Research on the Culture Technique of Multiplication and Rooting for Sorbus sibirica Tissue Culture Seedlings

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Abstract. Sorbus sibirica has an important timber value, medicinal value and ornamental value, but the traditional breeding methods propagation coefficient is very low, in order to shorten the Sorbus sibirica breeding cycle in a short time and get a lot of adult, this test used in vitro reproductive technology Sorbus sibirica seedlings. Using primary culture plantlets success as a raw material, researched for multiplication phase and rooting stage, mainly to explore the best Sorbus sibirica multiplication and rooting culture training program, used five different ratios of propagation medium formulation 4 different ratios were rooting medium formulations. Sorbus sibirica multiplication and rooting plantlets were induced research aspects, the results were: MS + 6-BA 0.5 mg / L + NAA 0.12 mg / L + KT 0.2 mg / L + IBA 0.05 mg / L formula most favorable seedling propagation; 1/2MS + IBA 0.5 mg / L formula most favorable seedlings rooting rate of 100%, the average number of roots reached 4.6 and thick roots.

Introduction

Sorbus sibirica, Rosaceae, Sorbus, deciduous trees, fruit has a high consumption and medicinal value.With the continuous improvement of people's living standards, Siberian sorbus market demand is also growing, but Siberian sorbus traditional breeding methods of reproductive coefficient is very low. In Russia and Japan has been cultivated a fine timber forest lines and excellent economic ornamental garden lines, is a rare set of fruit, wood and ornamental in a precious tree species. Which contains great potential economic benefits. With the development of in vitro culture technology, the characteristics of fast, efficient and excellent quality, it is of great significance to try to adopt the tissue culture technology to carry out Siberian sorbus breeding.

The results showed that the rooting of four different combinations of rooting medium was selected to select the best hormone concentration in combination with the report on the tissue culture of Sorbus sibirica.

Materials and methods

Test time, place. The experiment in June 2016 to December 2016 in Jilin Agricultural Science and Technology College flower tissue culture laboratory.

Test materials. Culture seedlings, so in the selected best hormone concentration of the formula to add different concentrations of activated carbon, to compare the best amount of activated carbon, the test according to the concentration gradient six kinds of activated carbon were set up to improve the rooting rate of the tissue culture of Siberian sorbus.

proliferation of tissue culture seedlings. The tissue culture seedling of Siberian sorbus is derived from Liaoning Forestry Research Institute. Room was cultured for about 10 days to start the transfer .

Test equipment. Autoclave(Shenyang Kerui Xinghua Glass Instrument Co., Ltd.), refrigerator (US song Electric Co., Ltd.), Ultra-clean table (Sujie purification equipment company), drying oven (Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences), wipes, baskets,

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cups, glass wicks, pipettes, gauges, test tubes, measuring cups, beaker, glass rods, pipettes, ear washes, petri dishes, reagent bottles, alcohol lamps and so on glasswares.

The above instruments, utensils and glassware are provided by Flower Tissue Culture Laboratory of Jilin Agricultural Science and Technology University.

Test Drugs. WPM，$Ca(NO_3).4H_2O$，KH_2PO_4，K_2SO_4，NAA，6-BA，KT，GA_3，$NaClO_3$，84 Disinfectant，$HgCl_2$，Benzalkonium bromide, alcohol, glutaraldehyde, white sugar, distilled water and so on drugs.

Test Methods. Selection of basic medium. The modified medium was selected as the basic medium, and the modified scheme was as follows: the calcium nitrate tetrahydrate [$Ca(NO_3).4H_2O$], potassium dihydrogen phosphate [KH_2PO_4], potassium sulfate [K_2SO_4 (KH_2PO_4) 340 mg, potassium sulfate [K_2SO_4] 742.5 mg. The original formula of sucrose is replaced by white sugar, the amount of calcium chloride [$Ca(NO_3).4H_2O$] 695 mg, potassium dihydrogen phosphate [KH_2PO_4] 340 mg, potassium sulfate [K_2SO_4] 742.5 mg; 20g / L; the amount of agar added in the medium was 6 g/L and the pH was adjusted to 5.6.

Adding plant hormones and activated carbon ratio. IBA Density： Y1: 1/2 WPM + IBA 0.2 mg/L；Y2: 1/2 WPM + IBA 0.4 mg/L；Y3: 1/2 WPM + IBA 0.6 mg/L；Y4: 1/2 WPM +IBA 0.8 mg/L. Each recipe inoculated 50 bottles, in the same culture conditions for cultivation, and finally compare the most conducive to the root of a formula ratio.

Activated carbon ratio：On the basis of the comparison of the two concentrations of IBA, six kinds of activated carbon were added with different concentration of active carbon, and J1: 1/2 WPM (modified); J2: 1/2 WPM (modified) + best IBA addition + activated carbon 0.1%; J3: 1/2 WPM (modified) + best IBA addition + activated carbon 0.1%, J5: 1/2 WPM (improved) + best IBA addition + activated carbon 0.2%; J6: 1/2 WPM (improved) + best IBA addition + activated carbon 0.15% IBA + activated carbon 0.25%. Each recipe was inoculated with 50 bottles, and the optimum amount of activated carbon was compared under the same conditions.

Training process and conditions. After 10 days of cultivation from Sorbus sibirica planted from Liaoning Provincial Academy of Forestry, the seedlings were grown from the tissue culture seedlings, and the tender stems were cut into 1.0 cm pieces on the clean bench. To add different concentrations of IBA ratio of modified 1/2 WPM medium, the bottle of 4, each recipe 50 bottles, observe the rooting situation. Finally, the best addition of the best concentration of IBA concentration, the best formula in the addition of different concentrations of activated carbon, the same method will be transferred to six different concentrations of activated carbon medium, the regular observation of the rooting situation.

The culture conditions were as follows: the humidity was maintained at 80% ~ 85%, the temperature was 25 ℃, the light intensity was 1500-2000 Lx, and the photoperiod was 16 h/8. The incubation conditions were 121 ℃, 131 kPa and 20 min.

Results and Analysis

Different rooting conditions of IBA rooting medium

Table 3-1 Rooting of different concentrations of IBA rooting medium

Formula	IBA//mg/L	Number of bottles/bottle	Rooting days/d	Rooting rate/%	Average root numbers	Root form
Y1	0.2	50	16	23	2.6	The roots of a large number of calli, only a small amount of roots
Y2	0.4	50	13	72	2.8	There was a small amount of callus in the roots
Y3	0.6	50	10	85	3.8	Root no callus, root stout
Y4	0.8	50	14	84	4.1	Root no callus, root stout

It can be seen from Table 3-1 that the rooting rate of Y3 and Y4 is high, reaching 80%, indicating that the addition of IBA in the modified 1/2 WPM formula can reach the level of 0.6 mg / L, The number of days of rooting, Y3 began to take root on the 10th day, and Y1 began to take root on the 16th day, indicating that IBA amount is too small, is not conducive to rooting; comprehensive comparison of root morphology, Y3, Y4 were Y1, Y2 formula good effect , The base of the seedling can quickly become rooted, and the growth of the root is thick, the average number of roots more. And Y3, Y4 formula, although the root of the morphological difference is not large, but the rooting rate Y3 is greater than Y4, and Y3 root time earlier, you can save the rooting cycle, so the comprehensive comparison: Y3 (improved 1/2 WPM + IBA 0.6 mg / L), it was suggested that 0.6 mg / L IBA was the best on the modified 1/2 WPM minimal medium, and it was suitable for rooting culture of Siberian sorbus.

Effects of different activated carbon additions on rooting of tissue culture seedlings

Table 3-2 Effects of different activated carbon additions on rooting of siberian safflower tissue culture seedlings

Formula	IBA// mg/L	Activated carbon addition/%	Number of vaccinations /bottle	Rooting days/d	Aversge root numbers	Rooting rate/%
J1	0.6	0	50	11	3.9	84
J2	0.6	0.05	50	10	4.5	93
J3	0.6	0.10	50	8	4.6	98
J4	0.6	0.15	50	9	4.2	96
J5	0.6	0.20	50	10	4.2	93
J6	0.6	0.25	50	11	4.0	85

It can be seen from Table 3-2 that the small amount of tissue culture seedlings is inoculated into six kinds of medium with different activated carbon, and the rooting effect is good, indicating that the addition of IBA 0.6 mg / L is correct , But compared with Y2, Y3, Y4, Y5, Y6 and Y1 (control), the rooting rate was improved, indicating that the addition of activated carbon in the culture medium can effectively improve the rooting rate of tissue culture seedlings, which and Yao Lili published in 2006 (Y2, Y3, Y4, Y5, Y6). With the increase of the amount of activated carbon, the rooting rate increased first and then decreased, which indicated that the rooting rate of Y2, Y3, Y4, Y5 and Y6 was different. Increasing the amount of activated carbon can increase the tissue culture to take root, but it is not the addition of the better, there is a quantity of choice relationship. In the five additions, Y3 and Y4 had the highest rooting rates of 98% and 96% respectively, and then decreased to Y5. At Y6, the rooting rate reached 85%, and the difference was not added (Y1 was 84%) Large, so the amount of activated carbon is defined between 0.10% and 0.15%. (3 d) is also slightly larger than Y4, the average number of roots (4.6) is also slightly larger than Y3, and the comparison shows that Y4 is the same as the rooting rate of Y3, Y3 and Y4, (1/2 WPM (improved) + best IBA addition + activated carbon 0.1%) is the most suitable, that is to say, the average number of roots is better than the other. On the basis of the original optimal rooting medium formula, adding 0.1% activated carbon could greatly promote the rooting of Siberian Safflower tissue culture.

Conclusion and discussion

The results showed that the addition of IBA was beneficial to the rooting of seedling, but the degree of rooting was different in different dosage. The percentages of IBA0.4 mg/L and 0.6 mg/L were more than 80%, indicating that the modified 1/2 WPM formulation and the selection of the

auxin type IBA were correct. By comparing 0.4 mg/L and 0.6 mg/L IBA, the number of roots and the average number of roots were more, regardless of the number of days of rooting (0.6 mg/L IBA began to take root on day 10) , It is best to add 0.6 mg/L IBA on the modified 1/2 WPM minimal medium. In order to verify that the addition of activated carbon in the literature can effectively promote rooting, a comparative experiment with different concentrations of activated carbon is added to the modified 1/2 WPM + 0.6 mg/L IBA formulation. The results showed that the tissue culture seedlings were inoculated into the supplement. After the activated medium on the medium, the rooting effect is good, verify the correctness of the literature. But the different additions and rooting rates were improved, but the degree of rooting was different, but the increase of activated carbon was not the best. With the increase of activated carbon, the rooting rate increased first downward trend. Through the comprehensive comparison, 1/2 WPM (modified) + IBA addition 0.6 mg/L + activated carbon 0.1% is the most suitable, that is, adding 0.1% activated carbon can greatly promote the Siberian safflower tissue culture seedlings rooting.

Due to the relationship between time and energy, this experiment only studied the effect of the amount of hormone added and the amount of activated carbon on the rooting of Siberian Safflower Tissue, which was confined to the rooting stage of the culture chamber and the addition of other substances in the rooting stage. No impact and the survival rate after the bottle is not discussed, remains to be further studied. Siberian sorbus is a common woody tree species in the northeastern region. There are relatively few studies on its medicinal value. For the research on its cultivation, especially in the rapid propagation of tissue culture, this experiment is based on the data of the predecessors The results showed that the effect of activated carbon on the rooting of tissue culture seedlings was the conclusion, hoping to provide the theoretical basis for the tissue culture of Siberian sorbus. During the course of the experiment, especially in the pre-no-activated carbon comparison process, 0.8mg/L IBA there are individual seedlings appeared vitrification phenomenon, due to individual seedlings, this experiment did not carry out one-step experimental analysis, most people think that glass The phenomenon is due to the large humidity in the bottle and poor ventilation in the culture chamber. In this study, it is found that the high concentration of IBA bottle is easy to cause vitrification. It is hoped that this phenomenon can be further verified. In conclusion, it is hoped that the conclusions of the trial will provide a basis for the rapid propagation of Siberian sorbus, in order to accelerate the industrial breeding of Siberian sorbus.

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