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GENETIC TRANSFORMATION OF AVOCADO

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ABSTRACT

An important advantage for using genetic transformation to improve perennial fruit trees is that a single horticultural trait can be altered, without significantly affecting the phenotype of the cultivar. Embryogenic avocado cultures have been transformed with different gene constructs in order to address different breeding objectives. Avocado is being transformed with genes encoding for different antimicrobial proteins, including chitinase, glucanase, the antifungal protein (AFP) and magainin, and the first greenhouse planting of transgenic plants containing the gene for AFP has been established. As part of a strategy for controlling avocado sunblotch disease, embryogenic cultures have been transformed with the pac1 ribonuclease gene from yeast. In order to control fruit ripening and extend the shelf life of avocado fruit, embryogenic cultures have been transformed with the gene for SAM hydrolase, a bacterial gene that mediates the breakdown of S-adenosylmethionine (SAM), a precursor of ethylene.

Key Words: avocado, somatic embryo, genetic transformation

INTRODUCTION

Certain breeding objectives of avocado are difficult to achieve in a timely manner, due to the long juvenile period of the species. Genetic engineering strategies as they are applied to vegetatively propagated fruit species, can target specific characteristics of the plant. Hence, it is feasible to alter a cultivar for one or more traits without altering the phenotype of the clone. Genetic engineering of avocado is limited only by the availability of genes that are associated with specific horti-

cultural traits. Relatively few genes have been isolated from avocado, and they are associated with fruit ripening, i.e., ethylene biosynthesis. Several mRNAs increase during avocado fruit ripening, i.e. messages for cellulase (Christofferson *et al*., 1984), cytochrome P-450 oxidase (Bozak *et al*., 1990), and polygalacturonase and ACC oxidase (Dopico *et al*., 1993). Different cDNAs associated with fruit ripening have been isolated: polygalacturonase pAVOpg (Kutsunai *et al*., 1993) and ACC oxidase or pAVOe3 (McGarvey *et al*., 1992).

Avocado has been genetically transformed with certain genes that mediate specific horticultural traits.

• Fruit ripening. Avocado fruit is strongly climacteric (Kays, 1997). Ethylene acts as a natural triggering mechanism for the induction of the respiration climacteric (Kays, 1997), and also regulates fruit ripening by coordinating the expression of several genes. Mexican and Guatemalan avocados are unable to ripen while the fruit are still attached to the tree and can remain on the trees accumulating oil for 2-4 months after reaching maturity (Whiley, 1992). This can prolong avocado supply by "on-tree storage", but can cause alternate bearing in subsequent years. The fruit of West Indian and West Indian X Guatemalan hybrids mature, ripen and drop if not harvested at maturity, and fruit cannot be stored on the trees (Whiley, 1992). To ensure availability of fruit year-round in tropical zones, several avocados cultivars each with a different harvesting season must be grown. There is therefore no uniform standard for the tropical avocado.

Ethylene production can be reduced by blocking the activity of specific genes involved in ethylene biosynthesis. Blocking or lowering ethylene biosynthesis has not been attempted with woody tree species; however, this could increase on-tree storage of tropical avocados and prolong shelf-life. Theologies *et al*. (1992) reported that the cloning of the genes in tomato, e.g., ACC synthase and ACC oxidase, enabled the regeneration of plants bearing fruit with extended shelf-life using antisense technology. SAM hydrolase from bacteriophage T3 encodes S-adenosylmethionine hydrolase (Bestwick at al., 1991), and catalyzes the conversion of SAM to methylthioadenosine (Good et al., 1994). Efendi (2003) has described the transformation of embryogenic avocado cultures with SAM hydrolase, which is under the control of an avocado fruit ripening-specific cellulase promoter. The gene would be expressed in mature green fruit just as they would normally start to ripen.

• Disease resistance

Avocado sunblotch is caused by the avocado sunblotch viroid (ASBVd), which replicates in chloroplasts (Semancik and Szychowsky, 1994). There is no known resistance to this disease within avocado and its close relatives. Procedures for eliminating pathogens from plants include regeneration from the nucellus and from micrografts. Although viroid infections of grape and citrus can be eliminated by micrografting, recent studies (Suarez et al., in press), using RT-PCR as a sensitive diagnostic tool, have demonstrated that ASBVd persists in avocado plants regenerated from the nucellus and in micrografted plants. An alternative strategy for controlling viroid diseases involves genetic transformation with the pac1 ribonuclease gene from *Schizosaccharomyces pombe* (Sano *et al*., 1997). We are attempting to transform avocado using a gene construct containing pac1 with a transit peptide gene that would result in the expression of the pac1 gene in the chloroplasts.

As part of a strategy to enhance resistance to root (Phytophthora root rot) and foliar diseases of avocado, a number of genes whose expression is associated with antimicrobial activity have been inserted into embryogenic avocado cultures, including those for chitinase, β-1,6-glucanase and the antifungal protein. These genes are activated as part of the host defense mechanism, and the proteins are referred to as pathogenesis-related proteins. Another gene of interest encodes the defense peptide magainin 2, which has been isolated from the skin of the African clawed frog. Magainin 2 consists of a short α-helix peptide, and has been shown to be effective against fungi and gram positive and negative bacteria.

METHODOLOGY AND RESULTS

Embryogenic cultures were induced on semi solid $B5^-$ medium (Gamborg et al., 1968), MS (Murashige and Skoog, 1962) minor salts, 0.41 μM picloram and (in mg $liter^{-1}$) thiamine HCl (4), myo-inositol (100) and sucrose (30,000) (Witjaksono and Litz, 1999a). Cultures were maintained on semi solid MSP, MS basal medium, with 0.41 μM picloram, the organic components of $B5^-$ and agar (8,000) (Witjaksono and Litz, 1999a). Embryogenic cultures were transferred into liquid MS3:1P medium, MS basal medium with 60 mM inorganic nitrogen (75% of N as NO_3^- and 25% as NH_4^+) supplemented with organic addenda (Witjaksono and Litz, 1999b) and the other components of MSP. Petri dishes were sealed with Parafilm® and maintained in darkness at room temperature (25ºC). Suspension cultures in Erlenmeyer flasks were sealed with aluminum foil and Parafilm, and incubated under ambient laboratory conditions at 125 rpm.

Embryogenic suspension cultures were genetically transformed with various gene constructs (Cruz-Hernandez et al., 1998). Embryogenic cultures were transformed with *A. tumefaciens* strains EHA101 or EHA105 accordingly: 1) *AFP* incorporated in pGPTV containing *uidA*, *bar* (glyphosate resistance) and the CaMV 35S promoter (Raharjo et al., unpublished data); 2) β-1,3-glucanase and chitinase cloned in pGPTV with *uidA*, *bar* and the CaMV 35S promoter (Witjaksono et al., unpublished data). 3) In addition, a complex transformed somatic hybrid consisting of 'Gwen' with *chalcone synthase* and *nptII* + 'Hass' with AFP, *bar* and *uidA* was selected on medium containing phophinothricin (PPT) and kanamycin sulfate. 4) Embryogenic cultures have been genetically transformed with pAG4092 harboring SAM hydrolase to block ethylene biosynthesis, with *nptII* and under the control of an avocado fruit-specific cellulase promoter (Efendi, 2003).

DISCUSSION AND CONCLUSIONS

Transformed plants with the AFP, AFP+chalcone synthase and SAMase genes have been regenerated with the agency of micrografting (Raharjo and Litz, this proceedings). Transgenic plants with AFP are currently in greenhouse trials.

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