N.Z. Agronomy Society
Special Publication No. 5

Paper 17

DEVELOPMENT OF HIGH AMYLOSE MAIZE PRODUCTION IN AUSTRALIA

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ABSTRACT

A high amylose open pollinated maize cultivar was developed by seven cycles of mass selection (seed parent only) to test the feasibility of high amylose starch manufacture and use in Australia. High amylose starch has been successfully produced since 1981, and a substantial local demand for the product has been identified.

A breeding plan to meet current and long term needs for cultivars with genetic diversity and higher productivity is outlined. This plan provides for continuous population improvement of two divergent parent populations for the purpose of producing an inter-population hybrid high amylose cultivar.

KEYWORDS

Amylose-extender locus, mass selection, starch production, synthetic.

INTRODUCTION

Amylose is the linear polysaccharide component of normal maize starch. The linear (amylose) and branched (amylopectin) molecular forms of starch have very different physical properties, and starches composed of only one component have many specialised uses in both food and non-food industries for which the natural mixed starch is unsuited. The high energy cost of fractionation makes separation of amylose and amylopectin from mixed starch sources non-commercial. In maize, separation can be achieved, in part, by use of gene mutants. Waxy maize, based on homozygosity for a recessive allele (*wx*) at the waxy locus, has been developed and widely used as a source of pure amylopectin starch. Recessive alleles at the loci su_1, su_2, *du*, and *ae* modify starch composition in the opposite direction, i.e. toward a high amylose content. Amylomaize cultivars, homozygous for allele *ae* at the amylose-extender locus, and with an amylose content of about 60%, have been developed and grown on a small scale in USA (about 6400 ha in 1975; Creech and Alexander, 1978) as a source of high amylose starch.

In this report we desribe our efforts to develop high amylose maize production in Australia. This development has progressed from a small feasibility study in 1980/81 to a contract production area of about 4000 ha in 1984/85. High amylose maize production is likely to continue as a high value component (in terms of returns per hectare) of the Australian maize industry. This development is not a major innovation, but is presented as an instance in which industry motivation required plant breeding innovation, namely the development of an appropriate high amylose cultivar at a low research and development cost.

THE NEED FOR A HIGH AMYLOSE STARCH SOURCE

The project to breed a high amylose maize cultivar and produce high amylose starch was developed informally, beginning in about 1974, in anticipation that a local market could be found for this starch form. There was no industry demand and little known usage of imported high amylose maize starch before our first commercial production in Australia in 1981.

Industry reasons for embarking on this project were:

- High amylose starch was an obvious and complementary addition to Corn Products — Fielders existing production of normal corn starch, waxy starch, and modified waxy starch.
- Genetically modified starch forms, from waxy and amylomaize, could provide a hedge against any future Food and Drug Administration (USA) and National Health and Medical Research Council (Australia) regulations restricting the use of chemically-modified starch in human foods.
- Between 1965 and 1980 many new food and non-food uses of high amylose starch were devised and patented (Young, 1984; Table 1) and it seemed likely that a broad Australian industry demand for high amylose starch could be developed. Local production of high amylose maize and high amylose starch was needed to test this proposition.

Table 1. Development of new applications of high amylose starch (Young, 1984).

Year	Patent descriptions for new uses Non food	Food
1961	Amylose yarn	
	Paint thickener	
	Cigar binder	
	Water soluble film	
	Foundry core binder	Extruded confectionery products
	Cement binder	
	Low moisture film	
	Corrugating adhesive	Coating canned fruit
	Multiply packaging	Cooked food thickener
	Encapsulating agent	Transparent dried fruit coating
1970	Paper reinforcing	Confectionery - creme centres
	Surgical thread, bandages	Bread quality, antistaling
	Meat packaging film	Confectionery, gums and jellys
	Biodegradable film	Gelatin replacement
	Hydrogels for slow release drugs	Tomato paste texturising
	Textile printing, and marking ink	Deep fried food coating
	Glass fibre sizing and reinforcement	Coating french fried potato
	Tablet manufacture	Edible foams
	Air freshener gels	Low calorie bread
		Pudding thickener
		Crisp pastry dough
		Pizza pastry dough additive
1980	Slow release herbicide packaging	Canned fruit thickener
		Extrusion products

THE PLANT BREEDING RESPONSE TO INDUSTRY NEEDS

Usually there are at least two sources of cultivars for local production of a new crop. In the present study these were:

- Introduction of amylose-extender maize inbred lines from USA for production of a high amylose hybrid — a reasonable plant breeding and genetics research effort was allocated to high amylose development in USA between 1946 and 1970. A number of public, amylose-extender inbred lines, usually the backcross derived versions of popular normal maize inbreds, were developed at the University of Missouri, and at Purdue University, Indiana, (Henderson, 1980).
- Rapidly developing an amylose-extender maize population.

The latter option was chosen, for the following reasons:

- The commercial uncertainty of a local high amylose maize industry did not permit an allocation of resources for an inbred maintenance, hybrid seed production, and testing programme.
- A high amylose synthetic cultivar could be developed rapidly (three plant generations) from available stocks, and this avoided the time lag involved in procurement, quarantine, propagation, seed increase, and hybrid seed production based on introduced amylose-extender inbred lines from USA.
- A synthetic variety, though expected to have a lower yield potential than a hybrid, was appropriate for testing the feasibility of local production and end use of high amylose maize starch.
- A synthetic variety avoided the need for detailed, accurate forecasting of future seed requirements, because low cost seed can be retained to satisfy the needs for any future level of grain production. (It was this aspect of our planning that permitted a rapid expansion of the area sown in the 1982/83 and 1983/84 growing seasons).
- A broadly-based synthetic cultivar might enable high amylose maize to be grown in a wide range of environments, thus providing flexibility for securing contract acreages.

DEVELOPMENT OF SU AMYLOMAIZE HIGH AMYLOSE MAIZE SYNTHETIC

A synthetic variety with allele frequency, q (*ae*; amylose-extender greater than 0.97 was developed by the breeding plan described in Fig. 1. The SU Amylomaize cultivar was developed by hybridisation, random mating,

Figure 1. Breeding plan for development of the high amylose maize population, SU Amylomaize.:

Time period	Breeding procedure	
1974	Hybridisation	*ae/ae* ♀♀ x *Ae/Ae* ♂♂ MG Co-op/W22 FR Synthetic
		↓
1975-1976	Random mating 3 cycles	*Ae/ae* population selection of *ae* phenotype kernels
		↓
1977-1985	Mass Selection (seed parent only) 7 cycles 500 selected ears/cycle 1.25% retention	SU Amylomaize p(*Ae*) = 0.03; q(*ae*) = 0.97 selection for yield/plant stalk strength earlier maturity kernel phenotype ear height husk leaf extension

and mass selection (seed parent only) in sequential steps between 1974 and 1985. In this cultivar, the major source of variability for characters other than endosperm type was the FR synthetic. This synthetic population was developed earlier by intercrossing inbred lines 13B, 21H, 23TR, 25TR, H548. These inbreds were developed from Australian open pollinated maize varieties, and four had been used in double cross maize hybrids. The FR synthetic is a relatively late maturing variety, broadly adapted to the coastal production areas of the New South Wales north coast and southeast Queensland, and is characterised by gene frequencies p(Rf1) = 0.9 and q(rf1) = 0.1 at the Rf1 locus (restoration of fertility to Texas cytoplasm maize), and p(Pwr) = 0.62 and q(Pww) = 0.38 at the *P* locus (pericarp and cob colour).

A feature of the breeding plan in Fig. 1 is the choice of mass selection (seed parent only) for continuous improvement of SU Amylomaize. A number of effective directional selection procedures for improvement of maize populations have been well documented (Hallauer and Miranda, 1981). Mass selection (seed parent only) was chosen because:

- Mass selection is an inexpensive breeding procedure. Commercial uncertainty of a high amylose maize industry at the inception of this programme precluded an allocation of funds for high amylose maize improvement. However, using mass selection, a low cost, feasible scheme combining a cycle of selection with annual replacement of breeder seed was developed.
- Mass selection permitted individual plant selection at low cost for simultaneous improvement of ear size, ear height, maturity, husk cover and tightness, stalk strength, and amylose-extender kernel phenotype. Selection for this array of characters was necessary because the hybrids made to initiate the synthetic population were the rather wide cross of USA corn belt germplasm with Australian maize.

Low cost mass selection (seed parent only) integrated with breeder seed replacement has been carried out for seven cycles. In each cycle, the number of selected ears required was set at 500, to provide sufficient breeder seed and to permit selection at moderate truncation levels for each of the plant characters listed above; the total population size was not predetermined. Selection was by phenotypic evaluation of individual plants in an isolated foundation seed production area of about three hectares. Usually the required number of ears was obtained from an area of about one hectare. On this basis the retention fraction was about 500/40,000 or 1.25%. The truncation level achieved for individual characters was not recorded, but an approximate value can be estimated by assuming equal selection intensity for each of the six characters. Then, the truncation level for individual characters is given by $0.0125^{1/6} = 0.48$. This is equivalent to a selection intensity of 0.81 (Falconer, 1981).

In the SU Amylomaize programme, residual plants after mass selection are bulk harvested as a foundation seed lot. Annual replacement of breeder seed, designed to maintain the *ae* allele frequency at greater than 0.95, is integrated with mass selection. In each generation, the amylose extender phenotype kernals from the 500 selected seed ears are composited to obtain the planting seed for a one hectare field that is the population for the next cycle of mass selection and for breeder seed production.

As a check on the maintenance procedure, the relative frequency of alleles *Ae* (normal endosperm) and *ae* (amylose-extender) has been calculated from the proportion of heterozygous (*Ae/ae*) plants detected among the selected group of ears in each generation of mass selection. These data are given in Table 2, and show that this method of producing breeder seed has been effective in keeping the frequency of the *ae* allele high.

Table 2. Estimated allele frequencies at the *Ae* (Amylose-extender) locus in selected populations, and an unselected population, of SU Amylomaize.

Population	Year grown	Allele frequencies *Ae*	*ae*
C0	1978	0.03	0.97
C3	1982	0.02	0.98
C4	1983	0.02	0.99
C5	1984	0.01	0.99
C5[1] Commercial	1984	0.06	0.94
C6	1985	0.03	0.97

[1] Commercial field planted with C4 breeder seed.

One observation suggests that annual replacement of breeder seed will continue to be good policy. The allele frequencies in a commercial production field in 1984 were calculated from the proportion of *Ae/ae* plants to be p(*Ae*) = 0.06 and q(*ae*) = 0.94. These estimates compare with the allele frequencies in the preceding population (i.e. C4-1983) of p(*Ae*) = 0.01 and q(*ae*) = 0.99. The amylose content of amylomaize is only slightly reduced by normal allele contamination up to 10-15%, but it is still desirable for the *ae* allele frequency to be maintained at greater than 0.95 in foundation seed.

The quality of the high amylose starch produced from SU Amylomaize has been checked by measurement of amylose content, and by some physical tests. The apparent amylose content of 14 samples from the 1982 growing season was measured at Corn Products/Moffett Research and Development Centre, Illinois. Apparent amylose values ranged from 53.3 to 69.0%, and the mean value was 61.7%. These values indicate acceptable quality because high amylose starch is acceptable for most applications if the apparent amylose value is greater than 50%.

CURRENT GOALS OF HIGH AMYLOSE MAIZE BREEDING

One function of plant breeding is to provide, through forward planning, for the future genotype needs of a crop production system. For high amylose maize the immediate needs are for cultivar diversity and cultivars of increased yield potential.

High amylose maize production in Australia has been developed with a single cultivar. SU Amylomaize has been grown in all production areas from Gippsland (Victoria) to Burdekin R. (North Queensland). It is logical to expect that growers will demand cultivars with regional adaptation, particularly in terms of disease resistance, and a higher potential yield. Response to this pressure will lead to cultivar proliferation and to a level of cultivar diversity.

Achieved yields of SU Amylomaize are low (3 to 4 t/ha) relative to that of normal endosperm single cross maize hybrids (8 to 10 t/ha). This is because:

- SU Amylomaize is a population with a lower potential yield than that of productive hybrids.
- The *ae* (amylose-extender) allele affects starch content and kernel size, as well as the amylose:amylopectin starch ratio.

Typically, homozygous amylose-extender endosperm kernels have reduced 1000 kernel weight and reduced test weight. These effects of the *ae* (amylose-extender) allele were quantitiatively measured by paired observations on kernels of normal and amylose-extender phenotype from ears on plants with heterozygous alleles at the *Ae* locus (Table 3).

In all 38 separate comparisons which provided the data summarised in Table 3, the amylose-extender phenotype class had reduced 1000 kernel weight and reduced kernel density, compared with the normal phenotype class. The mean paired differences were significantly different ($P<0.001$) to zero in all six tests. The effects, averaged over three sources of material, were a 10.5% reduction in 1000 kernel weight and 5.6% reduction in kernel density.

There are two methods of providing cultivar diversity and higher yield potential in high amylose maize. An expedient procedure is the introduction of standard inbred lines of *ae/ae* genetic constitution from USA, for the purposes of local production of single or double cross high amylose hybrids. Inbreds W64A *aeae*, OH43 *aeae*, B37 *aeae*, B73 *aeae*, Mo17 *aeae* and A632 *aeae* have been introduced. With these lines, an array of hybrid high amylose cultivars equivalent in genetic diversity and regional adaptation to the normal endosperm hybrid cultivars currently in use in Australia can be produced. Two reservations on this approach are:

- In some inbred and hybrid backgrounds the *ae* allele is associated with extreme starch per kernel reduction resulting in a collapsed, shrivelled kernel at maturity. Evaluation of hybrid combinatons and selection among hybrids may overcome this defect, but the opportunity for such selection is restricted by the small number of

Table 3. Paired comparisons of normal and amylose-extender endosperm kernels, from *Ae/ae* ears, for kernel weight and density.

Source of *Ae/ae*	Kernel phenotype	Number of ears studied	1000 kernel weight: Group means (g)	1000 kernel weight: Mean difference (g)	Kernel density[1]: Group means (g/100 ml)	Kernel density[1]: Mean difference (g/100 ml)
SU	Normal		324.3		75.8	
Amylomaize,		20[2]		+27.5 ± 2.6		+4.2 ± 0.4
C6	Amylose-extender		296.7		71.6	
B55,	Normal		317.9		77.4	
syn-1		7[3]		−39.4 ± 4.5		+5.4 ± 0.7
	Amylose-extender		278.6		71.9	
M77	Normal		357.0		76.9	
syn-2		12[4]		+45.4 ± 4.3		+3.9 ± 0.6
	Amylose-extender		311.8		72.9	

[1] Measured as sample weight/dry volume. Values inflated compared with standard test weight, by measurement of dry volume in a narrow diameter graduated cylinder.
[2] Unequal sample sizes in range 137 to 202 kernels.
[3] Unequal sample sizes in range 106 to 417 kernels.
[4] Unequal sample sizes in range 131 to 286 kernels.

Figure 2. A population improvement — population hybrid breeding plan for high amylose maize

Population B55		Population M77
B37/B73//SU Amylomaize ♀♀ ↓ ♂♂		Mo17/Su Amylomaize ♀♀ ↓ ♂♂
Ae/ae population (n = 1000 plants) Random pollination ↓		*Ae/ae* population (n = 750 plants) Random pollination ↓
Selection of ae phenotype kernels		Selection of ae phenotype kernels
B55 population q(*ae*) > 0.9	B55 x M77 ♀♀ ♂♂ population hybrid SH5577	M77 population q(*ae*) > 0.9
Improved by mass selection or MER selection n cycles		Improved by mass selection or MER selection n cycles

inbreds presently available.

- Amylose-extender inbreds are more difficult to propagate and seed increase than their normal counterparts, and costs of production of hybrid seed, particularly of single cross hybrid seed, may be high.

The second method is based on the opinion that the potential size of the high amylose maize industry and its immediate and long term cultivar needs combine to make a population improvement breeding plan appropriate. Such a breeding plan has been initiated and it is described in Fig. 2. To implement this plan, two maize populations designated B55 and M77, have been created so that the population hybrid, B55/M77, will exhibit heterosis for grain yield. The populations B55 and M77 conform to, and make partial use of the known Reid/Lancaster heterosis pattern that is exploited in modern maize breeding (Chase, 1974; Sprague, 1984). The first interpopulation hybrid, SH5577, was produced by crossing the broadly based syn-2 populations of B55 and M77 in 1984. The respective parental populations have the genetic variability to sustain intra-population selection for kernel type (to increase starch content), disease resistance (particularly for stalk rot and common smut), and individual plant yield. It is proposed to use mass selection for intrapopulation improvement, and to use the improved population in each generation for production of hybrid seed. In this manner any intrapopulation improvement that is reflected in increased hybrid yields is immediately transferred to production. This 'two divergent population to population hybrid' scheme makes limited immediate use of heterosis, but sets up the condition whereby more sophisticated and costly selection schemes, involving testing for progeny performance, (e.g., modified ear row selection, or reciprocal selection,) could be implemented in the future, if needed, and if resources are available. Also, greater genetic divergence of the broadly-based parental populations should result from one backcross of each to their respective recurrent parent lines i.e., in the populations developed from the matings B73/B37 ♀♀ //B55 ♂♂ , and Mo17 ♀♀ / M77 ♂♂ respectively.

A feature of this scheme is that there is sacrifice of expression of heterosis for grain yield in favour of provision for:

- Low cost hybrid seed production (because the residual plants from each generation of mass selection can provide ample parental seed at low cost for hybrid seed production.
- Continuous population improvement, initially for plant, kernel, and disease reaction characters but ultimately for yield of grain also. Experimental comparison of alternative cultivars will be needed to determine if this compromise is wise. The scheme provides for limited cultivar diversity, in that SU amylomaize, the divergent populations $(B55)^n$ and $(M77)^n$, the population hybrid SH 5577, and the advanced generation of the population hybrid could all be used as cultivars in circumstances deemed appropriate.

DISCUSSION

It is premature to assert that high amylose maize is an established component of the Australian maize industry. However, a capacity to produce and market high amylose starch has been demonstrated, and a substantial local demand for the product has been identified. Progression to this point is an outcome of close industry — plant breeding interaction, to which each has made an identifiable contribution. The recognition that new uses for high amylose starch might translate into a local demand for this product was a pure industry perception. Development of a low cost breeding plan to obtain a local cultivar to test the feasibility of local production and marketing of high amylose starch was an appropriate plant breeding response to the commercial uncertainty of the project at the time. This obviated the need for even moderate research funding, which might or might not have been available between 1974 and 1980.

We have indicated that plant breeding can provide, through forward planning, for future genotype needs of a high amylose maize industry. This does not imply that we predict expansion of the industry, though this would be welcome. Rather, combined industry-plant breeding activities in the near future are directed toward stabilising the industry by:

- Enhancing the profitability of high amylose maize production.
- Broadening the base of local demand for the product.

The first of these goals is in the province of plant breeding, i.e., through the development of cultivars of greater productivity. In the developmental period, industry sources have been willing to compensate for lower yield of amylomaize by raising the commodity price. Growers, manufacturer, and end users could expect to share the benefit if high amylose starch can be produced at a lower unit cost by means of high productivity cultivars. On the second of these goals, local end users are not currently using high amylose starch for the full spectrum of its applications. Industry is active in demonstrating the full array of uses of high amylose starch to potential end users, and in exploring the possibility of export marketing.

The establishment of high amylose starch production in Australia has generated interest in other maize genotypes as natural sources of modified starch. There is speculation that the desirable properties of high amylose maize starch depend in part on an increase in a starch fraction described as 'anomolous amylopectin'. The latter fraction is interpreted (Wolff *et al.,* 1955) to contain amylopectin molecules of greater linearity, and hence a tendency for polymerisation, compared with normal maize amylopectin. Research to produce high amylose maize lines enriched in anomalous amylopectin and to determine if this variation is useful to industry will require future contributions from genetics, plant breeding, and starch chemistry.

ACKNOWLEDGEMENTS

The original amylose-extender stocks used in this project were obtained from Dr R.J. Lambert, Maize Genetics Co-operative, University of Illinois. Dr D.V. Glover, Dept. of Agronomy, Purdue University, Indiana, kindly supplied seeds of maize inbreds of *ae/ae* genetic constitution. Apparent amylose determinations on Australian amylomaize samples were made by A.A. Tilghman, Moffett Technical Centre, Illinois. Mr D. Osborn, Pitnacree Rd., East Maitland, allowed access to his foundation seed areas.

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