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Sexual crosses of *Zymoseptoria tritici*

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Protocol status: Working

We use this protocol and it's working

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Prepare plant material

- 1 Use a susceptible variety. Sow wheat into plastic pots (e.g. 22×18×17cm, 50-60 seeds/pot). Note: smaller pots with less plants are also ok but, I guess, high plant density play an important role by keeping high humidity in between the plants. Wait 2 to 3 weeks till the third leaf is well developed. Note: normally we performed the inoculation @ day 17 after sowing.

Picture: 17 d old Drifter plants ready for inoculation. Note: the plants density should be higher

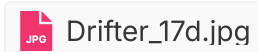
Greenhouse conditions

16 h light (6:00 – 22:00); 8 h dark (22:00 – 6:00)

Temperature: 18°C day, 15°C night

Humidity: 70%

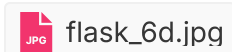
After 14 to 21 days (it depends on the growing conditions and the cultivar) the plants are ready for inoculation. Note: the third leaf should be developed.



Prepare fungal material

- 2 Inoculate 50 mL **YSB** liquid media (on 100 mL Erlenmeyer, EM) amended with 50 µg/mL kanamycin with the desired *Z. tritici* strains (e.g. inoculate the liquid media with 30-50 µl of spores from the -80°C stock). Note: choose isolates with "PCR-proof" contrary mating type and the desired characteristics. Note: the spore inoculum can also be produced on YMA plates. Shake the EM @ 120-140 rpm for 5 to 7 days (@ 18°C).

Picture: 6 days old inoculum of *Z. tritici* ready for be used for the inoculation



Inoculation

- 3 Transfer the inoculum from EM to 50 mL Falcon tube and centrifuge @ 3750 rpm⁻¹ for 10-15min (see picture). Gently decant. Resuspend the cell pellet into 20 to 30 mL water, vortex and count the spores (e.g. with a Thoma chamber).

Picture: Pelleted spores in 50 mL Falcon tube

Prepare total 60 to 100 ml mixed spore suspension, each strain in a conc. of 10⁶ cells ml⁻¹

¹. Note: the total volume depends on the number of pots and plants. Add 0,05 %

TWEEN20 to the spores solution (e.g. 50 µl into 100 mL spore solution). Inoculate the plants by spraying till “run off” (e.g. cosmetic sprayer).

Cover the pots with a plastic bag to ensure 100% humidity (see picture). Incubate for 48-72 h. After 48-72 h remove the plastic bags. Note: it is recommended to watering well the soil before spraying in order to have a good humidity during the incubation.

Picture: Plants covered with plastic after inoculation.



pellet_falcon.jpg



inoculated_plants.jpg

Incubation

- 4 After 2-3 weeks in green house pycnidia should be visible. Place the plants into pseudothecia maturation conditions by transferring the pots outside the greenhouse (spring and fall weather conditions work best). Cover the pots and the plants with a net, e.g. cheesecloth (see picture). Note: water the soil regularly.

Start to monitor for the release of ascospores after 2-3 weeks outdoors, then every week until release of the ascospores is observed. The leaves must start to degrade to get mature pseudothecia. Another possibility is to lay the infected leaves on the soil when pycnidia become visible. Water frequently, if necessary every 2-3 days for 3-5 weeks. Note: Release of ascospores must be checked every week.

Picture: Infected leaves on the soil.

Picture: Infected plants covered with cheesecloth outdoors.



infected_leaves.jpg



plants_outdoors_covered.jpg