Know-how for Horticulture™

Breeding for sweetness and tenderness in tropical supersweet corn

Ian Martin
Queensland Horticulture Institute

Project Number: VG96006

VG96006

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the Golden Circle Cannery Pty Ltd and Pacific Seeds Pty Ltd.

ISBN 0 7341 0433 2

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

Horticulture Australia

FINAL REPORT

HORTICULTURE AUSTRALIA LTD

Project VG96006

Nominated Completion Date: 30th June , 1999.
Extended Completion Date: 31st December, 2001

Breeding for sweetness and tenderness in tropical supersweet corn.

Ian Martin

Queensland Horticulture Institute
A Unit of A.F.F.S, Queensland Dept of Primary Industries.

PROJECT TEAM
Ian Martin, Terry McCarthy, Hazel Brown, Veronique Boissinot, Denis Persley, Ross Wright, Jeff Barnes (QHI), Allan Peake (Pacific Seeds)

VG96006

Ian Martin
Kairi Research Station
PO Box 27, Kairi
Queensland 4872
Australia
Phone/Fax: 07 40 958 419
Phone: 07 40 958 229
Email: Ian.Martin@dpi.qld.gov.au

Date of Report: 14th March, 2002.

Purpose of Report.

This report is a summary of research conducted over a five year period from 1996 to 2001 aimed at improving sweetness and tenderness in tropical super-sweet corn germplasm using several methods of genetic improvement.

It seeks to examine the feasibility of improving the two traits and to compare the relative effectiveness of the breeding methods.

It also makes recommendations about the breeding approach most likely to achieve desirable standards of sweetness and tenderness that would enhance the attractiveness of sweetcorn in the domestic market and facilitate the development of an export industry to Asian countries.

FUNDING SOURCES

1. Horticulture Australia Ltd

2. Golden Circle Ltd

3. Queensland Department of Primary Industries

4. Pacific Seeds

Disclaimer

Any recommendations contained in this publication do not necessarily represent current Horticulture Australia Ltd policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.

Table of Contents

Media Summary

VG96006: Breeding for sweetness and tenderness in tropical supersweet corn.

- The sweetcorn industry in tropical and subtropical climates in Australia has large potential but has suffered because of a lack of varieties with resistance to the severe disease and insect problems experienced in these areas.

- The release of supersweet hybrid H5 from the Horticulture Australia Ltd- funded breeding program at Kairi Research Station overcame many of the disease problems, particularly Johnson Grass Mosaic virus and Turcicum leaf blight. However H5 lacks tenderness and sweetness when compared with temperate-adapted hybrids.

- Horticulture Australia therefore decided to fund the current three year project, VG96006, with the aim of overcoming these deficiencies by selecting for sweetness and tenderness traits in tropical supersweet germplasm.

- Success in the project would enable an expansion of sweetcorn production in the tropical growing regions of Australia to service domestic and export markets. H5 is already being exported to Japan in canned form but much improved tenderness and to a lesser extent sweetness is needed to access the large volume markets in Japan.

- A successful outcome to the project would also improve the presentation of fresh sweetcorn in supermarkets during the lengthy periods when only tropical sweetcorn can be grown for reasons of disease pressure (JGMV) or climatic limitations (heat stress in summer months). Temperate hybrids cannot cope with these stresses.

- The project has demonstrated that it is possible to make good progress in selecting for sweetness and tenderness in tropical sweetcorn. For example sweetness rating has increased from a rating of 5.8 in H5 to 6.5 in H349, a hybrid produced during the project. In like manner tenderness has increased from 4.7 in H5 to 6.6 in H349, (rating scale is 1=poor, 9=excellent). These increases reflect a change from sub-standard to very good eating quality.

- The supersweet mutant gene *brittle1* was evaluated as an alternative to the standard mutant *shrunken 2*. We found that *brittle1* produced hybrids with less sweetness than those with the existing gene *shrunken2*. Tenderness was similar in the two groups of hybrids

- Backcrossing techniques were used to infuse temperate germplasm into the two tropical gene pools SHP1and SHP2 in an effort to increase sweetness and tenderness. This resulted in paired gene pools SHP11 and SHP12 (75% temperate), SHP15and SHP16 (50% temperate).

- The best hybrid from the 75% temperate pools (viz, H741) produced sweetness and tenderness comparable with Goldensweet, a popular commercial hybrid but lacked the kernel depth of Goldensweet. Indications are encouraging for the next generation of hybrids to have acceptable sweetness and tenderness but combined with greater disease and insect resistance and heat tolerance.

- Although several new tropical hybrids are promising, especially H206 and H349, we have not yet selected one for commercial release. H349 has excellent sweetness and tenderness and reasonable flag leaf but cob appearance is marginal.

- Combinations of tropical inbreds crossed with temperate inbreds to produce F1 hybrids has not yet proved to be a successful strategy. Tenderness was found to be lacking in the initial hybrids but this is likely to have been a reflection of the particular inbreds used. When superior inbreds are developed and crossed in the right combination successful hybrids may result. However this approach can lead to problems in seed production caused by wide divergence in flowering dates and disease in the temperate inbreds.

- Future research should concentrate on selecting for sweetness and tenderness in the two new sets of "heterotic pair" populations (SHP11/12, SHP15 /16). The former pair is more suited to the fresh market industry and the latter could serve either the fresh market or the processing (canning) industry. In addition to creating these populations we are initiating two gene pools directed more closely at the processing market. The requirements for this application include high yield and kernel recovery as well as high sweetness and tenderness.

- The breeding method recommended is recurrent selection of So plants using the "beaver breeding" technique for evaluation of sweetness and tenderness with tandem selection for yield and disease resistance on the derived S1 families. Using this method we can complete one cycle per year. Given that tenderness is highly heritable and under the control of relatively few genes, progress should be rapid. Elite inbreds can then be siphoned off to create new hybrids (see Appendix I).

Technology Transfer

1. Project Team Meetings

The project team members (listed on the cover page of this report) have met each year (sometimes twice a year) to view trial plots at Gatton Research Farm, present progress reports for each region and sub project, discuss technical aspects of results and plan future research. Reports and discussions have been compiled and circulated to team members.

2. Contributions to QFVG Annual Report

Regular contributions to the Queensland Fruit and Vegetable Growers Annual Technical Report have been made, describing progress of the project and significant findings.

3. Fresh market grower consultation

We have consulted with a major fresh market sweetcorn grower in the Lockyer Valley. He has inspected variety trials to convey the traits he considers are important in the fresh market trade. Pacific Seeds personnel have held field days for growers to display experimental hybrids and they talk regularly with growers.

4. Consultation with processors

Pacific Seeds breeder Allan Peake has participated in discussions with Golden Circle Cannery technical representatives and management to determine what they want in new improved hybrids. I have visited Golden Circle Ltd cannery during a visit from a major Japanese importer to become aware of the requirements for this lucrative export market.

5. Promis (Project management information system)

Regular milestone and progress reports have been uploaded into this computer-based system. Reports are available to scientists throughout Australia.

6. Horticulture Australia Ltd Annual Report

The project was selected for presentation in a HAL Annual Report. This article included a comment from a major grower on output of the project to date.

7. QFVG Meeting

I have attended a meeting of QFVG to present aspects of a proposed new project and to review progress of this current one.

Technical Summary

HRDC PROJECT VG96006: Breeding for sweetness and tenderness in tropical supersweet corn

I. Problem definition, reasons for undertaking research, objectives and strategies.

There is great potential to grow supersweet corn in the tropical areas of Australia. The sweetcorn breeding program at Kairi Research Station released the first successful tropical supersweet hybrid, H5 in the early 1990 s. It has been particularly popular in tropical and subtropical regions because of its resistance to Johnson Grass Mosaic virus and Turcicum leaf blight (Exserohilum turcicum). It also has good resistance to infestation by Heliothis ear worm (Helicoverpa armigera). However, H5, while ideal for the processing industry (canning) is considered to have limitations for the fresh cob market. It consistently rates as one of the toughest hybrids in "bite test" evaluations; it sometimes has poor tip fill and it has very pale husk leaf colour which causes consumers in supermarkets to perceive it to be less fresh than it actually is. Taste is thought be reasonable but there is considerable room for improvement.

This study aimed to improve sweetness and tenderness in tropical germplasm with the ultimate aim of releasing a commercial hybrid with superior eating quality to H5. Although sweetness and tenderness were nominated as the primary aims, improvement of visual characteristics such as husk leaf colour, cob shape and flag leaf are thought to be indispensable requirements for the fresh market. Five breeding methods were tested for their efficacy in improving sweetness and tenderness. They were,

1. Selection for sweetness and tenderness within existing tropical supersweet populations SHP1 and SHP2.

2. Use of supersweet mutant gene **brittle1 (bt1***)* as an alternative to the standard supersweet gene **shrunken2 (sh2).**

3. Direct crossing of tropical supersweet inbreds with temperate supersweet inbreds to produce commercial F1 hybrids combining disease resistance from the former and quality from the latter.

4. Introgression of temperate germplasm into the tropical populations SHP1 and SHP2 to create 50% temperate heterotic- paired populations (SHP15 and SHP16)and 75% temperate populations (SHP11 and SHP12) by crossing and backcrossing to temperate germplasm

5. Recurrent selection of S0 plants for sweetness and tenderness within the populations described above using the "beaver breeding" technique in which a portion of the cob is evaluated for sweetness and tenderness and the remainder left on the plant to produce mature seed for subsequent use of the progeny.

II. Findings

- **Selection within existing tropical supersweet populations** for sweetness and tenderness established that good progress was possible.As measured by taste panels, gains of 12% for sweetness and 40% for tenderness were recorded when hybrid H349 (isolated from cycles 3 and 4) was compared with H5 from cycle 0.

- The ***brittle1*** gene was found to have a sweetness rating 12.5% lower on average when a group of brittle hybrids was compared with a group of *shrunken2* hybrids. This result confirms other research with the bt1 gene. Tenderness was similar in both groups. However cob size, tip fill and cob shape were all enhanced in the brittle group

- **Combination of tropical and temperate inbreds to generate F1 hybrids** has produced hybrids satisfactory in all respects except for tenderness. We suspect the common tropical parent SHB17 is responsible for much of this toughness. If so, the use of improved tropical inbred parents in the next cycle could overcome this problem. However this method has the potential to reduce the effectiveness of favourable genetic factors in the final hybrid and to create problems in seed production.

- **Introgression of temperate germplasm into tropical populations** at varying levels led to hybrids with markedly better sweetness and tenderness than benchmark tropical hybrids but there were negative side effects in reduction of disease resistance, yield and insect resistance.

- **Recurrent selection on S0 plants** in the modified tropical breeding populations (some temperate germplasm) identified some elite individual plants for sweetness and tenderness. This method appears to offer the greatest promise of attaining the levels of eating quality currently present in the best temperate-adapted varieties.

III. Recommendations

- As a result of this work we feel that future work towards improving sweetness and tenderness should be focussed on improvement of ***shrunken 2*** genotypes rather than ***brittle 1*** types.

- **Selection within the newly-constructed populations** (SHP15 and 16) will probably afford us the best approach to combining disease resistance with sweetness and tenderness.

- **Selection in pure tropical populations** will not provide adequate visual characteristics (eg flag leaf) for the fresh market requirements but could be effective for the processing (canning) market because of the superior yield and kernel recovery attributes of the tropical populations.

- **Recurrent selection using S0 plants** to assess sweetness and tenderness and the derived S1 families to assess disease resistance and yield is likely to be the most effective and rapid breeding method. Tenderness (or at least pericarp thinness) is apparently controlled in partial dominant fashion and most disease resistances are simply inherited in dominant or at least additive function. Inheritance of sweetness is less well defined in the literature. As well as the major gene *sh2,* there are almost certainly a number of modifier genes involved. We are confident that the proposed method will effectively select for the trait.

1. Introduction

The potential for production of supersweet corn in subtropical and tropical regions of Australia is large. It is an industry that has considerable economic implications for Australia from both domestic and export aspects. Currently the sweetcorn industry in Australia is valued at $30 million annually at the farm gate. At retail level it is estimated to be about 4 times that amount. Yet Australians are not yet consuming sweetcorn in anything like the quantity that Americans do. When the quality of product displayed in supermarkets is improved from its present moderate level we can expect higher consumption to occur. Part of the reason for substandard quality in supermarkets is inadequate storage conditions but it is also true that some of our current commercial hybrids are deficient in eating traits, particularly sweetness and tenderness. Hence the purpose of this project.

In Queensland sweetcorn is produced in 3 main districts, one in the subtropics (Lockyer/Darling Downs) and two in the tropics (Bowen and Ayr). Production takes place in the period from August to May in the former and from June to September at Bowen and Ayr.

Prior to the release of the tropical hybrid H5, production in the Lockyer was seriously limited by the virus disease Johnson Grass Mosaic (JGM). H5, with its single dominant gene resistance to JGM, developed with the assistance of Horticulture Australia funding, enabled growers to extend the growing season by as much as 3 months. Using susceptible hybrids planting after December is not recommended as JGM increases in severity as the season progresses. The introduction of H5 means that growers can plant into March with success. H5 also possesses superior resistance to Turcicum leaf blight and better tolerance of heat stress than the temperate hybrids it replaced.

Unfortunately H5 and the tropical supersweet gene pools it was developed from tend to have tough pericarp and bland flavour. Thus while the market has been prepared to accept H5 at times of the year when temperate hybrids were not available (notably in the period from May to July), the markets have been reluctant to accept tropical hybrids at other times.

This project therefore had as its objective selection to improve the sweetness and tenderness of hybrids developed from the two broad genetic base tropical supersweet populations Supersweet Population 1(SHP 1) and Supersweet Population 2 (SHP2). Secondary, though important considerations were the improvement of husk leaf colour, cob shape and disease resistance. The breeding manipulations used to modify the original populations are discussed in Methods.

1.1 Tenderness

Tenderness in supersweet corn is closely correlated with the thickness of the pericarp layer. Ito and Brewbaker (1981) found a positive correlation of 0.98 between ratings established by "bite test" and pericarp thickness. They were able to achieve a reduction in bite test scores of 3.9% per year with selection based on bite test evaluation and using a mass selection method. They pointed out that the aleurone layer also made a contribution to perceived tenderness through its influence on kernel texture. The rate of gain convinced them that pericarp thickness is highly inherited and that any technique that measured it (even indirectly) would result in useful progress. In a later study (1991) they estimated heritability to be 55.2% and the number of genes involved to be in the range of 1.4 to 5.9, depending on the genotypes used. Helm and Zuber (1972) found pericarp thickness to be quantitatively inherited with thin pericarp dominant in one set of crosses but not in a second series. Heritability was high (>80%). Ho et al (1975), also found thinness dominant and heritability high (72%). A recent study by Wang and Brewbaker (2001) identified three quantitative trait loci (QTL) on chromosomes 1,2 and 6 that accounted for 37.3% of the measured variation in pericarp thickness and suggested that other QTL would be found with investigation of other recombinant inbred lines (RIL).

The implications of these findings for this project are that selection for thin and therefore tender pericarp by use of the bite test should be effective and that if inbreds with thin pericarp can be identified the trait should be dominant in hybrids containing them.

A possible explanation for the relatively tough pericarp encountered in our tropical supersweet breeding populations and hybrids is that the selection pressure exerted for good germination during the early phases of the program may have indirectly selected for thicker pericarp and thus the current lack of tenderness. (Genotypes with thicker pericarps have been found to have superior retention of metabolites within the kernel during the seed germination process and therefore to offer less support to soil borne fungi that infect seed and hence germination).

The consensus is that selection for tenderness based on the bite test does produce good genetic progress provided the assessments are made on a basis of standard cob maturity (that is harvest maturity determined by a constant number of days after mid silking). The number of days from silking to maturity depends on the temperatures experienced at the time of testing. Higher temperatures accelerate the progress to harvest maturity.

In breeding programs it is quite feasible to evaluate a portion of a self-pollinated cob using the bite test at the appropriate time after fertilisation and retain the remainder of the cob on the plant for generation advance if the rating is satisfactory. This accelerated breeding technique is colloquially referred to as "beaver breeding". We have used this technique in developing the basic populations for this study.

1.2 Sweetness

The ultimate objective was to release a commercial hybrid that would possess the ability of H5 to be grown under disease and heat stressed conditions but have an eating quality that approached that of the temperate supersweet hybrids. Although there are exceptions, most people seem to prefer a very sweet flavour in sweetcorn.

This sweetness is provided mainly by the sucrose content. The highest sucrose content for a single mutant gene is provided by the *shrunken 2 (sh2)* gene. It generates a sucrose content as high as 35% compared with 15% to 20% from the *sugary1* mutant used in ordinary sweetcorn (Creech, 1968). Higher sucrose contents can be generated by using combinations of sh2 with other mutant genes **such as the su1 gene** but this complicates the breeding process and can also impair germination percentages because of the reduction in starch content (Kaukis and Davis, 1986).

We have chosen to work mainly with the *sh2* mutant in this project although we did investigate the *brittle 1*(bt1) mutant as an alternative. Even amongst sh2 genotypes there are large differences in sweetness and flavour suggesting the influence of modifying genes. Our selection in this project is on these modifiers. We have also sought to select genotypes that maintain a high level of sweetness for an extended time after fertilisation. This characteristic has the virtue of permitting delays in harvesting with no appreciable reduction in sweetness . (The sh2 gene also inherently confers longer shelf life than most other mutants). By conducting the taste evaluations at 28 to 30 days after flowering (compared with the usual 25 days) we have sought to find genotypes with enhanced retention of sweetness in the field.

A distinction should be drawn between sweetness and taste. Sweetness is an important component of taste but taste also includes other flavours that can vary greatly. Some of them, such as the "beany" and "grassy" flavours are considered undesirable. Taste can also be taken to include texture. Texture can be watery, gritty or creamy, the latter being favoured. Creamy texture in sweetcorn is thought to be associated with a high level of water soluble polysaccharides. These compounds tend to be lacking in shrunken 2 genotypes.

A successful outcome in terms of the objectives described above would provide consumers in the major cities with a much better quality fresh sweetcorn product for a significant period of the year (May to July). It would also give the industry the potential to mount a large processing industry in tropical areas with year round growing capability and the potential to export to Asian markets.

1.3 Other important traits monitored during the course of the project.

1.3.1 Disease and insect resistance

Traits such as disease resistance and resistance to Heliothis ear worm are clearly important to growers and were therefore monitored in the project trial. Resistance to Heliothis is much better in tropical supersweet hybrids. Tropical germplasm has much heavier husk cover than temperate material. Brewbaker and Kim (1978) found that damage due to Heliothis was negatively correlated (r= -0.40) with numbers of husk leaves. Tightness of husk leaves, length of husk extension and tightness of husk closure are other factors contributing to Heliothis resistance.

1.3.2 Visual traits: Cob shape, flag leaf, kernel colour, tip fill.

These traits are important in the fresh market industry.

Although data was recorded for these traits we have not reported in detail on these factors because they were outside the main subject of the project.

2. Materials and Methods.

The project covers the work undertaken by two breeding programs, one at Kairi on the Atherton Tableland in north Queensland and a second at Gatton in the Lockyer Valley in south-east Queensland.

2.1 Selection for sweetness and tenderness in existing tropical *shrunken 2* germplasm

Numerous inbreds were available to the project from previous inbreeding in the original tropical populations SHP1 and SHP2. These are populations that utilize the *shrunken 2* gene, the most commonly-used super-sweet mutant in commercial sweetcorn. It produces a high level of sucrose, thus imparting an extremely sweet flavour. During the spring of 1996 sixty-five single-cross hybrids were generated by combining inbreds from SHP1 with inbreds from the counterpart SHP2. These were evaluated at Kairi Research Station in the autumn of 1997 with particular emphasis on quality characteristics. Elite hybrids from this initial evaluation were then tested at Bowen in 1998 and at Gatton on the research farm of Pacific Seeds Ltd. In the spring of 1998 a further 130 hybrids were generated with a later group of inbreds and tested these in the subsequent autumn. Once again the superior quality hybrids were re-tested at Bowen and Gatton of 1999.

2.2 Use of brittle 1 gene

Although the *shrunken 2 (sh2)* mutant is the most widely used super-sweet gene, the gene *brittle 1 (bt1)* is used commercially in Hawaii and Thailand. The *sh2* gene confers a sweeter taste than *bt1* because it produces a higher percentage of sucrose. However some consumers prefer the less sweet taste of *bt1* which also imparts a more "corn-like" flavour to its cultivars.

We had developed a series of inbreds containing the *bt1* gene and also possessed a group of inbreds from Hawaii with the gene. In the spring of 1997, forty-nine hybrids were generated. These were all produced from our own brittle inbreds (not Hawaiian) and were evaluated for sweetness and tenderness in the following year. Subsequently, in 1999, we tested a new group of 119 *bt1* hybrids at Kairi. These hybrids utilised inbreds from local and Hawaiian sourced. Promising hybrids from both these groups were further evaluated at Gatton.

2.3 Direct crossing of temperate and tropical inbreds to produce F1 cultivars

The breeding work at Pacific Seeds, Gatton, applied a different approach to put sweetness and tenderness into tropical supersweet cultivars. In this work elite tropical inbreds with the single dominant gene for Johnson Grass Mosaic disease, were crossed with temperate inbreds to generate F1 hybrids with disease resistance and hopefully with improved sweetness and tenderness. Inbreds were then developed from these F1 families and then crossed with a tropical inbred to generate hybrids for test.

2.4 Introgression of temperate germplasm to obtain sweetness and tenderness
Starting from the assumption that U.S.- bred temperate germplasm was the best source of quality traits, the decision was taken to develop populations with 75% temperate and 50% temperate and 25% genetic constitution.

This was achieved by taking the two tropical populations SHP 1 and SHP 2 (both using the shrunken 2 gene) and crossing them to temperate germplasm selected for sweetness and tenderness. This generated 2 new populations SHP 15 and SHP16 respectively (with 50% temperate:50% tropical genetic constitution). Subsequently these populations were back-crossed to their respective tropical counterparts to produce a further 2 populations with 75% tropical genetic constitution. These populations were designated SHP 13 and SHP 14. Populations 15 and 16 were also backcrossed to the respective temperate germplasm to produce populations SHP11 and SHP12 with 75%temperate germplasm.

These procedures were intended to achieve the combination of eating quality traits from the temperate hybrids with insect and disease resistance from the two tropical germplasm sources. They also provided sets of "heterotic-pair" populations with different dosages of the temperate germplasm (namely 25%, 50% and 75%) Results are presented to indicate the progress from application of the recurrent selection to populations SHP11/12 and SHP15/16 as measured by hybrids derived from them.

2.5 Recurrent selection for sweetness and tenderness based on S0 plant evaluation.
Having set up these genetically heterogeneous gene pools we then subjected them to recurrent selection for improvement of the target traits. It was decided to use selection based on S0 plants for sweetness and tenderness and to test the subsequent S1 lines for disease resistance. Evaluation of sweetness and tenderness of S0 plants used a technique known as "beaver breeding" that uses half the cob for evaluation and leaves the remainder for seed.

2.6 Assessment of sweetness and tenderness
Tenderness can be assessed by objective means such as use of a penetrometer or by use of a micrometer to measure the pericarp thickness. Both of these methods are somewhat time-consuming. Ito and Brewbaker (1981) found a high correlation (r=0.98) between pericarp micrometry and evaluation of tenderness by the "bite test" on a genotype comparison basis although the correlation for individual ears was low (r=0.24).

Since our interest was in evaluating numerous genotypes we elected to use the bite test method as the penetrometer method would have been too time consuming. In similar fashion we decided to evaluate sweetness by using a taste panel rather than and objective method such as measurement of sucrose. Also since taste has other determinants than sucrose it was considered to be a more appropriate method.

In all evaluations a panel of 3 assessors was used and each tasted 3 cobs per plot. Rating used was based on a 1 to 9 system where 1was least favourable for the trait in question and 9 most favourable. The mid-silk date of each plot was recorded and at the given time after that date (+28 days) we evaluated the cobs and recorded the data.

3. Results

3.1 Improvement of existing tropical germplasm through selection of inbred parents and superior combinations of inbreds.

3.1.1 Sweetness.

The data in Table1 illustrate the degree of improvement for sweetness that has been achieved through selection of inbreds with superior sweetness and the combination of these inbreds to produce hybrids. The hybrid H141, in 5 trials at the 3 trial sites has proved to be markedly sweeter (rating=6.66) than the existing commercial hybrid H5. H217 and H191 have also produced superior mean values for sweetness. Coefficient of variation for ratings amongst panel members were calculated for Bowen, 1998 and Kairi, 1999. They were reassuringly low suggesting good agreement amongst the panel members.

Table 1. Kernel sweetness (taste) of four super-sweet corn hybrids tested in five trials at three locations in three seasons (Rating scale: 1=bland , 9= sweet)

HYBRID	KAIRI 1997	GATTON 1998	BOWEN 1998	KAIRI 1999	GATTON 1999	**MEAN**
H141	**6.1**	**8**	**5.4**	**5.8**	**8**	**6.66**
H217	**6.1**	**6**	**NT**	**5.9**	**8**	**6.5**
H191	**5.6**	**8**	**6**	**6**	**6**	**6.32**
H5	**5.1**	**6**	**4.7**	**5.4**	**8**	**5.84**
LSD(0.05)	**1.1**		**0.9**	**0.9**		
C.V (%)	**9.5**		**8.4**	**8.5**		

NT = NOT TESTED

3.1.2 Tenderness

One of the main reasons for commencing this project was the extremely tough pericarp of tropical hybrid H5. Table 2 demonstrates this conclusively. A tenderness rating of 6 or better is desirable for a commercial sweet corn; H5 (mean=4.8) is well below the requirement. H141 was significantly (0.01) more tender than H5 in the trial at Bowen in 1998 and 25% more tender overall. On the two occasions that they were calculated coefficients of variation for ratings within the panel were low, suggesting a reasonable degree of agreement amongst panel members.

Table 2. Kernel tenderness ratings for four supersweet hybrids in five trials at three locations in three seasons. (Rating scale:1=tough, 9=tender)

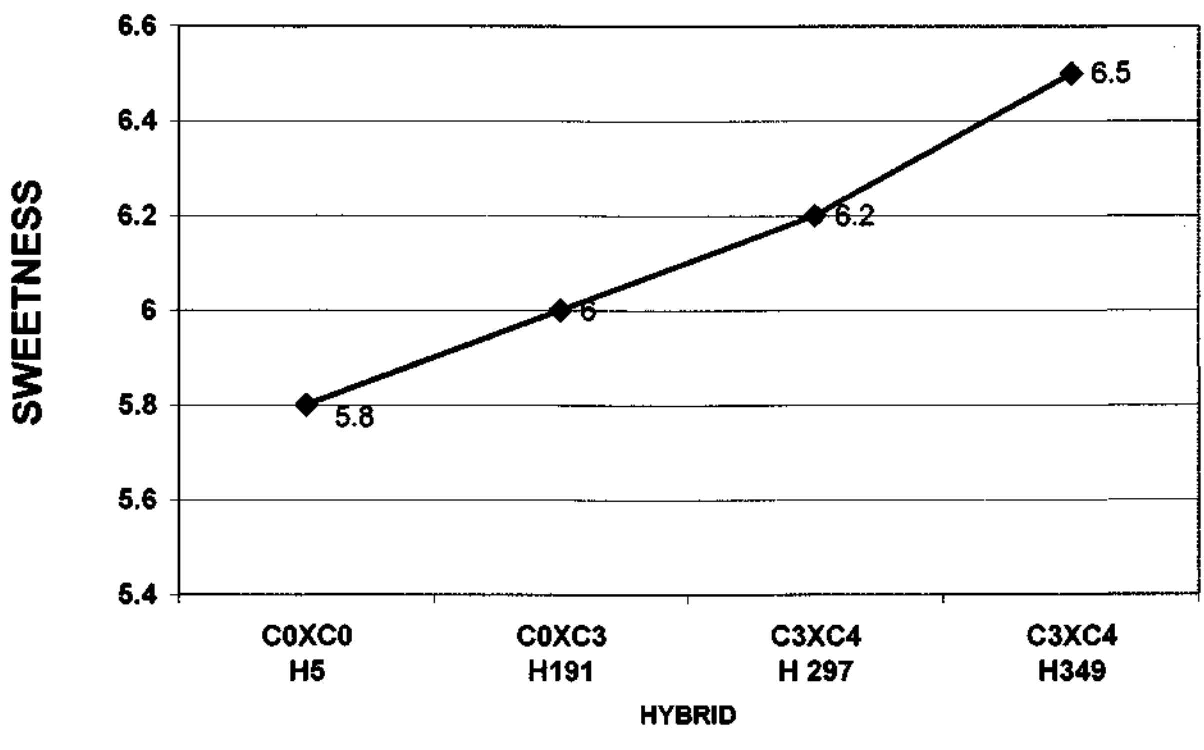
HYBRID	KAIRI 1997	GATTON 1997	BOWEN 1998	KAIRI 1999	GATTON 1999	MEAN
H141	**5**	**7**	**6.5**	**5.4**	**8**	**6.4**
H217	**5.8**	**3.6**	**NT**	**5.9**	**6**	**5.3**
H191	**5**	**7**	**4.3**	**4.4**	**6**	**5.3**
H5	**4.7**	**5.4**	**5.3**	**4.7**	**4**	**4.8**
LSD(0.01)	**1**		**0.9**	**1.3**		
C.V. (%)	**9.9**		**5.7**	**9.8**		

NT= NOT TESTED

3.1.3 Improvement in sweetness resulting from recurrent selection based on hybrid performance.

Figure 1 illustrates the improvement in sweetness ratings we have achieved through selection of superior inbreds over time. The hybrid H5 is a combination of two inbreds selected from the original (C0) selection cycle of the Populations SHP1 and SHP2. Hybrids H191, H297 and H349 are succeeding hybrids utilising inbreds from later cycles of the same two populations. For example H349 employs a Cycle 3 inbred from SHP1 and a Cycle 4 inbred from SHP2. All ratings presented were made in the same field trial.

Figure 1. Sweetness improvement in supersweet hybrids over four cycles of selection (Kairi,1999)



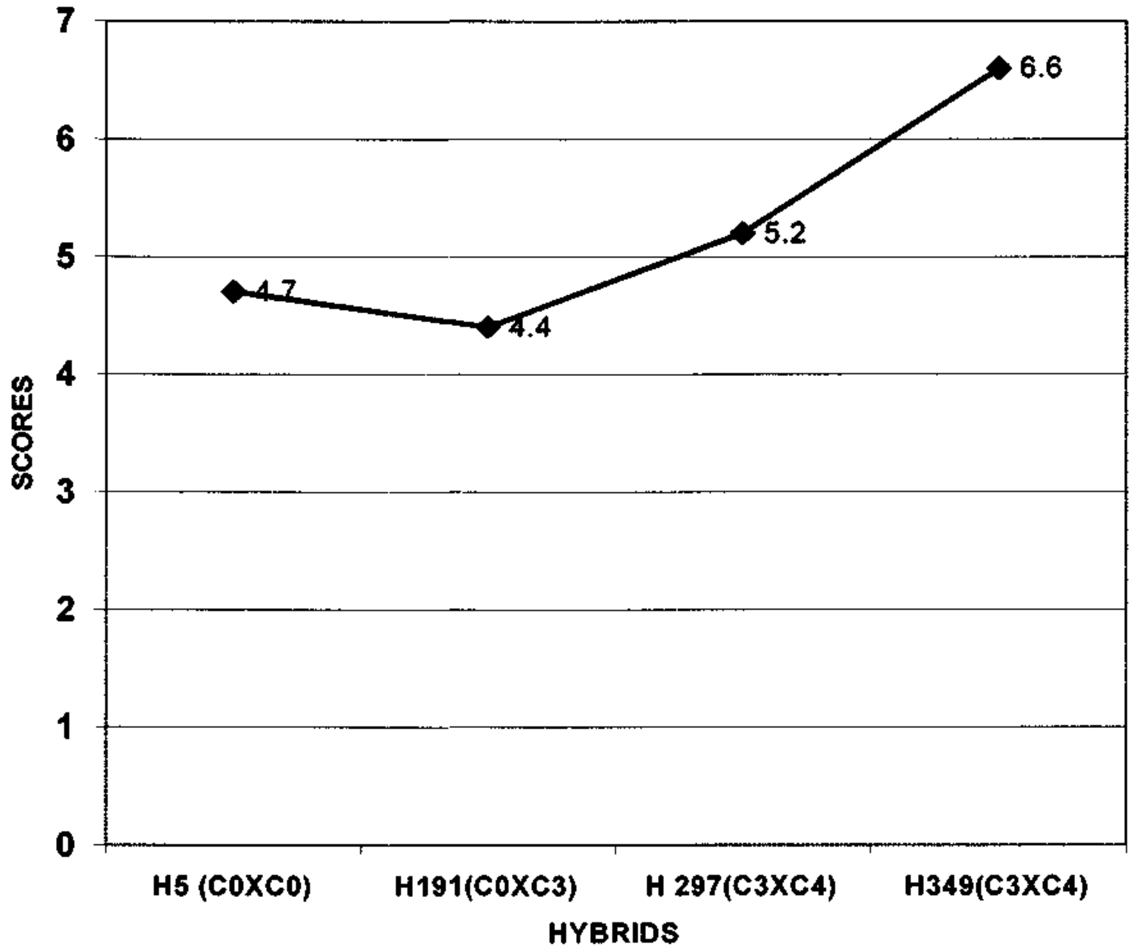
Improvement in sweetness rating from 5.8 to 6.5 gives an indication that genetic modification of sweetness is achievable in the existing tropical supersweet populations without evaluation for sweetness in the progenies during the inbreeding process. The improvement shown here has come about through observation and selection of inbreds based on their performance in hybrids rather than their sweetness and tenderness per se. Adoption of a systematic program of selection of individual plants, as discussed later, should achieve greater genetic gains.

3.1.4 Improvement in tenderness in tropical supersweet populations from recurrent selection based on hybrid performance.

Figure 2 presents data for hybrids constructed from inbreds from four cycles of selection in the supersweet populations SHP1 and SHP2. Hybrid H349, utilising inbreds from Cycle3 of SHP1 and Cycle4 of SHP2 had markedly more tender kernels (rating = 6.6) than hybrid H5 (rating = 4.7) that utilised inbreds from the initial cycles.

Gains of this magnitude lead one to suspect that minor genes must play a substantial role in modifying the effect of the major gene (sh2). It is encouraging that this degree of gain can be obtained without resorting to an intensive program of taste testing.

Figure 2. Tenderness scores for supersweet hybrids from four selection cycles (Kairi, 1999)



3.2 Use of the brittle 1 gene to improve sweetness and tenderness

The mutant supersweet gene *brittle 1* has been extensively used in some tropical areas, most notably Thailand and the state of Hawaii. Brewbaker in Hawaii has reported advantages for brittle hybrids for germination and shelf life of seeds. Taste panels have preferred the "corny" flavour of brittle to what some perceive as the excessive sweetness of *shrunken 2* cultivars. The literature had no insight to provide on the relative merits of the two genes with regard to pericarp tenderness. Given these facts it was judged useful to investigate whether the brittle1 gene could provide a path to superior sweetness or tenderness in tropical sweetcorn. One of the operational problems we faced was that it is difficult to compare the 2 mutant groups in one trial because intercrossing of the genes has the effect of mutual negation of the supersweet trait since both genes are recessive. This can be overcome by hand pollination of each plant that is assessed, but this consumes much time and effort. The second method is to plant two trials adjacently in a paddock and isolate them with buffer areas. We used this spatial isolation method in some trials but also tested brittles in discrete trials. The ideal way to compare the two mutants would have been with isogenic hybrids differing only in the mutant genes. Unfortunately we didn't have a set of isogenic inbreds to facilitate such a comparison. We therefore compared a group of *shrunken-2* hybrids with a group of hybrids using the *brittle-1* mutant.

Table 3. Comparison of brittle 1 mutant hybrids with shrunken 2 hybrids at Gatton in 1998

HYBRID	Mutant Gene	Sweetness	Tenderness	Cob Weight (gm)	Tip Fill	Cob Shape	Tip Cover
BT52	Brittle	6	8	248	6	6	4
BT76	Brittle	4	4	282	6	8	10
BT80	Brittle	6	4	292	8	6	2
BT81	Brittle	4	4	279	8	6	4
BT90	Brittle	6	8	258	8	8	6
BT91	Brittle	6	6	249	6	6	6
BT92	Brittle	6	6	250	6	6	4
BT106	Brittle	6	6	245	6	6	4
BT123	Brittle	6	8	245	4	6	6
Mean		**5.6**	**5.8**	**262**	**6.5**	**6.4**	**5.3**
H245	Shrunken	8	6	254	6	6	6
H284	Shrunken	6	6	251	6	6	6
H297	Shrunken	4	4	248	4	6	6
H141	Shrunken	8	8	207	6	6	6
H5	Shrunken	6	6	244	6	6	8
Mean		**6.4**	**6**	**240.8**	**5.6**	**6**	**6.4**

3.3. Direct crossing of tropical and temperate supersweet inbreds to produce commercial F1 hybrids as a means of improving sweetness and tenderness.

The thrust of Pacific Seeds Ltd breeding has been to cross elite tropical inbreds to a wide range of temperate inbreds, purportedly with quality eating traits. These hybrids are referred to as **intermediate** in recognition of their constitution of 50% temperate and 50% tropical germplasm. Later approaches have used a tropical inbred as one parent and an intermediate inbred (50% temperate:50% tropical) as the other

In most instances disease resistance genes are dominant or partially dominant in their action. Thus one would expect the resistance of the tropical inbreds to be strongly expressed in the F1 hybrids. This is certainly the case with the single dominant gene for resistance to Johnson Grass Mosaic virus. On the other hand there may be a penalty to pay for this strategy in that some of the desirable quality traits of the temperate parent seem to be diminished in the final hybrid. Many hundreds of hybrids have been screened in this approach.

Table 4 presents results from a trial at Gatton comparing a group of tropical hybrids with a group of intermediate hybrids. When the means for two groups are compared it is clear that introgression of temperate germplasm has not produced superior sweetness and that tenderness is in fact inferior in the intermediate group. However use of temperate germplasm has produced superior flag leaf , tip fill and percentage of marketable cobs.

Table 4. Comparison of full tropical supersweet hybrids with intermediate hybrids (50% temperate germplasm), Gatton.

HYBRID	Type	Sweet	Tender	Flag leaf	Tip fill	Cob shape	% marketable cobs
81021	intermediate	8	4	2	8	6	90
80106	intermediate	6	4	4	8	6	95
PAC 377	intermediate	8	6	6	8	8	85
Mean		**7.33**	**4.67**	**4.00**	**8.00**	**6.67**	**90.00**
H206	tropical	8	8	2	6	8	95
H191	tropical	6	6	2	8	8	70
H141	tropical	8	8	2	6	4	85
H5	tropical	8	4	2	6	8	80
Mean		**7.50**	**6.50**	**2.00**	**6.50**	**7.00**	**82.50**

NB 1. All ratings are on the basis of 1=poor, 9= excellent.

3.4. Introgression of temperate germplasm into tropical breeding populations as a means of improving sweetness and tenderness in tropical supersweet corn.

Introgression is the perhaps the most thorough method of utilising temperate germplasm to improve quality traits of the methods described here. It requires a lot of breeding effort. The breeding methods have been described previously and are diagramatically conveyed in Appendix I. They have led to the development of four sets of heterotic pair populations with varying levels of introgressed temperate germplasm.

Paired populations	% temperate germplasm
SHP1/SHP2	0
SHP13/SHP14	25
SHP15/SHP16	50
SHP11/SHP12	75%

During the 2001 season we conducted trials at Bowen and Kairi with groups of hybrids generated from each of these sets, with the exception of the SHP13/14 set. With this set we were able to use some inbreds in combination with full tropical inbreds giving a theoretical 12.5% temperate germplasm. Table 5 presents the results for the best hybrids within each germplasm group and compares their performance with successful commercial hybrids.

Table 5. Sweetness, tenderness and other quality traits in super-sweet corn hybrids having different levels of temperate germplasm in trials at Kairi Research Station, 2001) (Higher values indicate superior quality).

Hybrid	Temperate content (%)	Sweetness	Tenderness	Kernel colour	Kernel Depth
Dominion	100	6.7	7.0	6.1	11.0
Goldensweet	100	5.1	6.5	5.3	11.3
H741	75	5.8	6.3	5.2	9.3
H 581	12.5	5.7	6.0	6.0	9.6
H769	50	5.1	5.7	-	-
H5	0	5.5	4.7	6.3	10.4

NB: All ratings based on a 1 to 9 scale with higher ratings indicating superior performnace.

With the exception of H769 the results generally show improvement in sweetness and tenderness traits as the percentage of temperate germplasm is increased.

3.5 Recurrent selection on S0 plants for sweetness and tenderness in segregating populations.

Having constructed the sets of paired breeding populations referred to above, the logical progression is to select within the populations for quality traits. This has been carried out using what is commonly called the "beaver breeding" method. This entails self pollinating individual plants within the populations, then taste testing the pollinated cobs at the optimal time (generally 28 days after pollination). The taste testing procedure consumes half the cob and the remainder is left on the plant for the seed to mature. Sweetness and tenderness ratings are recorded and at harvest time the superior ears are harvested. The superior S1 progenies are then genetically recombined to create the next cycle of plants and the process repeated in a form of recurrent selection.

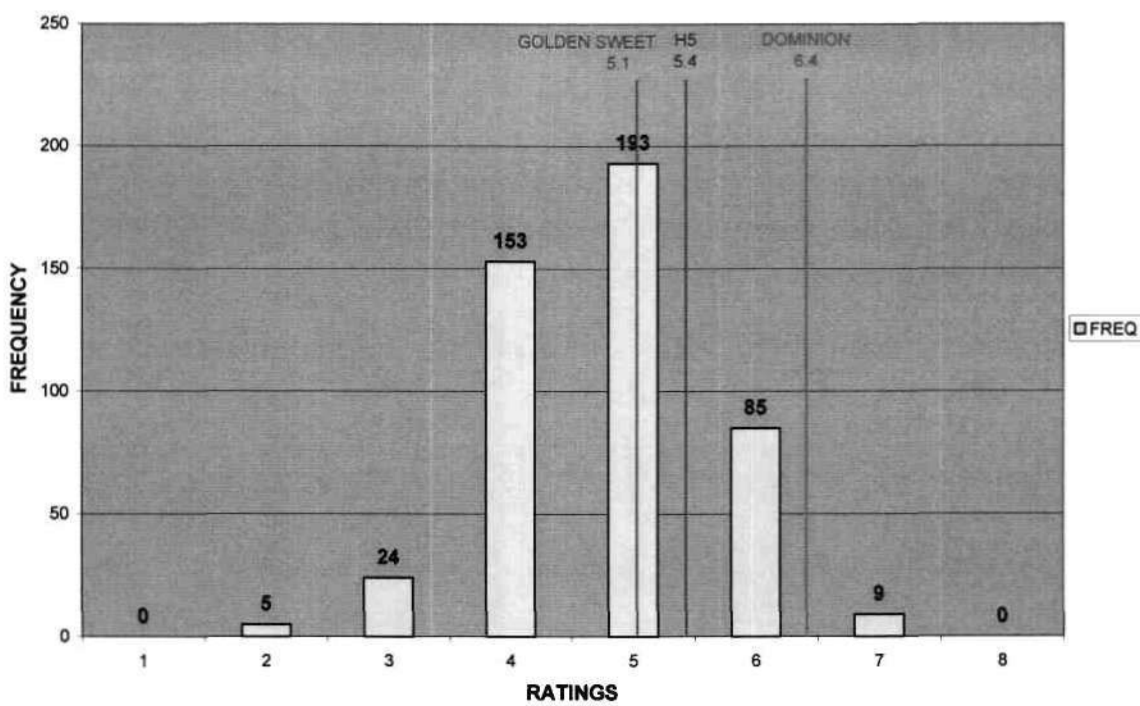
3.5.1 Sweetness.

This procedure has been applied to Populations 11,12, 15 and 16. Results obtained for sweetness and tenderness with Population 15 are presented in the frequency histogram in Figures 3 and 4 respectively.

The hybrid Dominion is recognised as probably the best quality hybrid in the world and is the one that the Japanese market demands. From a total of 466 S0 plants tested we recorded nine with superior sweetness to Dominion and a further 85 plants with comparable sweetness. When the comparisons are made with H5, our existing tropical hybrid (sweetness = 5.4) it is evident that we are in a position to make substantial gains over this hybrid.

The best of these progenies have now been recombined to produce an improved cycle for further selection. In addition we have self-pollinated them to isolate inbreds for eventual production of improved hybrids.

Figure 3. SWEETNESS RATINGS OF POPULATION 15 PROGENIES

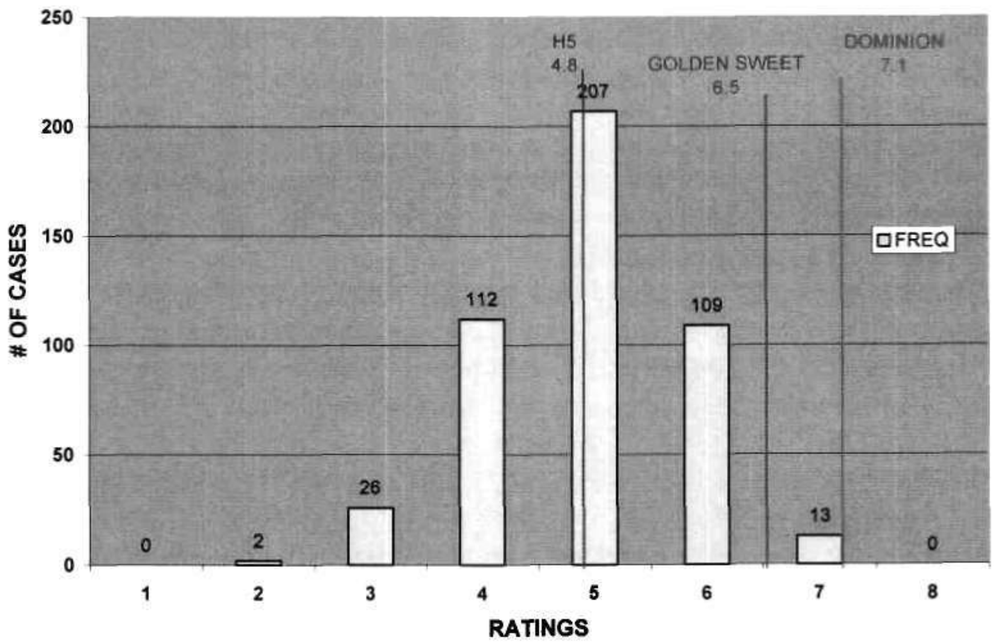


Given the potential to execute a cycle of selection per year it is likely we can make rapid changes in the sweetness mean for the population and almost certainly in the higher sweetness individuals. The progress for this trait will be monitored in future trials.

3.5.2 Tenderness.

Figure 4 illustrates the array of tenderness ratings we found in individuals in population SHP15. In similar fashion to the experience with sweetness ratings we were able to find individuals with comparable tenderness to Dominion, the acknowledged elite hybrid. From a total of 469 plants tested 122 (26%) had tenderness ratings of 6 or better. A rating of 6 would be acceptable in the Australian market but the volume Japanese import industry would require a rating in the vicinity of 7.

Figure 4 .TENDERNESS RATINGS OF POPULATION 15 PROGENIES



Tenderness is considered in some quarters to be more important for consumer acceptance than sweetness. On this basis it is surprising that H5 has achieved market acceptance. Nevertheless it is apparent that we have ample scope for improvement of tenderness within Population 15.

4. Discussion

4.1 Progress for sweetness and tenderness through selection of existing tropical material.

4.1.1 Sweetness.

Figure 1 (in Results) illustrates the progress that has been made during improvement in tropical material for the trait of taste (principally sweetness) since the initial hybrid H5 was released. H349, using one Cycle 3 inbred and one Cycle 4 inbred is significantly superior to H5 (P=0.1) which uses two Cycle 0 inbreds. This improvement demonstrates the feasibility of improvement in tropical supersweet germplasm.

It is generally considered that tropical material can not match temperate hybrids for eating quality. The data presented in Figure 1 at least place a question mark over this assumption. Although we did not have a temperate hybrid in the trial for direct comparison, the level of sweetness adjudged by the taste panel for H349 is comparable with the rating achieved by Dominion in more recent trials (see Fig 3).

These gains have come without a systematic effort to improve sweetness. Most of the improvement is attributable to evaluation of S3 or S4 inbreds in hybrid combination followed by recombination of the elite inbreds to commence the next cycle of selection. There has been no evaluation of sweetness during the inbreeding process.

If a recurrent selection method based on evaluation of S0 plants per se for sweetness with recombination of elite S1 progenies is adopted it could be expected to produce superior gains in a shorter period of time than the method used to obtain the results presented here. Four cycles of selection to produce H349 would have taken approximately 12 years.

The advantage for temperate hybrids for sweetness is more a reflection of the longer period of selection and far greater breeding effort invested in temperate germplasm rather than any inherent advantage over tropical germplasm

4.1.2 Tenderness

Lack of tenderness has been a major criticism of tropical supersweet corn, particularly as it applies to our original hybrid H5. There has been a tacit assumption that tropical super-sweet corn inherently has a tough pericarp. The progress from four cycles of selection reflected in the performances of the hybrids in Figure 2 refutes the idea that tropical sweetcorn is not capable of improvement for tenderness. H349, utilising C3 and C4 inbreds is significantly superior to earlier hybrids H5 and H191 at the probability level of $P=0.01$.

Whether selection for tenderness is associated with greater susceptibility to seed and seedling diseases, and consequently reduced germination, has yet to be established. A thinner pericarp could be expected to permit more leaching of metabolites from the seed and this is thought to encourage infection from soil-borne fungi.

Tenderness is strongly influenced by the length of time after kernel fertilization (Brewbaker, 1981) so one needs to be cautious about genetic gains. Brewbaker points out that there is also interaction of genotype with rate of maturation of the endosperm. Thus measuring tenderness at a fixed time after fertilisation would tend to favour those genotypes that are slow to mature. Despite these qualifications the order of magnitude of gain illustrated here gives some confidence that the gains are real.

The poor performances of H5 and H191 for tenderness is possibly related to the fact that both hybrids use the Cycle 0 inbred SHB17. There is a suggestion here that toughness might be inherited in dominant fashion. This would conflict with the findings of Helm and Zuber to the extent that they found pericarp thinness to be partially dominant. Tenderness is thought to be conditioned by relatively few genes, from two to five depending on the outcome of various studies. Brewbaker has found high heritability values for pericarp thickness and has estimated reductions of 8.3

microns per selection cycle. It is feasible to make good gains with recurrent selection for thinner pericarp using the bite test as the mechanism for evaluation. In more recent breeding endeavours we are in fact using this technique. We have been successful in producing one tropical inbred, SHA56, that is markedly superior to others and consistently generates hybrids with tender kernels.

4.2 Use of the brittle 1 gene.

The *bt1* gene appears to have produced a lower level of sweetness to that achieved with *sh2* (Table 3). This accords with findings from the program in Hawaii where a reduced level of sweetness was observed in isogenetic hybrids.

The gene also appears to have offered no improvement in tenderness when compared with sh2 genotypes, although this may be more of a function of the background genetics rather than the *bt1* gene per se. Similarly the advantages seen in characteristics such as cob size, tip fill and cob appearance are probably related to the full genome of the brittle gene pools rather than the gene itself.

It is difficult to justify parallel breeding programs with the two genes. For this reason, and given that *bt1* offers no quantum improvement in quality factors, we are unlikely to continue further with the brittle studies. The only place where they might find a place is in the processing industry where yield is important.

4.3 Crossing tropical with temperate inbreds to make F1 commercial hybrids.

On the basis of the data presented in Table 4, this method would appear to have limited potential. Sweetness in the three hybrids of 50% temperate: 50% tropical genetic constitution is comparable with 100% tropical material. Tenderness is inferior in the hybrids with one temperate parent. This can possibly be attributed to the fact that the tropical parent is a parent of H5, and that this inbred appears to condition a tough pericarp.

One must concede that this is a very limited set of data and that the failure to record better results could be a function of not having access to the best temperate germplasm. Other traits such as tip fill, flag leaf, cob shape and percentage marketable cobs are all generally better than pure tropical hybrids. As improved tropical inbreds become available, this approach has some prospect of producing commercial hybrids.

One problem that might be encountered is that the high levels of disease resistances observed in tropical inbreds (eg to Johnson Grass Mosaic and Turcicum leaf blight) may not be realised in crosses with temperate inbreds if gene action is not fully dominant. Also experience tells that use of a temperate parent in seed production plots can cause problems of obtaining flowering sychronisation with later flowering tropical inbreds and difficulties caused by greater disease susceptibility of the temperate parental inbred. For these reasons the method is not preferred.

4.4 Introgression of temperate germplasm as a means of improving sweetness and tenderness in tropical supersweet corn.

This is perhaps the most interesting and challenging approach to improving quality. It also has the most to offer from a long-term plant improvement viewpoint. It offers the chance to combine all the desirable traits in individual germplasm populations. It seeks to combine the multiple disease and insect resistance sources in tropical material with the superior sweetness and tenderness of the temperate germplasm.

The data presented in Table 5 from trials conducted at Kairi Research Station in 2001 suggest that a high level of temperate germplasm is necessary in order to approach the levels of sweetness and tenderness in the best temperate hybrids. By virtue of this fact the hybrids might no longer be considered to be what we call "tropical". For example H741 has quality exceeded only by the variety Dominion, the acme of eating quality, but H741 is 75% temperate in constitution. The germplasm set with 50% temperate germplasm has been disappointing in its quality. H769, the best of the group of 40 hybrids under test was considerably inferior to all but H5 in tenderness and had poor sweetness. It is anticipated that further testing of this group will reveal a better result.

A more extensive testing program with additional hybrids is needed to fully assess this approach. It is considered a logical conjoint activity will be to look at the potential for improving the newly-constructed populations through the medium of recurrent selection.

4.5 Recurrent selection for sweetness and tenderness in new supersweet populations containing infused temperate germplasm

Figures 3 and 4 are encouraging in that they show individual plants from SHP15 possessing sweetness and tenderness comparable with Dominion, a hybrid considered to have the best combination of those traits. In several cases some plants had both the desirable characteristics. A total of 466 ears were assessed for sweetness and 469 for tenderness. Sixty-nine S0 selfed ears were retained (14.7% selection intensity) as candidates for recombination in the next cycle. Agronomic performance of the derived S1 families will be used to decide the final families for recombination. Mean value for sweetness for the unselected group was 4.8 and for the selected group 5.6 representing a selection differential of 16.6%. For tenderness the mean ratings were 4.9 and 5.6 for a selection differential of 14.3%.

Since tenderness is highly heritable, selection and recombination of these plants in a repeated fashion (recurrent selection) at a selection intensity approximating 15% should bring about significant improvement in the mean of the populations SHP15 and SHP16. The duration of the cycle of one year creates the opportunity to achieve rapid improvement. Ito and Brewbaker (1981) achieved gains in kernel tenderness in the order of 3.9% per generation with mass selection despite the fact that this method does not provide control of the male parent.

Use of the method we have described should ensure progress in excess of that obtained with mass selection. A gain of 5% per cycle over 4 cycles would theoretically elevate the best hybrids to a tenderness rating in the vicinity of 7, similar to that achieved by Dominion.

Additionally the infusion of SHP15 and SHP16 elite progenies into tropical populations SHP1 and SHP2 is in progress as a means of integrating the quality factors in the former populations and the disease and insect resistance of the latter. The results of the selection procedures described above will be monitored and reported from trials over the next few years.

Bibliography of Cited Literature

1. Brewbaker, James L. and Soon Kwon Kim.1979. Inheritance of Husk Numbers and Ear
   Insect Damage in Maize. Crop Science 19: 32-36

2. Creech, Roy G. 1968 Carbohydrate synthesis in maize. Advances in Agronomy 20:275-322.

3. Helm, J.L and Zuber, M.S. 1972. Inheritance of pericarp thickness in corn belt maize. Crop Sci. 12:428-430.

4. Ho, L.C., Kannenberg, L.W., and Hunter, R.B. 1975. Inheritance of pericarp thickness in short season maize inbreds. Can. J. Genet. Cytol. 17:621-629.

5. Ito, Glenn M. and Brewbaker, James L. 1981. Genetic Advance through Mass Selection for Tenderness in Sweet Corn. J.Amer. Soc. Hort. Sci. 106(4):496-499.

6. Ito, Glenn M. and Brewbaker, James L. 1991. Genetic Analysis of Pericarp Thickness in Progenies of Eight Corn Hybrids. J.Amer. Soc. Hort. Sci. 116(6)1072-1077.

7. Kaukis, Karl and Davis, David W. 1986. Sweet corn breeding. In "Breeding Vegetable Crops" Avi Publishing Co.

8. Wang, B and Brewbaker, J.L. 2001. Quantitative trait loci affecting pericarp thickness of corn kernels. Maydica 46: 159-165.

APPENDIX I. Illustration of project breeding manipulations in creation of new breeding populations. (Counterpart populations SHP12 and SHP16 were set up in similar manner but not shown here).