2,4-Dichlorophenoxyacetic acid soaking promotes rooting in stem tip cuttings of *Hypericum perforatum*

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Abstract

Rooting and shoot growth were evaluated on stem tip cuttings of *Hypericum perforatum* receiving various auxin soaking treatments. Five-cm-long stem tips (0.08 g fresh weight in average) were soaked in IAA, I BA, NAA and 2,4-D solutions at 5, 25, 125 μM for 10 min, and then placed in a rockwool block for three weeks. A deionized water soaking treatment was served as a control. The results indicated that the stem tip cuttings of non-treated control rooted fairly well within 3 weeks. However, IAA and NAA soakings at the given doses inhibited the rooting of treated cuttings. The stem tip cuttings of *Hypericum perforatu* rooted and developed best when they were treated with a 5 μM 2,4-D solution. Moreover, 5 μM 2,4-D soaking tended to accelerate the rooting of stem tip cutting. Thus the 5 μM 2,4-D treated stem tip cuttings might be able to transplant earlier than non-treated cuttings.

Key words:

Auxin, stem cutting, *Hypericum perforatum*, IAA, IBA,

Introduction

Hypericum perforatum is a perennial herb, which contains many biologically active constituents such as hypericin and hyperforin, has been used as a medicinal plant in Europe and Asia for centuries (6). It is reported to have anti-inflammatory, antiviral, sedative, analgesic, diuretic and vulnerary properties, and has also been used for the treatment of depression (1, 14). *Hypericum perforatum* can grow in a variety of habitats, and this plant has been introduced into many regions of the world including North and South America, South Africa, Australia and New Zealand (21). However, an introductive trial showed that the *Hypericum perforatum* grew vigorously but didn't bloom, and therefore produced no seeds under the natural conditions of Taiwan (9). Thus, a commercially acceptable asexual propagation method must be established, if *Hypericum perforatum* is expected to grow and extent successfully in Taiwan.

Stem cutting is one of major vegetative propagation techniques used in the commercial production of many plant species (3, 13, 16, 25). The new generation and subsequent growth of

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adventitious roots in the stem tissue of cutting is a crucial process in vegetative propagation. Insufficient rooting would result in a huge economic loss in large-scale propagation industry. Auxins are commonly used in commercial cutting propagation as root-promoting chemicals (4, 18, 22). Auxin solutions are generally applied to the basal portion of cutting by means of a quick dip or an extended basal soak. Indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), depending on species, cutting conditions and application concentrations, are the two endogenous auxins that best promote rooting known for years (2, 22, 26). IBA promotes rooting more effective than IAA, and it is now used commercially worldwide to root many plant species (8, 13, 20). Two synthetic compounds 1-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), which capable of exerting auxin-like effect, are also used to promote rooting in asexual propagation (2, 3, 11, 24, 26). The objectives of this study were to examine the effects of IAA, IBA, NAA and 2,4-D on rooting and initial growth stem tip cuttings of *Hypericum perforatum.* The collected data would help us to develop a protocol for vegetative propagation of *Hypericum perforatum* for the future extension of this medicinal plant in sub-tropical climate of Taiwan.

Materials and Methods

Seeds of *Hypericum perforatum* L., which were obtained from the Seed of Change (Santa Fe, NM, USA), were planted at the experimental farm of department of Agronomy, National Chung Hsing University in August 2005. Three-month-old *Hypericum perforatum* were served as the stock plants for vegetative propagation (Fig. 1A).

For vegetative propagation, 5-cm-long stem tips (0.08 g fresh weight in average) were taken from

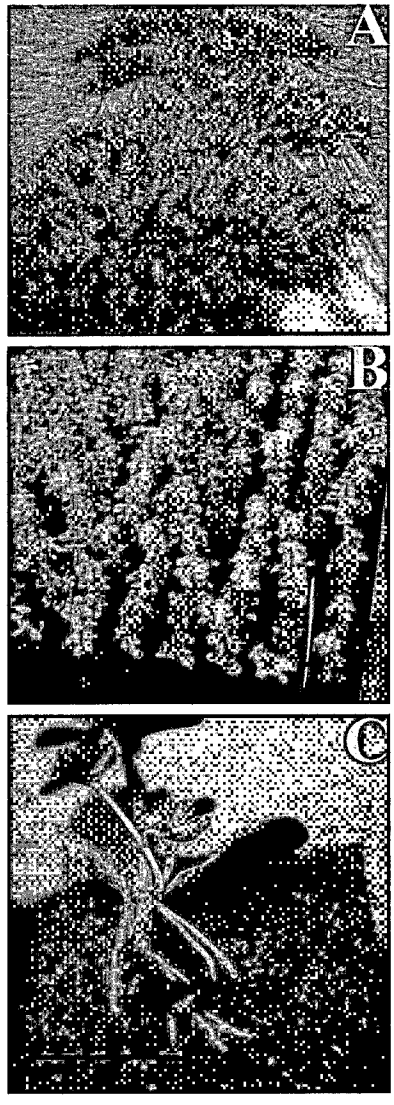

Figure 1. The field-grown *Hypericum perforatum* stock plants (A), the stem tip cuttings grown in rockwool block (B), and the developed root mass of cutting (C). Horizontal black bar represents 1 cm in length.

stock plants in the middle November 2005. Four different kinds of auxins, including IAA, IBA, NAA and 2,4-D, were used at 3 concentrations of 5, 25 and 125 μM. The basal 2 cm of the cuttings was soaked in an auxin solution for 10 min, and then placed in a rockwool block (2.5 x 2.5 x 2.5 cm) (Fig. 1B).

A deionized water soaking treatment was used as a control. All the treatments were replicated four times with 10 cuttings per replicate. The cuttings were placed under a mist system set at 60 min intervals for 10 min in a greenhouse at 25℃. Stem fresh weight, stem length, root number and root length were measured 1, 2 and 3 weeks after the experiment was started (Fig. 1C).

The experimental design was a randomized complete block design with four replications of 10 cuttings each. All data were subjected to an analysis of variance and when a significant ($P<0.05$) F ratio occurred for treatment effects, a least significant difference (LSD) was calculated.

Results

The analyses of variance for all cutting traits were presented in Table 1. Significant treatment effect was found for the number of root initiated, root length, cutting fresh weight and cutting height of 1-week-old cuttings. The effects of treatment, auxin

Table 1. The analysis of variance for cutting fresh weight (FW), cutting height, number of initiated roots and root length of 1-week-old, 2-week-old and 3-week-old *Hypericum perforatum* stem tip cuttings receiving various auxin soaking treatments.

1-week-old cuttings		F value			
Source of variation	DF	Root number	Root length	Cutting FW	Cutting height
Treatment	12	2.33**	2.40**	6.93**	8.52**
Auxin (A)	3	1.79	2.34	20.11**	15.24**
Concentration (C)	3	1.60	1.75	3.79**	10.11**
(A) x (C)	6	1.92	2.76**	1.92	4.37**
Error	147				
2-week-old cuttings		F value			
Source of variation	DF	Root number	Root length	Cutting FW	Cutting height
Treatment	12	5.48**	1.66	7.50**	4.39**
Auxin (A)	3	13.19**	1.14	21.26**	14.05**
Concentration (C)	3	1.26	3.61**	3.94**	1.32
(A) x (C)	6	3.74**	0.95	2.41*	1.09
Error	147				
3-week-old cuttings		F value			
Source of variation	DF	Root number	Root length	Cutting FW	Cutting height
Treatment	12	10.02**	3.91**	4.75**	11.70**
Auxin (A)	3	25.29**	5.20**	13.54**	38.95**
Concentration (C)	3	0.53	0.81	2.55*	0.88
(A) x (C)	6	7.12	4.82**	1.46	3.48
Error	147				

*, ** are significant at $P < 0.05$ and $P < 0.01$, respectively.

type and auxin concentration were also significant for cutting fresh weight and cutting height. Significant auxin type and auxin concentration interaction was detected only in root length and cutting height of 1-week-old cuttings (Table 1). Significant treatment and auxin type effects were also detectable for all the morphological traits of 2-week-old and 3-week-old cuttings, except for the root length of 2-week-old stem tip cuttings (Table 1). The effect of auxin concentration was found only in cutting fresh weight of 2-week-old and 3-week-old cuttings and in root length of 2-week-old stem tip cuttings (Table 1). Significant interaction between auxin type and auxin concentration was detected only in root number and cutting fresh weight of 2-week-old cuttings and in root length of 3-week-old cuttings (Table 1).

No rooting was observed for all the stem tip cuttings, including control (Fig. 2A) and auxin-treated materials (Fig. 2B), in first week (Table 2). However, both control and auxin-soaked cuttings successfully rooted in second week. An average of 8.3 roots per cutting initiated was found for control treatment (Table 2). Root number further increased

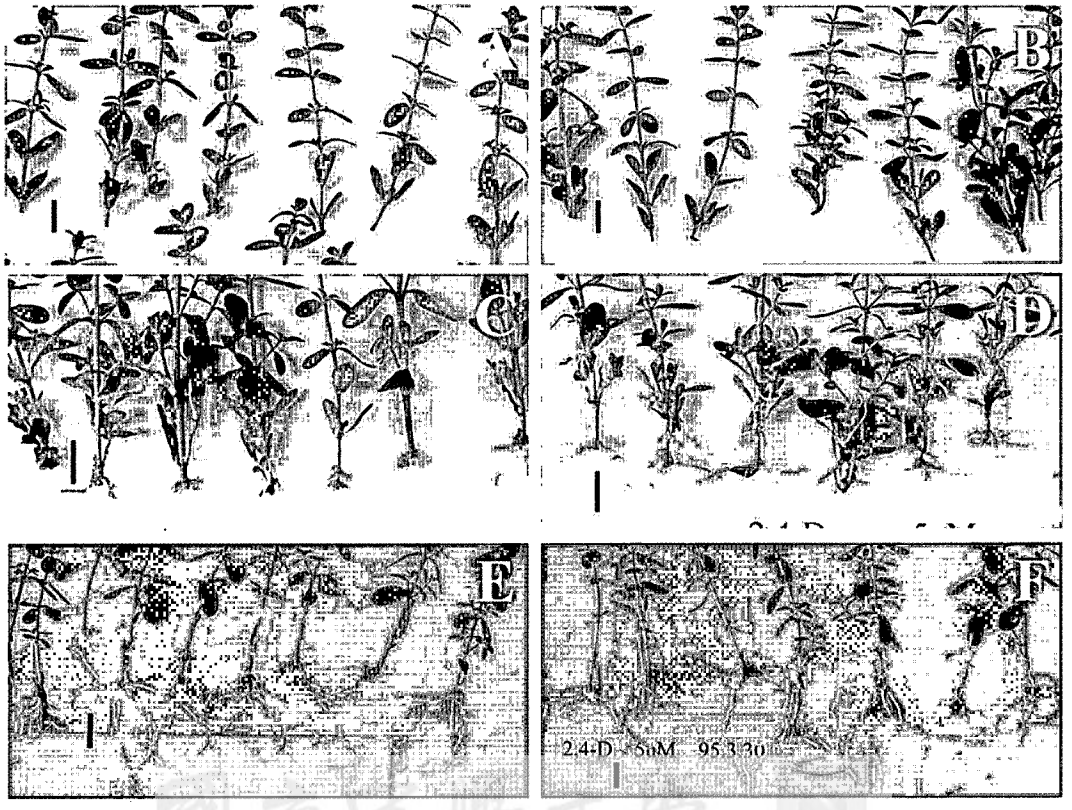

Figure 2. The root mass of control and 5 μM 2,4-D treated stem tip cuttings. (A) 1-week-old control cuttings (B) 1-week-old 5 μM 2,4-D treated cuttings (C) 2-week-old control cuttings (D) 2-week-old 5 μM 2,4-D treated cuttings (E) 3-week-old control cuttings (F) 3-week-old 5 μM 2,4-D treated cuttings. Vertical black bar represents 1 cm in length.

to 14 roots per cutting after 3 weeks for control (Table 2). However, the 5 μM 2,4-D was the only treatment significantly superior than control in rooting induction (Table 2) after 2 weeks of growth duration. This superiority in rooting induction of 5 μM 2,4-D was further intensified to 26.2 roots per cutting on 3-week-old cuttings as compared to that of control and other auxin treatments (Table 2). The 25 μM IBA also showed positive rooting response in comparison with control (Table 2). Surprisingly, Both NAA and IAA at three given concentration showed detrimental effects on rooting induction as compared to control (Table 2).

Table 2. The number of initiated roots (root number cutting^{-1}) of 1-week-old, 2-week-old and 3-week-old *Hypericum perforatum* stem tip cuttings receiving various auxin soaking treatments.

Treatment	Root number per cutting		
	1-week-old	2-week-old	3- weeks-old
Control (H_2O)	0	8.3± 3.3	14.0± 5.4
NAA 5 μM	0	6.4± 1.4	10.6± 3.3
NAA 25 μM	0.2± 0.4	6.2± 2.9	9.2± 3.2
NAA 125 μM	0	7.3± 1.5	9.7± 2.5
IAA 5 μM	0	5.1± 2.5	8.6± 3.4
IAA 25 μM	0.1± 0.3	4.0± 2.4	10.5± 4.2
IAA 125 μM	0	4.5± 4.3	11.4± 2.8
IBA 5 μM	0	6.9± 2.2	14.5± 4.7
IBA 25 μM	0	7.7± 2.6	22.0± 6.8
IBA 125 μM	0	9.9± 3.2	17.8± 5.8
2,4-D 5 μM	0.6± 1.3	12.3± 2.9	26.2± 6.7
2,4-D 25 μM	0	10.6± 3.0	13.3± 3.3
2,4-D 125 μM	0	6.2± 7.2	16.6± 10.2
$LSD_{0.05}$	0.3	2.9	4.7

Root length was highly variable among the treatments, as indicated by the relatively greater $LSD_{0.05}$ value, in comparison with that of other cutting traits (Table 3). As a result, no significant differences between auxin treatments and control were obtainable for the root length of 2-week-old cuttings. Similar trends were also found for the root length of 3-week-old cuttings, except for the 2,4-D treated cuttings that was 33% longer than that of control (Table 3). However, the majority of the cuttings receiving various types and levels of auxin soaking treatment produced less-developed root mass than that of control (data not presented). Only the 2-week-old and 3-week-old cuttings receiving 5 μM 2,4-D soaking treatment (Figs 2D and 2F) produced more root mass than their respective controls (Figs 2C and 2E).

Table 3. The root length (cm cutting^{-1}) of 1-week-old, 2-week-old and 3-week-old *Hypericum perforatum* stem tip cuttings receiving various auxin soaking treatments.

Treatment	Root length (cm)		
	1-week-old	2-week-old	3-week-old
Control (H_2O)	0	1.49± 0.91	4.53± 1.36
NAA 5 μM	0	1.49± 0.68	4.64± 1.21
NAA 25 μM	0	1.49± 0.72	5.12± 1.04
NAA 125 μM	0	1.08± 0.42	5.27± 0.97
IAA 5 μM	0	1.13± 0.47	3.69± 1.65
IAA 25 μM	0.01± 0.03	1.11± 0.79	4.47± 0.96
IAA 125 μM	0	0.62± 0.57	3.79± 0.87
IBA 5 μM	0	1.28± 0.51	3.96± 0.88
IBA 25 μM	0	1.42± 0.73	2.83± 1.10
IBA 125 μM	0	1.44± 0.68	4.40± 2.47
2,4-D 5 μM	0.06± 0.13	2.12± 0.89	6.04± 1.54
2,4-D 25 μM	0	1.50± 0.50	4.87± 1.78
2,4-D 125 μM	0	0.64± 0.61	3.10± 1.8 5
$LSD_{0.05}$	0.03	0.99	1.26

The fresh weight of control cuttings increased from 0.08 to 0.36 g cutting-1 after 3 weeks of growth duration (Table 4). The best fresh weight gains were found on the 5 μM 2,4-D treated cuttings, with the average fresh weights were 0.24, 0.30, and 0.41 g cutting^{-1} for 1-week-old, 2-week-old and 3-week-old cuttings, respectively (Table 4). Although 25 μM 2,4-D treatment gave the greatest fresh weight gain on 2-week-old cuttings, however, a minor growth retardation was found when growth duration was extended for one more week in comparison with 5 μ M 2,4-D treatment (Table 4). Both 25 and 125 μM IBA treatments gave a significant fresh weight gain on 3-week-old cuttings as compared to that of control. Nevertheless, notable growth inhibitions were found on NAA and IAA treated stem tip cuttings (Table 4).

As shown in Table 5, both 5 and 25 μM 2,4-D treatments slightly increased the height of treated cuttings after 1 week of growth duration, even though the enhancements did not reach the 5% statistically significant level. Similar responses were also obtainable on 25 μM IAA and 5 μM IBA treatments (Table 5). On the contrary, significant reductions in cutting height were noted for all other auxin treatments, particularly for 5, 25 and 125 μM NAA, as well as 5 and 125 μM IAA treatments (Table 5). For 2-week-old cuttings, only 25 μM 2,4-D showed significant cutting height enhancement response in comparison with control (Table 5). The other auxin treatments either showed statistically insignificant height enhancement or showed negative response as compared to control. Only 5 and 25 μM IBA, as well as 5 μM 2,4-D showed statistically significant cutting height enhancement on 3-week-old stem tip cuttings (Table 5).

Table 4. The cutting fresh weight (g cutting^{-1}) of 1-week-old, 2-week-old and 3-week-old *Hypericum perforatum* stem tip cuttings receiving various auxin soaking treatments.

Treatment	Cutting fresh weight (g)		
	1-week-old	2-week-old	3-week-old
Control (H_2O)	0.19± 0.05	0.24± 0.08	0.36± 0.11
NAA 5 μM	0.15± 0.04	0.21±0.05	0.26± 0.05
NAA 25 μM	0.10± 0.02	0.15± 0.02	0.23± 0.05
NAA 125 μM	0.11± 0.02	0.15± 0.02	0.27± 0.06
IAA 5 μM	0.13± 0.02	0.16± 0.02	0.25± 0.06
IAA 25 μM	0.17± 0.03	0.16± 0.02	0.27± 0.04
IAA 125 μM	0.14± 0.03	0.16± 0.02	0.25± 0.05
IBA 5 μM	0.20± 0.04	0.24± 0.06	0.33± 0.05
IBA 25 μM	0.19± 0.04	0.23± 0.06	0.39± 0.05
IBA 125 μM	0.18± 0.03	0.24± 0.08	0.39± 0.11
2,4-D 5 μM	0.24± 0.09	0.30± 0.09	0.41± 0.04
2,4-D 25 μM	0.21± 0.06	0.36± 0.09	0.34± 0.11
2,4-D 125 μM	0.18± 0.05	0.23± 0.05	0.33± 0.12
$LSD_{0.05}$	0.04	0.07	0.08

Table 5. The cutting height (cm) of 1-week-old, 2-week-old and 3-week-old *Hypericum perforatum* stem tip cuttings receiving various auxin soaking treatments.

Treatment	Cutting height (cm)		
	1-week-old	2-week-old	3-week-old
Control (H_2O)	8.25± 0.54	10.17± 1.12	13.31± 1.83
NAA 5 μM	7.66± 0.45	9.71± 1.25	10.40± 1.01
NAA 25 μM	7.13± 0.65	8.73± 0.95	9.41± 1.33
NAA 125 μM	7.28± 0.36	8.65± 1.05	12.04± 0.69
IAA 5 μM	7.53± 0.50	9.53± 1.03	11.18± 2.14
IAA 25 μM	8.42± 0.56	9.28± 0.73	11.66± 1.20
IAA 125 μM	7.51± 0.64	9.53± 0.65	11.47± 0.94
IBA 5 μM	8.40± 0.49	10.93± 1.16	15.66± 1.39
IBA 25 μM	8.12± 0.54	10.67± 1.16	15.26± 6.83
IBA 125 μM	7.91± 0.50	10.63± 1.01	14.89± 1.63
2,4-D 5 μM	8.51± 0.77	11.05± 0.96	15.18± 1.48
2,4-D 25 μM	8.69± 0.29	11.57± 1.35	14.58± 3.09
2,4-D 125 μM	7.61± 0.91	10.11± 1.74	12.52± 2.85
$LSD_{0.05}$	0.51	1.21	1.71

Discussion

IAA is the major endogenous auxin regulating the process of rooting in a wide range of plant cuttings (2, 5, 22, 26). However, IBA is also found in a variety of plants and tissues (2, 13). Biochemical analyses indicate that IBA acts primarily through its conversion to IAA in a process resembling β-oxidation (2, 26), though the roles for IBA independent of conversion to IAA have been proposed (12). In the present study, all the IBA treatments, particularly 25 μM IBA treatment, show better rooting promotion ability (Table 2) and subsequently better shoot growth than IAA treatments (Tables 4 and 5). These results are in agreement with other studies (8, 20, 13), in which they reported that when supplied to the rooting solution, IBA initiates lateral and adventitious roots more effective than IAA. The greater ability of IBA to promote rooting in comparison with IAA may result from its better stability both *in vivo* and in solution (12, 15). Moreover, IAA in plants forms inactive oxidation products following exposure to exogenous IAA (2, 20). After this irreversible oxidation, IAA level might drop below an optimal concentration for root initiation; therefore exogenous application of IBA becomes effective. Recent study of Ludwig-Muller et al. (13) confirmed that IBA is, not IAA, an important factor for rooting in *Arabidopsis*. Whether this result is also the case in *Hypericum perforatum* needs to be ascertained.

However, the lower level of endogenous IAA can not explain the poor rooting responses of IAA-treated cuttings as compared with control cuttings (Table 2). Overall results with stem tip cuttings (Table 2 and Fig. 2) suggest that *Hypericum perforatum* is an easy-to-root plant species. The stem tip of cuttings of *Hypericum perforatum* receiving no auxin treatment (control) produced an extensive root mass within 3 weeks (Fig. 2E). These results seem to indicate that an auxin treatment might be unnecessary for *Hypericum perforatum* stem tip cuttings. Trueman and Peters (23) reported that tip cutting is generally less auxin-responsive than lower segment cutting. The endogenous IAA is produced largely in shoot apical region, but the accumulated apical IAA may also be transported from young leaves and developing leaf primordia (26).

In this study, only the stem tip cuttings attached with young leaves were used for auxin treatments comparisons (Fig. 1B). It appears that the level of endogenous IAA produced and accumulated in the apical region of stem tip cutting of *Hypericum perforatum* might be adequate to promote rooting, even though the level of endogenous IAA was not determined in the present study. The failure of promoting adventitious roots resulted from the applications of exogenous IAA (Table 2) might be related to its biphasic effects. When present at low concentrations at the cellular sites of action, IAA usually stimulates roots initiation and root growth. However, inhibition of root growth generally occurs with increasing IAA concentrations (7). It appears that the exogenous applications of IAA become herbicidal when endogenous IAA supplies have already met the demand of stem tip cuttings (Table 4). On the other hand, the herbicidal level of IBA is higher than IAA (26), possibly because it is a slow release source of IAA (13). Besides, IBA is taken up and transported more slowly than IAA in a variety of systems, perhaps leaving more IBA at the plant base where it can affect root initiation (12, 13).

Both NAA and 2,4-D are two synthetic compounds capable of regulating root elongation

and root formation, and mimicking auxins in exhibiting a bisphasic mode of action (2, 3, 11, 24, 26). Low concentration of NAA has been shown to promote rooting better than IBA in several plant species (18, 19). Apparently it is not the case in stem tip cuttings of *Hypericum perforatum*. In the present study, NAA exhibits inhibitory effects at all tested concentrations, as indicated by the reduced root initiation and shoot growth (Tables 2, 4 and 5) in comparison with that of control. This might be due in part to the increased permeability of the tissue to NAA because it appears to be less dependent on auxin influx carriers for uptake into cells (10). Thus, the natural auxin IAA is rapidly catabolized, whereas the synthetic auxin NAA remains at high level within the cutting. The increased NAA level would exert herbicidal effect and inhibit root and shoot growths. The inhibitory effects of NAA might be associated with the induced ethylene synthesis and subsequent abscisic acid (ABA) accumulation, which would eventually lead to growth inhibition (7). On the other hand, basal soaking of 5 μM 2,4-D solution is of benefit for rooting of stem tip cutting of Hypericum perforatu (Table 2 and Fig. 2). The greater root mass of 5 μM 2,4-D treated cutting (Figs. 2E and 2F) would favor the better shoot growth as compared to that of control. Nevertheless, 125 μM 2,4-D solution would inhibit root initiation and elongation (Tables 2 and 3). Raghavan et al. (17) indicated that at high concentrations, 2,4-D also induced ethylene and ABA, leading to growth inhibition and senescence of Arabidopsis. Whether this notion could be applied to *Hypericum perforatu* needs to be ascertained.

Results from the present study provide a basis for the vegetative propagation of *Hypericum perforatum* by stem tip cuttings. It appears that the stem tip cuttings of *Hypericum perforatum* root easily within 3 weeks, even though no any types of auxin soaking are applied prior to planting. In fact, IAA and NAA soakings exhibit inhibitory effects on rooting of stem tip cutting. However, stem tip cuttings of *Hypericum perforatum* root best when they are treated with a 5 μM 2,4-D solution. The 5 μM 2,4-D treated cuttings initiate more roots and subsequently develop a better root system. Improved rooting for 5 μM 2,4-D treated cuttings is of benefit for shoot growth. Moreover, 5 μM 2,4-D soaking tends to accelerate the rooting of stem tip cutting. Thus, the 5 μM 2,4-D treated stem tip cuttings might be able to transplant earlier than non-treated control cuttings.

Acknowledgements

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