Study on Culture Technology of Viburnum in Vitro

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Abstract: Inoculating M. spores in 1 / 4MS culture, 30 days can induce the formation of D0.2cm protophyte, and 120-150d can form test-tube seedlings. Protophytes of D0.5cm were taken for proliferation and culture, and the effects of proliferation were studied. The factors, the conditions for the differentiation of sporophyte seedlings and the technology of sporophyte test-tube seedlings, the results showed that: at a temperature of 25 ℃, light 12hr · d -1, light intensity 2000lx, 30g · l -1 was added to the medium , Agar 6g · l -1, pH5.8 and other conditions, the most suitable medium for protophyte proliferation is 1 / 2MS + KT5.0 mg · l -1 + IBA1.0 mg · l -1 + KH 2 PO 4 200 mg · l -1 + Activated carbon 0.5%. The protophyte that is more suitable for the differentiation of sporophyte seedlings has the characteristics of loose texture, large flakes and thick flesh. At 28 ° C, 90-100% humidity, 4 shades of yin and 6 shades of awning or under the forest, coconut velvet was selected as the substrate for qualified single plant seedlings, and the survival rate was 97.6%.

1. Introduction

Osmunda [1], scientific name Osmunda japonic Thunb, is a perennial perennial herbaceous plant, belonging to the fern phylum, Asteraceae, Asteraceae, commonly known as the cat's head, cattle hair, and blue-stemmed moss. It is rich in nutrition, contains a variety of essential amino acids, vitamins and trace elements, is delicious, pollution-free, and pollution-free, and is loved by consumers at home and abroad. At present, the development of Weiwei in China is mainly based on the use of wild resources. Predatory harvesting is very destructive, causing the quality of dried Weiwei to degrade year by year, and wild resources are gradually depleted. Therefore, it is of great significance to cultivate high-quality seedlings and carry out artificial large-scale production.

2. Materials and Methods

2.1 Test Materials Weiwei Spores Were Collected from Yinjiaping Township and Changyang Huozhaoping Township, Yiling District, Yichang City, Hubei Province.

2.2 Test Method the Test is Divided into Four Stages:

(1)Induction of spore germination and protophyte formation: Select spore leaves for expansion and pick when the color changes from green to brown. Carefully place them in a smooth paper bag for immediate disinfection and inoculation or store in a 4-5 ° C refrigerator. The green spike-shaped spore leaves were cut out, soaked in 0.1% washing powder water for 3 minutes, rinsed with tap water for 10 minutes, and sterilized with 0.1% $HgCl_2$ for 8 minutes, washed with sterile water 4-5 times, blotted with paper to absorb water, and spiked spore-shaped Cut into 0.5cm long sections and inoculate three mediums [2,3] of 1 / 4MS, 1 / 2MS and MS respectively. Inoculate 10 sections per bottle and repeat 9 times for each treatment. Culture conditions: temperature 25 ℃, light 12hr d^{-1}, light intensity 2000lx, sucrose 30g · l^{-1}, agar 6g · l^{-1}, pH 5.8, observe and record the spore germination, filaments (false roots) and the original after inoculation Occurrence of the leaf body.

(2) Proliferation effect test of protophyte under different culture conditions: Inoculate D0.5cm protophyte pellets into 1 / 2MS + KT5.0 mg , Place it under different temperature conditions for cultivation and observe the effect of cultivation; inoculate D0.5cm protophyte pellets into three groups of medium with different inorganic salt concentration, different KT concentration, and different add-ons for cultivation.The other cultivation conditions are the same. (1) Observe and

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record the fold increase and growth of the original leaf body.

(3) Differentiation and growth test of sporophyte seedlings: D1.0cm, different growth conditions of the protophyte were inoculated with 1 / 2MS + KT5.0 + IBA1.0 + KH_2PO_4 200 mg • l^{-1} + Activated carbon 0.5% culture medium, observe and record the sporophyte seedling differentiation.

(4) Refining and transplanting test of test tube seedlings: Give the same temperature, light, humidity, different levels, different substrates and other seedling conditions, and test to find out the most suitable conditions for test tube seedlings to survive and grow.

3. Results and Analysis

3.1 Induction of Spore Germination and Protophyte Formation in Weiwei

The germination of spores inoculated on three media is shown in the table below.

Table 1 Observation of Spore Germination

Medium	7d observation of germination under a microscope	15d visual observation of germination
1/4MS	Germination	Green velvets on spore segments and in culture
1/2MS	A little sprouting	Some green velvets are visible
MS	Very few spores germinate	No green velvets

The spores started to germinate under the microscope after 5d, and a large number of spores germinated after 7d. After the culture continued for 15d, the green velvets appeared. The velvets grew into filaments (false roots), and the false roots and flakes continued in the future. After 30 days, protophylls with a diameter of about 0.2 cm can be formed. From the table above, it can be seen that the spore germination of Weimaran is related to the concentration of inorganic salts. When the concentration is high, the spore cells divide and affect its germination. When the concentration of inorganic salts is low, Promote cell division and accelerate its germination.

3.2 Factors Affecting Protophyte Proliferation

The protophyte was cut into D0.5cm protophyte pellets and inoculated into the following different media, and the proliferation of the protophyte was counted 60 days later.

3.2.1 Proliferation Effect of Protophyte from Different Culture Temperatures

After the protophyte was inoculated on 1 / 2ms + kt5.0 + iba1.0 medium, it was cultured under different temperature conditions.The growth status of the protophyte is shown in Table 2.

Table 2 Protophyte Growth At Different Culture Temperatures

Culture temperature	Increase in average diameter (cm)	Protophyte growth
18℃	0.44	Visible roots, golden color and tight texture
25℃	0.71	The flaky body is large and obvious, the color is dense green, and the texture is loose
30℃	0.55	The flakes are many and small, the color is yellow-green, and the texture is compact

It can be seen from Table 2 that different temperatures have a greater impact on the growth of the protophyte: at lower temperatures, the protophyte growing at the lower temperature is obvious, the color is yellowish, and the texture is compact, which indicates that the pseudoroot growth at low temperature Exuberant, sheet-like growth is slow, which is not conducive to the proliferative culture of the protophyte; higher temperature is also not conducive to the proliferation and growth of the protophyte. Experiments show that a temperature of 25 ° C is more suitable for the growth of the protophyte.

3.2.2 Proliferative Effect of Different Inorganic Salt Concentrations on Protophyte

Table 3 Protophyte Proliferation Under Different Inorganic Salt Concentrations

Medium	Increase in average diameter (cm)	Protophyte growth
1/4MS+KT5.0+IBA1.0	0.69	The flaky body is large and obvious, and the texture is loose
1/2MS+KT5.0+IBA1.0	0.71	The flaky body is large and obvious, and the texture is loose
MS+KT5.0+IBA1.0	0.68	The flaky body is large and obvious, and the texture is loose

It can be seen from Table 3 that the differentiation and growth conditions of the protophyte under different inorganic salt concentrations are not much different, and are suitable for the growth of the protophyte. It can be seen that the concentration of the inorganic salt in the medium has little effect on the growth and proliferation of the protophyte.

3.2.3 Proliferation Effect of Kt At Different Concentrations

Table 4 Protoplast Proliferation At Different Concentrations of Kt

Medium	Increase in average diameter (cm)	Growth Status
1/2MS+KT0.5+IBA1.0	0.26	Obvious pseudo-roots, few lamellae, compact leaf texture
1/2MS+KT2.5+IBA1.0	0.59	The flakes are obvious, there are fewer pseudoroots, and the texture of the original leaves is loose.
1/2MS+KT5.0+IBA1.0	0.71	The flaky body is large and obvious, and the texture is loose
1/2MS+KT6.5+IBA1.0	0.56	The flakes are mostly small and small, and the texture of the original leaf is compact.
1/2MS+KT8.0+IBA1.0	0.62	Many flaky bodies and small pieces, the texture of the original leaves is compact

It can be seen from Table 4 that different concentrations of the hormone KT have a greater effect on the proliferation and growth of the protophyte. When the KT concentration is in the range of 0.5 ~ 5.0 mg The growth rate of the leaf body is accelerated, and the growth of the sheet body is obvious; when the KT concentration is in the range of 5.0 to 8.0 mg · l^{-1}, as the KT concentration increases, the growth rate of the original leaf body slows down. This shows that when KT is 5.0 mg • l^{-1} is most conducive to the proliferative culture of the protophyte. The test also shows that when the KT concentration is lower than the IBA concentration, it is beneficial to the growth of the pseudoroot, and when the KT concentration is higher than the IBA concentration, it is beneficial The growth of flaky bodies.

3.2.4 Proliferation Effect of Different Appendages on Protophyte

Based on 1 / 2ms + kt5.0 + iba1.0 (ck), add different kinds of addendum [2,3] and culture, observe the effect of protophyte proliferation and growth, and record the results See Table 4.

Table 5 Proliferation of Protophytes under Different Addenda

addenda	Increase in average diameter (cm)	Protophyte growth
$KH_2PO_4$200 mg•l^{-1}	0.78	The flaky body grows particularly vigorously with a loose texture
KH_2PO_4 200 mg • l^{-1} + Activated carbon 0.5%	0.82	The flaky body grows particularly vigorously with a loose texture
CK	0.61	Sheets grow vigorously and have a loose texture

It can be seen from Table 5 that the addition of different kinds and certain concentrations of additives to the medium has a certain promotion effect on the growth of the protophyte. The reason may be that the demand for certain elements during the protophyte growth Large, activated carbon can absorb some harmful substances generated during the growth of the original leaf body in a test

tube, which is more conducive to the growth of the original leaf body.

3.3 Differentiation of Sporophyte

D0.5cm, different growth conditions of the protophyte were inoculated on 1 / 2MS + KT5.0 + IBA1.0 + KH_2PO_4 200 mg • l^{-1} + activated carbon 0.5% medium, after After 120 days of culture, sporophyte seedlings were grown, and the number of seedlings and growth statistics are shown in Table 5.

Table 6 Protophyte Differentiated Sporophyte Seedlings under Different Growth Conditions

Protophyte growth	Differentiated sporophyte number ※	Growth of sporophyte
Tight texture, distinct pseudoroots, few flaky bodies	2.5	Color yellow-green, small leaves, thin stems, small roots, average seedling height 1.5cm, root length 1.0cm
Tight texture, few false roots, many flakes and small pieces	6.5	Color green, small leaves, thin stems, small roots, average seedling height of 1.6cm, root length of 1.1cm
Loose texture, large flaky body, thick meat	10	Strong green color, large leaves, thicker stems, average seedling height of 1.6cm, root length of 1.2cm

※Refers to the average number of spore seedlings on the D1.0cm protophyte

It can be seen from Table 6 that the growth status of the protophyte has a great impact on the formation of sporophyte in vitro: the protophyte with obvious pseudoroots and few flakes is not conducive to the differentiation of sporophyte seedlings, and the texture is loose and flake Large, fleshy protophylls have a large number of sporophyte seedlings and are small.

These sporophyte test tube seedlings were cut with D0.5cm protophytes and transferred to 1 / 2MS + IBA0.5 + sucrose 2% medium.After about 30 days of culture, the sporophyte seedlings grew to When the height is about 3cm and the heart-shaped leaves are 3-6 pieces, when the root system is 3-4 and becomes thicker, you can move to the test site for seedling cultivation.

3.4 Refining and Transplanting of Test-Tube Seedlings

The tuber seedlings of Viburnum sporophytes are divided into four different levels①Eligible single plant seedlings: Roots are more than 3 thick, the leaves are more than 3cm in length, the leaves are complete, green, and the number of leaves is more than 3, and the plants are growing well and planted as a single plant. Single root seedlings: 1-2 root systems, but the upper part is fully developed and grows well, the leaf length is 2cm, the number of leaves is more than 3 leaves, and the single plant is planted. , Bring the protophyte, more than 5 seedlings, the upper part is well developed and robust, the single plant seedlings have a leaf length of more than 2cm and more than 3 leaves. Body, number of seedlings, upper part is good, robust, single plant seedlings are less than 2cm long, 2 leaves.) Choose four different substrates (① mother soil, ② coconut velvet, ③ 1 mother soil, 1 coconut velvet mixed ④ 5 parts of mother soil, 3 parts of sawdust, 2 parts of coal ash mixed with four kinds) Transplant, cover with moisturizing net + micro-membrane to keep moisture and cool, keep the temperature 20-28 ℃, humidity 90-100%, under the condition of scattered light, Test tube seedlings were subjected to refining experiments. After the planting, the growth of the young seedlings was observed daily, and the observation results at 20 days are summarized in Table 7.

Regardless of the transplanting medium, the survival rate of the qualified single plant seedlings is the highest, followed by the first-class bush seedlings and rooted single-plant seedlings, and the second-class bush seedlings have a lower survival rate. 79.6%, 70.6%. Regardless of the level of seedlings, the survival rate of coconut velvet smelting seedlings is the highest, followed by the mixture of coconut velvet mother soil and mother soil. : 93.7%, 89.4%, 86.3%, 54.5%. The qualified single seedlings planted in coconut velvet have the highest survival rate of 97.6%. The production can be adapted to local conditions, and the substrate can be selected for easy refining and low cost for refining. As long as the seedlings are carefully managed, the survival rate can reach

85% -98%.

Table 7 Survival Rates Of Test Tube Plantlets of Weiwei Transplanted in Different Grades and Substrates

Different levels Different substrates		qualified Single plant	Rooted Single plant	First level Cong Miao	Secondary Cong Miao	total
Mother earth	Number of original bowls	184	126	100	80	490
	Survival bowl number	168	102	95	58	423
	Average survival rate%	91.3	80.9	95.0	72.5	86.3
Coconut	Number of original bowls	84	54	100	80	318
	Survival bowl number	82	46	96	74	298
	Average survival rate%	97.6	85.2	96.0	92.5	93.7
1 mother earth 1 coconut	Number of original bowls	80	80	100	80	340
	Survival bowl number	76	68	94	66	304
	Average survival rate%	95.0	85.0	94.0	82.5	89.4
5 parts mother earth 3 parts sawdust 2 parts coal ash	Number of original bowls	47	63	100	80	290
	Survival bowl number	36	41	53	28	158
	Average survival rate%	76.6	65.1	53.0	35.0	54.5
in total	Number of original bowls	395	323	400	320	1438
	Survival bowl number	362	257	338	226	1183
	Average survival rate%	91.6	79.6	84.5	70.6	82.3

4. Results and Discussion

The results showed that: at a temperature of 25 ° C, 12hr · d^{-1} light, 2000lx light intensity, 30g · l^{-1} sucrose, 6g · l^{-1} agar, pH5.8, etc. Vegetable spores were inoculated on 1 / 4MS medium and cultured for 30 days to induce the formation of protophylls with a size of D0.2cm. 1 / 2MS + KT5.0 + IBA1.0 + KH_2PO_4 200 mg L^{-1} + activated carbon 0.5% medium is conducive to the proliferation of protophyte, adding a certain amount of KH_2PO_4 and activated carbon is beneficial to the proliferation of protophyte. Loose texture, flakes The number of sporophyte seedlings differentiated by large and fleshy protophylls is good and the quality is good.

It is generally believed that hormones play an important role in differentiation, and it can control the direction of differentiation. During the protophyte proliferation of Osmunda weaves, we made different combinations of kt concentrations and found that when the kt concentration was lower than the iba concentration When the concentration of kt is higher than the concentration of iba, it is conducive to the formation of flaky bodies. This is related to the high concentration of cytokinin or kinetin which is good for long shoots and the high concentration of auxin is good for long The viewpoint of roots is the same. We also conducted experiments on the proliferation effects of different inorganic salt concentrations on the protophyte, and the results showed that the concentration of inorganic salts had little effect on the growth and proliferation of the protophyte. Both are suitable for the growth and proliferation of protophytes.

References

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