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In vitro Shoot-Tip Grafting in Citrus

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In the majority of citrus-growing areas around the world, infections caused by viruses, viroids, bacteria, spiroplasmas and phytoplasmas, cause significant economic losses. They result in decline, loss of vigour, low yields and inferior fruit quality. They also limit the use of several elite rootstocks that are prone to a number of diseases. As a result, they may eventually turn into the primary production-limiting factors (Juarez *et al.*, 2015). Only preventative efforts, such as employing tolerant or resistant germplasm, excluding potential diseases from the citrus area and establishing new plantings using pathogen-free, high-quality nursery trees, are effective at controlling graft-transmissible infections. It is frequently difficult to find pathogen-free plants of many cultivars, so it is vital to rescue healthy plants from diseased ones. In such a situation, a technique known as shoot tip grafting emerged that can regenerate citrus plants free of all graft-transmissible diseases and without juvenile characteristics.

Concept

In vitro shoot-tip grafting (STG), also known as micrografting (Conejero *et al.*, 2013), involves grafting very tiny shoot tips (0.1-0.2 mm from top to bottom) from sick plants onto immature rootstock seedlings (Navarro, 1975; 1992). Since the little shoot tips are frequently pathogen-free, the procedure enables the recovery of non-juvenile plants that are completely free of all citrus diseases. Due to these benefits, STG is employed in the majority of citrus-growing regions to restore healthy plants for commercial propagation.

History

In vitro rootstock seedlings that were just two weeks old were grafted with shoot tips from diseased plants by Murashige *et al.* (1972) in order to save a few citrus trees. Some of these plants lacked juvenile characteristics and were free of the exocortis viroid. Navarro *et al.* (1975) conducted a thorough study of this process and gave it the name shoot tip grafting *in vitro* (STG). They then established a systematic procedure that allowed for a 30–50% incidence of effective grafts that were transplanted to soil, with a survival rate of over 95%. The resulting plants were mostly free of graft-transmissible diseases and lacked juvenile characteristics. Globally, STG has made it possible to recover a large number of healthy cultivars and plant hundreds of millions of healthy certified trees.

Principle

The procedure is based on following two principles which are given below:

1. The ability of shoot tips to regenerate whole plants
2. Part of the plant is usually free of microorganisms, even though the rest of the plant may be infected.

Hypotheses

1. Pathogens move through the vascular system that is not present in the meristematic cells of the shoot apex.
2. The intense activity of meristematic cells has also been considered as a limiting factor of virus replication by competing with them for the necessary molecules.

Technique of shoot tip grafting *in vitro*

The STG technique includes the following steps:

1. **Preparation of rootstock:** Rootstocks are prepared from seedlings generated via *in vitro* seed germination. The most popular rootstocks for STG are 'Troyer' or 'Carrizo' citranges (*Citrus sinensis x Poncirus trifoliata*).
2. **Preparation of scion:** Shoot tips can be removed from a wide range of sources, including vegetative flushes that are actively growing, dormant buds that are on field or greenhouse plants, and shoots that are formed by budwoods or nodal buds developed *in vitro.* Budwoods are cultured *in vitro* using the Murashige and Skoog (1962) plant cell culture salt solution, solidified with 1.2% Bacto Agar, at constant 32°C and subjected to 80 µE m^{-2} s^{-1} illumination for 16 hours per day. When separating shoot tips for STG, fresh flushes are created every 8 to 16 days.
3. **Grafting:** Under aseptic conditions, the rootstock seedling is taken out of the test tube and it is decapitated, leaving about 1.5 cm of the epicotyl. There are numerous ways to graft, but the greatest outcomes have been reported when grafting is done at the top of the decapitated epicotyl, with the shoot tip in touch with the vascular ring, or in an inverted-T incision (Navarro *et al.* 1975).
4. **Culture *in vitro* of grafted plants:** Plant cell culture salt solution is used as the liquid nutrient media in which micro grafted plants are cultured. Histological studies reveal that between the rootstock and the shoot tip, 3 days after grafting, there is already some callus growth, and at the graft union, 5 days after grafting, the callus is fully established. Seven days after grafting, the first signs of vascular differentiation are seen, and 11 days later, the shoot tip and the rootstock have a fully developed vascular connection. Successful grafts have 2-4 enlarged leaves by 4-6 weeks after grafting, at which point they can be planted in soil.
5. **Transfer to soil:** Before being put in soil, scions of successful grafts need to have at least two enlarged leaves. This stage is typically attained four to six weeks following grafting. Plants with micro grafts are transplanted into pots with citrus-growing-friendly synthetic soil mixes that have been steam sterilised. If the shoot tips are removed from adult plants, plants recovered by STG don't have juvenile characteristics. Within two years of grafting, they often blossom and produce fruit.

Applications of Shoot tip Grafting (STG)

STG is being routinely used for the following purposes:

1. The regeneration of somatic hybrids from defective embryos
2. Plants can regenerate from irradiated branches to create seedless variants.
3. Growing plants from extremely difficult-to-germinate haploid embryos. The International Citrus Genome Consortium employed STG to recreate the Clemenules haploid plant, which was then used to sequence the entire citrus genome.
4. Developing stable non-apomictic tetraploid plants, which are excellent for triploid breeding
5. The regeneration of transgenic plants from shoots that are extremely challenging to in vitro root. STG is now frequently used in the genetic transformation of citrus (Juarez *et al.*, 2015).

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