

“Hiogi” Rose Open Tissue Culture and Rapid Propagation Technology

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Nowadays, many countries are using tissue culture technology to breed the fine varieties of the rose. At present, this rapid propagation method is mature and has been factory-produced. It is important to speed up the upgrading of old varieties and rapidly popularize the famous new varieties. The role. There are many reports on routine tissue culture techniques for the Chinese rose, but the open-loop tissue culture techniques have not been reported.

The operation process of the “Filipino Fan” open-end tissue culture and rapid propagation technology is as follows.

First, the composition of the medium

1. Induction medium: MS + BA1 mg/L + S106 fungicide 0.3 ml/L.
1. Growth medium: MS + BA2 mg / L + NAA 0.2 mg/L + S106 Fungicide 0.3 ml/L.
2. Rooting medium: 1/2MS + NAA 0.1 mg/L + IAA1 mg/L + S106 Fungicide 0.3 ml/L.

Second, technical procedures

Sterilization of culture flask: According to the size and quantity of the culture flask, take the appropriate amount of tap water in the container; and then add 1 liter of S105 bactericidal powder according to the amount of water every 40 liters of tap water. Stir well. Soak it in a cleaned culture flask and cap for 30 to 40 minutes. Then take the culture bottle and the bottle cap and pour the water on the clean work surface for use.

The production of sterilization medium

Take 1 liter of medium as an example: take 1L of tap water and heat it in a rice cooker. (Because of heating and evaporation, the mother liquor is boiled to 1100 ml when it is packed. It is 1L after cooling, so it is equipped with 1 liter of medium. Take 1L of tap water), weigh 30g of sugar and 4g of agar; add it to rice cooker pot water; and dissolve it all, then add 100 times MS mother liquor: Among them: a large amount of element A: 10 ml, a large amount of element B: 10 ml, iron salt: 10 ml, Trace elements: 10 ml, organic matter: 10 ml, cytokinin 1 ml of BA1 ml per ml, boiled. Adjust pH 5.8 with 1 mol NaOH or HCl, and finally add 0.3 ml of S106 bactericide solution, continue to boil for 2 to 3 minutes to mix the bactericide in the medium, and then dispense.

Pick up the culture bottle before packing, so that the bottle mouth is pressed down firmly on the bottle wall and the bottle mouth water drops, so that the bottle mouth is placed on the work surface, then the medium is evenly dispensed, and finally the culture bottle is taken. Cover the water droplets attached to the bottle cap with force, and buckle it on the bottle mouth of the poured medium, and inoculate it.

Taking materials

In the spring or autumn, after the dry dew on the sunny day in the morning, the vigorously growing “Financial Fan” rose varieties of the year, which are semi-lignified and free of pests and diseases, are brought back to the laboratory.

Remove the leaves and prickles on the branches, cut off the upper stems of the branches and the lignified stems at the lower end, and select the middle semi-lignified full and ungerminated shoots with axillary buds for explants, cut into 2 cm long stalks segment.

Material processing

First use a little detergent to drip into a clean bottle, add the finished “Fiffan fan” rose explants stem segment, add two-thirds of the volume of the bottle of water to shake and wash for 5 to 6 minutes (also can be used Place it on a magnetic stirrer for 5 to 6 minutes. Pour off the water with foam in the bottle. Find a piece of medical gauze and pour it into the bottle. Rinse the tap water until it is free of foam.

Pour the tap water, transfer the stem of the “Filipino fan” rose to a bottle treated with S105 sterilizing solution, pour 75% alcohol for 8~10 seconds, pour the tap water, immediately pour out the water in the bottle, then pour Rinse with tap water once. Pour out and drain the tap water from the bottle, pour in 0.1% mercury solution and shake for 8~12 minutes (can also be placed on a magnetic stirrer for 8~12 minutes), pouring from the mercury solution to the pouring time. Add mercury water solution to the tap water, add tap water to wash and wash for not less than 3 minutes each time, and wash continuously for 5 - 6 times.

According to the number of explants, add 0.5 ml of S106 bactericide to 100 ml of cold boiled water to prepare explant treatment solution, and transfer the phenanthrene explants to the treatment liquid. The volume of the treatment liquid is “Philippines”. 3 to 4 times of the fan’s rose explants, soak for 1.5 to 2 hours (can also be placed on a magnetic stirrer for 1.5 to 2 hours, to be inoculated.

Vaccination

The tweezers and scissors are sterilized by sterilizer or immersed in 75% alcohol by alcohol burning. The left hand picks up the tweezers, the right hand picks up the scissors, and the tweezers pick up the “Feifan” rose explants from the treatment liquid. Stem section, dry treatment attached to the explants, cut off the section of the stalk bud stem with the sterilizing treatment solution, and open the buckle in the medium bottle cap inserted into the medium in the polarity direction, one bottle A “Filipino fan” rose stem section, tighten the cap, and re-soak the used scissors and tweezers in an alcohol bottle. Take out another set of scissors and tweezers and repeat until all the inoculations have been completed.

Training

Write the inoculated culture flask with a marker on the date of inoculation and the name of the variety, and transfer it to the culture chamber for cultivation. The culture room is illuminated for 12 to 16 hours/day, the light intensity is 1800 to 2000 LX, the temperature is 20 to 23°C and the culture is for 20 to 25 days. When the axillary buds are germinated to 1 to 2 cm, subculture can be carried out. During the cultivation period, the pollution was found to promptly remove the contaminated bottle from the culture chamber.

Subculture and culture

Making bactericidal proliferation medium

For example, take 1 liter of medium as an example: measure 1L of tap water and heat it in a rice cooker, weigh 30g of sugar and 4g of agar; add it to the rice cooker pot water, add all the solution and then add 100 times MS mother liquor, among which: a large amount of element A10ml, a large amount Element B10 ml, iron salt 10 ml, trace element 10 ml, organic 10 ml, cytokinin 1 ml of BA2 ml per ml, boiled 1 ml of NAA 0.2 ml per ml. Adjust pH 5.8 with 1 mol NaOH or HCl, and finally add 0.3 ml of S106 bactericide solution, continue to boil for 2 to 3 minutes to mix the bactericide in the medium, and then dispense.

Take the culture bottle before dispensing. The bottle mouth is pressed down firmly to the bottle wall and the bottle mouth water drops. Then the bottle mouth is placed on the work surface, the culture medium is evenly distributed, and finally the culture bottle cap is taken. The water drops attached to the bottle cap are buckled on the bottle mouth of the poured medium, and are to be coagulated and inoculated.

The tweezers and scissors are sterilized by sterilizer or immersed in 75% alcohol by alcohol burning. The left hand picks up the tweezers, the right hand picks up the scissors, and the tweezers pick up the “Feifan” rose explants from the culture bottle. Sprouting the stem segments, cutting the sprouts with scissors, and discarding the original stem segments. Open the buckle in the medium bottle cap and insert it into the medium in the polarity direction. Insert a bottle of 4 buds or new stems into the stem section, twist the cap, and re-soak the used scissors and tweezers in the alcohol bottle. Soak in. Take out another set of scissors and tweezers and repeat the operation until all the inoculations have been completed. Write the inoculated culture flask with a marker on the date of inoculation and the name of the variety, and transfer it to the culture chamber for cultivation. The culture room is illuminated for 12 - 16 hours/day, the light intensity is 1800 - 2000 LX, and the temperature is 20 - 23°C. After culturing for about 30 days, the buds should continue to be cut and budded for cultivation, until the target number of seedlings is reached, and then the rooting culture can be transferred.

Rooting culture

Making bactericidal rooting medium

For example, take 1 liter of medium as an example: take 1L of tap water and heat it in a rice cooker, weigh 15g of sugar and 4g of agar, add it to the rice cooker pot water, and then add 100 times of MS mother liquor after all dissolved: Among them: a large amount of element A5 ml, a large amount Element B5 ml, iron salt 10 ml, trace element 10 ml, organic 10 ml, 1 gram of NAA 0.1 ml per ml, 1 gram of IAA 1 ml per ml, boil. Adjust pH 5.8 with 1 mol NaOH or HCl, and finally add 0.3 ml of S106 bactericide solution, continue to boil for 2 to 3 minutes to mix the bactericide in the medium, and then dispense.

Before taking the bottle, take the bottle and apply it to the bottle wall and the bottle of water. Then, put the bottle mouth up on the work surface, then pour out the medium and evenly pack it. Finally, take the bottle cap and force it. The water drops attached to the bottle cap are buckled on the bottle mouth of the poured medium, and are to be coagulated and inoculated.

The tweezers and scissors are sterilized by sterilizer or immersed in 75% alcohol by alcohol burning. The left hand picks up the tweezers, the right hand picks up the scissors, and the tweezers pick up the “Feifan” rose buds from the culture bottle. Cut into single buds or two-leaf stem segments with scissors, open the medium bottle cap and insert it into the medium according to the polarity direction, insert 10 buds or cut the two-leaf stem segments into one bottle, twist the cap and use The scissors and tweezers are soaked in an alcohol bottle and soaked. Take out another set of scissors and tweezers and repeat the operation until all the inoculations have been completed. The inoculated bottle is written on the date of inoculation with a marker and transferred to a culture chamber for cultivation. The culture room is illuminated for 12 to 14 hours/day, the light intensity is 1800 to 2000 LX, and the temperature is 20 to 23°C. Cultivate for 25 to 30 days, until all the roots are grown, and when the seedlings are 3 cm high, the bottles can be transplanted and regenerated.

Management of rooting and transplanting seedlings and management of conventional tissue culture seedlings.

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