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Rapid Vegetative Propagation of *Curculigo orchioides* Gaertn. using Rhizome Cutting, Endangered Medicinal Herb

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***Abstract* – *Curculgo orchioides* is an endangered medicinal herb belonging to the family Hypoxidaceae. Natural regeneration of *C. orchioides* limited due to poor seed viability and germination. The effects of rhizome cutting position (rhizome bud, distal rhizome and end rhizome rhizome) on shoot regeneration and rooting ability of *C. orchioides* rhizome cuttings were investigated. The highest shoot regenerations were for the end rhizome segment whereas, the average shoot length for rhizome bud segments was highest comparing among treatments. Meanwhile, the rooting ability for were significantly higher in end rhizome segments than remaining treatments. This study to determine the best vegetative propagation protocol was carried out for *C. orchioides*.**

***Keywords* – Propagation, Curculigo Orchioides, Rhizome Cutting.**

I. Introduction

Curculigo orchioides Gaertn. (Hypoxidaceae) is one important traditionally medicinal plant in Vietnam. *C. orchioides* is mainly distributed under canopy *Pinus* forest [1]. The leaves, rhizome and tuberous root of *C. orchioides* are medicinally useful in Vietnam, China, India and Pakistan [1] [2] [3] [4]. Previous study suggested that the rhizome of *C. orchioides* was used antioxidant, hypoglycemic, hepatoprotective activity, antibacterial and antifungal activity, spasmolytic, aphrodisiac, diuretic, overcome impotence, antipyretic, antitumor activity, harmonizes the immune system, anti-inflammatory and anticancer principles [5] [6] [7] [8] [9] [10] [11] [12] [13] [14]. In traditional medicine Vietnam, *C. orchioides* was used for treatment of impotence male, less food, rheumatism urinary white female, neurasthenic [1]. Whereas, *C. orchioides* was used as a tonic for the decline treatment in physical strength in traditional medicine Chinese [2]. Further, in traditional medicine India, *C. orchioides* was used for preventing bone loss, and for treatment of various diseases including asthma and diarthrosis, as a tonic for jaundice, overcoming impotency [5] [6], [10].

C. orchioides mainly propagated from seeds in nature, as well as, it can also be propagated through rhizomes [15]. However, the seed of *C. orchioides* is low germination and *C. orchioides* of rhizome was always attacked by insects [16]. Thus, *C. orchioides* is the limiting factors for natural propagation. Furthermore, the demand using *C. orchioides* for medicine is constantly increasing. While, the quantity of *C. orchioides* in nature was quite erratic and not enough. Consequently, *C. orchioides* has led to the current endangered status [17]. Therefore, using vegetative propagated techniques to increase these plants vegetatively for conservation this plant is necessary.

There was some previous research on propagation *C. orchioides* using *in vitro* propagated [15] [16] [17] [18] [19]. Here researches were investigated the effect MS medium supplemented with hormone growth, such as NAA, 2, 4-D, IBA, BAP and KIN on shoot and rooting regeneration from leaf and rhizome explants [15] [16] [17] [18] [19]. However, the technology of *in vitro* culture requires modern equipment and is difficult to use for the community for the underdeveloped economy. In the technique of vegetative propagation outside the *in vitro* culture, the cuttings from branches, leaves, rhizome and roots are still used. Thus, we investigated effective different position rhizome cutting to shoot and rooting regeneration of rhizome explain of *C. orchioides*. The main objective of this study is comparing the effect of position rhizome cutting on shoot response, numbers and rooting of shoot in propagation of *C. orchioides*. The study will provide to best easy protocol to use vegetative propagation to create multiple nurseling for *C. orchioides* conservation in Vietnam.

II. Materials and Methods

The *C. orchioides* were assembled from Hue province understory natural forests, Vietnam (lat. 16°23´ N and long. 107°38´ E) and was classified by Dr. M.D. Tran at the Faculty of Forestry, Hue university's Agriculture and Forestry, Vietnam. *C. orchioides* rhizomes were washed thoroughly under running tap water (1 h) and soap. The rhizomes buds (RB), distal part of the rhizomes (DR) and end of the rhizomes (ER) were cut from materials. The rhizomes were also cut into pieces with each piece having a long following annual growth rhizome disc (Figure 1). The rhizome segments were placed under sand in greenhouse, maintained at 24°C day, and watered daily. Light in the greenhouse was reduced by the presence of a 50% grid cover set up above the supplemental lighting. 30 Rhizome cutting segments were raised for each treatment and all experiments were repeated three times.

Plantlets were then separated and planted on autoclaved garden soil (mixture of the garden soil: sand with the ratio 3: 1 in plastic bags. The saplings were put on a greenhouse which provided constant conditions of 25°C of day/night temperature, 50% of humidity and 8 h of photoperiod, and watered daily. Plants were then transplanted to field for evaluation.

Analysis of variance and Duncan's multiple range test was used for comparison among treatment means.

III. Results and Discussion

Effect of Different Rhizome Cutting position on number of Shoot Regeneration per Rhizome Cutting Segments

Number of shoot regeneration per rhizome segments differed significantly among treatment ($F = 52.472$, $p < 0.001$) (Table 1). A Duncan's test showed that the number of shoot regenerations were significantly higher in the ER of segments than in both RB and DR segments (Table 1).

Effect of different Rhizome Cutting position on Length of Shoot Regeneration per Rhizome Cutting segments

Comparing vegetative propagation average length of shoot regenerations per RCP segments among different treatments showed it was significantly differences among treatments ($F = 28.532$, $p < 0.001$) (Table 1). A Duncan's test showed that this average length of shoot regenerations was significantly worst in the ER segment comparing among treatment, whereas the length of shoot regeneration not differ between RB and DR of segment (Table 1).

Effect of different Rhizome Cutting position on number of Root Regeneration per Rhizome Cutting Segments

Number of root regeneration per RCP segment differed also significantly among treatment ($F = 18.583$, $p = 0.03$) (Table 1). Effect of RCPs on number of root regenerations were analysed by a Duncan's test showed that number of root regeneration in ER of segment was significantly highest comparing among RCP segments (Table 1).

Effect of different Rhizome Cutting Position on Length of Root Regeneration per Rhizome Cutting Segments

An examination of the length of root regeneration after 8 weeks treatment with different RCP segments showed that significantly differed among treatments ($F = 39.548$, $p < 0.001$) (Table 1). The result of a Duncan's analysis also showed that average root length regeneration in DR of segment was significantly highest comparing among RCPs (Table 1).

This is the first research therein effect RCPs on vegetative propagation shoot and rooting ability per RCP segment of *C. orchioides*. In our results, the shoot regeneration in ER of segment was significantly highest (Figure 2, Table 1). Thus, we suggest that ER position may be effective in vegetative propagation shoot regeneration from rhizome cutting of *C. orchioides*. In addition, the shoot length was significantly higher than in IBA alone treatment in all remaining treatments from all RCP segments, in which in RB segment was significantly highest. Similarly, Beasley [20] reported that shoot development in single-node segments excised from individual rhizome pieces was least at the proximal end with increasing activity toward the distal, as well as, multi-node sections, shoot development was least at the proximal end and greatest at the distal end in *Sorghum halepense* rhizome cutting. Hence, using ER post-harvest for *C. orchioides* vegetation propagating is economic efficiency.

In additionally, several early studies showed that IBA was more efficient in root ability of rhizome cutting. Nivot et al. (2008), used IBA treatment on root ability of rhizome sections that *Asarum canadense* produced two times and *Sanguinaria canadensis* three to four times more roots when treated, respectively, compared with the control. Further, Srivastava (2002) studied that a high cytokinin to auxin ratio has been regularly shown to elate shoot response and shoot development in *in vitro*. So, using hormone growths to creasing shoot regeneration and rooting ability in vegetation propagation of *C. orchioides* were needful for future.

IV. Conclusion

In our research, we investigated *C. orchioides* vegetation propagation using different rhizome cutting positions to examine changes in shoot regeneration and rooting ability of number and length per PCR. We showed that, the shoot and root regenerations of number in ER of segment were significantly highest comparing among treatment, whereas, average length of root per segment in DB of segments was significantly highest.

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References

[1]. T.L. Do, *Vietnamese Medicinal plants and Remedies*, Vietnam: Ha Noi Medicine Publishing House, 2004, pp. 1274.

[2]. Anonymous. 1979. *Jiyangsu college of new medicine, dictionary of Chinese traditional medicine*. People's press, Shanghai, 1363.

[3]. P.B. Bhamare, Traditional knowledge of plants for skin ailments of Dhule and Nandurbar districts, Maharashtra (India). *J. Phytol. Res*, 11(2), 1998, pp. 195-196.

[4]. N.S. Chauhan, and V.K. Dixit, 'Research Article Antihyperglycemic activity of the ethanolic extract *of Curculigo orchioides* Gaertn.,' *Phcog. Mag.*, *3*(12), 2007, pp. 237.

[5]. K.S. Rao, and S.H. Mishra, 'Studies on *Curculigo orchioides* Gaertn. for anti-inflammatory and hepatoprotective activities,' *Indian Drugs*, 33, 1996a, pp. 20-25.

[6]. K.S. Rao, and S.H. Mishra, 'Effect of rhizomes of *Curculigo orchioides* Gaertn on drug induced hepatotoxicity,' *Indian Drugs*, 33(9), 1996b, pp. 458-461.

[7]. I.I. Hejazi, R. Khanam, S.H. Mehdi, A.R. Bhat, M.M.A. Rizvi, S.C. Thakur and F. Athar, 'Antioxidative and anti-proliferative potential of *Curculigo orchioides* Gaertn in oxidative stress induced cytotoxicity: *In vitro*, *ex vivo* and in silico studies, '*Food Chem. Toxicol.*, 115, 2018, pp. 244-259.

[8]. S. Jaiswal, A. Batra and B.K. Mehta, 'The antimicrobial efficiency of root oil against human pathogenic bacteria and phytopathogenic fungi,' *J. Phytopathol*., 109(1), 1984, pp. 90-93.

[9]. C.A. Beasley, 'Development of axillary buds from johnsongrass rhizomes,' *Weed Sci.,* 18(2), 1970, pp. 218-222.

[10]. M.L. Dhar, B.N. Dhar, M.N. Dhawan, D.N. Mehrota and C. Ray, 'Screening of Indian plants for biological activity part-I,' *Indian J. Exp. Biol.*, 6, 1968, pp. 232-249.

[11]. T. Kayalvizhi, S. Ravikumar and P. Venkatachalam, 'Green synthesis of metallic silver nanoparticles using *Curculigo orchioides* rhizome extracts and evaluation of its antibacterial, larvicidal, and anticancer activity,' *J Environ. Eng*., 142(9), 2016, C4016002.

[12]. S. R. Kurma, and S. H. Mishra, 'Studies on *Curculigo orchioides* for anti-inflammatory and hepatoprotective activities,' *Indian Drugs*, 33, 1995, pp. 20-25.

[13]. V., R. Madhavan, V.,R. Joshi, A. Murali and S.N. Yoganarasimhan, 'Antidiabetic activity of *Curculigo orchioides*

root tuber,' *Pharm. Biol.*, 45(1), 2007, pp. 18-21.
[14]. K. S. Nagesh, and C. Shanthamma, 'An overview on tissue culture studies of *Curculigo orchioides* Gaertn: An endangered multi potential medicinal herb,' *J. Med. Plants Stud.*, 4(4), 2016, pp. 119-123.
[15]. H. A., Prajapati, S. R. Mehta, D. H., Patel and R. B. Subramanian, 'Direct *in vitro* regeneration of *Curculigo orchioides* Gaertn, an endangered anticarcinogenic herb,' *Curr. Sci.*, 84(6), 2003, pp. 747-749.
[16]. H.A., Prajapati, S.R., Mehta and R.B. Subramanian, *In vitro* regeneration in *Curculigo orchioides* Gaertn. An endangered medicinal herb, '*Phytomorphol.*, 54, 2004, pp. 85-95.
[17]. T. B., Nguyen, D. L. Tran, T. Nguyen, and V. D. Vu, *Vietnam Red data book, Part. II. Plants*. Vietnam: Science and Technics Publishing House, 2007.
[18]. C.B., Shende, V.S. Undal and U.S. Chaudhari, '*In vitro* propagation of *Curculigo orchioides* from rhizome bud,' *J. Agr. Tech.*, 8(1), 2012, pp. 353-362.
[19]. T. D. Thomas, 'Pre-treatment in thidiazuron improves the *in vitro* shoot induction from leaves in *Curculigo orchioides* Gaertn, an endangered medicinal plant,' *Acta Physiol. Plant* 29(5), 2007, pp. 455-461.
[20]. D. J. Woolley, and P. F. Wareing, 'The interaction between growth promoters in apical dominance. I. Hormonal interaction, movement and metabolism of a cytokinin in rootless cuttings,' *New Phytol.*, 71, pp. 781-793.
[21]. N. Nivot, A. Olivier and L. Lapointe, 'Vegetative propagation of five northern forest understory plant species from either rhizome or stem sections,' *Hort Sci.*, 43(5), 2008, pp. 1531-1537.
[22]. L. M. Srivastava, '*Plant growth and development: Hormones and environment,'* CA: Academic Press, San Diego, 2002.

Table 1. Effect of different cutting position on position on shoot and rooting ability of *C. orchioides* rhizome cuttings segments

Target	Rhizome cutting position			F	*P*
	RB	DR	ER		
Number of shoot regenerations	1.09 ± 0.01c	1.29 ± 0.11b	**2.19 ± 0.09a**	**24.472**	**< 0.001**
Length of shoot regenerations	4.21 ± 0.06a	4.56 ± 0.2a	3.12 ± 0.07b	**28.523**	**< 0.001**
Number of root regenerations	4.97 ± 0.28b	4.39 ± 0.17b	**6.4 ± 0.25a**	**18.583**	**0.003**
Length of root regenerations	4.73 ± 0.18b	**6.63 ± 0.29a**	3.80 ± 0.2c	**39.548**	**< 0.001**

RB = Rhizome bud, DR = Distal of the rhizome, ER = End of the rhizome. Lower-case letters in a cell show results of significant differences between treatments using Duncan's multiple range test (p <0.05). The same letters within each column and each row indicates no statistically significant differences. The values represent the mean (± SE) of three independent experiments. The highest statistically significant mean value was bold.

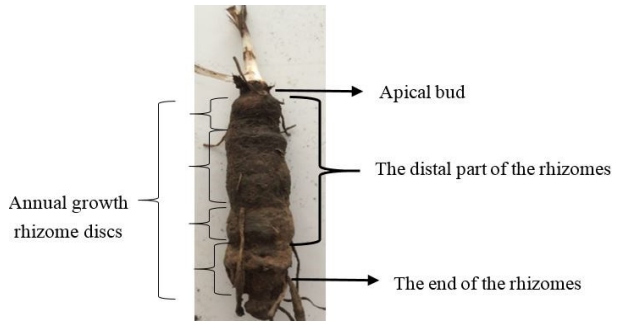

Fig. 1. The cutting position of *Curculigo orchioides* rhizomes.

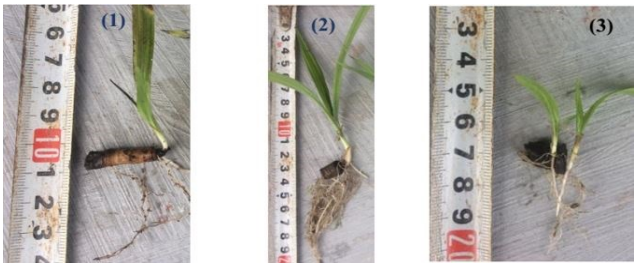

Fig. 2. Direct shoot and root regeneration from different *C. orchioides* rhizome cutting. (1), (2), (3) shows shoot and root regeneration from RB, DR, ER segment, respectively.

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