AMES, IOWA
Iowa State University

A sterilization protocol for field-harvested mature maize seed used for in vitro culture and genetic transformation

--Martinez, JC; Wang, K

In vitro tissue culture techniques require not only an aseptic work environment but also contaminant free starting materials (Dixon, Plant Cell Culture, a Practical Approach, IRL Press, 1985). Two common contaminants affecting in vitro-cultured tissues are bacteria and fungi. Enrichment of the media used for in vitro culture makes explants susceptible to these microorganisms (Leifert and Waites, Int. Soc. Plant Tissue Cult. Newsletter 60:2-13, 1990). Despite careful attention while applying sterilization techniques, contamination still may cause 100% loss from field-grown material due to large numbers of microorganisms present in the explants (Skirvin et al., In Vitro Cell Dev. Biol. Plant 34: 278-280, 1999). One of the first steps in the process of plant genetic transformation is to acquire sterile plant materials for in vitro tissue culture.

For maize, a well-established protocol exists for the sterilization of initial material when immature embryos are used (Frame et al., In Vitro Cell Dev. Biol. Plant 36:21-29, 2000). However, no effective sterilization protocols are reported for mature maize seeds, especially for field-harvested seeds that typically carry large amounts of air- and soil- borne pathogens. One strategy to overcome the contamination problem in mature seed sterilization is simply to start with large quantities of seeds, which increases the chances of obtaining adequate numbers of sterilized seed for in vitro culture. Alternatively, a method that prevents introduction of these contaminants into the laboratory in the first place would be ideal.

Here we report an efficient and reproducible seed sterilization method, which was developed specifically for mature maize seeds harvested from field-grown plants. The method includes three major disinfection stages. First, the maize seed surface is disinfected and soaked in sterile water, then the softened seeds are disinfected and the mature embryos isolated. Finally, the dissected mature embryos are disinfected before being placed on plant media for culturing. Using this step-wise sterilization method, an average of 98% sterile mature embryos are obtained, and plant vigor (measured by Seedling Growth Rate; ISTA, Seed Sci. Technol. 27(Suppl.):27-32, 1999) is not compromised compared to the non-sterilized control (Martinez, 2008).

Materials. 1) Mature maize seeds harvested from field-grown plants. 2) 80% Ethanol (~300 ml for ~200 seeds). 3) 50% bleach solution: mix 450 ml of commercial bleach (5.25% hypochlorite) with 450 ml of Millipore water containing 2 drops of the surfactant Tween-20. Use ~900 ml for ~200 seeds. 4) 15% bleach solution: mix 15 ml of commercial bleach (5.25% hypochlorite) with 85 ml of Millipore water containing 1 drop of the surfactant Tween-20. Use ~100 ml for ~200 dissected embryos. 5) Millipore water (autoclaved). 6) Petri dishes: 60 x 20 mm, 100 x 15 mm, 150 x 15 mm

Methods. First seed surface disinfection and seed softening: 1) Place 50 seeds in a 250 ml beaker along with a stir bar. 2) Add ~ 75 ml of 80% ethanol, cover with the aluminum foil and place the beaker on a stir plate. Stir at medium speed for 3 minutes. 3) Take the beaker to the flow bench and decant the ethanol into a liquid-waste container. 4) Add ~ 75 ml of 50% bleach solution, cover with the aluminum foil and stir for 15 minutes on medium speed. 5) In the flow bench, decant the bleach into the liquid-waste container. 6) Sterilize the seeds a second time by repeating Steps 4 and 5. 7) Rinse the seeds 5 times with sterile Millipore water (~ 75 ml each time). 8) After the last rinse, keep seeds in ~50 ml sterile water (just enough to cover the seeds--do not overfill the container), cover with aluminum foil and leave the beaker inside the flow bench for 24 hours. This treatment serves to soften the seed coat and endosperm for ease of embryo dissection.

Second seed surface disinfection and embryo dissection: 9) After 24 hours, sterilize the softened seeds once with 50% bleach solution for 2 minutes while stirring (covered). 10) Decant the bleach and rinse the seeds 5 times with sterile Millipore water. 11) Leave seeds covered with last wash so they do not dry out. 12) Move sterilized seeds to a 150 x 15 mm petri dish in groups of 3 or 4 for embryo dissection. 13) Dissect the mature embryo using bent nose forceps to hold the middle of the seed with the embryo facing up. First, cut longitudinally along both sides of the embryo to remove the endosperm flanks. Next cut the base of the seed away, including two thirds of the root (tip) portion of the embryo axis. Finally, cut away the remaining endosperm from the top end of the embryo being careful not to injure the apical end of the embryo. The remaining endosperm can then be peeled away from beneath the embryo. Gently lift the embryo away from the seed carcass using the scalpel and place it in a 60 x 20 mm petri dish containing 10 ml sterile distilled water. 14) Repeat Step 13 until you dissect all the seeds.

Embryo sterilization: 15) Using a sterile pipet, remove water from the petri plate containing the dissected embryos. 16) Add ~15 ml of 15% bleach solution to the petri plate, close the lid and place on a table top shaker (low speed) for 5 minutes. 17) Remove the bleach solution using a sterile pipet. 18) Rinse the embryos 4 times (15 ml each time) with sterile Millipore water. 19) Embryos are ready to be placed on a plant medium. Place about 10 embryos per 100 x 15 mm petri plate of medium. 20) Check the plates after 2, 3 and 4 days and if contamination is found, move uncontaminated explants to fresh medium.

Acknowledgements. We want to thank Bronwyn Frame, Susana Goggi and Christian Erik for comments and advice, Diane Luth, Susana Goggi, Bronwyn Frame and Thomas Loynachan for providing supplies, equipment and facilities when needed, and Javier Garcia, Jessica Zimmer and Anita Dutta for technical assistance and Bronwyn Frame for critical review of the protocol.

BEIJING, CHINA
Chinese Academy of Sciences

Studies on Zhuo-Zi No.1, a purple hybrid in maize (*Zea mays* L.)

--Wang, Y; Zeng, M

In this paper, we describe the research status quo on Zhuo-Zi No.1, a purple hybrid in maize (*Zea mays* L.). We have a breeding program for purple maize, using information about genetic loci summarized by Coe et al. (1977).

Please Note: Notes submitted to the Maize Genetics Cooperation Newsletter may be cited only with consent of authors.