Invited paper: Using synthetic wheats to breed cultivars better adapted to changing production conditions

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Abstract

Bread wheat is the most widely cultivated and consumed food crop. In the past decade its area of production has increased in rainfed regions. Agriculturally significant interactions have been observed between cultivars and agronomic practices. Bread wheat developed from two stages of intercrossing among diploid grass-like species. About 10,000 years ago the cross between *Triticum urartu* and *Aegilops speltoides* resulted in *T. dicoccoides* (wild emmer). In about 6,000 BC the domesticated version of this latter species, *T. dicoccum* (cultivated emmer) intercrossed with *Aegilops tauschii* (goat grass). This cross gave us *T. aestivum* (hexaploid bread wheat), while *T. dicoccum* itself evolved into *T. turgidum* ssp. *durum* (tetraploid durum wheat). Using cytological techniques bread wheat can be artificially recreated by intercrossing modern tetraploid durum wheat with present-day derivatives of goat grass. Groups in the USA (e.g. Kansas State University), Mexico (CIMMYT) and Australia (e.g. Department of Primary Industries, Victoria) have developed such "synthetic" hexaploids and are using them in (pre-) breeding. Despite some technical difficulties in intercrossing synthetic and modern bread wheats, many synthetic derivatives have been developed. A number of them have shown great promise in resistance to most major wheat diseases, tolerance to abiotic stresses such as drought, heat, waterlogging. It is even possible to develop high end-use quality derivatives that meet industry standards. Recent experiments carried out by DPI-Vic's SynERGE research group across Australia have identified synthetic wheat derivatives that outyield commercial varieties in rainfed conditions by 18-30%. At the molecular level these new synthetic derivatives have boosted genetic diversity.

Breeding and Agronomy

The two crops most widely consumed by humans are wheat and rice. In 2005, 220 and 155 million hectares were respectively grown around the world, producing 630 and 620 million tons. Of these two crops wheat is geographically the most widely adapted. It is planted from just above the northern polar circle (65?N), to the southern regions of Argentina (55?S), and in the equatorial regions of east and central Africa and the Andean region of Latin America. In the latter regions it is grown up to 3000 meters above sea level, while in the Netherlands it grown in reclaimed polders several meters below sea level. It is estimated that more than 75% of the world's population consumes wheat as part of their diet daily. In parts of northern Africa and in the newly independent republics in the Caucuses region, annual consumption per person is highest at around 200 kg (Pingali, 1999).

Since the mid 1970's most of the expanded adoption of new varieties has taken place in rainfed areas, rather than irrigated regions (Reynolds and Borlaug, 2006). Yield increases under irrigated production have remained around 0.5-1% per year, while in many rainfed environments annual production per hectare has increase by a surprising 1-3% (Trethowan et al., 2002). In many countries, this was achieved alongside the adoption of improved management practices including minimum or zero-tillage. Presently 11 north and south American countries apply conservation technologies on more than 70 million hectares to grow a plethora of crops, often including wheat in the rotation. These growers include small subsistence farms and commercial farms, which range in size from a fraction of a hectare to spanning several thousands of hectares (Roberto Peiretti, CAAPAS President (American Confederation of Farmers Organizations working for Sustainable Agriculture), pers. comm.).

While both genetic and agronomic progress in increasing yield and adaptation have both been very successful, studies on synergistic interactions between modern varieties and modern production methods remain rare (Trethowan et al., 2005). This is somewhat surprising as anecdotal evidence abounds that indeed there are agriculturally significant interactions between varieties and production methods, although

not always statistically significant ones when studied in trials sampling few years and locations. Nevertheless some breeders can be slow in modifying the agronomy in their breeding plots to reflect those in farmers' fields. In some cases the situation is the reverse, and breeders are using methodologies more advanced than the growers in the target region. However, increasingly growers are adopting new practices faster than the breeders, and the latter continue applying outdated husbandry to their plots (Roberto Peiretti, CAAPAS President (American Confederation of Farmers Organizations working for Sustainable Agriculture), pers. comm.). It is obvious that this approach may result in opportunities lost.

A new generation of wheat varieties is on the horizon that will be discussed below. As the traits are discussed, it will be obvious that agronomists and breeders should work closely together to enable these new genetic resources to translate optimally into more stable, increased production and return for growers. But first we present a bit of history on the origin of wheat.

Wheat's Parents

About 10,000 years ago in the Fertile Crescent region in western Asia, possibly somewhere in what is now southern Turkey, early wild wheat was domesticated by humans who were beginning to adopt sedentary agriculture (Feldman, 2001). The wheat plants they encountered were derivatives from a natural cross between two distantly related grasses. These wild grasses are known as *Triticum urartu* and *Aegilops speltoides*, and their rare intercrossing resulted in *T. dicoccoides* (wild emmer; AABB), bearing fairly large spikes or heads. When this cross originally took place is not well known, but this could have been some 30,000 years ago. It is also not known how many individual, unique events of such an intercross occurred (Simonite, 2006).

Three major problematic traits were resolved by human reselection within about 1000 years (8000-7000 BC; Feldman, 2001), constituting the 'domestication' of wheat, the so-called "domestication syndrome". 1) Uniform crop establishment and growth. Rare mutants with synchronous germination and maturity were identified, facilitating uniform stands and easy bulk harvest at the end of the season. Originally only a small proportion of seeds planted germinated around the same time in response to the first rains and grew into plants, with the remainder waiting until later rains. This is a good survival strategy, as a plant will not "know" whether the first rains will set through, but such a response would leave a modern farmer's field fairly bare, with a just a portion of the seed planted actually germinating and emerging. 2) Selection for indehiscent seeds. In wild emmer the grain-bearing spikelets on the spike are held together at the base by a brittle extension of the main stem called the 'rachis'. As the spike matures and dries, spikelets enclosing the grain drop to the ground, ready to germinate and establish a new generation. While understandable from the evolutionary standpoint of a wild plant ensuring that its progeny is naturally sown, this shattering of the spikelets did not facilitate easy harvest. Diverging genotypes were identified that did not have a brittle rachis, but a rachis that remained intact firmly holding onto the spikelets and grain, and allowing easy harvest. 3) Selection for threshable seed. Wild emmer and some of its later partially domesticated relatives had grains that were fused to the glumes (similar to flower petals), not unlike modern barley. This provided a measure of protection to the seed, but encumbered the threshing process, the aim of which is to obtain pure uniform flour ready for preparing bread products, which do not contain pieces of the hard inedible glume.

By 7,000 BC a new domesticated wheat type emerged called *T. dicoccum* (cultivated emmer), which proved a great success (Feldman, 2001). It quickly spread throughout the newly forming agricultural settlements as far south as northern and north-eastern Africa and moved both west into what is now Europe and eastwards into Asia proper, and eventually evolved into modern durum wheat (*T. turgidum* ssp*. durum)*, used for pasta products. It is likely that that emmer wheat evolved into distinct ecotypes as new environments were encountered as cultivation spread outwards from the Fertile Crescent. Human adoption, reselection and further domestication led to genetic bottlenecks that may have resulted in an expansion of ecotypes or landraces, but each less diverse than the original parental population.

Around 6,000 BC in what is now Iran, pollen from a third wild grass, the goat grass *Aegilops tauschii* (DD), pollinated a cultivated emmer plant (*T. dicoccum*) and naturally formed hybridised seed (Feldman, 2001). This is a rare occurrence and probably happened in the region near the Caspian Sea. This cross

resulted in the emergence of hexaploid wheat (*T. aestivum*; hexaploid genome = AABBDD), from which a product could be made that could be classed as a 'convenience' food. Unlike any other crop the flour of bread wheat contains gluten, which allows it to rise while being prepared and then maintain this expanded shape, resulting in various forms of 'bread'. Once made, these are easily transported and can be readily consumed.

On the one hand, bread wheat seems miraculously diverse, having sprung from the diploid genomes of three distinct grasses, *T. urartu* (AA), *Ae. speltoides* (BB) and *Ae. tauschii* (DD). However, upon closer examination it appears likely that genetic diversity in the cultivated emmer is likely to have been low as a result of geographical spread and selection by the time it encountered *Ae. tauschii*. With even more certainty we know that the cross between the female *T. dicoccoides* (tetraploid genome = AABB) and the male *Ae. tauschii* (diploid genome DD) happened very infrequently. Modern bread wheats contain either one or two versions (alleles) only of DD genome genes, which led to the theory that just one or two individual *Ae. tauschii* plants actually contributed DNA to modern bread wheats (Ogbonnaya et al., 2005). Seemingly contradictory, bread wheat's genetic foundation thus is both broad and narrow at the same time.

Modern bread wheat is a relatively young crop and has only experienced about 8,000 generations of evolution. Humans (*Homo* spp.), in comparison, whose ancestors have been around for 3.5 - 4.5 million years, have gone through about 300,000 generations of evolving and recombining genetic differences in the gene pool. This means that humans have had their genes reshuffled by a multiple of about 30 times more often than wheat.

Modern times

When we fast-track to the present day, we see this young and genetically relatively narrow crop becoming the major human staple on all inhabited continents. Breeders continue to find some genetic variability within the crop (originally present or evolved) that help them address many of the common constraints. But increasingly we find ourselves genetically constricted for the reasons detailed above. Abiotic production constraints such as drought, heat, frost, increasingly prove difficult to address satisfactorily using the present genepool. Likewise variable biotic constraints, exemplified by Take All, stem rust, stripe rust, soil-borne diseases and insects, present challenges that require increasingly quick responses. With climate change upon us (Hughes, 2003; Natural Resource Management Ministerial Council, 2004; Pittock et al., 2003), expanding the genetic diversity for such a major food crop as wheat seems both imperative and a great opportunity for Australia to help lead.

Reproducing Bread Wheat's Original Cross

As described above modern bread wheat was born out of a limited number of chance crosses combining the genetic diversity of a few individuals from three different grasses. Obviously derivatives of many other individuals from the same grass species that did not participate in the initial crosses might contain genes that could be beneficial to wheat in today's much changed growing environment. Some of the derivatives of those early wheat relatives are still around today. The question then is: can we cross genes from such wild relatives into wheat and in a sense recreate bread wheat all over again from scratch?

In the 1940's Kihara in Japan (1944) and McFadden and Sears in the US (1944, 1946) independently intercrossed various A-, B- and D-genome grasses with one another, in the quest to describe the original parents of durum and bread wheat. It is during this research 60 years ago that the roles of the various parental grasses in wheat's parentage, referred to above, were established. Following that period no known or widely successful attempt was made to expand genetic diversity in wheat using this knowledge of ancestry for breeding purposes until the 1980's, when the first articles appeared projecting great promise from such "synthetic" wheats (Gill et al., 1985). When a new synthetic cross is made using a tetraploid and *Ae. tauschii* accession, genetic diversity in bread wheat increases by about 50%, assuming no more than two original events of 'hexaploidization' took place. The current challenge is to make the best use of this new diversity in developing varieties for farmers and consumers.

Approximately at the same time (1985-1990) Cox and Mujeeb-Kazi embarked on identifying and applying genetic variation from wild wheats in a way that was relevant to modern breeding programs. Cox worked on winter wheats at the Kansas State University (KSU), in Kansas, USA, while Mujeeb Kazi was based at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, focusing on spring habit wheats. Both associated breeding programs soon saw the potential in these new hybrid crosses (Cox, 1995; Mujeeb-Kazi et al., 1996). In CIMMYT's wide crossing program most synthetics were produced using modern durum wheats (*T. turgidum* ssp*. durum)* as the AABB donor, while a few dozen combinations included *T. dicoccoides* and *T. dicoccum.*

Some of the first breeders and geneticists that started utilizing this novel diversity at KSU included Rollie Sears, Gina Brown Guedira and Allan Fritz. At CIMMYT from 1990 onwards Sanjaya Rajaram and Maarten van Ginkel among others were the first to incorporate primary synthetics in the active bread wheat breeding program. This was motivated by the high levels of resistance to Karnal bunt (*Neovossia indica* or *Tilletia indica*) in the synthetic hexaploids. At the same time in Australia, Lagudah (1986) initiated work on synthetics, followed by Eastwood (1995) under the supervision of Dr Gerald Halloran of the University of Melbourne.

Incorporating Novel Diversity from Wheat's Wild Relatives into Common Wheat

In the past 16 years, CIMMYT scientists have created 1014 spring habit synthetics wheats, and about 200 winter habit synthetics. Thousands of crosses were made between these synthetics and modern advanced lines, resulting in numerous synthetic derivatives. Present estimates are that more than one third of all new advanced bread wheat lines produced by the CIMMYT breeding program for irrigated and low rainfall areas around the globe are synthetic wheat derivatives.

How difficult is it to introduce such novel genetic diversity into ongoing bread wheat breeding programs? Four issues of potential concern, which can be resolved based on experience by scientists at CIMMYT, DPI-Victoria and others, are discussed below.

- Excessive glume and rachis hardness is observed in quite a large proportion of the synthetics. Their inheritance is not well understood. However, in practice it has been possible to restore ready threshability in synthetic derivatives by back-crossing them to common wheats, growing relatively large F2 populations and selecting for 'normal' spikes in the F3. Therefore this issue is easily addressed.
- Hybrid progeny from synthetic and common wheat crosses may die due to excessive necrosis at the early seedling stage. Pukhalskiy et al. (2000) summarize the distribution of the Ne1/ne1 and Ne2/ne2 hybrid necrosis alleles. When the alleles at both separate loci are present in a heterozygous state (as in an F1 from a cross involving complementary parents) seedlings will die as early as the one or two leaf stage (Worland et al., 1987). Frequencies of the alleles are different in bread wheats and durum wheats, and as a result when bread and durum wheats are crossed (notwithstanding problems associated with genome size discrepancies) the F1 progeny will often express hybrid necrosis. As synthetics are derived from crosses involving durum wheats (or their ancestors), crosses between synthetics and modern bread wheats (both hexaploid) on average will result in hybrid necrosis in 1-50% of the cases. Its effects can vary considerable; for instance, Wilson, Eastwood and Ogbonnaya (unpublished) crossed 163 CIMMYT synthetics as male parents with a Victorian wheat cultivar, "Goldmark". Of these, more than 90% were severely necrotic, suggesting that more than 140 of the synthetic hexaploids and/or their tetraploid parents carry the complementary necrotic gene to the one in "Goldmark". Unless the exact allele constitution of both intended parents is known, only experience will tell which combinations will not result in hybrid necrosis. The solution is to try various combinations between different varieties and synthetics.
- Synthetics themselves are often less than impressive compared to common wheats from an agronomic standpoint. A small proportion has high biomass and this increase appears to be transmissible in crosses to common wheat. But in other cases outstanding progenies can be derived from crosses with seemingly mediocre synthetics. Tanksley et al. (1996) have shown that wild tomatoes expressing complex traits at levels that overall are not up to modern breeding

standards, may contain component genes that in additive fashion can in fact improve modern breeding germplasm once introgressed. The same phenomenon appears to be operating in crosses involving synthetic wheats and modern wheats. The take home message is to persevere with the synthetics, possibly initially emphasizing those that have resulted in outstanding progenies in other programs.

- Issues related to end-use quality are also holding some breeders back from exploring the potential of synthetic wheats. When *Ae. tauschii* was first studied, its potential for improving quality or indeed compromising it through the introgression of undesirable genes was not clear (Lagudah et al., 1987). In recent years a number of publications have indicated that in fact the introgressed D-genome may improve wheat quality (Yueming et al., 2003). Gedye et al. (2004) reported the discovery of novel haplotypes for puroindoline in synthetic hexaploids with potential impact on kernel texture in wheat. They suggested that the new puroindoline alleles from *Ae. tauschii* all gave soft endosperm though textural differences were apparent, attributable to interactions between the durum and *Ae. tauschii* genomes. In contrast, Lillemo et al. (2006) found significant but small differences in grain hardness amongst the *Pina* and *Pinb* alleles using a larger collection of synthetics and its derivatives. The functional effects of the new *Ae. tauschii* derived puroindoline alleles in wheat quality is yet to be determined. The results of the evaluation of about 200 synthetics suggested that the *Ae. tauschii* parent has a much larger influence on the quality of the synthetic hexaploid than the durum parent (Ogbonnaya and Cornish, unpublished). About 20% of synthetic hexaploids could be classified as attaining the Australian hard and Australian prime hard quality classification based on examining the polymeric glutenins proteins (Table 1). This is consistent with the results by Lage et al. (2005), who reported significant genetic variation among synthetic hexaploids for protein content, SDS-S values, grain weight and plumpness. In summary, synthetic hexaploids with poor bread-making quality but otherwise outstanding traits, should be exploited by crossing and backcrossing to high quality conventional wheats, while other synthetics with novel proteins should be studied to determine their potential value.

Table 1 Suggested classification of 194 synthetic hexaploids according to %UPP (un-extractable polymeric protein) determined by size exclusion high performance liquid chromatography (SE-HPLC) (Ogbonnaya and Cornish, unpublished data).

%UPP	Predicted dough strength	Predicted Class	# Synthetics
<30	Very weak	AS	54
30-44	Weak	ASW	85
45-49	Intermediate	APW	25
50-54	Strong	AH	20
55+	Very strong	APH	10

However, besides the question whether novel genetic diversity for bread-making traits can be introgressed from *Ae. tauschii* through synthetics, the major concern among a number of breeders remains as to whether *any* synthetic derivatives can be extracted that have even acceptable bread-making quality. In the past five years at CIMMYT it has become abundantly clear that synthetic derivatives carrying excellent bread-making quality can indeed be bred if the common bread wheat parent(s) in the cross has good quality. HMW- and LMW-glutenin profiles of the parents are used to determine promising crosses, and to identify the best quality lines in their progeny. Nelson et al. (2006)

reported that some lines from the synthetic (W7985) x Opata ITMI population showed quality values consistently superior to those of the parental lines. Despite these observations by breeders at CIMMYT and elsewhere, some breeders remain sceptical about using synthetics to resolve non-quality related constraints in their target production environment, because they are afraid of compromising wheat quality.

Traits for which Novel Diversity from Synthetics Proves Highly Promising

Over the past decade CIMMYT has produced synthetics and their derivatives with exceptional expression for such traits (only one or two references provided) as:

Agronomic features

- Yield (components): yield under irrigated conditions (Villareal et al., 1994a).
- Yield under rainfed drought conditions (Reynolds et al., 1999).
- High thousand kernel weight (up to 65 grams; Calderini and Reynolds, 2000).
- High above ground biomass (Villareal et al., 1994a).
- High photosynthetic rate (Blanco et al., 2000).
- Other morphological traits (Villareal et al., 1994c).

Abiotic tolerances

- Tolerance to drought (Reynolds et al., 1999).
- Frost at flowering (Maes et al., 2001).
- Salinity (Schachtman et al., 1992).
- Waterlogging (Villareal et al., 2001).

Biotic resistances

- Resistance to the following diseases: leaf rust (Ma et al., 1995).
- Stripe rust (Assefa and Fehrmann, 2000).
- Stem rust (Marais et al., 1994).
- *Septoria tritici* leaf blotch (Arraiano et al., 2001).
- *Septoria nodorum* glume blotch (Loughman et al., 2001).
- Fusarium head blight (Mujeeb-Kazi et al., 2001).
- Tan spot (a.k.a. yellow leaf spot; Cox et al., 1992).
- *Helminthosporium* leaf blight (Mujeeb-Kazi et al., 2001).
- Powdery mildew (Kong et al., 1999).
- Karnal bunt (Villareal et al., 1994b).
- Cereal cyst nematodes (Eastwood et al., 1991).
- Root lesion nematodes (Thompson et al., 1999).
- Greenbug (Hollenhorst and Joppa, 1983).
- Hessian fly (Tyler and Hatchett, 1983).

Quality features

- Proteins (Williams et al., 1993).
- Glutenins (Pena et al., 1983; Pfluger et al., 2001).
- Zn efficiency (Cakmak et al., 1999).

Resistances to several of these abiotic and biotic stresses have also been found in the evaluation of these synthetics in Australia, some of which are discussed in more detail below.

Yield and Yield Components

Yield trials including synthetic derivatives carried out in the second half of the 1990's by CIMMYT bread wheat breeders under irrigated conditions revealed that their yield potential had reached that of the common wheats, despite trailing initially. Considering that this had been achieved in just 5-7 years, there was optimism that yields exceeding those of common wheats were not far off. In 2001-2003 synthetic wheats were identified that equalled the highest yielding common wheats, with some exceeding them.

During this period the first synthetic derivatives were included in CIMMYT's international yield trials and provided to hundreds of cooperators around the world. These trials started to identify some of the synthetic derivatives as globally competitive and expressing both specific and wide adaptation.

Adaptation to Drought Conditions

While yield potential under fully irrigated conditions appears to be boosted by genes from synthetic wheats, adaptation to drought conditions is only partly explained by 'yield potential'. During the second half of the 1990s synthetic derivatives were subjected to experimental drought conditions at CIMMYT, with a lot of this work led by Richard Trethowan. By 2002 the first such derivatives, including some that contained Australian varieties in their pedigrees were introduced by Francis Ogbonnaya of DPI Victoria into Australia.

Breeding for increased wheat yield in the diverse rain-fed Australian environments remains a challenge for plant breeders. The synthetic derivatives tested around Australia in rainfed conditions during the past four years were mostly those where primary synthetics had been backcrossed into Australian genetic backgrounds (i.e. commercial varieties). They showed up to 8-30% increased yield over both the Australian parents and the local modern check varieties (Dreccer et al., 2006; Ogbonnaya et al., 2006; Fig 1). Some of the higher yielding SBLs under optimal moisture conditions were also the best yielding under limited moisture. These results indicate that synthetic derivatives are a promising means of significantly improving wheat yield in more diverse and stressed environments than hitherto thought possible.

The chart shows Predicted yield (t ha-1) on the y-axis (ranging 3 to 7) versus sites Hor-R, Roma, Hor-I, BI, NA on the x-axis. Two regression lines are shown: Synthetic backcross line with $y = 0.6344x + 3.0406$, $R^2 = 0.9519$; and best local check with $y = 0.453x + 2.9586$, $R^2 = 0.6931$.
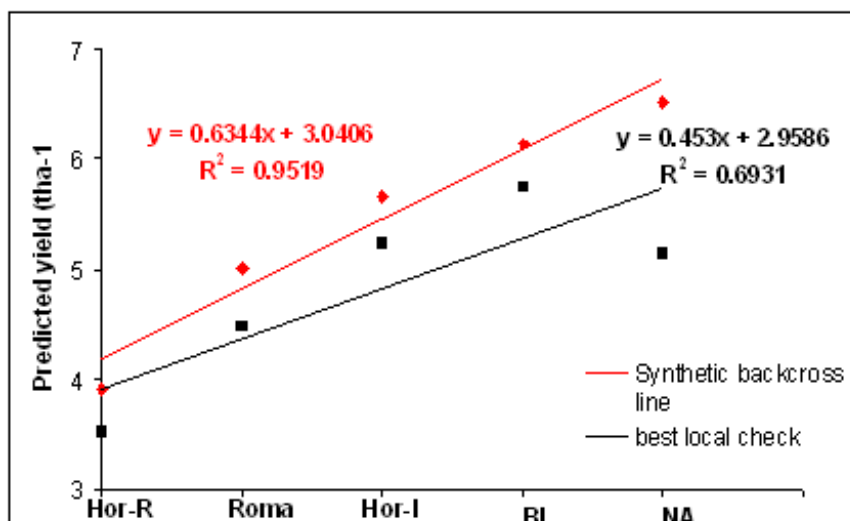
Figure 1. Yield of synthetic backcross derived lines and the best local check regressed against site mean yield at five sites across Australia in 2005. Hor-R, Hor-I, BI and NA represent Horsham rainfed, Horsham irrigation, Biloela and Narrabri.

Globally synthetic derivatives have been very impressive, outyielding local varieties by 5-40% under drought conditions in India, Pakistan, Ecuador, Australia and Argentina (Coghlan, 2006). CIMMYT and Australian scientists have studied the success of these synthetic wheats and suggest that this may be due to unusually deep and/or thicker roots, providing better access to soil water.

Heat Tolerance

Some of the synthetic derivatives showed tolerance to hot conditions (up to 35-40?C in Mexico) during grain filling. In Australia heat and drought tolerance are expected to become increasingly important as temperatures rise and become more variable (Hughes, 2003; Natural Resource Management Ministerial

Council, 2004; Pittock et al., 2003). Synthetics offer an avenue to explore genetic variation for adaptive traits, which could be used to mitigate the impact of fluctuating temperatures during the critical stages of grain growth and development.

Salt Tolerance

It is estimated that 20% of the irrigated land in the world is presently affected by salinity, excluding the regions classified as arid and desert lands (Yamaguchi and Blumwald, 2005). In Australia, sodicity and associated subsoil salinity may affect 30-60% of the in the 250-450 mm annual rainfall zone, in areas either subject or not to rising water tables (Rengasamy, 2002). Yields can be well below theoretical for the rainfall received, when subsoil salinity is present, and unused water at harvest is one its symptoms (Sadras et al., 2002). The cost of transient salinity and associated constraints in sodic soils for the farming economy in Australia has been estimated at ca. AU$1.5 billion per year (Rengasamy, 2002). In addition to the adoption of good agronomic practices such as the application of gypsum, genetic solutions must also be considered because of the limitations inherent in relying on management practices alone. For example, the incorporation of gypsum has had limited success because of the addition of salts to subsoil salinity. It has been argued that cropping in areas with subsoil salinity is sustainable, as there is no contact with groundwater. (Munns et al., personal communications), though the impact in areas with rising water tables associated with leaky agricultural systems is unknown. Therefore salt-tolerant germplasm should be sought as an economically feasible alternative to methods of soil amelioration. However, there is limited genetic variability in elite germplasm used in breeding programs amongst current bread wheat cultivars. Considerable genetic variation for salt tolerance has been found in synthetic hexaploids (Ogbonnaya et al., 2005; Prichard et al., 2002; Schachtman et al., 1992, 1991). In saline conditions, synthetic hexaploids exhibited significantly better Na^+ exclusion and maintained higher leaf K^+ compared to cultivated wheat varieties (Fig. 2).
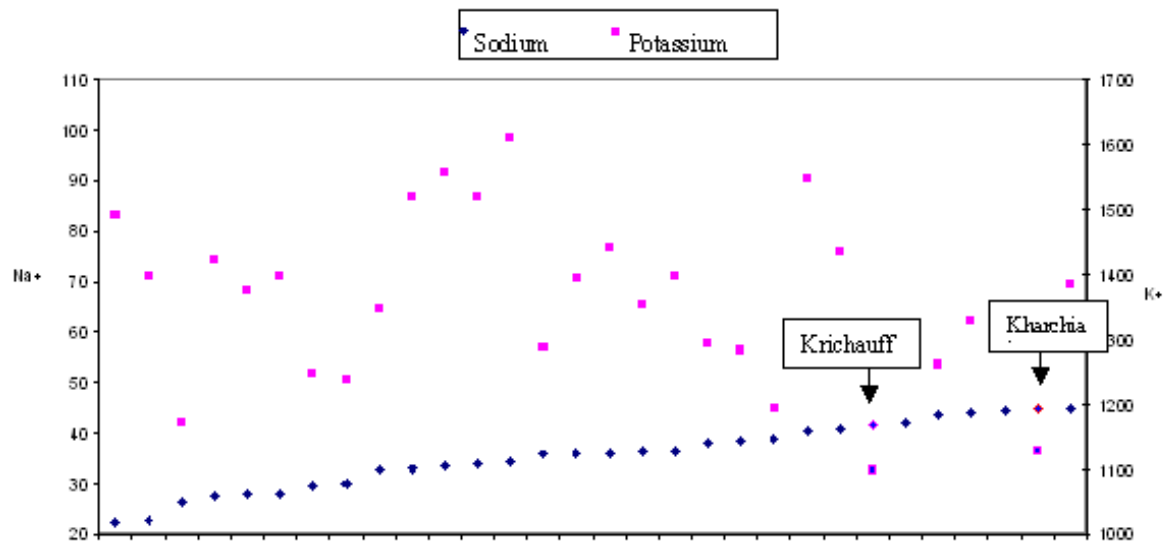

Figure 2. Predicted geometric mean sodium and potassium concentration of synthetic hexaploids showing the range of variation in sodium content of synthetic hexaploids against bread wheat tolerant cultivars, Krichauff and Kharchia. The X-axis represents the 28 synthetics studied. The left and right Y-axis represent sodium and potassium concentration, respectively.

A major locus, *Kna1* was identified on chromosome 4D that is linked to this mechanism in bread wheat (Dubcovsky et al., 1996). Much of the screening for salt tolerance to date has been based on the sodium exclusion mechanism. However, this mechanism alone does not explain the full spectrum of genetic variation that is available in the synthetics, suggesting that additional useful loci may be involved. Given the substantial level of variation in salinity tolerance in synthetics, there is an opportunity to uncover novel

mechanisms involved in conferring salinity tolerance. The discovery of novel mechanisms that may act additively and can be pyramided may substantially boost the salinity tolerance of bread wheat.

Tolerance to Pre-harvest Sprouting

Pre-harvest sprouting (PHS) reduces end use quality especially in environments with rainfall and high humidity during the harvest period. In a survey of more than 60 Australian wheat cultivars only one PHS tolerant genotype was found (unpublished data from Daryl Mares). Thus, PHS remains a major cause of wheat downgrading in Australia, especially in the north and some parts of western and southern Australia. Average losses across the Australian wheat crops are about $30-40 million annually (Abawi and White, 2000). PHS resistance is inherited as quantitative trait controlled by a large number of genes, which are significantly influenced by genetic background, environmental conditions and their interactions. Synthetic hexaploids possess a considerable amount of genetic variation for seed dormancy measured as germination index (GI; percentage of seeds germinated at a particular day after imbibition), a major trait associated with PHS tolerance. For example, the average germination index at day 7 (GI-7) of synthetic hexaploid was 0.29 (range 0.01 to 0.71) compared to the bread wheat susceptible cultivar, Annuello with GI-7 of 0.86. Imtiaz et al. (2006) reported the development of synthetic derived bread wheat lines highly resistant to pre-harvest sprouting that are either white-grained or red grained. Ogbonnaya et al. (2006b) also reported the identification of novel quantitative trait loci associated with seed dormancy in synthetic backcross derived lines.

Disease Resistance

Synthetic wheats also appear to be resistant to a large number of major wheat diseases. For some diseases (e.g. cereal cyst nematode, *Septoria tritici* leaf blotch, *Septoria nodorum* glume blotch, yellow leaf spot and *Helminthosporium* leaf blight) levels of resistance that approach immunity in limited experiments in Mexico and Australia have been found, which was not earlier thought possible. Introgression of such novel resistances into new varieties is expected to raise production as the negative effects on yield by these diseases may be almost eliminated.

Several diseases occur widely in commercial wheat crops in regard to which few if any advances are reported in the literature by breeders at all, because little if any genetic resistance is known to exist in the common wheat genepool. These diseases include several of the foot and root rots, such as Take All, *Pythium* and *Rhizoctonia*. Alongside the identification and implementation of linked molecular markers, moving novel resistance genes from synthetics into new varieties will allow profitable production in some areas in Australia presently considered as having very hostile soils. Some synthetics possess resistance to multiple diseases, providing breeders and researchers with the opportunity to use synthetics to develop germplasm with an array of disease resistances.

Stem rust was the single major wheat disease for centuries around the world including in Australia until 40-50 years ago when the disease was brought under control using a gene complex (i.e. several genes interacting) derived from a *T. dicoccum* variety called Yaroslav. Since the late 1990s a new strain of stem rust has arisen in Uganda (called Ug99) that posses a challenge to the Yaroslav gene complex. While its resistance is not fully negated, disease levels on the carrier plants are too high for farmers to find acceptable. Fortunately some of the synthetic wheats have proven highly resistant to this new strain. While the Ug99 strain has not yet reached Australia, we now know that novel resistance is available when we need it.

Table 2. Genetic variation in synthetics for various diseases expressed as a percentage of the total number evaluated (total numbers varied from 200-280; Ogbonnaya et al, unpublished data).

Disease classification	CCN	YLS-seedling	Leaf rust	P. neglectus	Septoria nodorum

|					leaf	glume
Resistant	6	2	18	7	-	3
Moderately resistant	-	51	29	-	40	16
Moderately susceptible	-	35	24	30	47	71
Susceptible	94	12	29	63	13	13

* CCN = cereal cyst nematode, YLS = yellow leaf spot and *P. neglectus* = *Pratylenchus neglectus*

Genetic Diversity

The effect of using synthetics in the ongoing CIMMYT wheat breeding program on inherent genetic diversity is large and positive (Warburton et al., 2006). Figure 3 depicts the history of genetic diversity in wheat breeding, and the following three phases are observed:

- The level of genetic diversity available in original landraces, representing the dawn of domestication.
- The level of genetic diversity once science-based breeding was adopted (1950' – 1990's), when diversity dropped.
- A return in genetic diversity to levels reminiscent of those of the landraces, once synthetic derivatives had been introduced (early 2000) into advanced lines coming out of ongoing breeding at CIMMYT.

Figure 3. Plot of a molecular measure of genetic diversity (the quadratic response of the Shannon diversity index (Y-axis)) over time (measured for each of seven Year Groups*). Each observation has ? standard error (Warburton et al., 2006).
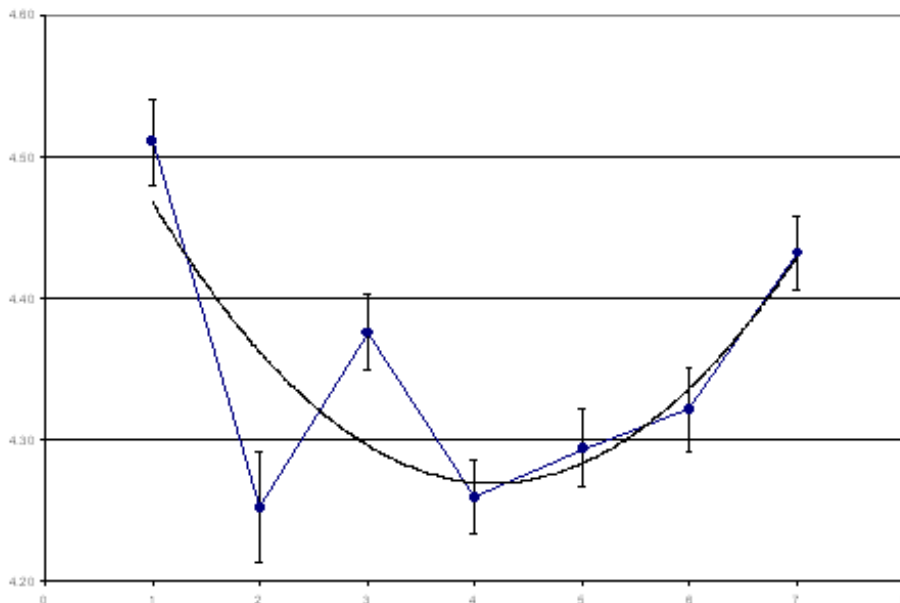
Year group 1 = Landraces.
Year group 2 = Cultivars released between 1950 & 1966.
Year group 3 = Cultivars released between 1967 & 1974.
Year group 4 = Cultivars released between 1975 & 1982.
Year group 5 = Cultivars released between 1982 & 1989.
Year group 6 = Cultivars released between 1990 & 1997.
Year group 7 = Breeding lines in advanced field trials for the years 2002 - 2003
and performing well; expected to be released within 0 & 3 years as cultivars.

Commercialization of Synthetic Derivatives

China is one country that quickly realised the potential of this novel genetic diversity. China has been using the CIMMYT-developed synthetic hexaploid wheat in research programs since 1995 in order to improve quality, yield potential, and disease resistance in the Sichuan province. Chinese scientists report that the synthetic wheats pass on their cross progenies such beneficial traits as large kernels, high spike weight, and resistance to new races of local stripe rust. Chinese breeders have created their own synthetic derivatives by crossing CIMMYT's primary synthetics with their local wheat varieties (first author; pers. comm.).

They released the first such self-made synthetic derivatives to farmers in 2003, and are currently testing five more. One of the two new varieties had the highest average yield (> 6t/ha) of any variety during two years of yield trials, outyielding the commercial check variety by 35%. This variety, named Chuanmai 42, has already become an important breeding parent also in other programs in China.

In Spain the first synthetic derivative from CIMMYT germplasm was pre-registered in 2003 under the name Carmona, and is fast growing, providing seed in a shorter period than most commercial cultivars. This will help wheat growers who often plant late in the year in southern Spain. The variety adapts to zero-tillage systems, and it fosters fewer foliar diseases, giving better yields, and high industrial end-use quality.

In many other countries around the world synthetic derivatives are in advanced stages of testing and will soon be officially released.]

Research and Promotion of Synthetic Wheat in Australia

Since 2000 the research team housed in the Plant Genetics & Genomics Platform within the Department of Primary Industries (DPI) in Victoria, has taken the lead in Australia in facilitating the introduction of novel diversity from synthetic wheats into ongoing breeding programs. As of this year the program has renamed itself SynERGE (Synthetic Enriched Resources for Genetic Enhancement), and established its own website/URL (http://bioinformatics.pbcbasc.latrobe.edu.au/synerge/index.html)

All synthetic wheat related research by this group is coordinated through the Molecular Plant Breeding Cooperative Research Centre (MPBCRC). Funding is provided by DPI, MPBCRC, Grains Research and Development Cooperation (GRDC), plus several others.

The exploitation of the wild relatives in the SynERGE program has occurred through 'recreating' synthetic hexaploid wheat from artificial hybridisation between its progenitor species, *T. turgidum* ssp. *durum* and *Ae. tauschii*, in addition to acquiring primary synthetics from CIMMYT.

The program seeks to provide the delivery of additional useful genetic diversity held in wheat's wild relatives to the Australian wheat breeding programs, acting as a conduit between national/global discovery and genetic resources sciences, and the breeding programs. The following processes are involved:

- Discovering and identification of novel genetic diversity

- Dissecting the genetic basis
- Introgression of novel genes
- Development of tools that will allow these novel genes to be rapidly exploited, including especially diagnostic or linked molecular markers
- Development of parental wheat germplasm with novel genetic diversity.

The key traits to be targeted are prioritised through direct and continuing consultation with Australian wheat breeders. Strong linkages exist between the Horsham-based SynERGE program and all major wheat research, breeding and industry partners in Australia and also to a large extent abroad. Outside Australia, the group has developed particularly strong linkages with CIMMYT in Mexico and the International Center for Agricultural Research in Dry Area (ICARDA) in Syria. Currently, the traits being targeted by SynERGE include improved resistances to pre-harvest sprouting, drought, salinity, cereal cyst nematode and yellow leaf spot. Several other abiotic and biotic constraints are being screened under the SynERGE umbrella through collaborative projects with others throughout Australia.

Conclusions

The following conclusions can be drawn from this brief expose on synthetic wheats and their potential.

- Wheat has both a diverse background, originating from the intercrossing of three distinct grasses, and a narrow base, as just a few individuals contributed their genes to wheat.
- The original cross that formed bread wheat can be recreated by scientists using conventional crossing between the grass-like parents of wheat. The result is called 'synthetic' wheat, and due to the genetic diversity among the parental grasses the new synthetic wheats may have many novel genes.
- Despite some surmountable technical difficulties these genetically diverse synthetic wheats can be crossed to common wheat in ongoing breeding programs, resulting in synthetic derivatives.
- Among the synthetic derivatives several have been shown to have high yields, large grains, deep roots, tolerance to abiotic and biotic stresses, and increased quality.
- Several years of trials throughout Australia have identified a number of synthetic derivatives that outyield the commercial varieties by 18-30%.
- At the molecular level a large increase in novel genetic diversity has been shown beyond present-day commercial varieties and similar in magnitude to old landraces.
- China was the first country to release a high-yielding synthetic derivative (i.e. Chuanmai 42) to its farmers.
- In Australia synthetic research is coordinated through the DPI-Victoria SynERGE research program, based at Horsham.

Acknowledgments

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