

Procedure transplanting plantlets from *in vitro* to greenhouse

1. Do rinse two times the rooting plantlets which are ready to transplanting with tap water and divide into groups by their height.
2. Planting plantlets into autoclaved medium in a planting tray. The medium must be slightly higher than the base.
3. Spraying a little bit of water around plantlets base area thoroughly so that the medium will tighten roots without the presence of air cavities.
4. Labeling the name tag (plant name, planting date, etc.) and put the planting tray into Reemay tents.
5. Spraying some amount of water hourly to Reemay tents and surrounding area thoroughly to increase relative humidity. Reemay tents should be vented every day early in the morning so the plantlets will have sufficient light.
6. In case of an occasionally high intensity of light, use the 90% polyethylene sunshade net on critical period during 10.00 a.m.–16.00 p.m. and then open the Reemay tents 10-15 cm little by little until the end of the 7th day after transplanting.
7. Use the 70% polyethylene sunshade net after critical period during 12.00–14.00 p.m. for 1 week.
8. After 1 week, use the 50% polyethylene sunshade net for transplanted and vigor plantlets.



In Vitro Plantlets Transplanting to Greenhouse

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A large number of *in vitro* plantlets do not survive after being transplanted due to the difference of the environment of *in vitro* culture and greenhouse. The *in vitro* plantlets are grown under relatively high humidity, a low level of light and an inadequate photosynthesis mechanism so plantlets are stressed and dead when transplanted to greenhouse where the environment is contrary to *in vitro* culture. Acclimatization is the most essential method to promote plantlets for survival and vigor.

The successful acclimatization technique can begin while plantlets are still in the *in vitro* culture, such as reducing relative humidity by loosening/uncapping the culture vessel or using a drilled lid, increasing level of light and light period per day to promote photosynthesis and making plantlets resistant to higher light level, higher temperature and disease infection. This technique promotes the development of the physiological and anatomical capacities of plantlets, such as opening and closing of stomata, rooting absorption, high potential of the leaf cells. All of these techniques serve as a preparation of plantlets to be ready for transplanting to greenhouse.

The first step for transplanting to greenhouse is to modify an environment possible as *in vitro* culture for plantlets gradually adjusting them to another environment. The relative factors of acclimatization are

Relative humidity

To increase relative humidity in greenhouse is the most important factor being achieved by using an automatic mist system along with placing plantlets in an enclosed area of polyethylene tents or Reemay (white spunbonded polyester fabric) tents that will retain water vapor to prevent wilting of plantlets.

Light

In the critical period (3-4 days after transplanting) should be shaded above Reemay tents by using 90% polyethylene sunshade net and reducing to 70 and 50%, respectively. That is optimal level of light for plantlets to grow up in greenhouse.

Temperature

Using an automatic mist system, shading, air circulation or air conditioner can reduce the temperature in greenhouse. Normal temperature for transplanting is about 13-30°C depending on the plant species.

Fertilizer

To add, after critical period macro- and/or micro-nutrients into the medium in which plantlets are transplanted is not advisable and, moreover, unpopular. A soluble complete fertilizer 21-21-21 diluted to a 1/4 to 1/2-strength of the recommended rate is rather applicable, being administered twice a week, and supplemented with trace elements once per week.

Medium

The medium must be an optimal standard growing medium; adequately supporting plantlets, pH 5.8-6.2, drainage, aeration and disease free (autoclaved medium).



Diseases

To avoid disease infection to plantlets an autoclaved medium, new containers and hygienic tools must be used. However, a fungicide or bacteria disinfectant of mild or moderate strength can be used in case of an infection and a regular cutting of damaged leaves.