Pre-emptive breeding to combine superior eating quality in tropical super sweet corn with resistance to major diseases

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Final Report

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HAL Project VG07198

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This report presents the process and outcomes of a five year project, which employed genetics and breeding approach for integrating disease resistance, agronomy and quality traits that enhances sustainable productivity improvement in sweet corn production. The report outlines a molecular markers based approach to introgress quantitative traits loci that are believed to contribute to resistance to downy mildew, a potentially devastating disease that threatens sweet corn and other similar crops. It also details the approach followed to integrate resistances for other major diseases such as southern rust (caused by *Puccinia polysora* Underw*)*, Northern Corn Leaf Blight (*Exserohilum turcicum)* with improved agronomy and eating quality. The report explains the importance of heterosis (hybrid vigour) and combining ability in the development of useful sweet corn hybrids. It also explains the relevance of parental performance to predict its breeding value and the performance of its hybrids.

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2 Media summary

Australia's sweet corn industry has huge potential to expand as long as it remains competitive in the international market. Tropical and subtropical environments are major areas of expansion. However, germplasm grown in such environments have poor eating quality compared to temperate types. Moreover, there are a number of diseases that can cause significant loses both in terms of yield and quality.

Selection for improved tenderness and flavor in tropically-adapted super sweet corn germplasm in the Kairi (North Queensland) breeding program has resulted in improvements in eating quality. Significant progress has also been made in terms of developing resistance to the most prevalent diseases of super sweet corn. Some of these diseases can cause as much as 75% yield losses in sweet corn. In some Asian countries downy mildew is reported to cause as much as 100% loss. Considering the increased difficulty to control by fungicide, breeding resistance remains to be the most feasible option. Downy mildew is a quarantinable disease presently not in Australia. If it finds its way into this country, it will be very damaging to the industry. Therefore, pre-emptive breeding is required to protect the local industry from any potential threats. To ensure the competitiveness of Australian sweet corn industry, it's necessary to develop hybrids that are not just productive but must also be profitable to grow.

Horticulture Australia Ltd in partnership with Queensland Department of Agriculture Fisheries and Forestry, supported a five year project aimed at the development of effective sources of resistance to the most important tropical diseases, and to develop hybrids that are superior to the current major tropical hybrid, Hybrix 5 in terms of productivity, quality and disease resistance.

In this project downy mildew resistance has been transferred from imported maize germplasm to local sweet corn using conventional breeding supported by use of molecular markers. Resistances to other leaf diseases were also introduced to the newly developed sweet corn lines.

More than 100 new hybrids were created and tested in different seasons and environments. A number of hybrids with distinct and improved yield, agronomy, disease resistance and quality traits were identified. Two extensively tested hybrids have been identified as candidates for commercial release. These hybrids showed excellent resistance to leaf blight and other disease. The hybrids showed better productivity (28% more marketable cobs than Hybrix 5), and better tenderness. One of the hybrids has smaller plant architecture and good stand establishment. The hybrids have been handed to our commercial partner, Pacific Seed Ltd. for semi-commercial testing.

3 Technical summary

The sweetcorn breeding program has developed superior inbred lines by incorporating the *shrunken (sh2)* gene into locally-adapted germplasm. This created the opportunity to identify superior hybrids with wider adaptation to tropical Australia and Asian countries. However, wider adoption of these hybrids and our success in marketing these hybrids will very much depend on our ability to incorporate gene(s) or QTLs that confer resistance against the major foliar diseases threatening the sweet corn industry. In particular the longer term development of the industry in tropical areas of Australia will require high levels of resistance to southern rust, also known as Polysora rust. Heavy southern rust infections may result in stunting, incomplete ear tip fill, and pustules on ear husks, reducing yield and marketability.

Downy mildew occurs predominantly in tropical and subtropical regions of Asia such as China, India, Indonesia, Pakistan, Thailand and Philippines. Genetic resistance is the most cost-effective means of controlling downy mildew and has also become essential since the emergence of races of mildew resistant to the systemic fungicide, metalaxyl. Progress in this aspect is pivotal to protecting the local industry from potential threats. Demonstrable potential for Downy mildews to evade the quarantine barrier mounted by biosecurity services in Australia is shown in the invasion by a relatively innocuous species of the disease, Java downy mildew that was first noted in the Northern Territory and has spread to Queensland on the wild sorghum species, *Sorghum plumosum*.

Five QTLs have been reported to explain a significant proportion of the phenotypic variance for downy mildew resistance. The most important ones were those located on chromosome 6. SSR markers were identified to track the inheritance of the QTLs that are linked to downy mildew resistance. SSR markers (*mmc*0241 and *bnlg*1154) were used to introgress downy mildew from downy mildew resistant maize lines. Three generations of marker assisted selections followed by three generations of selfing were employed to develop potentially downy mildew resistant lines. The lines have also been selected for agronomy and eating quality.

Downy mildew converted lines were crossed to southern rust resistant lines in an effort to combine resistances against both downy mildew and southern rust. Segregating generation was evaluated following pedigree selection procedures and lines with acceptable quality and agronomy were developed.

The potential of disease resistant lines in commercial hybrid production were assessed by generating more than 100 new hybrids. The hybrids were evaluated in different environments and seasons. Most of these hybrids showed improved disease resistance and eating quality. Two hybrids appear to have better quality and productivity compared to Hybrix 5.

Development and testing of new hybrids requires substantial time and resources. If there are ways to accurately predict the best possible cross combinations, development of hybrids becomes more efficient and less costly. We have assessed the potential of line *per se* performance and genetic distances estimated based on markers or agronomic traits to predict hybrid performance. None of these were able to accurately predict hybrid performance. Thus, creating and testing of hybrids appears to remain the only sure way to determine the commercial use of the newly developed lines.

4 General Introduction

In 2009/10, an estimated 66,700t of sweet corn was produced in Australia, 90% in NSW and QLD. The overall domestic retail value of fresh sweet corn was estimated at $107M (Horticulture Australia, 2011). The farm gate value of sweet corn produced in Queensland was predicted to reach $36M AUD in 2012/13 (Queensland's agriculture strategy, 2013). Most Australian grown sweet corn is still sold on the domestic market, but there is growing interest in the export markets of South-East Asia. Just over 40% of the Australian grown sweet corn was directed to some form of processing. At the same time, 17,220 tonnes of processed sweet corn products were imported (Horticulture Australia, 2011).

It appears that there are strong domestic and international drivers that encourage further expansions. However, growth of the industry ultimately depends on its competitiveness in the international markets. The processing industry particularly faces stiff competition from countries where labour is relatively cheaper.

In mid 90's the development and deployment of tropical sweetcorn hybrid (Hybrix 5) with the support of Horticulture Australia Ltd. has been instrumental for 241% increase of sweet corn production in 2005 compared to 1993 production (Franco-Dixon, 2009). This has been achieved by expanding the area of production by 150% during the same year (Franco-Dixon, 2009).

The most important feature that was critical for the success of Hybrix 5 (H5) was its excellent resistance against Johnson Grass Mosaic Virus (JGMV). Most temperate types of hybrids are very susceptible to JGMV. JGMV development is very severe in late plantings after December. The introduction of H5 permitted extension of planting windows well into March (Martin, 2002). Nevertheless, the eating quality, particularly tenderness and flavour of H5 are inferior compared to temperate hybrids. The acceptance of H5 has been restricted to times when temperate hybrids can't be grown successfully (Martin, 2002). Its huge plant architecture and long growing period also made it unsuitable for higher density and quick turnover of sweet corn crops. Noting these shortcomings, Horticulture Australia has supported projects which aimed at improving the agronomy and eating quality of tropical germplasm to facilitate the development and deployment of improved tropical hybrids. The purpose of this project was therefore to develop hybrids that excel H5's disease resistance, agronomic and eating qualities. It also aimed at generating inbred lines that can used as sources of resistances against major diseases.

5 Marker assisted backcrossing

5.1 Introduction

Philippine downy mildew of maize (PDM), caused by the oomycete *Peronosclerospora philippinensis* and brown stripe downy mildew (BSDM) caused by *Sclerophthora rayssiae* var. *zeae* are destructive diseases. In the Philippines, losses on sweet corn can be as high as 100% (George et al., 2003). Disease severity is highest in areas with a tropical climate that receive high rainfall. Downy mildew is considered to be a quarantined disease in Australia. However, if introduced it would have devastating effects on various industries including sweet corn. Genetic resistance is the most cost-effective means of controlling downy mildew and has also become essential since the emergence of races of mildew resistant to the systemic fungicide, metalaxyl.

Four countries, India, Indonesia, Thailand and Philippines have undertaken a joint QTLs mapping project aimed at identifying downy mildew resistance genes (George et al., 2003). Five QTLs have been reported to explain a significant proportion of the phenotypic variance for downy mildew resistance. The most important ones were those located on chromosome 6. SSR markers were identified to track the inheritance of the QTLs that are linked to downy mildew resistance. Effective resistance can be developed if these QTLs are introgressed into locally adapted germplasm. Progress in this aspect is pivotal to protecting the local industry from potential threats. The potential for Downy mildews to evade the quarantine barrier mounted by biosecurity services in Australia is demonstrable in the advent of a relatively innocuous species of the disease, Java downy mildew that was first noted in the Northern Territory and has spread to Queensland on the wild sorghum species, *Sorghum plumosum*. The purpose of this research was therefore to pre-emptively breed for downy mildew resistance using a marker assisted crossing approach.

5.2 Materials and methods

Two maize donor lines obtained from Philippines were used as sources of QTLs presumably linked to downy mildew resistance (DMR). Sequence information for five SSR markers co-localized with major QTLs associated with DMR (George et al., 2003) were obtained from the maize genome (http://www.maizegdb.org/ssr.php). Details of SSR profiling were described in (Solomon et al., 2012a). To assess the absence and presence of DMR QTLs in the local sweet corn lines and breeding populations, DNA samples from sweet corn and donor maize lines were amplified using five SSR markers (*mmc*0241, *phi*078, *bnlg*1702, *nc*013 and *bnlg*1154). These markers were mapped to chromosome six and were reported to be potential markers to introgress DMR (George et al., 2003). The maize lines were imported from Philippines, and are believed to be resistant to downy mildew (DM). The 10 sweet corn lines were developed from different breeding pools by Queensland Department of Agriculture, Fisheries and Forestry (DAFFQ) sweet corn breeding program. The six sweetcorn breeding populations; A=Pro1, B=Pro2, C=Pop15, D=Pop16, E=Pop21 and F=Pop24 represent three populations from the two breeding groups (heterotic pools).

Simplified backcross procedure was followed to convert sweet corn lines to possible DMR lines (Figure 1). In this procedure, F1 and BCF1s were assayed for the presence or absence of bands corresponding to the DMR QTL markers (Figure 2). Crosses possessing bands similar to the maize donor lines were selected. To eliminate maize only shrunken sweetcorn type kernels were selected (Figure 1). Three backcrosses without selfing were carried out. BC3 derived lines were selfed for three generations, and lines were evaluated for their eating quality, agronomy and phenology.

5.3 Results

The markers profile of sweet corn lines and breeding populations obtained from the DAFFQ breeding programs showed that four inbreds (1, 3, 6 and 7) and population D (Pop16) had positive amplification for marker *mmc*0241. Marker *phi078* which was detected only in one of the maize lines (M12) was also present in some sweet corn lines (1, 3 and 6) as well as population F(Pop24). Marker *bnlg*1702 was present in both maize lines, but none of the sweet corn lines except for line 2 and 10, which showed the presence of this marker. However, it was present in all populations except for population D (Pop16). Marker *nc013* was present in maize 11 and sweet corn line 5. It was also present in all populations except for

population A and D. *Bnlg1154* was not detected in any of the sweet corn lines or populations (Table 1).

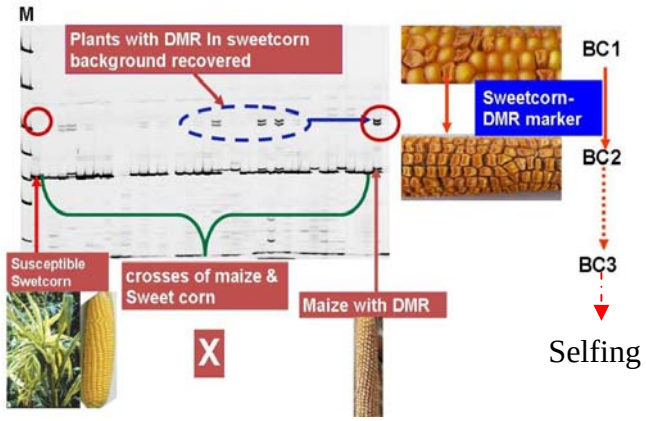

Figure 1. Schematic view of introgressing DMR from maize to sweet corn lines.

Table 1. Marker profiles of Maize donor lines (M11 and M12), sweet corn lines (1-10) and six sweet corn breeding populations (A-F).

Marker(bp)	**M11**	**M12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**A**	**B**	**C**	**D**	**E**	**F**
mmc0241(175)	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0
phi078(125)	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1
*bnlg1702(*175)	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1
*nc013(*100)	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1
Bnlg1154(150)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

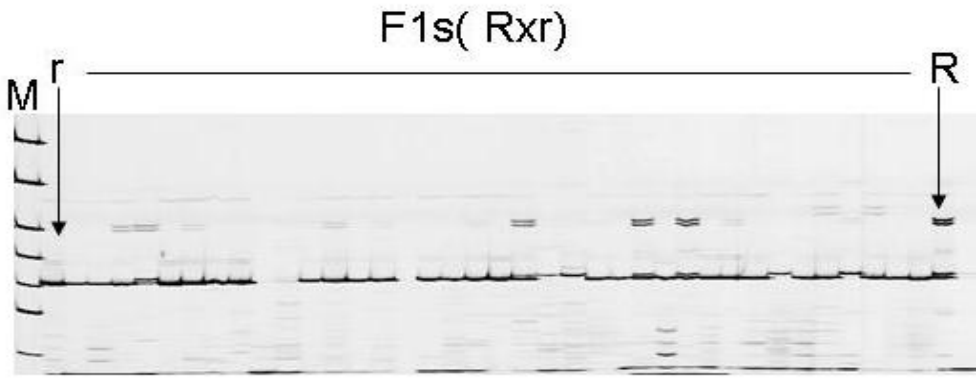

Figure 2. Example of identification of possible downy mildew resistant F1s using *Bnlg1154.* M=25bp fragment size marker, r= recurrent sweet corn parent, R= DMR donor parent (maize) and F1s= Crosses of sweet corn(r) and maize (R). PCR products were visualized using 4.5%(v/v) denaturing polyacrylamide (19:1 Acrylamide:Bis) gels with 10 TBE on Gelscan system.

To identify individuals with possible DM resistance, we used only two markers (*mmc0241* and *Bnlg1154*) since they were present in both maize lines and they were not very prevalent in our breeding materials. Only lines with clearly amplified bands (Figure 2) were selected. After three generations of marker assisted selections, individuals were selfed. During the process lines uniformity, eating

quality and other agronomic traits were also assessed. Ten lines were created from crosses involving two sweet corn lines and two maize lines.

5.4 Discussion

Markers *mmc0241, phi078, bnlg1702, nc013 and Bnlg1154* are linked to chromosome 6 and are believed to flank QTLs associated to DMR (George et al. 2003). According to George et al. (2003), the major QTL on chromosome 6 is located in a region containing other QTLs for disease and pest resistances including resistance to southwestern corn borer (SWCB), resistance to *Fusarium moniliforme*, maize dwarf mosaic virus (MDMV), and wheat streak mosaic virus (WSMV). The markers' profiling showed interesting diversity in the F1s and backcross generations. Interestingly some local materials seem to have DMR based on the markers profile. The presence of bands similar to the donor parents may indicate possible DM resistance. However, the presence of bands may not be due to identity by decedent since the mapping population in which the QTLs were located derived from bi-parental crosses. It's likely the donor parents were not the progenitors of the mapping population. Therefore, validation of the results is necessary by field testing the lines in an overseas location where DM is prevalent.

6 Integrating disease resistances with improved agronomy and quality

6.1 Introduction

Many diseases affect the quality and productivity of sweet corn depending on the location and seasonal conditions. The most important ones in tropical and subtropical Australia include southern rust and turcicum leaf blight (commonly called, Northern Leaf Blight or NLB).

Southern rust, also commonly known as Polysora rust, is caused by *Puccinia polysora* (Underw). Southern rust affects both field maize and sweet corn. In susceptible varieties of sweet corn, up to 50% yield loss can occur (Plant disease, 1991). Southern rust is associated with high temperatures, high relative humidity, and heavy rainfall. Apart from its effect on yield, it also causes significant damage to quality factors such as ear length, ear diameter, percentage of moisture, and percent soluble solids in the kernel. Heavy southern rust infections may result in stunting or even death of the plant resulting in incomplete ear tip fill, and pustules on ear husks, reducing yield and marketability. Patterns of genetic resistance to southern rust can be race-specific or general (Bailey et al., 1987). However, general resistance is thought to have played an important role in controlling the disease (Hu et al., 1997). Northern leaf blight (NLB), caused by *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs), is a prevalent disease of sweet corn (*Zea mays* L.). Reductions in the yield of sweet corn have been associated with severe necrosis or chlorosis of leaves in the upper two-thirds of the canopy (Pataky et al, 1998). Pataky et al. (1998) reported 2 to 8% reduction in yield for each 10% increase northern leaf blight infection. In some countries like India, up to 90% yield reduction in susceptible maize varieties has been reported. Dominant *Ht* of genes conferring monogenic resistance were described (Welz and Geiger, 2000).

Reciprocal recurrent selection has been shown to lead to the fixation of differentially selected alleles at molecular levels (Solomon et al., 2010). Recurrent selections for agronomic and eating quality traits simultaneously also help accumulate desirable phenotypes in the breeding pools. The purpose of this work

was therefore to integrate genetic resistance against economically important diseases including southern rust, Northern leaf blight (NLB) and downy mildew and develop hybrids with acceptable quality and agronomic characteristics following recurrent selections approach.

6.2 Materials and methods

6.2.1 Recurrent selection

Pro1 and Pro 2 gene pools were developed originally from tropical sweet corn with *shrunken 2* genetic backgrounds. They have undergone a number of selection cycles based on performance of S0 selfs. Every season 200 to 500 S0 were first selected for resistance against diseases including southern rust, NLB and other leaf and stem diseases. Only resistant types were self pollinated. Eating quality such as tenderness and flavour were assessed on half of the self-pollinated cobs at 21 to 24 days after silking. The remainders of the selfed cobs from S0 with acceptable eating quality were harvested when the cobs dried out. Selected S1s were then used to constitute the next selection cycle. To develop pure breeding lines, about 10% of the most promising S1 lines were subjected to pedigree selection for five generations following ear to row selection methods. Apart from disease resistance, agronomic traits such as plant uniformity, height, leaf architecture, and flowering times and tillering were considered as selection criteria.

6.2.2 Generation of F1 hybrids

The commercial values of inbred lines developed using recurrent and pedigree selection were evaluated by developing single cross hybrids. Single cross F1 hybrids were generated by crossing lines from the two heterotic pools. Fourteen hybrids were created using S5 inbreds from selection cycle one of Pro 1 and Pro2 populations. In order to assess the potentials of DM resistant lines generated through marker assisted backcrossing, a total of 55 hybrids were produced using S3 downy mildew resistant inbreds derived from a cross of maize and sweet corn lines and S4/5 lines from Pro 1 population and Pro2. All Pro 1 and 2 lines used in the crossing have promising resistance to northern leaf blight and southern rust resistance.

6.2.3 Evaluation of F1 hybrids

Experimental hybrids and a check hybrid, Hybrix 5 (H5) were planted at Gatton and Hermitage in double rows in 5 meter long plots. Disease observation plots were also established at Kari when necessary. Experimental hybrids were not replicated but the checks were replicated to estimate error variance. 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). Bite tests were used to rate flavour (sweetness) and tenderness. On scale of 1-10, 1 was given for grassy flavour and 10 for very sweet and juicy flavour. Kernel tenderness was also rated on a scale of 1-10 where 1 = very tough and chewy and 10 = very tender and crispy. In Table 2, are ratings for NLB= northern leaf blight (turcicum leaf blight) and PR= polysora rust (or southern

rust). Disease rating was in 1-9 scale; 1= complete resistance and 9=complete susceptibility.

6.3 Results

6.3.1 Recurrent selection

Two cycles of recurrent selections were completed on Pro 1 and 2 populations. The number of individuals grown and selected varied depending on the level of disease incidence and other eating quality traits. The general level of southern rust resistance was much higher than that of a temperate hybrid used in the guard rows. Some individual plants had almost no rust infection and are competitive with the levels in the more resistant maize hybrids. As a rule individuals with susceptibility scores > 3 in 1 to 9 scores were rejected. A total of 10 S5 lines were developed out of 660 S2 in PRO1- C7 with 98% of the lines rejected at various stages due to disease, agronomy and quality problems. Similarly, from the corresponding population, PRO2-C7, only 25 S5 lines were created from a total 840 S2 lines.

6.3.2 Performance of hybrids developed from the latest inbred series

The 14 hybrids along with check, Hybrix5 (H5), were evaluated at Gatton, Hermitage and Kairi for a number of agronomic, quality and disease resistance traits. Traits that were recorded including tillering, stand uniformity, plant architecture, number of marketable cobs, cob weight, flavour, tenderness and resistance to NLB **(**turcicum leaf blight) and southern rust (Polysora rust). The most important productivity, agronomy and disease resistance indicators are summarised in Table 2. Nearly 53% of the hybrids tested showed commercially acceptable stand uniformity better than the check hybrids. Except for two hybrids all of these hybrids did not show a significant tillering problem. Almost all of the hybrids showed better resistance to NLB compared to the check, H5. They also showed significantly better resistance to southern (or Polysora) rust (PR) (Table 2). However, in terms of productivity most of them showed lower number of marketable cobs. Averaged over Gatton and Hermitage, only two hybrids showed about 20% more marketable cobs compared to H5. At Hermitage only one hybrid (2368/2352) showed better tenderness rating compared to H5. At Gatton however eight hybrids showed significantly better tenderness compared to H5. Across both Gatton and Hermitage, 6 hybrids showed significantly better flavour compared to H5 (Table 2).

6.3.3 Performance of hybrids derived from DM and PR resistant lines

54 and 52 hybrids were tested at Gatton and Hermitage, respectively. They were compared against the check H5 at both sites. Agronomic and quality traits were recorded. Most S3 hybrids had a major tillering issue. The problem was worse at Gatton due to cooler temperature that occurred during the vegetative growing period. Nevertheless, similar signs of tillering problem were also observed at Hermitage. About 47% of the tested hybrids were better than, or as good as H5 in terms of uniformity and acceptability for commercial production. No sign of disease or northern leaf blight (NLB) infection was noted on the hybrids tested although some level of NLB infection was found on H5. 88% of the hybrids had more marketable cobs compared to H5, and the increase in number of marketable cobs was as high as 79% over H5. However, only about 33% of the hybrids showed greater than 10% improved flavour compared to the check, H5. 46% of the hybrids also showed greater tenderness (at least 10% better) compared to H5. Twelve hybrids were found that combined better number of marketable cobs with improved favour and tenderness (Table 3).

Number of marketable cobs between Hermitage and Gatton were fairly consistent with $R^2 = 0.52$ accounting for 52% of the total variation. The slope and the intercept for the regression analysis were also different from zero (Figure 3a). Performance for flavour however had no relationships at all (Figure 3b). Relationship between tenderness ratings at Gatton and Hermitage though statistically significant, was very weak. The relationship explained only 7.9% of the total variation (Figure 3c).

Table. 2. Some important agronomic and disease resistance scores for new series of hybrids created using S5 lines developed under recurrent and pedigree selection programs.

	MC (number cobs/plot)		Tenderness (1-10 rating)		Flavour (1-10 rating)		NLB	PR
Cross	Gatton	Hermitage	Gatton	Hermitage	Gatton	Hermitage	Gatton	Kairi
Hybrix5	18	16	6	5	6	5	5	4.5
2351/2370	12	19	7	5	7	7	1	2
2343/2363	22	19	7	5	8	7	1	1
2352/2370	6	13	7	5	6	7	1	3
2372/2344	10	9	8	5	7	6	1	2
2358/2330	12	3	7	4	7	4	1	1
2357/2344	7	NA	8	5	7	5	1	2
2368/2341	4	10	6	5	8	5	1	3
2363/2354	19	20	6	5	7	6	1	1
2368/2352	21	21	6	6	7	7	1	1
2370/2344	15	18	7	5	7	5	1	1
ProSC418	18	10	6.5	5.5	6	4.5	1	3
ProSC413	21.5	13.5	7.5	5	7	5.5	1	4
Mean	14	14	7	5	7	6	1	2

NLB= northern leaf blight (turcicum leaf blight) and PR= polysora leaf rust (southern rust). Disease rating is based on 1-9 scale, where 1=excellent resistance and 9=extremely susceptible. NA=not available.

Table 3. Across sites performance of hybrids developed from DM and PR resistant lines.

				Percentage over Hybrix 5		
Id	**MC**	**Flavour**	**Tenderness**	**MC**	**Flavour**	**Tenderness**
2345/2253	43	6	6	79	0	17
2342/2261	42	6	5	75	5	-2
2276/2336	41	5	6	71	-9	22
2349/2276	41	6	5	71	0	-7
2352/2262	40	5	5	67	-9	-12
2341/2256	40	6	6	65	14	12
2347/2282	40	6	6	65	9	7
2279/2347	39	6	4	63	0	-17
2334/2273	39	6	6	60	9	17
2329/2276	38	5	6	58	-5	17
2351/2257	38	6	5	58	9	-7
2354/2270	38	7	6	58	18	17
2353/2258	38	6	5	56	0	-2
2353/2261	38	6	5	56	14	-2
2265/2344	37	6	7	54	14	27
2326/2283	37	6	6	54	5	17
2351/2279	37	6	5	54	5	-7
2353/2259	36	4	4	50	-27	-22
2330/2264	36	7	6	48	27	17
2334/2274	36	6	7	48	14	27
2354/2282	36	5	5	48	-9	-2
2268/2344	35	7	6	46	18	17
2279/2353	35	6	5	46	14	-12
2254/2342	35	7	7	44	18	27
2347/2280	35	5	5	44	-5	2
2264/2350	34	6	6	42	0	12
2352/2259	34	6	6	42	9	7
2350/2282	34	6	6	40	5	12
2270/2342	33	6	6	38	5	17
2344/2255	33	6	6	38	14	12
2345/2257	33	6	6	38	5	12
2259/2346	33	6	6	35	5	22
2259/2342	32	7	6	33	27	17
2346/2256	32	5	5	31	-5	-2
2353/2253	32	6	5	31	14	2
2352/2282	31	5	5	29	-5	-2
2275/2341	31	6	6	27	9	12
2351/2282	31	6	5	27	14	2
2280/2341	30	6	5	23	9	2
2338/2253	30	7	7	23	18	27
2349/2255	29	5	5	21	-5	-7
2349/2282	29	7	5	21	23	2
2354/2272	29	6	5	21	0	-7
2276/2353	29	7	7	19	18	27
2281/2353	29	6	5	19	5	2
2347/2274	28	6	5	17	5	2
2269/2344	28	6	6	15	14	12
2336/2259	27	5	6	10	-5	7
2341/2258	23	6	7	-4	0	27
2338/2273	21	6	6	-15	14	7
2352/2270	18	6	6	-27	0	7
2348/2282	14	6	5	-42	9	-12
2336/2261	7	5	6	-71	-9	7
2337/2253	5	6	6	-81	0	17
Hybrix 5 (H5)	24	6	5			

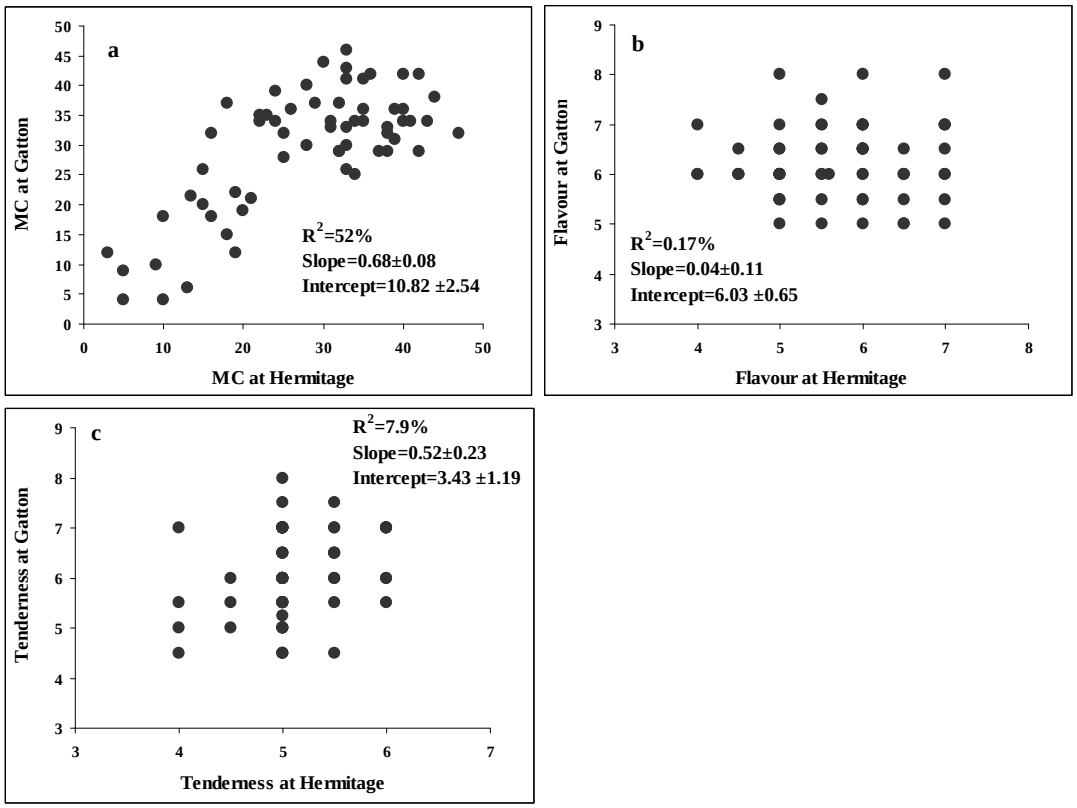

Figure 3. Correlation between the performance at Gatton and Hermitage for number of marketable cobs (MC) (a), flavour (b) and tenderness (c) for new generation of hybrids evaluated.

6.4 Discussion

There are a number of agronomic traits that determine the commercial feasibility of a hybrid. Number of marketable cobs depends on the size and the appearance of individual cobs. It is an important varietal selection criterion. Many experimental hybrids, particularly, crosses derived from DMR converted lines appear to be productive with yield advantage as high as 79% over the check, Hybrix 5 (H5). Some of these hybrids also showed significantly better eating particularly, tenderness and flavor than H5. However, none of the hybrids tested showed significantly quick flowering and physiological maturity compared to H5. In many sweet corn growing environments quick flowering and physiological maturity are vital to ensure quick turn-around of hybrids and flexibility in terms of planting and harvesting schedule. Some of the hybrids developed from the latest series of inbreds from Pro1 and 2 populations were quite smaller compared to H5 albeit this was achieved with reduced number of marketable cobs. Growers prefer smaller plant stature and low tillering so that higher density planting would be feasible. It's also important to note that the observed comparison was not based on replicated and multi location trials. Comparatively, number of marketable cobs (MC) showed consistent performance between sites indicating test result at one site may help assess the potential of the hybrids. However, significant intercept and relatively smaller R^2 value (Figure 3a) indicated that performance at one site may not accurately predict the potential of the hybrids. The quality traits of flavour and tenderness showed more inconsistent performance between sites indicating multi locations testing is needed to accurately assess the potential of the hybrids. Hence,

additional work is needed to further validate results and assess the stability of hybrids performance across different sites.

7 Combining ability of diverse sweet corn lines for yield and quality traits

7.1 Introduction

Combining ability is a measure of the relative ability of a genotype to transmit its performance to its F1 hybrids. Combining ability analysis helps estimate the breeding value of a parental line. The variance due to general combining ability (GCA) gives an indication about the roles of additive gene actions. Variance due to specific combining ability (SCA) provides an estimate of the effect of non additive type gene actions. Heterosis is the superior performance of the hybrids over the parental lines. It is a vital genetic phenomenon in commercial sweet corn production. Heterosis depends on the genetic background of parents. Breeders develop a number of inbred lines and generate numerous hybrid combinations. However, significant heterosis (hybrid vigour) is expressed only in some of the crosses. This is because the combining ability of inbred lines varies in cross combinations. Heterosis is a function of both general combining ability (GCA) and specific combining ability (SCA). GCA and SCA are estimated from replicated trials established over different target localities. The generation of inbred lines suitable for use in production of superior hybrids is costly and requires many years in traditional empirical breeding programs. Much of the developmental effort is devoted to field testing of newly created lines in various single cross combinations to identify those lines with superior combining ability.

Development of a reliable method of predicting hybrid performance without the need to generate and test hundreds of single cross combinations has been the goal of numerous studies in both self and cross pollinated crops (Boppenmeir et al., 1992; Melchinger, 1999; Solomon et al., 2007). However, reports on this matter have been controversial. Dias et al. (2004) examined 54 published works on the subject matter, and found that 28 of them reported positive associations between heterosis and genetic distance while 26 of them detected negative or inconclusive correlation between genetic distance and hybrid vigour. Melchinger (1999) believed that genetic distance predicts F1 performance and the efficiency of prediction was greater with crosses between inbred lines from the same heterotic-grouped pairs of populations than in crosses between inbreds from different heterotic pairs. In sweet corn, there are no clearly defined heterotic groups. Field testing of hybrids is more costly than it is in field maize where heterotic patterns are well established. We believe if marker based distance prediction can help identify superior F1s, our hybrid development program will be much more rapid and cost efficient.

The objectives of this research were to:

- assesses the combining ability of genetically diverse breeding lines and estimate the importance of heterosis in the expression of useful agronomic and quality traits
- understand the relationships between parents performance *per se* and F1 performance.
- investigate the role of SCA and Heterosis to superior F1 performance
- evaluate the genetic distance based prediction of F1 performance

7.2 Materials and methods

7.2.1 Development of F1 hybrids

Ten inbred lines derived from different breeding populations (Table 4) were crossed to generate 45 F1 hybrids in a half diallel mating design without reciprocals. Parents and F1 hybrids were evaluated across three locations.

7.2.2 Field experiments

Field experiments were established using ten parental lines, 45 F1 hybrids and commercial checks. Trials were established in southern Queensland at Hermitage (28º 21' S, 152º 10' E), north Queensland at Kairi (17º 22' S, 145º 57'E) and south east Queensland at Gatton (27º 33' S, 152º 33'E). Kairi is situated at 667 m.a.s.l. and is typical of a tropical high rainfall area. Hermitage (475 m.a.s.l.) represents a dry subtropical environment. Gatton (98 m.a.s.l.) represents a hot subtropical environment. Plots consisted of two rows 5 m long and 0.75 m spacing between rows. Plant density was maintained at approximately 45000 plants per hectare. Standard agronomic practices were applied to all trials. Trials were not sprayed for disease or insect pests. A number of qualitative and quantitative measurements were taken (see Solomon et al., 2012ab for details)

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7.2.3 Diversity assay

Leaf samples for parental lines were used for DNA isolation. DNA was extracted following a modified CTAB procedure. Polymorphic SSRs markers, uniformly distributed across the maize genome (http://www.maizegdb.org/ssr.php) were used for assessing genetic variability (see Solomon et al., 2012b).

7.2.4 Data analyses

The multilocations data were subjected to analysis of variance using Gardner and Eberhart's analysis III (Gardner and Eberhart, 1966). The variation among all entries was partitioned into general combining ability (GCA) and specific combining ability (SCA). Statistical analysis was done using the DIALLEL.SAS05 Program (Zhang et al., 2005). Mid parent heterosis was computed as: Mid-parent heterosis (MPH, %)=[$Progeny_{ij} - (p_i + p_j)/2/(p_i + p_j)/2)$] x100, where p_i = performance of inbred $_i$, p_j = performance of inbred $_j$, progeny $_{ij}$ = performance of a cross of inbred $_i$ and $_j$.

Genetic distance matrices were computed for all pairs of genotypes from binary data based on both shared and unique (SSR) bands using Jaccard's similarity methods (Jaccard, 1908). Jaccard's distance (GD) was calculated as 1-JCS (Jaccard's similarity coefficient). To calculate genetic distance matrices based on agronomic and quality traits, mean performances were first (normalized), *Z* transformed to standardized units. The agronomic dissimilarity matrices based on all measured traits were calculated using the Euclidean distance method (Kaufman and Rousseeuw, 1990). Linear regression analyses were used to determine associations and the strength of association among parameters.

7.3 Results

7.3.1 F1 performance and heterosis

Considerable variability was observed in the performance of F1 hybrids derived from the ten diverse sweet corn parental lines. Plant height and number of marketable cobs showed the maximum variability (Figure 4). The mean values for plant height was 188cm. On average F1s days to 50% silking was 72 days from the date of planting. Average over 45 hybrids, the mean number of marketable cobs was 3445 dozens/ha. Tenderness and flavour showed the smallest variability, with mean values being 5 and 6.7 on the average (Figure 4). Detailed comparison in the performance of F1 hybrids compared to their parental lines given for a range of traits (Solomon et al., 2012a. The level of mid parent heterosis was varied depending on the type of trait. The estimate of heterosis for plant height was in the range of 10 to 59 cm, and the average was 30.1cm. The amount of heterosis estimated for days to silking was in the range of -12.6 to -1.7 days after planting. On average days to silking decreased by about 7 days compared to the parental lines. Marketable cobs showed heterosis in the range of -11 to 82.3 with the mean value being 40.9 dozens/ha (Table 5).

Table 4. Pedigrees of ten inbred sweet corn parents with the *sh2* gene used in the diallel crosses.

Parent	Pedigree	Origin and description
P1	04-8/SHA3-2#6	100% Tropical (ex POP1)
P2	06/SHMA29-2-B45	50% Temperate (ex POP15)
P3	05-1/SHMA30-B13	50% Temperate (ex POP15)
P4	05-9/PROA1-B18	75% Tropical (ex PRO1)
P5	08-6/PROA22	75% Tropical (ex PRO1)
P6	07/SHB5	100% Tropical (ex POP2)
P7	05-3/PROB4-1	90% Tropical (ex PRO2)
P8	08-6/PROB16	90% Tropical (ex PRO2)
P9	05-1/SHMB8	50% Temperate (ex POP16)
P10	07-5/SHMB9	50% Temperate (ex POP16)

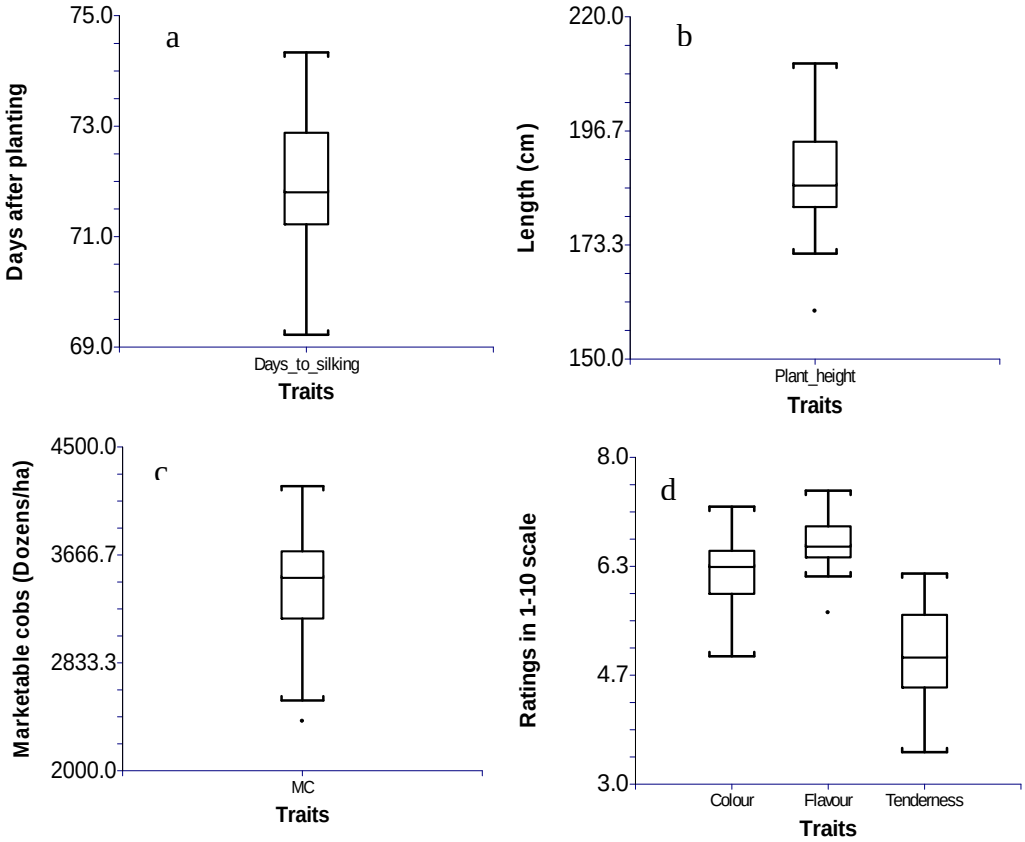

Figure 4. Summary of days to silking (a), plant height (b), number of marketable cobs(c) and quality traits (kernel colour, flavour and tenderness) (d) for 45 F1 tested across Gatton, Hermitage and Kairi, Queensland.

Table 5. Maximum, minimum and mean values for mid parent heterosis (%) computed from across three location trials of 10 inbreds and their F1 hybrids

Trait	Minimum	Maximum	Mean
Days to 50% silking (DS)	-12.6	-1.7	-6.9
Plant height (PH, cm)	4.8	35.6	21.9
Number of marketable cobs (MC, dozen / ha)	-11.5	82.3	40.9
Kernel colour (KC, 1-10 rating)	-19.4	39.9	6.2
Tenderness (TD, 1-10 rating)	-40.4	16.2	-15.4
Flavour (Flv, 1-10 rating)	-8.1	35.8	4

7.3.2 Parent and F1 performance relationships

The regression analyses showed that line performance *per se* and F1 hybrid performance is significantly related for all traits except for the number of marketable cobs. The intercept in the regression analysis for days to silking was not different from zero, but the slope was significantly different from zero and was very close to unity. However, the regression analysis showed that only 25% of the total variation was accounted for by the observed relationships (Table 6). Regression parameters for plant height were all significant and R^2 explained about 40% of the total variation. The regression mean square for tenderness was also significant. The intercept coefficient for tenderness was not different from zero. The slope was also significant although it was small in magnitude. All regression parameters for flavour were highly significant ($P<0.01$), but the slope was also

small. In both tenderness and flavour, the observed relationships explained very small fractions of the total variability (Table 6). It appears that parental lines with late silking time were likely to result in late silking hybrids (Figure 5a). Similarly taller inbred parents may give taller hybrids (Figure 5b). For quality traits data points were clustered at specific values. However, the general trend was more flavoursome and tender parents are likely to result in better hybrids (Figure 5c&d).

Table 6. Regression analyses for line *per se* and F1 performance relationships for days to 50% silking (DS), plant height, marketable cobs (MC), tenderness and flavour based on performance of 45 hybrids and 10 parental lines across three sites in Queensland.

	DS (days)	PH (cm)	MC (dozens/ha)	Tenderness (rating 1-10)	Flavour (rating (1-10)
Regression	47.15**	1970.11**	2153.153975	2.49*	1.28**
Intercept	12.56	101.31**	3524.30**	1.53	4.86**
Slope	0.90**	0.56**	-0.03	0.59*	0.28**
R^2	0.25	0.40	0	0.12	0.19

* and ** are significant at P=0.05 and 0.01, respectively.

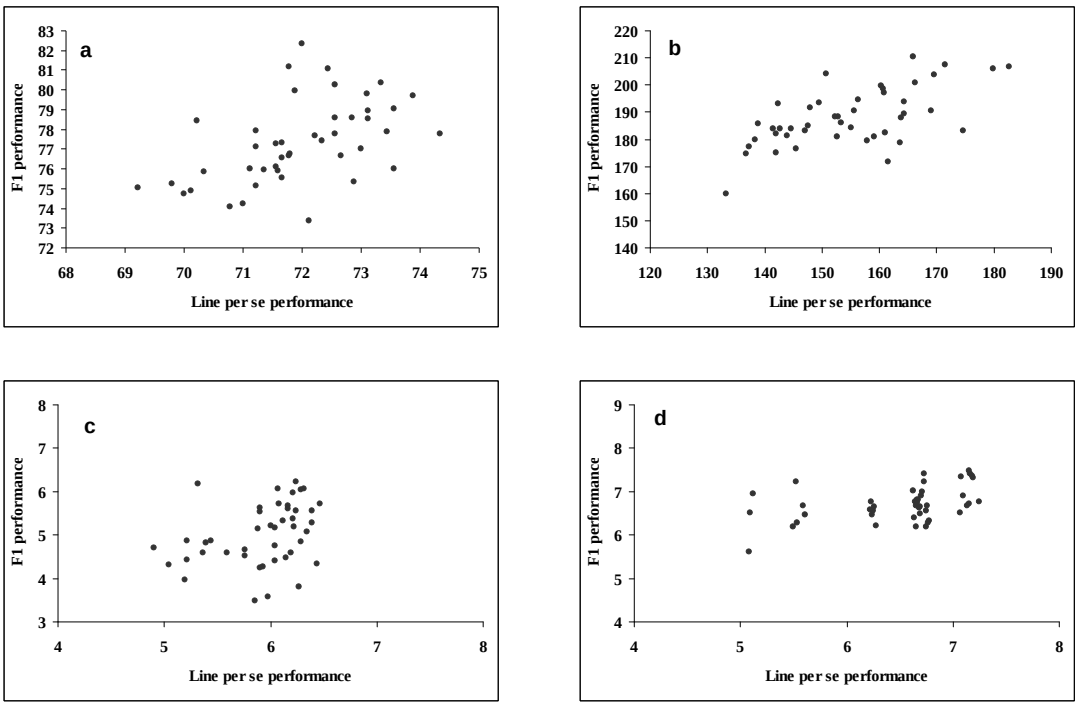

Figure 5. Relationships between line performance *per se* and F1 performance for days to silking (a), plant height (b), tenderness (c) and flavour (d) for 45 sweet corn hybrids evaluated across Gatton, Hermitage and Kairi in Queensland.

7.3.3 Combining ability and *per se* performance

Regression analyses for line *per se* performance and GCA effects are summarized in Table 7. Parental lines performance for days to 50% silking, MC and tenderness were not related to GCA effects. On the other hand plant height and flavour were strongly related to GCA effects. Smaller or negative GCA values often related to shorter height or reduced flavour (Figure 6). The observed relationship explained 67 and 48% for the total variability for plant height and flavour respectively (Table 7).

Table 7. Regression analyses for general combining ability (GCA) effects and line performance *per se* relationships for days to 50% silking (DS), plant height (PH), marketable cobs (MC), tenderness and flavour based on performance of 10 parental lines across three sites in Queensland.

	DS (days)	PH (cm)	MC (dozens/ha)	Tenderness (rating 1-10)	Flavour (rating (1-10)
Regression	1.68	233.07**	176.38	0.25	0.14*
Intercept	-10.28	-41.90**	31.51	-1.55	-0.82*
Slope	0.13	0.27**	-0.01	0.26	0.13*
R^2	0.36	0.67	0	0.19	0.48

* and ** are significant at P=0.05 and 0.01, respectively.

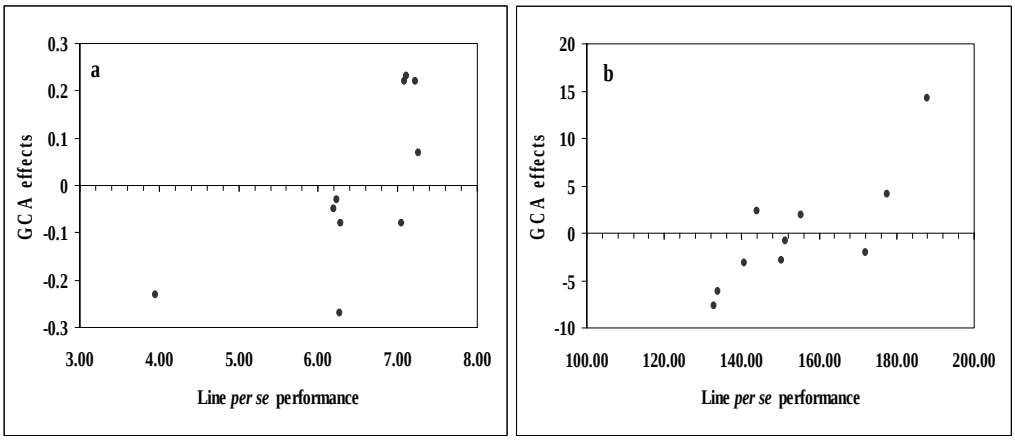

Figure 6. Relationships between line performance *per se* and GCA effects performance for flavour (a) and plant height (b) for 10 sweet corn inbreds evaluated across Gatton, Hermitage and Kairi in Queensland.

Heterosis (MPH) estimated based on the performance of mid parent values significantly related only to the number of marketable cobs and tenderness (Table 8). The observed relationships between MPH and F1 performance for the number of marketable cobs and tenderness were comparatively strong with R^2 = 0.62 and 0.75 for marketable cobs (MC) and Tenderness respectively (Table 8). For both MC and tenderness the intercept and the slope were significantly different from zero (P<0.01) (Table 8). Increased MPH resulted in increased F1 performances both for MC and tenderness (Figure 6). Reduced heterosis particularly for tenderness resulted in reduced tenderness in the hybrids (Figure 7b).

Table 8. Regression analyses for relationship between mid parent heterosis (MPH%) and F1 performance for days to 50% silking (DS), plant height (PH), marketable cobs (MC), tenderness and flavour based on performance of 45 F1 across three sites in Queensland.

	DS (days)	PH (cm)	MC (dozens/ha)	Tenderness (rating 1-10)	Flavour (rating (1-10)
Regression	0.76	81.85	4329418.89**	16.16**	0.22
Intercept	72.38**	183.93**	2831.18**	5.83**	6.68
Slope	0.06**	0.19	15.03**	0.05**	0.01
R^2	0.01	0.02	0.62	0.75	0.03

* and ** are significant at P=0.05 and 0.01, respectively.

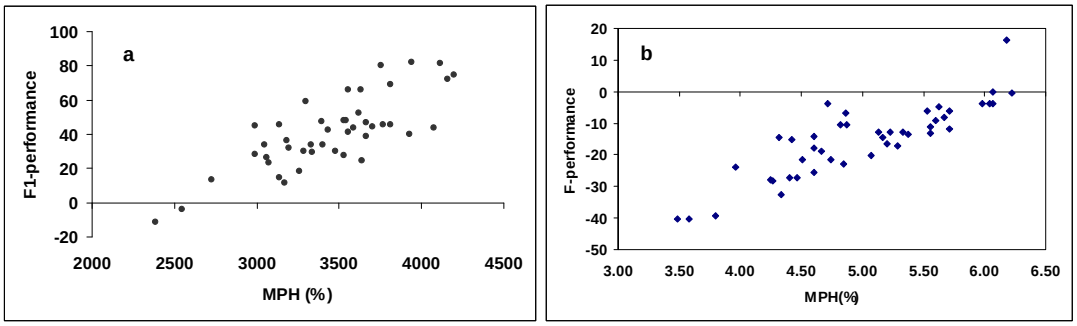

Figure 7. Relationships between F1 performance *per se* and midparent heterosis (MPH%) performance for number of marketable cobs (a) and tenderness (b) for 45 sweet corn hybrids evaluated across Gatton, Hermitage and Kairi in Queensland.

The relative importance of SCA and MPH to the performance of F1 was visualized by plotting the standardize values. Values were standardized about their mean and standard deviation to eliminate differences due to unit of measurements. Close overlap of values indicate close association. For traits like marketable cobs both SCA and MPH were very close to the F1 values (Figure 8a). On the other hand where the importance of MPH and SCA were negligible to the observed F1 flavour values, considerable disparity in the values was observed (Figure 8b). All correlations between all F1 and SCA were highly significant (P<0.01), except for flavour. However, the association between MPH and F1 performance was significant only for MC and tenderness. SCA values were significantly correlated to MPH estimates in all cases except for flavour (Table 9).

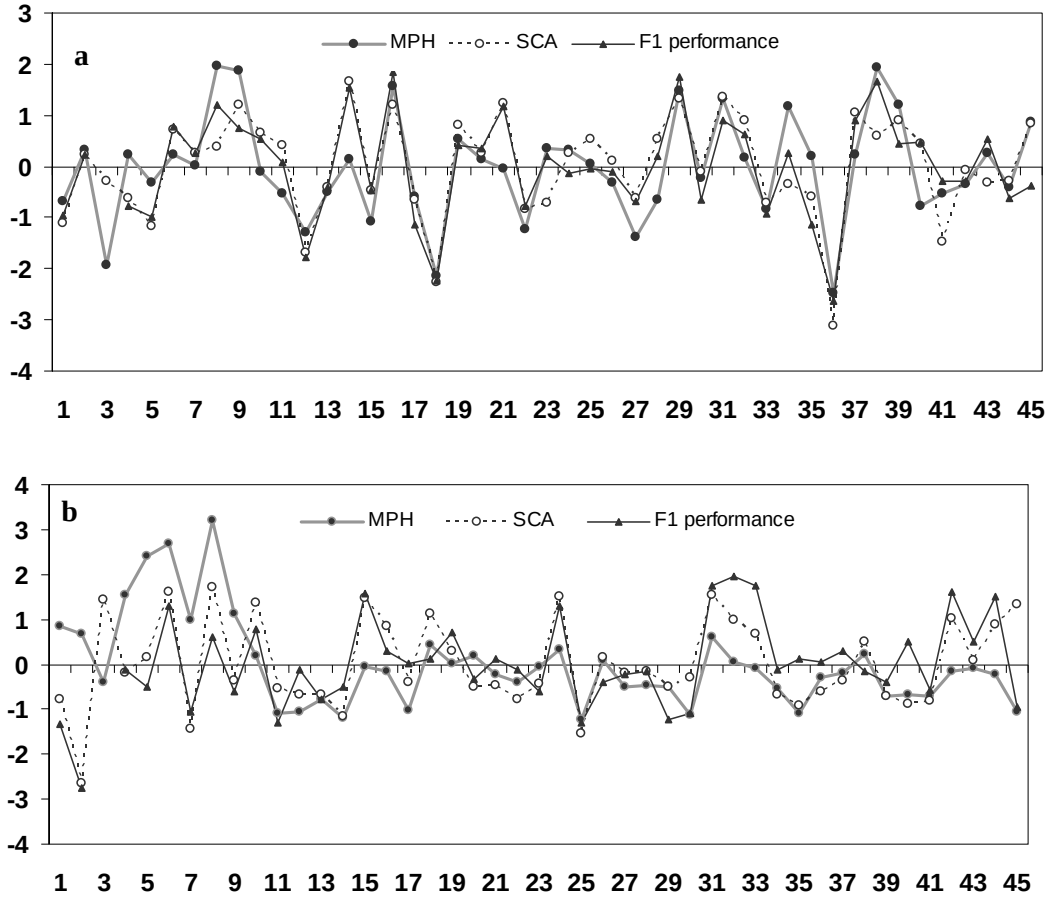

Figure 8. Correspondence of the standardized values of mid parent heterosis (MPH), specific combining ability (SCA) and F1 performance for marketable cobs (a) and flavour (b) based on the evaluation of 45 hybrids across three sites.

Table 9. Correlation coefficient for the relationship between F1 versus SCA, F1 versus MPH and SCA versus MPH based on the performance of 45 sweet corn hybrids across three sites.

	F1vs SCA	F1 vs MPH	SCA vs MPH
DS	0.55**	0.11	0.42**
PH	0.61**	0.13	0.59**
MC	0.88**	0.78**	0.72**
Tenderness	0.42**	0.86**	0.43**
Flavour	0.10	0.18	-0.01

** Significant at P= 0.01.

7.3.4 Performance of candidate hybrids

Initially the 45 hybrids were tested along with the check at Gatton, Kairi, and Hermitage. The top 20 were retested at Bowen and Gatton. Summary of some agronomic and quality traits are presented in Table 10. Cross 16 was significantly quicker than Hybrix 5 (H5). It was also significantly shorter than H5. The two

hybrids showed significantly better tenderness. They also gave 28% more marketable cobs than H5. The other distinct advantages of the two hybrids were their excellent resistance to Northern leaf blight. Their performances were verified by a subsequent autumn planting in observation plots at Gatton and winter planting at Bowen. About one kg of seeds from each of the hybrids and about 500 gm seeds of each of the parental lines was produced and given to our commercial partner, Pacific Seed Ltd. Pacific Seed Ltd will undertake further on-farm verification trials for possible commercialization.

Table 10. Some agronomic and quality traits for candidate hybrids based on the performance across three sites

Cross	DS (days)	DM(days)	Ph(cm)	MC	EL(cm)	Ear fill (%)	ED (mm)	FLV	TD	NLB
14	72	95	176	3543	21	88	49	7.2	6.0	1
16	70	92	182	3556	20	85	47	7.8	6.0	1
Hybrix 5	74	96	206	2778	22	88	48	6.2	4.1	5
$LSD_{0.05}$	2	2	17	665	2	8	4	1.3	1.7	

DS= days to 50% silking (number of days after planting), DM= days to physiological maturity (number of days after planting), Ph (plant height in cm), MC (number of marketable cobs (dozens/ha), (EL length of dehusked cobs in cm), ED (ear diameter in mm), FLV(flavour ratings in 1-10 scale; where 1= worst and 10 = best), TD (tenderness rating in 1-10 scale; where 1=worst and 10=best) and NLB ((northern leaf blight (turcicum) ratings in 1-9 scale; where 1=complete resistance and 9= leaf is dead)).

7.3.5 Genetic diversity of parental lines and its relation to F1 performance

The dendrograms from UPGMA cluster analyses based GD (distance based on SSR markers polymorphism) and PD (distance based on agronomic traits) distance matrices are shown in Figure 9 and 10. The cophenetic correlation between the dendrograms and the original data matrices showed a good fit with matrix correlation of $r = 0.84$ for the phenotypic data and $r = 0.91$ for the molecular data. The cluster analysis on the basis of GD revealed three groups (Figure. 9). The first cluster was composed of parents 7 and 8, derived from the PRO2 population with 90% tropical background. The second cluster was composed of five parents with sub-grouping of parents 9 and 10, originated from POP16 (population with 50% temperate genetic background). The other sub group in this cluster contained parents 4 and 5 derived from PRO1 (population with 75% tropical genetic background). Parents 1, 2 and 3 constituted the third cluster. Parent 2 and 3 were originated from the same population. Clustering on the basis of PD failed to produce clear grouping of parents. However, two major clusters and one outlier at the bottom of the dendrogram were discernible (Figure 10). The first cluster was composed of three parents (5, 6 and 8). Most of the parents fell in the second cluster. Two major sub groupings occurred in this cluster. Parents 9 and 10 were tightly linked and created one sub group. The parents 2, 4 and 7 created another sub group.

The predictive power of genetic distance estimates, based on molecular marker profile and phenotypic traits, were analysed by correlation of distance matrices with mean values. Genetic distance (GD) estimated from Jaccard similarity coefficient were related, but only weakly, to F1 performance for the quality trait of flavour ($r = 0.39$; $P < 0.05$). Genetic distance estimates on the basis of molecular marker and phenotypic traits did not correlate ($r = 0.16$; $P > 0.05$).

7.4 Discussion

F1 showed greater variability for agronomic traits like plant height and marketable cobs, whereas eating quality traits such as tenderness and flavour showed the smaller variation. This is expected since parental lines were selected for acceptable eating quality; it is possible that they had very limited variability in terms of flavour or tenderness.

Hybrid breeding in maize relies to a great extent on the development and improvement of heterotic groups. In theory, heterosis is dependent on parental genetic diversity (Fulconer and Mackay, 1996). MPH was also higher for agronomic traits compared to the quality traits (Table 5) suggesting that parental lines were more diverse for agronomic traits than they were for quality traits, this perhaps being due to the fact that most sweet corn inbreds are selected to meet basic requirements for quality and appearance. Heterosis could be exhausted if related inbreds are recombined repeatedly without the infusion of new genotypes to diversify the breeding pool (Revilla et al. 2000). Although R^2 values were generally small, line *per se* and F1 performance were significantly related in most cases (Table 6) indicating that careful choice of parental lines is important to produce commercially valuable F1 hybrids. This is particularly true for quality traits such as tenderness and flavour. However, the interdependence between parental and F1 performance were not that strong, indicating prediction of F1 from parental lines performance may not be dependable. Line performance *per se* and GCA effects was related only for plant height and flavour. The R^2 explained about 50% of the total variation (Table 7) indicating the breeding value of lines can be inferred based on a lines performance. F1 performance for MC and tenderness were strongly related to MPH (Table 8 & Figure 7) suggesting the value of F1 hybrids depends on the heterotic combination of the parental lines. MPH was also correlated to SCA for all traits except for flavour. Similarly F1 was correlated to SCA effects for all traits other than flavour suggesting both MPH and SCA affects F1 performance.

In this experiment genetic diversity of parental lines was studied using selected polymorphic SSR markers. Cluster analysis of the parental lines on the basis of marker data reflected the pedigree relationships of the inbreds. In Figure 9, the first cluster was composed of inbreds originated from PRO2 population synthesized mostly from tropical types. They were selected both for their good resistance against mosaic virus and leaf blight. However, they fell in different groups when clustering was done on the basis of phenotypic data. The phenotypic data used for clustering did not include disease assessment, suggesting these two inbreds may have different agronomic potential. Five parents formed the second cluster, nevertheless, further stratifications within the group more or less reflects the pedigree relationship of the lines. The last cluster, however, showed slight deviation from the observed pedigree relationships of the parents due to close linkages between parents 1 and 3 instead of 2 and 3 (Figure 9). Nevertheless, grouping of inbreds on the basis of phenotypic data did not reflect their pedigree relationships (Figure 10). Moreover, genetic distance estimates on the basis of molecular marker and phenotypic traits did not correlate (r = 0.16; P > 0.05). Morphological variability in sweet corn genotypes could be limited (Revilla and Tracy 1995). This could lead to poor separation of lines when classified on the basis of phenotypic data.

8 Technology transfer

8.1 Field day and updates to growers

Three candidate hybrids and commercial check were each planted in 6 rows of 12m long demonstrations plots. Seed companies, growers and high level mangers from DAFF Queensland, were invited and attended the field day in December 2012. We have consulted with major fresh market sweetcorn growers in the Lockyer Valley. Updates on research activities and progress were given to Rugby Farms and Mulgowie Farms in 2011 and 2012.

8.2 Publications and presentations

Two scientific papers were published in peer reviewed international journals in 2012. One publication discussed Combining ability, genetic diversity and heterosis in relation to F1 performance. It was published in plant breeding journal. The other one discussed genetic effects and genetic relationships among sweet corn lines and F1 hybrids. It was published in Euphytica. The publications are listed in the reference list of this report.

Research progress reports were presented to Horticulture and Forestry science management team meetings in 2010 and 2011. Presentation was also given to a breeders meeting in 2010.

9 Conclusions and recommendations

Excellent progress has been made in terms of building up disease resistance and improving eating quality using recurrent selection approaches. Also, marker assisted backcrossing has been used to introgress the presumed downy mildew resistance QTL(s). Nevertheless, the performance of downy mildew resistance materials have never been verified under field conditions. Therefore, the presence of the markers in the lines can only be used as an indicator for possible downy mildew resistance. To make use of the lines as sources of resistance in a breeding program where field screening is impossible in Australia due to the absence of the disease may be an unreliable approach. The DM resistant lines were also crossed with known sources of resistance to southern rust. The lines that were extracted from the combinations could be a good resistance source of both southern rust and downy mildew diseases. The new hybrids that were created from the latest series of inbreds showed promising results with respect to agronomic criteria and eating quality. However, most of them lack productivity suggesting further work is needed to combine the desirable features with productivity.

Considerable variability in terms of yield and agronomic traits were observed among the hybrids generated from diverse sweet corn lines. The variability observed for eating quality traits such as tenderness and flavour was relatively small. Future work needs to focus on identifying new sources of variability for eating quality.

A considerable level of heterosis was observed for most economically important traits indicating the usefulness of the parental lines in commercial hybrid production. However, the MPH for tenderness was negative suggesting hybrids were tougher than the inbreds, hence future improvement of tenderness should focus mainly on the enhancement of parents *per se* performance. Absence of any strong association between F1 performance, SCA, MPH and genetic distance for

analysed traits indicated that marker based prediction of any of these parameters in the current population is not feasible.

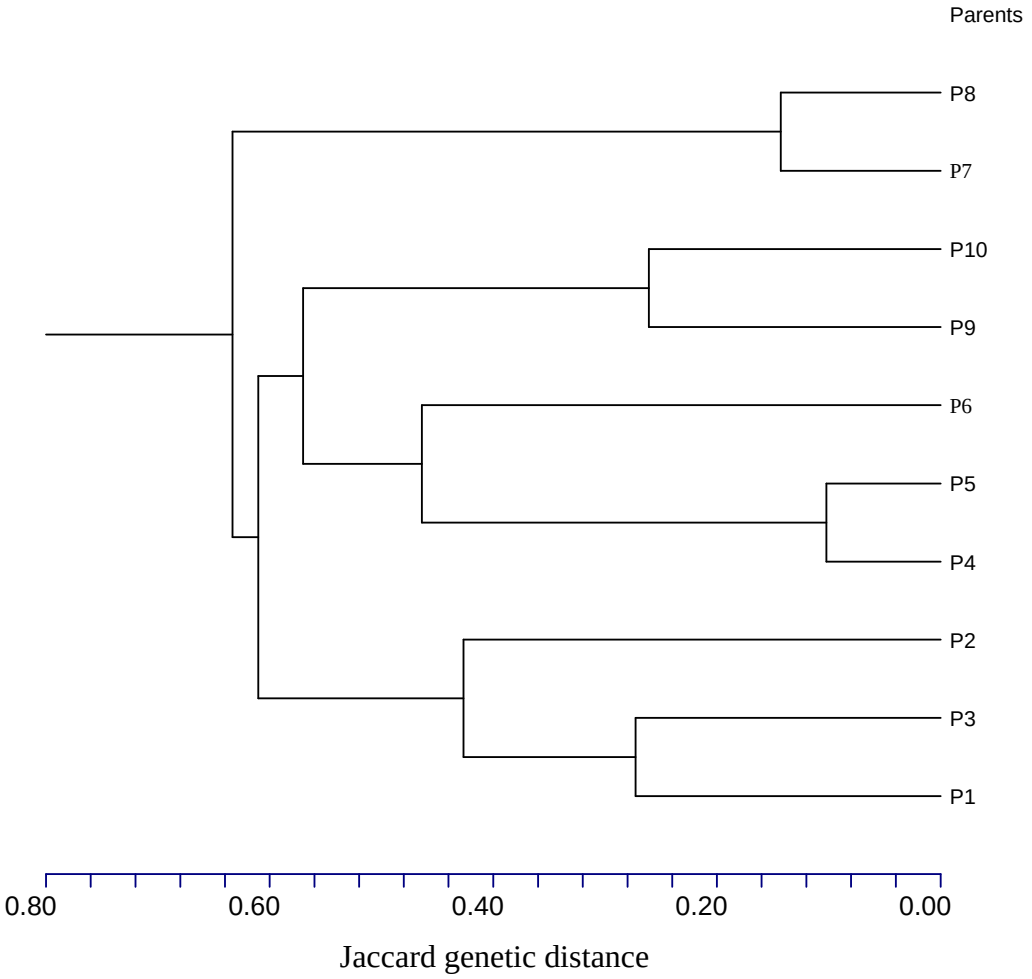

Figure 9. UPGMA cluster analysis revealing genetic relationships among 10 *sh2* parents using Jaccard genetic distance estimated based on 20 polymorphic SSR markers. See Table 1 for parents' origin and descriptions

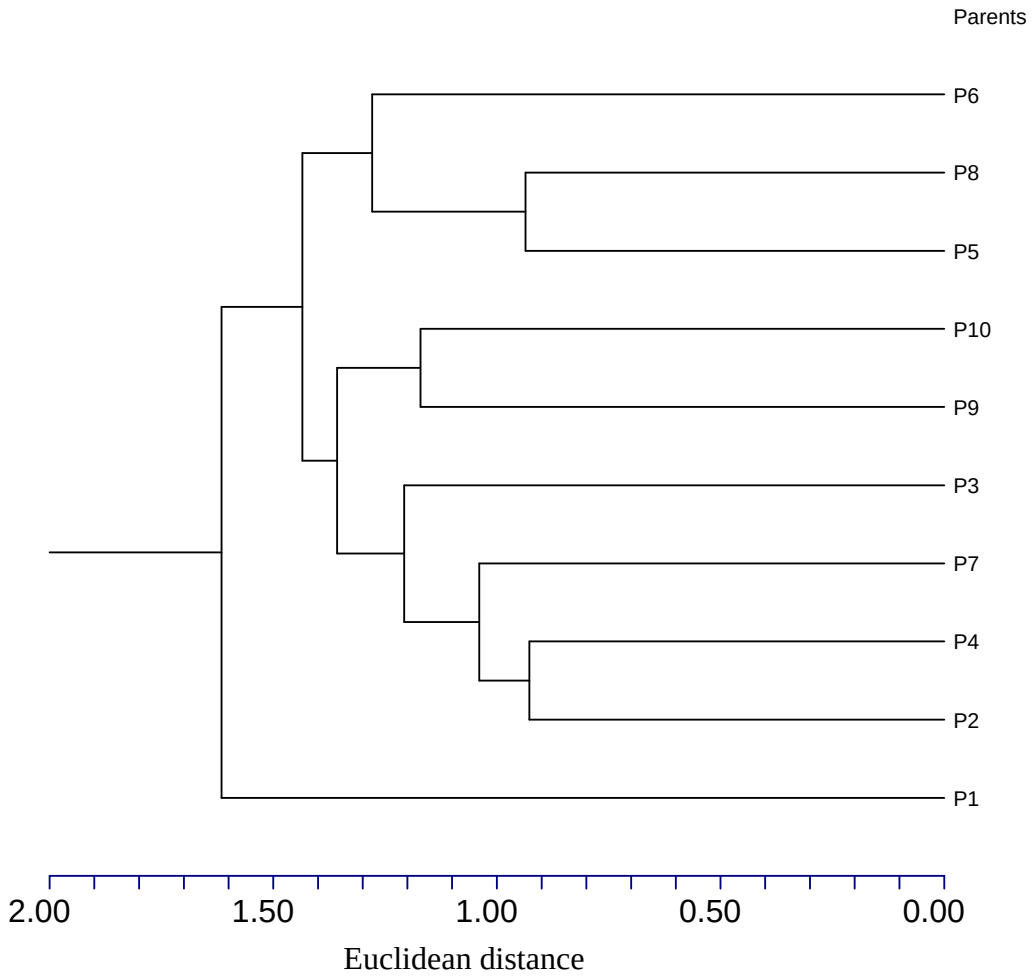

Figure 10. UPGMA cluster analysis revealing genetic relationships among 10 *sh2* parents using Euclidean distance (PD) estimated from normalize mean values of 21 phenotypic traits. See Table 1 for parents' origin and descriptions

10 Reference

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