Culturing and Crossing *Marchantia* - Plants

Basic culture condition is as follows if not otherwise mentioned.

- Light: continuous, 50 - 60 µmol photon m^{-2} s^{-1} (roughly same as the light intensity 30 - 40 cm under 2 or 3 white fluorescent tubes)
- Temperature: 22°C (growth is inhibited over 30°C)

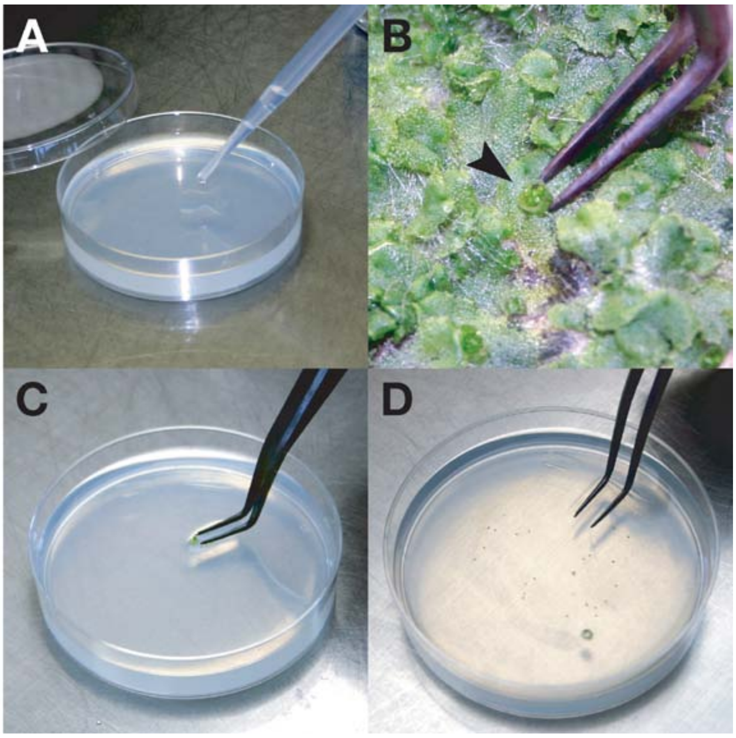
Marchantia can be grown on aseptic media or soil. Aseptic culture is suitable for maintenance and storage of lines and also for some types of experiment, but cross under aseptic condition has not been successful. It is currently necessary to grow plants open air to cross.

Aseptic culture

For aseptic culture of *Marchantia* plants, half strength Gamborg's B5 with vitamins is used. Sucrose (1% w/v) may be added to enhance growth and gemma production, but could affect morphology: thallus becomes less expanded and 'clumpy'. Either 1% w/v agar (inexpensive, but translucent) or 0.3% w/v Gellan gum (transparent) can be used to make plates. In Kohchi lab, about 1 cm-thick medium is set in disposable round plastic dishes (9 cm in diameter, 2 cm in depth). After transferring plant materials, breathable tape, such as 3M Micropore Surgical Tape 1/2in, is recommended to seal the plates.

Plants grown on sucrose-containing media under continuous light well produce gemmae, which are suitable for subculture described below.

- Put a couple of drops of sterile water in the center of a fresh plate (A)
- Harvest a cupule filled with gemmae (B)
- Transfer to the plate (C)
- Spread gemmae on the surface (D)



Marchantia thallus regenerates well, and thus cut pieces of thallus can be used for subculture. Meristems in such pieces continue to grow as they have been, and pieces without meristem regenerate. More growth can be expected with gemmae than with cut pieces.

Open air culture

Marchantia plants grow well on 3-4 cm-thick vermiculite soaked with 1/500-1/1000 Hyponex in Tupperware or other containers. It is critical to keep vermiculite moist. *Marchantia* itself seems tolerant to over-watering, but it often causes outbreak of fungi and algae and thus is not recommended. If plants grow well, give 1/500-1/1000 Hyponex every 2-3 weeks.

Thalli are susceptible to dessication. It is important to keep plants out of blow from air conditioners. On the other hand, excessive humidity causes morphological changes, sterility, and microbial problems. Use of an airtight container should be avoided unless it is absolutely necessary.

Cross

<u>Transition to reproductive phase</u>

Far-red (FR) light (730 nm) is essential for transition to reproductive phase in *Marchantia*. Regular white fluorescent tubes lack FR, and thus FR must be supplemented to induce the transition. From our experience, FR is continuously required during normal development of sexual organs. The following factors are required for the transition.

- long day
- FR light
- moderate temperature (15-22°C, higher temperature, over somewhere between 25-30°C, seems to inhibit the transition)

LED

LED is a superior source of monochromatic light with less energy consumption and longer life time, and has been applied to growing plants. Kohchi lab uses an LED system (FR-LED module:MIL-IF18(A), Module holder:MIL-U200, Controller:MIL-C1000T) purchased from a Japanese company, SANYO Electric Co. Ltd. The specification of the FR-LED are as follows:

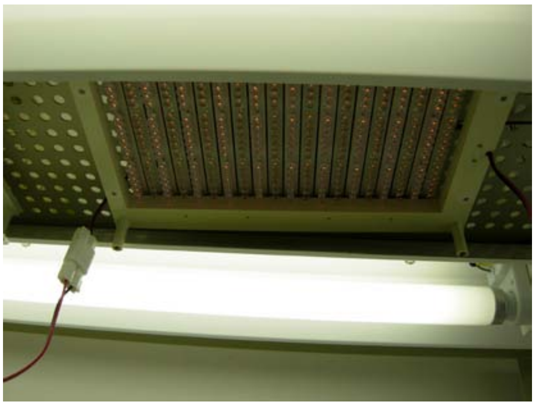
- Peak wavelength: 734 nm
- Half-band width: 13 nm

LED arrays are placed between white fluorescent tubes, and plants are put right under the arrays. FR-LED is very potent, but one bad thing is, it's relatively expensive

It usually takes about two weeks to visually recognize developing sexual organs. In the case of male plants, further couple of weeks are required to mature, *i.e.*, become capable of releasing sperm.

LED arrays and *Marchantia* plants.

LED array with a light-scattering plate.

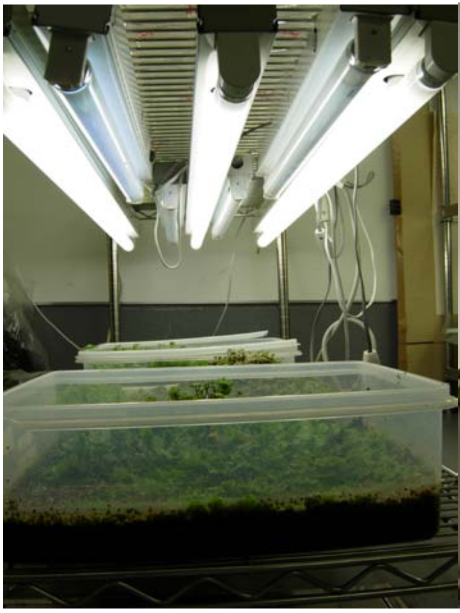


LED array without a light-scattering plate.

FR fluorescent tube

FR can be supplemented with a FR fluorescent tube. In Kohchi lab, two 20W FR tubes are added per one 40W white tube. In Japan, FR tubes are available from Toshiba (FL20S-FR-74, about 3,000 yen).

FR-rich fluorescent tubes, such as Panasonic FL40S-FR-P, are commercially available for plant growth or horticultural use, but we DID NOT get expected results with this type of light source.



Short blue-ish tubes are FR tubes

Incandescent lamp

80-100 W incandescent lamp is a good source of FR light. The appropriate distance between the light source and plants is 40 - 50 cm, but a large amount of infra-red (heat ray) generated by the lamp must be cut by water filter, for example. In Kohchi lab, a plastic square dish, such as Corning 431110, filled with 50 mM EDTA solution (antiseptic agent) is placed between the lamp and plants. Incandescent lamps generate really large amount of heat, and must be used in a room or growth chamber which has sufficient cooling capacity.

Natural light

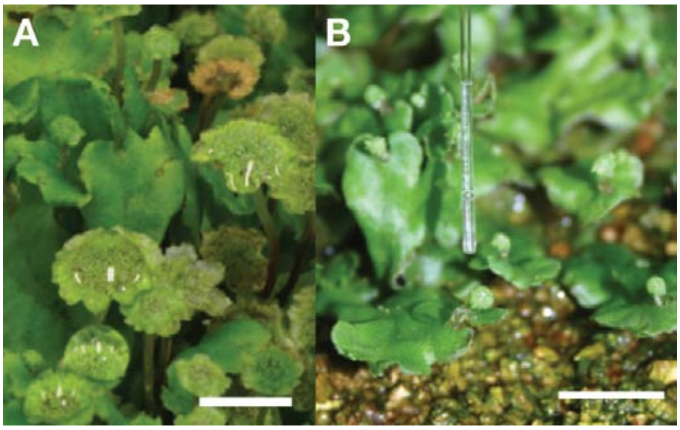
Natural light (sun light) contains FR light and induces transition to reproductive phase under long day condition. When day-length is not sufficient, it is necessary to supplement light with FR light.

Cross

In *Marchantia*, motile sperms fertilize eggs, and water is indispensable in this process.

Collecting sperm

Put a water droplet on an antheridiophore (>5 mm in diameter). After a few minutes, sperms are released into the droplet, which may be visible as "white cloudy stuff". If it is difficult to see, a transparent glass Pasteur pipette is a good choice to collect



sperm suspension. To check sperm motility, use a microscope with dark-field setup (or differential interference contrast or phase contrast) (200~400X).

Apply collected sperms to archegoniophores of which size is about 2~3 mm in diameter and height. It takes about one month for a sporangium to mature after fertilization.

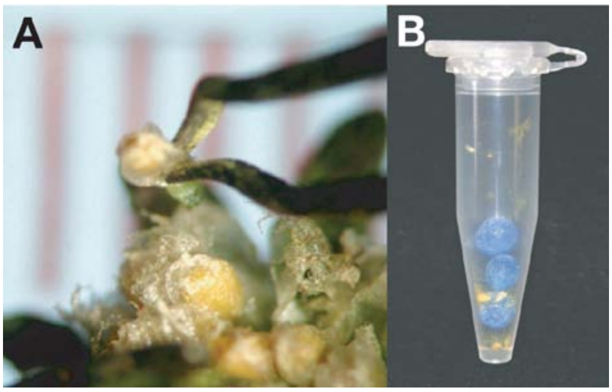
Note: A sporangium easily breaks and releases spores under dry condition. To prevent a sporangium from breaking, keep the entire plants humid by wrapping with plastic film or placing in a humid chamber, about three weeks after fertilization.

Storage methods

Spores last years after proper desiccation, and thus the most reliable form for long-term storage. However, cross is necessary to obtain spores, which is the major disadvantage of this method. Although further optimization is required for reliability, thalli and gemmae can be also stored for long time. Suspension-cells are maintained only by subculturing, and no other method has been tested.

<ins>Spores</ins>

Harvest sporangia in microtubes. With lid open, let spores dry for a couple of days in a container covered with paper towel. Dried spores can be stored in either a refrigerator or a freezer. Silica gel may be added to storage tubes, but not essential.



<ins>Thalli</ins>

Thalli grown on 0M51C agar plates can be stored at 4° C under dark. To prevent desiccation, each plate should be sealed with Parafilm, or alternatively, plates can be stored in a sealed plastic bag. Higher survival rate can be expected if younger thalli, or gemmae are stored. There is a case that thalli were successfully stored over five years. For long-term storage, microbial contamination must be appropriately controlled.

<ins>Gemmae</ins>

Appropriately dried gemmae last long (>2 years). It seems simply letting plants desiccated on plates or vermiculite. As dessicated, thalli die and their color turn brown, but often gemmae remain green in cupules (if not green, forget it). Harvest dried gemmae in microtubes and store in a refrigerator. Survival rate after storage in a freezer appears to be lower than those stored in a refrigerator.

IMPORTANT: This protocol has not been well established. Important lines should be stored in more than one methods. Further research is necessary to establish this approach.

Note: Gemma formation is suppressed under FR light or on medium without sucrose.

(K.T. Yamato, July 22, 2009)