Transgenic Plant Journal ©2009 Global Science Books

Genetic Engineering of Pineapple

Jaya R. Soneji* • **Madhugiri Nageswara Rao****

University of Florida, IFAS, Citrus Research & Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA

Corresponding authors: * **jayasoneji@yahoo.co.in** ** **jrs@crec.ifas.ufl.edu**

ABSTRACT

Pineapple is an important crop for tropical countries. It is consumed as fresh fruit as well as processed or canned, dehydrated and juice products. Even though it is grown in more than 82 countries around the world, there is a remarkable lack of commercial varieties. 'Smooth Cayenne' is the only cultivar which dominates the trade and pineapple industry. Conventional breeding has yielded very poor results making genetic engineering particularly suitable for genetic improvement of pineapple. Tissue culture regeneration has been widely reported in pineapple making genetic engineering more amenable. Genetic engineering also offers the means for manipulating horticulturally important traits without altering the cultivar phenotype. This review provides an overview of the genetic transformation efforts carried out in pineapple.

Keywords: *Agrobacterium tumefaciens*, *Ananas comosus* L. Merr., genetic improvement, particle bombardment, transformation
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **2iP**, 6-(γ,γ-Dimethylallylamino)purine **ACC**, 1-aminocyclopropane-1-carboxylic acid; ***acacs2***, 1-aminocyclopropane-1-carboxylic acid synthase gene; **B_5**, Gamborg *et al.* (1968) medium; **BA**, 6-benzyladenine; **BAP**, 6-benzylamino purine; ***bar***, bialaphos resistance; ***cp***, coat protein gene; **CH**, casein hydrolysate; **CM**, coconut milk; **GFP**, green fluorescent protein; **GUS**, β-glucuronidase; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **Kn**, kinetin; **MS**, Murashige and Skoog (1962) medium; **MT**, Murashige and Tucker (1969) medium; **MWP**, mealybug wilt of pineapple; **N**, Nitsch (1951) medium; **N6**, Chu (1978) medium; **NAA**, α-naphthaleneacetic acid; ***npt*II**, neomycin phosphotransferase II; ***ocs***, octopine synthase; **PCR**, polymerase chain reaction; **PMWaV-2**, Pineapple mealybug wilt associated virus-2; **PPO**, polyphenol oxidase; **PPT**, phosphinothricin; **RT**, reverse transcript; ***ubi***, polyubiquitin

CONTENTS

INTRODUCTION

Pineapple (*Ananas comosus* L., Merr., 2n=50), a perennial monocot herb, is economically the most important member of the family Bromeliaceae (Collins 1968). It is best suited to a mild tropical climate with temperatures between 16 and 32°C, is amenable to cultivation on large scale (Davey *et al.* 2007) and cannot withstand temperatures below freezing. World production of pineapple has shown a steady increase over the years due to the expansion of the pineapple industry in developing countries. The world production of pineapple is about 18,873,577 tonnes with a yield of about 197,495 hectogram per hectare (FAO 2008). Around 82 countries in the world produce pineapple in economic quantities with Thailand, the Philippines, Brazil, China, India, Costa Rica, Nigeria, Kenya, Mexico, and Indonesia producing the majority of world supplies of pineapple (**Fig. 2**). Costa Rica, the Ivory Coast, and the Philippines supply

BOX 1: Glossary of terms used in pineapple.

Axillary bud, bud formed in leaf axils (**Fig. 1H**); **Crown**, shoots from apical end of fruit (**Fig. 1A**); **Hapa**, slip at base of peduncle (**Fig. 1C**); **Peduncle**, fruit stalk (**Fig. 1D**); **Ratoon**, suckers bearing second or successive crops (**Fig. 1F**); **Slip**, leafy shoot from peduncle (**Fig. 1C**); **Stem disc**, a portion of the stem transversely cut into discs (**Fig. 1G**); **Sucker**, basal leafy shoot arising from bud under ground level (**Fig. 1E**); **Syncarp**, a fleshy compound fruit composed of the fruits of several flowers (**Fig. 1B**)

Received: 12 November, 2008. Accepted: 27 May, 2009.

Invited Review

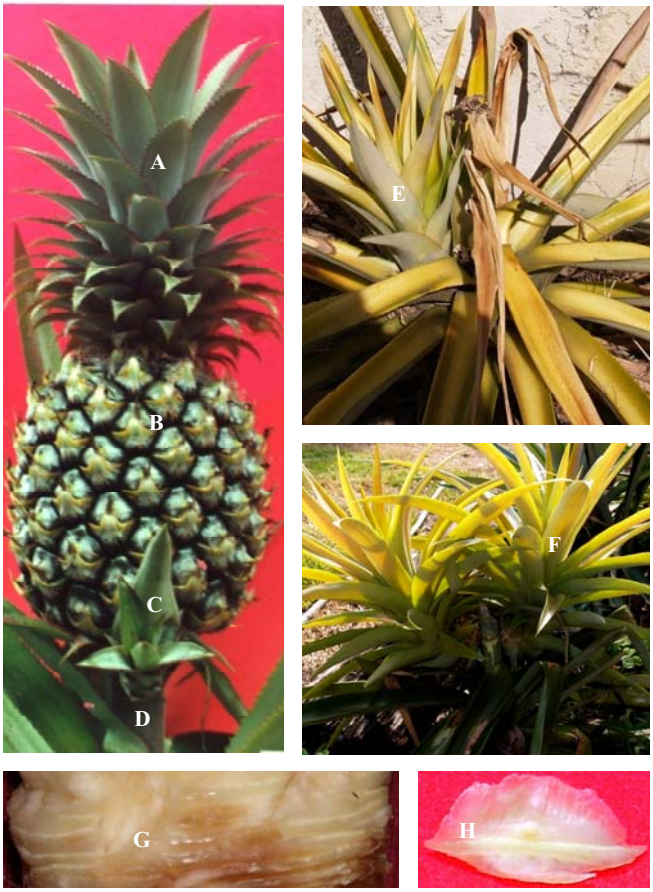

Fig. 1 Various parts of pineapple. (**A**) Crown. (**B**) Syncarp. (**C**) Slip/ Hapa. (**D**) Peduncle. (**E**) Sucker. (**F**) Ratoon. (**G**) Stem disc. (**H**) Axillary bud.

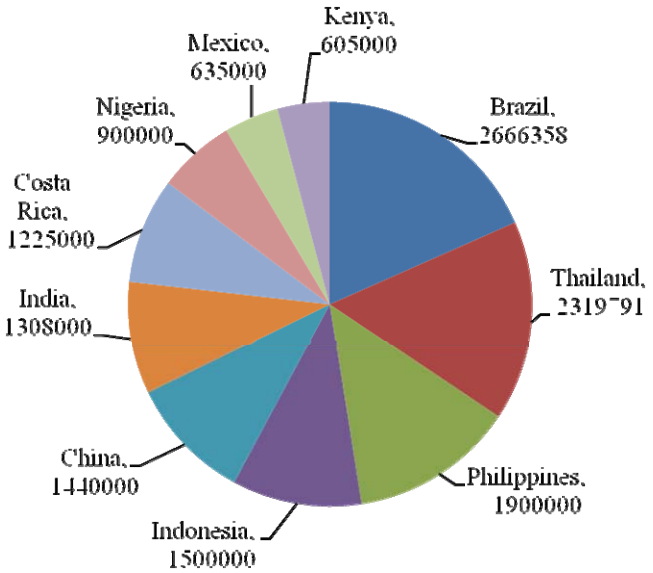

Fig. 2 Top ten pineapple producing countries of the world (the value is the production in tones, FAO 2008).

60% of the world's fresh pineapple exports whereas Thailand, the Philippines, and Indonesia supply 80% of the world's canned pineapple exports. Thailand and the Philippines also dominate world pineapple juice exports, accounting for more than half of total volume (Soneji and Nageswara Rao 2008).

The export value of pineapple and pineapple products from the producing countries was over US$665 million in 2004 (FAO 2008). 'Smooth Cayenne' accounts for around 70% of world pineapple production although other cultivars such as 'Red Spanish', 'Perolera', 'Pernambuco', 'Primavera', 'Del Monte Gold', 'Hawaiian King', 'Hilo', 'Honey Gold', 'Queen', 'Singapore Spanish' and 'Sugarloaf' are also grown. 'Smooth Cayenne' is the progenitor of most of the cultivars that are used for canning, production of processed products and fresh consumption (Firoozabady *et al.* 2006). Of recent, interest in developing *Ananas* selections specifically for the ornamental market has increased (Sanewski 2008). Breeding programs have been initiated using parental combinations of *A. comosus* var. *comosus*, *A. comosus* var. *bracteatus*, *A. comosus* var. *ananassoides*, *A. comosus* var. *erectifolious* and *A. macrodontes* (Sanewski 2008; Souza *et al*. 2008). Several hyrbids have been selected with specific characteristics to be used as pot plants, cut flowers, landscape plants and ornamental mini fruits (Souza *et al*. 2008). Selected lines include hybrids having a bright pink or red syncarp, dark red-brown foliage and a dwarf, clumping habit (Sanewski 2008).

Though a number of intraspecific and interspecific crosses have been carried out encompassing the many aspects of productivity, fruit quality, and pest and disease resistance, the heterozygous nature of pineapple cultivars and the consequent strong segregation and recombination have limited the success of hybrid breeding (Carlier *et al*. 2007; Botella and Smith 2008). Biotechnological approaches such as genomics and genetic engineering may be able to overcome the constraints of breeding programs. In pineapple, limited amount of genomics research has been carried out. Molecular markers have been developed to study genetic relationships among the different *Ananas* species and with other members of the Bromeliaceae family (Kato *et al*. 2004; Paz *et al*. 2005). The unique pineapple genome maps published so far are the genetic maps of molecular markers including the morphological trait 'piping' (Carlier *et al*. 2004, 2006). Genetic engineering appears to be a promising strategy since it allows transferring a single gene, or a few genes, without substantially altering the initial genome. In this review, the role of genetic engineering in pineapple genetic improvement has been discussed.

ECONOMIC IMPORTANCE

Pineapple yields many products making it a versatile plant. The edible portion of the fruit that constitutes about 60% of the fresh fruit contains approximately 85% water, 0.4% protein, 14% sugar, 0.1% fat and 0.5% fiber (Samson 1980). The fruit is rich in vitamins A, B and C (**Table 1**). Besides being used as a fresh fruit, it offers considerable scope for canning. The fruit is utilized for preparation of juice, jam, candy and as crystallized glace fruit. The juice has 75-83% sucrose and 7-9% citric acid on a dry weight basis (Davey *et al.* 2007). Pineapple is also exploited in many other ways. Pineapple juice is taken as a diuretic, as an antidote for seasickness and as a gargle for sore throat. The juice of the leaf is used as a purgative and vermifuge (Morton 1987). Its juice is also utilized, although in small quantities, for the manufacture of alcohol, calcium nitrate, citric acid and vinegar. The dried waste after juice extraction is used as

Table 1 Food value per l00 g of edible portion of pineapple fruit

Nutritional value	Per 100 g of edible portion
Moisture	81.3-91.2 g
Carotene (Vitamin A)	0.003- 0.055 mg
Thiamine	0.048 - 0.138 mg
Riboflavin	0.011- 0.04 mg
Niacin	0.13 - 0.267 mg
Ascorbic acid (Vitamin C)	27.0 - 165.2 mg
Iron	0.27 - 1.05 mg
Crude fiber	0.3 - 0.6 g
Phosphorus	6.6 - 11.9 mg
Calcium	6.2 - 37.2 mg
Ash	0.21- 0.49 g
Nitrogen	0.038 - 0.098 g
Ether extract	0.03 - 0.29 g

Source: Soneji and Nageswara Rao 2008

cattle feed. The fruit also contains bromelain, a proteolytic enzyme that has many therapeutic uses and is also used for tenderizing the meat, chill proofing beer, is added to gelatin to increase its solubility, is used for stabilizing latex paints, and in leather-tanning process (Morton 1987; de la Cruz-Medina and Garcia 2007). The fibres from leaves yield a strong white silky fibre that is used for making a fine fabric called "pina" cloth (Samson 1980) and is also used as cordage. Pineapple fibre has been processed into paper with remarkable qualities of thinness, smoothness and pliability (Collins 1960). Some chimeric forms of pineapple are marketed as ornamental plants (Davey *et al.* 2007) and a small market exists for the flowers of some genotypes (Ko *et al.* 2008).

IN VITRO REGENERATION OF PINEAPPLE

A number of researchers have reported plant regeneration *via* organogenesis and embryogenesis in pineapple (**Table 2**). Pineapple was first micropropagated *in vitro* by Aghion and Beauchesne (1960). Shoot apices of pineapple have been cultured using different growth regulators at various

Table 2 Studies on *in vitro* regeneration in pineapple.

Explant(s)	Cultivar	Basal medium	Growth regulator(s)	Response	Reference
Shoot tips	Cayenne	MS	30 mg l^{-1} adenosine	Plants and protocorm-like bodies	Mapes 1973
Shoot tips	Kew	Knudson with N micro-elements	1.0 mg l^{-1} NAA	Plantlets	Lakshmi Sita *et al.* 1974
Terminal buds	Market cultivar	MS	1.8 mgl^{-1} NAA, 2.0 mg l^{-1} IBA, 2.1 mg l^{-1} Kn	Plantlets	Mathews *et al.* 1976
Young syncarps, axillary buds, crowns and slips	NA	MS	10.0 mg l^{-1} NAA, 10.0 mg l^{-1} BA	Callus regeneration	Wakasa *et al.* 1978
Axillary buds	Market cultivar	MS	1.8 mgl^{-1} NAA, 2.0 mg l^{-1} IBA, 2.1 mg l^{-1} Kn	Multiple shoots	Mathews and Rangan 1979
Basal region of *in vitro* obtained shoot buds	Market cultivar	MS	400 mg l^{-1} casein hydrolysate (CH), 15% (v/v) CM, 10.0 mg l^{-1} NAA	Callus regeneration	Mathews and Rangan 1981
Hybrid embryos	Kew X Queen	MS	0.1 mg l^{-1} IBA, 0.1 mg l^{-1} BA	Callus regeneration	Srinivasa Rao *et al.* 1981
Axillary buds	NA	MS ½ strength MS	25% (v/v) CM 1.0 mg l^{-1} BA	Multiple shoots	Zepeda and Sagawa 1981
Axillary buds	Cayenne, Red Spanish and Perolera	MS	2.0 mg l^{-1} NAA, 2.0 mg l^{-1} BA	Multiple shoots	de Wald *et al.* 1988
Meristems	Red Spanish	MS	0.1 mg l^{-1} 2,4-D, 0.5 mg l^{-1} BA	Multiple shoots	Liu *et al.* 1989
Lateral buds	Queen and Smooth Cayenne	MT	2.0 mg l^{-1} NAA, 2.0 mg l^{-1} IAA, 2.0 mg l^{-1} Kn	Plantlets	Fitchet 1990
Apical crown region	Queen and Smooth Cayenne	MT	40.0 mg l^{-1} NAA, 15% (v/v) CM, 400 mg l^{-1} CH	Callus regeneration	Fitchet 1990
Axillary buds		MS	0.5 mg l^{-1} BA, 0.2 mg l^{-1} IAA	Multiple shoots	Cote *et al.* 1991
Shoot apices	Kew	MS	0.02 mg l^{-1} NAA	Plantlets	Hirimburegama and Wijesinghe 1992
Axillary buds	Cayenne, Red Spanish and Perolera	MS	2.0 mg l^{-1} NAA, 2.0 mg l^{-1} BA	Multiple shoots	Moore *et al.* 1992
Etiolated nodal explants	Cayenne	N6	5.3 mg l^{-1} Kn or 4.5 mg l^{-1} BA	Multiple shoots	Kiss *et al.* 1995
Lateral buds	Primavera and Perolera	MS	2.0 mg l^{-1} IAA, 1.0-3.0 mg l^{-1} BA	Multiple shoots	de Almeida *et al.* 1996
Hybrid zygotic embryo	Serrana Smooth Cayenne X Perolera	MS	1.0 mg l^{-1} NAA transferred to 0.3 mg l^{-1} NAA, 2.1 mg l^{-1} BA	Callus regeneration	Benega *et al.* 1996a
Unfertilized ovules	Serrana Smooth Cayenne, Pina blanca, Red Spanish, Perolera	MS	5:1 ratio of dicamba with BA	Callus regeneration	Benega *et al.* 1996b
Leaf bases	Smooth Cayenne, Red Spanish	MS	2.5 mg l^{-1} dicamba, 0.5 mg l^{-1} BAP	Callus regeneration	Daquinta *et al.* 1996
Leaf or shoot bases	*Ananas comosus* Merr. 'varigatus'	½ strength MS	1.0 mg l^{-1} NAA, 1.0 mg l^{-1} BA	Nodular tissue regeneration	Teng 1997
Plants obtained from axillary buds	Smooth Cayenne	MS	0.3 mg l^{-1} NAA, 2.1 mg l^{-1} BA, 1.0 mg l^{-1} paclobutrazol	Multiple shoots	Escalona *et al.* 1999
Leaves	Red Spanish	MS	0.2 mg l^{-1} 2,4-D, 0.1 mg l^{-1} Kn	Callus regeneration	Garcia *et al.* 2000
Basal region of *in vitro* obtained shoot buds	Queen	MS	0.2 mg l^{-1} 2,4-D, 0.2 mg l^{-1} 2iP	Callus regeneration	Soneji 2001
Leaf bases	Phuket	MS	0.5 mg l^{-1} 2,4-D, 2.0 mg l^{-1} BA	Plant regeneration	Sripaoraya *et al.* 2001
Axillary buds	Queen	MS	1.8 mgl^{-1} NAA, 2.0 mg l^{-1} IBA, 2.0 mg l^{-1} Kn	Multiple shoots	Soneji *et al.* 2002a
Basal part of the leaf	Queen	MS	0.2 mg l^{-1} 2,4-D, 0.2 mg l^{-1} 2iP	Protuberances, shoots	Soneji *et al.* 2002b
Bases of leaves	Smooth Cayenne	½ strength MS with B5 vitamins	0.5 mg l^{-1} NAA, 1.5 mg l^{-1} BA	Shoots	Firoozabady and Gutterson 2003
Axillary and terminal buds	Phuket	MT	2.0 mgl^{-1} NAA, 2.0 mg l^{-1} IBA, 2.0 mg l^{-1} Kn	Multiple shoots	Sripaoraya *et al.* 2003
Leaf bases	Phuket	MS	3.0 mgl^{-1} picloram	Embryogenic callus	Sripaoraya *et al.* 2003
Leaf bases		MS	1.0 mg l^{-1} BA	Plant regeneration	Perez *et al.* 2006
Protocorm-like bodies		MS	0.5 mg l^{-1} BA	Plant regeneration	Perez *et al.* 2006

2,4-D, 2,4-dichlorophenoxyacetic acid; 2iP, 6-(γ,γ-Dimethylallylamino)purine; B5, Gamborg *et al.* (1968) medium; BA, 6-benzyladenine; CH, casein hydrolysate; CM, coconut milk; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kn, kinetin; MS, Murashige and Skoog (1962) medium; MT, Murashige and Tucker (1969) medium; N, Nitsch (1951) medium; N6, Chu (1978) medium; NAA, α-naphthaleneacetic acid; NA, not applicable

Fig. 3 ***In vitro*** **axillary bud culture in pineapple**. (**A**) Excised axillary bud cultured *in vitro*. (**B**) Multiple shoots arising from axillary bud. (**C**) Rooting of *in vitro* grown shoots. (**D**) Tissue cultured pineapples established in the field.

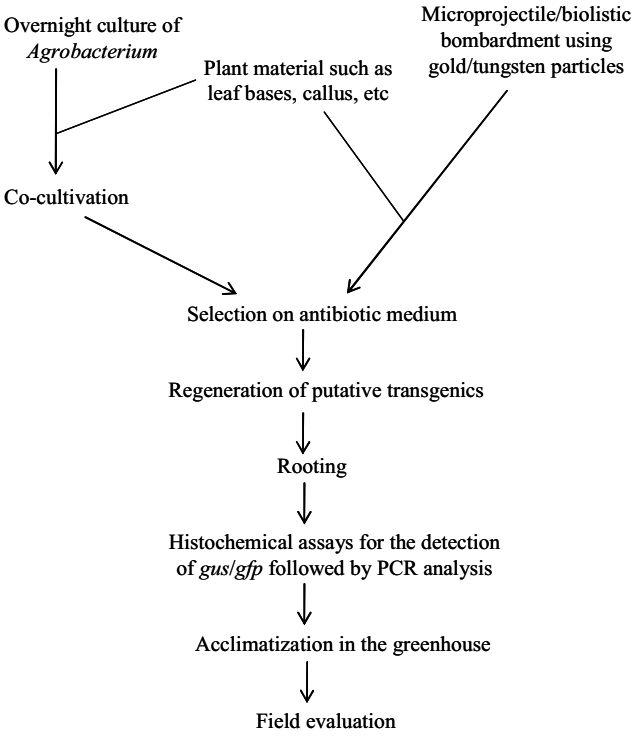

Fig. 4 Flow chart depicting the various steps involved in pineapple transformation.

concentrations to study their growth and to achieve bud proliferation for developing a rapid propagation method (Mapes 1973; Hirimburegama and Wijesinghe 1992; Albuquerque *et al.* 2000). Plantlets have also been obtained from shoot meristems excised from slips (Lakshmi Sita *et al.* 1974). Crown tips from mature fruits have been micropropagated to obtain plantlets (Rahman *et al.* 2001). Stem disc containing axillary buds has been cultured (Poh and Khoon 1975). Axillary buds from the crowns of mature fruit (Soneji *et al.* 2002a; **Fig. 3**), or both lateral and axillary buds (Cabral *et al.* 1984; de Wald *et al.* 1988) have also been cultured. Leaves have been used as explants for the propagation of pineapple. They have either given rise to shoot buds directly (Soneji *et al.* 2002b) or indirectly *via* callus formation (Daquinta *et al.* 1994, 1996; Garcia *et al.* 2000). Callus has been established from a number of explants such as young syncarps, axillary buds, crowns and slips (Wakasa *et al.* 1978; Wakasa 1989), hybrid embryos (Srinivasa Rao *et al.* 1981), lateral bud and meristem tips of crowns (Liu *et al.* 1989), crown sections with or without buds (Lapade *et al.* 1988), basal region of *in vitro* obtained shoot buds (Soneji 2001) and leaf explants (Soneji *et al.* 2002b). Synthetic seeds of pineapple were first produced by the encapsulation of tiny (2-3 mm) *in vitro* grown shoots (Soneji *et al.* 2002c). Microshoots of pineapple have also been encapsulated for the purpose of short term storage (Gangopadhyay *et al.* 2005).

TRANSFORMATION OF PINEAPPLE

Pineapple genetics is not well understood. It is a self-incompatible and highly heterozygous plant with a 2-year time between successive fruit generations; therefore conventional breeding to improve fruit quality is difficult (Pickergill 1976). It is one of the few crops in which all cultivars are derived from spontaneous mutations and natural evolution without controlled breeding (Osei-Kofi *et al.* 1997). Genetic engineering is an attractive strategy with great potential to improve the horticultural characteristics of pineapple varieties by introducing very specific traits without altering other agronomic attributes (Smith *et al.* 2002).

For genetic engineering, foreign DNA can be introduced into plant cells by either vector-mediated transfer or direct transfer, both of which essentially involve precise tissue culture methods. Transformation of pineapple has been achieved by co-cultivation with *Agrobacterium tumefaciens* as well as by microprojectile bombardment (**Fig. 4**). *Agrobacterium*-mediated transformation (**Table 3**) has been used for genetic engineering of pineapple embryogenic cultures (Isidron *et al.* 1998; Firoozabady and Gutterson 1998), leaf bases (Graham *et al.* 2000a) and morphogenic callus (Espinosa *et al.* 2002) while microprojectile bombardment (**Table 4**) has been used to transform embryogenic suspension cultures (Nan *et al.* 1996), leaves (Sripaoraya *et al.* 2001), protocorm-like structures (Nan and Nagai 1998) and callus (Espinosa *et al.* 2002). Pineapple has also been transformed with reporter genes, *gus* (β-glucuronidase) and *gfp* (green fluorescent protein), as indicators to optimize the conditions for transient and stable gene expression (Ko *et al.* 2000). Among the most important traits of interest for cultivar improvement are disease and pest resistance (fungal, bacterial and viral diseases, insects and nematodes), improvement of fruit quality (sweetness, acidity, texture, nutrition and ripening characteristics), and control of flowering (Firoozabady *et al.* 2006). Most of the work in pineapple improvement *via* genetic transformation has been attempted in 'Smooth Cayenne', a cultivar of importance to the processing industry.

Heart and root rot resistance

Phytophthora species cause heart and root rot in pineapple leading to great losses (Kamoun 2001). Heart rot in pineapples can be caused by both *P. cinnamomi* and *P. nicotianae*. Plants of all ages are attacked, but young crowns are most susceptible. The first symptom is a color change of the heart leaves to yellow or light, coppery brown and later on the heart leaves wilt, causing the leaf edges to roll under, turn brown and eventually die. *P. cinnamomi* is the main pathogen that causes root rot in pineapples. All leaves show color changes similar to those caused by heart rot. The outer leaves also become limp and die back from the tips. Once

Table 3 Transgenes introduced in pineapple *via Agrobacterium*-mediated transformation

Character introduced	Explants	Plasmid	Promoter-transgene-terminator	Reference
Herbicide tolerance	Embryogenic tissues		*als* *nptII*	Firoozabady *et al.* 1997
Transient assay	Leaf bases		35S *CaMV -gus-ocs* 35S *CaMV -gfp-nos* *nos-nptII-nos* *SCSV*4-*nptII-SCSV*5	Graham *et al.* 2000a, 2000b
Heart and root rot resistance	Callus from young leaves	pHCA58	*ocs*-35S *CaMV*-rice actin I-*chitinase-nos* 35S *CaMV-ap24-nos* *ubi-bar-nos*	Espinosa *et al.* 2002
Heart and root rot resistance	Callus from young leaves	pHCA59	*ocs*-35S *CaMV*-rice actin I-*chitinase-nos* 35S *CaMV-gluc-nos* *ubi-bar-nos*	Espinosa *et al.* 2002
Heart and root rot resistance	Callus from young leaves	pTOK233, pIG121Hm	35S *CaMV-uidA-hph-nos* *nos-nptII-nos*	Espinosa *et al.* 2002
Nematode resistance	Stem segments and leaf bases		Modified rice *cystatin* (*ubi9-d86*) *protease inhibitor*	Sipes *et al.* 2002
Fungal resistance	Leaf bases of *in vitro* grown shoots	pMSI186	*ubq3-MSI-99-nos* *ubq3- nptII -nos*	Mhatre 2003
Transient assay	Embryogenic cell clusters, embryogenic tissues	pALS1301	*smas-gus-nos* *ubi1-surB-ocs*	Firoozabady *et al.* 2006
Transient assay	Embryogenic cell clusters, embryogenic tissues	pNPT0402	*ubi1-nptII-nos* *ubi1-surB-nos*	Firoozabady *et al.* 2006
Control fruit ripening	Embryogenic cell clusters, embryogenic tissues	pPO7022b	*ubi1-surB* *smas-acacs2-ocs*	Firoozabady *et al.* 2006
Control of flowering	Embryogenic cell clusters, embryogenic tissues	pPO7127	Enhanced 35S *CaMV-acacs3-ocs*	Firoozabady *et al.* 2006
Transient assay	Embryogenic cell clusters, embryogenic tissues	pPO7123	Enhanced 35S *CaMV-gus-ocs*	Firoozabady *et al.* 2006
Mealybug wilt resistance	Leaf bases	pCAMBIA 1300	*PMWaV*-2 *coat protein*	Perez *et al.* 2006
Control of flowering	Stem segments and leaf bases		*smas-acacs2-ocs* *ubi-surB-utr*	Trusov and Botella 2006

Table 4 Transgenes introduced in pineapple *via* microprojectile bombardment.

Character introduced	Explants	Plasmid	Promoter-transgene-terminator	Reference
Transient assay	Leaf bases		35S *CaMV-gus-ocs* 35S *CaMV-gfp-nos* *nos-nptII-nos* *SCSV*4-*nptII-SCSV*5	Graham *et al*. 2000a, 2000b
Herbicide tolerance	Leaf bases	pAHC25	*ubi-gus* *ubi-bar*	Sripaoraya *et al*. 2001
Blackheart resistance	Callus initiated on leaf bases	pART7	35S *CAMV-ppo-ocs* 35S *CAMV-nptII*-35S *CAMV*	Ko *et al*. 2005, 2006
Blackheart resistance	Callus initiated on leaf bases	pART7	35S *CAMV-opp-nos* *ubi1-ppo-ocs* 35S *CAMV-nptII*-35S *CAMV*	Ko *et al*. 2005, 2006
Blackheart resistance	Callus initiated on leaf bases	pART7	35S *CAMV-gus-ocs* 35S *CAMV-nptII*-35S *CAMV*	Ko *et al*. 2005, 2006
Blackheart resistance	Callus initiated on leaf bases	pBS247	*SCSV4-gus-SCSV5* *SCSV4-nptII-SCSV5*	Ko *et al*. 2005, 2006
Blackheart resistance	Callus initiated on leaf bases	pGEM	*ubi1-gfp-nos* 35S *CAMV-nptII*-35S *CAMV*	Ko *et al*. 2005, 2006
Mealybug wilt resistance	Protocorm-like bodies	pCAMBIA 1300	*PMWaV*-2 *coat protein*	Perez *et al*. 2006

this happens, the root system is dead and plants can easily be pulled from the ground. Fruit from diseased plants are small and unmarketable. Plants can recover if symptoms are recognized early and treated immediately. However, if roots are destroyed right back to the stem, they cannot regenerate (de Matos 1995).

Attempts have been made to introduce antifungal genes such as *chitinase* (*chi*) and *ap24* into pineapple genome for gaining resistance to *P. nicotianae* var. *parasitica* (Espinosa *et al.* 2002; Yabor *et al*. 2006). The *chi* gene product degrades chitin, an essential compound of most of the fungal cell walls (Broglie *et al.* 1986; Schlumbaum *et al.* 1986) while the *ap24* gene codes for a wide-spectrum antifungal protein which destabilizes the fungal membrane potentials (Singh *et al.* 1989; Woloshuk *et al.* 1991). Espinosa *et al.* (2002) described a complete protocol for *Agrobacterium*-mediated transformation (**Table 3**) of pineapple using regenerable pineapple callus obtained from young leaves (**Table 2**) and LBA4404 (pTOK233) and AT2260 (pIG121Hm) strains (Hiei *et al.* 1994) of *Agrobacterium*. The plasmid pHCA58 contained a class-I bean *chi* gene (Broglie *et al.* 1986) and the tobacco *ap24* gene (Melchers *et al.* 1993) while the plasmid pHCG59 contained the *chi* gene and a class-I tobacco β-1,3-glucanase (*gluc*) gene (Ohme-Tagaki and Shinshi 1990). Both the plasmids carried the bialaphos resistance (*bar*) gene for resistance to phosphinothricin (PPT). Their study resulted in a 6.6% efficiency of transgenic plant recovery (**Table 5**). No reports are available on whether these transgenic plants were tested for their resistance to heart and root rot under field conditions. However, Yabor *et al*. (2006), under greenhouse conditions, studied the biochemical side effects of introduction of *bar, chi* and *ap24* genes into pineapple.

Attempts have also been made to produce fungal resistant transgenic pineapples. For this leaf bases of *in vitro* shoots of pineapple were transformed with *Agrobacterium*

Table 5 Various methods used for the calculation of transformation efficiency in pineapple.

Method used for calculation of transformation efficiency	Transformation efficiency	Reference
For *Agrobacterium*-mediated transformation		
(Number of PPT-resistant shoots/Number of regenerated shoots) X 100	1.8 - 6.6%	Espinosa *et al.* 2002
Number of resistant lines g^{-1}	1 - 60 resistant lines	Firoozabady *et al.* 2006
Events g^{-1} tissue	0 - 210 events	Firoozabady *et al.* 2006
For particle bombardment		
(Number of explants producing transgenic callus/Number of explants bombarded) X 100	0.2 - 0.8%	Smith *et al.* 2002
(Total number of discrete spots/Total number of callus pieces bombarded) X 100	0.56 - 1.19%	Ko *et al.* 2006

strain EHA105 harboring the plant expression vector pMSI168 containing MSI-99, a substitution analogue of magainin, which is an antimicrobial peptide. The transformed leaf bases were cultured on regeneration medium (Soneji *et al.* 2002b, **Table 2**) supplemented with 50 mg l^{-1} Kanamycin and 400 mg l^{-1} Cefotaxime. Six percent of leaf bases produced callus and only 2% produced direct multiple shoots. Transgenic plants were established first in cups and later in pots in the green house. The transformed status of the transgenic plants was determined by Southern hybridization of polymerase chain reaction (PCR) products and reverse transcription (RT)-PCR (Mhatre 2003). The transformation efficiency obtained as well as the resistance of these transgenics to fungus has not been reported as yet.

Mealybug wilt resistance

Mealybug wilt of pineapple (MWP) is a serious problem found in all pineapple growing regions of the world. The disease is characterized by severe leaf tip dieback, downward curling of the leaf margins, and reddening and wilting of the leaves that can lead to total collapse of the plant (Hu *et al.* 2005). Two types of wilt are common in pineapple, "quick wilt" and "slow wilt". "Quick wilt" is observed when a large colony of mealybugs feeds on pineapple for a short period and is characterized by discoloration of leaves to yellows or reds and the loss of rigidity in leaves. "Slow wilt" occurs after the development of a large colony of mealybugs on pineapple. It shows fewer color changes, however, the leaves get covered with mealybug feeding sites, leaf tips turn brown, outer leaves droop and the leaf will be flaccid to the touch. Both types cause the collapse of roots by the invasion of saprophytic organisms or by drying up the root (Rohrbach *et al.* 1988).

MWP is caused by Pineapple mealybug wilt associated virus-2 (PMWaV-2) infection and mealybug feeding. Perez *et al.* (2006) engineered the PMWaV-2 coat protein (*cp*) gene in sense and inverted repeat orientations into pCAMBIA 1300 transformation vector. They used *Agrobacterium*-mediated genetic transformation to introduce *cp* gene in sense orientation into the leaf bases of pineapple. Primary transformants from leaf bases were regenerated (**Table 2**) with or without the addition of 16 mg l^{-1} of hygromycin B in the regeneration medium. They also used biolistic bombardment of protocorm-like bodies of pineapple to introduce *cp* gene in inverted repeat orientation. The primary transformants from protocorm-like bodies were cultured on regeneration media (**Table 2**) supplemented with increasing antibiotic concentration of 16 to 25 mg l^{-1} of hygromycin B. Seven lines of putatively transgenic pineapple plants that were resistant to PMWaV-2 infection were produced after multiple challenges with viruliferous mealybugs. Gene constructs have also been developed using RNA-mediated resistance technology to develop transgenic pineapple resistant to MWP. These transgenics have been tested twice in bioassays in the greenhouse and have shown no MWP symptoms and were PMWaV negative (Hu *et al.* 2005).

Nematode resistance

The most devastating pathogen in the pineapple industry is the reniform nematode *Rotylenchulus reniformis* (Rohrbach and Apt 1986; Starr and Page 1990). Nematode control poses a severe problem due to the semi-perennial nature of pineapple and the lack of natural resistance to nematodes in cultivars of this crop (Sipes and Schmitt 1994). Nematode infection can cause losses of up to 40% of the first fruit crop and 80-100% of subsequent ratoon crops (Schenck 1990; Sipes and Schmitt 1994).

Attempts have also been made to introduce nematode resistance into pineapple (Rohrbach *et al.* 2000). Stem segments and leaf bases of low acid pineapple variety MD-1 were transformed using AGL0 strain of *A. tumefaciens* to introduce a modified rice cystatin (*ubi9-d86*) protease inhibitor (**Table 3**). Around 22 transgenic lines were developed (Sipes *et al.* 2002) and compared to wild type pineapple plants for growth and reproduction of reniform nematode. Nematode infection reduced plant growth in both the wild type and transgenic plants. Reproduction of nematode on transgenic plants was less than that on wild type. However, the range of nematode reproduction per plant was greater on the transformed plants than on the wild type plants suggesting chimerism within the transformed pineapple plants (Sipes *et al*. 2002).

Herbicide tolerance

Pineapple production and commercialization is restricted in many parts of the world by pests and diseases, the short shelf life of harvested fruit and the lack of effective weed control (Sripaoraya *et al.* 2001). Genes for herbicide tolerance have not been identified in pineapple or its wild relatives making conventional breeding for herbicide-tolerant cultivars impossible. The only available alternative is the genetic engineering of pineapple varieties to introduce herbicide tolerance.

Firoozabady *et al.* (1997) were the first to report genetic transformation of embryogenic tissues using the disarmed strain of *Agrobacterium*, C58C1, harboring a binary vector carrying either an *als* gene, conferring resistance to the selective herbicide chlorsulfuron or the neomycin phosphotransferase (*nptII*) gene which confers resistance in plant cells to the antibiotics neomycin, kanamycin and geneticin. About 30 transformed callus lines were obtained per gram of fresh weight of the embryogenic calli inoculated with bacterium. A number of plants from several independently transformed lines have been transferred to the greenhouse to evaluate their genetic stability.

Sripaoraya *et al.* (2001) utilized microprojectile-mediated delivery of the plasmid AHC25, carrying the *gus* reporter gene and the *bar* gene for herbicide tolerance to transform leaf bases of pineapple cultivar 'Phuket'. The bombarded leaf bases were cultured on regeneration medium (**Table 2**) which also contained 0.5 mg l^{-1} PPT. Regenerated plants were assessed *in vitro* for their tolerance to the commercial herbicide Basta™, containing glufosinate ammonium as the active component, by adding 0-20 mg l^{-1} to the agar medium. Transformed plants remained green while non-transformed plants either died on treatments above 3 mg l^{-1} or were chlorotic/necrotic. The same test was repeated for transgenic plants after they were acclimatized under glasshouse conditions for 75 days. Transgenic plants sprayed with Basta™ containing concentrations of glufosinate ammonium up to 1400 mg l^{-1} remained healthy and retained their pigmentation. Six month old glasshouse

plants were planted in a field and after 210 days were sprayed with Basta™ containing concentrations of glufosinate ammonium up to 4000 mg l^{-1}. The transgenic plants were found to be tolerant to all concentrations of the herbicide. Fruit yield and quality were also not affected by transgene insertion and expression (Sripaoraya *et al*. 2006). The generation of herbicide-tolerant pineapple will facilitate more efficient weed control in this widely cultivated tropical crop (Davey *et al.* 2007). Sripaoraya (2007) has carried out studies on inheritance of transgene between transgenic and non-transgenic pineapple cultivars 'Pattavia' and 'Phuket' using direct and reciprocal crosses as well as selfing. He obtained seeds and plantlets from direct and reciprocal crosses of transgenic plants and 'Pattavia' while all self and both direct and reciprocal crosses between transgenic plants and 'Phuket' did not give any seed. GUS expression was used for checking for the presence of the transgene (*bar* gene) in leaves of plantlets from hybridization. Out of 125 plantlets obtained from the 'Pattavia' and transgenic plant crosses, 71 showed positive GUS expression and 54 were negative for the gene. Chi-square analysis of plants resistant and sensitive to Basta™ herbicide showed a 1:1 ratio, which follows Mendel's Law of inheritance for a pair of genes controlling the trait. Further work is being carried out to evaluate their resistance to Basta™ herbicide.

Control of flowering

Flowering is one of the most important processes in plant ontogeny, consisting of the transition from vegetative growth to generative development that ultimately allows reproduction (Trusov and Botella 2006, 2008). To synchronize flowering, pineapple growers usually select planting material by size/weight (Reinhardt and Medina 1992) and, once plants reach maturity, usually a year after planting, treat them with a number of flowering-inducing agents (Bartholomew 1977; Reid and Wu 1991). However, a fraction of the crop (ranging from 5 to 30% and reaching up to 70% under certain conditions) still manages to flower ahead of schedule, a phenomenon known as 'natural flowering' or 'environmental induction' (Min and Bartholomew 1996). Natural flowering of pineapple is not synchronized. This is a highly undesirable characteristic of pineapples grown worldwide, causing disruption in harvest scheduling and market supply, increasing harvest costs due to multiple harvests of the same field, and resulting in significant harvest losses (Min and Bartholomew 1996). Improved control over flowering and fruit ripening would allow harvesting to be achieved in a single pass, and would also increase the feasibility of mechanical harvesting.

Although pineapple fruits are non-climacteric, both ethylene biosynthetic genes are up-regulated in the flesh of pineapple fruits during ripening (Cazzonelli *et al.* 1998, 1999). An 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (a key enzyme in the pathway that leads to formation of ethylene in plants) gene that may be involved in floral initiation has been cloned (Botella *et al.* 2000). The 1-aminocyclopropane-1-carboxylic acid synthase (*acacs*) gene from pineapple was expressed in meristematic cells and activated to induce flowering under certain environmental conditions such as low temperatures and photoperiod (Ko *et al*. 2008). Pineapple plants were modified by the insertion of additional copies of *acacs2* gene which encodes for isoforms of ACC synthase that already occurs in pineapple (Firoozabady *et al.* 1997). Its silencing in pineapple could suppress flowering until it is induced artificially. This might facilitate synchronization of fruiting and ripening, and enable mechanized harvesting. Stem segments and leaf bases of low acid pineapple variety MD-1 were transformed using AGL0 strain of *A. tumefaciens* to introduce ACC antisense (*ubi9*). In order to enhance gene expression in pineapple, an intron derived from the chalcone synthase (*CHS-A*) gene of *Petunia hybrida* (Koes *et al.* 1989) was inserted into the central region of the waxy leader. This promoter–leader–intron structure was linked to the 5′ end of a 0.97 kb fragment of an incomplete cDNA copy of the *acacs2* message (Botella *et al.* 2000). A total of seven transgenic lines were produced. After transformation, clonal propagation in tissue culture was used to produce a total of 111 plants for line 1 and 108 for line 2 (Trusov and Botella 2006). Transformed pineapple plants containing genetic constructs to inactivate the ripening-related ACC synthase were evaluated under field conditions (Botella *et al.* 2000; Botella and Fairbairn 2005). Trusov and Botella (2006, 2008) analyzed the flowering dynamics for the first generation of transgenic plants grown directly from tissue culture and showed that transgenic plants in both lines had a lower number of flowering plants over the first 6 month period after planting. They performed a second field trial using vegetatively propagated progeny of the plants used in the first trial. They reported high basal levels of *acacs2* signal in the early flowering transgenic plants, probably due to the constitutive expression of the inserted *acacs2* fragment. Auxin treatment of these plants results in even higher levels due to the enhanced expression of the endogenous gene in addition to the *acacs2* RNA pool produced from the inserted transgene. In the late flowering transgenic plants, however, *acacs2* levels were almost undetectable and auxin induction fails to produce any increase in transcript levels, indicating that both the native *acacs2* gene as well as the inserted transgene had been silenced. Their study proved that silencing of the *acacs2* gene using genetic engineering techniques can be successfully used to control natural flowering in commercial situations, thereby addressing a major problem faced by the pineapple industry.

Improvement of fruit quality

Blackheart is the major postharvest limitation to pineapple production. It is characterized by a distinct browning of the core and flesh of the affected fruit (Teisson *et al.* 1979). It is a physiological disorder induced by exposure of pineapples to low temperatures. It occurs after continuous cool storage (three days at temperatures below 21°C) or low temperatures during fruit development (less than 25°C during the day or less than 20°C during the night combined with low light). This exposure to low temperatures stimulates polyphenol oxidase (PPO) activity leading to the discoloration of the pulp of the pineapple (Graham *et al.* 2000a; Rohrbach *et al.* 2000). As there are no obvious external symptoms of blackheart disorder, affected fruit is often not detected until it is sliced after purchase, resulting in considerable consumer dissatisfaction (Teisson *et al.* 1979; Stewart *et al.* 2002).

Stewart *et al.* (2001) cloned a *ppo* gene from pineapple fruits under conditions that produce blackheart. Ko *et al.* (2006) used callus initiated on leaf bases cultured on medium described by Wakasa *et al*. (1978, **Table 2**) for particle bombardment. Two plasmids (pDH-kan^R and pBS420) expressing the *nptII* selectable marker gene and plasmids (pART7.35S.GUS, pBS247.SCSV4.GUS and pGEM-Ubi-GFP) expressing the *gus*, *gfp* or *ppo* genes were used in these experiments (**Table 4**). Constructs were designed containing the PINPPO1 gene in a sense (*ppo*) and sense/antisense (*opp.ppo*) orientation in pART7. Large-scale shoot regeneration was initiated approximately 8 months after bombardment of callus pieces. They obtained an average transformation efficiency (**Table 5**) of 0.56% for the *ppo* construct with the production of 14 independent transgenic lines and over 1,700 plants. The *opp.ppo* construct, on the other hand, showed a transformation efficiency of 1.19% and produced 8 independent transgenic lines with over 1,000 plants. The *ppo* gene has been silenced in transformed plants and transgenic plants are under field evaluation (Gomez-Lim and Litz 2004).

SAFETY RISKS AND CONCERNS

Pineapple is found only under cultivation and does not occur naturally. 'Smooth Cayenne', the most dominant cultivar, is not a competitive colonizer of natural ecosystems (Ko *et al*. 2008). Possible ways of dispersal of transgenic pineapples is by pollen escape or dispersal of clonally propagated plants by the assisted movement of vegetative parts by humans or large animals. Monitoring of the cultivated pineapple plots for 2 years after trails for volunteers has been made essential by the regulatory agencies. These volunteers can be destroyed by spraying with suitable herbicide followed by rotary hoeing. Pineapple is basically pollinated by humming birds, occasionally by honey bees or pineapple beetles (Purseglove 1972). The pollen grains are not dispersed by wind (Kerns *et al*. 1968). Seed production in pineapple is also very low (Coppens d' Eeckenbrugge and Duval 1994). Pineapple survives poorly in the natural environment, posing no real risk associated with pollen escape (Ko *et al*. 2008). The stability of the transgene and its expression is also of great importance. As it is consumed as a fresh as well as processed fruit, transgenic pineapple will fall under the scrutiny of food regulatory agencies. Data, such as toxicology, allergenicity, effects on nutritional qualities, etc., on each transgenic line have to be developed. Though biotechnological approaches have considerable potential for the agronomic improvement of pineapple, consumer acceptance is the most important issue (Davey *et al*. 2007).

FUTURE PROSPECTS

Pineapple is the third most important tropical fruit in world production. Despite considerable efforts in pineapple breeding programs, limited success has been achieved due to the high heterozygosity among the domesticated varieties. Genetic engineering has the potential to unlock an entirely new round of genetic improvements by transferring specific traits from other species to pineapple. Much of the work involving genetic transformation of pineapple is proprietary, and has not been published (Gomez-Lim and Litz 2004). Most of the genetic engineering programs have focused on nematode resistance, pineapple mealybug wilt virus resistance, resistance to fungal diseases, herbicide tolerance, flowering, fruit ripening control, and blackheart resistance. This research would be of great importance in improving the pineapple cultivars especially 'Smooth Cayenne' which is widely used throughout the world.

The development of pineapple biotechnology is dependent on the availability of a number of molecular tools. Though, pineapple is a major fruit crop, there have been few molecular genetic studies which have been able to isolate and/or characterize very few genes. Recent advances in genomics and bioinformatics have the potential to revolutionize the field of breeding and genetics through targeted manipulations of traits. This will enhance our understanding of structural and functional aspects of plant genomes leading to the integration of basic knowledge in ways that can augment our ability to improve crop plants. The integration of these modern technologies in pineapple crop improvement and biotechnology will offer promise of greatly improving the cultivars that are grown through precise and targeted manipulations of the genome. The construction of dense genome maps of molecular markers is of paramount importance for the further isolation, via positional cloning, of genes of interest for pineapple improvement. This is of particular significance regarding those genes that are uniquely known and detected by their phenotypic expression in plants (Carlier *et al*. 2007). The information generated through this would have great potential in pineapple genetic improvement and/or genetic engineering programs. However, the usefulness of the new genetically improved and/or engineered pineapples must be proved by its performance in field trials or target environment(s) to verify the function and productivity of the traits as well as acceptance by farmers, growers, quality managers and consumers.

REFERENCES

Aghion D, Beauchesne G (1960) Utilization de la technique de culture sterile d'organes pour obtenir des clones d'*Ananas*. *Fruits* **15**, 464-466

Albuquerque CC, Camara TR, Menezes M, Willadino L, Meunier I, Ulisses C (2000) Cultivo *in vitro* de ápices caulinares de abacaxizeiro para limpeza clonal em relação à fusariose. *Scientia Agricola* **57**, 363-366

Bartholomew DP (1977) Inflorescence development of pineapple (*Ananas comosus* L. Merr.) induced to flower with ethephon. *Botanical Gazette* **138**, 312-320

Benega R, Isidron M, Arias E, Cisneros A, Martínez J, Torres I, Hidalgo M, Borroto CG (1996a) *In vitro* germination and callus formation in pineapple hybrid seeds (*Ananas comosus* (L.) Merr.). *Acta Horticulturae* **425**, 243-246

Benega R, Isidron M, Arias E, Cisneros A, Martínez J, Companioni L, Borroto CG (1996b) Plant regeneration from pineapple ovules (*Ananas comosus* (L.) Merr.). *Acta Horticulturae* **425**, 247-250

Botella JR, Fairbairn DJ (2005) Present and future potential of plant biotechnology. *Acta Horticulturae* **622**, 23-28

Botella JR, Smith M (2008) Genomics of pineapple, crowning the king of tropical fruits. In: Moore PH, Ming R (Eds) *Genomics of Tropical Crop Plants*, Springer, pp 441-451

Botella JR, Cavallaro AS, Cazzonelli CI (2000) Towards the production of transgenic pineapple to control flowering and ripening. *Acta Horticulturae* **529**, 115-122

Broglie KE, Gaynor JJ, Broglie RM (1986) Ethylene-regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*. *Proceedings of the National Academy of Sciences USA* **83**, 6820-6824

Cabral JRS, Cunha GAP, Rodrigues M (1984) Pineapple micropropagation. *Anais do VII Congresso Brasileiro de Fruticultura* **1**, 124-127

Carlier JD, Coppens d'Eeckenbrugge G, Leitão JM (2007) Pineapple. In: Kole C (Ed) *Genome Mapping and Molecular Breeding in Plants (Vol 4) Fruits and Nuts*, Springer-Verlag, Berlin, pp 331-342

Carlier JD, Nacheva D, Coppens d'Eeckenbrugge G, Leitão JM (2006) Genetic mapping of DNA markers in pineapple. *Acta Horticulturae* **702**, 79-86

Carlier JD, Reis A, Duval MF, Coppens d'Eeckenbrugge G, Leitão JM (2004) Genetic maps of RAPD, AFLP and ISSR markers in *Ananas bracteatus* and *A. comosus* using the pseudotestcross strategy. *Plant Breeding* **123**, 186-192

Cazzonelli C, Cavallaro A, Botella JR (1998) Cloning and characterization of ripening-induced ethylene biosynthetic genes from non-climacteric pineapple (*Ananas comosus*) fruits. *Australian Journal of Plant Physiology* **25**, 513-518

Cazzonelli C, Cavallaro T, Botella JR (1999) Searching for the role of ethylene in non-climacteric fruits. In: Kanellis AK, Chang C, Klee H, Bleecker AB, Pech JC, Grierson D (Eds) *Biology and Biotechnology of the Plant Hormone Ethylene*, Kluwer Academic Publishers, Dordrecht, pp 29-30

Chu CC (1978) The N_6 medium and its application to another culture of cereal crops. In: *Proceedings of a Symposium on Plant Tissue Culture*, 25-30 May, 1978, Science Press, Beijing, pp 43-50

Collins JL (1960) *The Pineapple: Botany, Cultivation and Utilization*, Interscience Publishers Inc., New York, 294 pp

Collins JL (1968) *The Pineapple*, Leonard Hill, London, 295 pp

Coppens d' Eeckenbrugge G, Duval MF (1994) Utilization of pineapple genetic resources in breeding. *Tropical Fruits Newsletter* **12**, 3-5

Cote F, Domergue R, Folliot M, Bouffin J, Marie F (1991) Micropropagation *in vitro* de l'ananas. *Fruits* **46**, 359-366

Daquinta MA, Castillo R, Lorenzo JC, Cobo I, Escalona M, Trujillo R, Borroto C (1994) Formación de callos en piña (*Ananas comosus* (L.) Merr). *Revista Brasileira de Fruticultura* **16**, 83-89

Daquinta M, Cisneros A, Rodriguez Y, Escalona M, Perez C, Luna I, Borroto C (1996) Somatic embryogenesis in pineapple (*Ananas comosus* L., Merr). *Pineapple News* **3**, 5-6

Davey MR, Sripaoraya S, Anthony P, Lowe KC, Power JB (2007) Pineapple. In: Pua EC, Davey MR (Eds) *Biotechnology in Agriculture and Forestry (Vol 60) Transgenic Crops V*, Springer-Verlag, Berlin, Germany, pp 97-127

de Almeida WAB, de Matos AP, da Souza AS (1996) Effects of benzylaminopurine (BAP) on *in vitro* proliferation of pineapple (*Ananas comosus* (L.) Merr.). *Acta Horticulturae* **425**, 235–239

de la Cruz-Medina J, Garcia HS (2007) Pineapple post-harvest operations. In: Mejia D (Ed) Compendium on post-harvest operations. Available online: http://www.fao.org/inpho/content/compend/text/ch33/AE614e01.htm#1.2

de Matos AP (1995) Pathological aspects of the pineapple crop with emphasis on the Fusariosis. *Revista de la Facultad de Agronomía* **21**, 179-197

de Wald MG, Moore GA, Sherman WB, Evans MH (1988) Production of pineapple plants *in vitro*. *Theoretical and Applied Genetics* **71**, 637-643

Escalona M, Lorenzo JC, Gonzalez B, Daquinta M, Gonzalez JL, Desjardins Y, Borroto CG (1999) Pineapple (*Ananas comosus* L. Merr.) micropropagation in temporary immersion systems. *Plant Cell Reports* **18**, 743-748

Espinosa P, Lorenzo JC, Iglesias A, Yabor L, Menéndez E, Borroto J, Hernández L, Arencibia AD (2002) Production of pineapple transgenic plants

assisted by temporary immersion bioreactors. *Plant Cell Reports* **21**, 136-140

Firoozabady E, Gutterson N (1998) Genetically transformed pineapple plants and methods for their production. *International Patent Application number* PCT/US98/03681

Firoozabady E, Gutterson N (2003) Cost-effective *in vitro* propagation methods for pineapple. *Plant Cell Reports* **21**, 844-850

Firoozabady E, Heckert M, Gutterson N (2006) Transformation and regeneration of pineapple. *Plant Cell, Tissue and Organ Culture* **84**, 1-16

Firoozabady E, Heckert M, Oeller P, Gutterson N (1997) Transformation and regeneration of transgenic pineapple plants. In: *5th International Congress of Plant Molecular Biology*, 21-27 September, 1997, Singapore, p 1358 (Abstract)

Fitchet M (1990) Organogenesis in callus cultures of pineapple (*Ananas comosus* L., Merr). *Acta Horticulturae* **275**, 267-274

Food and Agriculture Organization (2008) FAOSTAT Database. Available online: http://faostat.fao.org

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**, 151-158

Gangopadhyay G, Bandyopadhyay T, Poddar R, Gangopadhyay S, Mukherjee KK (2005) Encapsulation of pineapple microshoots in alginate beads for temporary storage. *Current Science* **88 (6)**, 972-977

Garcia GP, Perez MI, Benega R (2000) Analysis of somaclonal variation in pineapple (*Ananas comosus* (L.) Merr.) plants regenerated from callus in the field. *Pineapple News* **7**, 8-10

Gomez-Lim MA, Litz RE (2004) Genetic transformation of perennial tropical fruits. *In Vitro Cellular and Developmental Biology – Plant* **40**, 442-449

Graham M, Ko L, Hardy V, Robinson S, Sawyer B, O'Hare T, Jobin M, Dahler J, Underhill S, Smith M (2000a) The development of blackheart resistant pineapples through genetic engineering. *Acta Horticulturae* **529**, 133-138

Graham M, Smith M, Hardy V, Ko L (2000b) A method of transformation. International Patent Application No. PCT/AU00/01350

Hiei Y, Otha S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa*) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *The Plant Journal* **6**, 271-282

Hirimburegama K, Wijesinghe LPJ (1992) *In vitro* growth of *Ananas comosus* L. Merr. (pineapple) shoot apices on different media. *Acta Horticulturae* **319**, 203-208

Hu JS, Sether DM, Metzer MJ, Pérez E, Gonsalves A, Karasev AV, Nagai C (2005) Pineapple mealybug wilt associated virus and mealybug wilt of pineapple. *Acta Horticulturae* **666**, 209-212

Isidron PM, Benega GR, Cisneros PA, Arias VE, Lorenzo FJC, Espinosa AP, Borroto NCG (1998) Application of biotechnological and traditional methods in Cuban pineapple breeding program. In: *Third International Pineapple Symposium,* 17-20 November, 1998, Pattaya, Thailand, p 45 (Abstract)

Kamoun S (2001) Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. *Current Opinion in Plant Biology* **4**, 295-300

Kato CY, Nagai C, Moore PH, Francis Z, Kim MS, Steiger DL, Ming R (2004) Intra-specific DNA polymorphism in pineapple (*Ananas comosus* (L.) Merr.) assessed by AFLP markers. *Genetic Resources and Crop Evolution* **51**, 815-825

Kerns KR, Williams DF, Smith JB (1968) A review of seediness in pineapple. PRI Report No. 124, Hawaii

Kiss E, Kiss J, Gyulai G, Heszky LE (1995) A novel method for rapid micropropagation of pineapple. *HortScience* **30**, 127-129

Ko HL, Graham MW, Hardy VG, Jobin M, O'Hare TJ, Smith MK (2000) Transformation of pineapple using biolistics. In: *6th International Congress of Plant Molecular Biology*, 18-24 June, 2000, Quebec, Canada, p 64 (Abstract)

Ko HL, Hardy VG, Jobin-Decor M, Campbell PR, Eccleston KL, Graham MW, Smith MK (2005) The introduction of transgenes to control blackheart in pineapple: biolistics vs *Agrobacterium* transformation. In: Bennett IJ, Bunn E, Clarke H, McComb JA (Eds) *Contributing to a Sustainable Future. Proceedings of the Australian Branch of the IAPTC&B*, Perth, Western Australia, 21-24 September, 2005, pp 195-202

Ko HL, Campbell PR, Jobin-Decor MP, Eccleston KL, Graham MW, Smith MK (2006) The introduction of transgenes to control blackheart in pineapple (*Ananas comosus* L.) cv. Smooth Cayenne by microprojectile bombardment. *Euphytica* **150**, 387-395

Ko HL, Sanewski GM, Smith MK (2008) Pineapple. In: Kole C, Hall TC (Eds) *Compendium of Transgenic Crop Plants (Vol 5) Transgenic Tropical and Subtropical Fruits and Nuts*, Blackwell Publishing, Oxford, UK, pp 109-129

Koes RE, Spelt CE, Vandenelzen PJM, Mol JNM (1989) Cloning and molecular characterization of the chalcone synthase multigene family of *Petunia hybrida*. *Gene* **81**, 245-257

Lakshmi Sita G, Singh R, Layer CPA (1974) Plantlets through shoot tip cultures in pineapple. *Current Science* **43**, 724-725

Lapade AG, Veluz AMS, Santos IS (1988) Cloning of the Queen variety of pineapple (*Ananas comosus* L., Merr) through the callus. *The Nucleus* **26 (1)**, 14-16

Liu JL, Rosa ME, Lizardi E (1989) Smooth leaf (spineless) Red Spanish pineapple (*Ananas comosus*) propagated *in vitro*. *Journal of Agriculture University of Puerto Rico* **73**, 301-311

Mapes MO (1973) Tissue culture of bromeliads. In: *Proceedings of the International Plant Propagation Society* **23**, 47-55

Mathews VH, Rangan TS (1979) Multiple plantlets in lateral bud and leaf explant *in vitro* cultures of pineapple. *Scientia Horticulturae* **11**, 319-328

Mathews VH, Rangan TS (1981) Growth and regeneration of plantlets in callus cultures of pineapple. *Scientia Horticulturae* **14**, 227-234

Mathews VH, Rangan TS, Narayanswamy S (1976) Micro–propagation of *Ananas sativus in vitro*. *Zeitschrift für Pflanzenphysiologie* **79**, 450-454

Melchers LS, Sela-Buurlage MB, Vloemans SA, Woloshuk CP, Van Roekel JSC, Pen J, Van den Elzen PJM, Cornelissen BJC (1993) Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and β-1,3-glucanase in transgenic plants. *Plant Molecular Biology* **21**, 583-593

Mhatre M (2003) Transformation of pineapple leaf bases with the magainin gene analogue, MSI-99. *Pineapple News* **10**, 10

Min XJ, Bartholomew DP (1996) Effect of plant growth regulators on ethylene production, 1-aminocyclopropane-1-carboxylic acid oxidase activity, and initiation of inflorescence development of pineapple. *Journal of Plant Growth Regulation* **15**, 121-128

Moore GA, de Wald MG, Evans MH (1992) Micropropagation of pineapple (*Ananas comosus* L.). In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry (Vol 18) High-Tech and Micropropagation*, Springer, Berlin, pp 460-469

Morton J (1987) Pineapple. In: Morton J (Ed) *Fruits of Warm Climates*, Miami, FL, pp 18-28

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Murashige T, Tucker DPH (1969) Growth factor requirements of citrus tissue culture. In: *Proceedings of the First International Citrus Symposium* **3**, 1155-1161

Nan GL, Nagai C (1998) Genetic transformation of pineapple (*Ananas comosus* [L.] Merr.) *via* particle bombardment. *In Vitro Cellular and Developmental Biology – Plant* **34**, 55A

Nan GL, Nagai C, Moore PH, Sun SSM, Sipes BS, Paull RE, Rohrbach K (1996) Tissue culture and genetic transformation studies on pineapple. *In Vitro Cellular and Developmental Biology – Plant* **32**, 102A

Nitsch JP (1951) Application of different macro and micro nutrients in the study for a balanced basal medium. *American Journal of Botany* **38**, 566-577

Ohme-Tagaki M, Shinshi H (1990) Structure and expression of a tobacco β-1,3-glucanase gene. *Plant Molecular Biology* **15**, 941-946

Osei–Kofi F, Amoatey HM, Lokko Y (1997) Improvement of pineapple (*Ananas comosus* L., Merr.) using biotechnology and mutation breeding techniques. In: Report of II FAO/IAEA Research Coordination meeting on "*In vitro* techniques for selection of radiation–induced mutants adapted to adverse environmental conditions, 15–19 April 1996, Cairo, Egypt, pp 23-27

Paz EY, Gil K, Rebolledo L, Rebolledo A, Uriza D, Martínez O, Isidrón M, Simpson J (2005) AFLP characterization of the Mexican pineapple germplasm collection. *Journal of the American Society of Horticultural Sciences* **130**, 575-579

Perez EA, Ma H, Melzer MJ, Sether DM, Borth WB, Cheah K, Nagai C, Hu JS (2006) Development of transgenic pineapple plant with resistance to PMWaV-2. *Pineapple News* **13**, 41-42

Pickergill B (1976) The pineapple. In: *Fruit Culture in India*, Indian Council of Agricultural Research, New Delhi, pp 168-175

Poh TLW, Khoon CB (1975) Tissue culture of pineapple. In: *Proceedings of the National Plant Tissue Culture Symposium*, Kuala Lumpur, Malaysia, pp 51-58

Purseglove JW (1972) *Tropical Crops - Monocotyledons I*, John Wiley and Sons, New York, 607 pp

Rahman KW, Amin MN, Azad MAK (2001) *In vitro* rapid clonal propagation of pineapple, *Ananas comosus* (L.) Merr. *Plant Tissue Culture* **11**, 47-53

Reid MS, Wu MJ (1991) Ethylene in flower development and senescence. In: Mattoo AK, Suttle JC (Eds) *The Plant Hormone Ethylene*, CRC Press Inc., Boca Raton, FL, pp 215-234

Reinhardt D, Medina VM (1992) Growth and fruit quality of Perola and Smooth Cayenne pineapple cultivars. *Pesquisa Agropecuaria Brasileira* **27**, 435-447

Rohrbach K, Apt WJ (1986) Nematode and disease problems of pineapple. *Plant Disease* **70**, 81-87

Rohrbach KG, Beardsley JW, German TL, Reimer NJ, Sanford WG (1988) Mealybug wilt, mealybugs, and ants on pineapple. *Plant Disease* **72 (7)**, 558-565

Rohrbach KG, Christopher D, Hu J, Paull R, Sipes B, Nagai C, Moore P, McPherson M, Atkinson H, Levesley A, Oda C, Fleisch H, McLean M (2000) Management of a multiple goal pineapple genetic engineering program. *Acta Horticulturae* **529**, 111-114

Samson JA (1980) *Tropical Fruits*, Longman Press, London, 250 pp

Sanewski GM (2008) Breeding *Ananas* for the cut-flower and garden markets. *Pineapple News* **15**, 48

Schenck S (1990) Correlations of *Rotylenchulus reniformis* population densities with 1,3-di-chloropropene dosage rate and pineapple yields. *Journal of Nematology* **22**, 753-739

Schlumbaum A, Match F, Vogeli U, Boller T (1986) Plant chitinases differ in antifungal activity. *Nature* **324**, 325-367

Singh NK, Nelson DE, Kuhn D, Hasegawa PM, Bressant RA (1989) Molecular cloning of osmotin and regulation of its expression by ABA and adaptation to low water potential. *Plant Physiology* **90**, 1096-1101

Sipes BS, Schmitt DP (1994) Evaluation of pineapple, *Ananas comosus*, for host plant resistance and tolerance to *Rotylenchulus reniformis* and *Meloidogyne javanica*. *Nematropica* **24 (2)**, 113-121

Sipes B, Nagai C, McPherson M, Atkinson H, Christopher D, Hu J, Paull R, Rohrbach K, Moore P, Oda C, Wood P, Conway M (2002) Pineapple genetically modified for resistance to plant-parasitic nematodes. *Phytopathology* **92**, S76

Smith MK, Ko H-L, Hamill SD, Sanewski GM (2002) Pineapple transformation: managing somaclonal variation. *Acta Horticulturae* **575**, 69-74

Soneji JR (2001) Tissue culture studies in pineapple (*Ananas comosus* L., Merr.) and molecular characterization of variants. PhD thesis, University of Mumbai, India, 129 pp

Soneji JR, Nageswara Rao M (2008) Pineapple genetic improvement and biotechnology. In: Thangadurai D, Tripathi L, Vasanthaiah HKN, Jasso DC (Eds) *Crop Improvement and Biotechnology*, Bioscience Publications, India, pp 101-117

Soneji JR, Rao PS, Mhatre M (2002a) Somaclonal variation in micropropagated dormant axillary buds of pineapple (*Ananas comosus* L., Merr.). *Journal of Horticultural Science and Biotechnology* **77 (1)**, 28-32

Soneji JR, Rao PS, Mhatre M (2002b) Plantlet regeneration from leaf explants of pineapple (*Ananas comosus* L., Merr.). *Journal of Plant Biochemistry and Biotechnology* **11 (2)**, 117-119

Soneji JR, Rao PS, Mhatre M (2002c) Germination of synthetic seeds of pineapple (*Ananas comosus* L. Merr.). *Plant Cell Reports* **20**, 891-894

Souza FVD, dos Santos Cabral JR, dos Santos-Serejo JA, Ricardo FF, dos Santos Nepomuceno O, de Souza EH (2008) Evaluation of new hybrids of ornamental pineapples. *Pineapple News* **15**, 48-49

Srinivasa Rao NK, DoreSwamy R, Chacko EK (1981) Differentiation of plantlets in hybrid embryo callus of pineapple. *Scientia Horticulturae* **15**, 235-238

Sripaoraya S (2007) Inheritance of transgene of genetically modified pineapple. *Pineapple News* **14**, 22

Sripaoraya S, Marchant R, Power JB, Davey MR (2001) Herbicide-tolerant pineapple (*Ananas comosus*) produced by microprojectile bombardment. *Annals of Botany* **88**, 597-603

Sripaoraya S, Marchant R, Power JB, Davey MR (2003) Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). *In Vitro Cellular and Developmental Biology – Plant* **39**, 450-454

Sripaoraya S, Keawsompong S, Insupa P, Power JB, Davey MR, Srinives P (2006) Genetically manipulated pineapple: transgene stability, gene expression and herbicide tolerance under field conditions. *Plant Breeding* **125**, 411-413

Starr JL, Page SLJ (1990) Nematode parasites of pineapple. In: Luc M, Sikora RA, Bridge J (Eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, CAB International, Oxon, UK, pp 519-538

Stewart RJ, Sawyer BJB, Bucheli CS, Robinson SP (2001) Polyphenol oxidase is induced by chilling and wounding in pineapple. *Australian Journal of Plant Physiology* **28**, 181-191

Stewart RJ, Sawyer BJB, Robinson SP (2002) Blackheart development following chilling in fruit of susceptible and resistant pineapple cultivars. *Australian Journal of Experimental Agriculture* **42**, 195-199

Teisson CI, Combress JC, Martin-Prevel P, Marchal J (1979) Internal browning of pineapples: III) symptoms, IV) biochemical approach. *Fruits* **34**, 315-338

Teng WL (1997) An alternative propagation method of *Ananas* through nodule culture. *Plant Cell Reports* **16**, 454-457

Trusov Y, Botella JR (2006) Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple [*Ananas comosus* (L.) Merr.]. *Journal of Experimental Botany* **57**, 3953-3960

Trusov Y, Botella JR (2008) Silencing of the ACC synthase gene ACACS2 causes delayed flowering in pineapple. *Pineapple News* **15**, 57

Wakasa K (1989) Pineapple. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry Trees* (Vol 5), Springer, Berlin, pp 13-29

Wakasa K, Koga Y, Kudo M (1978) Differentiation from *in vitro* culture of *Ananas comosus*. *Japanese Journal of Breeding* **28 (2)**, 113-121

Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, Van den Elzen PJM, Cornelissen BJC (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *The Plant Cell* **3**, 619-628

Yabor L, Arzola M, Aaragon C, Hernandez M, Arencibia A, Lorenzo JC (2006) Biochemical side effects of genetic transformation of pineapple. *Plant Cell, Tissue and Organ Culture* **86**, 63-67

Zepeda C, Sagawa Y (1981) *In vitro* propagation of pineapple. *HortScience* **16**, 495