Chapter 10

TRANSFORMATION OF BANANA USING MICROPROJECTILE BOMBARDMENT

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1. INTRODUCTION

1.1. Importance and Origin of Bananas and Plantains

Bananas and plantains (Musa spp.) are grown throughout the humid tropics and
subtropics where they are of great importance both as a subsistence crop and as a
source of domestic and international trade. World production is estimated at more
than 100 million tonnes annually (1), the majority of which is grown by
smallholders for their own consumption and/or traded locally. The total area under
production is estimated at over 5.5 million hectares (1) with the major growing
regions being Asia, Africa and Latin America.

The natural range of wild Musa species is from the northern end of Australia to
southern China and west to India. Edible, seedless banana and plantain cultivars are
derived from intra- and interspecific hybridization of the two seeded, wild diploid
species, Musa acuminata (A genome) and M. balbisiana (B genome). These hybrids
are believed to be the result of naturally occurring crosses which were then selected
by early farmers in the Asian region. The haploid genome of both M. acuminata and
M. balbisiana consist of 11 chromosomes, however, the acuminata genome has been
estimated as being slightly larger, 610 Mbp cf. 560 Mbp for balbisiana (2). Many
edible hybrids are parthenocarpic, female sterile and triploid with the relative
contribution of each species to the genome being annotated by either A or B. Dessert
bananas are usually AAA, plantains AAB and cooking bananas ABB. In recent
times, breeding programs have also generated tetraploid hybrids. For brevity, all
groups will be referred to collectively as bananas.

1.2. Need for Genetic Improvement and Limitations of Conventional Breeding

Banana breeding programs have largely focussed on generating pest and disease
resistant cultivars while at the same time retaining acceptable yield and fruit quality.
The two major fungal diseases of banana are Sigatoka leaf spots (Mycosphaerella
spp.) and Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*). Viruses have a significant impact on banana production and include banana bunchy top virus (BBTV), banana bract mosaic virus (BBrMV) and banana streak virus (BSV). Among the many pests affecting banana, nematodes, particularly the burrowing nematode (*Radopholus similis*), are the most serious. Various strains of the bacteria, *Pseudomonas solanacearum* infect the vascular tissue (Moko and blood disease) and fruit (Bugtok) of banana. The significance of these pests and diseases in particular banana-growing regions varies depending on banana cultivar, distribution of the pathogen and cultural practices. Due to infertility, triploidy and long generation time, very few conventionally bred cultivars have reached commercial release (3). There have been significant successes in breeding disease resistant plantains for use by subsistence growers (4, 5) however, problems have been encountered with BSV. Many cases of BSV infection in these new hybrids are believed to be the result of a viral sequence being integrated into the genome of a commonly used breeding parent which is reactivated by the hybridization and/or tissue culture process (6). As a result of the aforementioned difficulties encountered with conventional breeding, a great amount of effort has been directed towards improving banana through manipulation of somatic cells and more recently genetic engineering (3).

### 1.3. Genetic Transformation of Banana

Around the world, banana biotechnology programmes are mainly focused on genetically engineered disease resistance although other applications such as delayed ripening (7) and edible vaccines (8) have also been investigated. Transgenic fungal resistance strategies include the use of antifungal proteins (9) and resistance genes (R-genes) derived from wild banana (10). Transgenic virus resistance strategies include posttranscriptional gene silencing (11), use of mutated viral replication proteins (12) and a novel approach utilizing viral activation of suicide genes (13).

Although there has been one published report of banana transformation targeting meristematic tissue rather than a regenerable cell culture (14), most banana transformations systems involve the use of embryogenic cell suspensions (ECSs). This is due to the low efficiency and chimerism experienced when transforming meristems.

ECSs have been generated using a range of explants including corm tissue (15), leaf bases (15), immature zygotic embryos (16), meristems (17) and immature male (18) and female (19) flowers. Currently, ECSs are most commonly derived from immature flowers or from ‘scalps’, the term used for meristems derived from a particular form of proliferating shoot culture (16). Both these techniques appear to be applicable to a wide range of genotypes including seeded diploids, edible triploids and also recently developed tetraploids derived from breeding programmes (20,21). If there is convenient access to field-grown bananas, immature flowers are the preferred explant for initiation of ECSs because (a) embryogenic callus can be induced directly from floral explants rather than requiring periods of up to 9 months to generate the ‘scalps’ from which embryogenic cultures are then initiated and (b)