Horticulture Innovation Australia

Final Report

Transforming tenderness and eating quality in tropical sweetcorn through introgression of tender germplasm

Aldo Zeppa The Department of Agriculture and Fisheries (DAF)

Project Number: VG10105

VG10105

This project has been funded by Horticulture Innovation Australia Limited using funds from the Australian Government and the following organisations:

HSR Pty Ltd Crookham Australia P/L T/As Snowy River Seeds

Horticulture Innovation Australia Limited (Hort Innovation) makes no representations and expressly disclaims all warranties (to the extent permitted by law) about the accuracy, completeness, or currency of information in *Transforming tenderness and eating quality in tropical sweetcorn through introgression of tender germplasm*.

Reliance on any information provided by Hort Innovation is entirely at your own risk. Hort Innovation is not responsible for, and will not be liable for, any loss, damage, claim, expense, cost (including legal costs) or other liability arising in any way (including from Hort Innovation or any other person's negligence or otherwise) from your use or non-use of *Transforming tenderness and eating quality in tropical sweetcorn through introgression of tender germplasm*, or from reliance on information contained in the material or that Hort Innovation provides to you by any other means.

ISBN 0 7341 3658 7

Published and distributed by: Horticulture Innovation Australia Limited Level 8, 1 Chifley Square Sydney NSW 2000 Tel: (02) 8295 2300 Fax: (02) 8295 2399

Contents

Summary
Keywords
Introduction
Methodology9
Parental crosses
Pedigree selection
Preliminary evaluation of F1 hybrids9
Data analyses
Outputs 11
Outcomes
Segregating generation evaluation
Segregating generation evaluation
Segregating generation evaluation 12 Variability of hybrids created using the new inbred lines 12 Comparative performance of the new hybrids 15
Segregating generation evaluation 12 Variability of hybrids created using the new inbred lines 12 Comparative performance of the new hybrids 15 Evaluation and Discussion 19
Segregating generation evaluation. 12 Variability of hybrids created using the new inbred lines 12 Comparative performance of the new hybrids 15 Evaluation and Discussion 19 Recommendations 21
Segregating generation evaluation.12Variability of hybrids created using the new inbred lines12Comparative performance of the new hybrids15Evaluation and Discussion19Recommendations21Scientific Refereed Publications.22
Segregating generation evaluation.12Variability of hybrids created using the new inbred lines12Comparative performance of the new hybrids15Evaluation and Discussion19Recommendations21Scientific Refereed Publications.22Intellectual Property/Commercialisation23
Segregating generation evaluation.12Variability of hybrids created using the new inbred lines12Comparative performance of the new hybrids15Evaluation and Discussion19Recommendations21Scientific Refereed Publications22Intellectual Property/Commercialisation23References24
Segregating generation evaluation.12Variability of hybrids created using the new inbred lines12Comparative performance of the new hybrids15Evaluation and Discussion19Recommendations21Scientific Refereed Publications.22Intellectual Property/Commercialisation23References24Acknowledgements.25

Summary

The sweetcorn breeding program conducted by Queensland Department of Agriculture and Fisheries (DAF), has contributed significantly to the Australian sweetcorn industry by breeding hybrids that can produce standard sweetcorn for both processing and fresh consumption. The DAF program has focused primarily on tropical sweetcorn. Snowy River Seeds Ltd has one of the largest private sweetcorn breeding programs in the southern hemisphere and develops hybrids suitable for production in tropical, subtropical and temperate environments.

The most significant aspect of the tropical germplasm developed by DAF is better disease resistance compared to the tenderer, temperate germplasm. Tropical germplasm has bigger plant stature that makes is less efficient in terms of productivity. Quite often, tropical germplasm also requires longer growing periods making it less flexible in terms of planting and harvesting. There is very limited genetic variation for ear and cob related characteristics.

There is more genetic variability in terms of tenderness in temperate sweet corn than in tropical germplasm collections held by DAF. Introgression of tender Snowy River germplasm to the tropical gene pool should improve the chance of combining superior agronomy with improved eating quality. Combining improved eating quality with superior resistance to diseases e.g. northern corn blight (turcicum leaf blight), Johnson grass mosaic virus (JGMV), polysora rust (*Puccinia polysora* or southern rust) will be of significant benefit to the sweetcorn industry at Gatton, Bowen and in the Burdekin where temperate hybrids are the varieties of choice in the main part of the growing season. This project is aimed to develop tropical hybrids with significantly improved eating quality and disease resistance, thus expanding production areas and planting windows.

Selected tropical materials based on their agronomic characteristics and resistances to diseases were crossed to selected Snowy River lines with superior eating quality. About 100 bi-parental crosses were generated. Inbred lines were developed based on a 'shuttle' breeding approach where screening of segregating generations was switched between tropical and subtropical environments. This improved the chance of exposing the segregating genotypes to wider environmental and disease situations, facilitating faster development of widely adapted inbreds. Screening of F2s at Gatton was complemented by growing spreader rows susceptible to JGMV. The development of uniform polysora and northern corn blight infections at Kairi was also enhanced by growing spreader rows every 20 entries.

A series of 21 new inbred lines with the desired features were developed. Quick flowering and reduced plant stature, improved cob appearance, kernel colour and tenderness were introduced from temperate germplasm coupled with excellent resistance to JGMV, polysora rust, and northern corn blight introgressed from the tropical background. The disease score of the selected lines were all below 3 in 1 to 9 scale where 1= complete resistance and 9=completely susceptible. Preliminary analyses of the commercial values of the lines were determined by creating 267 F1 hybrids (112 in 2013-14 and 155 in 2014-15). Preliminary observation of the hybrids at Gatton showed that some of the new hybrids revealed up to 30% reduction in height compared to the tropical hybrid (Hybrix 5), and were comparable to the Goldensweet Improved hybrid.

Some of the hybrids were also quicker to flower by as much as seven days compared to Hybrix 5 and were similar to both Goldensweet Improved and Garrison (temperate hybrids). Some of these hybrids produced more marketable cobs compared to the temperate hybrids. They showed better husk ratios compared to Hybrix 5 indicating they had reduced layers of husks making them easy for de-husking. Tenderness was improved by as much as 40% when compared to Hybrix 5, and their flavour was as good as both the tropical and temperate hybrids.

Over 160 inbred lines were provided to our project partner, Snowy River Seeds. Some of these inbreds are the parental lines of some of the promising hybrids identified in the preliminary hybrid testing stage. About 20 inbred lines were found to combine excellent resistance against diseases including JGMV, with good agronomy and eating quality. Snowy River Seeds will develop different hybrids based on the various combinations of inbred lines and evaluate them in their major sweetcorn production environments.

Keywords

Sweet corn, disease resistance, Johnson grass mosaic virus, shrunken (sh2) gene

Introduction

Tropical maize germplasm seems to have broad genetic diversity and adaptation. They are excellent sources of resistance for diseases such northern corn blight (*Exserohilum turcicum* or turcicum leaf blight), southern rust (*Puccinia polysora*), maize mosaic virus (MMV) and the closely related Johnson grass mosaic virus (JGMV). Since maize is originated from tropics and sub-tropics where it is grown primarily for human consumption, it also has better grain quality and resistance or tolerance to major pests (Hallauer and Carena 2014).

However, tropical maize has also undesirable features such as excessive plant height and high cob insertion, longer growing period (lateness), low yield potential, poor harvest index, and poor husk cover (Abadassi and Hervé, 2000). Sweet corn is widely used for human consumption throughout the world. It is an important source of fibre, minerals, and certain vitamins and carotenoids (Lertrat and Pulam, 2007). Sweet corn eating quality is determined by its unique combination of flavor, texture and aroma. Sweetness is the most important factor in consumer satisfaction with sweet corn (Evensen and Boyer, 1986). It makes up most of the flavor component and depends largely on kernel sucrose content. Texture is determined primarily by pericarp tenderness, levels of water soluble polysaccharides (phytoglycogen), and the kernel moisture content (Lertrat and Pulam, 2007).

Different endosperm genes are used in sweet corn improvement to increase sugar and decrease starch content. *Shrunken (sh2)* and *brittle (bt)* genes, located on chromosomes 3 and 5 respectively, are classified as Class 1 mutants involved in large reductions in starch and increase in sugar. The *sugary1 (su1)* and *sugary enhancer1 (se)*, located on chromosomes 4 and 2 respectively are Class 2 mutants that alter the types and the proportion of polysaccharides in the endosperm (Lertrat and Pulam, 2007). The predominant type of sweet corn grown in Australia contains the *Shrunken (sh2)* due to the higher level of sugar and relatively longer shelf life.

Sweet corn breeders around the world have been using standard breeding approach to explore the available genetic variability in temperate and tropical germplasm to develop commercially useful hybrids. There are also a few attempts to explore temperate germplasm to enhance the performance of tropical germplasm and vice versa. Temperate germplasm generally has better eating quality, particularly, tenderness but are susceptible to diseases. Tropical germplasm can augment temperate germplasm by providing disease resistance and better tolerance to higher temperatures. On the other hand, quick flowering, reduced plant stature, reduced husk layers and generally improved tenderness can be introduced to tropical germplasm from the temperate types.

Over many years the DAF program achieved improved genetic resistance against Johnson grass mosaic virus (JGMV), southern rust and northern corn blight. Successful incorporation of disease resistance into susceptible lines will greatly improve the adaptation range of Snowy River Seeds hybrids both locally and internationally. Our recent investigation into the genetic variation and heritability of tenderness and flavour showed that rapid genetic progress to combine superior eating quality and agronomy will only be possible through introgression of genetically divergent germplasm to our germplasm collection (Solomon et al., 2012). This collaborative research will provide the opportunity to expand the genetic base of our breeding materials thereby increasing the chance of producing superior

elite materials. This will enhance the pace of development of commercial hybrids to benefit Australian sweet corn growers. The purpose of this project was therefore to combine temperate and tropical germplasm with the aim of identifying germplasm that combine desirable features from the two germplasm pools.

Methodology

Parental crosses

Eleven tropical and 10 temperate parental lines were assembled to generate about 100 different cross combinations. The tropical lines were originated from various breeding pools and were genetically diverse (Solomon et al. 2012). The temperate germplasm was obtained from our collaborator, Snowy River Seeds. Reasonable genetic diversity is expected in the temperate germplasm as well. To enhance flowering synchronization, three staggered plantings were done. Tropical lines were first planted. After a week both the tropical and the temperate germplasm were planted, and then a week after the second planting, temperate germplasm were replanted. A minimum of five crosses for each biparental combination was conducted. A total of 500 individual crosses were made.

Pedigree selection

F2s were generated from selfing of at least five F1 plants. Minimum of 400 plants were grown from each F2. A total 40,000 F2 individuals, about 400 per cross, were grown in the segregating screening nurseries. The seasonal growing conditions from late plantings were particularly favourable for the development of various diseases e.g. Johnson grass mosaic virus (JGMV), maize stripe virus, northern corn blight and common rust. The infection development of JGMV was excellent. It was further assisted by forage sorghum planted every 20 rows. The sorghum was regularly trimmed back to promote continuous supply of disease inoculum to late flowering materials. This helps minimize disease escape due to late flowering. Strict selection pressure was further applied based on phenology and agronomy.

Some crosses showed genetically more promising material than others, and in a few cases no selection was made at all due to poor agronomy. In other cases, however, up to 20 outstanding F2s were selected. F3 were subsequently grown in a tropical environment to screen for tropical adaptations and also resistance against polysora rust (southern rust). Selected F3s were grown in head to rows to raise F4 families. F4 families were grown in double rows at Gatton in autumn and spring. Disease pressure was maintained by growing spreader rows. Selection was made from the most uniform and agronomically superior families. Selected families were also crossed to generate F1 hybrids.

Preliminary evaluation of F1 hybrids

More than 250 single cross hybrids were created using F4 and F5 inbreds. Crosses were made between unrelated pedigrees. The F1 hybrids and three commercial standards were tested in an observation trial with two rows of 5 m long plots. All relevant agronomic and quality data were recorded on plot basis. Total fresh marketable ears (MC) were counted per plot when the moisture content of the

kernels declined to 70–75% (Olsen et al. 1990). The cobs were considered marketable if husked ear length was >20 cm with >10 cm of edible kernel (length of unhusked ear). No significant lodging and disease or earworms incidence occurred and was therefore not recorded (Kwabiah 2004).

Agronomic traits such as plant height (cm) were measured from ground level to the base of tassel. Random samples of three marketable cobs were collected to determine: ear weight with husk (ear_weight_husked) and without husks (ear_weight_dehusked) expressed in gram (g). Husk ratio values were determined as husk_ratio = 100 x (husk weight / ear weight with husk) and expressed in %. Ear_length (cm) and Ear_diameter (mm) were determined from de-husked ears. Ear_fill (cm) used to measure the length of ear completely filled with well-developed kernels.

Kernel depth (mm) for kernels on an ear was determined by measuring the overall length of kernels from the mid-section of each ear. Kernel colour (KC) on unhusked ear was rated on 1 to 10 scale. Rating was based on uniformity and intensity of the kernels colour on the ears. A rating of 1 was given to uniform whitish kernels colour and 10 was given to uniform and bright yellow colour. Bite tests were used to rate flavour (sweetness) (Flv) and tenderness (TD). This was on a scale of 1-10, 1 was for grassy flavour and 10 for very sweet and juicy flavour. Kernels tenderness was also rated on a scale of 1-10 where 1 = very tough and chewy and 10 = very tender and crispy. Cob tip cover, flag leaf, husk and silk appearance were evaluated on a scale of 1–5, where 1 = poor, 3 = good and 5 = excellent.

A subsequent series of 155 different hybrids were produced in a hybrid production nursery established in September 2014 at Gatton, and 21 inbreds were used in a variety of combinations to produce hybrid seed for a field trial including agronomy and quality testing. Varying amounts of seed was produced, but generally in ample quantity and of good quality to plant a significant hybrid trial in order to assess the potential of the parental inbred material. In late 2014 there was significant uncertainty about the future of a requested project extension. Without assured funding, it was impossible to plan for ground preparation (and the associated costs) for a February planting at Gatton. At that stage, the hybrid seed was still in the nursery and had not been harvested, shelled or graded. In early 2015, uncertainty continued about the project extension, and the decision was taken to return all hybrid seed to Snowy River Seeds for their continued evaluation. Snowy River Seeds subsequently planted the 155 hybrids at Bowen as single row plots (5. 5m length) in a modified augmented design and evaluations made using a different rating scale (See Appendix 1).

Data analyses

The data from the F1 hybrids observation trial were used to analyze the variability pattern of the F1s. Principal component analyses based on the variance and covariance relationships of 14 different agronomic and quality traits was applied. To avoid biases due to different scale of measurements, the observation data were first normalized. Since the data did not allow for statistical significance tests, performance of test hybrids was expressed as percentage of increase or decrease in relation to the commercial checks. Data from the most promising hybrids were presented in form of bar charts for key agronomic and quality traits.

Outputs

68 selected F5 and F6 inbreds returned to collaborative partner Snowy River Seeds in July 2014 for further evaluation.

179 inbreds planted in a spring 2014 nursery at Gatton were characterized and underwent further selection. Subsequently 163 F5 and fixed line inbreds were returned to Snowy River Seeds in February 2015.

155 different hybrids were produced in a hybrid production nursery at Gatton in spring 2014 from 21 inbred parents in varying combinations. These were evaluated at Bowen in 2015 by Snowy River Seeds. Five hybrids identified as worthy of further evaluation when compared with commercial checks.

Outcomes

Segregating generation evaluation

In most of the seasons, the level of disease pressure was very good, particularly JGMV at Gatton. Since materials were advanced at different seasons, it is possible to say that they were exposed to various types of diseases that occur at different seasons. Kairi provided 'hotspots' for diseases like polysora rust, common rust, and northern corn blight. Nevertheless, the level of infection for common rust and corn blight at Gatton was also very high particularly in spring plantings. Apart from the level of disease resistance, strict selection pressure was further applied based on phenology and agronomy. At Kairi variability in terms of vigour and photoperiod requirements was also very high. Emergence and stand establishment were also considered during the selection. At F2 stage, about 40,000 plants or about one hectare of F2 plants were grown. Only 558 F3 families were created. At F4, a total of 230 families were planted, but only 114 lines were advanced to F5.

Variability of hybrids created using the new inbred lines

Four groups were created based on the analyses of the first two principal components. Section I & II represent hybrids that are not similar to both tropical hybrid (Hybrix 5) and the temperate/subtropical hybrids (Garrison and Goldensweet Improved). Section III represents hybrids that were very close to the temperate hybrids (Garrison and Goldensweet Improved). Section IV showed hybrids which appeared to be tropical like Hybrix 5.

The two principal components however explain only a small proportion of the total variability (about 40%) (Fig. 1a). The biplot of PC1 and PC3 produced similar compartments. However, most of the hybrids resembled more the tropical hybrid, Hybrix 5 than the temperate hybrids (Garrison and Goldensweet Improved). There were also many hybrids that didn't resemble either the temperate or tropical hybrids. The amount of variability explained by PC1 and PC3 was about 37% (Fig. 1b). Most of the hybrids fell in section II and III. Section I possesses hybrids that were related neither to Garrison and Goldensweet Improved nor to Hybrix 5, and section III contains hybrids which were related to the tropical type. Comparatively, PC2 and PC3 plot showed a smaller number of hybrids that were related to Hybrix 5. The two axes, PC2 and PC3, however explained less than 30% of the observed variability (Fig. 1c).

Traits with the highest eigenevector (> 0. 3) that contributed most to PC1 were ear diameter, ear length, ear fill, ear weight (husked & dehusked). These traits contributed about 59% of the eigenevalue of PC1. On the other hand, days to silking, tasseling, flavour and tenderness were more important to PC2, and explained 82% of the eigenevalue of PC2. Kernel colour, marketable cobs and flavour contributed to 65% of eigenevalue of PC3 (Table 1).



Table 1. Contribution of traits to the three major principal components based on the principal component analyses using normalized values of traits measured from observation trials conducted in Autumn 2014 at Gatton

	PC1	PC2	PC3
Days_to_Silking	-0. 23365	0. 47595	-0. 0836
Days_to_Tasseling	-0. 23541	0. 49233	-0. 14683
Ear_Diameter (mm)	0. 3158	0. 11097	0. 19738
Ear_Length (cm)	0. 39466	0.0714	-0. 28157
Ear_fill (cm)	0. 37283	0. 12114	-0. 27981
Ear_weight_dehusked (g)	0. 48592	0. 16093	0. 00683
Ear_weight_husked (g)	0. 40254	0. 26716	-0. 04335
Husk_ratio	-0. 00938	0. 22133	-0. 28841
Kernel_colour (1-10 rating)	0. 04204	0.04942	0. 47925
Kernel_depth (mm)	0. 17893	0. 12968	0. 21557
Marketable cobs (No. cobs/plot)	0. 21427	-0. 09861	0. 3601
Plant_height (cm)	0. 08375	-0. 17341	0. 26361
Flavour (1-10 rating)	-0. 08505	0. 38083	0. 37176
Tendernes (1-10 rating)	-0. 05755	0. 38556	0. 27621
Eigenvalue	3. 31	2. 12	1. 85
Percentage of variation (%)	24. 11	15. 44	13. 49

Fig. 2 summarizes the observed variability and the frequency distribution of the hybrids tested in the observation trial with respect to key agronomic and quality traits. The observed number of marketable cobs ranged from 10 to 40 cobs per 2 row x 5m long plots. Most of the hybrids showed number of marketable cobs less than the commercial check, Goldensweet Improved (Golden SI). However, 15 hybrids showed higher number marketable cobs compared to Garrison. The tropical hybrid, Hybrix 5, was far more superior in terms of productivity. Only six hybrids showed higher number of marketable cobs compared to Hybrix 5 (Fig. 2a).

Most of the hybrids showed plant height that fell between Garrison and Hybrix 5, ranging from 145 to 245 cm. However, a few crosses also showed height less than Garrison. The majority of the hybrids, nevertheless, showed height taller than Goldensweet Improved (Golden SI) but shorter than Hybrix 5 (Fig. 2b). Only two hybrids were quicker to flower compared to Garrison and Golden SI. Most of the hybrids were quicker than Hybrix 5 but slower than Garrison and Golden SI. However, the difference between Garrison/Golden SI and Hybrix 5 was only 7 days (Fig. 2c).

Husk ratio measures the weight of the husk to the weight of stripped ears; therefore, it's an important indicator of the recovery of consumable portion of the cobs. It can also be used as an indicator of the ease of husk removal. The tropical hybrid, Hybrix 5, showed the maximum husk weight ratio. Garrison showed the smallest husk weight ratio. Most of the tested hybrids showed ratios between Garrison and Golden SI (Fig. 2d). As far as tenderness is concerned, Hybrix 5 was the toughest. Garrison and Golden SI were the most tender hybrids. Most of the new crosses showed one point improvement when compared to Hybrix 5. There were also a considerable number of hybrids that showed two points improvement over Hybrix 5 and less than one point compared to Garrison and Golden SI (Fig. 2e). In terms of flavour, there were no differences among the checks. However, a number of hybrids showed flavour ratings better than the checks (Fig. 2f).

The observed variability for some agronomic and quality indicator traits is summarised in Table 2. The weight of ear with husk varied from 732 to 1282g, with an average value of 992g. Hybrix 5 showed the heaviest cob among the checks. However, substantial weight of Hybrix 5 cob was attributed to the husk weight as evidenced from a lower ear weight_dehusked. The dehusked cob weight ranged from 248 to 815 g, Garrison being the heaviest among the checks. Ear diameter varied from 39 to 52 mm, and Golden SI showed the biggest girth. Hybrix 5 showed the longest ear with 20cm, the observed variability for this trait was 15 to 22 cm. The range for ear fill (ears filled completely with well-developed kernels) varied from 13 to 22 cm, and Hybrix 5 showed the longest well filled ear.

Golden SI and Garrison seemed to have slightly opened tip cover compared to Hybrix 5. The observed tip cover ratings for the trial varied from 1 to 5, where 1 is completely open (ear tip protruding), and 5 is tightly covered tip where the ear is buried deep into the husk. Flag leaf appearance is also an important visual quality indicator of freshness. It is particularly useful for fresh consumption. The hybrids in the trial rated between 0 (no flag leaf) to 5 very long deep green flag leaf. Hybrix 5 lacked flag leaf whereas Golden SI showed the best flag leaf rating.

Cob appearance was rated in 1- 5 scale, where one was unattractive cob appearance with very rough, pale, and disease or weather affected husk cover, and 5 is smooth and shiny green and clean husk cover. Hybrix 5 and Garrison were rated 3, whereas Golden SI was rated 5. The range observed in the trial was between 1 and 5 indicating considerable variability for cob appearance. Kernel colour ratings varied from 4 to 8, the average being 6. 5. Hybrix 5 showed the worst ratings (5) among the checks.

Row formation was rated in 1-5 scale; where 1 was given when kernels on the cobs appeared with no

pattern whatsoever. A rating of 5 was given when kernels appeared in straight line from the base to the tips with an even appearance on the ear. The ratings varied from 1 to 5, and all commercial checks were rated as 4. The number of kernel rows in a cob varied from 14 to 20, Garrison and Golden SI had higher number rows compared to Hybrix 5. Kernel depth indicates the size of kernel. The observed variability ranged from 5 to 10, and Garrison and Golden SI showed deeper kernels than Hybrix 5.

Comparative performance of the new hybrids

Ranking of hybrids based on marketable cobs identified five top performing hybrids with excellent level of disease resistance and suitability for commercial production. Compared to the commercial check, Hybrix 5, two hybrids showed 5 and 10% more marketable cobs. Compared to Garrison, five hybrids showed increase in marketable cobs ranging from 5 to about 20%. The largest increase was observed when comparison was made against Goldensweet Improved, 30 to 40% more marketable cobs (Fig 3a). These hybrids also showed considerable reduction in height when compared to the tropical hybrid (Hybrix 5). The reduction ranges from 10 to 30% (Fig. 3b). However, when compared to Garrison they were slightly taller (10-20%).

In terms of flowering time, there were differences of two days in most cases when comparison was made between the elite hybrids and temperate hybrids (Goldensweet Improved and Garrison) (Fig. 3c). The selected hybrids also showed better ear recovery, lower husk weight ratios particularly when compared Hybrix 5. The reduction in husk weight was as high as 120% when compared to Hybrix 5. However when compared to Garrison they showed slightly higher husk weight. The increase in husk weight varied from about 10 to 30% when compared to Garrison (Fig. 3d). As far as flavour is concerned the selected hybrids were more flavoursome compared to all check hybrids (Fig. 3e). One hybrid showed 35% improvement compared to all checks.

The selected hybrids were also tenderer particularly when compared against Hybrix 5. The increase in tenderness varied from 20 to 40% when compared to Hybrix 5. Two hybrids showed marked reductions in tenderness when compared against Goldensweet Improved and Garrison (Fig. 3f).



Fig. 2. Frequency distribution of the performance of hybrids for (a) number of marketable cobs, (b) plant height, (c) days to flowering (silking), (d) husk weight ratio, (e) tenderness and (f) flavour. Based on observation trial conducted in autumn 2014 at Gatton.



Fig. 3. Comparison of some selected hybrids against tropical hybrid (Hybrix 5), subtropical/temperate hybrids (Garrison and Goldensweet Improved) (a) for number of marketable cobs, (b) for plant height, (c) for days to flowering (silking), (d) for husk weight ratio, (e) for tenderness and (f) flavour based on observation trial conducted in autumn 2014 at Gatton.

Traits	Garrison	Golden SI	Hybrix 5	max	min	Mean
Ear_weight_husked (g)	1000	1150	1262	1282	732	991.6
Ear_weight_dehusked (g)	737	697	644	815	348	619. 3
Ear_Diameter (mm)	49	51	45	52	39	45. 4
Ear_Length (cm)	18	18	20	22	15	18. 8
Ear_fill (cm)	18	18	19	22	13	17. 8
Tip cover	3	3	4	5	1	2.4
Flag leaf appearance	3	5	0	5	0	1.7
Cob appearance	3	5	3	5	1	3. 0
Kernel colour	8	7	5	8	4	6.5
Kernel Row Formation	4	4	4	5	1	3. 8
Kernel Row Number	18	18	16	20	14	17.0

Table 2. Descriptive statistics for some traits measured from observation trial conducted in Autumn2014 at Gatton

Interestingly, of the 155 hybrids tested at Bowen in April 2015 (with commercial comparators Hybrix 5, Galaxy and Astronaut), Snowy River Seeds has identified five as worthy of further testing straight away, which is excellent (Table 3; Appendix 1). Snowy River will aim to bulk these up after bulking up the inbred parents. Mr Damien Courtier, breeder from Snowy River is looking forward to making more crosses to these inbreds with some of Snowy River's elite inbreds in a spring nursery.

Cv and/or Hybrid ID	Female inbred pedigree	Male inbred pedigree	Pericarp	Texture	Flavour	Comment
Hybrix 5		-	2	1	1	Good length, tip blanking, bad taper, no quality tall plant
Galaxy	SRSH292	SRSH99	3	2	2	Good length, excellent tip fill, good rows, nice looking plant
Astronaut	SRSH99	SRSH291	3	3	2	Short, excellent tip fill, good kernel, pale, looks good
75x3	D10 x SW10_109-4-3- B2	D1 x SW3_3-8- 1-B1	4	3	2	Short, excellent tip fill, ok quality, see again
76x21	D1 x SW3_3-7-1- B2-B3	D2 x SW7_18-2- 2-B3	3	2	2	Short, excellent tip fill, good plant style, good plant health, see again
44x3	D4 x SW11_44-6- 1-B4	D1 x SW3_3-8- 1-B1	3	2	2	Short, some tip blanking, ok plant, interesting maturity, see again
44x75	D4 x SW11_44-6- 1-B4	D10 x SW10_109-4-3- B2	3	2	2	Short, excellent tip fill, ok quality, see again
75x28	D10 x SW10_109-4-3- B2	D3 x SW3_25-4- 2-B2	2	3	2	Short, good tip fill, good quality, looks good, see again.

Table 3. Selected hybrids and commercial checks from a hybrid set tested at Bowen 2015

Evaluation and Discussion

Sweet corn in Australia grows in central NSW, Lockyer valley in Queensland, and central west and Riverina districts of NSW. The central west and Riverina districts of NSW produce more than 50% of Australia's sweet corn (Sweet corn information kit, 2005). South Queensland produces from spring to autumn and New South Wales produces summer and autumn crops, while the Ord River in Western Australia and north Queensland have winter production.

These diverse growing environments and seasons require different hybrids. To ensure reliable supply of sweet corn, both for fresh consumption and processing, expanding planting windows is very important. One of the major challenges of temperate sweet corn hybrids is susceptibility to diseases such as Maize dwarf mosaic virus (MDMV) and the closely related Johnson grass mosaic virus (JGMV). The main effects of these diseases is that they stunt plant growth, delay silking, and causes up to 70% yield loss (Gregory and Ayers, 1982) Yield losses are due to a reduction in photosynthetic rate due to chlorophyll loss, and an elevation in respiration rate due to virus replication (Gates and Gudauskas, 1969).

In USA, nearly two thirds of all commercial sweet corn hybrids are susceptible to MDMV (Pataky et al., 2011). Resistance is conferred by a single gene or tightly linked genes on the short arm of maize chromosome 6 (Jones et al., 2007). In the mid-1990's in Australia development of tropically adapted hybrids, possessing a major dominant gene (Mv) for general resistance against JGMV (Brewbaker 2003) has made possible the expansion of sweet corn production in environments where temperate type hybrids fail due to diseases such as JGMV (Franco-Dixon 2009).

In this work emphasis was given to screening JGMV susceptibility, as it is believed that resistance gene for MDMV would be transferred from the tropical germplasm. The segregating generations were evaluated under JGMV pressure. Disease development was assisted by using susceptible forage sorghum and screening materials at different seasons and sites. Significant proportions of the segregating populations were rejected primarily due to their susceptibility to JGMV. We believe that the inbred lines developed from this project have reliable resistance against JGMV.

Resistance against other foliar diseases such as northern corn blight (turcicum leaf blight) and polysora rust (southern rust) was also screened. Inbred lines developed in this project were also thoroughly evaluated for various agronomic traits, particularly, plant stature and flowering time. It's important to develop inbred lines that are smaller in plant stature and quicker in flowering but productive. This will maximize resource use efficiencies such as water and fertilization. Quick flowering also allows growing more crops in a year, and provides flexibility in terms of the time of planting and harvesting.

Hybrids developed from the new series of inbreds showed considerable variability in terms of agronomic and quality traits. Grouping these hybrids based on principal component analyses revealed that hybrids with similar performances with tropical hybrid, Hybrix 5 and temperate/subtropical hybrids fell in different compartments (Fig. 1), indicating important agronomic and quality traits could be recovered after disease resistance was introduced. Line *per se* and F1 performance were significantly related for most agronomic and quality traits in sweet corn (Solomon, 2014) suggesting that careful choice of parental lines is important to produce commercially valuable F1 hybrids. Hence, choice of parental lines based

on optimum combination of agronomic, quality and disease resistance traits is vital to develop commercially acceptable hybrids. The tropical hybrid, Hybrix 5, appears to be very productive (Fig. 2a), however, larger plant size as indicated by higher plant height (Fig. 2b) and higher husk ratio (Fig. 2d) made it less efficient in terms of producing usable cobs without too much waste. The bigger plant size and higher biomass production obviously is associated with longer growing period, although, the range in days to flowering was not more than a week (Fig. 2c). It's interesting to note that from the limited crosses we made, by introgressing temperate and tropical germplasm, it was possible to reduce the growth duration, shorten plant height and reduce the husk weight ratios.

The commercial values of some of the lines that were developed in this project were assessed by creating experimental hybrids and evaluating the hybrids in an observation trial in comparison with commercial checks. Some of the best performing hybrids also showed better productivity in terms of marketable cobs especially when compared against the temperate/subtropical types (Goldensweet Improved and Garrison) (Fig. 3a). In terms of important agronomic traits such as plant height and flowering time significant progress has been made particularly when comparison was made between the new hybrids and the tropical hybrid (Hybrix 5) (Fig. 3b & c). Similarly, eating quality, particularly, tenderness was improved when compared to Hybrix 5 (Fig. 3e) indicating introgression of temperate germplasm can enhance the most critical quality parameter namely, tenderness.

Recommendations

This project is a preliminary work which aimed at developing parental lines that could be used to create commercially useful hybrids. Therefore, at this stage field demonstration will be irrelevant as more work is needed to create more hybrids and conduct replicated trials to assess adaptation and stability. However, the breeding nurseries and experimental hybrids were visited by Mr. Damien Courtier, breeder from Snowy River Seeds, our partner in the project.

The materials developed in this project exhibited excellent resistance against JGMV. The 'shuttle breeding' approach we followed also allowed exposure of the segregating generation to various environmental conditions and perhaps different pathogen races. Lines were selected based on their overall *per se* performance, therefore can have a good potential to generate commercially useful hybrids. Preliminary observations on the crosses made using limited combinations of the developed inbred lines showed that some crosses have the potential to combine good attributes both from the tropical and subtropical germplasm. However, the result was based on an unreplicated trial and without rigorous statistical tests. Therefore, it should only be considered as preliminary information.

The inbreds developed in this project can be used to create commercial hybrids by crossing lines from different pedigrees or crossing them to lines originated from different sources. Alternatively, they can be used as sources of useful traits such as disease resistance to improve other defective parental lines. Therefore, the germplasm created in this project is a good asset both for the collaborator seed company, Snowy River Seeds and the DAF breeding programs.

Scientific Refereed Publications

None

Intellectual Property/Commercialisation

The material produced from this project is co-jointly owned by DAF, HIA and Snowy River Seeds.

The next steps in the commercialization process are as follows:

- A Material Transfer Agreement is in place between DAF and Snowy River Seeds for 165 inbreds.
- Seed of approximately 165 inbreds will be planted in early November 2015, and pollination will occur at the end of December 2015-early January 2016.
- Observations on these inbreds e.g. disease resistances will be made by Snowy River.
- Five hybrid combinations (previously identified in the project as being possibly superior) will be generated from a planting of inbreds at Bowen RF in May 2016
- Hybrid seed produced from the May 2016 planting will be planted in larger plots for evaluation in spring 2016, when a decision will be made on which hybrids will be commercialised
- Some hybrids may also be produced by crossing inbreds from this project with Snowy River hybrids
- Royalties are payable from the project agreement. If two inbreds from the VG10105 project are used to produce hybrids, higher royalty levels are payable. If one inbred from the project is used as a parent, lesser royalties are payable.

References

- Abadassi, J., Hervé, Y., 2000. Introgression of temprate germplasm to improve an elite tropical maize population. Euphytica 113, 125-133.
- Brewbaker, J. L., 2003. Corn production in the tropics: the Hawaii experience. University of Hawaii, Manoa.
- Evensen, K. B., Boyer, C. D., 1986. Carbohydrate composition and sensory quality of fresh and stored sweet corn. Journal of American society for horticultural science 111, 734-738.
- Franco-Dixon, M. A., 2009. An Ex-Post Economic Analysis of the Hybrix5 Sweet Corn Breeding Program in Queensland. Annual Conference of the Australian Agricultural and Resource Economics Society, Australia, Cairns.
- Gates, D. W., Gudauskas, R. T., 1969. Photosynthesis, respiration, and evidence of a metabolic inhibitor in corn infected with maize dwarf mosaic virus. Phytopathology 59, 575–580.
- Gregory, L. V., Ayers, J. E., 1982. Effect of inoculation with maize dwarf mosaic virus at several growth stages on yield of sweet corn. Plant Dis. 66, 801–804.
- Hallauer, A. R., Carena, M. J., 2014. Adaptation of tropical maize germplasm to temperate environments. Euphytica 196, 1-11.
- Jones, M. W., Redinbaugh, M. G., Louie, R., 2007. The Mdm1 locus and maize resistance to Maize dwarf mosaic virus. Plant Dis. 91, 185–190.
- Kwabiah AB. (2004) Growth and yield of sweet corn (Zea mays L.) cultivars in response to planting date and plastic mulch in a short-season environment. Scientia Horticulturae 102: 147-166.
- Lertrat, K., Pulam, T., 2007. Breeding for increased sweetness in sweet corn. International Journal of Plant Breeding 1, 27-30.
- Olsen, J. K., Blight, G. W., Gillespie, D., 1990. Comparison of yield, cob characteristics and sensory quality of six supersweet (sh₂) corn cultivars grown in a subtropical environment. Aust J Exp Agric. 30:387–393.
- Pataky, J. K., Williams II, M. M., Headrick, J. M., Nankam, C., du Toit, L. J., Michener, P. M., 2011. Observations from a quarter century of evaluating reactions of sweet corn hybrids to disease nurseries. Plant Dis. 95, 1492–1506.
- Solomon, F., 2014. Pre-emptive breeding to combine superior eating quality in tropical super sweet corn with resistance to major diseases. Final Report for VG7198, Horticulture Australia.
- Solomon, K.F., Martin, I., Zeppa, A., 2012. Genetic effects and genetic relationships among shrunken (sh2) sweetcorn lines and F1 hybrids. Euphytica 185:385-394.
- Sweet corn information Kit., 2005. Reprint-information current in 2005. Queensland Government.

Acknowledgements

The strategic co-investment funding contributed by Snowy River Seeds Ltd to enable this work to proceed is gratefully acknowledged.

The contribution of the following Key Research Personnel is also gratefully acknowledged:

Veronique Keating	Field Technician, Kairi Research Facility
Charlie Nastasi	Research Assistant, Kairi Research Facility
Ian Martin	Senior Researcher, Post retirement associate, Kairi Research Facility

Appendices

APPENDIX 1

Trial Results from Bowen, 2015 Planting date: 22nd April, 2015 Evaluation date: 13th July, 2015 Checks: H5, Galaxy, Astronaut Trial layout: modified augmented design, 5.5m plot length, single row Results: H5



Galaxy



	s	t	e	7	7	-	0	0	7	-	-	+	~	*	×	р	+	-
		es es	യ	c	c	-	0	0	0	0			Φ	Ø	Ø	Ø	Ø	-
2015 BOWEN IT	_	s	-	s	s	ß	σ	σ	٤	8	σ	σ	-	7	-	-	×	8
cross/ pedigree:	*	s	1	*	*	Q	1	1	2	1	-	1	5	2	5		-	<
11161	52	49	1	1	2	2	19.9	5.6	17	3	4	3	2	13	2	3	2	2
SRSH 292* X SRSH 99																		
comments: good length excellent t	ipfil	l goo	d rowi	ing ni	ce pla	ant 1	ooks goo	d										

Astronaut



	00	-	Ð	7	7	-	0	0	7	7	-	-	~	*	*	P	-	-
	N <u>12</u> 2	2	20	c	c	_	0	0	0	0	-	23	Ð	Φ	Ð	Ø	Ø	_
2015 BOWEN IT	-	cn	7	s	S	a	a	σ	×	٤	Ρ	p	7	7	٦	7	×	a
cross/ pedigree:	*	0	â	~	*	9	1	1	7	1	-	1	-	7	2		1	<
11312	52	50	1	1	2	2	17.7	5.4	19	3	4	3	2	13	3	З	3	2
SRSH 99 X SRSH 291*	1																	
comments: short excellent tipfill	good	kern	el sty	yle pa	le lo	oks g	ood											

ZHY089BA



	60	-	0	ч	7	-	0	C	7	7	+	t	*	*	×	O	-	-
	-	a	8	c	c	-	0	0	0	0			Φ	æ	e	e	Ð	\simeq
2015 BOWEN IT		60	2	s	S	2	ъ	0	W	W	P	p	-	-		-	×	8
cross/ pedigree:	*	(n	E.	*	*	9	E	1	5	ा	Ť.	1	5	5	5		-	<
75 x 3	49	48	1	0	2	2	19.3	5.4	18	2	5	3	2	14	3	4	3	2
D10 x SW10_109-4-3-B2 / D1 x SW3_	3-8-1-B1																	
comments: a little short exc	ellent tip	fill	ok qua	ality	margi	nal h	sp worth	anothe:	r look									

ZHY104BA



	60	-	e	7	7	-	0	0	-	-	-	-	*	*	x	P	-	-
		ß	8	=	=	-	0	0	0	0		-	Φ	Ø	Ð	Ð	e	
2015 BOWEN IT	-	s	-	S	5	2	0	ъ	×	W	P	p	-	7	~	7	×	2
cross/ pedigree:	*	s	T.	*	*	g	1	1	D	1	f	1	D	5	Þ	1775	t	<
76 x 21	52	49	1	1	2	1	17.7	5.3	15	3	5	3	2	13	2	3	2	2
D1 x SW3_3-7-1-B2-B3 / D2 x SW7	18-2-2-B3	fill	margin	al hs	n goo	d pla	nt style	good p	lant he	alth	worth	anot	her l	ook				

ZHY194BA



	^o	-	e	T	7	-	0	0	-	7	~	+	~	~	*	P	-	-
	-	8	20	=	E	-	0	o	0	0		277	Ð	Ø	Ð	e	0	-
2015 BOWEN IT	<u> </u>	00	-	s	\$	2	0	σ	W	W	q	P	-		-	-	×	2
cross/ pedigree:	*	S	1	*	*	9	1	1	2	Ę.	-	1	2	٦	⊐	-	-	<
44 x 3	52	49	1	1	1	1	19.6	5.1	14	3	3	3	3	13	2	3	2	2

ZHY197BA



a c	L S	с . м	- 0	0	0	0	_	- <u></u> -	Φ	Φ	Ð	0	Ð	-
- v	\$	10	100											
		0,	m 0.	9	\$	W	σ	σ	7	~	7	~	×	œ
	*	*	o	j.	D	Ĕ	-	1	2	5	۵.	-		<
L 5	5	2	2 19.5	5.3	16	4	5	3	2	14	2	3	2	2
		. 5	5 2	5 2 2 19.5	5 2 2 19.5 5.3	5 2 2 19.5 5.3 16	5 2 2 19.5 5.3 16 4	x x 0 1 3 1 5 2 2 19.5 5.3 16 4 5	5 2 2 19.5 5.3 16 4 5 3	5 2 2 19.5 5.3 16 4 5 3 2	x <td>5 2 2 19.5 5.3 16 4 5 3 2 14 2</td> <td>x x<td></td></td>	5 2 2 19.5 5.3 16 4 5 3 2 14 2	x <td></td>	

ZHY224BA

	s	-	Ð	ъ	ч	-	0	0	7	-	+		*	*	X	p	-	-
		cu	3	⊑	=	-	0	0	0	0			Φ	Φ	Φ	Ð	æ	-
2015 BOWEN IT	_	co.	7	s	ŝ	23	0	σ	×	W	q	q	7	~	٦	7	×	8
cross/ pedigree:	~	ŝ	i.	ѫ	*	g	T	1	Þ	Ű.	-	1	5	⊐	5	-	÷	<
75 x 28	49	48	1	1	1	2	18.0	5.4	18	3	4	3	2	13	2	2	3	2
D10 x SW10_109-4-3-B2 / D3 x SW3_2	25-4-2-B2	2	122		8	a 1					20							

comments: a little short good tipfill good quality marginal hsp looks quite good worth another look

All Results:

Key: silk - days from planting to 50% mid silk Tassel - days from planting to 50% mid pollen shed

Ear position - position of ear in relation to stem 1=beside stem, 3= perpendicular to stem

Husk cover – amount of cover from tip to top of cob 0= exposed, 5 = 5 fingers wise

Husk tightness - how tight husk is around tip 1- very loose, 5= very tight

Flag – length of flag leaf 1= none to very short, 5= 25cm long

Cob length - length in cms Cob width - width in cms

Row number - number of rows around cob

Row style - straightness of rowing 1= very jumbled, 5 = all very straight

Tipfill – kernels right to the tip of the cob 1 = blanking greater than 3cms, 5 = filled to the very tip

Tipstyle – shape of cob 1 = very tapered, 5 = very cylindrical

Kernel colour – 1 = very pale, 5 = deep orange colour Kernel depth – depth in mm

Kernel style - shape of kernel 1 = very blocky, 5 = tapered and refined

Pericarp, Flavour, Texture - subjective quality parametres 1-5 rating 1= poor, 5= excellent

APPENDIX 2



Plate 1. Effects of JGMV infection which is causing severe stunting of susceptible plants.



Plate 2. Forage sorghum used as JGMV spreader rows within sweet corn blocks.