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Biotechnology Strategies for Improving Avocado

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Abstract

The development of advanced breeding lines and cultivars of perennial fruit crops, such as the avocado, by conventional plant breeding methods has been a slow process for several reasons: (1) trees have long juvenile periods, usually three to twenty years, depending on the species, (2) controlled pollinations are very difficult to achieve with many fruit trees, (3) post pollination fruit drop is often excessive, (4) most trees are genetically heterogeneous, and (5) many years are required in order to evaluate progeny from controlled pollinations. In recent years, biotechnology has rapidly impacted breeding programs of many horticultural crops; however, breeding objectives of perennial fruit crops have only just begun to be addressed. In this review, we will attempt to demonstrate the potential of biotechnology for improving avocado.

Key Words: Avocado, Persea, biotechnology, genetic engineering

Introduction

Like most tropical and subtropical fruit, the most widely grown avocado cultivars have resulted from openly pollinated seedling trees. Due to their generally superior fruit qualities, these selections have gained wide consumer acceptance, and a very few cultivars, e.g., 'Hass' and 'Fuerte', now represent the market standards for fruit

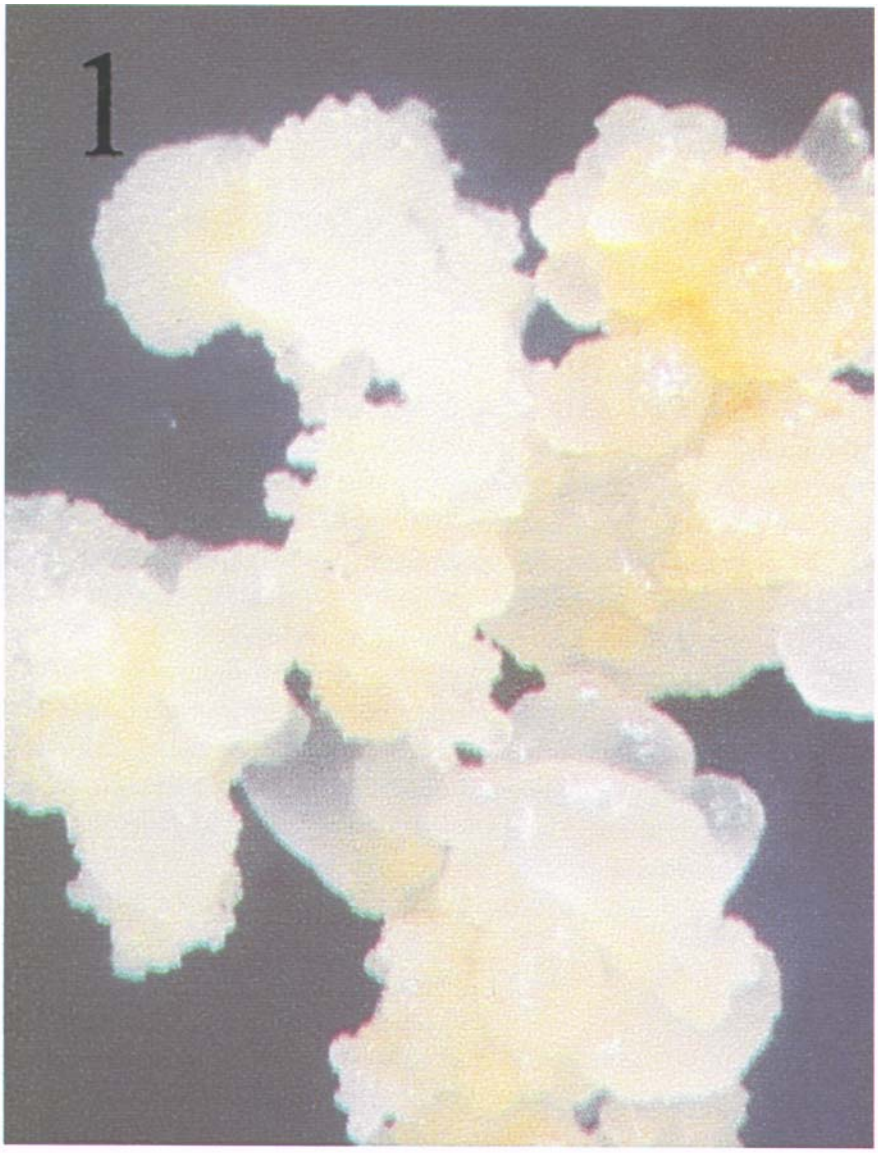

Figure 1. An embryogenic avocado cultures, consisting of proembryonic cells and masses.

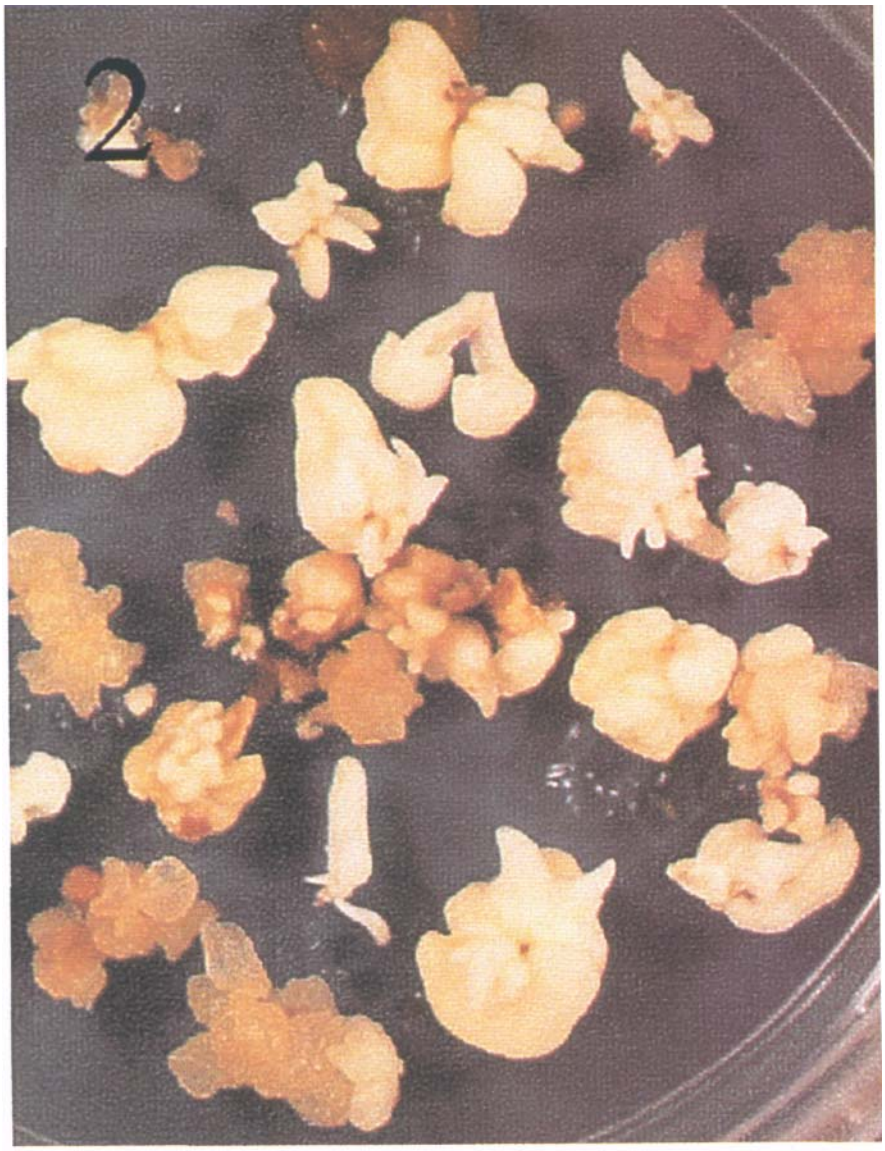

Figure 2. Mature avocado somatic embryos.

quality and appearance. Recent avocado cultivar releases, therefore, are measured by their performance in comparison with these selections. Inevitably, therefore, perennial fruit breeding programs attempt to develop advanced cultivars with most of the qualities of 'Hass', but with some additional qualitative changes, for example, resistance to pests and pathogens, better storage quality, more compact tree architecture, etc. Because of the great heterogeneity in segregating seedling populations of trees, the probability of finding a plant with the quality of its parent is very low.

Plant breeding and genetics have been revolutionized in the past few years by molecular techniques for gene cloning and the development of efficient methods for regenerating many of the major seed-propagated field and horticultural crop species from single cells. There has not been a comparable advance with perennial fruit crops.

The most significant constraint for applying modem plant breeding tools to tree crops is the perceived difficulty in developing a complete regeneration system for most woody plants from cell and tissue cultures.

Protocols for manipulating avocado rootstock and scion selections in vitro have been developed; we have presented a brief overview of the current status of this work, and have speculated on the directions that avocado improvement is likely to take in the near future.

Developing biotechnology strategies for improving avocado rootstocks

• Regeneration of avocado from cell and tissue culture

Genetic engineering of plant species can only be achieved if there is an efficient regeneration procedure from cell cultures. With avocado and other perennial tree species, this involves the reduction of the whole plant to a single cell that itself has the potential for regenerating the complete tree. Woody plants have traditionally bee characterized as recalcitrant in this respect, and have been difficult to regenerate. However, a few important subtropical tree crop species have been regenerated, e.g., citrus (Rangaswamy, 1958) and mango (Litz *et al,* 1982) from the nucellus, and the longan (Litz, 1988) from leaves. Witjaksono (1997) reported that it is also possible to regenerate avocado from the nucellus, a maternal tissue in the young developing seed.

• Somatic embryogenesis

The pattern of regeneration of the avocado from cell cultures is referred to as somatic embryogenesis, i.e., embryos that develop from non-germ cells of the plant. Implicit in this regeneration pathway is that the tree is rejuvenated. Avocado somatic embryos are morphologically identical to seeds with an important exception: they are clonal. Embryogenic avocado cultures (Figure 1) are induced on a highly defined plant growth medium, which contains minerals, sugars, vitamins and the plant growth substance picloram (Mooney & van Staden, 1987; Pliego Alfaro & Murashige, 1988; Witjaksono & Litz, 1999a, b). These cultures resemble callus; however, they are undifferentiated cultures that consist of single (proembryonic) cells, each of which can form a single somatic embryo, and small groups of cells (proembryonic masses), which can form clusters of somatic embryos. When grown under optimum conditions, embryogenic cultures can provide an almost unlimited supply of single cells and groups of cells. Each of the cells within an embryogenic culture has the potential of forming a somatic embryo (Figure 2).

Avocado can be propagated by somatic embryogenesis (Figure 3), but propagation is much more efficient if the shoots that emerge from germinating somatic embryos are removed and micropropagated using standard procedures (Figures 4 and 5) (Witjaksono et al., 1999a). Avocado clones are rejuvenated by somatic embryogenesis, and the juvenile period can be six to seven years before flowering occurs. Therefore, this procedure, however, efficient, is unlikely to replace

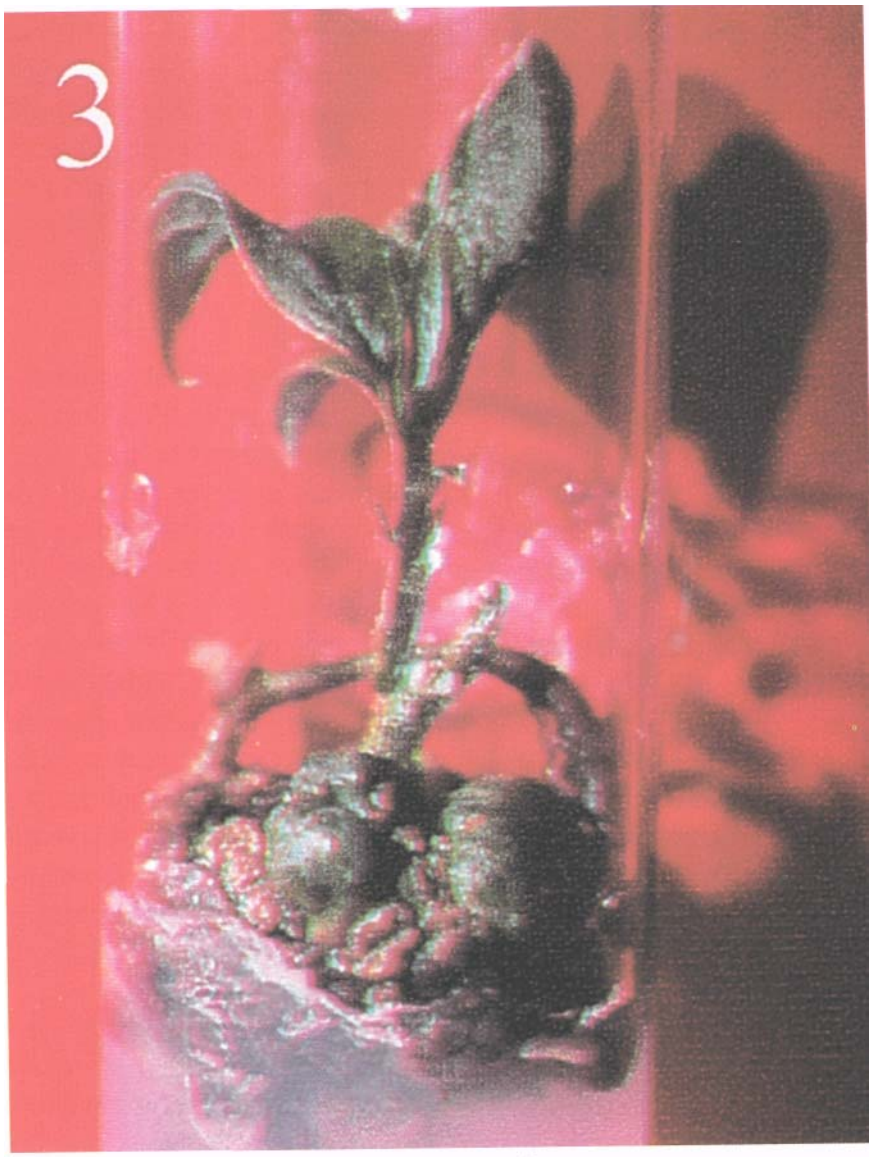

Figure 3. Regenerated avocado plantlets.

Figure 4. Micropropagated avocado shoots.

conventional methods for vegetatively propagating scion selections. On the other hand, somatic embryogenesis could potentially be utilized as a highly efficient method for propagating new and existing rootstock selections.

Embryogenic avocado cells can also be the basis for modifying avocado using genetic engineering. For example, each somatic embryo originates from a single cell. The modification of a single gene within an embryogenic cell will also be reflected in the expression of this gene in the somatic embryo and in the regenerated plant.

• Genetic transformation

The transfer of a gene from one species to another is referred to as genetic transformation, and has been demonstrated with avocado (Cruz Hernandez et al., 1998). This procedure is based upon the infection of embryogenic avocado cells with a

disarmed strain of the crown gall bacterium, *Agrobacterium tumefaciens.* The *Agrobacterium* has been genetically engineered, and contains two microbial genes, neophophate transferase which confers resistance to the antibiotic kanarnycin, and the reporter gene, 8-glucuronidase (GUS), both of which are mediated by the 35S promoter, i.e., both genes are expressed at all times or constitutively throughout the transformed organism. The presence of the kanamycin resistance gene is critical for selection purposes, because the initial transformation event may involve only a few cells in a population of many thousand. When putatively transformed and nontransformed cells are grown in the presence of rate-limiting concentrations of kanamycin, only transformed cells are able to grow. Transformed cultures can be stained specifically for GUS activity, and for this reason, GUS is therefore essential for following the course of transformation within a culture.

There have been relatively few attempts to clone important genes in the avocado. Until today, most of the genes that have been isolated from avocado are in some way involved with the ripening of avocado fruit. For example, some of the genes that control softening of avocado fruit have been cloned (Christiansen et al., 1984; Dopico et al., 1993; Kutsunai et al., 1993). Although the control of ripening of California avocado fruit is perhaps not the highest research priority; however, crop improvement is undergoing rapid change as genes that control: 1) fruit ripening, 2) resistance to fungal diseases, 3) resistance to virus diseases, 4) resistance to insect pests, and 5) resistance to herbicides have been identified and transferred from one species to another. At this tune, the first generation of transgenic plants that incorporate the traits above have altered the economics of staple crop production, i.e., soy bean, maize, canola, etc. We are attempting to transfer genes into avocado which express "pathogenesis-related" proteins. These genes, including plant defensins, glucanase, chitinase, the antifungal protein gene, etc., could provide higher levels of resistance to fruit and soil-borne pathogens. Our major goal is to increase the resistance of avocado rootstocks to Phytophthora root rot.

The next generation of transgenic plants will be more sophisticated, and will be altered for more complex horticultural traits, including architecture, cold tolerance, fruit quality, *etc.* (Litz & Witjaksono, 1999).

• Somatic hybridization

A major limitation in plant breeding is the genetic isolation of certain important species from related species that have potentially interesting traits. This isolation, when it is caused by sexual incompatibility, normally cannot be overcome by conventional breeding methods. In order to overcome incompatibility barriers between plant species, the procedure of somatic hybridization has been developed to produce asexual hybrids. This procedure has important implications for improvement of avocado rootstocks.

The major constraint for avocado production is Phytophthora root rot. Control of this disease is based upon the use of avocado rootstocks that are tolerant of the pathogen, chemical control and soil remediation through the use of organic mulches. Although high

Figure 5. Rooted avocado micropropagated plants.

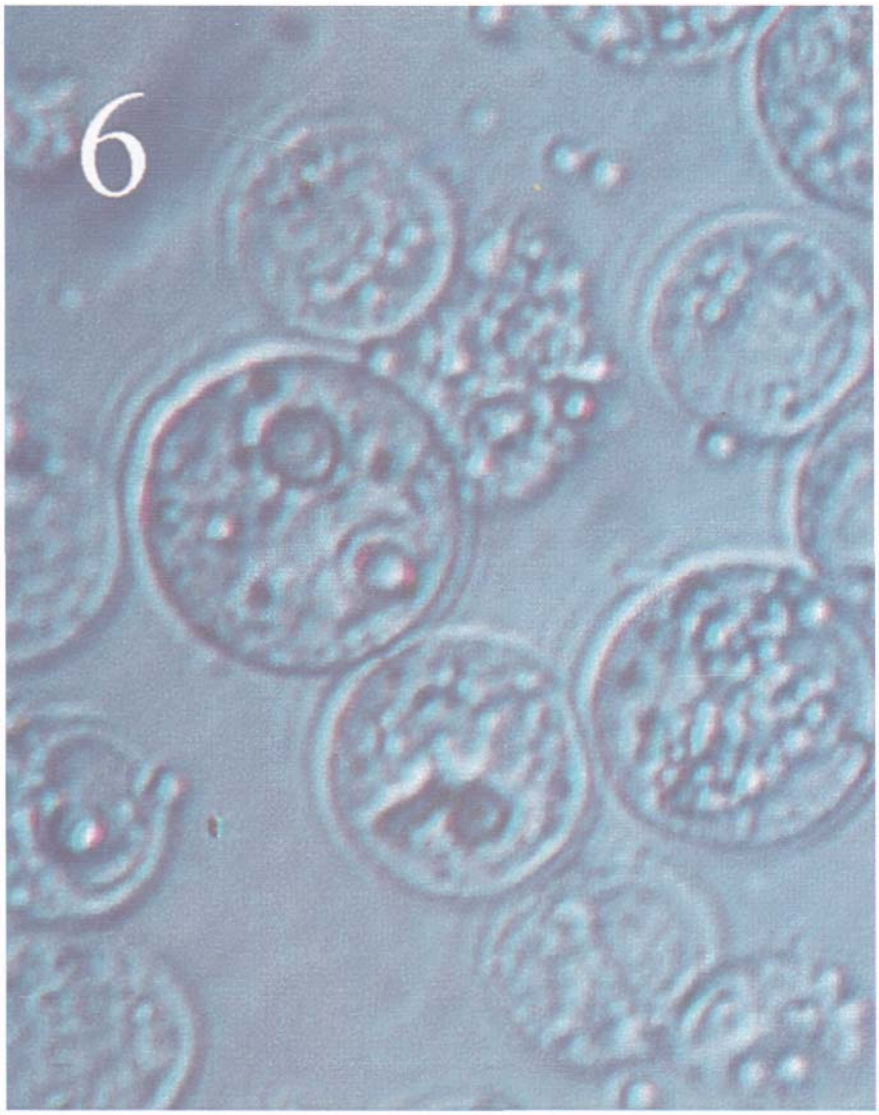

Figure 6. Protoplasts isolated from embryogenic avocado cultures.

levels of resistance to Phytophthora root rot do not occur within *Per-sea americana,* other *Persea* species have been shown to be resistant (Bergh & Lahav, 1996). The resistant species are in the subgenus *Eriodaphne,* and include, among others, *Persea borbonia, P. cinnerascens* and *P. pachypoda.* These *Persea* species would seem to be ideal rootstocks for the avocado; however, the Phytophthora root rot-resistant species are sexually and graft incompatible with the avocado. Somatic hybridization is therefore a potential strategy for overcoming the incompatibility between the Phytophthora root rot- resistant species in the subgenus *Eriodaphne* and the avocado. This procedure is dependent on the ability to regenerate plants from protoplasts. Protoplasts are plant cells from which the cellulose walls have been removed, leaving a fragile membrane-bound cell.

- **Protoplast isolation and culture**

. Witjaksono et al. (1998) demonstrated that protoplasts could be isolated efficiently from embryogenic avocado cultures. Embryogenic cells and cell clusters are incubated in a liquid plant growth medium supplemented with enzymes that degrade the cell wall and fortified with an osmoticum to prevent the protoplasts from bursting upon their release. Following the removal of cell debris by differential centrifugation, the protoplasts can be plated in semi-solid plant growth medium or suspended in liquid medium (Figure 6). After a few days, cell walls develop and enclose the protoplast, and the newly formed cells begin to divide; somatic embryos eventually develop from the resulting cell clusters (Figure 7) and plants can be recovered.

A source of protoplasts for the Phytophthora root rot-resistant *Persea* species is the mesophyll layer of young leaves (Witjaksono, 1997). Leaf protoplasts do not possess regenerative potential (Figure 8); however, when they have been fused with embryogenic protoplasts, the hybrid protoplast is embryogenic (Figure 9). Fusion of the two types of protoplasts is accomplished in a liquid plant growth medium with a high osmolarity and containing polyethylene glycol, which stimulates fusion of the protoplasts.

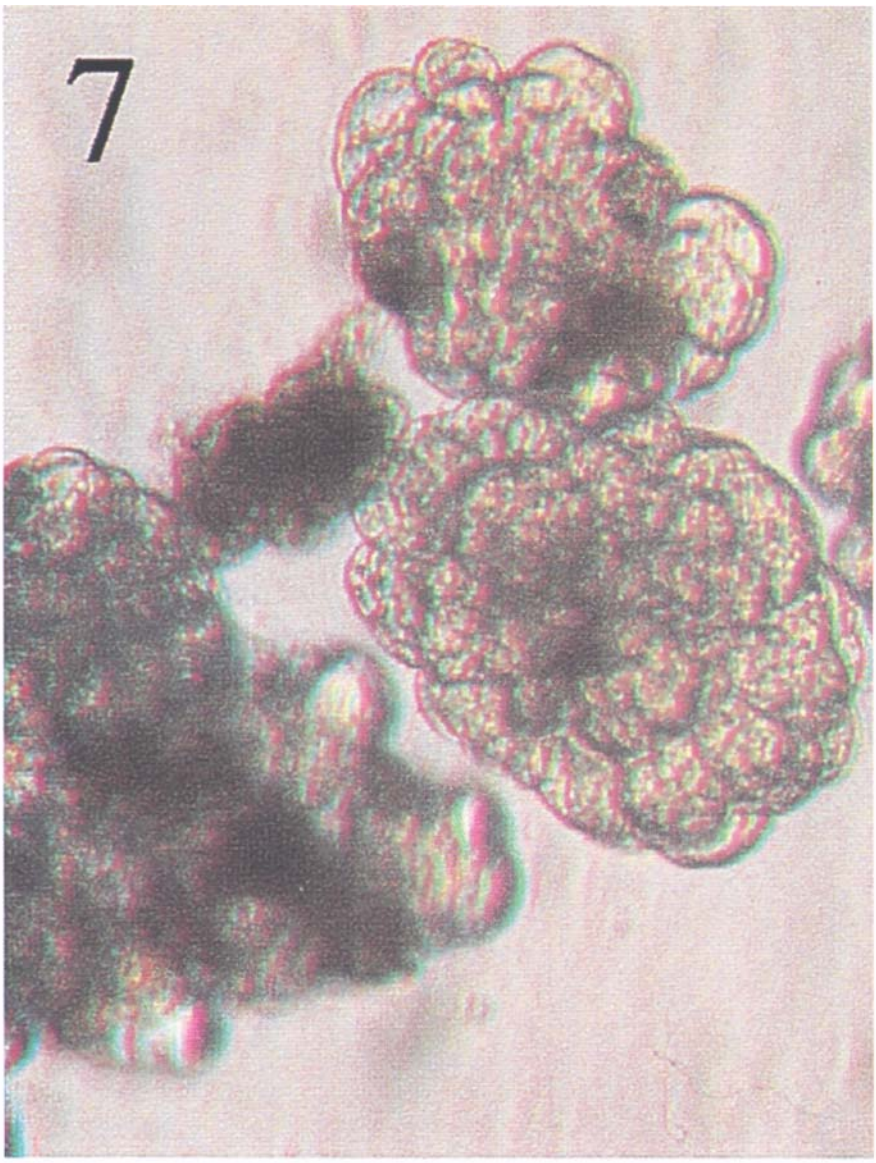

Figure 7. Proembryonic masses derived from an embryognic avocado protoplast.

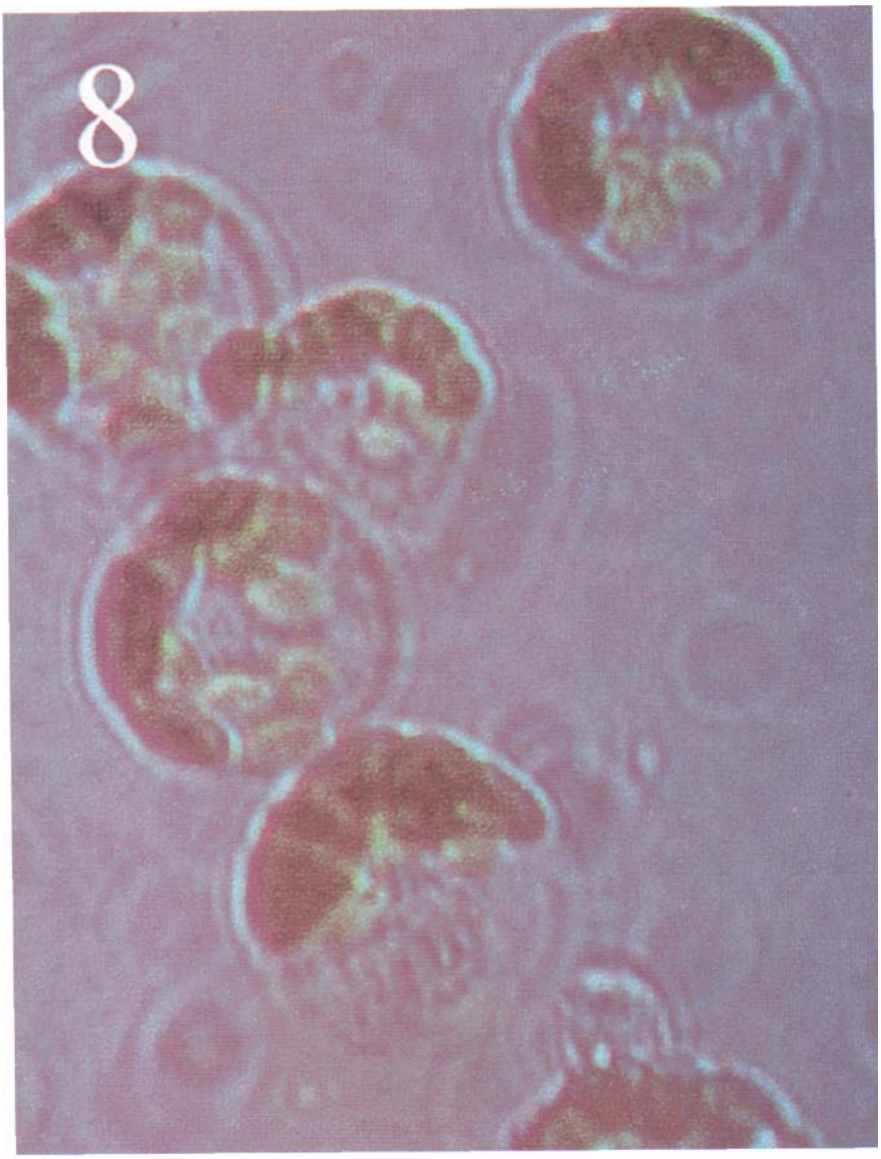

Figure 8. Isolated protoplasts from leaves of a wild Persea species.

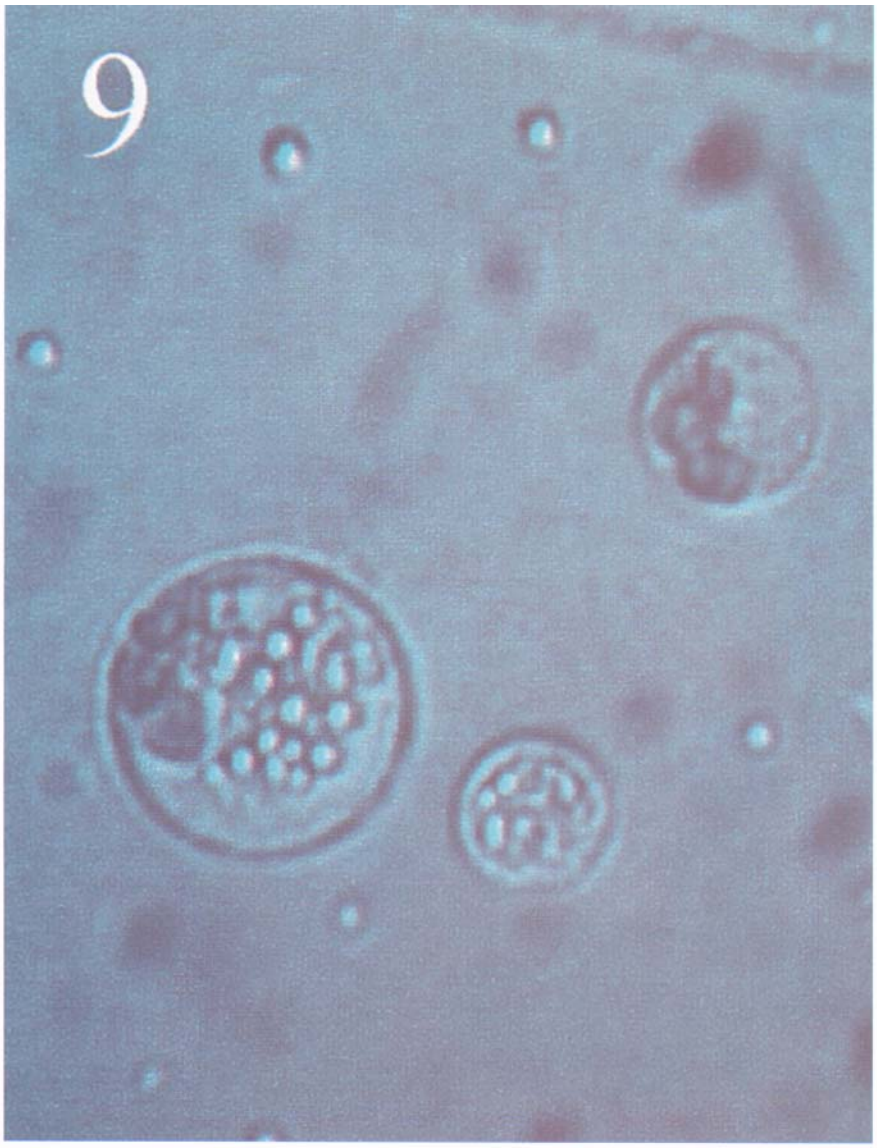

Figure 9. Fusion of an embryogenic avocado protoplast with a wild Persea leaf protoplast.

Conclusions

The development and release of avocado scion and rootstock cultivars by conventional breeding methods is a slow process due to the heterogeneity of the species and the long period of time required to evaluate seedling selections. Biotechnology can accelerate this process in some ways and provide a new dimension for increasing interesting genetic diversity. It is possible to alter existing cultivars for specific horticultural traits. Instead of breeding for improved cultivars, it is possible to make the existing cultivars better. This revolution is currently targeting existing avocado rootstocks for enhanced resistance to Phytophthora root rot. Three strategies are being utilized: (1) developing an efficient regeneration system (somatic embryogenesis) from

cell cultures that can be used either for propagating rootstocks or for genetic manipulation at the cell level, (2) genetic transformation of embryogenic cell cultures with genes that are associated with disease resistance, and (3) somatic hybridization in order to create artificial hybrids of avocado and Phytophthora root rot-resistant *Persea* species.

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