Morphological Characteristics of Genetically-Modified Pineapple Fruit

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INTRODUCTION

Genetic engineering is an attractive method for changing a single characteristic of 'Smooth Cayenne' pineapple, without altering its other desirable attributes. Techniques used in pineapple transformation, however, such as tissue culture and biolistic-mediated or *Agrobacterium*-mediated gene insertion are prone to somaclonal variation, resulting in the production of several morphological mutations (Smith et al., 2002). Fruit mutations can include distortion in fruit shape (round ball, conical, fan-shaped), reduced fruit size, multiple crowns, crownless fruit, fruitless crowns, and spiny crown leaves (Dalldorf, 1975; Sanewski et al., 1992). The present paper describes the variability in fruit-shape mutations between transgenic and non-transgenic fruit, and its subsequent impact on organoleptic characteristics.

METHODS

Mature pineapple fruit ('Smooth Cayenne') from transgenic and non-transgenic tissue-cultured plants grown under sub-tropical conditions were harvested in September 2002. Fruit were derived from plants from non-transgenic callus tissue-culture, biolistic-mediated transformation (1-2 genes inserted), or *Agrobacterium*-mediated transformation (1-2 genes inserted), as part of a program to develop a 'blackheart-resistant' pineapple. Transformations were performed as described in Graham et al. (2000). Transformation 'events' from each treatment were selected, from which replicate plants were regenerated and multiplied using standard tissue culture techniques (Smith et al., 2002).

Fruit were held for 3 weeks at 10°C + 1 week at 23°C to develop blackheart symptoms. Fruit were assessed for size, shape, and flesh colour. The pulp was removed, juiced and a sample measured for total soluble solids and titratable acidity. The impact of transformation technique on the percentage of 'normally-shaped' fruit was assessed. The impact of fruit shape on TSS, TA, skin and flesh colour was assessed. Data from 'normally-shaped' fruit was then analysed separately. The covariants, skin colour at harvest, flotation index, and fruit weight, were used to minimise statistical variance associated with plant size or fruit maturity at harvest.

RESULTS

Both non-transformed and transformed tissue cultured plants resulted in a range of fruit shapes and sizes. These were divided into: normal, stunted, small, large, small spherical, spherical, slightly fanned, fanned, small conical, and conical. In general, normal fruit were the predominant fruit-shape, although biolistically-transformed fruit containing 2 introduced genes (GUS and *nptII*, or PPO and *nptII*) tended to have a lower percentage of normal fruit (Table 1). Biolistically-transformed fruit containing only 1 gene (*nptII*) however behaved similarly or slightly better than non-transformed fruit. *Agrobacterium*-transformed plants generally produced a similar percentage of normal fruit to non-transgenic plants.

Fruit-shape had a significant impact on TSS, TA and internal colour (Table 2). Normally-shaped fruit, fanned and large fruit had highest TSS, and conical fruit lowest. Normally-shaped fruit, fanned, small spherical, spherical, and small fruit had highest TA, and conical and large fruit lowest. Slightly fanned fruit had a slightly higher hue angle than normal fruit, giving fruit pulp a slightly greener colour. It is possible that this may have been related to the closer proximity of the skin to the flesh at measurement.

'Normal' fruit from each transformation 'event' were analysed for TSS, TA, and internal colour (hue angle). TSS, TA and internal colour were generally similar for all 'events' and tissue-cultured plantlets. The exceptions to this were three events with significantly lower TSS, two events with significantly lower TA, and one event with significantly higher TA (Table 1).

DISCUSSION

The range of fruit shapes and sizes derived from both transformed and non-transformed plants, particularly in the first generation after tissue culture, illustrates the difficulty in selecting a commercially acceptable transgenic pineapple fruit. From the present trial, variation associated with callus-derived tissue cultured plants can account for up to 40% fruit exhibiting abnormal shape and size. However first generation *ex vitro* plants are likely to exhibit epigenetic changes that do not always carry through to subsequent vegetative generations (Huxley and Cartwright, 1994; Smith and Hamill, 1996). The present trial was also not conclusive as to how biolistic- or *Agrobacterium*-mediated transformation might impact on the percentage of abnormal mutations, although fruit generated through biolistics (2 genes inserted) had 83-100% mutation in four out of six events.

Fruit-shape was observed to have significant impact on both the flavour (TSS, TA) and colour (hue angle) of pineapple flesh. In comparison to normal fruit, conical fruit had significantly lower TSS and TA, which may indicate retarded fruit development. Large fruit on the other hand had low TA, but normal TSS, possibly indicating advanced maturity.

Normally-shaped fruit from different events generally had similar TSS, TA and internal colour. Exceptions to these appeared in random events, either biolistic- or *Agrobacterium*-mediated. It is possible that integration of transgenes may have disrupted genes associated with TSS and TA development. Further trials however are planned to determine the relative stability of the observed traits in subsequent generations.

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Table 1. Effect of transformation 'event' on fruit-shape mutation, TSS and TA.

Event	%Normal fruit[a]	TSS[b]	TA[b]
Tissue culture (non-transformed)	65(17)	11.48def	1.11cd
Tissue culture (non-transformed)	63(16)	11.54efg	1.14d
Biolistic (*nptII*)	88(16)	10.96cde	1.10bcd
Biolistic (*nptII*)	90(10)	9.96ab	0.97abc
Agrobacterium (*nptII*)	88(8)	10.72bcde	0.97abc
Biolistic (*nptII* + GUS)	0(2)	-	-
Biolistic (*nptII* + GUS)	17(6)	10.81bcdef	1.45e
Biolistic (*nptII* + GUS)	60(5)	11.28bcdefg	1.38de
Biolistic (*nptII* + PPO)	0(1)	-	-
Biolistic (*nptII* + PPO)	0(9)	-	-
Biolistic (*nptII* + PPO)	79(14)	10.46abc	1.01abc
Agrobacterium (*nptII* + GUS)	100(1)	8.3a	0.70a
Agrobacterium (*nptII* + GUS)	67(6)	12.86g	0.93abc
Agrobacterium (*nptII* + PPO)	47(15)	10.53bcd	0.96ab
Agrobacterium (*nptII* + PPO)	56(16)	10.66bcde	0.99abc
Agrobacterium (*nptII* + PPO)	54(13)	10.76bcde	0.98abc
Agrobacterium (*nptII* + PPO)	71(14)	10.83bcde	1.08bcd
Agrobacterium (*nptII* + PPO)	57(14)	10.96bcde	1.18d
Agrobacterium (*nptII* + PPO)	58(12)	12.16fg	1.18d

[a]Total number of fruit shown in parentheses; [b] Normal fruit only

Table 2. Effect of fruit-shape on TSS, TA and flesh colour (hue).

Fruit-shape	TSS	TA	Hue angle (flesh)
Normal	10.94c	1.06c	102.9a
Small	9.73b	0.99bc	103.8ab
Large	10.30bc	0.71ab	102.2ab
Small spherical	10.19b	0.99bc	103.1a
Spherical	9.09b	0.98bc	103.8ab
Small conical	9.9abc	0.76abc	103.0ab
Conical	6.77a	0.66a	103.0ab
Slightly fanned	9.87b	0.96b	104.4b
Fanned	10.37bc	0.97bc	104.0ab
Stunted	7.70ab	0.92abc	103.1ab

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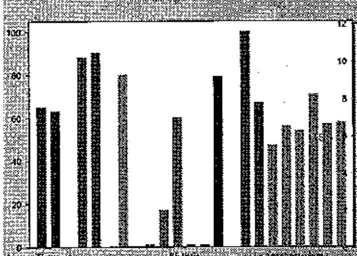
Introduction

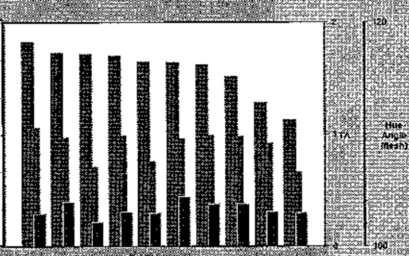
Genetic engineering is an attractive method for changing a single characteristic of 'Smooth Cayenne' pineapple. However, techniques used in pineapple transformation, such as tissue culture and biolistic-mediated or *Agrobacterium*-mediated gene insertion are prone to morphological mutations (Smith et al., 2002). Fruit mutations include distortion in fruit shape, reduced fruit size, multiple crowns, crownless fruit, fruitless crowns, and spiny crown leaves (Dalldorf, 1975; Sanewski et al., 1992). The present paper describes the impact of gene insertion on fruit-shape and factors affecting taste and appearance.

Experimental procedure

•Fruit from transgenic (biolistically transformed or *Agrobacterium* transformed) and non-transgenic tissue-cultured plants were harvested.
•Fruit were assessed for size and shape, and the impact of transformation technique on the percentage of 'normal' fruit.
•Flesh from differently-shaped fruit was measured for total soluble solids (TSS), titratable acidity (TA), and flesh colour (hue angle).
•The impact of fruit shape on TSS, TA, and flesh colour was assessed.
•Data from 'normally-shaped' fruit was then analysed separately to assess impact of transformation on TSS, TA, and flesh colour.



Impact of tissue culture and gene insertion technique on shape mutation



Impact of fruit shape on TSS, TA and internal flesh colour

Transgenic and non-transgenic Smooth Cayenne pineapples being 'bagged' to prevent pollen transfer

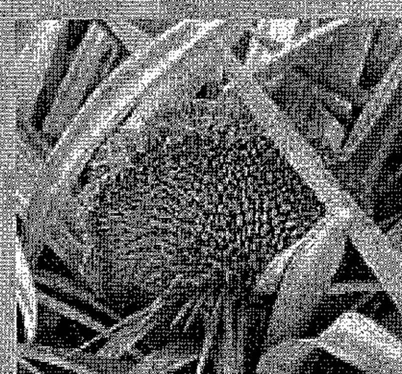
Results

Callus culture and biolistic gene insertion increases shape mutation
•Shapes included: normal (predominant shape), stunted, small, large, small spherical, spherical, slightly fanned, fanned, small conical, and conical.
•Biolistic fruit containing 2 introduced genes had a lower percentage of normal fruit.

Significant impact of shape on flavour and flesh colour
•Normal, fanned and large fruit had highest TSS, and conical fruit lowest.
•Normal, fanned, small spherical, spherical, and small fruit had highest TA, and conical and large fruit lowest.

Little impact of gene insertion on flavour and flesh colour
•Normally-shaped transgenic and non-transgenic fruit had similar TSS, TA and internal colour.

A fan-shaped Smooth Cayenne pineapple mutation



Conclusions

•Callus culture (non-transgenic) can account for up to 40% of shape mutations.
•Biolistic gene insertion may increase mutational rate, while *Agrobacterium*-mediated transformation has little effect. Fruit generated through biolistics (2 genes inserted) had 83-100% mutation in four out of six events.
•Fruit-shape had significant impact on both flavour (TSS, TA) and colour (hue angle) of pineapple flesh.
•Normally-shaped transgenic and non-transgenic fruit generally had similar TSS, TA and internal colour. Exceptions to these appeared in random events, either biolistic- or *Agrobacterium*-mediated. It is possible that integration of transgenes may have disrupted genes associated with TSS and TA development.

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