MAY 10, 2023

Protocol status: Working
We use this protocol and it's working

Created: Apr 26, 2023

Last Modified: May 10, 2023

PROTOCOL integer ID: 81046

Spore production of the wheat stripe rust pathogens in control growth cabinet.

Ramawatar Nagar[1]

[1]ICAR-National Institute for Plant Biotechnology

Ramawatar Nagar
ICAR-National Institute for Plant Biotechnology

ABSTRACT

Rust are biotrophic pathogens that can only be grown on the living organism and can not be grown in a lab on artificial media. Hence, the only tissue available for biological experiments are spores. Spores are complex structures that are not suitable for many biological experiments, for example, nuclei extraction and high molecular weight DNA extraction. Spores can be germinated without the need of the host. While germinating, spores from nuclei get into the germinating tube. The germinating tube can then be used for extracting nuclei and high molecular-weight DNA. However, the spore germination is not consistent. The proportion of germinated spores can vary a lot from one trial to another. To get consistent spore germination, I standardized conditions which are discussed here in the protocol. Similarly, extracting nuclei from the generation spore is not stringent forward ether. After a lot of trials with different tissue disruption methods and other conditions, I could get rust nuclei extracted and purified/enriched with Fluorescent-activated cell sorting (FACS).

Freshest spores germinate: It is not just the freshly collected spores, but the time between the sporulation and spore collection also affects spore germination. PST spore germination decreases progressively from the time of sporulation to the time of collection to finally putting them for germination. Spores collected between one or two days of sporulation give the best spore germination.
Therefore, enough plants should be infected to get the desired amount of spores in a single harvest within one or two days of sporulation.
Determination of good spore germination: I judge spore germination based on the recovery of ungerminated spores during the washing of mycelium mass with 0.1 % Triton X-100. To remove the germinated spores, from the mycelium, I wash the mycelium mass with 0.1 % Triton X-100 in MillQ water and filter through the 100-micron mash. The ungerminated spore filter through 100-micron mash, while the mycelium mass forms a clump which stays on the top of the filter. The amount of ungerminated spore recovered in the 100-micron filtration and the size of the mycelium clump give a fair indication of how good the germination was.
Spread of spores on the water surface: PST spore germination is also affected by how thinly the spore spread on the water surface. The thinner the spore spreads, the better the spore germination. Thinner spore-spared spores would have more access to the moisture and therefore will take part in the germination. With so many trials and run, I realized that 400-milligram spores on a Pyrex baking tray of size 33X23XX5 cm (13X9X2 inches).
Drowning of spores while spreading on the water surface also affects the spore germination: spores should be very gently spared on the water surface. Spores drown in water and never germinate; therefore to get a good spore germination, it should be kept in mind to spread spores as gently as possible. To spread spores gently as possible, I prepared a spore dispense with the help of a 50 mL falcon tube and a piece of 100-micron nylon mash. With this spore dispenser, the drawn spores are the least.
Humidity: humidity and moisture also affect spore germination. To ensure a good humid condition, I keep the germination tray in an enclosed box and spray a lot of water before closing the box.
Germination time: PST spore germination is not synchronized and germination increase with the time of incubation. I determine spore germination by the amount of ungerminated spore recovered during the washing of mycelium mass with 0.1 % Triton X-100. The recovery of the ungerminated spore reduces with incubation time over 18–24 hours. The least amount of ungerminated spore is recovered at 36 hours of incubation. Incubation over 36 hours further reduces the recovery of ungerminated spores but affects the nuclei preparation. Nuclei preparation from the spore kept for more than 48 hours turn out to be very clumsy and cloudy. To conclude, the best germination archived between 24-36 of incubation at 10 C.

MATERIALS

OTHER MATERIAL:

Pyrex baking tray (33X23XX5)
-Seeds: Morocco wheat variety (susceptible to Pst79)
-Spores: Pst79 (Pathotype: 104E137A-)
-Soil: Martin mix
-Grow regulator: Maleic hydrazide (initial concentration: 270g/L final concentration: 1.1g/L)
-Fertilizer: 1 spoon in 1L of water
-Talc (Sigma)
Water bath at 42C
Plastic containers

1 Spore Production

1.1 For spore production we normally use 30 pots and sow around 10-15 seeds per pot. The seeds shouldn't be sow very deep in the soil.

1.2 Put them in control growth cabinet and add some fertilizer to the surface of each pot. After that don't water the plants for 5 days.

1.3 After 4-5days or when the seeds germinate and the coleoptiles are around 2-3cm long, add 20ml of Maleic hydrazide to each pot. The soil should be dry.

1.4 When the first leaf is 10 cm long, water the plants again and now you can infect the leaves.

2 Infection

2.1 Before infection fill plastic container with 2-3 cm of water. Put~15 pots per container and take them to the old building for infection.

2.2 From the -80C spore stock, take

3 Spore collection

> **Note**
>
> Before and during spore collection make sure no water makes contact with the spores. That could trigger germination and therefore reduces the viability of spores

3.1 After 12 days of infection collect spores in a long beaker. This first batch is going to be pale because is more talc than spores.

Fig1: Plants after 12 days if infection

3.2 Tape the tubes in the speedvac in the second floor, without setting a temperature. Run for 20 min.

3.3 Label tubes and place them at -80C

3.4 After 14, 16 and18 days after infection repeat above steps. The amount of spores should be higher and brighter now on.

4 5-1ml of spores and put them in the water bath for 4min

4.1 Mix the spores with talc in a small beaker (in a final volume of 20ml) until is homogenous

4.2 For infection spray water in the leaves and then spray spores. Repeat that twice

4.3 Close containers and place them in the fridges (old building), the temperature should be 9C

4.4 Keep container for 2 days in the fridge then move pots to cabinet #24 in the CEF.