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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 29375-2026

Replacing GB/T 29375-2012

Technical code of practice for the propagation of virus-free potato in-vitro plantlets

马铃薯脱毒试管苗繁育技术规程

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Table of Contents

Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1.Structure and Drafting Rules of Standardization Documents". Drafting. This document supersedes GB/T 29375-2012 "Technical Specifications for Virus-Free Test-Tube Seedling Propagation of Potatoes" and is consistent with GB/T 29375-2012. Compared to previous versions, aside from structural adjustments and editorial changes, the main technical changes are as follows:

a) A definition for "test-tube seedlings" has been added (see 3.8);

b) The phrase "Regular disinfection, using a

2.1 ratio of 40% formaldehyde and potassium permanganate, pour 10mL of 40% formaldehyde solution into 5g of..." has been deleted. The original text was changed to "The inoculation room and culture room should be kept clean, and fumigation should be carried out weekly." Skip the disinfection step. Disinfection can be achieved through methods such as chlorine dioxide or ozone fumigation in a sealed environment, or ultraviolet light irradiation combined with 75% ethanol spray. (See 4.3.1,

4.4.1 in the.2012 edition);

c) The UV lamp time has been changed from "20 min" to "30 min" (see 4.3.3,

4.4.3 in the.2012 version);

d) The section on using greenhouses as cultivation rooms has been removed (see

4.4.6 in the.2012 edition);

e) Added the requirement to "inspect the seedling growth status daily, promptly clean up any contamination, and disinfect the culture room" (see 4.3.6);

f) Added detection methods for viruses and viroids, and removed "detection according to the methods in Appendices A and B of GB 18133-2000" (see...). 6.1.2 (

5.1.2 in the.2012 version);

g) The specific method for artificially breaking dormancy, "soaking seeds in a 1% thiourea 5 mg/L gibberellin solution for 5 minutes," and the germination method have been deleted. "Surface disinfection can be achieved by soaking in a 0.1% carbendazim solution for 30 minutes or by spraying with 75% ethanol, and then placing it in the dark at 25°C." Germination; or sowing in sterilized fine sand with a moisture content of 20% (water containing 5 mg/L carbendazim) (see

4.1 Layout

4.1.1 There are no pollution sources in the surrounding area, and the conditions for isolation are met.

4.1.2 The layout of the production workshop should follow the principles of "clean environment, easy operation, and convenient disinfection".

4.1.3 The entrance should have an air shower system and be equipped with changing rooms, washing rooms, preparation rooms, sterilization rooms, storage rooms, inoculation rooms, and culture rooms. Each room should... isolation.

4.1.4 The culture room has sufficient natural or artificial light.

4.1.5 A schematic diagram of the layout of the virus-free seedling production workshop is shown in Figure 1. Figure

4.2 Equipment and Reagents

4.2.1 Configuration room equipment and appliances. see Appendix A, A.1.

4.2.2 Equipment and utensils for cleaning and sterilization rooms. see A.2.

4.2.3 Equipment and instruments for the vaccination room. see A.3.

4.2.4 Equipment and instruments for the culture room. see A.4.

4.2.5 Reagents. See A.5.

4.2.6 Shoot tip culture medium. see Appendix B.

4.2.7 Murashigi-Skug medium (MS medium). see Appendix C.

4.2.8 Propagation medium. see Appendix D.

4.2.9 Preservation of culture medium. see Appendix E.

4.3 Hygiene Requirements

4.3.1 The inoculation room and culture room should be kept clean and disinfected at least once a week. Chlorine dioxide disinfectant, ozone fumigation, or ultraviolet disinfectant can be used. Disinfection is achieved through a combination of external light irradiation and 75% ethanol spray.

4.3.2 The vaccination room should be filtered and ventilated before vaccination.

4.3.3 When using a laminar flow hood, the UV lamp should be turned on 30 minutes in advance. During inoculation, turn on the fan, turn off the UV lamp, and clean the laminar flow hood surface and... The inner walls were disinfected by wiping with 75% ethanol.

4.3.4 Tweezers, scissors, scalpels, dissecting needles, and other tools should be sterilized before use, and the parts that come into contact with plant material should be cauterized or inserted into a high-pressure area each time. Sterilize in a warm sterilizer and use after cooling.

4.3.5 Staff should wear sterilized work clothes, wash their hands thoroughly, and wipe their hands and work surfaces with 75% ethanol during the operation.

4.3.6 Inspect the growth of the test-tube seedlings daily, remove any contamination promptly, and disinfect the culture room.

5.Procedure for propagating virus-free potato seedlings in vitro The potato virus-free in vitro seedling propagation procedure includes nine stages. The shoot tip culture stage is further divided into four steps; in the second stage, the seedlings are examined... If the test results confirm the absence of (virus-like) viruses, stages 3, 4, 5, 6, 7, and 8 can be omitted. The program flowchart is shown in Figure 2.

6.1 Selection of Detoxification Materials

6.1.1 Field Selection From the budding stage to the flowering stage, select healthy plants with typical traits of the original variety and strong growth vigor, mark them, and harvest from the high-yielding plants. Tubers free from disease spots, insect infestation, and mechanical damage, and conforming to the characteristics of the variety, are selected from marked individual plants as virus-free material. Alternatively, tubers with the original characteristics can be selected during the seedling stage. The tips or axillary buds of the lower short branches of healthy plants with typical varietal traits were used as virus-free

materials.

6.1.2 Virus detection and screening Selected tubers or plants were tested for potato spindle tuber viroid (PSTVd) according to the identification method of GB/T 31790, and potato X... Virus (PVX) was detected according to the identification method of GB/T 36833, and potato virus Y (PVY) was detected according to the identification method of GB/T 36816. Potato M virus (PVM) was detected according to the identification method of GB/T 36846, as well as Potato Leaf Roll Virus (PLRV) and Potato A virus. PVA and Potato S virus (PVS) were detected using reverse transcription polymerase chain reaction (RT-PCR). Blocks free of PSTVd were screened. Stems or plants can be used as basic materials for stem tip virus removal; if virus-free tubers or plants are detected, they can be directly propagated.

6.1.3 Germination treatment and virus inactivation The procedures for germination treatment and virus inactivation are as follows:

a) Potato tubers can be sprouted naturally or through human intervention to break dormancy and encourage sprouting.

b) For potato tubers containing S virus or X virus, place them in an incubator at (37±1)°C when the new shoots reach 2cm~3cm to inactivate the virus. 3 to 4 weeks.

6.2 Shoot tip culture

6.2.1 Preparation of shoot tip culture medium The procedure for preparing shoot tip culture medium is as follows:

a) See Appendix B for the formulation of shoot tip culture medium.

b) Quickly dispense the prepared shoot tip culture medium into containers and seal them.

c) Place the dispensed containers in an autoclave and autoclave at 121°C (

1.1 kg/cm^2) for 20 minutes. Cool and let stand for 3-5 days. Contaminated culture medium is kept on standby.

6.2.2 Material disinfection After treatment 6.1.3, take the buds 2-3 cm long from the tubers, remove the outer leaves, rinse with running water for 30 minutes, and transfer to the inoculation room for ultra-clean work. Soak the sample in 75% ethanol for 30 seconds, rinse with sterile water, then soak in 1% sodium hypochlorite for 7-10 minutes, and rinse four times with sterile water. 5 times.

6.2.3 Shoot tip stripping and inoculation Using a 40x binocular dissecting microscope, remove the outer leaves and peel away the stem tip until a smooth, semi-circular growing point is exposed. Use a scalpel or dissecting needle to cut off the graft. The growing points of 1-2 leaf primordia (0.1mm-0.3mm) are quickly placed on the surface of the shoot tip culture medium. The mouth of the container is sterilized with an alcohol lamp and sealed. Please indicate the serial number, variety name, and vaccination time.