Embryo Rescue and Embryo Excision – What's the Difference?©

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Embryo rescue and embryo excision are both procedures that expand our abilities to propagate plants sexually, but in very different ways: (1) the types of difficulties they enable us to overcome, (2) the stage of the embryo's development when the procedure is performed, and (3) the conditions the embryos are placed into once removed from the developing fruit.

EMBRYO RESCUE

Embryo rescue is used to expand our ability to produce viable offspring from wide crosses (i.e., distantly related parents) by "rescuing" embryos that would otherwise have a high likelihood of aborting on the mother plant during development. These rescued embryos are placed under artificial conditions conducive to continued development. Embryo dormancy mechanisms are not in place at the time the procedure is done and the technique is sometimes used to circumvent complex embryo dormancy.

The embryos are very young at the time they are rescued, usually at the heart stage or smaller. Ovule culture is a specific type of embryo rescue which is done at an even earlier stage where the entire ovule is removed due to the difficulty of working with the tiny embryo. As an example, in *Abelia* this is done 5 to 6 weeks after pollination, when the ovule is about 2 mm long and the embryo is less than 1 mm.

The procedure of embryo rescue is done entirely under sterile conditions. The exterior of the ovary is surface sterilized. For *Abelia,* the ovules are soaked in a 70% ethanol solution, then in a 5% bleach solution. In ovule culture, the ovary walls are cut away and the ovule is removed. The ovules is placed in a petri dish on a nutrient medium such as Woody Plant Medium or Linsmeier-Skoog medium containing agar, a carbohydrate source, and sometimes organic compounds and growth regulators. The petri dish is sealed to prevent microbial contamination and dessication. For embryos rescued at a later stage or in taxa with larger embryos, the ovule wall is also removed and the naked embryo is placed on the nutrient medium. As embryos develop to the torpedo stage, or after dissecting torpedo-stage embryos out of ovules, they are recultured onto fresh basal medium without plant growth regulators or organic compounds. The embryos are then placed under lighted conditions and germinate in 6 to 8 weeks. Some embryos germinate in the original medium while still within the ovule, but rarely develop into normal plants.

EMBRYO EXCISION

Embryo excision is used to remove physical barriers to germination (hard seed coat and impervious integuments; the latter aka. testae) and, thus increase germination percentage, and uniformity in time to germination, and reduce time to germination.

The technique is especially useful in the study of seed dormancy and viability.

Embryos are excised within a few weeks of their normal maturation time. Frequently, slightly immature fruit are chosen to avoid seed dormancy conditions that exist in mature embryos. In various maple species (*Acer* spp.) this is about 20 weeks after pollination (±2 weeks).

Because embryos at this stage have almost fully functional cuticles and defense mechanisms, as well as stored energy reserves, sterile technique is rarely necessary. The fruits are surface sterilized and soaked to make the tissues more pliable. The seed coat (pericarp) and integuments are removed and the embryos are placed on a filter paper in a petri dish. The paper is moistened with either water or a dilute solution of a plant growth regulator of choice. The dishes sealed with Parafilm are then placed under light or in darkness depending upon the germination requirements of the taxon. The embryos will germinate in 3 to 10 days if they are not dormant or if the proper plant growth regulator has been used to overcome the dormancy. Embryos requiring an after-ripening will not germinate under this system.

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