

TISSUE CULTURE ESTABLISHMENT GUIDE



PREPARATION

Surface Sanitization	Thoroughly wash and sanitize all surfaces, tools, buckets, etc. to remove algae, insects and potential pathogens.
Temperature	74-80°F (23-27°C) constant (avoid temperatures below 68°F (20°C))
Light	14-20 hour daylength (extension is necessary in winter months) Ideal light amount is 70–100 micromol m ⁻² s ⁻¹ (350-500 fc) for a total of 5 moles of light per day. Shading may be required.
Media and Planting	Media should supply physical support, good porosity and the ability to close around the roots providing good stem/root to soil contact. Dümmen Orange recommends Quick Plug horseshoe plug to wrap the roots and prevent damage when planting.
Pot Size	Larger propagation trays, 4"(11cm)-6"(15cm) pots. Choose appropriate for species.

PLANTING/ACCLIMATIZATION

Loosen Agar	Use a spatula to loosen up the agar around the roots so the plants can be pulled from the cells without root damage
Wash Roots	Use room temperature water to wash away the agar, keep the plants in water.
Transplanting	Place the plantlets gently into pre-wet media and press gently so the media closes around the roots and the plantlets are anchored. A poorly seated plantlet will dry out.
Misting During Transplant	Use a spray bottle to keep the plants moist during the planting process prior to placing the trays in the high RH propagation area.
Misting or Fog	Maintain 70-185% relative humidity for the first 7-10 days without adding extra moisture to the media (use short bursts of mist), fogging is ideal. Plantlets should never be allowed to wilt while the new leaf cuticles form. Plants can also be covered with a tent or remay cloth.

WEANING/HARDENING

After Establishment	Following 7-10 days of high humidity and high temperatures begin to acclimate the plants to drier greenhouse conditions and brighter light. Temperatures can begin to step down if the species requires it. Do not make large jumps in environmental conditions, go slow.
Light	Can be begin to slowly add more light each day until plants are fully hardened, working up to 200-300 micromol m ⁻² s ⁻¹ (1000-1500 fc) for a total of 10+moles per day.
Fertility	Begin to feed as normal for the crop class after 7 days.

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