

Project report to Defra: Wheat Breeding AR 0914

Introduction

Traditional plant breeding programmes rely on selecting genotypes in optimal conditions: in the absence of weeds, diseases and pests and with peak nutrient availability. This breeding approach has produced many successful pedigree line bred varieties for non-organic production systems. However, organic agriculture has suffered from a lack of varieties adapted to the environmental variability on organic farms (Wolfe *et al.*, 2008). Furthermore, there is a need to reduce costly inputs in non-organic agriculture and to prepare for the impacts of climate change.

In organic and low input conditions, the variability of the environment has a far greater influence on yield than the choice of variety (Wolfe *et al.*, 2008). This can lead to a lack of stability in performance that has been demonstrated in many studies (e.g. Soliman & Allard, 1991). Physical mixtures of complementary varieties provide an improved ability of a crop to buffer variation in soil, climate and disease and weed pressures (Wolfe, 2001, Didon & Rodriguez, 2006).

The advantage of diversity in cereal variety mixtures has been well demonstrated (Finckh *et al.*, 2000, Finckh and Wolfe, 2006). However, there are practical limits to the number of genotypes that can be used, usually no more than three or four, which limits the potential for buffering against environmental variability.

This project was designed to overcome the problem of limited genetic variability by developing crop material directly from segregating populations of half-diallel intercrosses involving up to 20 parents in the form of 'evolutionary breeding' (Suneson, 1956).

Objectives

The overall objective of this project was to increase the sustainability and competitiveness of both non-organic and organic farming systems by developing genetically diverse wheat populations that respond rapidly to on-farm selection for improved productivity and yield.

The five main objectives, as stated in the contract, were:

1. To generate six distinct, highly heterogeneous composite cross populations of winter wheat for further development and selection. The populations will comprise: one with parental material selected for good milling potential, one with parents selected for high yield potential and one comprising both sets of parent material. Each of these populations will then be split to either include or exclude heritable male sterility.
2. To evaluate the performance and evolution of composite cross populations over time under a diverse range of environmental conditions and identify characteristics that confer improved productivity in these environments.
3. To track the genetic changes that accompany selection, so providing a better understanding of the assemblages of traits that underlie improved productivity in diverse environments.
4. To provide genetically diverse crop material for further selection by farmers and as a resource for future publicly funded research.

5. To disseminate the results to the scientific community and industry.
Objectives One, Two, Four and Five have been met fully. Objective Three is ongoing.

Methods

Selection of parents

Six composite cross populations of winter wheat were created in 2002 by John Innes Centre (JIC). The twenty parental cultivars were selected from data of both published and unpublished studies, and from experience of the consortium partners. Key criteria for selection included a diverse genetic base and the potential for robust performance under low input agronomic conditions. The parent cultivars were selected in summer 2002 in two categories: high yielding (Bezostaya, Buchan, Claire, Deben, High Tillering Line, Norman, Option, Tanker, Wembley) ‘Yield’ varieties, and high bread making quality (Bezostaya, Cadenza, Hereward, Maris Widgeon, Mercia, Monopol, Pastiche, Renan, Renesansa, Soissons, Spark, Thatcher) ‘Quality’ varieties. Bezostaya was included in both categories as it was known as both high yielding and high quality in Russia, where it was grown successfully over many years.

Creation of CCPs

All 20 parents were crossed together in a complete half-diallel to produce all individual 190 F₁ cross combinations. The F₁ seed was harvested, germinated and grown to maturity in a glasshouse. All ears were bagged to ensure self-pollination, and the F₂ seed from each of the individual F₁ plants of each cross was harvested and bulked for each cross. From these F₂ bulks three separate ‘foundation’ composite cross populations (CCPs) were started by bulking F₂ seed from the individual crosses. The first was synthesized from the 66 crosses between varieties with good milling potential (Q), the second synthesized from the 36 crosses for varieties identified as having high yield potential (Y) and the third synthesized from the 99 crosses between Y and Q parents (YQ). In addition, male sterile CCP populations (CCPms) were generated by artificially hybridizing all the above parents to characterized genetic males sterile lines (as females) obtained from two sources, RAGT and CIMMYT, shown below:

RAGT lines (Shango derivatives)

JB Plant 1

JB Plant 2

NWH 65

F2/F3 Sterile Bulk Population 2/77

CIMMYT lines

F1TOPDMSO102 7 TURACO DMS

F1TOPDMSO102 10 GALVEZ S 87 DMS JB

F1TOPDMSO102 12 CUMPAS T88 DMS

F1TOPDMSO102 14 NING8201 DMS

The F₂s of these crosses were bulked as above to create QCCPms, YCCPms and YQCCPms, which together with QCCP, YCCP and YQCCP, gave six CCPs available as starting material for field evaluation.

Creation of mixtures

In order to compare the performance of mixtures of homozygous lines with that of heterozygous populations, parental seed of equal proportions was also mixed in the same categories as those used to create the populations to provide a Yield, Quality and Yield-Quality Mixture. Unfortunately the Norman seed that was used in the mixtures

subsequently turned out to have been contaminated. Therefore the Yield and the Yield-Quality Mixtures contained a small amount of an unknown genotype.

Seed bulking

The six CCPs, three mixtures and all parental varieties (apart from Norman) were drilled in single replicate plots of varying size at four locations in October 2003. There was enough seed available in autumn 2004 to begin replicated field trials.

Field trial site description

Trial sites consisted of two organic sites (Wakelyns Agroforestry (WAF), in Suffolk (52°39'N, 1°17'E); Sheepdrove Organic Farm in Berkshire (SOF) (51°41'N, -1°52'E)) and two non-organic sites (Metfield Hall Farm (MET), continuous wheat, adjacent to WAF in Suffolk (52°41'N, 1°29'E); and Morley Farm (MOR), an experimental farm in Norfolk managed by The Arable Group (TAG) (52°56'N, 1°10'E)). Experiments took place in different fields within the rotations (or areas of fields) in each year on soils with clay content in the range 13-40 %. Soil type was medium to heavy at Metfield, Morley and Wakelyns and light to medium at Sheepdrove. The preceding crop was always grass-clover or grass-vetch-clover ley at Wakelyns; grass-clover at Sheepdrove; winter wheat at Metfield; and winter oil seed rape at Morley.

Assessment and analysis

Twenty two assessments were carried out each year on all plots at all sites throughout their growth cycle, from emergence to final yield and quality. CCPs and mixtures were analysed in relation to their contributing parents (Y, Q and YQ category). The Genstat package was used to analyse all data. Standard ANOVA and REML meta analysis was used to analyse individual year's data. However, to analyse the reliability and stability of the populations and mixtures (for various attributes such as yield and quality), VNSI International developed a new method of stability analysis, 'GEstability', based on the cultivar-superiority measure of Lin & Binns (1988), which is explained below.

Results

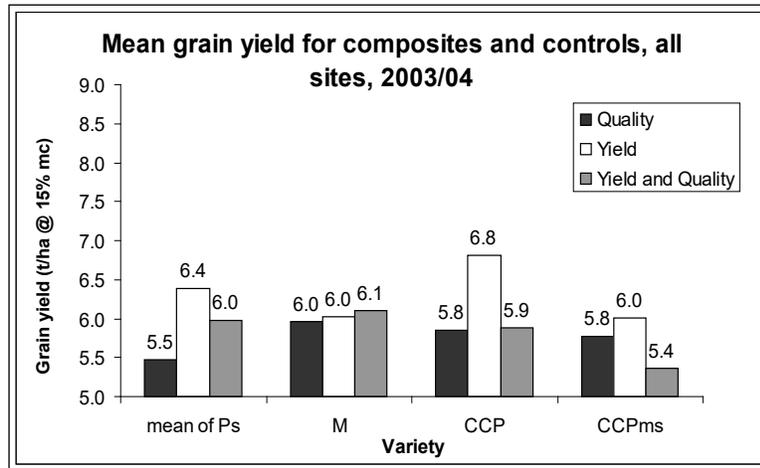
Trial year one (2004/05) summary

The first year of replicated trials was completed in the cropping year 2004/05. In trial year one, the composite cross populations (CCPs) were at an early stage of environmental adaptation (see Phillips and Wolfe, 2005). The selected data presented demonstrates the variability across parent cultivars, mixtures and CCPs, and across field sites.

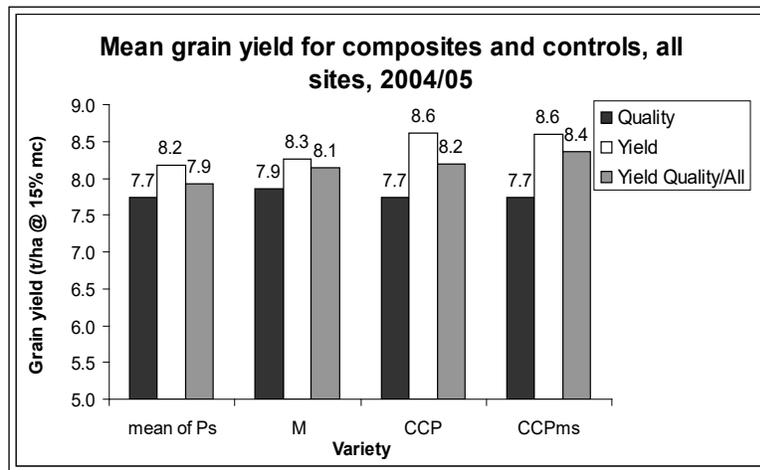
Grain yield

In both organic and non-organic systems there was a tendency for the mixtures and CCPs to produce a greater grain yield than the means of their parent cultivars (Figure 1a and 1b). This was more strongly evident at the organic sites.

a)



b)



Figures 1a & b. Mean grain yields for categories of Composite Cross Populations (CCPs), Composite Cross Populations with male sterility (CCPms), Mixtures (M) and Parents (P), all sites, in cropping years 2003/04 and 2004/05. Genotypic range is indicated for high yielding (Y), high quality (Q) or both high yielding and high quality (YQ).

For 2005, analysis of non-organic systems showed that the grain yield of YCCPms was significantly higher than QCCPms ($p = 0.022$) and in the organic systems, both the YCCP and the YQCCP out-yielded the QCCP ($p = 0.013$) (Table 1).

CCP(ms) category	Grain yield (t/ha @ 15% mc)	
	Organic system	Non-organic system
YQ	6.4 ^b	10.1
Q	5.7 ^a	9.8 ^b
Y	6.3 ^b	10.9 ^a

Table 1. Mean grain yield in non-organic (s.e.d. = 0.231) and organic (s.e.d. = 0.229) systems for categories Y (yield), Q (quality) and YQ (yield quality), 2004/05. Values indicated by the same letter in the same column are not significantly different ($p < 0.05$)

Comparing organic and non-organic sites, there was a clear difference in relative performance of the modern varieties, in that they produced high yields under non-organic conditions but relatively poor yields under organic conditions. Importantly, the populations exhibited a greater stability of yield across organic sites compared to their parents and, to their physical mixtures. However, this effect was not evident under non-organic conditions.

Trial year 2 (2005/06) summary

The replicated field trials of 2005/06 included additional populations which had been exchanged among sites within systems. These multi-site populations provided a means of detecting any early site specific adaptation.

Grain yield

Although the CCPs performed within the ranges of the parents, they often yielded higher than the means of the parents. The YCCPs had higher yields ($P > 0.05$) than the QCCPs at all sites. However, the YQ population yields did not differ significantly from the Y population yields at three of the four sites (MET, SOF & WAF), demonstrating an improvement in performance of the YQ relative to the Y populations since 2004/05.

At all sites except SOF, there were significant differences among the yields of the populations, mixtures and parental means; the mean of the parents was always significantly lower than the yields of the populations, and the mixtures. At both non-organic sites, the mixtures significantly out-yielded the populations, but this was not true at organic sites.

Quality indicators

There were highly significant ($P < 0.001$) differences in protein concentration among the varieties, mixtures and populations at all sites. When categories (Q, Y or YQ) were examined, significant differences were found at all sites; the Q category had the highest protein concentration and the Y category the lowest. For Hagberg Falling Number (HFN), there were significant ($P < 0.005$) differences between categories at two of the sites (MOR & SOF); the Y category had significantly lower HFNs than the other categories.

Trial year three (2006/07) summary

Populations and mixtures

The yields of the populations and the mixtures are compared to the relevant parent means in Table 2 for the third season of replicated trials.

Table 2. Yields of the Y, Q and YQ populations, without or with male sterility, and the mixtures, as a percentage of the category parent means in 2006/07. Values of less than 3% above or below 100 are unlikely to be significant.

	Non-Organic			Organic		
	Y	Q	YQ	Y	Q	YQ
Population	103	103	101	102	103	109
Population with male sterility	101	99	100	107	105	104
Mixture	105	104	103	100	105	105

Although the yield gains from the populations and mixtures are relatively modest for the three years, they are consistent (see Table 2) with the larger gains tending to occur under organic conditions.

Integrated data from three years of trials

Organic conditions

All the populations consistently yielded more than the means of their parents and the YQ and Q mixtures out-yielded their parental means (Table 3). The YQ category was on average closer in yield to the Y than the Q category.

Non-organic conditions

The yield differences between populations, mixtures and parental means were smaller compared to those in organic conditions, although the CCPs and mixtures did consistently out-yield the means of their parents. However, in the CCPms category only the YCCPms out-yielded the mean of its parents. The mixtures consistently yielded more than the populations. All the YQ categories were consistently intermediate between the Y and the Q categories (Table 3).

Table 3. Mean grain yield (t/ha @ 15% moisture content) for categories Y Q and YQ for three years at non-organic and organic sites.

	Organic			Non-organic		
	Y	YQ	Q	Y	YQ	Q
Parents	5.44	5.30	5.15	9.89	9.41	8.94
CCP	5.57	5.62	5.18	10.17	9.47	9.17
CCPms	5.75	5.44	5.32	10.01	9.39	8.84
Mixtures	5.44	5.49	5.35	10.37	9.73	9.33

While the mean yields of the populations and mixtures are comparable to the mean parent yields, no indication of the stability of performance of these genotypes has been indicated. This aspect is considered in detail below (cultivar superiority).

Genotype by environment interactions

In agro-ecosystems the effect of the environment on crop performance can be divided into the systems, sites and years. Inspection of the variances in the analyses of yield of all populations, mixtures and parents indicated a large component due to system but the effects of site and year were smaller.

Over three years, the yields of parents, mixtures and populations stayed at similar levels at MET although the mixtures tended to become more variable. In contrast, at MOR yields of populations and mixtures decreased to parental levels, although the populations provided the most reliable performance. At SOF there was little change over time except that mixtures tended to perform with greater stability over the three trial years. The populations were consistently better than mixtures for yield. At WAF the yields of populations and mixtures tended to increase with time.

Parent varieties

Varietal yields were closely correlated between the two non-organic sites, MET and MOR ($r = 0.94$, $P < 0.001$). Correlation of yields between the two organic sites was also significant, but at a lower level ($r = 0.60$, $P < 0.01$).

Stability

Under variable agronomic conditions, stability of yield (or other characters) over sites and years is of optimal importance to the farmer compared to mean yield alone. We therefore needed a measure that combined absolute performance with stability across environments. On the advice of VSNI International the cultivar-superiority measure of Lin & Binns (1988) was used for this purpose. For each genotype, this is the sum of the squares of the differences between its mean in each environment and the mean of the best genotype in that environment, divided by twice the number of environments. Genotypes with the smallest values have higher, more stable yields (or other measured character).

Cultivar superiority of combined factors

Cultivar superiority for all varieties, mixtures and populations was determined for seven developmental characters which contribute to the final performance of the crop (establishment, early crop cover, canopy cover, tillering, head density, harvest index and green leaf area) and five key characteristics important to the farmer (yield, protein, TGW, specific weight and total harvested biomass) here termed 'harvest characteristics'. For each characteristic, the cultivar superiorities of the varieties, mixtures and populations were scaled to the appropriate parental mean, and then summed in the development and harvest categories. This provides a measure of cultivar superiority across a range of important characteristics and environments.

Confirming the central thesis of this project, both populations and mixtures proved their buffering capacity and displayed good cumulative cultivar superiority particularly for harvest characteristics relative to their pure line parents. For all characters in both systems, the mixtures and populations tended to have better than average cumulative cultivar superiority (Table 4 and 5). This was particularly notable for harvest relative to developmental characteristics.

Harvest characteristics

In the YQ category (22 entries), the YQ mixture, YQCCP and YQCCPms had the top three cumulative cultivar superiorities in both organic and non-organic systems, and the QCCP, QCCPms and Q mix performed best for cumulative cultivar superiority (out of 15) under organic conditions. In non-organic conditions the Q mix and QCCP performed better than all other genotypes for cumulative cultivar superiority, with the QCCPms in seventh place. For the Y category, the populations and mixture are in the top five genotypes, and under non-organic conditions, three of the best four genotypes for cumulative cultivar superiority (Table 4).

The mixtures were consistently lower ranking than the populations under organic conditions in all categories. However under non-organic conditions the mixtures were ranked first in the Q and Y categories, but in the YQ category the mixture was second to

the YQCCP. The CCPms were lower ranking than the CCPs in both systems for all categories except in the Q category of organic systems.

Development characteristics

Performance of the populations and mixtures was more variable for development characteristics under both systems, although they were generally better than average (Table 4 and 5). The CCPs outperformed the CCPms in all categories under both systems, except in the Q category under non-organic conditions.

In contrast to the performance of the populations and mixtures, some varieties, although performing well in one system, performed considerably less well in the other. See for example in the Y category for harvest characteristics, Buchan (performing well under non-organic but poorly under organic conditions) and Wembley (where the reverse was true) (Table 4 and 5).

In relation to popular modern varieties, for example Claire, a commonly grown variety in organic systems, the populations and mixtures gave a superior performance in harvest characteristics. Similarly, Hereward a common variety grown under non-organic conditions was outperformed by the Q mix and QCCP.

Non-organic					
Category	Variety	Development	Category	Variety	Harvest
YQ	Mercia	2.20	YQ	YQ CCP	1.02
YQ	Claire	2.84	YQ	YQ mix	1.07
YQ	Soissons	3.57	YQ	YQ CCPMS	2.23
YQ	Hereward	4.07	YQ	Buchan	2.58
YQ	YQ mix	4.11	YQ	Soissons	2.77
YQ	Wembley	4.58	YQ	Pastiche	2.80
YQ	Deben	4.79	YQ	Mercia	2.87
YQ	Buchan	4.84	YQ	Hereward	3.22
YQ	Pastiche	5.04	YQ	Option	3.32
YQ	YQ CCP	5.13	YQ	Monopol	3.72
YQ	YQ CCPMS	5.47	YQ	Renan	4.11
YQ	HTL	5.91	YQ	HTL	4.16
YQ	Cadenza	5.91	YQ	Spark	4.18
YQ	Option	5.94	YQ	Wembley	4.63
YQ	Spark	6.39	YQ	Claire	4.72
YQ	Renan	6.42	YQ	Renesansa	5.22
YQ	Monopol	7.08	YQ	Tanker	5.72
YQ	Tanker	7.13	YQ	Deben	5.98
YQ	Maris Widgeon	10.29	YQ	Bezostaya	6.85
YQ	Thatcher	10.30	YQ	Maris Widgeon	7.04
YQ	Bezostaya	16.61	YQ	Cadenza	7.10
YQ	Renesansa	19.11	YQ	Thatcher	13.99
Category	Variety	Development	Category	Variety	Harvest
Q	Mercia	1.94	Q	Q mix	1.59
Q	Soissons	3.22	Q	Q CCP	1.80
Q	Hereward	3.60	Q	Soissons	2.80
Q	Pastiche	4.65	Q	Pastiche	2.82
Q	Q mix	4.68	Q	Hereward	3.09
Q	Cadenza	5.19	Q	Mercia	3.10
Q	Spark	5.40	Q	Q CCPMS	3.32
Q	Renan	5.83	Q	Renan	3.81
Q	Monopol	5.92	Q	Monopol	3.82
Q	Q CCPMS	6.58	Q	Spark	4.19
Q	Maris Widgeon	8.68	Q	Renesansa	4.46
Q	Thatcher	8.88	Q	Bezostaya	5.67
Q	Q CCP	12.62	Q	Maris Widgeon	6.43
Q	Bezostaya	14.06	Q	Cadenza	7.19
Q	Renesansa	16.63	Q	Thatcher	12.61
Category	Variety	Development	Category	Variety	Harvest
Y	Claire	3.03	Y	Y mix	2.04
Y	Y CCP	3.40	Y	Y CCP	2.27
Y	Wembley	4.05	Y	Buchan	2.47
Y	Y mix	4.61	Y	Y CCPMS	3.31
Y	Y CCPMS	4.90	Y	Option	3.41
Y	Deben	5.10	Y	Claire	4.93
Y	Buchan	5.13	Y	HTL	5.07
Y	Option	5.90	Y	Tanker	5.15
Y	Tanker	7.04	Y	Wembley	5.17
Y	HTL	7.55	Y	Deben	5.51
Y	Bezostaya	18.21	Y	Bezostaya	8.27

Table 4. Cultivar Superiority scaled to category parental mean and summed for seven development characteristics (establishment, crop cover, canopy cover, tillering, head density, green leaf area, and harvest index), and for five harvest characteristics (yield, protein concentration, total biomass, thousand grain weight and specific weight) for Composite Cross Populations (CCPs), Composite Cross Populations with male sterility (CCPms), Mixtures (mix) and parent varieties, at non-organic sites. Genotypic range is indicated for high yielding (Y), high quality (Q) or both high yielding and high quality (YQ) categories.

Organic					
Category	Variety	Development	Category	Variety	Harvest
YQ	Claire	2.68	YQ	YQ CCP	1.88
YQ	YQ CCP	3.32	YQ	YQ CCPMS	1.89
YQ	Deben	3.37	YQ	YQ mix	2.15
YQ	Soissons	3.76	YQ	Wembley	2.77
YQ	Buchan	4.37	YQ	Renan	2.95
YQ	YQ CCPMS	4.49	YQ	HTL	2.99
YQ	Cadenza	4.85	YQ	Spark	3.28
YQ	Spark	4.85	YQ	Pastiche	3.51
YQ	Pastiche	4.94	YQ	Maris Widgeon	3.53
YQ	Wembley	4.94	YQ	Monopol	3.56
YQ	YQ mix	5.02	YQ	Thatcher	3.58
YQ	HTL	5.34	YQ	Soissons	4.06
YQ	Mercia	5.82	YQ	Hereward	4.30
YQ	Hereward	6.13	YQ	Claire	4.98
YQ	Option	6.53	YQ	Deben	5.91
YQ	Renan	7.59	YQ	Option	6.52
YQ	Tanker	8.72	YQ	Renesansa	6.54
YQ	Thatcher	8.80	YQ	Mercia	7.06
YQ	Maris Widgeon	9.54	YQ	Bezostaya	7.08
YQ	Monopol	10.90	YQ	Tanker	7.19
YQ	Renesansa	12.58	YQ	Cadenza	7.49
YQ	Bezostaya	17.28	YQ	Buchan	7.68
Category	Variety	Development	Category	Variety	Harvest
Q	Soissons	3.23	Q	Q CCPMS	1.39
Q	Spark	4.12	Q	Q CCP	2.05
Q	Cadenza	4.16	Q	Q mix	2.08
Q	Pastiche	4.27	Q	Renan	3.04
Q	Q mix	4.51	Q	Monopol	3.28
Q	Hereward	5.33	Q	Thatcher	3.46
Q	Q CCP	5.57	Q	Maris Widgeon	3.47
Q	Mercia	5.68	Q	Spark	3.55
Q	Renan	6.46	Q	Pastiche	3.88
Q	Q CCPMS	6.64	Q	Hereward	4.62
Q	Thatcher	7.36	Q	Soissons	4.65
Q	Maris Widgeon	8.05	Q	Renesansa	6.07
Q	Monopol	9.37	Q	Bezostaya	7.30
Q	Renesansa	10.82	Q	Mercia	8.29
Q	Bezostaya	15.16	Q	Cadenza	8.39
Category	Variety	Development	Category	Variety	Harvest
Y	Claire	2.90	Y	Y CCP	2.16
Y	Deben	3.48	Y	Wembley	2.43
Y	Y CCP	3.88	Y	Y CCPMS	2.78
Y	Y CCPMS	3.92	Y	HTL	3.03
Y	Y mix	3.97	Y	Y mix	3.43
Y	Buchan	4.68	Y	Claire	4.30
Y	Wembley	5.34	Y	Deben	4.79
Y	Option	6.31	Y	Option	5.87
Y	HTL	7.35	Y	Tanker	6.13
Y	Tanker	8.14	Y	Buchan	6.62
Y	Bezostaya	17.80	Y	Bezostaya	6.84

Table 5. Cultivar Superiority scaled to category parental mean and summed for seven development characteristics (establishment, crop cover, canopy cover, tillering, head density, green leaf area, and harvest index), and for five harvest characteristics (yield, protein concentration, total biomass, thousand grain weight and specific weight) for Composite Cross Populations (CCPs), Composite Cross Populations with male sterility (CCPms), Mixtures (mix) and parent varieties, at organic sites. Genotypic range is indicated for high yielding (Y), high quality (Q) or both high yielding and high quality (YQ) categories.

Baking quality

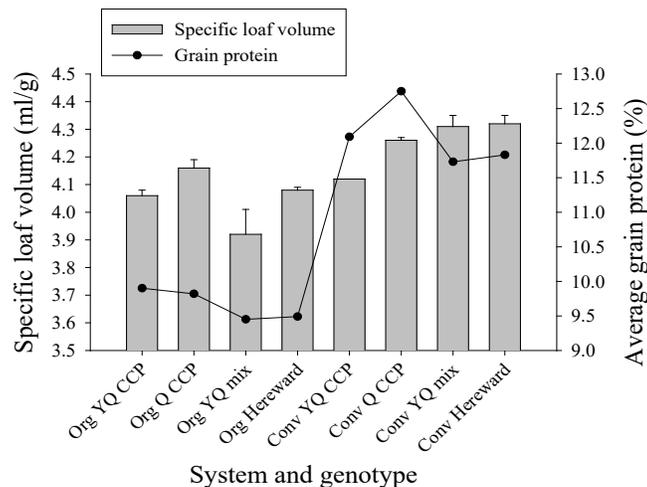
Samples of grain from the Q and YQCCPs, the YQ mix and Hereward from the organic and non-organic sites, harvested in 2007, were sent to CCFRA Technology Ltd for baking tests using the standard Chorleywood bread making process. Measures of bread quality included Specific Loaf Volume (the loaf volume to mass ratio).

Mean grain protein concentrations of the samples grown under organic (org) conditions were lower than those grown under non-organic (conv) conditions (Figure 2). However, the populations tended to have slightly higher grain protein than the mixtures and Hereward under both systems. Between the two populations, the QCCP protein was higher than the YQCCP under non-organic but not organic conditions.

Grain protein trends were not reflected in the specific loaf volume results (Figure 2). The QCCP produced higher specific loaf volumes than the YQCCP. This trend is seen in both systems, with the specific loaf volumes of the CCPs being slightly higher at the non-organic sites due to the higher grain protein concentrations.

The results for the YQ mixture are less clear. Despite a slightly lower grain protein concentration in the mixture than the YQCCP in both systems, in the non-organic system it produced better specific loaf volume than its CCP equivalent, whereas in the organic system the loaves were inferior. Similarly, the Hereward at non-organic sites performed better than the CCPs in terms of specific loaf volume, but less well at organic sites.

Figure 2. Specific loaf volume (ml/g; bars) and grain protein concentration (%; points) of organic (org) and non-organic (conv) YQ and Q composite cross populations (CCP), a physical mixture of the parents (YQ mix) and the variety Hereward. The error bars indicate the standard deviation.



Genetic characterization of the Foundation CCPs

The genetic composition of each of the parents was determined using multi-allele simple sequence repeat (SSR) genetic markers across the wheat genome and combined with the information on the numbers of seeds of each cross which had been bulked into the foundation CCPs. This allowed us to establish the starting allelic composition of the foundation CCPs. The 20 parental lines identified were characterised for 72 SSRs known

to be highly polymorphic for single loci and spread across the genome. All SSR methodologies are as described in Simmonds et al. (2008). Table 6 shows an example of the allelic constitution of the parents for 8 multi-allelic SSRs on the homoeologous group 1 chromosomes of wheat. For example, for locus *Xgwm140*, Thatcher has a unique band not found in other parents.

	gwm357 1AS	psp3027 1AC	psp3000 1BS	gwm011_1BC	psp3100_1BL	gwm140 1BL	gwm337 1DC	gwm458 1DC	gdm111_1DL
Bezostaya	120	164	217	191	173		187	112	212
Buchan	122	162	237	nd	177	264	189	116	208
Cadenza	124	164	249	211	187	252	185	112	206
Claire	124	162	237	193	167	237	189	116	197
Deben	122	162	237	193	167	237		114	208
Hereward	124	162	237	193	181	243	189	114	208
High Tillering Line	120	164		nd	177	295		110	208
Maris Widgeon	124	160	237	193	167	257	185	116	208
Mercia	122	160	237	193	181	250	185	100	210
Monopol	124	162	237	193	181	243	185	112	208
Norman	122	160	237	195	167	235	185	116	208
Option	122	162	237	195	177	264			208
Pastiche	122	160	237	195	167	235	185	116	210
Pegassos	118		237	213	181			110	208
Renan	120	164	237	191	159				208
Renesansa		164	237	191 + 195	187	237		116	nd
Soissons	122	164	213	193	181		191	114	210
Spark	122	164	237	199	187		189	114	206
Tanker	120	162	237	nd	173	237		112	208
Thatcher	122		237	195	185	246	189	112	208
Wembley	124	162	237	193	185	303	187	114	206
Xi19	122	164	249	211	187	252		112	nd
Alleles	4	4	4	7	7	10	5	5	5

Table 6. Allelic constitution of the parents for eight multi-allelic SSRs on the homoeologous group 1 chromosomes of wheat. The markers used are at the top, and the number and colour of a cell indicate the size of the band amplified in the different parents at that locus.

Combining all marker data together also allowed a similarity dendrogram to be formulated to look at the genetic relationships among the parents of the CCPs (Figure 3).

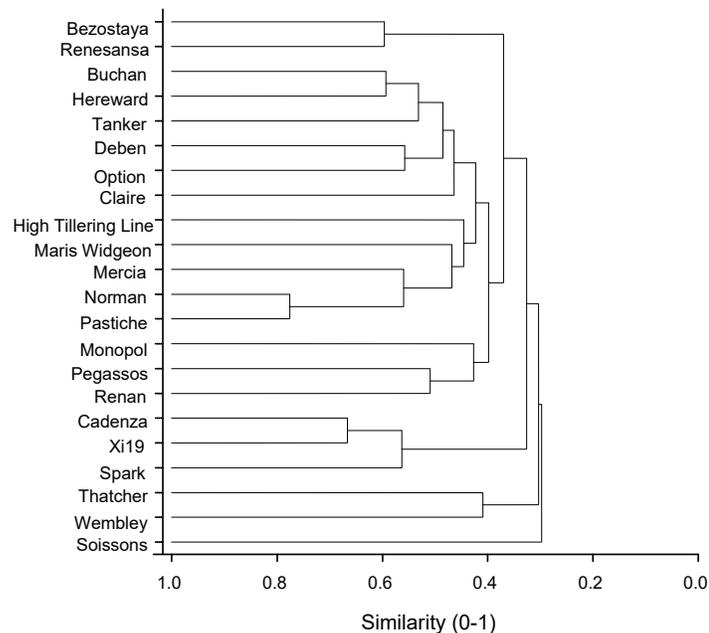


Figure 3. Dendrogram of the parental varieties used in the creation of the composite cross populations.

From the seed numbers of each F₂ pooled into each foundation CCP, it was possible to establish the starting allelic constitution of each CCP at each locus examined. For example, Table 7 shows starting allele frequencies in the YQCCP for one such marker *Xgwm46*, where Wembley has a unique allele.

Table 7. Starting YQCCP allele frequencies for marker *Xgwm46* on chromosome 7B

Variety	Allele	Starting Frequency (%)
Buchan	170	5.75
Cadenza	170	4.61
Claire	170	7.91
Deben	170	7.05
HTL	170	5.67
MWidgeon	170	5.45
Mercia	170	4.6
Norman	170	4.96
Renesansa	170	3.03
Spark	170	8.23
Tanker	170	6.15
Thatcher	170	3.62
		67.03
Bezostaya	174	4.93
Hereward	174	2.09
Option	174	6.11
Pastiche	174	2.47
Renan	174	5.26
Soissons	174	5.86
		26.72
Wembley	145	3.8

Genetic characterisation of the CCPs after four generations of cultivation

Following cultivation of the six CCPs under both organic and non-organic conditions, the genetic composition of the CCPs was tested to look at allele changes that might indicate adaptation to different agronomic conditions. To do this, a subset of 20 SSRs of the original 72 SSRs was used to analyze the genetic composition of the CCPs (Table 8).

Primer name	Chromosome position
gwm011	1BC
Psp3100	1BL
gwm337	1DC
gwm526	2BL
gwm120	2BS
gwm539	2DL
wmc56	3BC
gwm610	4AC
psp3103	4DC
gwm165	4DL
gwm186	5AL
gwm213	5BC
gwm234	5BS
gwm583	5DL
gwm190	5DS
barc134	6BL

gwm626	6BL
gwm469	6DS
gwm46	7BC
gwm44	7DS

Table 8. The markers chosen for CCP F₇ characterisation.

A single parental type may be identified by a unique allele or combinations of alleles in haplotypes so that its contribution to the advanced CCP can be quantified. Changes in allele frequencies may indicate specific selection of this component in the CCP, and comparisons can be made between the different CCPs grown under different input and farms within agronomic practices. These allele changes can also be compared to phenotypic measurements on the CCPs to look at correlations of alleles with adaptation.

Samples of 384 individual plants of the F₆ generation of each YQCCP were hand harvested from the populations at SOF, WAF, MET and MOR. Simple agronomic measurements were taken from each plant (plant height, tiller number, plant biomass, leading spike spikelet number, leading spike grain weight, leading spike grain number, whole plant grain weight). From the threshed F₇ harvested grain, a sample of ten grains was germinated to produce leaf material for DNA extraction. As a test of the concept of measuring genetic changes, the 384 DNA samples from the WAF YQCCP and SOF YQCCP were the first to be analyzed for allele composition. As an example, Table 9 shows the allele constitutions of a sample of individuals from the SOF F₇ YQCCP at *Xgwm46* on chromosome 7B.

Plant		
YQSOF181	170	
YQSOF182	174	
YQSOF183	170	
YQSOF184	170	
YQSOF185	170	
YQSOF186	174	
YQSOF187	170	
YQSOF189	170	
YQSOF190	145	170
YQSOF191	145	
YQSOF192	170	
YQSOF193	170	
YQSOF194	170	
YQSOF195	170	
YQSOF196	174	
YQSOF197	170	
YQSOF198	145	
YQSOF20	170	174
YQSOF200	174	
YQSOF202	170	174
YQSOF203	170	174
YQSOF205	170	
YQSOF206	170	174
YQSOF207	170	
YQSOF208	170	
YQSOF209	170	

Table 9. The allele constitutions of a sample of individuals from the SOF F₇ YQCCP at *Xgwm46* on chromosome 7B.

As expected, there are heterozygous plants in the CCP. However at this locus, there were 14 heterozygous and 313 homozygous individuals recorded out of the 327 plants with unambiguous classification, a slight excess of heterozygotes. This was also found at other loci and may indicate a heterosis effect of heterozygotes under these conditions. Allele frequency distributions for all 20 loci have been obtained for the SOF YQCCP and WAF YQCCP. To evaluate the extent of genetic changes, the allele frequencies in the foundation CCPs and F₇ CCPs were compared over all 20 loci. Figure 4 shows the changes for the locus discussed above, *Xgwm46*.

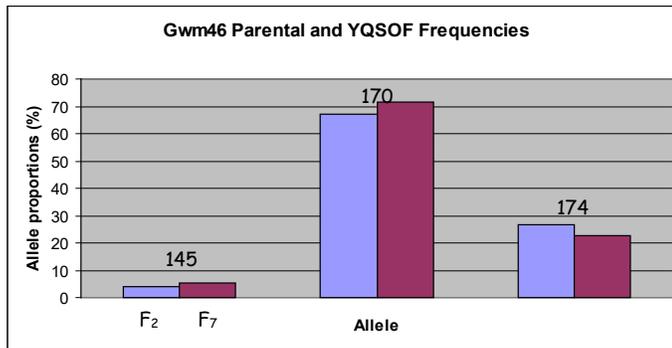


Figure 4. The changes for the locus *Xgwm46* on chromosome 7b.

For this locus, the frequency distributions in the F₇ generation SOF YQCCP have not changed over the period of cultivation, indicating an apparent lack of selection on this particular genome region. Comparisons can also be made between the allele frequencies in the two populations grown at SOF and WAF, for locus *Xgwm46* (see Figure 5) Again, no significant changes are observed at this locus.

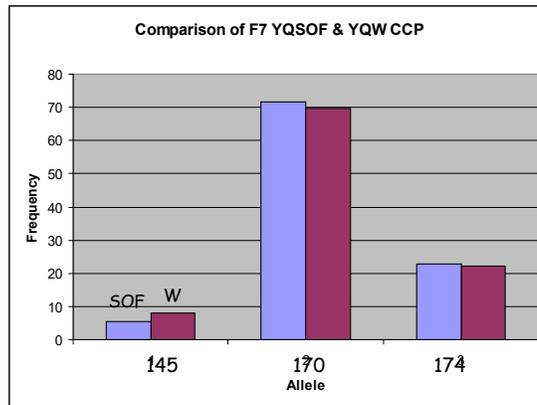


Figure 5. Allele frequencies in the YQCCP grown at SOF and WAF, for locus *Xgwm46*.

In addition to examining single loci, it is possible to look at changes for genomic regions flanked by two markers, giving haplotypes for a genomic region. Where two loci are linked together, the haplotype frequencies give better discrimination between varieties, as more parental haplotypes are distinct. Figure 6 shows the haplotype for the markers on chromosome 5D for the parents and the F₇ SOF YQCCP. It can be seen that two new

haplotypes have appeared (168-211, 170-213) the latter from recombination between Wembley and one of the other parents carrying allele 213.

Again the foundation and F₇ CCP haplotype frequencies can be compared to look at the genetic changes in the CCP. Figure 6 shows the SOF YQCCP data for the 5D haplotypes. Again no dramatic changes in gene frequency are observed, although haplotype 8 (166-215) from Norman has decreased considerably. However, given the low starting frequency, this could be due to genetic drift rather than selection.

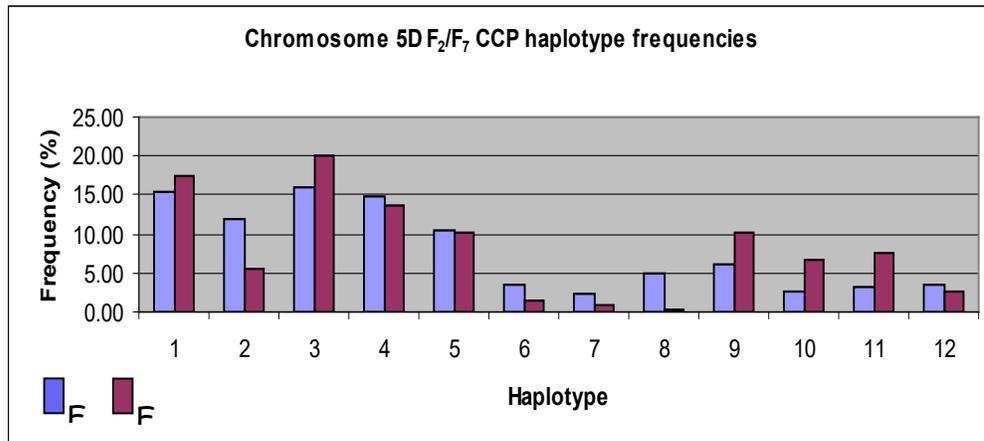


Figure 6. The haplotype for the markers on chromosome 5D for the parents and the F₇ SOF YQCCP.

Discussion

Genetic analyses

The use of multi-allelic SSRs is a useful tool in following the evolution of the CCPs as they are grown in different sites and systems. The data to date has identified some preliminary trends:

1. The genetic variation in the foundation CCPs is being maintained in the F₇ CCPs.
2. Variation in gene frequencies has occurred, but no dramatic changes that would indicate strong selection against alleles or haplotypes, have been observed.
3. Only one allele has been observed to be eliminated, *Xgwm539* on chromosome 2D, from Renesansa in the WAF YQCCP, albeit from a low starting frequency. Interestingly, this allele is linked with the gene *Ppd-D1b* for photoperiod insensitivity, and may indicate selection against early flowering types in this CCP.
4. There appears to be a higher frequency of heterozygotes than expected (> 1 out of 64), which may indicate heterozygous advance due to heterosis.

Performance analyses

Population and mixture yields over years and sites showed a slight increase relative to their parental mean. This does not however take into account the stability of performance of these diverse crops stands relative to pure line varieties. Therefore a new comprehensive analysis tool was developed by VSNI International in order to incorporate overall performance for both yield, and other characteristics, and their stability.

Three years of field selection under non-organic and organic conditions has resulted in the populations and mixtures outperforming the pure line varieties for harvest characteristics under both organic and non-organic systems. They also tended to perform better than average for development characteristics. These results fully justify the aims of this project, showing that diverse crop stands can provide improved buffering against environmental variation compared to pure line varieties for a range of characteristics, particularly among those grouped in the 'harvest' category. Economically, the use of these populations and mixtures presents a more practical and safe strategy than growing a range of parent varieties for the risk averse farmer (and particularly the organic farmer).

While populations and mixtures displayed good performance across systems, some pure line varieties showed remarkable changes in ranking between organic and non-organic conditions. For example, Buchan performed well in non-organic conditions and poorly in organic, while the reverse was true for Wembley. This highlights the risk of selecting varieties for organic systems based on their performance in non-organic trials, and also the variation in buffering capacity of single genotypes.

In populations and mixtures, the better performance for harvest compared to development characteristics show that the summation of the development characters, which are used as standards across the scientific community, do not provide a true forecast of harvest performance. Further analysis is required to gauge the value of these, or other, development characteristics for their contributions to individual harvest characteristics.

The population approach, using diversity to buffer environmental variation, was originally thought to have greatest potential application for organic and low-input systems. However these results suggest a similar benefit for non-organic agriculture, particularly with increasing climatic variation and the trend of reducing oil-based inputs.

Under organic conditions the populations performed better than the mixtures in the majority of cases. This is hypothesised to be the result of the greater genetic variability in the populations. Under non-organic conditions, the mixtures tended to be slightly superior in all but two cases. This may be because the mixtures are comprised mainly of genotypes bred for high input conditions; in the populations these gene complexes have been broken and have not undergone intentional selection, which may have reduced their potential performance under non-organic conditions.

These populations have not been subjected to mass or any other form of intentional selection, and yet have still performed well. The follow-on Sustainable Arable LINK supported Wheat Breeding LINK Project (LK 0999), however, incorporates mass selection and a potential for 'breeder selection' by farmers.

The genetic diversity generated by the populations was maintained over seven generations (in the results seen so far) indicating that these populations have not yet been exposed to extreme environmental variation. An extension to such variation in England is already in progress as part of the Wheat Breeding LINK (LK 0999) project (24 sites x 4 years). However, a strong indication of population's resilience was gained when YQCCP

seed from the 2004 harvest was trialled at the Agricultural Research Institute of the Hungarian Academy of Sciences. In the first year many plants died as a result of the cold continental winter conditions, but the surviving plants were harvested and sown the following year, and subsequently out-yielded the local varieties.

The heritable male sterility present in the CCPms causes out-crossing in each generation. Although sterility was present at a low level initially, and decreased over the three years, it is likely to delay adaptation and stabilisation of the CCPms. This may also explain the generally inferior performance of the CCPms relative to the CCPs, in these early generations of adaptation, although their performance compared to pure line varieties was still favourable.

The baking test results showed that loaf volume for the populations was maintained under organic conditions despite reduced protein concentration. This suggests that the populations grown organically may be better for bread making than modern varieties bred for high input but grown organically. Moreover, under non-organic conditions, populations may provide potential for producing bread from low input crops.

Implications

The validation of the population approach in wheat breeding should lead to an increased use of these diverse crop stands in UK agriculture. However there are practical issues associated with the registration, distribution and use of populations in the current legal system. The growing of mixtures avoids legal complications since the plant breeders' rights (PBR) can be applied to the individual components of the mixture, which abide by the DUS (Distinct, Uniform, Stable) and VCU (Value for Cultivation and Use) systems. However, no legislation exists for populations, so there is no legal framework to trade grain on the open-market. The populations do not abide by the DUS or VCU system, but such heterogeneity, as discussed above has major advantages. These issues are now under discussion with various stakeholders including Defra Plant Varieties and Seeds Division and other relevant bodies at the EU level, as part of LK 0999.

The highlights of the Wheat Breeding project were presented and discussed at a technical transfer event '*Diversity for Adversity*' held at NIAB, Cambridge, on March 31st 2008, attended by a number of stakeholders covering a range of expertise from arable farming and plant breeding to seed legislation.

Future work

The Wheat Breeding LINK (LK 0999) project will address the issues raised above. AR 0914 has created and developed various populations for potential commercial use, and begun to understand management issues relating to the complex genetic diversity found in such crops. The Wheat Breeding LINK project will take this further by trialling different selection techniques in order to drive population performance in certain directions e.g. to improve yield and baking quality. In partnership with farmers across the country, populations will be tested under different soil, management and weather conditions to their adaptability and stability. Links in the food chain (millers, bakers, maltsters etc) will be trialling grain from appropriate populations for its processing

capabilities. Further, discussions are underway with various bodies in the UK and Europe in order to develop a suitable framework for the production, distribution and marketing of the populations on a commercial scale.

Seed from the most genetically diverse of the CCPs (YQCCPms) has been integrated into project years two and three of Defra LINK funded project LK 0970 (Wheat LINK). The populations are a component of the research aimed at improving the agronomy of winter wheat; the CCPs are compared to a commonly grown winter wheat (Hereward) with a winter wheat bred for low-input conditions (Aristos). The different varieties/populations are compared across a range of agronomic variables, including seed rate, drilling arrangement, weeding and clover bi-cropping. Additional work in this project, which has focused on the root development of the CCP compared to the variety Aristos, has identified some clear differences in root length density. More detailed work is necessary to determine the potential complementation between genotypes within a CCP that may be taking place below ground as well as above ground.

Interest in the population approach from other institutes across Europe led to seed samples of the CCPs being sent to Denmark, France, Germany, Hungary, Switzerland and Turkey through the SUSVAR COST action 860. In addition, CCPs have been obtained from research carried out in Hungary, which are being grown in a pilot trial in the UK. The aim of these pilot trials is to further investigate the stability of CCPs in a wide range of climates to include Hungary (Agricultural Research Institute of the Hungarian Academy of Sciences), Germany (University of Kassel) and France (INRA). These groups are actively involved in planning a potential EU project to study the wider adaptation of the populations over a larger area of Europe. A second potential project involves creation of populations based on diploid and tetraploid parents and relatives of cultivated wheat in order to extend greatly the potential variability available in the crop.

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