Chapter 15

REGENERATION AND GENETIC TRANSFORMATION OF APPLE (MALUS SPP.)

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1. INTRODUCTION

1.1. The Importance of Apple in World Fruit Production

In terms of world production, apple ranks in the top three of major deciduous, citrus and tropical fruits, grapes and berries. In 2001, just over 61 million metric tonnes of apple fruit was produced, accounting for 14% of total world fruit production. Oranges and bananas, the other top three members, account for 14 and 15% of world fruit production, respectively (1). Over the past decade production has grown while demand has remained static, so producer returns have been diminishing in real terms for a number of years.

One strategy taken to maintain/increase profitability over the past 20 years has been to introduce novel varieties derived from traditional breeding and mutant ‘sports’ found in the orchard, such as, Fuji and Gala and its sport, Royal Gala. Worldwide, these varieties are steadily replacing older varieties such as Red Delicious and Granny Smith. However, as volumes increase the premium they return steadily erodes. Conventional breeding will remain a key part of future strategy for profitability but the length of time required to breed and test new cultivars (10-20 years), even with marker assisted selection, as well as the investment required to achieve market penetration/acceptance are major barriers. Such problems are further compounded by changing trends in food consumption towards new exotic fruits and convenience foods eroding demand for traditional foods such as apples (1). On the other hand, novelty is not always desirable. Such is the case for bittersweet apple cultivars used for juice/cider production as well as rootstocks. These types of apple tend to be well established and valued for their existing characters, which are often lost/ altered through conventional breeding. There are also many old cultivars that might be commercially viable if it were not for a few negative traits e.g. susceptibility to a particular disease, poor storage qualities or skin finish. Therefore conventional breeding is not so attractive when, in many cases, alteration of only a few traits is desired.

1.2. Opportunities for Cultivar Improvement by Genetic Modification

Genetic engineering offers new opportunities to add value and to increase productivity in a vastly shorter time frame (2-5 years) than that required by
conventional breeding. Targets on the production side include disease resistance (e.g. fireblight, scab, *Phytophthora* and powdery mildew), post harvest characters, pest resistance (e.g. insect/nematode pests), tree architecture (including control of vigour), flowering time, reducing biennialism, and stress resistance (e.g. to water/salt/heat stress). Most of these requirements are region-specific and so not all need to be deployed in a particular locality. Consumer-orientated traits include improving health characteristics, eliminating allergenicity, altering flavour components and colours, and providing novel benefits such as increased protection from tooth decay or immunisation.

Assuming further advances in sequencing and outputs from the human genome, future possibilities may include matching an individual’s genotype with diet. This will create many new opportunities, and possible pitfalls, for apple producers. Current public opinion towards genetic engineering in many countries outside the USA indicates that consumer-benefiting traits may be better received than the genetically engineered crops currently under cultivation, which are seen by many consumers as benefitting producers only. This is an expression of our personal view and experience. We also would like to stress that modification of many production orientated traits result in indirect benefits to the consumer in terms of reduced pesticide inputs, reduced pollution and other effects that reduce impacts on the wider environment.

1.3. Genetic Modification of Apple

Greensleeves was the first apple cultivar to be transformed (2) and with slight modifications regularly returns transformation frequencies between 8-16% on a per explant basis (S. Bulley, F. Wilson, A. Passey and S. Vaughan, unnpublish.). The method is based on the leaf disk transformation method using disarmed strains of *Agrobacterium tumefaciens*, originally described by Horsch et al. (3). The transgenes in the transformed apple plants produced this way displayed stable patterns of expression in fruit and Mendelian segregation in the progeny (4). To date, variations of this method have been used to transform many different dessert apple varieties (scions) and a number of rootstocks (Table 1).

Factors that are important for successful transformation in Greensleeves include a reliable method for regenerating shoots from leaf tissue, and the use of the hypervirulent *A. tumefaciens* strain EHA101 otherwise known as A281 (25). The hypervirulence of EHA101 is encoded in a region of pTiBo542 outside the T-DNA (26). Other strains have been used successfully with Greensleeves but do not give such high transformation frequencies in terms of blue stained callus with a construct containing the 35S-*gusA* reporter gene (25). In other cultivars, EHA101 has been found to be the most effective strain for transfection and transformation from four strains tested (7, 27).

This chapter updates a previous chapter (28) and includes changes in the protocol. It outlines the method of leaf disk transformation of the apple cultivar Greensleeves (2) with modifications as described (5). This can be used as a starting point when attempting to transform a variety where transformation has not yet been reported. With this in mind, the relative importance of each step is discussed and