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Full Length Research Paper

Effects of different media on the transplantation of *Huperzia serrata* (Thunb.) Trev.

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Wild *Huperzia serrata* was transplanted to moss, sand, corrosion soil, experimental garden soil, vermiculite mixed with perlite (1:1), and corrosion soil mixed with garden soil (1:1). The highest survival rate (90.0%) was observed in the moss medium, the second highest rate (53.3%) was detected in corrosion soil medium, and the lowest rate was found in garden soil medium. The physiological indices of *H. serrata* in response to the six media were also determined. The dry/wet ratio of *H. serrata* was inversely correlated with the survival rate across the different media, whereas the chlorophyll content and root activity of the species were positively correlated with it. The findings suggest that moss is the optimal medium for the transplantation of *H. serrata*.

Key words: *Huperzia serrata*, media, transplantation.

INTRODUCTION

Huperzia serrata (Thunb.) Trev. is a member of the Huperziaceae family. The entire *H. serrata* plant is also called *Qiancengta* in China. The sporangia of *H. serrata* are kidney-shaped, light yellow and axillary. Spores spread out when the sporangia mature (Guo et al., 2008). Gemmules abound on the upper part of the plant, and these can develop into new plants after landing. However, *H. serrata* rarely propagates via gemmules.

H. serrata has been used for detumescence, detoxification, and analgesic therapy (Medicinal Flora Compiling Group of Zhejiang Province, 1980). One of its effective chemical compounds is huperzine A (Figure 1). Research has shown that huperzine A has strong inhibitory activity on cholinesterase, which significantly affects Alzheimer's disease in clinical care (Ma and Wu, 2000). Unfortunately, overexploitation of wild plants for commercial purposes and difficult cultivation conditions has rendered *H. serrata* practically extinct. Plant tissue culture and cuttage have hence been used to propagate *H. serrata* (Ma and Gang, 2008). Yang et al. (2008) and Liang (2010) established the tissue culture system of *H.serrata* selecting shoot tips as explants. Sheng et al. (2000) and Tan et al. (2010) adopted branches of *H. serrata* to establish its cutting propagation technique separately. Despite such efforts, many problems in the transplantation and domestication of *H. serrata* still exist (Shen et al., 2002; Sun et al., 2008; Zhou et al., 2009). In this study, we investigated the effects of different media on the transplantation of *H. serrata* to determine the most promising environment for its manual cultivation.

MATERIALS AND METHODS

Wild *H. serrata* samples were collected from Jianshi, Enshi, Hubei Province, China, and were identified by Professor Suo-nan Wei of Northwest University. Plants that were 10 to 15 cm high were specifically selected.

Transplant experiment

Different media [moss, sand, corrosion soil, experimental garden soil, vermiculite mixed with perlite (1:1), and corrosion soil mixed

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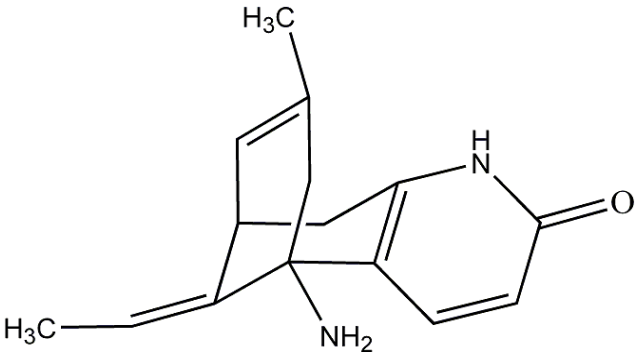

Figure 1. Chemical structure of huperzine A. Huperzine A is the effective chemical compound of *H. serrata*.

Figure 2. Growth of *H. serrata* in different media.During the transplant experiment it was found out that the plants (*H. serrata*) in moss grew better than those in the other media.

with garden soil (1:1)] were packed into pots, into which *H. serrata* was then carefully planted; this process was repeated 30 times for each medium. The media were soaked initially and watered every 7 days subsequently. The humidity, temperature, and light intensity in the greenhouse were 50 ± 5%, 20 ± 2°C and 2000 lx, respectively. After 90 days, the growth status of each plant was recorded and its physiological indices were determined.

Determination of dry/wet ratio

As previously described (Yan and Liang, 2002), the *H. serrata* plants were wholly and carefully taken out of the media. The plants were quickly washed with running water, and the residual water was absorbed with filter paper. They were measured, and their fresh weight was recorded. The plants were then oven-dried to constant weight at 60°C, and their dry weight was recorded.

Determination of chlorophyll content

Based on the method reported by Chen (2002), chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) were extracted from leaves with 80% acetone, and the optical density values of the extracts were determined at 645 and 663 nm, respectively, 80% acetone as control. The total content of chlorophyll is (Chl-r) = Chl-a + Chl-b.

Determination of root activity

Freshly sampled root segments weighing 200 mg were incubated at

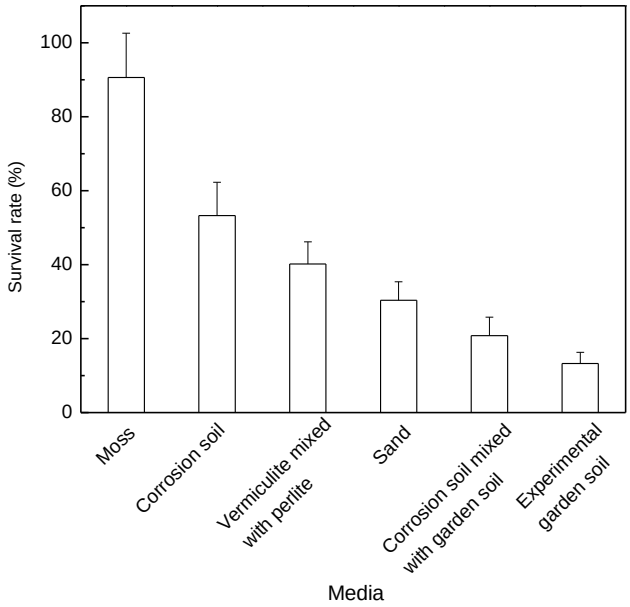

Figure 3. Effects of different media on the survival rate of transplanted *H. serrata*. *H. serrata* was transplanted to moss, sand, corrosion soil, experimental garden soil, vermiculite mixed with perlite (1:1), and corrosion soil mixed with garden soil (1:1). After 90 days, the survival rate of each medium was recorded.

25°C in 5 ml of potassium phosphate buffer (pH 7.0) containing0.4% (w/v) 2,3,5-triphenyltetrazolium chloride in the dark for 16 h (Steponkus and Lanphear, 1967) to determine the root activity of *H. serrata*. After the samples were washed three times with distilled water, intracellular insoluble formazan was extracted twice with 5 ml of 95% ethanol at 80°C for 20 min; the ethanol extracts were subsequently combined. After the extract mixture reached 10 ml, its absorbance was determined at 485 nm. Root activity was expressed as A_{485}/200 mg.

Statistical analysis

The design of all experiments was a complete randomized block, and the each physiological index determination experiment consisted of five replicate. All the experiments were repeated twice, and the data were presented by average ± standard error (SE).

RESULTS

Effects of different media on survival rate

A significant number of plants withered 3 months of post-transplantation. However, the plants in moss grew better than those in the other media (Figure 2). The differential survival rates among the six media (Figure 3), with the highest value obtained from the plants grown in moss and the lowest obtained from those grown in experimental garden soil, observed the following order: moss (0.900) > corrosion soil (0.533) > vermiculite/perlite (0.400) > sand (0.300) > corrosion soil/garden soil (0.200) > experimental garden soil (0.133).

Table 1. Effects of different media on the dry/wet ratio of transplanted *H. serrata*.

Medium	Dry/wet ratio
Moss	0.360 ± 0.021
Sand	0.392 ± 0.032
Corrosion soil	0.362 ± 0.047
Experimental garden soil	0.600 ± 0.023
Vermiculite mixed with perlite	0.378 ± 0.028
Corrosion soil mixed with garden soil	0.512 ± 0.045

After the survival rate of transplanted *H. serrata* was calculated, plants were wholly and carefully taken out of the media, this process was repeated 10 times for each medium. The plants were quickly washed with running water, and the residual water was absorbed with filter paper, their fresh weight was recorded. The plants were then oven-dried to constant weight at 60°C, and their dry weight was recorded.

Table 2. Effects of different media on the chlorophyll content of transplanted *H. serrata*.

Media	Chl-a (mg/g)	Chl-b (mg/g)	Chl-r (mg/g)
Moss	4.38 ± 0.55	11.71 ± 0.74	16.09 ± 0.62
Sand	3.03 ± 0.53	10.39 ± 0.69	13.42 ± 0.64
Corrosion soil	5.30 ± 0.39	10.14 ± 0.58	15.44 ± 0.55
Experimental garden soil	4.30 ± 0.35	6.24 ± 0.46	10.54 ± 0.44
Vermiculite mixed with perlite	4.56 ± 0.28	9.08 ± 0.77	13.64 ± 0.46
Corrosion soil mixed with garden soil	4.77 ± 0.31	7.90 ± 0.63	12.67 ± 0.51

Based on the method reported by Chen (2002), chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) were extracted from leaves with 80% acetone, and the optical density values of the extracts were determined at 645 and 663 nm, respectively, using 80% acetone as control. Then the chlorophyll content of transplanted *H. serrata* calculated by the following formula: Chlorophyll a, C_a=12.7A663 - 2.69A645 (mg/L); Chlorophyll b, C_b = 22.9A645 - 4.68A663 (mg/L); total chlorophyll, $C_r = C_a + C_b$ (mg/L).

Effects of different media on dry/wet ratio

Table 1 showed that the experimental garden soil had the highest dry/wet ratio, followed by the corrosion soil/garden soil mixture; the lowest ratio was obtained from the plants grown in moss. These findings contrasted to the survival rates.

Effects of different media on chlorophyll content

Table 2 shows that the different media significantly affected the chlorophyll content of *H. serrata* leaves after transplanting. Leaves of *H. serrata* grown in moss had the highest levels of Chl-a and Chl-b, whereas those grown in experimental garden soil had the lowest. The Chl-b content was higher than the Chl-a content for each medium.

Effects of different media on root activity

The 2,3,5-triphenyltetrazolium chloride method was used in determining the root activity of *H. serrata* grown in the six media to study the mechanism of their effects on the transplant survival rate of the species. *H. serrata* grown in moss demonstrated the highest root activity, followed by *H. serrata* grown in corrosion soil; *H. serrata* grown in experimental garden soil performed worst (Table 3).

DISCUSSION

The existing knowledge on the transplantation of *H. serrata* is very limited. Lin (2009) reported that transplant survival rates of *H. serrata* from different areas significantly differed and were much closely correlated with the transplanting media. By measuring physiological and biochemical indices after transplantation, we analyzed the effects of different media on the survival rate of *H. serrata*. We found out that moss is the optimal medium for transplantation of this species.

The root activity of *H. serrata* was positively correlated with the transplant survival rate (Tables 1 and 3). The groups with high root activity had high survival rate, whereas those with low root activity had low survival rate. The high content of chlorophyll in the moss medium and the low content in the experimental garden soil medium suggest that chlorophyll content directly affected the transplant survival rate of *H. serrata* (Table 2).

We also found out that *H. serrata* grown in moss had the lowest dry/wet ratio, indicating that high water content benefits transplanting. On the contrary, *H. serrata* grown in experimental garden soil, which had the highest

Table 3. Effects of different media on the root activity of transplanted *H. serrata*.

Media	Root activity (A_{485}/200 mg)
Moss	0.751 ± 0.032
Sand	0.630 ± 0.041
Corrosion soil	0.700 ± 0.056
Experimental garden soil	0.337 ± 0.042
Vermiculite mixed with perlite	0.632 ± 0.021
Corrosion soil mixed with garden soil	0.510 ± 0.026

Freshly sampled root segments weighing 200 mg were incubated at 25°C in 5 ml of potassium phosphate buffer (pH 7.0) containing 0.4% (w/v) 2,3,5-triphenyltetrazolium chloride in the dark for 16 h (Steponkus and Lanphear, 1967) to determine the root activity of *H. serrata*. After the samples were washed three times with distilled water, intracellular insoluble formazan was extracted twice with 5 ml of 95% ethanol at 80°C for 20 min; the ethanol extracts were subsequently combined. After the extract mixture reached 10 ml, its absorbance was determined at 485 nm. Root activity was expressed as A_{485}/200 mg.

dry/wet ratio, performed poorly in yellow leaves and stem shrinkage (Figure 2). Water supply thus proved to be very important in the transplantation of *H. serrata*. These findings reveal that the dry/wet ratio is a vital indicator of survival rate after transplantation.

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