VG96010
Pumpkin Varietal Improvement

Mark Herrington, *et al*
Queensland Horticulture Institute

Know-how for Horticulture™

VG96010

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Partnership in horticulture

PUMPKIN VARIETAL IMPROVEMENT

FINAL REPORT
HRDC Project No VG96010

(1 July 1996 to 29 February 2000)

Mark E. Herrington *et al.*

Queensland Horticulture Institute

PUMPKIN VARIETAL IMPROVEMENT

HRDC PROJECT No. VG96010

(incorporating, through project amalgamation, HRDC project No. VG517)

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Purpose:-
This report outlines the activities and outcomes of the above project. The project was designed to breed pumpkin cultivars resistant to virus and etch and thereby reduce losses caused by these diseases in the Australian industry.

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DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

Queensland Fruit & Vegetable Growers

TABLE OF CONTENTS

Media Summary 3

Technical Summary 4

1.0. General introduction 6
- 1.1. Jarrahdale type
- 1.2. Butternut type
- 1.3. Diseases

2.0. General materials and methods 7
- 2.1. Virus survey
- 2.2. Virus isolates
- 2.3.0. Breeding and selection
  - 2.3.1. 'Jarrahdale' type
  - 2.3.2. 'Butternut' type

3.0. General results 8
- 3.1. Virus survey

4.0 Developing and releasing 'Dulong QHI' - a virus resistant 'Jarrahdale' type 9
- 4.1. Selection history

4.2.0. Sensory evaluation – introduction 9
- 4.2.1. Sensory evaluation - material and methods
- 4.2.2. Sensory evaluation - results
- 4.2.3. Sensory evaluation – discussion

4.3.0. Managing quality defects- Inheritance of resistance to 'bone' – introduction 14
- 4.3.1. Managing quality defects – Inheritance of resistance to 'bone' - materials and methods
- 4.3.2. Managing quality defects – Inheritance of resistance to 'bone' - results and discussion

4.4.0. Confirmation of virus resistances 16

4.5.0. Field performance 17

5.0. Discussion on developing and releasing 'Dulong QHI' – a virus resistant 'Jarrahdale' type 18

6.0. Developing and releasing 'Sunset QHI' – a virus and etch resistant 'Butternut' type 19
- 6.1. Selection history

6.2. Technique development - glasshouse techniques for assessing reaction to etch 20

6.3.0. Inheritance of etch resistance – introduction 21
- 6.3.1. Inheritance of etch resistance - material and methods
- 6.3.2. Inheritance of etch resistance - results and discussion

6.4. Managing quality defects - stem adhesion 23

6.5. Field performance, etch and virus resistances 23

6.5.1. Field performance, etch and virus resistances - results and discussion 23

7.0. Discussion on developing and releasing 'Sunset QHI' – a virus and etch resistant 'Butternut' type 26

8.0. Technology Transfer 27

8.1. General

8.2. Specific Publications

9.0. Recommendations 28

9.1. Recommendations for future R and D

9.2. Recommendations for practical application to industry

10.0. Literature Cited 29

APPENDIX 1. Project photographs

Media summary

The grey 'Jarrahdale' and 'Butternut' type pumpkins are major culinary pumpkins in Queensland. Infection by mosaic viruses viz, papaya ringspot virus (PRSV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) on both pumpkin types and surface etch of 'Butternut' pumpkin cost the Queensland industry $4m annually.

The key project components were:-
Survey the distribution of viruses.

Understand the inheritance of resistance to etch in the 'Butternut' type.

Breed 'Jarrahdale' and 'Butternut' type pumpkins with resistance to viruses and etch.

The key outcomes were:-
ZYMV and PRSV were common in coastal Queensland, while PRSV-W dominated in sub-coastal areas. WMV was regularly found in all areas. These viruses are less problematic in southern states but ZYMV can occur anywhere implying some seed transmission.

The inheritance of etch resistance is complex; the best families should be selected and both parents should carry resistance if hybrid cultivars are required.

'Dulong QHI' (PBR application number 97/309), a grey 'Jarrahdale' type pumpkin, was released. It should be commercially available in July 2000. It combines excellent resistance to three viruses, good fruit quality, high productivity, and desirable fruit size.

'Sunset QHI' (PBR application number 2000/021), a 'Butternut' type is in the final testing and commercialisation stages with seed companies. It should be commercially available in July 2001. 'Sunset QHI' combines high etch resistance, good virus resistance, good fruit quality and high productivity.

Conclusions
These high quality virus and combined etch and virus resistant cultivars 'Dulong QHI' and 'Sunset QHI' should eliminate the annual losses of $4m to these diseases. This should reduce the effective overuse of land, water and concomitant pesticide application.

Recommendations for future R and D

The virus resistances in 'Dulong QHI' were fairly readily transferred together, suggesting a possible close linkage. Studies to identify and sequence the gene/s responsible could provide valuable intellectual property and is recommended.

The development of a range of virus resistant pumpkin types from these cultivars should be rapidly pursued.

Recommendations for practical application to industry

Pumpkin growers should trial these cultivars.

'Dulong QHI' and 'Sunset QHI' have resistance to the most important viral diseases of pumpkins in the tropics and subtropics. Because these resistances were incorporated by traditional breeding methods these cultivars offer important advantages in marketing, especially in the developing international 'organic produce' market. Using these cultivars to pursue this market is recommended.

Technical summary

We aimed to develop cultivars of pumpkin (*Cucurbita maxima* and *Cucurbita moschata*) resistant to papaya ringspot virus (PRSV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV). In the case of Butternut (*Cucurbita moschata*) the resistance was to be combined with resistance to etch (*Didymella bryoniae*).

The grey 'Jarrahdale' type pumpkin *(C. maxima)* is a major culinary pumpkin in Queensland. Losses to the mosaic viruses PRSV, ZYMV and WMV are estimated at $3m in Queensland and also occur in Northern Territory and Western Australia. Surface etch and mosaic are common diseases of 'Butternut' pumpkin (*Cucurbita moschata*). Affected fruit are rendered unsaleable and losses are estimated at $2m annually. Etch is associated with soil borne pathogens *Fusarium roseum* and *Didmyella bryoniae*. These monetary losses also reflect the overuse of pesticides and the natural resources of soil and water.

Virus distribution was surveyed using ELISA for identification of virus.

The inheritance of resistance in breeding lines of *C. maxima* to a quality defect known as 'bone' was determined in field studies where cut mature fruit of parental, hybrid and segregating generations were rated for percent of surface area showing brown colouration. Partially developed base populations of *C. maxima* were advanced through mass selection for fruit type, productivity, quality and resistance to viruses. The advanced population was compared with commercial cultivars in field trials and by a sensory panel.

Both ZYMV and PRSV were common in coastal Queensland, while PRSV-W dominated in sub-coastal areas. WMV was regularly found in all areas. These viruses are less problematic in southern states but ZYMV can occur anywhere implying some seed-borne transmission.

The inheritance of resistance to 'brown bone' did not fit a simple genetic model well, but was described by additive gene effects in a generation means analysis. Heritability was low although poorly estimated. To avoid 'bone', selection should be on a family basis.

'Dulong QHI' (PBR application number 97/309), a grey 'Jarrahdale' type pumpkin *(C. maxima)*, was released and is expected to be commercially available in July 2000. It combines excellent virus resistance (<1% leaf area affected compared to 80-95% for 'Jarrahdale' for PRSV-W, ZYMV and WMV), good fruit quality (overall acceptability score 54 similar to West Australian Grey 48 and Brian's Grey 53, although less ($P<0.05$) than Sweet Grey 63) and high productivity (38 t/ha, similar ($P> 0.05$) to West Australian Grey 48t/ha, Brian's Grey 51 t/ha and Sweet Grey 42 t/ha). Fruit size (3.9kg) is similar ($P> 0.05$) to Sweet Grey (4.1kg).

The inheritance, in *C. moschata*, of resistance to etch was determined in field studies. Partially developed base populations were advanced through backcrossing and mass selection for fruit type, productivity, quality, etch resistance and resistance to ZYMV and PRSV-W. The advanced population was compared with commercial cultivars in field trials.

The inheritance of etch resistance in the 'Butternut' type was best described as complex with additive epistasis, additive and dominance gene effects. Estimates of heritability were poor and selection should be on a family basis. In hybrid cultivars both parents should carry etch resistance.

'Sunset QHI' (PBR application number 2000/021), was developed and is currently in the final testing and commercialisation process with seed companies. It is expected to be commercially available in July 2001. 'Sunset QHI' combines in a 'Butternut' type high etch resistance (0.3% fruit surface affected compared with 15% for 'Butternut Large', $P<0.05$), high virus resistance, good fruit quality and high productivity (46 t/ha compared with 27 t/ha for 'Butternut Large', $P<0.05$).

The release of the high quality virus and combined etch and virus resistant cultivars 'Dulong QHI' and 'Sunset QHI' is expected to eliminate the annual losses of $4m to these diseases. These reduced losses can effectively reduce land and water usage and pesticide application.

The resistances in 'Dulong QHI' were fairly readily transferred together, suggesting that they may be closely linked or result from the same gene. Linkage studies to identify and perhaps sequence the gene/s responsible could provide valuable intellectual property. The rapid development of a range of virus resistant *C. maxima* pumpkin types is now possible from this advanced germplasm and should be pursued.

'Dulong QHI' and 'Sunset QHI' have resistance to some of the most important viral diseases of *Cucurbita spp.* in the tropics and subtropics. Because these resistances were incorporated by traditional breeding methods (ie not genetically engineered) these cultivars offer an important advantage in marketing, especially in the developing international 'organic produce' market.

1.0. GENERAL INTRODUCTION

We aimed in this research to develop commercial cultivars of pumpkins (*Cucurbita maxima* and *Cucurbita moschata*) resistant to viruses of major national and international significance. In the case of Butternut (*Cucurbita moschata)* this resistance was to be combined with resistance to etch (*Didymella bryoniae)*.

1.1. 'Jarrahdale' type

The grey 'Jarrahdale' type pumpkin *(C. maxima)* is a major culinary pumpkin in Queensland. Production from the pumpkin group is valued at in excess of $11m annually with interstate exports. Losses to papaya ringspot virus (PRSV) and zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) are estimated at $3m in Queensland and have also occurred in NT and WA. These monetary losses also reflect the overuse of pesticides and the natural resources of soil and water.

Early infection with PRSV-W or ZYMV results in 100 per cent loss of marketable yield in pumpkin and zucchini. Later infections result in lower losses but time of infection is beyond a grower's control. The viruses are aphid transmitted. While the experimental host range extends beyond cucurbits commercial losses are essentially confined to cucurbits. ZYMV may have a low level of seed transmission. This was the likely means of entry of the virus to Australia.

Cultural control measures such as increased aphicide application, roguing, elimination of alternative hosts and altered cropping schedules, while important, are generally considered an impractical means of mosaic virus control except in certain areas. Reflective plastic mulch has given best cultural control of some aphid-transmitted viruses in other crops but its effectiveness has varied with the aphid species present. It would not be practical with the usual vine-type pumpkin. Resistance would be the single most effective way to reduce losses and increase the efficiency of resource usage. Genetic improvement especially for virus resistance in the Jarrahdale type pumpkin was identified as a high priority area by the industry (HPIDC 16-05-94).

Previous traditional breeding work by QDPI incorporated PRSV, ZYMV and WMV resistance in a pumpkin breeding line 'Redlands Trailblazer' which unfortunately lacked in local consumer acceptability. However these resistances could be used to produce a better cultivar. Associated base populations had been established. These required further selection and development. The project was initiated to reduce losses due to virus by the development of virus resistant cultivars, building on previous projects and by the extension of information on virus control methods.
Virus resistance would reduce losses ($3m) in 'Jarrahdale' type pumpkin, and effectively reduce resource usage and pesticide application.

1.2. 'Butternut' type

Surface etch and mosaic are common diseases of butternut pumpkins (*Cucurbita moschata*). Affected fruit are rendered unsaleable. Etch is associated with soil borne pathogens *Fusarium roseum* and *Didmyella bryoniae*. The condition appears to be worsened by rainfall and overhead sprinkler irrigation. The incidence of affected fruit varies but is commonly in the order of 50%. Sources of resistance had been identified in QDPI breeding populations (Loader *et al.* 1996).

Mosaic is caused by virus (PRSV-W, ZYMV, WMV) infection transmitted by aphids, but resistance was available in a non-commercial *Cucurbita moschata* type from Nigeria. Apart from breeding resistant cultivars there is no economical, chemical or cultural control measure for these diseases in 'Butternut'. Presently it is not feasible to replace the butternut type with other pumpkin types which do not succumb to etch as the value of butternut pumpkin consumption is greater than all other pumpkin types combined (pers. com. L. Loader). Consequently this project was also initiated to reduce losses due to virus and etch in 'Butternut' pumpkins by the development of resistant cultivars.

Etch and virus resistance would reduce annual losses ($1.0m) in the 'Butternut' type pumpkin.

1.3 Diseases

For more detailed information on virus transmission and resistances please refer to Provvidenti *et al.* 1984, Greber *et al.* 1987, Provvidenti and Hampton 1996, Herrington *et al.* 1991, and Maluf *et al.* 1986. For more detailed information on *Didymella* (etch) epidemiology and resistances please refer to Bala and Hosein 1986, Hawthorne 1989, Zitter and Drennan 1995, Berstrom *et al.* 1982, Zitter and Kyle 1992, Sharrock and Parkes 1990, Zhang Yi Ping *et al.* 1995.

2.0. GENERAL MATERIAL AND METHODS

2.1. Virus survey

To determine the extent of virus incidence and problems, samples of infected cucurbits were collected in surveys during October 1995 in the Atherton Tableland, Bowen-Burdekin, Bundaberg and Lockyer regions with (6,7,17,6) samples respectively. Virus was detected and identified using ELISA. These data have been augmented with information from subsequent *ad hoc* enquiries.

2.2 Virus isolates

Initial virus isolates were supplied by Denis Persley (QHI):-
Papaya ringspot virus type W (PRSV-W), isolate DB-1 (Plant Pathology accession 505),
Zucchini Yellow Mosaic Virus, isolate G4 (Plant Pathology accession 289),
Zucchini Yellow Mosaic Virus, isolate K (Plant Pathology accession 293),
Watermelon Mosaic Virus, North Queensland isolate (Plant Pathology accession 369).

The isolates were subsequently maintained by subculturing:- PRSV-W in pumpkin 'Queensland Blue', ZYMV in pumpkin 'Jarrahdale' and WMV in zucchini 'Regal Black'.

2.3. Breeding and selection

Field and glasshouse cultural and inoculation techniques were similar to those described previously (Herrington *et al.* 1989, Loader *et al.* 1996)

2.3.1. 'Jarrahdale' type

Generally there were two plantings per year in South Queensland and one in North Queensland.

Seed sown in peat-vermiculite seedling mix in cells, then transplanted to field approximately 10 days to 18 days after sowing. Irrigation was overhead in South Queensland but at Bowen (North Queensland) trials were trickle irrigated. Nutrition was maintained by fertiliser applications based on soil tests or the current local recommendations, pest and disease treatments were applied as required. Spacings varied from 3 to 5m between rows and from 1 to 3 m between plants within rows. Where necessary expanded carborundum-dusted cotyledons of seedlings were inoculated on two consecutive days with infective leaf sap prepared 1:10 w/v symptomatic leaves and 0.1M sodium potassium phosphate buffer containing 0.1% sodium sulphite. Plants were field planted 1 or 2 days after final inoculation.

'Jarrahdale', 'Redlands Trailblazer' and F1 ('Jarrahdale' X 'Redlands Trailblazer') both inoculated and un-inoculated were planted as controls when assessing levels of virus resistance.

2.3.2. 'Butternut' type

Generally there were two plantings per year in both South Queensland and North Queensland.

Seed was commonly direct-sown in North Queensland while in South Queensland it was generally sown in peat-vermiculite seedling mix in cells then transplanted to field approximately 10 days to 18 days after sowing. Irrigation was overhead in South Queensland and on the Atherton Tableland (North Queensland), but at Bowen irrigation was by trickle. Nutrition was maintained by fertiliser applications based on soil test or the current local recommendations. Pest and disease treatments were applied as required. Spacings varied from 2 to 5m between rows and from 0.75 to 2 m between plants within rows. Where necessary expanded carborundum-dusted cotyledons of seedlings were inoculated on two consecutive days with infective leaf sap prepared 1:10 w/v symptomatic leaves and 0.1M sodium potassium phosphate buffer containing 0.1% sodium sulphite. Plants were field planted 1 or 2 days after final inoculation.

'Butternut Large', 'Nigerian' and F1 ('Butternut Large' X 'Nigerian') both inoculated and un-inoculated were also planted as controls when assessing levels of virus resistance, and 'Butternut Large', LL6.06 or LL12.05 (advanced selections developed by L. Loader, Loader *et al.* 1996) were planted as controls when assessing levels of etch.

3.0. GENERAL RESULTS

3.1. Virus Surveys

Both ZYMV and PRSV are common in coastal Queensland, while PRSV-W dominates in sub-coastal areas. WMV is regularly found in all areas (Table 3.1.). These viruses of cucurbits are less problematic in Southern states but ZYMV can occur anywhere, implying some seed-borne transmission.

Table 3.1. Occurrence and frequency of ZYMV, PRSV and WMV in cucurbits.

Area	PRSW-W	ZYMV	WMV
Far North Queensland (Mareeba, Qld)	Predominant	Occasional	Common
Dry tropics (Bowen-Burdekin, Qld)	High levels	Dominant	Common
Central Queensland (Emerald, Qld)*	Predominant	Not observed yet	Common
Bundaberg (Qld)	Frequent	Frequent	Common
Southern inland (Chinchilla, Qld)*	Predominant	Not observed yet	Common
Lockyer (Gatton, Qld)	Frequent	Frequent	Common
Ord River (Kununurra, WA)	Frequent	Frequent	Common

*Provisional data

4.0. DEVELOPING AND RELEASING 'DULONG QHI' – A VIRUS RESISTANT 'JARRAHDALE' TYPE

4.1. Selection History

Prior to the commencement of the project QDPI had developed a breeding (segregating) population of grey 'Jarrahdale' type pumpkins through field work at Redlands, Maroochy, and Gatton Research Stations.

Previous breeding history involved controlled and open-pollination followed by selection at each stage: *C. maxima* 'Queensland Blue' (Selected Strain) was crossed with *C. ecuadorensis* followed by three backcrosses to 'Selected', 'Large'(Yates Seed Co), and 'Wallworks' strains of 'Queensland Blue', followed by 2 generations of self-pollination and 2 generations of open-pollination, selected separate plants crossed to 'Jarrahdale' (Yates) and to 'W19' [a selection of parentage similar to above] and the resultant progeny were intercrossed, followed by a generation of self-pollination, intercrossed resultant selections, then 1 generation of self-pollination, out-crossed to 'Jarrahdale' (New World), then 7 generations of open pollination (in which initial population included the population of the above crossed with 'Jarrahdale' (SPS)). The original seed parent and all commercial parents in the ancestry were characterised by susceptibility to PRSV-W, ZYMV and WMV.

This variable population was carrying unfixed genes for advantages such as resistance to 3 viruses, high fruit quality and productivity but they were also carrying disadvantageous genes for skin cracking and internal colouration 'bone'.

In 1995 the project commenced with two generations of self-pollination (in 1995, 96) then one generation of open-pollination as combined lines 3214 and 3218. From these lines, through open pollination a uniform stable line known as 3287 was selected to become 'Dulong QHI'. Selection criteria included resistance to PRSV-W, ZYMV and WMV, yield, grey skinned fruit, and good flesh and consumer characteristics.

4.2.0. Sensory Evaluation - Introduction

It is important that a released cultivar has a consumer acceptability at least as good as or near to that of the current commercially available lines if the market is to be expanded or maintained in competition with other products. Throughout the development process of 'Dulong QHI' lines had been assessed to keep the acceptability at a marketable level, however no formal evaluation had been conducted. Therefore to ensure consumer acceptability 'Dulong QHI' was compared in sensory evaluations to a range of 'Jarrahdale' type pumpkins considered to be important on the market.

4.2.1. Sensory Evaluation - Materials and Methods

Sensory evaluations were conducted on four grey skinned 'Jarrahdale' type pumpkin cultivars using an 'experienced panel' at Centre for Food Technology (CFT), Hamilton, Queensland. The four cultivars viz, 'Dulong QHI', 'West Australian Grey', 'Sweet Grey' and 'Brian's Grey' produced at Bowen Research Station were evaluated in four replicates during October 1998 by between 21 and 24 panellists recruited from the staff at the Centre for Food Technology. The panel was experienced in sensory profiling techniques but had no specific training in pumpkin characteristics or knowledge of the sample backgrounds. The response of this panel was considered to be 'an indication of probable consumer response', although the results cannot be considered those of a true consumer panel since the panel includes a high proportion of technical staff.

At each session pieces, approximately 3 cm cubes, from four fruit of each cultivar were steamed for 18 minutes.

At any evaluation session, panellists evaluated two random pieces of cooked sample from each of the four cultivars in a balanced order according to a randomised block design using standard *hedonic-rating, and intensity rating scales* (AS 2542.2.3). Stephen Nottingham, (Senior Research Scientist), supervised the design and conduct of taste panels in the modern sensory evaluation laboratory at CFT.

The fruit appearance was scored on separate samples of four fruit, either whole (external) or cut (internal).

On the acceptability scales *(hedonic-rating)* the left value (0) corresponded to dislike very much and the right value (100) corresponded to like very much, these scales included *external and internal appearance, cooked appearance,* overall acceptability, texture acceptability and flavour acceptability.

On *intensity rating scales* that nominated a specific characteristic and scored the panellist's perception of relative intensity, the characteristics were scored from (0) low, to (100) high). For example the flesh colour was scored from yellow (0) to orange (100), brightness of colour from dull (0) to bright (100); texture characteristics, moistness from very dry (0) to very moist (100), and softness from very firm (0) to very soft (100); and intensity of flavour characteristics, sweetness from none (0) to very strong (100) and typical pumpkin from none (0) to very strong (100).

Data were recorded on 150 mm line scales using computerised sensory analysis (Compusense *five*© V 3.0, Compusense Inc. Canada). Provision was also made for panellists to describe internal and external characteristics and cooked flavour and texture using a common set of descriptors and their own words.

Scores for linear scales were averaged across panellists for each replicate prior to analysis of variance. Where there was a significant ($P<0.05$) difference between cultivars for a particular attribute then pair-wise comparisons were made using the least significant difference procedure.

4.2.2. Sensory Evaluation - Results

'Dulong QHI' was the most liked, or not statistically different from the most liked, cultivar for flavour, texture, cooked appearance, external appearance and internal appearance. However for overall acceptability (based only on tasting) 'Sweet Grey' was the best.

Overall acceptability - 'Sweet Grey' had the most liked overall acceptability (based on tasting) and, with the possible exception of 'West Australian Grey', cultivars were in the 'liked' range, although only marginally. 'Dulong QHI' was similar to 'West Australian Grey' and 'Brians Grey' ($P>0.05$). Overall acceptability patterns strongly reflected flavour acceptability which was reflected by softness and sweetness (Tables 4.2.1. and 4.2.2.).

Flavour acceptability - 'Dulong QHI' was acceptably well flavoured. Flavour acceptability of 'Dulong QHI' was between 'Sweet Grey' and the other cultivars, being statistically similar to both groups (Tables 4.2.2. and 4.2.7.).

Sweetness - 'West Australian Grey' was less sweet than other cultivars (Table 4.2.1.).

Typical pumpkin flavour - There was no difference ($P>0.05$) among the cultivars in the intensity of pumpkin flavour although 'Sweet Grey' had the highest value (Table 4.2.1.).

Texture acceptability - 'Dulong QHI' was acceptably well textured. Texture acceptability of 'Dulong QHI' was between 'Sweet Grey' and the other cultivars, being statistically similar to both groups (Tables 4.2.2. and 4.2.6.).

Softness - 'Dulong QHI' softness was about mid-range, neither soft nor firm. Softness of 'Dulong QHI' was between 'Sweet Grey' and the other cultivars, being statistically similar to both groups (Table 4.2.1.).

Moistness -All cultivars had a similar mid-range of moistness (Table 4.2.1.).

Cooked appearance acceptability - Dulong QHI had one of the most liked cooked appearances (Tables 4.2.2. and 4.2.5.). All cultivars were in the liked to well-liked range with 'West Australian Grey' highest and similar to 'Dulong QHI'. Interestingly 'Sweet Grey' had the least liked, but still a good cooked appearance.

Colour (of cooked sample) - 'Dulong QHI' had the most orange colour (Table 4.2.1.).

Brightness (of cooked sample) - Brightness was similar among cultivars (Table 4.2.1.).

Uncooked appearance - Uncooked appearance of 'Dulong QHI' was good (Table 4.2.2.).

Internal appearance acceptability - There was no difference among cultivars, although some panellists considered the skin of 'Sweet Grey' was too thick and some thought 'Dulong QHI' was too dark (Table 4.2.4.).

External appearance acceptability - 'Dulong QHI' and 'Sweet Grey' were the best, 'Brians Grey' and 'West Australian Grey' were considered too large (Table 4.2.3.).

4.2.3. Sensory Evaluation - Discussion

The results of the sensory panel have established that 'Dulong QHI' has a good combination of important characteristics and should be readily acceptable to consumers.

Sweetness, colour and softness are the attributes that seem to contribute most to the liking of pumpkin and 'Dulong QHI' has acceptable levels of these. The score for overall acceptability of 'Dulong QHI' is similar to two of the three other cultivars ie marginally above acceptable. Such scores could probably be expected because pumpkins are not known for their high sensory appeal to consumers. However further improvements could be made as evidenced by the higher score of 'Sweet Grey' and this should be considered in future cultivar development.

Table 4.2.1. The mean score for the appearance, texture and flavour characteristics of the four pumpkin varieties.

Cultivar	Brightness [ns]	Colour	Moistness [ns]	Softness	Sweetness	Typical Pumpkin flavour[ns]
Dulong	57.8 a	78.2 b	47.0 a	46.3 ab	41.5 b	56.1 a
West Australian Grey	62.7 a	63.6 ab	44.4 a	39.9 a	32.4 a	52.8 a
Sweet Grey	55.8 a	53.1 a	53.1 a	51.0 b	49.2 b	61.9 a
Brians Grey	59.3 a	56.1 a	51.4 a	49.8 b	41.8 b	56.1 a
LSD (P=0.05)	7.5	14.8	7.4	8	8.7	8.1

ns – not significant (P>0.05). a,b – means not followed by a common letter are significantly different. (P<0.05).

Table 4.2.2. The mean acceptability scores for the appearance, texture, flavour and overall assessment of the four pumpkin varieties.

Cultivar	External appearance	Internal appearance [ns]	Cooked appearance	Texture	Flavour	Overall
Dulong	62.5 c	60.5 a	64.0 bc	57.8 ab	57.7 ab	54.4 a
West Australian Grey	53.8 b	60.2 a	66.3 c	53.5 a	53.1 a	48.2 a
Sweet Grey	62.7 c	59.0 a	57.2 a	62.7 b	65.3 b	63.0 b
Brians Grey	43.2 a	56.9 a	60.2 ab	52.6 a	55.4 a	53.1 a
LSD (P=0.05)	6.6	11.1	5.1	5.8	8.1	7.1

ns – not significant (P>0.05). a,b – means not followed by a common letter are significantly different. (P<0.05).

Table 4.2.3. Frequency count of the external descriptors used to describe the four pumpkin varieties.

Descriptor	Dulong QHI	West Australian Grey	Sweet Grey	Brians Grey
Too large	2	54	3	74
Too small	2	0	3	2
Uniform shape	49	37	61	19
Non-uniform shape	24	24	8	39
Greyish colour	34	33	61	38
Greenish colour	41	41	13	25
Matty/webbed	10	27	8	42
Pale	6	19	41	26
Dark	16	2	2	1
Smooth	15	14	82	5
Large crevices	36	47	2	32
Blemishes /discoloured	52	46	42	69
Mottled	41	39	7	40
Other	8	9	5	14

Table 4.2.4. Frequency count of the internal descriptors used to describe the four pumpkin varieties.

Descriptor	Dulong QHI	West Australian Grey	Sweet Grey	Brians Grey
Too pale	4	14	22	28
Too dark	15	2	0	4
Uneven colour	39	47	43	58
Even colour	37	29	28	23
Poor flesh/seed ratio	14	35	12	50
Good flesh/seed ratio	61	33	67	30
Translucent	8	3	11	3
Skin too thick	3	1	14	1
Other	16	12	11	14

Table 4.2.5. Frequency count of the cooked appearance descriptors used to describe the four pumpkin varieties.

Cultivar	Dry	Moist	Mushy	Even colour	Uneven colour	Fibrous	Sheen	Skin fading	Pale	Dark	Other
Dulong QHI	18	43	4	58	21	24	14	4	1	38	7
West Australian Grey	27	37	4	57	24	26	15	1	7	13	3
Sweet Grey	21	41	8	21	53	13	9	20	13	15	5
Brians Grey	25	39	6	42	34	29	9	9	12	13	2

Table 4.2.6. Frequency count of the texture descriptors used to describe the four pumpkin varieties.

Cultivar	Creamy	Smooth	Grainy /gritty	Stringy	Floury/powdery	Pasty	Crunchy	Uniform	Non-uniform	Other
Dulong QHI	14	22	37	24	23	15	22	34	19	5
West Australian Grey	17	12	39	47	17	13	32	24	22	4
Sweet Grey	29	44	18	19	20	14	15	30	23	4
Brians Grey	16	21	33	52	16	17	21	22	27	1

Table 4.2.7. Frequency count of the flavour desciptors used to describe the four pumpkin varieties.

Sample	Bland	Creamy	Earthy	Harsh /sharp	Nutty	Peppery /spicy	Bitter	Other
Dulong QHI	39	24	19	2	18	6	7	7
West Australian Grey	45	15	24	3	20	7	5	11
Sweet Grey	21	43	15	2	29	2	2	9
Brians Grey	40	22	21	4	28	6	4	10

4.3.0. Managing Quality Defects - Inheritance of resistance to 'bone' - Introduction

Low levels of brown coloured areas in the flesh of pumpkin fruit have been observed in 'Jarrahdale' and high levels were observed in an experimental hybrid of a commercial company. Similar brown coloured areas had been observed at times in our breeding population since the first hybrids with *C ecuadorensis* were made in 1978. This condition was considered to be similar to the 'bone' or 'woodiness' which was common in older cultivars such as 'Queensland Blue' and 'Ironbark'. The condition appeared to be expressed mostly in hot weather, but its occurrence and intensity varied with season, lines, plants within lines and even fruit within plants within lines. Earlier work had tentatively suggested that Calcium and Magnesium accumulated in the brown tissue due to tissue break-down and that a lack of Potassium and perhaps Copper may contribute to its expression. It was also noted that insect damage caused callusing similar to white' bone' in some fruit.

Susceptibility to the condition had proved a difficult characteristic to eliminate. Some families were noted which seemed to more consistently produce the condition. These were crossed with lines that appeared to rarely produce the condition and used to better understand the inheritance of resistance to 'bone', as an aid in this and future selection procedures.

4.3.1. Managing Quality Defects - Inheritance of resistance to 'bone' - Materials and Methods

Seed of parental, hybrid and segregating generations were sown in seedling trays 22-10-97 and field planted at Maroochy Research Station Nambour on 16 November 1997. Spacings were at 1.5m between plants within rows and 5.6 m between rows with initially 2 to 20 planting positions per plot and 3 to 6 blocks.

During the last 8 weeks temperatures at the level of fruit were 27^{O}C average, 51^{O}C maximum, 16^{O}C minimum.

Fruit were harvested at maturity 25 February 1998, and assessed for occurrence of brown 'bone'. Symptoms on cut fruit were rated for percent (length) of surface area showing brown areas and as required converted to ratings from 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30%. Plants were classified as resistant if the average score of fruit on plant was less than 5% and the maximum extent of 'bone' of any fruit was less than 15%. Within lines brown 'bone' ratings were analysed using the method of residual maximum likelihood (REML) with structure fruit within plants within blocks. The blocks term was then dropped because the estimate of the variance component for blocks was negative or near zero. As there were significant differences between variances for different lines ($P<0.05$) individual pairwise 't' tests were conducted using the estimates of the individual line means and their standard errors from the REML analyses.

4.3.2. Managing Quality Defects - Inheritance of resistance to 'bone' - Results and Discussion

Although when the F1 is ignored a digenic model with complementary gene action explains much of the data, the complete data did not fit a simple genetic model well (Table 4.3.1.). The inheritance of resistance was described by additive gene effects (Table 4.3.2.) in the generation means analysis. Environmental variation as determined from the pooled variance of P1, P2 and F1 (0.4 on fruit rating data), probably acting through sampling error due to few fruit in some families was substantial. This high variation precluded good estimates of heritability. (It is however also possible that the parents used in the production of test generations had residual heterozygosity which contributed to the variation and the lack of fit of segregation ratios). Selection should continue to be on a family basis.

Table 4.3.1 Segregation for resistance to 'brown bone'.

Generation	Number of plants Resistant	Number of plants Susceptible	Expected ratio (res:susc)*	Number of plants total
P_S (3224)	1	8	0:1	9
P_R(Resistant 3221)	4	0	1:0	4
F_1(3178)	5	5	0:1	10
F_2(3222)	37	31	7:9 (Pr 0.08)	68
BC_1 P_S(3225)	0	42	0:1	42
BC_1 P_R(3223)	30	11	3:1	41

Plants classified as resistant if the average score of fruit on plant was less than 5% and maximum of any fruit was less than 15%. Symptoms on fruit were rated from 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30%. Planted Maroochy Research Station, Nambour, 16 November 1997, harvested and assessed 25 February 1998. * Expected ratio under digenic inheritance with complementary gene interaction with probability of calculated Chi squared.

Table 4.3.2. Mean severity of symptoms of 'brown bone' in fruit of pumpkin plants and values of parameters (with std error) estimated by analysis of generation means from fruit of field grown plants.

Generation	Severity of symptoms (with sem)	Number of fruit assessed	Parameter	Parameter estimates
P_S (3224)	2.13 (0.42) ab	28	*m*	1.4 (0.17) *
P_R(Resistant 3221)	0.14 (0.14) d	7	*a*	1.4 (0.15) *
F_1(3178)	1.42 (0.47) abc	35	*d*	0.0 (0.35) ns
F_2(3222)	1.30 (0.14) b	170	*aa*	-
BC_1 P_S(3225)	2.41 (0.16) a	140	*ad*	-
BC_1 P_R(3223)	0.70 (0.13) cd	94	*dd*	-
			Chi Square(3 df)	7.24 (P=0.07)

* Parameter's value was significantly different from zero; :n.s, not significantly from zero. Parameters for additive dominance model as per Basford and Delacey 1979, severity of symptoms followed by a letter in common are not significantly different (P=0.05). Symptoms on fruit were rated 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30% following harvest.

4.4.0. CONFIRMATION OF VIRUS RESISTANCE

'Dulong QHI' was developed for high levels of resistance to major viruses in sub-topical and tropical Australia. Final glasshouse inoculations were conducted to demonstrate these resistances.

Seed was sown in mix of peat and gravel in 125mm pots on 3-11-1999 in randomised design. Carborundum-dusted cotyledons were inoculated on 10 and 11-11-1999 with 1:10 w/v infected leaves (of 'Jarrahdale' pumpkin for ZYMV, of 'Queensland Blue' pumpkin for PRSV-W, and of 'Regal Black' zucchini for WMV) : buffer. Reaction was assessed on 24-11-1999 as the percentage of the area of the youngest expanded leaf (leaf 4) which as chlorotic and or deformed.

Table 4.4.1. Reaction of *C. maxima* cultivars to inoculation with PRSV-W, ZYMV or WMV.

Virus	Parameter	'Dulong QHI'	'Jarrahdale'	'Redlands Trailblazer'
Papaya ringspot virus type W	**Median score**	**0.05**	**90**	**0.1**
	Minimum score	*0*	*60*	*0*
	Maximum score	*0.5*	*95*	*0.1*
	Number of plants	*16*	*10*	*9*
Zucchini yellow mosaic virus (isolate G4)	**Median score**	**1.5**	**95**	**0.1**
	Minimum score	*0.1*	*90*	*0.1*
	Maximum score	*10*	*98*	*0.5*
	Number of plants	*10*	*10*	*10*
Zucchini yellow mosaic virus (isolate K)	**Median score**	**1**	**95**	**0.5**
	Minimum score	*0.1*	*90*	*0.1*
	Maximum score	*7*	*99*	*1*
	Number of plants	*10*	*10*	*7*
Watermelon mosaic virus	**Median score**	**0.1**	**85**	**0**
	Minimum score	*0*	*75*	*0*
	Maximum score	*0.5*	*90*	*0.1*
	Number of plants	*11*	*11*	*8*

Resistance of 'Dulong QHI' to PRSV-W, ZYMV and WMV was high (Table 4.4.1.), being substantially better than the commercial control, 'Jarrahdale', and similar to the resistant breeding line 'Redlands Trailblazer', although the latter may have very slightly better resistance to ZYMV. These levels of resistance should make a major contribution to minimizing losses to virus in commercial grey pumpkin crops.

4.5.0. FIELD PERFORMANCE

To be competitive a cultivar must perform satisfactorily in all characteristics and or excel in at least one area so that on balance there is a net improvement in profitability to the grower. 'Dulong QHI' had excellent resistance to viruses. It was grown in field trials at Maroochy (1999) and Bowen Research Stations (1998 and 1999) to confirm its performance was satisfactory relative to other cultivars.

Table 4.5.1. Field performance at Bowen Research Station 1998

Cultivar	Marketable Yield (t/ha)	Fruit Size (kg) of Marketable Fruit
Dulong QHI	38 a	3.9 c
Jarrahdale West Australia Grey	48 a	5.9 b
Brians Grey	51 a	7.8 a
Sweet Grey	42 a	4.1 c
F test	ns	**
LSD (P=0.05)	*(11)*	0.4
sem	3.62	0.14

Sown seedling cells:1/4/98, field:15/4/98, harvested 19-26/08/98. Bowen Research Station, 1998. Within columns values followed by a letter in common are not significantly different (P>0.05).

Table 4.5.2. Field performance at Bowen Research Station 1999

Cultivar	Total Yield (t/ha)	Marketable Yield (t/ha)	Fruit Size (kg) of Marketable Fruit
Dulong QHI	75 b	67 a	4.3 d
Eudlo QHI	73 bc	68 a	5.3 c
Jarrahdale SPS	87 ab	80 a	6.2 b
Brians Grey	84 ab	77 a	7.1 a
Sweet Grey	61 c	53 b	3.8 de
Hybrid Maverick	88 a	80 a	5.5 c
Redlands Trailblazer	76 ab	71 a	3.6 e
F test	**	**	*
LSD (P=0.05)	12.9	14	0.59
sem	4.43	4.6	0.203

Sown seedling cells:7/4/99, field:23/4/99, plot size 12m x 6m, 16 plants/plot, spacing 1.6m, harvested 10-17/9/99 Bowen Research Station. Within columns values followed by a letter in common are not significantly different (P>0.05).

Table 4.5.3. Characteristics of fruit at Maroochy Research Station 1999

Cultivar	Fruit Size (kg)	Fruit Length (mm)	Fruit Diameter (mm)	Fruit length/ diameter ratio (mm)	Thickness of Flesh (mm)
Dulong QHI	3.2 b	120 a	226 a	0.54 a	50 a
Eudlo QHI	3.5 b	142 b	224 a	0.64 b	47 a
Redlands Trailblazer	2.1 c	134 a	175 b	0.76 c	31 b
Jarrahdale	4.6 a	148 b	239 a	0.62 b	51a
LSD (P=0.01)	0.76	17	21	0.060	9

Within columns values followed by a letter in common are not significantly different (P>0.01)

'Dulong QHI' had good yields of medium sized fruit. Production and fruit size at Bowen were similar to 'Sweet Grey' in 1998 (Table and 4.5.1.). and between 'Jarrahdale' and 'Sweet Grey' in 1999 (Table 4.5.2.). Flesh thickness was also good (Table 4.5.3.). The highest yielding cultivars tended to have the larger fruit. 'Eudlo QHI' (Table 4.5.3.) was initially identified as a good cultivar and in trials had good yields but fruit were too variable, some had exceptionally pale fleshed fruit, variable seed colour and skin tended to crack. It was therefore not included in further studies.

5.0. DISCUSSION ON DEVELOPING AND RELEASING 'DULONG QHI' – A VIRUS RESISTANT 'JARRAHDALE' TYPE

"Dulong QHI' combines excellent virus resistance, good fruit quality and high productivity. It is also important to note that the virus resistance of 'Dulong QHI' is to the three most important viral diseases of cucurbits in tropical and sub-tropical Australia viz, papaya ringspot virus, zucchini yellow mosaic virus, and watermelon mosaic virus. From the point of view of market development it is also important to note that these resistances were incorporated without resorting to 'genetic engineering'. The resistance was incorporated by traditional breeding techniques. This feature should be an important advantage in the developing 'organic produce' market both domestically and internationally.

The fact that the three resistances were fairly readily transferred together suggests that they may be closely linked or even result from the same gene. While studying the inheritances of these resistances in a way to elucidate such linkages was beyond the scope of this project it is suggested that further inheritance studies leading to the identification of and perhaps sequencing of the gene responsible would provide valuable intellectual property. Similarly the moderately rapid development of a range of virus resistant *C. maxima* pumpkin types is now possible from this advanced germplasm and this objective should be pursued.

The project objective of developing a virus resistant, high quality 'Jarrahdale' type pumpkin to eliminate the annual losses of $3m caused by virus was achieved with the release of 'Dulong QHI'. These reduced losses will effectively lessen land and water usage and pesticide application. 'Dulong QHI' is protected by Provisional Plant Breeders Rights (Application number 97/309), and is expected to be commercially available in July 2000.

6.0. DEVELOPING AND RELEASING 'SUNSET QHI' – A VIRUS AND ETCH RESISTANT 'BUTTERNUT' TYPE

6.1. Selection History

While evaluating progeny in our 'Butternut' breeding program in the late 80's we noted a lack of etch symptoms in selected progeny when compared to the few commercial Butternuts planted as controls. Open- and sib-pollinated seed from single plants of these populations formed the basis for further evaluation and selection.

Etch resistance was confirmed in the selected base populations. The initial evaluation of potentially etch-resistant families produced two families where only 13-14% of fruit were etched; which was much lower ($P<0.05$) than the 65% for commercial 'Butternut'. Similarly on a per-plant basis only 22% plants of the most resistant families had at least one fruit with etch compared ($P<0.05$) to 87% of plants of the commercial 'Butternut'.

The etch tolerant base population 3704 was developed from:- *C. moschata* 'Butternut' X *C. ecuadorensis* followed by three backcrosses using 'Butternut' as the male parent, then 3 generations of mass selection for virus resistance and quality (producing lines 1180-89), followed by sib-pollination of single plant selections (1732, 1733). Selection within and among families for etch resistance and fruit type was imposed by L Loader and continued through 2 generations of modified self-pollination (Loader *et al.* 1996). Etch incidence on fruit was then only 2.5 and 11.3% etched fruit on the most resistant selected lines; which was much lower than the 74% ($P<0.05$) for commercial 'Butternut'. In another trial with similar progeny 40% of the resistant plants compared with 78% of control plants showed some etch on at least one fruit. However the average severity (% surface affected) of symptoms on the fruit was much lower 3% in the resistant line compared to commercial 24% ($P>0.05$). This was followed by two 2 generations of open-pollination in isolation with mass selection for etch tolerance and fruit quality to produce line 3074.

The virus tolerant base population 3095 was developed from:- *C. moschata* 'Nigerian'[1984 Plant Dis. 68:443-446] x *C. moschata* 'Butternut Large' followed by one backcross to 'Butternut Large' with selection for resistance to PRSV_W and ZYMV.

Subsequently selected plants of 3095(bulk) and 3074 were crossed. Then followed three back crosses to selections of 3074, one or two generations of self-pollination with selection for resistance to virus and etch (BL3392) and one or two generations of open-pollination. The advanced line was tested as BL3392.

6.2. Technique development – glasshouse techniques for assessing reaction to etch

Evaluating resistance to etch would be more efficient if there was available a glasshouse technique which correlated well with field responses. Because none were readily available, we made preliminary investigations of various glasshouse inoculation techniques once a *Didymella bryoniae* culture had been isolated from etch affected 'Butternut' pumpkins grown at Mareeba in 1998. The identity of the isolate was confirmed by John Alcorn (isolate i.d. no. N11622).

1. Spore suspension

Initial inoculations were tried with a spore suspension (in sucrose (0.1%) and hydrolysed casein (0.05%) with 0.01% wetting agent) sprayed onto the leaf after wounding with a carborundum slurry. The spore inoculation concentration was 2.5×10^5 spores/mL. After inoculation, plants were individually covered with a plastic bag to keep up humidity and placed in the glasshouse. Plastic bags were removed after 72 hours and the plants remained in the glasshouse. Assessments were made after 7 days using a rating scale devised by Zhang *et al.* (1995).

This method was not very successful - although some lesions were produced (on both control and inoculated leaves – indicating some mechanical damage from the wounding), they seemed to "stop" and not proceed further. Possible reasons for this may have been insufficient inoculum or conditions in the glasshouse were too hot and dry once the plastic bags were removed. In addition, the rating scale was not sensitive enough to describe any differences between infection levels. The fungus does not produce spores very readily in culture, so to circumvent this it was decided to use a slurry of the culture and agar plate and wound by sandwiching the first true leaf between sandpaper.

2. Sandpaper and Slurry Spray

Inoculation of a variety of lines using sandpaper wounding and spray inoculation of an agar plate slurry of *Didymella* culture (2 plates of fungus grown on Butternut Malt Agar [BMA] {modified from Cucumber Malt Extract Agar (St. Amand and Wehner, 1995)} in 100 mL sterile water) was tried. Controls were sprayed with BMA only (no culture). Plants were placed in the humidity chamber overnight prior to inoculation and inoculated as early as possible in the morning, this time giving best conditions for infection process because of guttation fluids (St. Amand and Wehner, 1995). Plants were placed back in the humidity chamber after inoculation and removed to the glasshouse after 72 hours and then assessed 7 and 12 days after inoculation. Incubation temperatures ranged from 25-35+^{O}C.

Table 6.1. Summary of assessments after 12 days following sandpaper/slurry inoculation of 'Butternut' leaves'

Line	Inoculation	% leaf dead	% petiole dead	suscept./resist. rating
3272	+	73	36	R
3272	0	8	0	
3276	+	62	22	R
3276	0	3	0	
3271	+	100	94	HS
3271	0	6	0	
3348	+	94	56	S
3348	0	3	0	
3349	+	83	34	MR/MS
3349	0	2	0	
3257	+	93	65	S
3257	0	1	0	
3278	+	100	90	HS
3278	0	9	0	
Butternut Large (Yates)	+	90	60	S
Butternut Large (Yates)	0	3	0	

R = resistant; S = susceptible; HS = highly susceptible; MR/MS = moderately resistant tor moderately susceptible. + = inoculated BMA plus culture, 0 = control inoculated BMA only.

With the above trial, there was considerable variation in the percentage infection of plants within the same family e.g. for line 3272 a selection from the resistant population LL6.06/3119, percent leaf death ranged from 30-100% (for 20 plants) while percent petiole death ranged from 0-100% (for 20 plants) at the 12 day assessment. This variation is not considered to be primarily due to genetic variability within the seedling population.

A large portion of this variability was be due to variation in the amount of inoculum and wounding each plant received, as it is difficult to standardize both these factors with the method used. Because of this, a slurry injection method was tried.

3. Slurry injection

This involved using a syringe to inject a known quantity (0.1 mL) of agar slurry +/- culture into the base of each first true leaf and down the hollow leaf stem/petiole. At approx. 7 days after inoculation it was possible to observe yellowing of the inoculated leaf and stem compared with the controls. At 14 days after inoculation, some of the inoculated leaves had died while others were at various stages of yellowing with or without lesions and unhealthy but still not shrivelled and dead.

Table 6.2. Effect of injection of inoculum

	Inoculation	Average Disease Rating
Replicate 1	0	0.3
	+	4.2
Replicate 2	0	0.3
	+	4.6
Replicate 3	0	2.6
	+	7.4
Replicate 4	0	0.7
	+	5.0

Rating scale:-0= no visible signs of infection; 1= slight yellowing/chlorosis, not completely green; 2= slightly more definite yellowing/chlorosis; 3= distinctly chlorotic but no lesions or cleared areas on leaves; 4= distinctly chlorotic with less than 10% of leaf area affected by lesions or cleared areas; 5= distinctly chlorotic with at least 10% but less than 30% of leaf area affected by lesions or cleared areas; 6= distinctly chlorotic with 30% of leaf area affected by lesions or cleared areas; 8= leaf dead/shrivelled, with at least 50% of petiole/leaf stem dead; 10= both leaf and petiole completely dead and shrivelled full length of petiole to leaf axil.

The conclusion from this trial was that it was too slow, still had the same variation between plants of the same line as the sandpaper wounding method and had no advantages over that method so that the sandpaper wound and spray method was the preferred method for screening. Because the results were not able to give confidence in glasshouse screening without substantially more work and resources we continued working using the field screening. However further work to correlate field and glasshouse response is desirable if future selection work is to be conducted.

6.3.0. Inheritance of etch resistance - introduction

Although we had progressed with the development of resistant lines the inheritance of etch was poorly understood. However, such information is very useful to help determine the best strategy for cultivar development and release. We therefore examined the inheritance of resistance to etch in field trials.

6.3.1. Inheritance of etch resistance - material and methods

Seed of parental, hybrid and segregating generations were planted on Southedge Research Station, Mareeba on 25 and 26 March 1998. Spacings were at 1m between plants within rows and 3 m between rows with initially 6 and 10 planting positions per plot and 3 replicates.

Fruit were harvested at maturity on 2 and 3 June, and assessed for reaction to etch on 6 to 12 June 1998. Symptoms on fruit were rated for percent surface area etched and as required converted to ratings of 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30%. Plants were classified as resistant if there was more than one fruit available for evaluation, plus the average score of the fruit on the plant was less than 5% and the maximum extent of etch of any fruit was less than 20%.

Where the performance of generations among the families derived from resistant lines, eg 3119 and 3074, were consistent the data were pooled for the Generation Means Analysis.

6.3.2. Inheritance of etch resistance - results and discussion

The data did not fit well any simple genetic model (Table 6.3.1.). The inheritance of resistance was complex with additive epistasis as well as additive and dominance gene effects (Table 6.3.2.). Environmental variation as determined from pooled variance of P1, P2 and F1 (1.6 on fruit rating data), probably acting through sampling error due to few fruit being produced on any single plant, was substantial. This high variation precluded good estimates of heritability. (It is however also possible that the parents used in the production of test generations had residual heterozygosity which contributed to variation).

Selection should be on a family basis, as was practiced, and open-pollinated cultivars may be the most suitable for initial release. If hybrid cultivars are to be produced with the resistant populations currently available both parents will need to carry etch resistance, further seed lines would need to be produced to aid in such a hybrid strategy.

Table 6.3.1. Average rating of severity of etch, fruit size and segregation of families of plants for etch resistance.

Line	Generation	Number of plants Resistant	Number of plants Susceptible	Number of plants total	Etch rating	Ave Wt (kg) per fruit
3069	Butternut	2	23	25	2.24 a	0.95 cd
3257	F1[Pa(3074)xBN]	3	11	14	1.64 ab	1.32 a
3263	F2[Pa(3074)xBN]	27	12	39	0.68 d	1.14 abcd
3258	BCRr[Pa(3074)xBN]	18	9	27	0.83 cd	1.25 ab
3261	BCR[Pa(3074)xBN]	6	0	6	0.28 d	1.40 a
3262	BCS[Pa(3074)xBN]	2	5	7	1.98 ab	1.15 abcd
3200	F1[Pb(3119)xBN]	3	15	18	1.84 ab	1.02 bcd
3266	BCR[Pb(3119)xBN]	31	8	39	0.67 d	0.94 cd
3268	BCS[Pb(3119)xBN]	11	17	28	1.45 bc	1.30 ab
3273	RPb(3119)	19	6	25	0.49 d	0.75 d

Plants classified as resistant if there was more than one fruit available for evaluation, plus the average score of fruit on plant was less than 5% and maximum of any fruit was less than 20%. Symptoms on fruit were rated from 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30%. Planted Southedge Research Station 25 and 26 March 1998, harvested 2 and 3 June, and assessed 6 to 12 June 1998 Mareeba. Within columns values followed by a letter in common are not significantly different (P>0.05).

Table 6.3.2. Mean severity of symptoms of etch on fruit of 'Butternut' like populations and values of parameters (with std error/deviation) estimated by analysis of generation means from fruit of field grown plants.

Generation	Severity of symptoms (with sem)	Parameter	Parameter estimates
P_S (Butternut)	2.2 (0.39)	*m*	-0.43 (0.51) ns
P_R(Resistant 3119)	0.48 (0.28)	*a*	0.86 (0.17) *
F_1	1.8 (0.26)	*d*	2.22 (0.71) *
F_2	0.70 (0.25)	*aa*	1.77 (0.58) *
$BC_1 P_S$	1.52 (0.23)	*ad*	-
$BC_1 P_R$	0.69 (0.10)	*dd*	-
		Chi Square(2 df)	0.03(P=0.98)

Parameter's value was significantly different from zero; n.s, not significantly different from zero Parameters for additive dominance model as per Basford and Delacey 1979. Symptoms on fruit were rated 6-12 June 1998 from 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30% following harvest on 25 and 26 March 1998, Southedge Research Station, Mareeba.

6.4. Managing quality defects – stem adhesion

For obvious marketability reasons it is important that fruit of advanced lines have high external quality. Adhesion of the stem to the fruit during development was observed at high frequency in an advanced line. This defect occurs at an early developmental stage and is obvious by the time flower buds open. To avoid carrying this defect breeding lines were evaluated for the presence of stem adhesion and etch resistance in a field trial 1997.

Table 6.4.1. Incidence of etch and stem adhesion on selected 'Butternut' type populations 1997.

Treatment	Percent fruit with etch	Percent fruit with stem adhesion	Plants with at least one etched fruit	Plants with at least one fruit with stem adhesion	Total number of plants
6.06	11	16	4	6	12
7.06	21	18	5	4	11
9.03	35	4	7	2	12
12.05	17	0	4	0	11
17.03	7	7	1	1	12
18.11	3	5	1	2	11
19.09	20	4	5	2	12
21.01	27	0	2	0	3
22.07	25	13	1	2	5
1749	0	0	0	0	11
3186	29	1	6	1	12
3188	37	0	5	0	7
3189	28	2	6	1	12
3190	77	2	19	2	20
Butternut Large	86	4	47	8	48
Butternut Large	86	0	11	0	12

3186= BC2{etch-resistant, virus-susceptible}=[3074(plant3) X3095sib]-best shape and size,;3188= BC2{etch-resistant virus-susceptible}=[3074(plant1) X3095sib]; 3189= BC2{ etch-resistant virus-susceptible }=[3095plant2 X3074plt?]-light and dark green, Bn shape-slight pear; 1749= 099('Nigerian') selfed; 3190= Seln of 'Butternut' from SA; 'Butternut Large' from Yates Seed Company. Evaluated Sown seedling cells:27/10/97, field:13-14/11/97, harvested 5-10/2/98 Southedge Research Station, Mareeba.

Commercial 'Butternut Large' expressed etch at high frequency but also had a small percentage of fruit with stem adhesion (Table 6.4.1.). Although some lines had little or no stem adhesion eg 12.05 or etch eg 18.11, up to 50 percent of plants of some lines expressed stem adhesion and 37 % of fruit expressed etch. Line 12.05 was selected for further development. The original source of virus resistance 1749 (ie self-pollinated 'Nigerian' [1984 Plant Dis. 68:443-446]) is especially interesting because it expressed neither etch nor stem adhesion and so may provide an additional source of tolerance to etch.

6.5. Field performance, etch and virus resistances

Etch reactions were evaluated as in replicated field trials at Mareeba on soil with a history of high etch incidence. Virus resistance was evaluated as percent leaf area affected 2 weeks after inoculation of carborundum dusted cotyledons with virus infective sap in the glasshouse. Yield was evaluated at Bowen Research Station.

6.5.1. Field performance, etch and virus resistances - Results and Discussion

The family BL3392 from which 'Sunset QHI' has been extracted by further selection had high levels of resistance to etch, had fruit size similar to 'Butternut Large', high levels of virus resistance and high productivity (Tables 6.5.1- 4.). Fruit are rather similar to 'Butternut Large' although slightly narrower and slightly longer and slightly more bulbous.

Table 6.5.1. Performance of 'Butternut' type selections at Southedge Research Station, Mareeba 1999.

code	Line	Etch Rating	Fruit size (kg)	Yield (t/ha))
9	Butternut Large	1.75 g	1.40 ab	21.0 bcd
10	LLadvanced 6.06	0.17 ab	1.78 de	28.5 de
11	LLadvanced 12.05	0.18 abc	1.76 cde	21.5 bcd
1	3367	0.15 ab	2.06 efgh	22.0 bcd
2	3369	0.37 abcd	2.21 gh	31.6 e
3	3377	0.17 ab	1.21 a	16.7 ab
4	3380	0.52 abcde	1.51 bcd	19.8 abc
5	3381	0.01 a	1.50 bcd	17.8 ab
13	3385	0.43 abcde	1.96 efgh	22.6 bcd
14	3386	0.24 abcd	2.20 fgh	17.3 ab
15	3387	0.93 ef	2.26 h	23.4 bcd
16	3388	0.83 def	1.44 abc	20.3 bc
6	3392(~Sunset QHI)	0.12 ab	1.45 ab	24.1 bcde
7	3393	0.63 bcde	1.93 efg	19.9 abc
8	3395	1.28 fg	1.85 ef	12.3 a
12	3402	0.71 cde	1.89 ef	27.1 cde
	F	**	**	**
	LSD (P=0.05)	0.27	0.31	7.8

Symptoms on fruit were rated from 0=0%, 1=0<x≤5, 2=5<x≤15, 3=15<x≤30, 4=x>30%, following field Sowing:14/4/99 and harvest 21/07/99, from Southedge Research Station, Mareeba. Plant spacing, 1.0m in rows, 2.0m between rows, 10 plants/plot. Within columns values followed by a letter in common are not significantly different (P>0.05).

Table 6.5.2. Performance of 'Butternut' type selections at Bowen Research Station 1999.

Code	Line	Marketable yield (t/ha)	Total yield (t/ha)	Fruit size (kg, marketable)
1	Butternut Large (Yates)	27.2 def	30.6 c	1.87 fgh
17	Butternut Large (SPS)	24.9 ef	30.1 c	2.05 defg
3	3367	39.3 abcd	43.1 ab	2.50 bc
4	3369	43.3 ab	49.3 a	2.97 a
5	3377	36.7 abcdef	39.7 abc	1.50 i
6	3380	45.7 a	47.9 a	2.21 cdef
7	3381	36.6 abcdef	39.3 abc	1.87 ghi
11	3385	22.2 f	34.1 bc	2.76 ab
12	3386	32.7 abcdef	37.7 abc	2.88 a
14	3387	32.5 abcdef	39.3 abc	2.48 bc
16	3388	27.8 def	30.4 c	1.71 hi
9	3392 (~Sunset QHI)	46.6 a	49.1 a	1.98 efgh
13	3393	32.3 bcdef	34.3 bc	2.32 cd
15	3395	36.8 abcdef	40.0 abc	2.35 cd
8	3402	40.7 abc	43.6 ab	2.27 cde
2	3435	37.0 abcde	40.4 abc	2.43 bc
10	3436	30.4 cdef	37.9 abc	1.90 fgh
	F	**	*	**
	LSD (P=0.05)	12.2	11.9	0.34

Sown seedling cells:2/5/99, field:21/5/99, harvested 15/10/99. Bowen Research Station, 1999. Within columns values followed by a letter in common are not significantly different (P=0.05). Plots 4.5x3m, 6 plants/plot; plant spacing, 0.75m

Table 6.5.3. Characteristics of fruit of 'Butternut' type selections at Mareeba.

Line	Flesh thickness (button) mm	Flesh thickness (side-wall of cavity) mm	Width of seed cavity (mm)	Bulb width (mm)	Width body of fruit (mm)	Fruit length (mm)	Length/ width (body) ratio	Bulb/ width (body) ratio
3367	17.5 g	26.4 fgh	78.6 def	131 defg	123 gh	187 cde	1.5 ab	1.08 abc
3369	13.3 abc	28.8 h	82.2 efgh	140 gh	129 h	176 bcd	1.4 a	1.10 abcd
3377	13.8 abcde	19.5 a	65.8 ab	105 a	92 a	195 e	2.2 e	1.16 bcdef
3380	14.2 bcde	22.0 bc	65.5 a	110 ab	100 ab	217 f	2.2 e	1.10 abcde
3381	12.0 a	23.9 cde	67.3 ab	115 bc	116 cdefg	159 a	1.4 a	1.00 a
3392 (~Sunset QHI)	15.0 cdef	23.5 bcd	67.9 abc	115 bc	103 abcd	177 bcd	1.7 bc	1.12 bcdef
3393	17.6 g	26.1 efg	76.5 cde	129 def	108 bcde	224 f	2.2 e	1.22 fg
3395	17.0 g	26.6 fgh	72.5 bcd	126 de	105 bcde	215 f	2.1 de	1.23 fg
Butternut Large	12.7 ab	22.3 bc	69.9 abc	114 bc	116 defgh	163 ab	1.4 a	1.00 a
LLadvanced 6.06	17.3 g	23.3 bcd	86.9 gh	134 efg	103 abc	194 e	1.9 cd	1.31 g
LLadvanced 12.05	15.7 defg	25.5 def	71.7 abc	123 cd	105 abcde	190 de	1.9 c	1.21 efg
3402	15.8 efg	23.4 bcd	80.4 efg	127 def	122 fgh	175 bc	1.4 a	1.05 ab
3385	16.4 fg	25.5 def	83.6 fgh	135 fg	117 efgh	178 cd	1.5 ab	1.16 cdef
3386	16.4 fg	28.5 gh	83.2 efgh	140 gh	125 gh	174 bc	1.4 a	1.14 bcdef
3387	16.5 fg	27.8 fgh	87.9 h	144 h	127 gh	176 bc	1.4 a	1.14 bcdef
3388	13.7abcd	21.1 ab	88.3 h	131 def	110 bcdef	157 a	1.4 a	1.20 def
LSD (P=0.05)	2.0	2.4	6.8	8.8	13	14	0.2	0.11

Sown field:14/4/99, harvested 21/07/99. Southedge Research Station, Mareeba, 1999. Within columns values followed by a letter in common are not significantly different (P=0.05). Plots 10 x 5m, 10 plants/plot; plant spacing, 1.0m in rows 2.0m between rows.

Table 6.5.4. Reaction of 'Butternut' type selections to inoculation with a combination of PRSV-W and ZYMV.

	13 days			17 days			
Cultivar	**Median score**	Minimum score	Maximum score	**Median score**	Minimum score	Maximum score	**Number of plants**
Sunset QHI*	2.0	0.1	40	10	0.5	40	39
Butternut Large	90	85	95	80	60	95	8
Nigerian	0.1	0	0.5	0	0	0	10
F1 (Nigerian x Butternut)	0.8	0.1	5	20	5	40	8

Carborundum-dusted cotyledons were inoculated PRSV-W and ZYMV on 8/02/2000, 1:10 w/v. Assessed on 13 days (21-02-2000) and 17 days (25-02-2000) after inoculation as the percentage of the area of the youngest expanded leaf (leaf 3, at 13 and leaf 4 at 17 days) which as chlorotic and or deformed. * Sunset was assessed over 4 component selections.

Because most other families in the selected populations also had high levels of resistance virus and to etch and high productivity there is confidence that 'Sunset QHI' will perform well in commercial production.

7.0. DISCUSSION ON DEVELOPING AND RELEASING 'SUNSET QHI' - A VIRUS AND ETCH RESISTANT 'BUTTERNUT' TYPE

'SUNSET QHI' combines high etch resistance, high virus resistance, good fruit quality and high productivity in a 'Butternut' type pumpkin. It is important to note that as with 'Dulong QHI' the virus resistance of 'Sunset QHI' is to at least two of most important viral diseases of cucurbits in tropical and subtropical Australia viz, papaya ringspot virus and zucchini yellow mosaic virus. Preliminary observations suggest that 'Sunset QHI' may also carry Watermelon Mosaic Virus resistance but this has not been formally tested. The resistances to PRSV and ZYMV were incorporated without resorting to 'genetic engineering'. The resistances were incorporated by traditional breeding techniques. This feature should be an important advantage in accessing the developing domestic and international 'organic produce' markets.

The project's objective of releasing a high quality etch and virus-resistant 'Butternut' type pumpkin to eliminate the annual losses of $2m to etch and virus by the development of an etch and virus resistant cultivar was achieved through the production of 'Sunset QHI'. These reduced losses can effectively reduce land, energy and water usage and limit pesticide application. 'Sunset QHI' is protected by Provisional Plant Breeders Rights (application number 2000/021), and is currently in the final testing and commercialisation process with seed companies. It is expected to be commercially available by July 2001.

8.0. TECHNOLOGY TRANSFER

8.1. General

- Sixty sample seed packs (approximately 150 seed each) of 'Dulong QHI' were distributed to growers through individual contact, State agencies, marketing groups and consultants in Queensland, New South Wales and Western Australia.

- Seed of 'Dulong QHI' was supplied to four seed companies for evaluation, pending commercialisation.

- Trials evaluating advanced selections of the Grey pumpkin type were conducted on farms on Atherton Tableland.

- Approximately 50 sample seed packs (approximately 100 seed each) of 'Sunset QHI' will distributed to growers through individual contact, State agencies, marketing groups and consultants in Queensland, New South Wales and Western Australia, when seed becomes available later in 2000.

- Growers, Seed company reps, and consultants inspected trials at Maroochy, Bowen and Southedge Research Station Mareeba approximately annually.

8.2. Specific Publications

- Persley, D. and Herrington, M. (1996). Virus disease in pumpkins - hope for the future. *Queensland Fruit and Vegetable News*, March 7. p12-13.

- 'Towards better Butternut pumpkins' - Press release October 1998 into numerous media outlets eg 'The Gympie Times'.

- 'Disease resistant pumpkins cut losses' - Press release February 1999 into numerous (approximately 32 nation wide) media outlets including ABC Radio, 'Good Fruit and Vegetables', 'Queensland Fruit and Vegetable News', 'Queensland Country Life', 'The Australian' and 'The Courier Mail' and 'The West Australian'.

- Herrington, M., Loader, L., Prytz, S., and Slade, A. (1999). Incorporating etch and virus resistance into Butternut Pumpkin. *In* '11th Australian Plant Breeding Conference Proceedings Vol 2 Adelaide South Australia 19-23 April 1999', ed. P. Langridge, A. Barr, G. Auricht, G. Collins, A. Granger, D. Handford, and J. Paull, p 58-59.

- 'Virus resistant pumpkins could benefit growers and consumers' - Press release December 1999, eg 'Good Fruit and Vegetables' February 2000, 10 (9) p5.

- Herrington, M. E. (1999). 'Dulong QHI'. *Plant Varieties Journal*, 12 (4) p51-53.

9.0. RECOMMENDATIONS

9.1. Recommendations for future R and D

- The resistances in 'Dulong QHI' were fairly readily transferred together, suggesting a possible close linkage. Linkage studies to identify and perhaps sequence the gene/s responsible could provide valuable intellectual property and is recommended.

- The development of a range of virus resistant pumpkin types from these cultivars should be rapidly pursued.

9.2. Recommendations for practical application to industry

- Pumpkin growers should trial these cultivars.

- 'Dulong QHI' and 'Sunset QHI' have resistance to the most important viral diseases of pumpkins in the tropics and subtropics. Because these resistances were incorporated by traditional breeding methods these cultivars offer an important advantage in marketing, especially in the developing international 'organic produce' market. Using these cultivars to pursue this market is recommended.

10.0. LITERATURE CITED

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APPENDIX 1. Project photographs

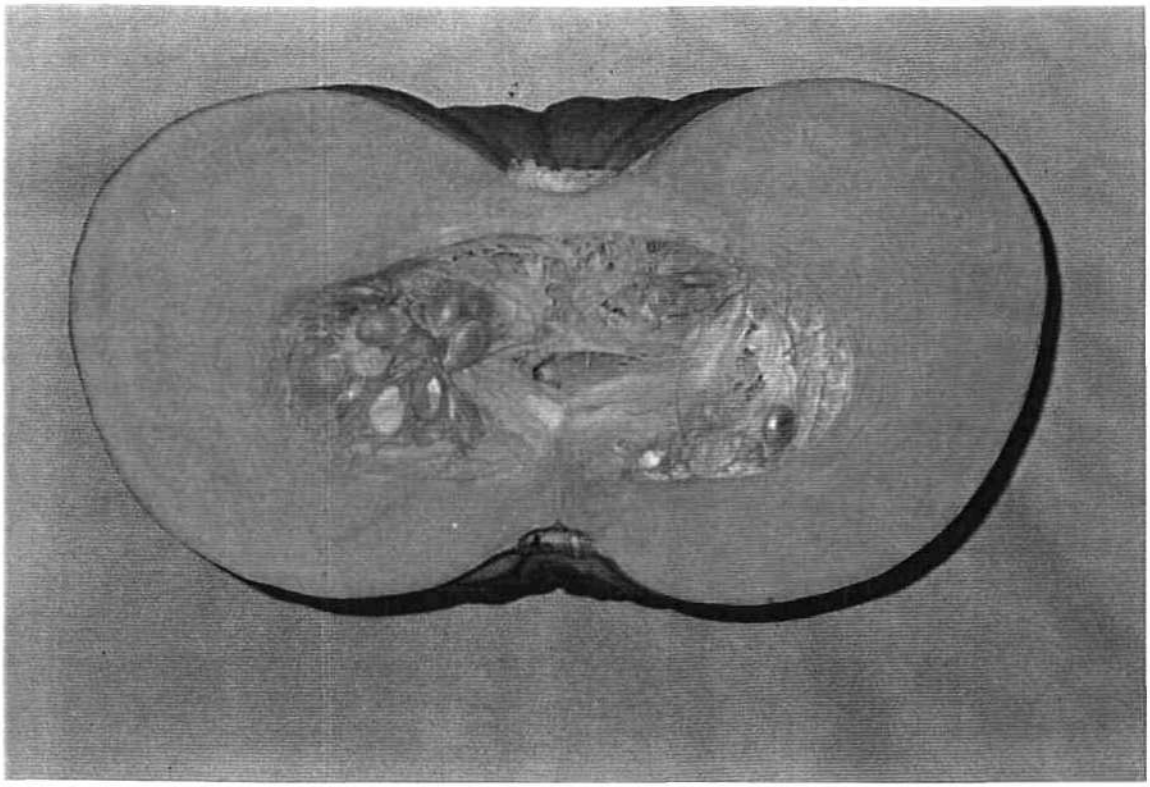

Figure 1. Cut fruit of 'DULONG QHI' - a multiple virus resistant pumpkin developed in the project.

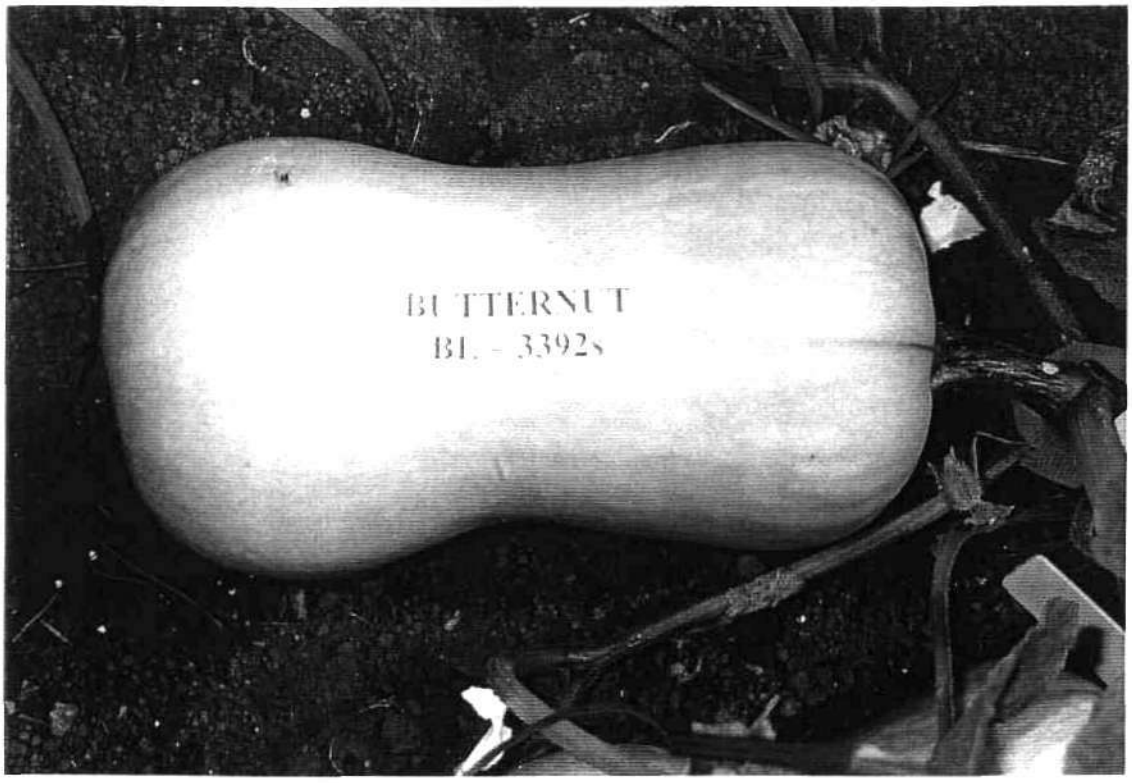

Figure 2. Fruit of 'SUNSET QHI' – a multiple virus and 'etch' tolerant Butternut pumpkin developed in the project.

Figure 3. Dark green knobbly fruit of 'Nigerian' the source of multiple virus resistance used in the Butternut pumpkin breeding.

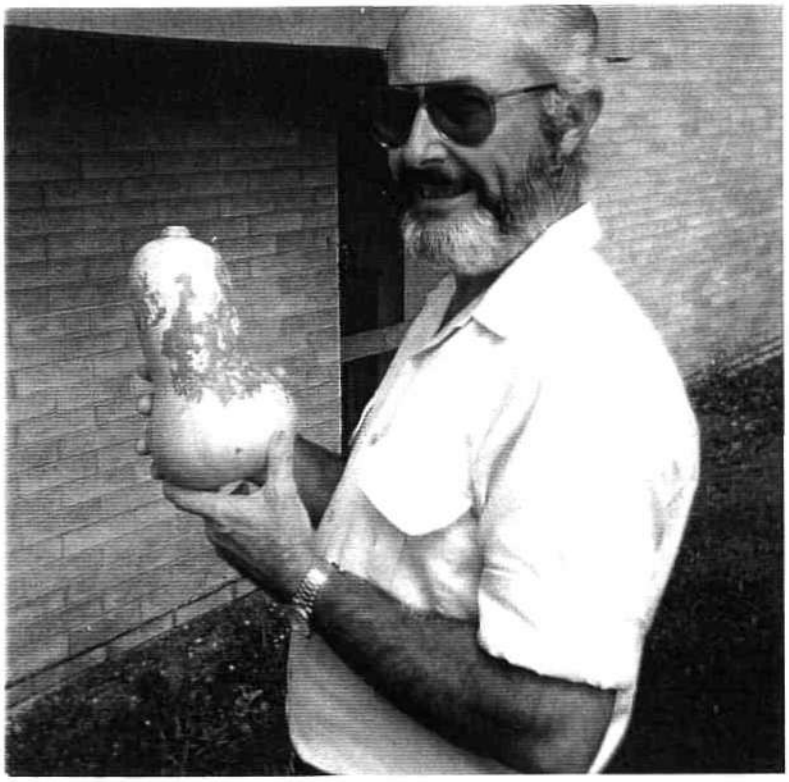

Figure 4. Lester Loader – a member of the project team, holding an 'etch' affected Butternut pumpkin.

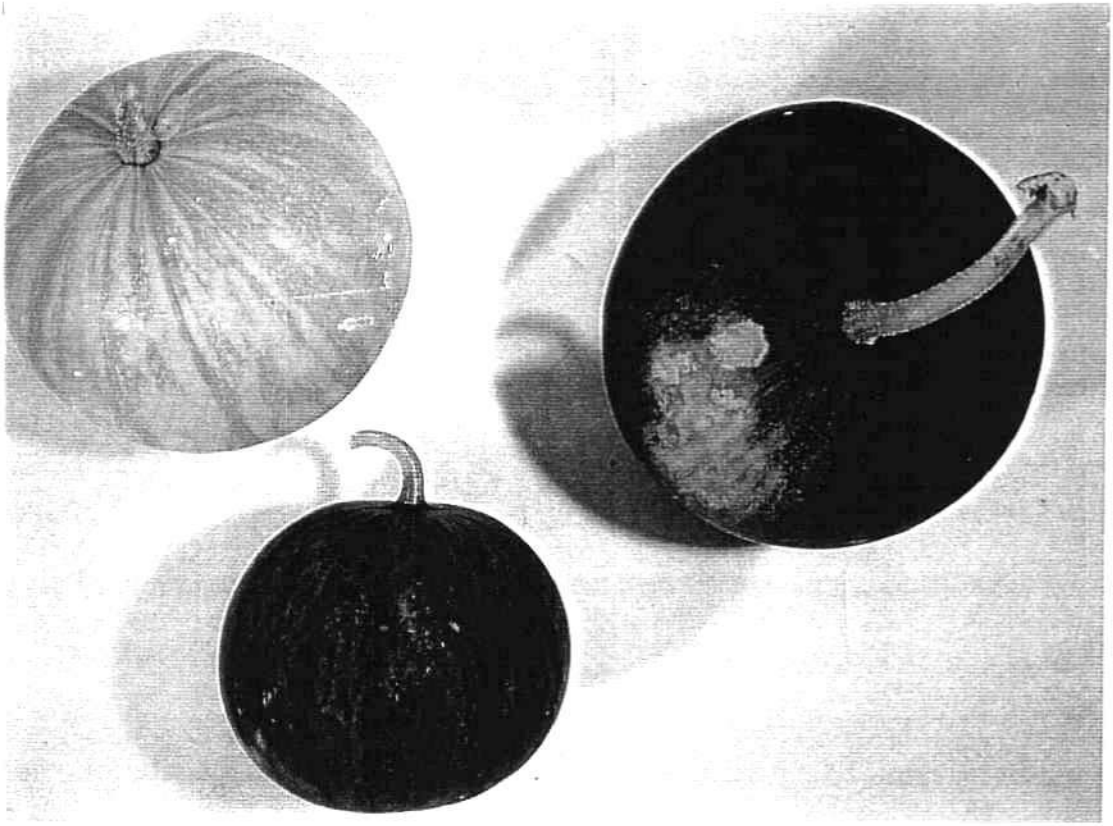

Figure 5. Fruit of *C. ecuadorensis* the original source of virus resistance used in the development of pumpkin variety DULONG QHI.

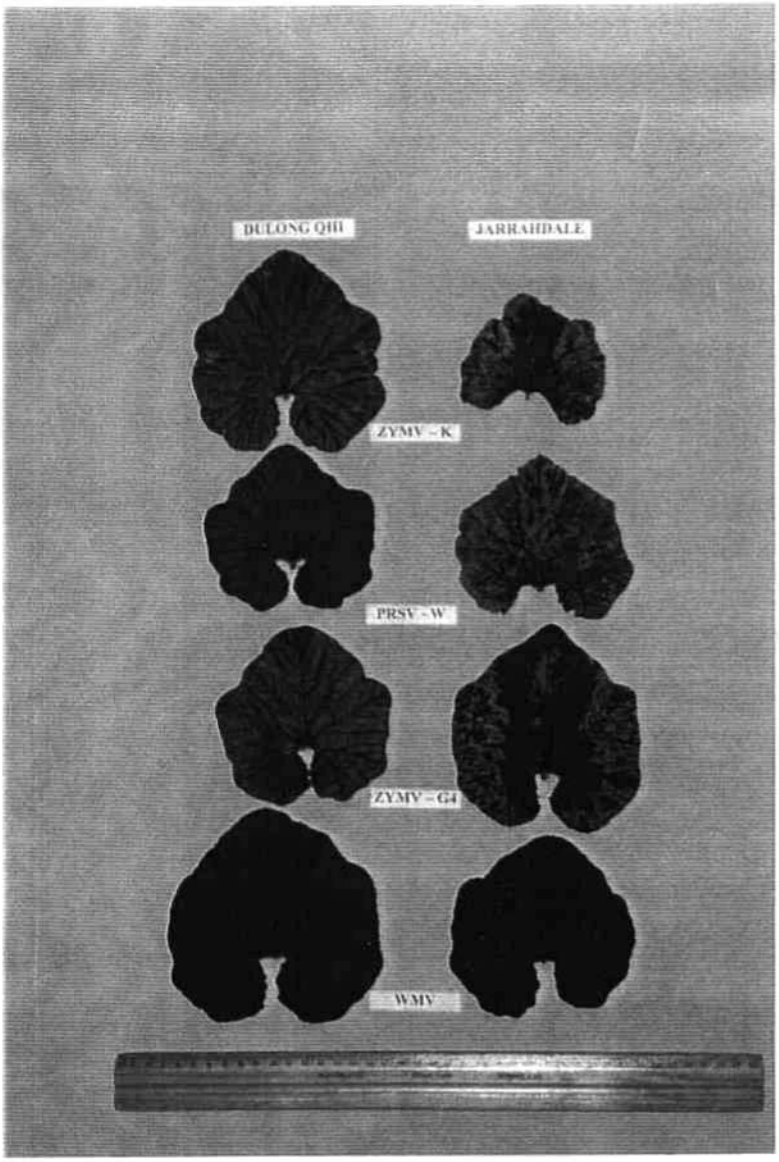

Figure 6. Leaves of the pumpkin varieties DULONG QHI (on left) and Jarrahdale (right) showing resistance to virus in DULONG QHI.