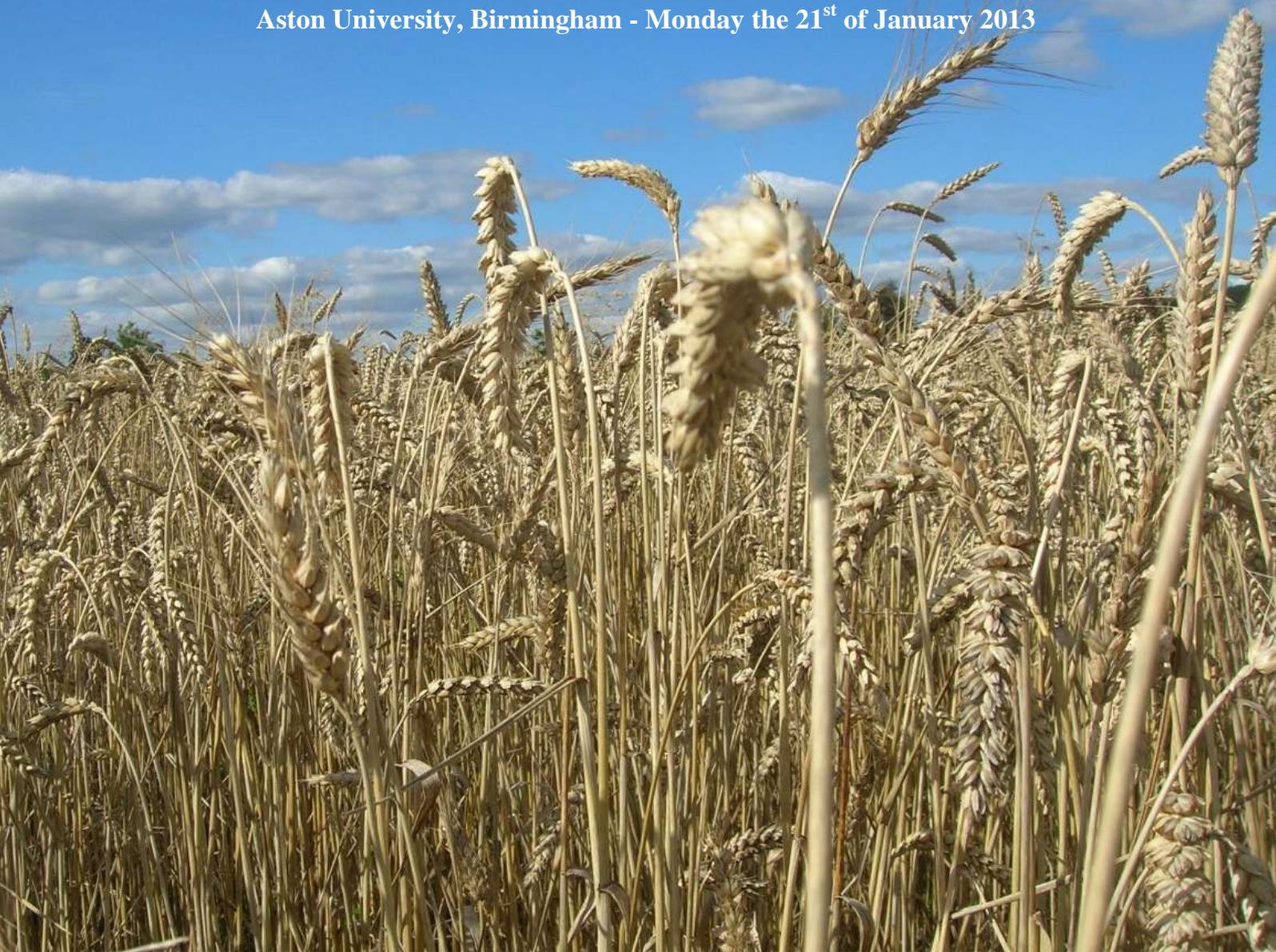


# International Symposium on Evolutionary Breeding in Cereals

Aston University, Birmingham - Monday the 21<sup>st</sup> of January 2013



Editors: Thomas F. Döring, Sally A. Howlett, Louisa R. Winkler, Martin S. Wolfe

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*Photograph: Winter wheat composite cross population growing at Duchy Home Farm, UK, August 2011. Picture taken by Oliver Crowley.*

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## Preface

Evolutionary plant breeding has a long history, but has so far not become part of mainstream breeding research, nor has it been implemented in practice to any substantial degree.

However, over the last decade, research in evolutionary plant breeding has markedly intensified. For example, there are currently major research projects on-going in this area, including the EU funded project SOLIBAM, the Wheat Breeding LINK project in the UK, and the Danish Biobreed project. Also, a new 3-year international research project called COBRA on this topic is due to start in March 2013. Funded by the CORE Organic 2 Eranet the project brings together over 40 partner organizations from 18 European countries.

In addition, interest in evolutionary plant breeding is growing among farmers, breeders and policy makers. In fact, there are currently encouraging developments in the imminent revision of seed legislation in Europe that could lead to more room for evolutionary plant breeding approaches in the future.

This renewed interest in evolutionary plant breeding is partly due to the recognition that mainstream plant breeding is limited in terms of its engagement with end users, i.e. farmers and growers. More urgently however, effects of climate change on agricultural production have become more noticeable and there is also a growing awareness of increasing resource constraints; together, these will create more stressful growing conditions for agricultural crops. With this background, it is now being recognized that crops need to be able to cope with more variable, contrasting, fluctuating, and generally more unpredictable growing conditions.

To be able to deal with this large and increasing environmental variability, plant breeding needs to become more decentralized and diversified. Evolutionary plant breeding offers great potential in this respect. The contributions collated from this symposium explore this potential as well as the limitations of evolutionary plant breeding. While they only show a part of the on-going research activities in Europe, we hope that these proceedings provide inspiration both for further research and for implementation in practice.

Hamstead Marshall & Fressingfield, March 2013

Thomas Döring and Martin Wolfe

# Evolutionary wheat breeding in the Netherlands

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**Key words:** evolutionary breeding, wheat, organic agriculture, yield stability, baking quality

## Abstract

Although the Netherlands have a strong position in vegetable breeding, not much cereal breeding is conducted as only one company has survived the consolidations. Organic wheat growers rely on a limited number of varieties that were developed about 20 years ago as only very few varieties combine good baking quality with adaptation to Dutch growing conditions. Varieties developed by organic breeders in Germany and Switzerland seem adapted to Dutch conditions. Still, this means a dependence on varieties developed for other climatic conditions. The total organic wheat acreage in the Netherlands (approx. 2000 ha) is too small to sustain a wheat breeding programme.

The question is whether evolutionary wheat breeding can be an alternative approach (Philips and Wolfe 2005). An added value of the evolutionary breeding approach is that increased diversity helps deal with biotic (pests, diseases) and abiotic (e.g. drought) stresses and more heterogeneity in soil fertility (Finckh 2008). Furthermore, genetic diversity would also allow such crops to co-evolve with changes in climate and adapt themselves to new growing environments (e.g. different farms).

Wageningen University is partner in a European “cycling” project wherein a winter wheat composite cross population (CCP) bred in the UK (Döring et al. 2010) is cycled to different European growing sites every year. We have replanted all populations every year next to the newly received populations and have analysed their progress in adaptation by comparing them for yield, yield components and traits related to vegetative growth.

However, evolutionary breeding based on natural selection is not expected to improve traits related to baking quality (Dawson et al. 2008). To overcome this drawback, a combination of evolutionary and directional selection is proposed (Murphy et al. 2005). Therefore, a second project in the Netherlands is being conducted with spring wheat to provide more information on possible solutions. It studies a modified evolutionary breeding approach together with Getreidezüchtungsforschung Dottenfelderhof (GD). In this modified evolutionary breeding scheme, CCPs are grown at a farmer field in the Netherlands; in the second generation, ear progenies selected at the farm are grown at the breeding station and tested for quality traits. Subsequently, the best ear progenies are mixed again and the optimised CCPs are returned to the farmer for further on-farm selection. In this presentation we will provide an update on the status of both projects.

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# Evolutionary plant breeding in Italy

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**Key words:** evolutionary plant breeding, climate change, wheat, barley, participatory research, organic varieties

## Abstract

In Italy organic cereal farmers are increasingly looking for varieties (e.g. old varieties or landraces) adapted to their agricultural and traditional processing systems (Bocci et al, 2011). They are also facing the challenge of a changing climate, which year after year becomes more evident (Hertsgaard, 2012). However, the present legal framework, market requirements and the loss of skills of selecting on the farm make it difficult for organic farmers to do so. Having this in mind, in the winter 2010 AIAB with the support of ICARDA set up an evolutionary plant breeding project, within the framework of the European research project SOLIBAM ([www.solibam.eu](http://www.solibam.eu)).

Farmers, even organic ones, are decreasingly looking at the crop and at the individual plants in the field. Therefore the first aim was to stimulate farmers to start again to look at diversity in their cereals fields and to select individual plants to eventually obtain more suitable varieties or populations. The materials given to farmers were segregating populations of durum wheat, soft wheat and barley developed at ICARDA. Farm days were organised each year on the participating farms, with the objective to bring back diversity to the farms and to see what kind of reaction farmers show.

This paper will present the results of the 2 years activities on the targeted crops, pointing out opportunities and bottlenecks of starting an evolutionary plant breeding project. The main question addressed by the presentation will be: what are the conditions that can influence the participation of farmers in research activities?

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# Diversity to improve wheat cropping systems: evidence from field trials in Italy using evolutionary populations

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**Keywords:** Composite cross populations, living mulch, planned agro-biodiversity.

## Abstract

Under reduced levels of external inputs wheat production is often limited by low yield, high weed pressure and highly heterogeneous environments. This is further exacerbated by unpredictable climatic fluctuations. Here, we tested the hypothesis that organic and low input wheat production may be improved by the deliberate introduction of diversity into the crop stand (Newton et al. 2009). Field experiments were carried out to test the efficacy of increasing both genetic and species diversity of the crop stand to support economically acceptable yields, increased yield stability and weed reduction. Genetic diversity was increased by using varietal mixtures or evolutionary populations, while species heterogeneity was achieved by including a leguminous living mulch. The experiment was carried out within an organic stockless rotation, included in the MASCOT (Mediterranean Arable System Comparison Trial) long-term experiment (Bàrberi & Mazzoncini 2006) on an alkaline silty-loam with high weed pressure. Four Composite Cross Populations (CCPs) were grown since 2010-11 (generation zero) and compared to a mixture of four modern commercial varieties (MMV), a mixture of four traditional Italian varieties (MHV) and two pure line controls. Two of the CCPs were provided in 2010 by the Organic Research Centre, UK, as F9 and two were provided in the same year by the Agricultural Research Centre of the Hungarian Academy of Sciences, as an F6 and an F8 respectively. The treatments were sown either as wheat only (PC) or with a *Trifolium subterraneum* additive living mulch (LM) in 2010/11, 2011/12 and 2012/13.

Contrary to the mixtures, the CCPs were harvested and re-sown in either the PC or LM system after bulking seeds from the respective treatments in the previous season. Crop establishment, yield, yield components, wheat phenology, wheat and weed growth were assessed. In year 1, the crop was sown very late (09.02.11), due to abnormally intense autumn rainfall; establishment was poor and yield was low, especially in the LM system. In year 2, by contrast, the trial was sown much earlier (15.10.11) and the average grain yield was 250% higher than in year 1, with no differences observed between the PC and LM systems. Seeds left from generation zero of each CCP were also sown alongside the trial. The year 3 experiment was sown on 21.10.12. The MHV yielded less than the MMV and one of the pure lines in year 2. However, no clear differences were observed between CCPs, mixtures and pure lines with respect to yield and weed suppression, neither in year 1 nor in year 2. Nevertheless, these CCPs were supposedly not adapted to our environment and climate, and were subjected to an unusually short growing season in year 1.

From the data so far, we suggest that innovations at the genetic level may be the most direct way to improve low input and organic wheat production. In this context, sowing heterogeneous populations is of value in maintaining or stabilizing yields. In 2012-13 we will also compare different generations of CCPs at both early and late sowing time, to test the hypothesis that CCPs may change and improve their performance due to environment and management-related evolutionary processes.

## Acknowledgements

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## Adaptive winter wheat populations in the UK: selected results

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**Keywords:** wheat, mixtures, populations

### Abstract

For the past two centuries, a short period in the evolution of cultivated wheat, plant breeding has focused increasingly on the selection of pure line varieties, protected for breeders in a rigid legal framework. This has been effective because of relatively uniform environmental conditions assisted by a range of inputs, the resources for which have been regarded as unlimited. The resulting large-scale monoculture is having numerous negative effects on agricultural and non-agricultural ecosystems together with stagnation in output. Of the various ways in which this situation can be improved, we have been involved in exploiting variety mixtures (e.g. Wolfe 1985) and, more recently, in the development of composite cross populations (CCPs) (Phillips & Wolfe 2005, Döring et al. 2010) through evolutionary plant breeding (Suneson 1956).

Based on 21 parent varieties selected in 2001, a set of CCPs focused on yield, quality or the combination was grown under organic or non-organic conditions in the UK and in other environments across the European mainland. Without artificial selection, the general tendency was for the populations to gain height significantly, relative to their parents, associated with a modest yield gain, resulting in reduced harvest index but greater, overall, nutrient use efficiency. As expected, there was a gain in resilience relative to the average of the parents.

However, within the UK, there was little evidence of local adaptation or even of adaptation to farming system (see Knapp et al., this volume). This may have been because the effects of environmental changes from season to season were greater than the within-season differences. However, the great potential for adaptation within the populations was evident when CCP YQ was grown in Hungary, under winter conditions more severe than those experienced during the selection and evolution of the parents. After only season of poor yield, the population recovered to produce a high yield in the following season.

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# Creation of populations adapted to organic farming and on-farm biodiversity management: PPB programme on bread wheat in France

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**Key words:** participatory research, organic breeding, bread wheat, on-farm management

## Abstract

Organic farming needs research on concepts and methods for selection and biodiversity conservation. This project aims to address these needs by developing new methods, knowledge and resources specific to organic farming. High diversity among organic systems requires more diversity among and within cultivars to enable them to cope with environmental variations. This strategy increases resilience and stability of cultivars in space and in time (Wolfe et al. 2008). In France, we have developed populations in a participatory plant breeding programme (PPB) in collaboration with Réseau Semences Paysannes (Farmer Seed Network, RSP) (Dawson et al. 2011).

Our project started in 2005 with the creation of 90 populations by crosses from a broad genetic range. In 2012, there are 26 farms in France hosting those populations. The research team works in collaboration with the farmers in order to provide information for their selections. Researchers and farmers collectively conduct evaluations of the populations and make decisions about selections.

In a mass-selection programme undertaken by the farmers on these populations, we analyzed the selection differential and response to selection and estimated realized heritability ( $h^2_r$ ) for agronomical and morphological traits. Selection differential and response to selection were found positive for most of the traits of interest to the farmers.

We also studied genotype-by-environment (GxE) interactions in the network of farms using two statistical approaches: the Finlay Wilkinson and AMMI models (Gauch et al. 2006, Nabugommu et al. 1999). Genotype by environment interactions were found highly significant for many traits. Using farmers' practices and pedoclimatic data, we identified groups of farms that share similar profiles.

These results will help us to understand the impact of the selection methods and organization on the evolution of the populations and the creation of new crops. Each year, these results are summarized in a report sent to the farmers in the programme prior to sowing, which helps to guide their selection process.

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# Using SNP markers and morphological data to compare diversity between generations in composite cross populations of landraces and cultivated barley (*Hordeum vulgare*)

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**Keywords:** *Hordeum vulgare*, broadening of genetic base, heterozygosity, genetic variation, phenotype, local adaptation

## Abstract

Modern crop varieties have been bred in order to provide high yields under high input management, which has led to a relatively lower genetic diversity in modern cultivars compared to wild or traditional crop varieties. This depletion of variation present in modern crops is believed to have a big effect on traits involving response to environmental pressures (Suneson 1956, Allard 1988, Goldringer et al. 2006, Harlan 1921, Ibrahim & Barrett 1991, Paillard et al. 2000).

In order to study whether a higher amount of variation would create crops that are better in dealing with their environment and to measure their genetic diversity, we have been developing highly heterozygous populations by hybridizing approximately 25 lines of barley (*Hordeum vulgare*) landrace and modern cultivar material in four generations. We have measured life history traits (height, seed number, ear number) and genotyped up to 384 SNP (single nucleotide polymorphism) markers per individual for every generation. The aim of this project was to measure the stability of different traits between generations and sites and to see whether the morphological traits of the more heterozygous populations (F4) showed less variability than those of the less heterozygous ones (parental).

We found that there was a higher degree of heterozygosity but similar amounts of genetic variation present in later generations relative to earlier ones. We found variable outcomes for morphological measurements: for example, traits like plant height and grain number showed less variation in later generations, whereas ear length variability remained stable but the population average increased over time. In the coming years, we will test these composite cross populations under field conditions at multiple sites for life history traits and output but also for disease resistance and nitrogen use efficiency.

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# Genetic analysis of evolving winter wheat populations reveals reversion to wild type

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**Key words:** competition, composite cross population, plant height, vernalization

## Abstract

In evolutionary plant breeding, a genetically diverse crop population is subjected to local natural selection. In this process, local adaptation may occur through natural selection, and crop yields may be more stable because of buffering effects of genetic diversity (Döring et al. 2011). By re-sowing diverse crop populations for several generations at one site, genotypes that are well adapted to prevailing biotic and abiotic environmental conditions are expected to increase in frequency through natural selection. The aim of this study was to test if natural selection can be detected at the genetic level in differently managed composite cross populations (CCPs) of winter wheat.

CCPs, produced by intercrossing 12 milling and 9 feed varieties in a half diallel, and pooling at F<sub>2</sub>, were grown for 11 generations at two organic and two conventional sites in South England without any artificial selection. Twenty of the parental varieties and about 400 individuals of each population at generation 7 and 11 were genotyped with 20 microsatellites and 20 SNP markers, including “perfect markers” for height (*Rht*), vernalization (*Vrn*) and photoperiod sensitivity (*Ppd*), and were phenotyped for yield component traits. Allele frequencies of the founding population were calculated based on the marker analysis of the parental lines and the number of F<sub>2</sub> seeds of each cross that went into the founding population. Spatial differentiation among sites and temporal differentiation (compared to the founding population) were investigated using principal component analysis (PCA) and F<sub>ST</sub>- statistics. Changes of allele frequencies were tested against simulated distributions based on estimated effective population size to test if any observed changes were due to genetic drift or natural selection.

Results showed that no adaptation to management or to sites could be detected, i.e. the allele frequencies did not change in significantly different ways at the four sites. However, signs of natural selection were observed in the same direction at all four sites. Five markers showed significantly greater changes of allele frequencies than expected under pure genetic drift. At the loci for height, *Rht-B1* and *Rht-D1*, the dwarfing alleles decreased in frequency and, at the loci for photoperiod sensitivity, *Ppd-B1* and *Ppd-D1*, the insensitive alleles decreased in frequency. The marker detecting the wheat-rye translocated chromosome, *IB-IR*, showed selection against the introgression.

The main reason for lack of adaptation to management conditions or location, as observed in this study, is seen in strong year-to-year variability of environmental conditions at each site. The selection process that could be observed in the same way at the four locations suggests that adaptation took place towards growing in a mixed stand population rather than to environmental conditions. At the 5 loci where significant changes of allele frequencies could be detected, there was selection for the wild-type alleles and against mutant alleles that were important during the last four decades in conventional wheat breeding. Selection for increased height can be explained by competition for light. Thus, competition in a diverse population selects for stronger intra-specific competitors, which could be detrimental to crop production but probably positive for weed suppression.

## Acknowledgements

We are grateful for research grants from DEFRA, UK through the Sustainable Arable LINK programme (LK0999).

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# Study of linkage disequilibrium evolution in a wheat MAGIC population

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**Keywords:** linkage disequilibrium, MAGIC, wheat

## Abstract

At an accelerating pace, advances in technology are providing an increasing number of molecular markers to the cereal geneticist. Most of these newly produced markers first need to be mapped before they can be used in breeding programmes. Although classical bi-parental segregating populations can be used for marker mapping, they are limited in value when mapping high numbers of markers, both because of a low rate of polymorphism and due to the limited probability of recombination between increasingly tightly linked markers. New populations based on multi-parental crosses and higher levels of recombination have been developed recently, for example MAGIC populations (Multi-parent Advanced Generation Inter-Cross), which overcome these drawbacks.

The MAGIC wheat population we are studying is composed of 1100 SSD (Single Seed Descent) lines resulting from 12 cycles of random crosses of 62 founders with a large genetic base. These random crosses were facilitated by the integration of a nuclear male sterility allele (*ms1b*, Probus donor) in the population. This population has been genotyped with an Illumina array of 9k SNPs (Single Nucleotide Polymorphisms), using a set of markers spread across the genome (Chao et al. 2010). The MAGIC parents were also genotyped with 3000 SNPs developed on chromosome 3B (Paux et al. 2006). Polymorphism and quality checks resulted in the selection of 6480 SNP markers from the 9k set, and 2690 SNP markers on the 3B chromosome.

Before mapping markers, the genetic structure and linkage disequilibrium (LD) present in SSD lines and parental lines was assessed, confirming the efficiency of the 12 cycles of random crossing. Following this, the markers were mapped by means of a new method based on LD analysis, using the 3B dataset on the MAGIC parental panel. The quality of the method was assessed using previously mapped markers, or using 9k data on the SSD.

The validity of the population for both marker mapping and quantitative trait locus (QTL) detection is discussed with regard to these first results.

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# Improved quality and disease management in diverse populations

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**Key words:** Composite cross populations, disease resistance, near infrared spectroscopy, protein content

## Abstract

Improved biodiversity is one of the key principles in organic farming and therefore organic plant breeding seeks alternatives to the currently dominant pureline monocultures. Evolutionary plant breeding of composite cross populations (CCPs) has been proposed as a breeding tool for organic plant breeding, where a highly heterogeneous population of offspring is made by mixing a large number of segregating lines from different parents (Döring et al. 2011).

Natural selection will, to some extent, reduce traits conferring major disadvantages in a population if these have a high heritability and a significant impact on seed reproduction. It will not, however, necessarily improve baking quality, negative traits with a strong interaction with the environment or minor negative traits. There may be a need, therefore, for improved maintenance breeding and selection to increase yield, disease resistance and quality in populations. Protein content, as a measure of quality, can be improved in a population by seed sorting. Traditional sorting equipment achieved this based on physical characteristics, e.g. seed gravity; novel technologies for high throughput sorting of seed for hardness and protein content are now available based on image analysis and Near Infrared Spectroscopy (NIRS).

Regarding disease resistance in plants, if two parents carry two different resistance genes to a disease, one quarter of the offspring may end up being susceptible due to simple Mendelian distribution. In this way, even after crossing multiple resistant parents, a CCP may end up having higher susceptibility to some diseases than the parental lines. Each cross should therefore be grown, assessed and selected for resistance to relevant diseases individually before eventually combining them to form a CCP. Some diseases, e.g. *Fusarium* and smut, can produce mycotoxins which have deleterious effects on seed quality even at low levels without a significant impact on grain yield. Hence, resistance to such diseases will not be sufficiently increased by natural selection under common field conditions. In such cases, selection pressure can be augmented artificially by inoculation with fungi to increase infection incidence under controlled conditions. In this way, inoculation can be considered as a breeding tool to improve quality parameters in CCPs. In the CORE2 funded COBRA project, starting in March 2013, these strategies will be tested and further developed.

Agrologica have grown CCPs of wheat (jointly developed by the Organic Research Centre and the John Innes Centre UK) since 2007. In parallel, 250 new crosses from 30 parents were made to develop a new population improved resistance to common bunt (*Tilletia caries*). Research on this material is now under way in the BIOBREED project (Steffan et al. in press, Steffan et al. this volume). Based on the practical experience from growing CCPs at Agrologica, some considerations for future development of this breeding strategy can be drawn.

## Acknowledgements

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# Is mass selection a tool to improve quality in winter wheat composite cross populations?

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**Key words:** composite cross population, grain density, grain size, mass selection, protein content

## Abstract

The genetic diversity within composite cross populations (CCPs) of cereals potentially offers optimised crop performance on organic farms, where functional diversity can be used to replace the role of chemical inputs (Döring et al. 2011); however, such a high genotypic heterogeneity inevitably contains individuals that may be functionally redundant or sub-optimal. We therefore asked whether it is possible to enhance performance of a CCP by selecting an elite sub-population to suit a specific end-use, while retaining sufficient diversity to confer adequate buffering capacity against unpredictable growing conditions.

Two CCPs were subjected to three methods of mass selection over four generations of winter wheat. The wheat was grown at one organic and one conventional site in Suffolk, UK. Dark grain colour was selected using an optical colour sorter; grain size was selected using sieves; and grain density was selected using a gravity separator.

Dark coloured grain had significantly greater protein content (% dry basis) than light coloured grains at both organic and non-organic sites (average difference 0.9%), but there was no difference when compared to the unselected population from which the colour fractions originated. Moreover, while protein content in the *progeny* of the dark fractions tended to be greater than that of the light fractions, the difference was small (average difference 0.3%) and not statistically significant.

Selecting for grain size resulted in an average difference in thousand grain weight (TGW) of 11.7g between the fraction of the CCP with smaller grains and the fraction with larger grains. A significant difference was retained in subsequent generations, with the TGW in the progeny of the large sized fraction being on average 4.5g greater than TGW in the small sized fraction.

The gravity separator was able to separate the CCP into a heavy fraction and light fraction with an average difference in TGW of 9.25g. However, there was no difference upon harvesting of subsequent progeny.

This paper shows that the phenotypic diversity present in a CCP of winter wheat can be subjected to mass selection using simple methods that are available on-farm. The results indicate that phenotypic traits which can be subjected to mass selection are linked to desirable quality characteristics such as grain protein and TGW. However, heritability appears to be weak, implying limited use as an on-farm breeding tool within the framework of evolutionary plant breeding.

## Acknowledgements

We are grateful for research grants from DEFRA, UK through the Sustainable Arable LINK programme (LK0999).

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# From landraces to mixtures and to composite cross populations: a range of initial populations to start evolutionary participatory plant breeding

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**Key words:** genetic variability, crossing scheme, participatory breeding, mixtures, farmer varieties.

## Abstract

The development of varieties for organic agriculture and the conservation of biodiversity are two major issues in the context of environmental degradation due to intensive agricultural practices and the predicted impacts of climate change on agricultural systems. Organic farming often leads to environmental conditions which are more variable for the plants since chemical inputs are not used. While modern crop varieties fit the needs of conventional farming in industrialized countries, they often are not adapted to agronomic practices that decrease the use of inputs and fossil energy. To succeed in developing varieties adapted to these kinds of heterogeneous environments, participatory plant breeding (PPB) has been implemented in several cases (Dawson et al. 2011). Decentralized selection is needed in order to conduct direct selection in the target environments. This has been proved more efficient than the more indirect method of selecting in a favourable environment and growing the crop in a stressful environment. In addition, participation of farmers is required to benefit from their experience and expertise in varietal evaluation in their particular environment (Ceccarelli et al. 2001). Several strategies can be applied to start a new breeding scheme such as crossing, mixing or selecting within the available landraces or locally adapted populations. This will depend on the objectives and selection criteria of the farmers, on the diversity of available germplasm and also on the farmers' breeding approach. Choosing a specific scheme will then impact on the management and breeding of the population in subsequent generations. For instance, it is expected that after crosses, strong segregation will occur in the early generations leading to a profusion of phenotypic variants; then, the timing and intensity of selection applied needs to be determined according to the traits observed. Meanwhile, mixing landraces or pure lines will not generate many new genotypes in the first generations, but the composition still may vary in subsequent generations depending on natural selective pressures.

Here we report on several cases of early-stage wheat PPB projects in France that cover the whole range of initial population structures and thus of management in the following generations. All starting populations or mixtures were chosen through a collaborative approach with different farmers or groups of farmers. Unexpectedly, most farmers had precise ideas on the type of populations they wanted to work with, leading to a diversity of situations: single landraces, simple or complex mixtures of landraces or pure lines, biparental crosses, random crosses between landraces through the use of a male sterility gene. We describe the strategies and consider the consequences for evolutionary PPB.

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# Coupling genetic and eco-physiologic models to simulate temporal evolution of cultivar mixtures and composite populations

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**Keywords:** competition, compensation, facilitation, 3D plant model, fitness

## Abstract

There is an urgent need to change the current agricultural model in order to reduce its environmental impact. Studies in the literature which assess strategies based on increasing within-field genetic diversity to promote resilience to stresses in low-input agricultural systems are limited. Our team is therefore currently carrying out research to address this gap by developing models which are able to simulate the functioning of heterogeneous crops. Based on an interdisciplinary collaboration involving population genetics and ecophysiology, our aim is to model the development and the genetic evolution of a genetically heterogeneous crop, taking into account individual plant architecture and plant-to-plant interactions.

The main objectives are i) to develop an individual-based model coupling ecophysiology and population genetics, ii) to set parameters with regard to experimental data on wheat populations, and iii) to study the impact of developmental traits on the fitness of individuals in a heterogeneous crop. The approach will be based on the Open-Alea plant modeling platform (Pradal et al. 2008) for eco-physiological aspects, and upon Simupop (Peng & Amos 2008) for the population genetics layer. The initial efforts will address the impact of wheat architecture on - competition for light between individual plants. Beyond the specific crop model, we propose to build a generic framework to simulate the development of a heterogeneous population over a cropping period and its evolution over generations of re-sowing.

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# Phenotypic comparison of winter wheat mixtures and composite cross populations

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**Keywords:** composite crosses, evolutionary breeding, organic breeding

## Abstract

One of the central requirements of evolutionary plant breeding is high genetic diversity, which could make crops both more adaptable and stable in response to environmental conditions (Wolfe et al. 2008). This genetic diversity can be created using (complex) variety mixtures or Composite Cross Populations (CCPs). CCPs offer an inherently higher degree of diversity than mixtures, but current seed legislation in Europe, while allowing the use of mixtures, does not provide a framework for trading CCPs. To assess the case for changing this scenario we asked whether CCPs (made from various parental varieties) are phenotypically different from their corresponding mixtures (derived from the same parents).

In 2011 two evolving winter wheat CCPs (ORC YQ CCP, 20 parents, F<sub>10</sub>; NIAB Elite CCP, 8 parents, F<sub>4</sub> and two variety mixtures (ORC YQ Mix, NIAB Elite Mix, both derived from the same parental cultivars and being of the same generational age as the corresponding CCPs) were sown on two sites in the UK. A randomised complete block design was used with three replications per site. The sites were (i) Wakelyns Agroforestry in Suffolk (organically managed) and (ii) The Morley Arable Research Centre in Norfolk (conventionally managed). Assessments included establishment and percentage crop and weed cover (early spring 2012), percentage of diseased leaf area and senescence on the flag leaf (July), plant height, tiller density, lodging and ripening time (August) as well as grain yield and thousand grain weight. Analyses of variance were carried out comparing the ORC YQ CCP and the NIAB Elite CCP with their corresponding mixtures. The analysis included all possible combinations of the explanatory variables, their interactions and 18 response variables. Step-wise model reduction was employed using Akaike's information criterion (AIC) for model evaluation.

The AIC-best models revealed significant differences between CCPs and mixtures in the mean number of plants/m<sup>2</sup>, with the mixtures showing an average of 18.7 plants/m<sup>2</sup> more than the CCPs, although this did not translate into a yield increase. There was also a highly significant difference between CCPs and mixtures in the average straw height, with the CCPs being on average 12.6 cm taller than the mixtures. There were no significant differences between the CCPs and the mixtures regarding any of the other response variables. Our results show that CCPs did not differ from their respective mixtures in most phenotypic traits, but that they were consistently taller than the mixtures. While tall straw can lead to increased lodging risk, it confers greater competitiveness against weeds by decreasing light penetration through the canopy and can act as a passive resistance mechanism against *Fusarium* head blight of wheat (Mesterhazy 1995). Mechanisms for the observed difference in plant height between CCPs and mixtures are currently unclear but might include founder effects.

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# Baking quality of two winter wheat CCPs in the UK

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**Key words:** baking test, composite cross population, Hagberg Falling Number, protein

## Abstract

For epidemiological and agronomic reasons, efforts are being made to increase the genetic diversity of major crop species including cereals. One such effort is the generation and testing of winter wheat composite cross populations (CCPs) (Döring et al. 2011). However, increasing the genetic diversity within a wheat crop might also dilute certain desired traits. In particular, parent varieties included in CCPs might vary in parameters associated with baking quality; in this case, including many parents in the CCP to increase genetic diversity could theoretically conflict the aim of maintaining high baking quality, as parents of poorer baking quality are included. We asked how genetic diversity affects the bread-making quality of two wheat populations differing in their genetic diversity and the baking quality of their parents.

Two winter wheat CCPs were created in 2002, one from 20 parent varieties selected to combine high yield and good milling quality characteristics (YQ CCP) and one from a subset of 12 of these varieties selected specifically for high milling quality (Q CCP) (Döring et al. 2010). These CCPs were grown at multiple sites in the UK. Baking tests began when the populations reached generation F<sub>6</sub>. Grain samples of the two populations were obtained from several organic and non-organic farms across England. Tests were replicated over three years by two bakeries, each with their own milling and baking method. We hypothesised that the dilution of quality characteristics in the more diverse YQ population would make it less suitable as a bread-making wheat. Parameters tested included Hagberg Falling Number (HFN) and protein content of the flour. In addition, loaf height (tin loaf) was measured as an indicator of loaf quality, as it reflects the dough's ability to rise (a higher rise is considered more desirable). All sample identities were coded and unknown to the bakers.

The YQ and Q CCPs were comparable to each other in their HFN and loaf height, but differed significantly in their protein content with the Q CCP being higher in protein. Qualitative analyses by the bakers reflected the protein results more strongly than the HFN and loaf height results: bakers tended to favour the Q CCP over the YQ CCP, and where a high-protein commercial control was included, they favoured it over the Q CCP.

Our results show that the question of which parameters should be used to evaluate baking quality and what goals to set in milling wheat breeding programmes remains controversial. Further, the study suggests that protein content could be an important contributor to performance, but that high-diversity wheat CCPs (such as the YQ CCP) can still perform well, producing dough which rises as high as that from commercially-used wheat varieties. Including a proportion of parents with lower baking quality in a CCP might therefore be a way of breaking potential trade-offs between yield and baking quality.

## Acknowledgements

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# Common bunt resistant wheat composite cross populations

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**Keywords:** wheat composite cross populations; common bunt; protein content; genetic diversity; molecular marker; organic agriculture

## Abstract

Utilising diverse populations instead of genetically homogeneous varieties is expected to lead to a number of advantages in cereal production. These include reduced epidemics of plant diseases, improved weed competition and better exploitation of soil nutrients, resulting in improved yield stability. However, a number of challenges must be met before diverse wheat populations can be introduced into commercial wheat production: one of these is the development of breeding technologies based on mass selection which enable breeders and farmers to improve specific traits in populations and maintain diversity at the same time. BIOBREED is a project which commenced in Denmark in 2011 to meet these challenges for wheat population breeding. The project focuses on the development of tools and methods for mass selection of traits relevant for organic and low input production, where it is expected that the highest benefits of utilizing diverse populations can be achieved. BIOBREED focuses on three main aspects of wheat population breeding for organic and low input production systems: i) common bunt (caused by *Tilletia caries*) resistance, ii) selection for improved protein content and iii) the influence on population diversity of different selection pathways. Thirty three crosses were made between 23 common bunt resistant winter wheat varieties in order to generate two composite cross populations (CCPs). Progeny of all crosses was bulked in the F<sub>3</sub> to constitute the first population (*Pop.No.Sel*). Prior to the creation of the second population (*Pop.Sel*), 20 head rows of each of the F<sub>3</sub> of the parental crosses were inoculated with common bunt. From these segregating lines, 160 that showed high levels of resistance to common bunt were used to create *Pop.Sel* in generation F<sub>4</sub>. Afterwards the two populations were grown with and without inoculation with common bunt in order to i) select for bunt resistance and ii) to be able to compare the effect of heavy bunt infection on diversity. Preliminary results show a higher level of common bunt resistance in *Pop.Sel* (6% infection) than in *Pop.No.Sel* (20% infection) in the first year.

Prior to sowing the F<sub>5</sub> seed of population *Pop.Sel*, all seeds were sorted individually for protein content using a *BoMill IQ Grain Quality Sorter 1002S*. The 10% of seeds with the highest and the 10% of seeds with the lowest protein content were selected to generate two new populations (*Pop.Sel.high.Protein* and *Pop.Sel.low.Protein*). All four populations (*Pop.No.Sel*, *Pop.Sel*, *Pop.Sel.high.Protein* and *Pop.Sel.low.Protein*) and their parental lines were sown in a randomized complete block trial at two locations in Denmark in order to compare yield and quality parameters such as protein content and baking quality, and to assess their stability across environments. Results are expected for the summer 2013.

Research to date has not yet answered the question, ‘how much diversity is needed in populations?’ BIOBREED aims to quantify the levels of diversity in wheat CCPs after the different selection steps of i) cultivation with and without common bunt inoculum, and ii) sorting for single seed protein content. Molecular markers will be used to describe the influence of these different selection pathways on population diversity. 90 SSR markers — about two markers per chromosome arm — will be used to describe the initial genetic diversity of the 23 parental lines. F<sub>6</sub> seed of the different populations will be analysed with the same markers and population diversity after different selection pathways will be quantified.

## Acknowledgements

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BoMill Grain Sorter: [http://bomill.com/BoMill We Add Value to Your Grain/Products.html](http://bomill.com/BoMill>WeAddValueToYourGrain/Products.html)

# Winterkill in 2011/12: Effect on winter wheat pure line varieties and winter wheat composite cross populations in the F<sub>11</sub>

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**Keywords:** Evolutionary Breeding, Composite Crosses, Modern Landraces, Organic Breeding

## Abstract

In order to be able to adapt to changing environmental conditions, crop plants need a certain degree of diversity (Stevens 1942, Finckh 2008). The development of genetically diverse populations (composite cross populations, CCPs) instead of breeding entirely homogeneous pure line varieties by standard pedigree breeding methods is a strategy that aims at creating flexible varieties. This breeding approach, termed evolutionary breeding (Suneson 1956) was 'rediscovered' for organic plant breeding at the end of the 20<sup>th</sup> century and has since resulted in the development of the concept of modern landraces (Murphy et al. 2005).

Three winter wheat CCPs were created in 2001 in the UK consisting either of 20 modern wheat parents (called A population), a subset of 12 high quality parents (Q), or a subset of 9 high yielding parents (Y). Seed of the F<sub>4</sub> was transferred to the University of Kassel in 2005 and the populations have been grown since then under organic and conventional conditions in well separated large plots (>100 m<sup>2</sup>) in two parallel sets (12 populations total) without artificial selection applied. In the conventional system fungicides and insecticides were not applied in order to expose the populations to natural pest and disease pressure. In addition, since the F<sub>8</sub> two A populations have been maintained as broadcast sown populations without mechanical weed control. In 2011/12 the F<sub>11</sub> of all 14 populations were compared in a replicated field trial to the mixture of the 20 parents and the three commercial wheat cultivars Achat, Akteur, and Capo. The parent varieties were also grown in unreplicated plots for seed multiplication. In addition, the broadcast populations and the organic A populations were sown early with an undersowing of white clover to check for their ability to deal with an undersown legume.

A three week period of heavy frost in February 2012, followed by 6 weeks drought after an unusually mild winter, resulted in winterkill in 16 out of the 20 parent varieties. Only the four varieties 'Bezostaya', 'Monopol', 'Renan' and 'Hereward' survived with more than 100 plants per m<sup>2</sup>. As it would have been impossible to manage the plots over the summer they were abandoned. Similarly, all early sown plots were killed during the frost and had to be abandoned.

In contrast to the parental varieties, the other entries (i.e. CCPs, mixture of the 20 parents and the three reference varieties) in the replicated field trial recovered, resulting in low but acceptable yields. The number of ear bearing tillers was not significantly different between CCPs and varieties; however both of these groups showed considerable potential to compensate the substantial losses caused by winterkill. It is not surprising that the variety 'Bezostaya' with its Ukrainian background, and 'Monopol' and 'Renan' with a continental background showed a better performance than all English varieties (with the exception of 'Hereward'), which suffered considerably during the extreme cold.

Sufficient seeds of the F<sub>10</sub> have been saved to permit a second year of field trials comparing the F<sub>11</sub> populations in 2012/13, allowing for two years of data collection. In addition, it will be interesting to see the populations in comparison with F<sub>12</sub> populations, which will also be grown in the field in 2012/2013.

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# Minutes from the plenary discussion: Taking evolutionary breeding into the future

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Martin Wolfe outlined how the presentations of the symposium showed that crop populations can be more resilient, stable and better able to buffer against environmental stresses. Plant breeding for the organic sector should make use of this approach but needs legislation to support it.

David Wolfe then gave a short report on the history of the current EU seed law. The first manifestation of the law was as the Directives of 1966. The main obstacle to the marketing of internally diverse populations is set by the criteria of Distinctness, Uniformity and Stability (DUS), originated in 1970. New Directives introduced a Common Catalogue of seed based on national lists. Until today, only varieties listed on the Common Catalogue can legally be marketed, and accession to the list requires varieties to conform to DUS. The underlying rationale is that this requirement will achieve greater productivity across EU if members adhere to this.

This was also highlighted by the legal case in 2012 with Baumaux who took Kokopelli to court because Kokopelli was selling 'off-list' seeds originating from organic agriculture. The European Court of Justice endorsed the rationale of current law. While international law on this (FAO) ITPGR allows a non-DUS type approach it does not require it.

A reformulation of the EU seed law is currently underway, with the proposal to repeal all 12 existing Directives and replace them with a single Regulation. In consultations, European stakeholders have reported satisfaction with the principles underlying the existing Directives but supported simplification and consolidation: thus, the new system as proposed would resemble the present one in many details.

Nonetheless, there is a proposed article, 15 (3), which introduces a space for additional rules allowing production and marketing of varieties not on the Common Catalogue. This means that the Commission could adopt further measures through delegated acts in future years which would allow propagation and marketing of varieties in addition to those within the previous system which do not conform to DUS.

If accepted in its current form, the draft would take several years to come into force and the delegated acts would be a subsequent process. The challenge now facing those breeders interested in populations and other internally diverse varieties is to influence the drafting of criteria applied to varieties regulated by the delegated acts. Carefully handled, this is an important and promising opportunity.

In the ensuing discussion about potential criteria, a difficulty was seen in the fact that the benefit of populations is their dynamic flexibility, which makes them inherently unpredictable. For example, it would not be possible to use allele frequencies in the identifying description of a population unless the allele was a completely neutral one not expected to alter in response to the situation in which a population was grown.

One idea brought forward was to use history and traceability instead, i.e. to provide the pedigree of a population in terms of how it was produced and its cultivation history since then. This could further extend to a system of certified traceability, where an assessor inspects a crop in the field and certifies that it is the population as claimed, based on the pedigree and history. The creators of populations could also supply data from some years of cultivation of that population. A replacement of the DUS would then be the description of the population by its parents, and how it has been maintained since the first crosses. The traceability itself would be certified rather than the population genetics.

In this scenario of certified traceability, a potential seed buyer would have access to information on the parentage of the population and there would be data from the first few generations of multiplication of the population. Seed buyers would have access to a range of populations with different parentage. If information was not available about the parents it would apparently increase the risk for the buyer; but behind this would be notion of including 'the commons' as part of the common heritage of humanity. In fact, enshrining the value of the commons could be part of the legislation; breeders should then be able to incorporate landraces and less-described varieties, so in the ideal scenario, the legislation would not demand that all components of a population were registered varieties.

The participants agreed that it is more promising to concentrate on using historical rather than future or expected features of populations as their definitive characteristics, both because of their dynamism

and because two or more populations could conceivably overlap in terms of their genetic content and performance in the field, causing them to be difficult to distinguish except by their history.

It was suggested that a group of characteristics could be specified which are expected to appear in the population and together function as a unique identifier. However, it would not be possible to specify the frequency with which each characteristic occurs.

A general difficulty was seen in the approach to specify range of values within which a population would be expected to fall, and then to market it based on whether it sits in this range; this approach would need to take into account the impact of environment as this can change magnitude of parameters widely.

It was said that the issue of ‘reliability of the characteristics of the seed’ will have to be addressed to ensure criteria for populations make them safe for their markets. However, it was responded that populations change from year to year and that the ability to cope with through fluctuating conditions is what make this approach reliable. It was then pointed out that on the other hand demonstrating their resilience would need several years to prove and would be very expensive.

Further a “Demonstrated Added Value” criterion was suggested, i.e. testing the population under defined conditions (even if that includes a range of conditions) relevant to its proposed end-usage and to prove that it performs in accordance with the breeder’s specifications, using parameters defined by the breeder. This system could resemble the existing VCU (Value for Cultivation and Use) criteria.

It was pointed out that over-formalization might discourage many members of the target market of populations who, like the farmers in Réseau Semences Paysannes, could by nature be quite experimental.

One way of overcoming such difficulties might be to make the legislation very light-touch, placing the risk in the hands of the buyer of the seed. This would, in theory, make exchanges feasible at small scale and in the context of local markets where, for example, sellers and buyers have some shared knowledge which obviates the need for extensive documented quality assurances.

It was pointed out however, that the legislation must be composed in such a way as to satisfy other actors in the industry such as breeding companies, so the formulation must be considered from the perspectives of these actors. In relation to this, it was noted that the proposed system of populations provides for the protection of variety development, because varieties are still very valuable in the process of population generation.

It was further brought forward that the introduction of the delegated acts was done with the intention of creating a channel for the outputs of public investments like the CCPs. There have been discussions of an experimental permission to market populations, possibly quite soon, e.g. one year’s time. The Commission has agreed to consider a temporary experiment to allow populations to be marketed (according to the Standing Committee meeting October 2012).

The two challenges before this temporary experiment can be done are a) to define criteria in place of DUS that are more appropriate to populations but also protect the buyers/growers of seed, b) to define criteria which sufficiently distinguish the populations from normal commercial seed and which protect seed producers and breeders, ensuring that breeders’ rights are protected.

It was noted that the preamble to the current directives mention the value of agrobiodiversity, genetic resources and environmental conservation, but this is not followed through in the body of the legislation. This could be used as a lever in future discussions. Further, the UK authorities may be preparing to run an ‘experiment’ whereby the marketing of population seed is permitted during a limited window of time, but there is no information about when this might be. It was suggested that a process needs to be in place with DEFRA to promote this point within UK and that other countries need to do the same with their ministries. It was also argued that scientific data is necessary to support the case and that a certification system would be needed for quality control.