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Study on Stem Cutting Propagation of *Gynostemma Pentaphyllum* (Thunb.) Makino in Hoa Binh Province

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Abstract

Gynostemma pentaphyllum (Thunb.) Makino is a medicinal plant distributed mostly in the mountainous areas of Vietnam. *G. pentaphyllum* contains more than 189 types of saponin which can be used for the treatment of various diseases. Currently, due to over-exploitation, this plant species is under threaten. Our research aimed to study a suitable method for plant propagation by stem cutting techniques to promote the growth and development of *G. pentaphyllum*. Six experiments were conducted to (i) evaluate the established seedling rates of different *G. pentaphyllum* varieties, and choose the best variety to (ii) evaluate the established seedling rate influenced by different factors, *viz.* stem cutting position, cutting season, growing in the selected substrates, stem cutting length, number of buds per stem, and application of α-NAA. The results showed that among five varieties, G2 which was collected in Mai Chau district disclosed the highest ability to shooting, the highest number of roots per stem cutting, the shortest nursery days, the highest shooting rate, and the highestestablished seedling rate. We also determined that using middle-aged plants for stem cuttings, at 15-20cm in length, with 2 nodes and grown in Spring and Winter cropping seasons, using growing substrates with a mixture of 50% of black sand and 50% of soil, and plus α-NAA 50ppm in 3-5min produced the highest explant rate. The established seedlings at the time of explant were 15-20cm in height, containing 4-5 leaves and at least 9-10 roots per cutting, and were 30-32 days.

Keywords

Gynostemma pentaphyllum, stem cutting, propagation

Introduction

Gynostemma pentaphyllum (Thunb.) Makino belongs to the Cucurbitaceae family. *G. pentaphyllum* is distributed mostly in the mountainous areas, in shaded and humid places at 300-3200 meters above sea level. In Vietnam, *G. pentaphyllum* was found naturally in

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Phanxipang mountain, Sapa district, Ha Giang, Cao Bang, Hoa Binh, and Ninh Binh provinces (Do Tat Loi, 2004). *G. pentaphyllum* has been used in both traditional and modern medicine during the last five centuries. More than 189 types of saponin have been isolated and identified from this plant as known as gypenosides saponin (Gyps) (Li *et al.*, 2016). *G. pentaphyllum* contains pharmacological properties, therefore can be used for the treatment of various diseases such as inflammation, hepatitis, cancer, and cardiovascular disease; for heat-clearing, detoxification, antitussive, heart palpitation, fatigue syndrome, chronic bronchitis, and expectorant for relieving cough (Wu *et al*., 1998; Blumert & Liu, 1999). In Japan, *G. pentaphyllum* is indicated as a medicine and has been used as a diuretic, antipyretic, anti-inflammatory, and tonic (Chen, 2000; Tanner *et al.*, 1999).

Due to overexploitation, these plant species are currently under threaten. Therefore, the protection, growth, and development of this plant species are very important. Methods and optimal growing conditions for growing this plant were specified in a study of Guo & Wang (1993). They reported that the ideal soil was well-aerated and retained water, such as a fertile sandy loam with more than 30cm deep, rich in humus, nitrogen, and phosphorus with an optimal pH of 6.5-7.0, and 15-30^{o}C air temperature. The plant preferred shady areas. Under these optimal conditions, the blossoming and bearing of fruits increased and the total saponin content was the highest. Moreover, this herb could be collected every 20-30 days instead of 75-90 days in the other conditions in subtropics and tropics. In a high-yield plot, 4000-5000kg of dried herb may be collected every hectare (Guo & Wang, 1993).

To date, most studies of these species have focused on the extraction, chemistry, and pharmacology, while the study on the asexual propagation technique of this plant remains unclear. Pollinated by the insect, the seedlings from seeds are punny, whilst propagation by stem cutting provides quickly and true-to-type plants. However, this technique still depends upon many factors such as cutting age, stem cutting position, plant growth regulator, cutting season, and growing substrates, etc. The present study was conducted to determine the appropriate method for plant propagation by stem cutting technique.

Materials and Methods

Materials

Five varieties of *Gynostemma pentaphyllum* (Thunb.) Makino were collected and used in this study; of which, VB-GCL1 variety was collected in Sapa district of Lao Cai province; VB-GCL2 and VB-GCL3 varieties were collected in Mai Chau district and Da Bac district of Hoa Binh province, respectively; VB-GCL4 and VB-GCL5 varieties were collected in Nghe An province and Son La province, respectively. All of them were then grown in Biopharm Company Ltd., Hoa Binh province (No. 187, Group 17, Tan Binh, Hoa Binh, Vietnam from January 2018 to January 2019).

Methods

Six different experiments were conducted to evaluate the effect of different factors on the established seedling rate of each *G. pentaphyllum* variety as follows.

*Experiment 1: Evaluating the established seedling rates of some varieties Gynostemma pentaphyllum (*Thunb.) Makino

Five treatments were the five varieties, among them, G1 variety was the control treatment.

Experiment 2: Studying the effect of cutting season on the established seedling rate

Four treatments included Spring cropping season (February 15, 2018, TV1), Summer cropping season (May 15, 2018, TV2), Autumn season (August 15, 2018, TV3), and Winter cropping season (November 15, 2018, TV4).

Experiment 3: Studying the effect of growing substrate(s) on the growth and development of stem cutting

Three treatments included 100% black sand (control treatment, CT1), 100% soil (CT2), and a mixture of 50% black sand + 50% soil (CT3).

Experiment 4: Studying the effect of stem cutting position on the established seedling rate

Three treatments included stem cutting positions from apical cutting (> 1m, VT1), middle cutting (50 to 100cm, VT2), and basal cutting (0 to 50cm, VT3) of the stem.

Experiment 5: Studying the effect of stem cutting length and number of buds on established seedling rate of G2 variety

Three treatments consisted of 10cm (one bud per stem, CT1), 15cm (two buds per stem, CT2), and 20cm (three buds per stem, CT3)

Experiment 6: Studying the effect of α-NAA on the established seedling rate *and growth of stem cutting*

G. pentaphyllum was subjected to treatment with three concentrations of α –NAA, *viz.*, α –NAA 0ppm (100% of water, CT1), α –NAA 50ppm in 10min (CT2), and α –NAA 100ppm in 10min (CT3).

The experimental design was a randomized complete block design (RCBD) with threee replications for all of the experiments. A one-year-old plants of G2 variety were used as the plant material for experiments 2, 3, 4, 5, and 6. Each treatment contained 30 stem cuttings (middle-aged stems, except for experiment 4), the stem cutting length was from 15 to 20cm (except for experiment 5), which contained two buds and 1-2 leaves per stem. α -NAA was applied beforehand at 50ppm (except for experiment 6) in 3-5min and placed in 100% of black sand (except for experiment 3), starting from February 15, 2018 (except for experiment 2). Stem cutting was kept in greenhouse conditions with higher than 85% of humidity and covered by black nylon to achieve 50% reduction in light intensity.

We defined the established seedling that was 20-25cm long, containing 4-5 leaves, and 9-10 roots per stem, and aged 30-32 days.

Data collection

Five plants per treatment were observed every 15 days at 15 days and 30 days after growing. Duration in the nursery (days), the percentage of shooting (%, number of shooting stem per total stem), established seedling rate (%, number of seedling per total stem cutting), plant height (cm, from the surface to the top of the highest bud), number of leaves per plant (number of leaves at explant time), and number of roots per stem (number of roots per stem which were longer than 7cm) were measured.

Data analysis

Data were analyzed using IRRISTAT version 5.0. The data were subjected to one-way analysis of variance (ANOVA). Pairwise comparisons of means were performed with Least significant different (LSD).

Results and Discussion

Evaluation of the established seedling rate of different *G. pentaphyllum* varieties

G. pentaphyllum was carefully observed during the propagation process. Results showed that different varieties had different propagation durations. Propagation duration is one of the most important criteria for arranging the cropping system. The propagation duration of a variety consists of different stages, each lasts a certain length of time, depending on the characteristics of the variety and environmental conditions. Duration in the nursery, established seedling rate, shooting rate and rooting rate, number of roots per stem, shoot length, and number of leaves per stem of the five *G. pentaphyllum* varieties are given in **Table 1**.

The duration from cuttings to the first shooting (days until first shooting) of the five different *G. pentaphyllum* varieties was significantly different, ranging from 7 to 13 days. The G5 variety had the longest duration until the first shooting (13 days), whereas the G1, G3, and G4 variety had the shorter duraions until the first shooting (7 to 8 days) compared to the other varieties.

The shooting rate was calculated by the number of new shoots per total number of stem cutting. This indicator illustrated the initial regeneration ability of different *G. pentaphyllum* varieties. Results showed that the G2 (93%) and G3 (94%) obtained the highest shooting rates compared to the other three varieties, whilst G4 variety (83%) had the lowest shooting rate.

Table 1. Effect of different *G. pentaphyllum* varieties on the shooting rate, rooting rate, and established seedling rate

Treatments	Days until first shooting (days)	Shooting rate (%)	Rooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
VB-GCL1	8c^{d}	90^{b}	86^{c}	15.0^{c}	17.0^{b}	2.7^{d}	83.0^{bc}	32
VB-GCL2	10^{b}	93^{a}	92^{b}	18.3^{b}	22.5^{a}	4.7^{a}	88.0^{a}	32
VB-GCL3	8^{cd}	94^{a}	94^{a}	22.7^{a}	22.0^{a}	4.0^{b}	85.0^{b}	32
VB-GCL4	7^{d}	83^{d}	82^{d}	12.7^{d}	12.5^{c}	3.6^{c}	79.0^{c}	32
VB-GCL5	13^{a}	89^{bc}	92^{b}	17.7^{b}	12.0c	2.3^{e}	87.0^{ab}	32
$LSD_{0.05}$	1.35	2.02	1.85	3.53	1.17	0.30	2.85	-
CV%	7.70	1.19	1.10	2.17	2.50	3.10	1.79	-

Note: VB-GCL1: G. pentaphyllum varieties collected in Sa Pa, Lao Cai province
VB-GCL2: G. pentaphyllum varieties collected in Mai Chau, Hoa Binh province
VB-GCL3: G. pentaphyllum varieties collected in Da Bac, Hoa Binh province
VB-GCL4: G. pentaphyllum varieties collected in Nghe An province
VB-GCL5: G. pentaphyllum varieties collected in Son La province
Values followed by different letters within a column indicate significant differences at the 5% level.

Each variety of *G. pentaphyllum* had different shoot lengths and number of leaves per stem cuttings. Shoot length ranged from 12.07cm to 22.03cm. In which, the shoot length of G2 (22.5cm) and G3 (22.03cm) was significantly higher than that of the other varieties. In addition, the number of new leaves per stem cutting of the studied varieties ranged from 2.33 leaves to 4.67 leaves per plant. Again, the G2 and G3 varieties had the highest numbers of leaves (4.67 and 4.00 leaves per plant, respectively).

The rooting rate was calculated as the total number of new roots per total number of cuttings in each variety. While the shooting rate indicated the initial regeneration, the rooting rate indicator showed the long-term regeneration ability of the *G. pentaphyllum* varieties. In particular, the G3 variety had the highest rooting rate (94%), whereas G4 had the lowest rate (82%), the other varieties had the rooting rates ranging from 86% to 92%. At the time of explant, the number of roots of the *G. pentaphyllum* of all varieties met the standard for explanting. The number of roots of different varieties ranged from 12.67 roots to 22.67 roots. G3 variety (22.67 roots) attained the highest number of roots, while G4 variety (12.67 roots) had the lowest number of roots compared to the other varieties.

The established seedling rate was the main indicator to determine the regeneration capacity of *G. pentaphyllum*. Thereby, the established seedling rate in G2 variety (88%) was the highest, followed by G5 (87%), G3 (85%), G1 (83%), and G4 (79%).

It can be seen that at the time of explanting, the seedling standard was 20-25cm in height, had 4-5 leaves per stem, and was 30-32 days old.

Effect of cutting season on the established seedling rate, growth, and development of *G. pentaphyllum*

Seasonal crop, in which temperature and precipitation change during a year, causes the changes in photosynthesis and respiration production of the plant system, thus, it might affect the rooting rate of the cutting stem (Satpal *et al.*, 2014). Each plant species has an optimal period for the best rooting as well as growth whichalso depends on the variety. The ecological requirements of *G. pentaphyllum* are cool and cold conditions. Our result showed that the Summer cropping season (stem cuttings in May) had high temperatures and thus affected the survival rates and the established seedling rate of the *G. pentaphyllum*. Indeed, the established seedling rate in Summer (75.5%) was significantly lower than that in the Winter cropping season (83.33%).

The viability of seedlings at the time of explanting also showed that seedlings grow better under spring and winter cropping seasons, performed via shooting rate, shoot height, and number of leaves per stem cutting indicators. Specifically, shoot length under the spring and winter conditions (26.17cm and 26.62cm, respectively) was significantly higher than that under the summer (17.62cm) and the autumn conditions (17.91cm). Similarly, the number of leaves in the spring and winter was also significantly higher than that in the two other crop seasons. Furthermore, the total duration in the nursery in spring and winter was only 28 days, while being 32 days in the summer and the autumn **(Table 2)**.

Hence, the spring cropping season (cutting around February 15) and winter cropping season (cutting around November 11) were suitable for the cutting and propagation of *G. pentaphyllum*.

Effect of growing substrates on the growth and development of stem cutting

The quality of the growing substrates also plays a very important role in the rooting capacity of stem cutting. A growing substrate should be considered an integral part of the propagation system. A qualified growing substrate should be able to provide enough moisture and be strong enough to hold the stem cuttings during the rooting period. Different types of growing substrates had a significant effect on rooting rate, established seedling rate, number of roots per stem cutting, shoot length, and number of leaves per stem cutting of *G. pentaphyllum*. The results are shown in **Table 3**.

The substrate of 100% black sand (CT1) gave the highest rooting rate (92.00%) and shooting rate (93.33%), whereas the substrate of a mix of 50% of black sand and 50% of soil (CT3) resulted in the highest number of roots per stem (11.92%), shoot length (20.61cm), and established seedling rate (85.17%). Duration in the nursery of CT1 was similar to CT3 (with a total of 30 days). Among the substrates, the substrate of 100% of soil (CT2) showed the lowest results of rooting rate (87.33%), shooting rate (85.33%), number of root per stem (7.50 roots), shoot length (18.24cm), and established seedling rate (80.50%) when compared to the other substrates.

Therefore, the appropriate substrate for stem cuttings was CT3 (50% sand and 50% soil). Similarly, Singh *et al.* (2011) also showed that among seven types of growing substrates, the mixture of soil, sand, and farmyard manure improved the survival percentage (82.33%) and lead to a higher rooting percentage (64.26%).

Effect of stem cutting position on the growth and development of G2 variety

Table 4 shows the effect of stem cutting position on the duration in the nursery, propagation rate, and survival process of the five-leaf *G. pentaphyllum*. Middle-aged stem cuttings showed the shortest period of propagation (7 days) compared to those of the basal cutting (10

Table 2. Effect of cutting seasons on the shooting rate, rooting rate, and established seedling rate of *G. pentaphyllum*

Treatment	Rooting rate (%)	Shooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
TV1	95.00^{a}	93.33^{a}	9.42^{b}	26.17^{b}	5.42^{a}	82.50^{ab}	28^{b}
TV2	88.33^{b}	85.33^{b}	11.25^{a}	17.62^{d}	4.50^{b}	75.50^{bc}	32^{a}
TV3	85.33^{bc}	85.00^{b}	9.92^{b}	17.91^{c}	4.67^{ab}	79.17^{b}	32^{a}
TV4	80.83^{bc}	88.17^{ab}	12.17^{a}	26.62^{a}	5.08^{a}	83.33^{a}	28^{b}
CV%	*4.90*	*9.20*	*4.60*	*2.30*	*4.60*	*10.00*	*3.10*
$LSD_{0.05}$	*6.66*	*6.67*	*0.78*	*0.26*	*0.36*	*7.12*	*1.85*

Note: TV1: Spring season (February 15), TV2: Summer season (May 15), TV3: Autumn season (August 15), and TV4: Winter season (November 15). Values followed by different letters within a column indicate significant differences at the 5% level.

Table 3. Effect of growing substrates on shooting rate and established seedling rate of stem cutting

Treatment	Rooting rate (%)	Shooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
CT1	92.00^{a}	93.33^{a}	10.09^{b}	17.44^{c}	5.17^{a}	82.50^{ab}	30^{a}
CT2	87.33^{b}	85.33^{b}	7.50^{c}	18.24^{b}	5.75^{a}	80.50^{b}	20^{b}
CT3	90.33^{ab}	88.00^{b}	11.92^{a}	20.61^{a}	5.25^{a}	85.17^{a}	30^{a}
CV%	*2.90*	*4.20*	*3.60*	*2.3*	*11.5*	*5.00*	*4.84*
$LSD_{0.05}$	*4.12*	*3.54*	*0.61*	*0.31*	*1.10*	*3.12*	*2.92*

Note: CT1: 100% black sand (control treatment), CT2: 100% soil, CT3: 50% black sand + 50% soil. Values followed by different letters within a column indicate significant differences at the 5% level.

Table 4. Effect of stem cutting position on shooting days, established seedling rate, and rate of survival of *G. pentaphyllum*

Treatment	Days until first shooting (days)	Shooting rate (%)	Rooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
Basal cutting	10^{a}	92^{b}	90^{b}	5.14^{b}	20.0^{b}	3.67^{b}	82^{b}	29
Middle cutting	7^{b}	97^{a}	95^{a}	7.34^{a}	22.5^{a}	4.25^{a}	86^{a}	29
Apical cutting	11^{a}	88^{c}	89^{bc}	3.63^{c}	18.1^{c}	3.30^{c}	70^{c}	29
$LSD_{0.05}$	*2.92*	*1.89*	*1.51*	*0.53*	*1.17*	*0.3*	*3.3*	-
CV%	*1.38*	*0.65*	*0.73*	*2.17*	*2.5*	*3.1*	*4.1*	-

Note: Values followed by different letters within a column indicate significant differences at the 5% level.

days) and the apical cutting (11 days). The propagation rate and the explanting rate of the middle-aged cuttings were the highest (86%), followed by those of the basal-cuttings (82%) and the apical (70%). It suggests that the position of cuttings is a factor affecting the growth and development of the five-leaf *G. pentaphyllum*.

Shoot length of treatment using middle-aged stem cuttings (22.52cm) at the time of explanting was higher than that of the basal-cuttings (20.04cm) and the apical cuttings (18.14cm). In addition, at the time of explanting, middle-aged stem cuttings had 4.25 leaves per plant, the highest, at a significant level of 5%. Cuttings from the middle-aged stems performed better in all rooting parameters than a basal cutting plant. It can be concluded that middle-aged stem cuttings should be considered to produce high quality planting stock material (Amri *et al*., 2010). Hartman & Kester (1983) reported that the differences in propagation ability of apical, middle, and basal cuttings could be due to a high concentration of endogenous root promoting substances in the apical cuttings which arised from the terminal buds and also higher number of active cells which were capable of becoming meristematic.

Effect of stem cutting length and number of buds on the growth and development of *G. pentaphyllum* G2 variety

G. pentaphyllum is a herbaceous plant belonging to the Cucurbitaceae family, a climbing vine. Under normal conditions, *G. pentaphyllum* has the rooting ability at each leaf-bearing node. We conducted experiments to select the optimal number of buds per stem cutting for the asexual propagation of this medicinal plant by stem cutting. The results are presented in **Table 5**.

Results showed that treatment of 15cm length with two buds per stem (CT2) and treatment of 20cm length with three buds per stem (CT3) were the best treatments for the growth of *G. pentaphyllum* G2 variety. It showed the highest rooting rate (94.27% and 91.67%, respectively) and shooting rate (84.17%). Similarly, the explant rates in CT2 and CT3 (82.50% and 83.33%) were much higher than that of CT1 (47.5%).

The number of buds per stem cuttings affected the number of roots per stem cutting and rooting rate. After 28 days of cutting, the highest number of roots per stem cutting was seen in CT3 (14.17 roots), followed by CT2 (11.25 roots), and the lowest was observed in CT1 (6.84 roots). Furthermore, the higher shoot length and number of leaves per stem cutting were seen in CT2 and CT3. The duration in the nursery in these two treatments was shorter compared to CT1. Therefore, CT2 and CT3 were more suitable for the growth of *G. pentaphyllum.* Regarding economic efficiency, the length of stem cutting and its number of buds in CT2 (15cm and two buds per stem) was well recommended for the propagation of *G. pentaphyllum*. The results were similar to Ninh Thi Phip (2013) who used the main stem with 15-20cm length optimal for growth of *Polyscias fruticose* L. stem cutting.

Effect of α-NAA on the established seedling rate and growth of stem cuttin

Among many types of plant growth regulators, auxins are famous for their effectiveness and significance in stimulating root initiation in stem cuttings of most plants. Root length increases due to the enhancement of carbohydrates hydrolysis, synthesis of new proteins, cell enlargement, and cell division induced by the application of auxins. High carbohydrate and low nitrogen levels have been reported to favor root formation (Satpal *et al.*, 2014). The most reliable auxin in stimulating root production in cuttings stem is indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), and naphthalene acetic acid (NAA). Of these, NAA is commercially widely used in many plant species and was found to be more stable and effective in most cases (Totaan, 2019). The root production was found to increase rapidly at lower NAA concentrations.

In our research, the application of plant growth regulators on stem cuttings had a significant effect on the propagation rate, the rate of explanting, and the shortening of the time in the nursery (**Table 6**). Treatment of α-NAA at 50ppm (CT2) increased rooting rate (92.5%) and explanting rate (86.67%), while shortening explanting time (27 days) compared to non-α-NAA (CT1) and 100ppm of α-NAA (CT3). Treatment of α -NAA for cuttings had a significant effect on the number of roots per stem cutting of the plant after cuttings. In other words, the concentration of α -NAA treatment had a different effect. The treatment of CT2 had a much higher effect than those in CT3 and CT1. Indeed, CT2 showed a much higher effect than the two other treatments in all of the growth indicators, *viz.*, number of roots per stem cutting (6.70 roots), shoot length (23.90cm), rooting rate

Table 5. Effect of stem cutting length and number of buds on the growth and development of *G. pentaphyllum*

Treatment	Rooting rate (%)	Shooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
CT1	78.33^b	58.33^b	6.84^c	16.32^c	4.59^b	47.50^b	35^a
CT2	91.67^a	84.17^a	11.25^b	20.91^a	5.50^a	83.33^a	28^b
CT3	94.17^a	84.17^a	14.17^a	20.51^a	5.33^a	82.50^a	28^b
CV%	3.90	7.90	4.00	3.30	3.9	7.30	1.10
$LSD_{0.05}$	6.00	8.54	0.74	0.42	0.35	7.75	1.0

Note: CT1: 10cm (one bud per stem,), CT2: 15cm (two buds per stem), CT3: 20cm (three buds per stem). Values followed by different letters within a column indicate significant differences at the 5% level.

Table 6. Effect of α-NAA on the established seedling rate and growth of stem cutting

Treatment	Rooting rate (%)	Shooting rate (%)	Number of roots per stem	Shoot length (cm)	Number of leaves per stem	Established seedling rate (%)	Duration in nursery
CT1	72.50[bc]	83.4[ab]	5.50[bc]	12.2[c]	3.55[b]	70.67[bc]	33[a]
CT2	92.50[a]	90.2[a]	6.70[a]	23.9[a]	4.05[a]	86.67[a]	27[b]
CT3	79.25[b]	80.4[bc]	6.45[ab]	22.6[b]	3.75[b]	75.83[b]	28[b]
CV%	5.70	4.8	4.50	9.50	6.80	6.90	4.30
$LSD_{0.05}$	8.0	9.2	0.48	0.48	0.45	5.35	2.0

Note: CT1: α –NAA 0 ppm (100% of water), CT2: α –NAA 50 ppm in 3-5 minutes, CT3: α –NAA 100 ppm in 3-5 minutes. Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment.

(92.50%), and the number of leaves per stem cutting (4.05 leaves), the highest established seedling rate (86.67%), and the shortest duration in the nursery (27 days).

Thus, the treatment of α-NAA at a concentration of 50ppm (CT2) was suitable for the growth of *G. pentaphyllum*.

Conclusions

All *G. pentaphyllum* varieties in the experiments were able to produce vigor roots with a large number of roots. The G2 variety which was collected in Mai Chau district of Hoa Binh province showed the high ability to propagate with many emergence roots, fast rooting time, good propagation, and high rate of explanting. Using middle-aged stems for cuttings at 15-20cm length, with two buds in spring and winter cropping seasons and a mixture of 50% black sand and 50% soil as a growing substrate plus α-NAA 50ppm in 3-5min showed the highest explant rate and growth. The seedling standards as dermined in this research were 15-20cm in height, with 4-5 leaves per plant, containing at least 9-10 roots per cutting, and aged 30-32 days.

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