). The genus is mostly made-up of aromatic perennial herbs, which are distributed throughout the globe in Africa, Southern Europe, South-west Asia, Sri Lanka, Australia and Southern India (Lourens et al., 2008). South Africa harbours approximately 245 species (Lourens et al., 2004). *Helichrysum odoratissimum*, commonly known as imphepho is one of the mostly used and traded indigenous species in South Africa (Williams et al., 2000; Dold and Cocks, 2002; Zantanta et al., 2022). Plant material of *H. odoratissimum* are used traditionally to treat ailments related to cardiac, gastrointestinal, inflammation and psychological conditions (Lourens et al., 2008; Akinyede et al., 2021; Nkemzi et al., 2022). There is a growing interest in *Helichrysum* species, since the species possess phenolic compounds with antioxidant, anti-inflammatory and antimicrobial activity (Nkemzi et al., 2022; Zantanta et al., 2022); and can be used in dermatological and cosmetic products (Ribeiro et al., 2015; Twilley et al., 2021). Harvesting from the wild lack sustainability and may lead to variation, reduction, or loss of the active components in the material due to adulteration (Lubbe and Verpoorte, 2011; Street and Prinsloo, 2013; Van Wyk and Prinsloo, 2018). Therefore, a standardised propagation system is needed to ensure a sustainable supply of good quality plant material with high bioactivity. Cultivation makes it possible to obtain homogeneous and standardized raw materials (Węglarz et al., 2022); which is crucial for successful commercialization of this plant.

The species of the genus *Helichrysum* are naturally propagated by seeds and by resprouting from underground roots (Giovannini et al., 2008). The success of natural propagation depends mainly on the

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response of the propagule towards the interference of various external factors (Krichen et al., 2014). Plant production by seeds is advantageous when genetic variability in propagules is desired, but with many biochemical, physical, or morphological obstacles present (Malele et al., 2021). Efforts to improve seed germination in the genus *Helichrysum* have received attention from several workers (Willis and Groves, 1991; Afolayan et al., 1997; Doussi and Thanos, 1997; Grzesik and Nowak, 1998; Sawilska, 2007; Picciau et al., 2019; Boi et al., 2022). The authors mainly focused on factors affecting seed germination of various *Helichrysum* species. Mott (1972) and Willis and Groves (1991) reported constant temperature of 20 °C and alternating temperature of 20 to 10 °C as optimum temperatures for *H. apiculatum* and *H.cassinianum* seeds. Afolayan et al. (1997) reported a higher seed germination percentage (34%) at 25–30 °C under continuous light in *H. aureonites.* The authors also reported improved seed germination (45.2%) after treatment with gibberellic acid (GA_3), however, germination was still low. Willis and Groves (1991) found that seeds of *H. apiculatum* require a short period of exposure to high temperature to overcome innate dormancy, while Picciau et al. (2019) reported that *H. microphyllum* seeds does not require specific dormancy breaking treatments to achieve higher germination rate.

Even though seed germination studies on the genus have been undertaken, there is still limited scientific data on the germination of *H. odoratissimum*. Furthermore, lower seed viability and poor germination as reported on other species of the genus *Helichrysum* (Afolayan et al., 1997) may hamper propagation and commercial production of *H. odoratissimum*. The aim of the study was to determine the optimal germination conditions of *H. odoratissimum* by assessing the seed germination capacity at different temperature regimes and determine if pre-treatments can improve seeds germination.

Vegetative propagation is often considered or recommended as an alternative to seed propagation where plants that are true to type are needed, especially for commercialization (Hartmann et al., 2002). Among the various vegetative propagation methods, propagation by stem cuttings is one of the most viable techniques to enable the multiplication of genotypes of interest, to obtain crop uniformity, with the added advantage of a relatively low cost when compared to other methods (Gomes and Krinski, 2018). The success of stem cutting propagation is determined by the rooting ability of the clone and can be affected by various factors (Hartmann et al., 2011; Malele et al., 2021). Endogenous hormone concentration, plant regulators, juvenility, seasonality, and environmental conditions during rooting are the most important (Hartmann et al., 2011). In a study by Dragovic (2009), the removal of 1 cm of the epidermal layer from the lower part of the cutting increased the rooting percentage of *H. devium* and *H. obconucum*. According to Ayanoğlu et al. (2002), rooting success of *H. melaleucum* depended on the time of harvest, with semi hardwood cutting made in May (late spring) resulting in 4% rooting, while cuttings made in August (summer) showed 60% rooting success. Bryksa-Godziszand and Pawełczak (2010) achieved a higher rooting percentage (94%) without auxin application on in vitro *H. arenarium* root explants. Clonal propagation of various *Helichrysum* species through stem cuttings is substantially reported and represents a potentially useful approach towards species alternative propagation, conservation and sustainable use but there is a need for standardizing, especially for *H. odoratissimum*. In the current study, the effect of factors such as season, cutting position, growth media and plant growth regulators (PGR) were investigated.

This study was done in collaboration with members of a heritage site called Mothong African Heritage situated at Mamelodi, Gauteng province, which cultivate and protect traditional medicinal plants. Therefore, this study aimed at optimizing production of *H. odoratissimum* using generative and vegetative propagation methods to establish a supply chain for the cosmeceutical products from this plant. The results of this study will help Mothong African Heritage community and other growers.

2. Materials and methods

Two separate experiments were conducted to optimise propagation of *H. odoratissimum* (seed propagation and vegetative propagation). The seed germination experiment was carried out in a laboratory at Tshwane University of Technology, in controlled low-temperature incubators (Labcon™ 220v, 50 Hz), fitted with Lasec® thermometers (GLAA504.11IMYJ, −10/110 °C). *Helichrysum odoratissimum* seeds were purchased from a commercial outlet, Silverhill Seed Nursery, Cape Town, South Africa. The seeds were regarded as fresh seeds, as they were harvested during summer. Results of a preliminary study conducted on the fresh seeds, under normal germination conditions, showed 30% germination which is considered very low but in the range as other species of the same genus. The rest of the seeds were then stored for about three months under normal seed storage conditions (4°Celsius); until they were used in the experiment. Vegetative propagation experiments were carried out at a commercial nursery, Afriflowers, in Cullinan, Pretoria; under 40% shade net. The measured average day/night maximum and minimum temperatures during the experimental period were 27 °C and 16 °C (spring), 29 °C and 18 °C (summer), 25 °C and 16 °C (autumn) and 20 °C and 11 °C (winter). *Helichrysum odoratissimum* plants (rooted cuttings) were purchased in March 2019 from Nkosi Indigenous Nursery, KwaZulu-Natal and were planted as donor plants.

2.1. Preparation and implementation of the experiment

2.1.1. Propagation by seeds

The experiment was conducted during March–April 2020 and 2021 for 46 days at five controlled and constant temperature regimes (10 °C to 30 °C with 5 °C increments). A standard seed viability test was conducted by soaking seeds in tap water for 10 min and floating seeds were regarded as not viable. Seeds were cleaned by immersing them in 1% sodium hypochlorite for fifteen minutes and thereafter rinsing five times with autoclaved distilled water as described by Makena et al. (2018). A Youden square design was used to test the effect of temperature, twenty seeds were used as an experimental unit, replicated three times. Germination tests were performed in 6 cm diameter Petri dishes on top of two layers of filter paper, saturated with 5 ml of autoclaved distilled water as described by Kumar et al. (2011). Seeds were incubated under continuous darkness as to follow regenerative method used by the Mothong community since the community cover seeds with growth medium during seed sowing but exposed to normal light during observations. Germination was monitored (under the microscope) daily during the first seven days and then at three-day intervals until the last day of observation. Seeds were considered germinated once the radicle had protruded at least 2 mm from the testa. The germinated seeds were counted and removed to avoid a recount. Nine germination indices were used to compare the germination data for representation and accuracy (Table 1).

After subjecting *H. odoratissimum* seeds to cold stratification period, the germination percentage was lower than 60%, therefore three seed priming methods namely smoke water, cold water, hot water and control were investigated. A Youden square design (5 temperature treatments replicated three times) was used with temperature as a main factor and priming treatments were randomised within each temperature. The smoke primer disks containing a combination of natural substances (not mentioned by the supplier) were purchased from SANBI, Kirstenbosch. A disk was soaked in 50 ml of distilled water until the disk changed colour from green to white to get 100% solution of smoke water (Brown et al., 2004) in which the seeds were soaked overnight before sowing. Other priming methods

Table 1
Germination indices measured.

Germination indices	Formula	Formula description	Reference
Time for the first germination (T_0)	Which expresses the time of germination of faster seeds		
Time for the last germination (T_g)	Which expresses the time of germination of slower seeds.		
G% at T_0	Total germination percentage at the first day of germination observation		
Time to 50% germination ($T_{50\%}$)	$Y = a/1 + e(-x-b/c)$	a = maximum germination percentage; b = turning point; c = slope of the line; x = time (days); y = germination%.	Al-Ahmadi and Kafi, 2007
Germination percentage (G%)	GP=NT*100/N	NT is the total number of germinated seeds in each treatment and N is the total number of seeds used in the treatment	Mavi et al., 2010
Mean germination duration (MGD)	MGD=Σ(t*n)/Σn	t is the day on which germination was observed and n is the number of seeds germinated on day t.	Ranal and Santana, 2006; Mavi et al., 2010.
Mean germination rate (MGR)	MGR=CV/100=1/T	T is the mean germination time and CV is the coefficient of velocity.	F. Al-Ansari and Ksiksi, 2016
Germination rate index (GRI)	GRI=G1/1 + G2/2+..+Gi/i	G1 is the germination percentage on day 1, G2 is the germination percentage on day 2; and so on.	Shmueli and Goldberg, 1971
Germination index (GI)	GI=(20*N1)+(19*N2)+. . .+(1*N20)	N1, N2. . .N20 is the number of seeds germinated on the first, second and subsequent days until the 20th day and the multipliers are weights given to the days of the germination.	F. Al-Ansari and Ksiksi, 2016

were cold water; where seeds were soaked in cold water containing ice cubes until ice cubes melted, hot water; where seeds were soaked in hot water (50 °C) until the water cooled down as described by Yadav et al. (2011) with slight adjustments and control where seeds were not subjected to any priming method. The same germination procedure used in the temperature experiment was followed.

2.1.2. Propagation by stem cuttings

A randomized complete block design, with three replicates, was used to investigate the effect of different seasons, cutting positions, growth media and plant growth regulators (PGR) on survival percentage, number of green leaves, number of buds, rooting percentage, number of roots and root length per cutting. Cuttings were severed and planted in four different seasons: winter (June), spring (September), summer (December) and autumn (March). The seasonal experiments took 3 months under the shade nets and cuttings were hardened off outside for a week before terminating the experiment.

Two cutting positions (apical and median cuttings) were assessed. To assess the effect of plant growth regulators; Dynaroot™ No.1 (active ingredient: 4-Indole-3-butyric acid (8 g/kg) and a concentration of 300 mg/L of Salicylic Acid), Dip & Root™ B2 (active ingredient: 4-Indole-3-Butyric Acid (IBA) 10 g/Lt and 1-NaphtaleneAcetic Acid (NAA) 5 g/Lt) and control were tested. About 0.5 – 1 cm of the base of cuttings was dipped in Dynaroot™ No.1 or soaked for approximately 5 s in the diluted Dip & Root™ B2 concentration (4 drops in 10 ml of tap water) before cuttings were planted while the control, were untreated cuttings. Both treated and untreated cuttings were then planted in 128-hole seedling trays. Before use, all growth media mixtures were tested for water holding capacity using the method described by Naeth et al. (1991). Three different types of growth media with different water holding capacity were tested, namely: M1 - equal parts of perlite, vermiculite and sand (1:1:1) with 25% water holding capacity, M2 - bark compost and coco peat (2:1) with 75% water holding capacity and high nutrients (Aswath and Pillai, 2004) and (M3) - pine bark, vermiculite and sand (2:1:1) with 49% water holding capacity). Swelankomo (2004) recommended the use of growth media which is light, has good drainage and is well composted for the propagation of *H. odoratissimum,* therefore growth media 3 was tested. Five cuttings were used as an experimental unit. To complete all 4 trials, a total of 2160 cuttings were propagated. The experiment was watered on two days interval for 20 min using overhead sprinklers and monitored weekly. Data was collected on the last day of the experiment on number of buds, number of green leaves, number of roots per cutting, length of the longest root (cm), rooting percentage and survival percentage.

2.1.3. Statistical analysis

All variables measured were subjected to factorial analysis of variance (ANOVA) to test for the significant main effects and to examine the interaction effect between the factors using SAS version 9.3 statistical software. Data were analysed within the framework of the General Linear Model (SAS, 2011). The Shapiro-Wilk test was performed to test for normality (Shapiro and Wilk, 1965). The pattern of difference between means was analysed using Fishers' protected least significant difference test (LSD) at a 5% level of significance (Snedecor and Cochran, 1967). In vegetative propagation trial, data was analysed seasonally to test for heterogeneity and then analysed over seasons with weighted data but since clear seasonal differences were observed, only the combined data is reported and discussed. Data was expressed as mean ± standard deviation of triplicate measurements.

3. Results

3.1. Seed propagation

3.1.1. Effect of temperature on seed germination

Temperature at which seeds were incubated had a significant effect on all the germination indices measured (Appendix A). *Helichrysum odoratissimum* seeds were able to germinate at all temperature regimes tested, with significantly higher germination percentage at 15 °C which was not significantly different to the germination percentage obtained at 20 °C (Table 2). Although seeds incubated at 15 °C started germinating (T_o) later than seeds incubated at 20 °C and 25 °C, the seeds obtained the highest germination percentage (57,78%), had lower mean germination duration (8 days) (MGD) with the mean germination rate (MGR) of 0.2031 and about 33,82 germination rate index (GRI) and were able to reach 50 percent of the germinated percentage ($T_{50\%}$) earlier than seeds incubated at other temperature regimes. Seeds incubated at 10 °C took significantly longer to start germinating (7,75 days) and were the last to stop germination (T_g) (26,58 days), however, the seeds obtained a significantly higher germination percentage compared to seeds germinated at 30 °C, which was not significantly different from that of seeds germinated at 20 °C and 25 °C (Table 2). The germination index mean (GI) was high at 15 °C and 20 °C, while 30 °C had the lowest.

3.1.2. The interactive effect of temperature and priming methods on seed germination

There was a significant interaction between temperature and presowing treatments on all germination indices measured except on last day of germination (T_g), time to 50 percent germination ($T_{50\%}$) and mean germination duration (MGD) (Appendix A). Seeds primed

Table 2
Germination indices of *Helichrysum odoratissimum* at different incubation temperatures.

	10 °C	15 °C	20 °C	25 °C	30 °C	$LSD_{(Pr=0.05)}$
T_0 (days)	7,75 ± 4,6 a	5 ± 3,0 c	3,22 ± 2,3 d	3,67 ± 2,9 d	6,28 ± 4,6 b	**0,889**
T_g (days)	26,58 ± 15,7 a	20,92 ± 13,6 bc	23,67 ± 15,0 ab	20,17 ± 13,6 bc	18,33 ± 13,4 c	**3551**
$T_{50\%}$ (days)	22,999 ± 4,3 a	8606 ± 1,9 c	18,389 ± 8,3 ab	14,746 ± 3,5 bc	12,524 ± 3,2 bc	**8,0903**
T_0G%	15,14 ± 12,6 b	23,89 ± 19,0 a	7,78 ± 7,8 c	5,69 ± 4,4 c	6,67 ± 6,6 c	**3999**
G%	48,61 ± 30,6 bc	57,78 ± 34,9 a	53,47 ± 34,5 ab	45 ± 29,5 c	22,08 ± 21,6 d	**6164**
MGD (days)	14,24 ± 8,6 a	8,39 ± 5,2 c	11,6 ± 7,9 b	10,61 ± 6,8 b	11,11 ± 7,3 b	**1906**
MGR	0,2514 ± 0,19 ab	0,2031 ± 0,14 bc	0,2544 ± 0,23 a	0,1656 ± 0,14 c	0,055 ± 0,08 d	**0,04,944**
GI	162,1 ± 103,9 c	276,6 ± 170,3 a	225,5 ± 155,6 b	195,4 ± 133,9 bc	93,1 ± 95,6 d	**40,1**
GRI	18,3 ± 11,8 c	33,82 ± 21,2 a	30,42 ± 22,2 ab	25,42 ± 18,4 b	*10,96* ± d	**5935**

T_o: Time to the first germination, **T_g**: Time to the last germination, **$T_{50\%}$**: Time to 50% germination, **T_oG%**: Total germination percentage at the first day of germination, **G%**: Final germination percentage, **MGD**: Mean germination duration, **MDR**: Mean germination rate, **GI**: Germination index and **GRI**: Germination rate index. [Mean value (± standard deviation) with the same letter in a row are not significantly different from each other].

with smoke water had significantly higher germination percentage (G%) and mean germination rate (MGR) compared to all other pretreatments, regardless of the temperature used (Table 3).

Seeds incubated at 15 °C obtained similar good germination index (GI) and germination rate index (GRI) values irrespective of the pretreatment used (excluding hot water). However, at 20 °C, smoke water treatment significantly improved germination percentage (92%), mean germination rate (MGR) (0.594), germination index (GI) (362) and germination rate index (GRI) (48.33) (Table 3). Ten and 30 °C are not suitable temperatures for the germination of *H. odoratissimum* seed as none of the germination values irrespective of pretreatment could compare to the best values obtained.

3.2. Vegetative propagation

3.2.1. Effect of main factors on growth parameters of Helichrysum odoratissimum cuttings

All parameters measured were significantly affected by the season in which cuttings were taken. Cuttings planted in autumn performed significantly better in all parameters measured, while cuttings planted in summer resulted in the lowest values for all parameters measured (Table 4). The cutting position significantly affected the number of buds, number of roots and length of the roots produced by the cuttings. Apical cuttings had significantly more buds (2,7) and roots (21,9) and significantly longer roots (7 cm), compared to median cuttings (Table 4). The application of Dynaroot™ on cuttings significantly improved all root growth parameters as well as the survival of cuttings compared to the control, however, there was no significant difference on the number of buds and root length of the two plant growth regulator (PGR) treatments (Table 4). Cuttings planted in growth media 2 had a significantly higher survival percentage (57,2%) and rooting percentage (50,7%) compared to other media. The number of roots per cutting were similar in growth media 1 and growth media 2 and the root length of cuttings planted in growth media 2 and growth media 3 did not differ significantly. On the other hand, cuttings in growth media 1 scored the lowest in most parameters except on the number of roots as compared to cuttings planted in growth media 3 (Table 4).

3.2.2. Interaction between season and cutting position

The interaction between season and cutting position significantly affected all parameters measured except the number of roots produced (Appendix B). For most seasons, apical cuttings obtained higher values (although not always significant) than median cutting

Table 3
Interaction between pre-sowing treatment and temperature on germination indices of *Helichrysum odoratissimum*.

Temperature	Priming	T_o (Days)	G% at T_o	T_g (Days)	$T_{50\%}$ (Days)	G%	MGD (Days)	MGR	GI	GRI
10 °C	**Control**	10,33 ± 1,0 a	21,67 ± 7,5 b	33 ± 2,5	27,61 ± 2,2	58 ± 8,7 efg	19,21 ± 3,3	0,26 ± 0,07 cde	188,3 ± 41,1 e	21,32 ± 4,4 efg
	Cold water	10 ± 0 a	25,56 ± 6,8 b	37 ± 0	21,62 ± 3,4	64 ± 11,9 def	18,44 ± 3,0	0,31 ± 0,12 cd	223,3 ± 53,7 de	26 ± 7,2 de
	Smoke water	10,67 ± 1,3 a	13,33 ± 12,7 c	36,3 ± 1,3	19,76 ± 2,9	73 ± 14,3 bcd	19,30 ± 1,7	0,43 ± 0,16 b	236,9 ± 42,2 de	25,86 ± 4,5 de
	Hot water	0 g	0 e	0	0	0 j	0	0 h	0 g	0 h
15 °C	**Control**	6,67 ± 1,0 bc	42,22 ± 17,1 a	23 ± 7,5	7 ± 0,01	78 ± 9,3 bc	9,52 ± 1,1	0,23 ± 0,05 def	401,3 ± 57,7 a	49,58 ± 8,4 a
	Cold water	7 ± 0,1 b	27,78 ± 7,1 b	30,3 ± 7,3	7,91 ± 1,34	70 ± 10,0 cde	11,66 ± 2,0	0,23 ± 0,07 def	325,2 ± 55,3 abc	39,13 ± 6,8 abc
	Smoke water	6,33 ± 1,3 bc	25,56 ± 14,0 b	30,3 ± 3,2	10,91 ± 1,1	83 ± 7,5 ab	12,37 ± 1,7	0,35 ± 0,08 bc	380,0 ± 48,4 a	46,57 ± 8,4 ab
	Hot water	0 g	0 e	0	0	0 j	0	0 h	0 g	0 h
20 °C	**Control**	5 ± 1,5 cde	11,11 ± 11,1 cd	27,3 ± 6,8	15,97 ± 7,5	66 ± 9,5 def	13,71 ± 4,5	0,23 ± 0,07 def	288,1 ± 100,2 bcd	38,05 ± 17,8 abc
	Cold water	3,7 ± 1,3 ef	7,78 ± 3,6 cde	32 ± 6,8	23,35 ± 11,9	57 ± 7,1 fg	14,94 ± 5,6	0,19 ± 0,07 ef	251,8 ± 72,2 cde	35,30 ± 11,0 bcd
	Smoke water	4,22 ± 1,6 def	12,22 ± 5,6 cd	35,3 ± 4,5	15,85 ± 5,05	92 ± 6,1 a	17,76 ± 3,6	0,59 ± 0,12 a	362,0 ± 86,1 ab	48,33 ± 14,9 a
	Hot water	0 g	0 e	0	0	0 j	0	0 h	0 g	0 h
25 °C	**Control**	6 ± 1,5 bcd	8,33 ± 5,0 cd	23,3 ± 3,3	16,43 ± 0	51 ± 12,1 gh	14,47 ± 1,5	0,16 ± 0,06 f	215,1 ± 64,5 de	25,75 ± 9,5 de
	Cold water	5,67 ± 2,9 bcd	8,89 ± 2,2 cd	31,3 ± 10,4	12,45 ± 0	56 ± 15,3 fgh	14,46 ± 5,1	0,20 ± 0,10 ef	240,4 ± 91,1 de	32,69 ± 15,8 cde
	Smoke water	3 ± 0,7 f	5,56 ± 1,6 cde	26 ± 7,0	14,96 ± 0	73 ± 10,9 bcd	13,52 ± 3,0	0,30 ± 0,13 cd	326,0 ± 33,5 abc	43,25 ± 2,1 abc
	Hot water	0 g	0 e	0	0	0 j	0	0 h	0 g	0 h
30 °C	**Control**	9,33 ± 2,0 a	10,56 ± 5,8 cd	27 ± 9,1	0	24 ± 7,8 i	16,63 ± 3,2	0,05 ± 0,03 gh	89,0 ± 25,5 f	9,72 ± 2,8 gh
	Cold water	6,78 ± 4,1 bc	5 ± 0 de	22,3 ± 9,0	0	21 ± 13,7 i	14,12 ± 5,4	0,03 ± 0,04 h	90,0 ± 63,6 f	11,62 ± 7,6 fgh
	Smoke water	9 ± 3,3 a	11,11 ± 8,2 cd	24 ± 10,6	12,52 ± 0	44 ± 12,5 h	13,68 ± 2,5	0,14 ± 0,13 fg	193,1 ± 77,5 e	22,51 ± 14,8 ef
	Hot water	0 g	0 e	0	0	0 j	0	0 h	0 g	0 h
$LSD_{(Pr=0.05)}$		**1778**	**7997**	**NS**	**NS**	**12,327**	**NS**	**0,09,889**	**80,2**	**11,87**

T_o: Time to the first germination, **G% at T_o**: Total germination percentage at the first day of germination, **T_g**: Time to the last germination, **$T_{50\%}$**: Time to 50% germination, **G%**: Final germination percentage, **MGD**: Mean germination duration, **MDR**: Mean germination rate, **GI**: Germination index and **GRI**: Germination rate index. Means (± standard deviation) with the same letter in a column show no significant differences.

Table 4
The effect of main factors on growth parameters of *Helichrysum odoratissimum*.

	Treatments	Survival%	No: Green leaves	No: Buds	Rooting%	No: Roots	Root length (cm)
Season	**Autumn**	67,1 ± 1,19 a	12,8 ± 4,38 a	3,5 ± 1,58 a	63,1 ± 1,14 a	46,4 ± 1,20 a	8,2 ± 2,25 a
	Spring	38,7 ± 1,02 b	11 ± 4,74 b	1,9 ± 1,05 b	37,41,03 b	22,3 ± 1,01 b	6,5 ± 2,95 b
	Winter	55,9 ± 1,10 b	7,5 ± 3,13 c	1,9 ± 0,95 b	40,2 ± 1,48 b	24,7 ± 1,31 b	5,6 ± 2,60 c
	Summer	33,9 ± 1,50 c	7,1 ± 3,46 c	1,8 ± 1,16 b	33,3 ± 1,14 b	12,8 ± 1,06 c	5,2 ± 2,54 c
	$LSD_{(Pr=0.05)}$	**7,8388**	**1,1118**	**0,3398**	**7,4897**	**3,6921**	**0,6862**
Cutting position	**Apical**	48 ± 1,44	10 ± 4,71	2,7 ± 1,59 a	43 ± 1,37	21,9 ± 1,69 a	7,0 ± 2,78 a
	Median	46 ± 1,22	09 ± 4,48	2,1 ± 1,19 b	42 ± 1,22	15,3 ± 1,49 b	5,9 ± 2,80 b
	$LSD_{(Pr=0.05)}$	**NS**	**NS**	**0,2378**	**NS**	**1,8723**	**0,4793**
Growth regulator	**Dynaroot™**	54 ± 1,26 a	9 ± 4,45	2,2 ± 1,35 ab	50,3 ± 1,22 a	22,1 ± 1,73 a	6,8 ± 2,89 a
	Dip & Root™	48 ± 1,38 b	9,8 ± 4,87	2,4 ± 1,38 a	44,4 ± 1,32 b	18,1 ± 1,61 b	6.7 ± 2,71 a
	Control	39 ± 1,31 c	10 ± 4,45	1,6 ± 1,55 b	32,9 ± 1,26 c	13,6 ± 1,36 c	5,9 ± 2,85 b
	$LSD_{(Pr=0.05)}$	**5,9167**	**NS**	**0,2921**	**5,8448**	**2,2987**	**0,5908**
Growth media	**M1**	40,9 ± 1,44 b	10 ± 4,60	2,3 ± 1,40	37,3 ± 1,37 b	19,7 ± 1,70 a	6,1 ± 2,64 b
	M2	57,2 ± 1,25 a	9,5 ± 4,17	2,4 ± 1,43	50,7 ± 1,26 a	19,2 ± 1,73 a	6,9 ± 2,94 a
	M3	42,8 ± 1,23 b	9,5 ± 5,04	2,4 ± 1,46	39,9 ± 1,21 b	16,1 ± 1,45 b	6,4 ± 2,86 ab
	$LSD_{(Pr=0.05)}$	**5,9161**	**NS**	**NS**	**5,8445**	**2,2965**	**0,5882**

Mean value (± standard deviation) with the same letters in a column for each main factor is not significantly different from each other. **M1** - perlite, vermiculite and sand (1:1:1), **M2** - bark compost and coco peat (2:1) and **M3** - pine bark, vermiculite and sand (2:1:1).

in all parameters measured except in winter where median cuttings had more green leaves and buds, and in summer where median cuttings had a significantly higher survival and rooting percentage as compared to apical cuttings (Table 5). Apical cuttings taken in autumn significantly outperformed cuttings taken in all other seasons irrespective of cutting position.

3.2.3. Interaction between season and growth media

Similarly, the interaction between season and growth media significantly affected all parameters except root length (Appendix B). Cuttings planted in growth media 1 in autumn performed better in all parameters measured except on number of roots which were significantly higher on cuttings planted in M2 (55,4) (Table 6). No significant differences were observed on number of green leaves and buds produced by cuttings planted in all growth medium and rooting percentage of cuttings planted in M2 and M3 during autumn (Table 6). A significantly higher survival percentage was observed in cuttings planted in growth media 1 during autumn (78%), if compared to M3 (63%) and M2 (59%) (Table 6). However, cuttings planted in M2 during winter and summer had significantly higher survival percentage than those planted in other media in the same season. Growth media 3 resulted in higher values for almost all parameters in spring. During winter and summer, M2 gave the highest results for all parameters although not always significantly higher (Table 6). While cuttings planted during summer had the lowest in almost all the growth parameters measured (Table 6).

3.2.4. Interaction between season and plant growth regulator

There was a significant interaction between season and PGR on the number of green leaves and roots produced by *H. odoratissimum* cuttings (Table 7). Cuttings planted in autumn generally had a higher number of leaves and roots whether PGRs were used or not. In general, all PGR treatments including the control significantly improved the number of roots produced in autumn compared to most other treatment combinations (Table 7). Plant growth regulator application had no significant effect on number of roots during spring, but Dynaroot™ and Dip & Root™ significantly improved number of roots in winter compared to the control. Dynaroot™ also significantly improved the number of roots in summer compared to the control. Cuttings planted in winter and summer produced a significantly lower number of green leaves compared to cuttings planted in autumn and spring, for both treated and untreated cuttings (Table 7). During autumn, cuttings treated with Dynaroot™ produced a significantly lower number of green leaves compared to cuttings treated with other PGR's.

3.2.5. Interaction between growth media and plant growth regulator

The interaction between growth media and PGR significantly affected the survival and rooting percentage of *H. odoratissimum* cuttings. Cuttings planted in M2 and treated with PGR's generally performed better, with significantly higher survival and rooting percentages compared to the control and all other combinations, except for the M3 with Dynaroot™ combination (Fig. 1a-b). The use of growth regulators significantly improved survival and rooting percentage in cuttings planted in M2 and M3 compared to the control, although the effect of Dip & Root™ was significantly lower than that of Dynaroot™ in M3 (Fig. 1a,b). The application of PGR's had no significant effect on the survival percentage of cutting in M1 although the application of Dynaroot™ significantly improved the rooting

Table 5
Interactive effect of season and cutting position on growth parameters of *Helichrysum odoratissimum* cuttings.

Season	Cutting position	Survival (%)	No of green leaves	No of buds	Rooting (%)	Root length (cm)
Autumn	Apical	78,5 ± 0.9 a	13,8 ± 3,6 a	4,3 ± 1,1 a	71,1 ± 1,0 a	8,5 ± 2,5 a
	Median	55,93 ± 1,2 bc	11,7 ± 4,8 b	2,6 ± 1,5 b	55,2 ± 1,1 b	7,9 ± 1,9 ab
Spring	Apical	49,6 ± 1,1 cd	11,8 ± 4,2 b	2 ± 0,9 cd	48,5 ± 1,1 bc	7,4 ± 2,8 b
	Median	27,8 ± 0,7 e	10,2 ± 5,1 b	1,8 ± 1,1 cd	26,3 ± 1,7 e	5,6 ± 2,9 c
Winter	Apical	60,7 ± 1,2 b	7,2 ± 2,3 c	1,8 ± 0,9 cd	46,7 ± 1,1 bc	6,1 ± 2,1 c
	Median	51,1 ± 1,0 bcd	7,7 ± 3,2 c	2,1 ± 0,9 bc	33,7 ± 1,0 de	4,1 ± 2,7 d
Summer	Apical	24,6 ± 1,2 e	6,4 ± 3,7 c	1,9 ± 1,5 cd	23,5 ± 1,2 e	5,7 ± 2,8 c
	Median	43 ± 1,5 d	7,7 ± 3,2 c	1 ± 0,8 d	43 ± 1,5 cd	5,5 ± 2,3 c
$LSD_{(Pr=0.05)}$		**11,086**	**1,5777**	**0,4818**	**10,592**	**0,5877**

Mean value (± standard deviation) with the same letters in a column were not significantly different.

Table 6
Interactive effect of season and growth media on growth parameters of *Helichrysum odoratissimum* cuttings.

Season	Growth media	Survival (%)	No of green leaves	No of buds	Rooting (%)	No of roots
Autumn	M1	78 ± 1,1 a	14 ± 4,3 a	3,6 ± 1,3 a	75 ± 1,0 a	48,6 ± 1,0 b
	M2	59 ± 1,2 cd	12,7 ± 4,1 ab	3,5 ± 1,6 a	55 ± 1,2 b	55,4 ± 1,3 a
	M3	63 ± 1,1 bc	11,9 ± 4,5 ab	3,2 ± 1,7 a	59,4 ± 1,1 b	35,7 ± 0,8 c
Spring	M1	28 ± 0,9 gh	11 ± 3,3 b	1,7 ± 0,5 cd	28,3 ± 0,9 de	25,1 ± 1,2 d
	M2	39 ± 1,1 fg	9,0 ± 4,4 c	1,5 ± 0,8 d	35 ± 1,1 d	21,4 ± 0,8 d
	M3	49 ± 0,9 def	12,4 ± 5,1 ab	2,4 ± 1,2 b	48,9 ± 0,9 bc	21,2 ± 0,9 d
Winter	M1	51 ± 1,2 cdef	7 ± 2,4 cde	1,8 ± 1,0 cd	39,3 ± 1,2 cd	24,8 ± 1,1 d
	M2	75 ± 0,9 ab	8,1 ± 2,6 cd	2,1 ± 0,9 b	49,4 ± 1,2 bc	25,7 ± 0,9 d
	M3	42 ± 0,9 efg	6,8 ± 3,3 de	1,8 ± 0,8 cd	32,8 ± 0,9 d	23,4 ± 1,2 d
Summer	M1	18 ± 0,8 h	7 ± 3,3 cde	1,6 ± 1,0 cd	17,7 ± 0,8 e	13,1 ± 1,3 e
	M2	55 ± 1,4 cde	7,9 ± 3,6 cd	2 ± 1,3 bcd	53,1 ± 1,5 b	14,2 ± 1,0 e
	M3	29 ± 1,3 gh	5,8 ± 3,0 e	1,5 ± 1,0 d	29,1 ± 1,3 de	10,7 ± 1,5 e
$LSD_{(Pr=0.05)}$		**13,577**	**1,9457**	**0,5945**	**12,972**	**6,4315**

Mean value (± standard deviation) with the same letters in a column did not differ significantly from each other. M1 - perlite, vermiculite and sand (1:1:1), M2 - bark compost and coco peat (2:1) and M3 - pine bark, vermiculite and sand (2:1:1).

percentage compared to the control and Dip & Root™ application in M1 (Fig. 1a,b).

3.2.6. Season, cutting position and growth media

The length of the roots produced was significantly affected by the interaction of season, cutting position and media. Generally, cuttings planted in autumn had the longest roots as compared to cutting planted in other seasons regardless of the cutting position and growth media used (Fig. 2). While median cuttings planted in winter produced shorter roots irrespective of the growing media used. Apical cuttings planted in M2 during autumn produced significantly longer roots (9,9 cm) compared to other treatments but were not significantly different from the length of roots produced by the apical cuttings planted in M3 (8,3 cm) during the same season (Fig. 2). Median cuttings planted in M1 during winter (2,4 cm) produced the shortest roots although not significantly different from a number of other treatment combinations.

4. Discussion

4.1. Seed propagation

Germination is a complex process composed of many biological steps and is highly temperature dependent (Manoto et al., 2004). Previous studies have shown that temperature is one of the most critical factors affecting the percentage and speed of germination (Bewley and Black, 1994; Verma et al., 2010; Makena et al., 2018; Guo et al., 2020; Makhaye et al., 2021). According to Dove (2010) temperature affects germination in three primary ways: moisture uptake, hormone production, and enzyme activity. The germination percentage usually increases linearly with temperature up to an optimal temperature, after which the germination percentage decreases (Dove, 2010).

In the present study *H. odoratissimum* seeds incubated at 15 °C and 20 °C obtained significantly higher germination percentage (57,78% and 53,47%) compared to seeds incubated at 10 °C, 25 °C and 30 °C. These results corroborate previous findings by Guo et al. (2020) that the favourable germination temperature range of most perennial plants is from 10 °C to 20 °C. Similar results were reported by Mott (1972) and Willis and Groves (1991) where higher

Table 7
Interactive effect of season and plant growth regulator on growth parameters of *Helichrysum odoratissimum* cuttings.

Treatments		No of green leaves	No of roots
Autumn	Dynaroot™	11,2 ± 3,56 cd	55,3 ± 1,16 a
	Dip & Root™	13,6 ± 4,92 ab	47 ± 1,20 b
	Control	13,7 ± 4,23 a	36 ± 0,93 c
Spring	Dynaroot™	11,7 ± 4,98 bc	23,9 ± 1,18 e
	Dip & Root™	9,8 ± 5,46 d	22,4 ± 0,90 ef
	Control	11,6 ± 3,33 cd	20,1 ± 0,89 efg
Winter	Dynaroot™	7,1 ± 2,19 e	30,5 ± 1,12 cd
	Dip & Root™	7,8 ± 2,90 e	25,2 ± 0,94 de
	Control	7,6 ± 3,23 e	14,8 ± 0,85 ghi
Summer	Dynaroot™	6,5 ± 3,59 e	16,7 ± 1,24 fgh
	Dip & Root™	7,8 ± 3,29 e	12 ± 1,20 hi
	Control	7,3 ± 3,55 e	8,5 ± 0,85 i
	$LSD_{(Pr=0.05)}$	**1,9344**	**6,4748**

Mean values (±standard deviation) with the same letters in a column did not differ significantly from each other.

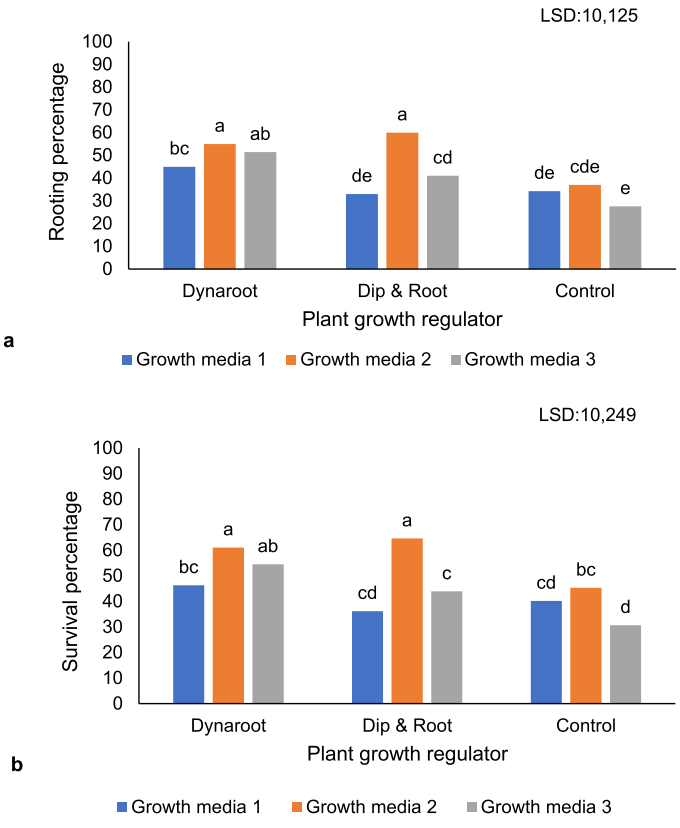

Fig. 1. The interactive effect of growth media and plant growth regulator on rooting percentage a) and survival percentage b) of *Helichrysum odoratissimum* cuttings.
Means with the same letters did not differ significantly from each other.

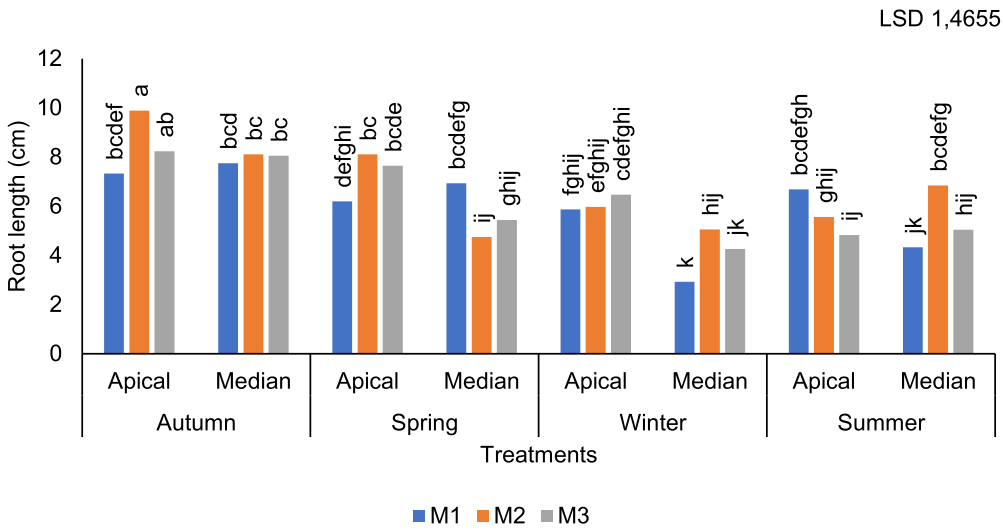

Fig. 2. Interactive effect of season, cutting position and growth media on root length of *Helichrysum odoratissimum* cuttings. Means with the same letters did not differ significantly from each other.

germination was observed in *H. cassinialum* and *H. apiculatum* seeds incubated at a constant temperature of 20 °C and an alternating temperature of 20 °C/10 °C, respectively. High temperatures have been reported to affect the membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994; Krichen et al., 2014), while lower temperatures have been found to reduce metabolic activity in the seed (Kulkarni et al., 2006). This could explain the low germination percentage observed in seeds incubated at 30 °C and the slower germination observed at 10 °C.

Low temperatures lower enzyme activities, reduce the rate of protein synthesis in the embryo and damage ripening corn seed, while higher temperatures increase respiration rate and decrease seed weight (Makena et al., 2018). The temperature of 15–20 °C was considered the optimal germination temperature for *H. odoratissimum* seeds because all the parameters evaluated in the current study were favoured by these treatments, resulting in the best values for most parameters. Higher GI (276,6) and GRI values (33,82) generally indicate a shorter germination time for a given germination percentage (Schellenberg and Biligetu, 2015) were also observed at 15 °C in the current study.

Smoke has been reported to stimulate germination and improve the growth and yield of numerous species (Brown et al., 2003; Light and van Staden, 2004; van Staden et al., 2004, 2006; Light et al., 2009; Chumpookam et al., 2012). Smoke water used in the current study significantly improved the mean germination rate and the germination percentage at most temperatures compared with other priming methods at the same temperatures. The higher germination percentage obtained may be attributed to the presence of the butenolide compound [3-methyl-2*H*-furo (2,3-c) pyrant-2-one] in the smoke primer (Merritt et al., 2006; Merrit et al., 2007; Commander et al., 2008). Butenolide not only enhances the rate and germination percentage but can also widen the environment window in which germination can occur (Light et al., 2009). It is believed that smoke water or butenolides act in a similar mode as growth hormones (Taylor and van Staden, 1996; Kulkarni et al., 2006; Sparg et al., 2006, 2010; Kulkarni et al., 2011). Smoke water may also be acting on the seed coat in a way comparable to scarification, by allowing the dormant embryo to efficiently imbibe water and take up oxygen; as suggested by Egerton-Warburton (1998) and Chumpookam et al. (2012). The combination of smoke water treatment and incubation at 20 °C (92%) resulted in higher germination percentage although not significantly different from smoke water/15 °C combination (83%). Temperature ranges 15 °C and 20 °C may have sped up the chemical reactions taking place in the seed and may have speeded up cell division when the plant embryo is growing. While smoke water may have overcome the detrimental effects of different temperatures.

Generally, soaking seeds in cold water reduced germination percentage by 1,8% compared to control in this study. It is believed that the cell membranes of some seeds became rigid and ruptured therefore impended germination. This corroborates with the observation by Amoakoh et al. (2017). The stimulation of seed germination by heat is usually due to the seed coat melting or cracking caused by high temperatures (Kamal and Behere, 2002) that make the seed coats permeable to both water and oxygen, breaking the exogenous dormancy (Keeley,1987; Keeley and Fotheringham, 2000). However, after rupturing the seed coats, high temperatures can damage the embryo, making germination impossible (Arán et al., 2017; García-Duro et al., 2019). The seeds in this study were soaked in boiled water until the water cooled down, this may have damaged the seed embryo resulting in no germination.

4.2. Propagation by stem cuttings

Since a cutting is in a thermodynamically unfavourable state from the time it is prepared, it is therefore affected by several factors (Haissig, 1986; K. Grigoriadou et al., 2020). Apart from the plants' intrinsic abilities, several physiological and environmental factors influence the success of vegetative propagation and sometimes various pre-treatments are needed to facilitate rooting and increase success.

Seasonality is considered one of the main factors affecting the physiological condition of plants (Pigatto et al., 2018). The physiological state of the cutting during the season in a year, rather than the season itself also influences the performance of the cuttings (Agbo and Omaliko, 2006). Differences in rooting percentage, survival ability and cutting quality; owing to the season in which cuttings were planted were evident in the current study. In general apical cuttings planted during autumn produced a significantly better survival percentage, rooting percentage and quality cuttings with more vegetative buds, green leaves, roots and longer roots, while cuttings planted during summer had the lowest values in most cases for all parameters measured.

The combination of autumn and apical cutting position resulted in significantly higher (78,5%) survival percentage and (71%) rooting percentage, while summer/apical cutting combination resulted in lower (24,6%) survival and (23,5%) rooting percentage. Lower ambient temperatures in autumn may have led to less transpirational loss

from cuttings, while cuttings planted during high-temperature seasons may have increased transpirational loss and stress, causing the death of cuttings before root initiation could occur (Hartmann et al., 1997; Swarts et al., 2018); especially in apical cuttings which had more leaves and therefore a larger surface area. The performance of cuttings planted in spring may be related to the persistence of dormancy effects (plants die down in winter and resprout in spring) and a scarcity of carbohydrate substances in the stems because of new growth (Harfouche et al., 2007).

Different cutting positions from the shoot influence the overall quality, rooting ability and subsequent growth habit of rooted stem cuttings (Saifuddin et al., 2013; Nogemane, 2017; Swarts et al., 2018). Use of apical cuttings in the current study resulted in higher survival percentage and better rooting, especially when cuttings were taken and planted in autumn. According to Leakey (2004) and Malele et al. (2021), leafy cuttings depend on current photosynthates produced during propagation, while hardwood cuttings depend on the hydrolysis and availability of carbohydrates stored within the stem tissues. Apical cuttings were taken from relatively actively growing, younger, leafy shoots. This may have given the apical cuttings an advantage as they were able to produce more photosynthates from the higher number of leaves present in the cuttings while median cuttings were taken from older shoots, with only two leaves per cutting. These observations are consistent with the statements by Leakey (2004), Smalley et al. (1991) and Wassner and Ravetta (2000) indicating that during rooting, the production of photosynthates facilitated by the presence of auxin in the leaves may be important in inducing rooting. Furthermore, median cuttings became moribund very quickly in the current study, resulting in most median cuttings failing to root because of the death of the leaves on the cutting, due to rotting, necrosis, bleaching or to leaf abscission. Since early differentiation and growth of leaf buds depends on food reserves available in the cutting, root formation allows the plant to absorb nutrients and water. However, where root formation lags behind shoot proliferation and survival rates usually decline.

Plant growth regulator application is one of the most common pre-treatments used to enhance the rooting of cuttings. Auxin has been reported to play a central role in the determination of the rooting capacity of cuttings, by enabling the faster production of rooted cutting material which is essential for vegetative propagation (Fogaça and Fett-Neto, 2005; Zhao et al., 2014). Auxin is known to increase rooting percentage and rooting time together with uniformity of rooting (Hartmann et al., 2002). Both PGRs, Dip & Root™ and Dynaroot™ used in the current study improved survival percentage and rooting percentage of cuttings planted in the two media (M2 and M3), with the better water holding capacity compared to control. Only Dynaroot™ had the same effect in medium 1. Similar results have been reported on other species of the Asteraceae family (Kassahun and Mekonnen, 2011; K. Grigoriadou et al., 2020). In the study by Kassahum and Mekonnen (2011), the application of PGR demonstrated a significant influence on the propagation ability of *Stevia rebaudiana* which produced more roots than control, while there was an increase in the number of roots per cutting in a study by K. Grigoriadou et al. (2020) on *Carlina diae* stem cuttings. The application of IBA increases the content of physiologically active sugars needed to provide energy for meristematic tissues and later for root primordia and root formation (Husen, 2008).

The type of substrate mixture used to grow cuttings had a significant effect on survival and rooting of *H. odoratissimum* cuttings. Rooting and survival percentage was higher in the compost with coco peat substrate (M2), compared to cuttings planted in other growth media although not always significant. The results are supported by the findings of King et al. (2011) where a mixture containing peat improved rooting percentage and number of roots per cutting on bald cypress cuttings. The higher water holding capacity and higher cation exchange capacity of the coco peat and compost medium (M2), can be very helpful in initiating rapid root development as compared to other two mixtures. In the current study, the three ways combination of season (autumn), cutting position (apical cutting) and growth media (bark compost and coco peat) resulted in significantly longer roots.

5. Conclusion

Considering the results of the two types of propagation techniques used in the current study, both techniques may be used for large-scale multiplication of *H. odoratissimum* although further investigations are needed on seedling establishment. The information provided in this study can be used as a practical contribution to the establishment of a commercial nursery for the cultivation of *H. odoratissimum*. Practically when propagated sexually, to achieve a higher germination percentage; seed may be primed with smoke water and sown during autumn (15–20 °C). When propagated vegetatively using stem cuttings, both apical and median cuttings may be used, planted during autumn using growth media with high water holding capacity and high nutrients and it would be ideal to apply PGR to improve rooting but *H. odoratissimum* cuttings may root without PGR application. The physiological conditions of stock plants are crucial for the initiation of the rooting process of cuttings (Zhao et al., 2014). This study, therefore, suggests that the best season to propagate *H. odoratissimum* is the beginning of autumn when it is believed that *H. odoratissimum* donor plants are in optimal physiological condition for vegetative propagation. The interaction between different factors such as season and growth media; season and cutting position must be taken into consideration as certain types of growth media and different cutting positions perform differently due to seasonal changes.

Declaration of Competing Interest

Authors of this article do not have any conflict of interest over the article.

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Appendix A. *Helichrysum odoratissimum* seed germination ANOVA table

Variates	Variables	Df	Sum of squares	Mean square	Variance ratio	Pr>*F*
T_0	**Temperature**	4	502.756	125.689	36.22	<**0.001****
	Pre treatment	3	1632.861	544.287	156.83	<**0.001****
	Temp*Pre	12	242.222	20.185	5.82	<**0.001****
G% at T_0	**Temperature**	4	8535.28	2133.82	30.38	<**0.001****
	Pre treatment	3	9056.11	3018.70	42.99	<**0.001****
	Temp*Pre	12	4725.83	393.82	5.61	<**0.001****
T_g	**Temperature**	4	1502.70	375.67	6.78	<**0.001****
	Pre treatment	3	29,290.80	9763.60	176.27	<**0.001****
	Temp*Pre	12	1169.70	97.47	1.76	**0.092**^{NS}
$T_{50\%}$	**Temperature**	4	989.45	247.84	10.23	**0.0002*****
	Pre treatment	2	13.99	6.99	0.29	**0.7521**^{NS}
	Temp*Pre	6	231.06	38.51	1.59	**0.2064**^{NS}
G%	**Temperature**	4	27,810.3	6952.6	41.67	<**0.001****
	Pre treatment	3	133,833.9	44,611.3	267.35	<**0.001****
	Temp*Pre	12	11,967.5	997.3	5.98	<**0.001****
MGD	**Temperature**	4	636.211	159.053	9.96	<**0.001****
	Pre treatment	3	7522.531	2507.510	157.08	<**0.001****
	Temp*Pre	12	372.897	31.075	1.95	**0.059**^{NS}
MGR	**Temperature**	4	0.965886	0.241471	22.49	<**0.001****
	Pre treatment	3	2.967753	0.989251	92.13	<**0.001****
	Temp*Pre	12	0.684763	0.057064	5.31	<**0.001****
GI	**Temperature**	4	682,409.	170,602.	24.16	<**0.001****
	Pre treatment	3	2,320,887.	773,629.	109.54	<**0.001****
	Temp*Pre	12	305,952.	25,496.	3.61	**0.001****
GRI	**Temperature**	4	12,312.14	3078.03	19.90	<**0.001****
	Pre treatment	3	36,050.29	12,016.76	77.67	<**0.001****
	Temp*Pre	12	5745.27	478.77	3.09	**0.004****

T_o: Time to the first germination, **T_g**: Time to the last germination, **$T_{50\%}$**: Time to 50% germination, **$T_oG\%$**: Total germination percentage at the first day of germination, **G%**: Final germination percentage, **MGD**: Mean germination duration, **MDR**: Mean germination rate, **GI**: Germination index and **GRI**: Germination rate index. [^{NS} = Not significant ($p \geq 0.05$), * and **= Significant ($p<0.05$ & $p<0.01$)].

Appendix B. Summary of analysis of variance (ANOVA) on the effect of season, cutting type, growth media, plant growth regulator and epidermal layer on the growth parameters of *Helichrysum odoratissimum* cuttings

		Survival			No of green leaves			No of buds		
	DF	Mean square	F value	Pr>*F*	Mean square	F value	Pr>*F*	Mean square	F value	Pr>*F*
Season	3	46,7,551,614	46,76	<0.0001***	677,952,218	52,1	<,0001***	59,4,059,638	48,69	<,0001***
Cutting	1	0,6,208,266	0,62	0,4314 ns	24,096,239	1,85	0,1752 ns	26,5,364,723	21,75	<,0001***
Season & cutting	3	20,1,242,137	20,12	<0.0001***	55,216,908	4,24	0,0062**	19,0,805,626	15,64	<,0001***
Media	2	17,8,480,525	17,85	<0.0001***	11,21,693	0,86	0,4239 ns	0,3,629,934	0,3	0,7430 ns
Season & media	6	12,7,322,803	12,73	<0.0001***	46,409,045	3,57	0,0022**	3,0,401,186	2,49	0,0240*
Cutting and media	2	0,8,076,485	0,81	0,4469 ns	20,749,359	1,59	0,2057 ns	0,8,180,611	0,67	0,5127 ns
Season, cutting & media	6	1,7,547,261	1,75	0,1084 ns	18,87,083	1,45	0,1974 ns	0,9,835,037	0,81	0,5663 ns
PGR	2	12,8,553,054	12,86	<0.0001***	32,187,156	2,47	0,0869 ns	4,5,472,431	3,73	0,0258*
Season & PGR	6	0,610,157	0,61	0,7222 ns	28,922,937	2,22	0,0426*	2,1,912,749	1,8	0,1017 ns
Cutting & PGR	2	2,290,871	2,29	0,1030 ns	4,14,064	0,32	0,7278 ns	0,1,486,175	0,12	0,8854 ns
Season, cutting & PGR	6	1,5,050,099	1,51	0,1763 ns	19,408,087	1,49	0,1829 ns	1,6,421,086	1,35	0,2386 ns
Media & PGR	4	3,2,445,576	3,24	0,0126*	28,966,993	2,23	0,0676 ns	0,2,862,979	0,23	0,9186 ns
Season, media & PGR	12	1,1,430,406	1,14	0,3250 ns	13,741,547	1,06	0,3997 ns	1,1,067,431	0,91	0,5409 ns
Cutting, media & PGR	4	0,7,672,996	0,77	0,5473 ns	14,073,205	1,08	0,3669 ns	2,2,211,462	1,83	0,1264 ns
Season, cutting, media & PGR	12	1,1,628,619	1,16	0,3097 ns	17,061,238	1,31	0,2146 ns	1,1,767,715	0,96	0,4843 ns
Epidermal	1	0,0,062,846	0,01	0,9369 ns	47,457,371	3,65	0,0576 ns	7,0,702,982	5,79	0,0170*
Season & epidermal	3	1,7,062,733	1,71	0,1659 ns	13,80,304	1,06	0,3670 ns	0,6,613,512	0,54	0,6541 ns
Cutting & epidermal	1	0,2,308,597	0,23	0,6313 ns	51,448,112	3,95	0,0482*	5,8,595,605	4,8	0,0296*
Season, cutting & epidermal	3	0,9,759,002	0,98	0,4045 ns	10,388,768	0,8	0,4962 ns	1,5,483,061	1,27	0,2862 ns
Media & epidermal	2	1,050,287	1,05	0,3512 ns	33,647,168	2,59	0,0779 ns	0,4,696,465	0,38	0,6810 ns
Season, media & epidermal	6	1,6,691,869	1,67	0,1284 ns	4,446,863	0,34	0,9141 ns	0,1,943,996	0,16	0,9870 ns
Cutting, media & epidermal	2	0,2,403,646	0,24	0,7865 ns	10,977,327	0,84	0,4317 ns	1,519,254	1,25	0,2902 ns
Season, cutting, media & epidermal	6	0,341,446	0,34	0,9145 ns	4,853,809	0,37	0,8955 ns	0,6,072,228	0,5	0,8097 ns
PGR & epidermal	2	3,9,495,846	3,95	0,0203*	46,261,066	3,55	0,0305*	3,707,466	3,04	0,0502*
Season, PGR & epidermal	6	1,024,442	1,02	0,4095 ns	19,414,004	1,49	0,1827 ns	2,0,487,352	1,68	0,1279 ns
Cutting, PGR & epidermal	2	1,2,100,282	1,21	0,2997 ns	32,655,465	2,51	0,0839 ns	2,599,402	2,13	0,1215 ns
Season, cutting, PGR & epidermal	6	1,6,980,741	1,7	0,1213 ns	25,092,452	1,93	0,0781 ns	1,7,178,679	1,41	0,2133 ns
Media, PGR & epidermal	4	0,636,654	0,64	0,6367 ns	9,965,155	0,77	0,5487 ns	0,4,279,877	0,35	0,8433 ns
Season, media, PGR & epidermal	11	0,6,078,585	0,61	0,8352 ns	13,449,534	1,03	0,4183 ns	1,2,936,366	1,06	0,3956 ns
Cutting, media, PGR & epidermal	4	1,0,653,664	1,07	0,3739 ns	5,374,435	0,41	0,7992 ns	1,21,143	0,99	0,4126 ns
Season, cutting, media, PGR & epidermal	9	0,7,886,034	0,79	0,6622 ns	4,220,279	0,32	0,9663 ns	0,9,048,021	0,74	0,6705 ns

		Rooting percentage			No of roots			Root length (cm)		
	DF	Mean square	F value	Pr>F	Mean square	F value	Pr>F	Mean square	F value	Pr>F
Season	3	33.7170604	33,72	<0.0001***	145,1,250,304	145,13	<0.0001***	169,7,216,445	35,18	<,0001***
Cutting	1	0.0535567	0,05	0,8172 ns	32,9,741,538	32,97	<0.0001***	83,2,284,341	17,25	<,0001***
Season & cutting	3	18.3928005	18,39	<0.0001***	2,059,669	2,06	0,1070 ns	16,0,274,827	3,32	0,0209*
Media	2	11.8982664	11,9	<0.0001***	6,4,967,628	6,5	0,0019**	28,7,388,811	5,96	0,0031**
Season & media	6	11.4272957	11,43	<0.0001***	3,403,325	3,4	0,0033**	3,9,518,835	0,82	0,5562 ns
Cutting and media	2	1.3035505	1,3	0,2732 ns	0,2,452,653	0,245	0,7827 ns	0,0,227,508	0	0,9953 ns
Season, cutting & media	6	1.6309334	1,63	0,1384 ns	1,2,603,812	1,26	0,2777 ns	19,9,569,578	4,14	0,0006***
PGR	2	17.7933875	17,79	<0.0001***	30,4,336,334	30,43	<0.0001***	32,5,662,611	6,75	0,0015**
Season & PGR	6	1.2334276	1,23	0,2891 ns	2,3,678,718	2367	0,0315*	5,4,463,274	1,13	0,3470 ns
Cutting & PGR	2	1.5893118	1,59	0,2059 ns	2,015,104	2015	0,1362 ns	2,2,428,321	0,46	0,6289 ns
Season, cutting & PGR	6	1.7675241	1,77	0,1056 ns	1,2,766,293	1276	0,2699 ns	5,1,930,338	1,08	0,3781 ns
Media & PGR	4	3.4228914	3,42	0,0094**	0,1,711,795	0,171	0,9529 ns	4,5,114,643	0,94	0,4447 ns
Season, media & PGR	12	1.1813776	1,18	0,2959 ns	0,4,782,134	0,478	0,9257 ns	5,6,633,916	1,17	0,3044 ns
Cutting, media & PGR	4	1.8943295	1,89	0,1115 ns	2,2,909,419	2,29	0,0612 ns	10,2,375,491	2,12	0,0797 ns
Season, cutting, media & PGR	12	1.3354780	1,34	0,1976 ns	0,8,589,562	0,858	0,5898 ns	7,9,554,893	1,65	0,0913 ns
Epidermal	1	0.2057266	0,21	0,6505 ns	0,9,376,573	0,937	0,3341 ns	17,3,297,031	3,59	0,0596 ns
Season & epidermal	3	1.7232482	1,72	0,1624 ns	0,1,873,662	0,187	0,9049 ns	3,7,758,958	0,78	0,5049 ns
Cutting & epidermal	1	0.4971612	0,5	0,4813 ns	6,0,381,499	6038	0,0149*	88,8,869,212	18,43	<,0001***
Season, cutting & epidermal	3	1.3435372	1,34	0,2605 ns	1,3,610,891	1361	0,2561 ns	3,3,321,133	0,69	0,5587 ns
Media & epidermal	2	1.0558613	1,06	0,3492 ns	0,4,980,133	0,498	0,6085 ns	4,9,649,992	1,03	0,3593 ns
Season, media & epidermal	6	1.5694976	1,57	0,1559 ns	0,299,403	0,299	0,9366 ns	7,5,546,412	1,57	0,1592 ns
Cutting, media & epidermal	2	0.2798922	0,28	0,7561 ns	1,5,214,415	1521	0,2211 ns	9,5,521,047	1,98	0,1409 ns
Season, cutting, media & epidermal	6	0.3966185	0,4	0,8810 ns	0,9,809,694	0,98	0,4394 ns	2,4,372,302	0,51	0,8039 ns
PGR & epidermal	2	4.6353090	4,64	0,0104*	1,2,311,226	1231	0,2943 ns	0,5,250,678	0,11	0,8969 ns
Season, PGR & epidermal	6	0.5906988	0,59	0,7377 ns	0,2,693,901	0,269	0,9507 ns	5,7,780,544	1,2	0,3092 ns
Cutting, PGR & epidermal	2	2.4093646	2,41	0,0917 ns	0,0,066,205	0,006	0,9934 ns	4,070,436	0,84	0,4317 ns
Season, cutting, PGR & epidermal	6	0.9477422	0,95	0,4612 ns	0,7,571,761	0,757	0,6045 ns	8,4,049,139	1,74	0,1133 ns
Media, PGR & epidermal	4	0.4159514	0,42	0,7971 ns	1,1,085,147	1108	0,3539 ns	3,7,823,366	0,78	0,5368 ns
Season, media, PGR & epidermal	11	0.5162896	0,52	0,9037 ns	1,2,457,131	1245	0,2595 ns	11,2,662,847	2,34	0,0103*
Cutting, media, PGR & epidermal	4	1.6855239	1,69	0,1534 ns	0,5,024,301	0,502	0,7340 ns	4,841,312	1	0,4069 ns
Season, cutting, media, PGR & epidermal	9	0.8211164	0,82	0,6286 ns	1,076,649	1076	0,3812 ns	7,4,479,198	1,54	0,1446 ns

NS = Not significant ($p \geq 0.05$), * and **= Significant ($p<0.05$), ***= Highly significant ($p<0.0001$).

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