

Proceedings

Workshop on the role of Marker Assisted Selection in breeding varieties for organic agriculture

BIOEXPLOIT



EUCARPIA

25 - 27 February 2009
Wageningen
The Netherlands



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H. Østergård, E.T. Lammerts van Bueren, L. Bouwman-Smits (Eds)

25-27 February 2009
Wageningen
The Netherlands

Østergård, H., Lammerts van Bueren, E.T., Bouwman-Smits, L. (Eds), 2009

Proceedings of the BioExploit/Eucarpia workshop on The role of Marker Assisted Selection in breeding varieties for organic agriculture, 25-27 February, 2009, Wageningen, The Netherlands, 59p

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Introduction

Aim

The aim of the workshop is to

- facilitate a broad discussion with invited speakers on the state of the art and progress in relation to whether, when and how breeding programs for organic agriculture can benefit from Marker Assisted Selection (MAS).
- summarise this into a policy paper edited by the organisers. This paper will identify and clarify key issues based on the presentations and a participant-driven SWOT analysis of role of MAS in breeding for organic agriculture.

Proceedings with abstracts of oral presentations and posters will be handed out at the beginning of the workshop.

Background

The workshop is a follow-up of the discussion on MAS in plant breeding programs for organic agriculture, organised by COST SUSVAR and ECO-PB in January 2005 in Driebergen, the Netherlands. Now it is time for an update and further deepening of the issues involved since, in the meantime, science has made progress, practical breeders have gained more experience with MAS, and questions for breeding for organic agriculture may be more articulated. This progress has been supported by the EU project BioExploit (Exploitation of natural plant biodiversity for the pesticide-free production of food, <http://www.bioexploit.net/>) developing efficient and rational breeding strategies using e.g. MAS, and members of the EUCARPIA Section Organic and Low-input Agriculture (www.eucarpia.org).

The workshop will discuss basic selection principles as well as contrast breeding strategies according to organic principles and MAS in a few cases combining major crops and important traits.

Participants

- Breeders and researchers involved and/or interested in plant breeding for organic agriculture with or without detailed knowledge on molecular techniques.
- Policy makers and opinion leaders among the stakeholders.

Scientific organisers

Edith Lammerts van Bueren, Louis Bolk Institute, Netherlands and Hanne Østergård, Risø-DTU, Denmark.

Local organisers:

Edith Lammerts van Bueren/Louis Bolk Institute and Liesbeth Bouwman/Wageningen University and Research Centre

Supporting bodies:

BIO-EXPLOIT and EUCARPIA Section Organic and Low-input Agriculture

Programme BioExploit/Eucarpia Workshop

Wednesday February 25th

11.00h – 13.00h registration

12.00h Lunch

Session I: Introduction on principles and perspectives of breeding for Organic Agriculture (OA) and of Marker Assisted Selection (MAS)

13.00h Opening

Hanne Østergård, Biosystems Dep., Risø DTU, Technical University of Denmark, Roskilde, Denmark.

13.10h Organic plant breeding – A general overview

Heinrich Grausgruber, BOKU, Institute of Agronomy and Plant Breeding, Vienna, Austria

13.40h Direct or indirect selection for breeding for Organic Agriculture

Julie Dawson, INRA - UMR de Génétique Végétale, Gif sur Yvette, France

14.10h QTL x E x M: combining crop physiology and genetics

Paul Struik, WUR, Centre for Crop Systems Analysis, Wageningen, The Netherlands

14.40h Tea break

15.10h Potentials of MAS in general: genetics, crops, traits, economy

Anker Sørensen, Keygene, Wageningen, The Netherlands

15.40h General discussion

16.30h Poster session and drinks

18.30h Dinner

Thursday February 26th

8.15h Coffee

Session II: Breeding for disease resistance traits in wheat

8.30h Required characteristics for organic wheat varieties with respect to disease resistance
Maria Finckh, University of Kassel, Department of Ecological Plant Protection, Witzenhausen, Germany

9.15h How MAS is included in wheat breeding programmes for disease resistance
Jens Weyen, SU-Resistenzlabor, SAATEN-UNION, Germany

10.00h Discussion

10.30h Coffee break

Session III: Breeding for qualitative disease resistance in potatoes to late blight

11.00h Tracing resistance genes in potato by MAS in a professional breeding program
Guus Heselmans, C.Meijer B.V., Rilland, The Netherlands

11.45h Organic potato breeding creates added value
Niek Vos, organic farmer-breeder, Kraggenburg, The Netherlands

12.30h Discussion

13.00h Lunch

Session IV: Breeding for baking quality in wheat

14.00h Application of markers when breeding for baking quality
Stine Tuvevsson, SW Seed, Svalöv, Sweden

14.45h Is heterogeneity an advantage or a disadvantage in breeding for baking quality in wheat?
Martin Wolfe, The Organic Research Centre, Elm Farm, United Kingdom

15.30h Discussion

16.00h Tea break

Session V: Breeding for quality trait (taste) in tomato

16.30h Tomato breeding for taste by Oldendorfer Saatzucht
Ulrike Behrendt, Oldendorfer Saatzucht/Kultursaat e.V., Holste, Germany

17.15h Application of MAS in tomato breeding programs for taste
Sjaak van Heusden, Wageningen UR Plant Breeding, Wageningen, The Netherlands

18.00h Discussion

18.30h Dinner

Session VI: Organic principles as seen from IFOAM

20.30h Principles of Organic Agriculture
Louise Lutikholt, International Federation of Organic Agriculture Movements (IFOAM), Bonn, Germany

Friday February 27th

8.15h Coffee

Session VII: The future role of MAS in breeding for organic agriculture

8.30h Participant-driven SWOT analysis based on the previous days's presentations and discussions as well as the experience of the participants. This to identify and clarify key issues of role of MAS in breeding for organic agriculture. The outcome of this group work will give the basis for a policy paper on these issues edited by the organisers.

Facilitator: Hanne Østergård, Risø DTU, Denmark.

10.30h Coffee break

11.00h Conclusions and Follow-up

Moderator: Edith Lammerts van Bueren/LBI, The Netherlands

12.00h Closure and lunch

Oral presentations

**Session I: Introduction on principles and perspectives of
breeding for Organic Agriculture (OA) and of Marker
Assisted Selection (MAS)**

Organic plant breeding – A general overview

Heinrich Grausgruber

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Introduction

The share of agricultural land and farms managed following guidelines of organic agriculture increased continuously in the last two decades. Today organic farming is practiced in more than 130 countries of the world. In the 27 member states of the European Union 4% of the land is under organic management (Willer et al. 2008). As likely as not this upgrowth will continue since the growth rates of the global organic food and beverages market increased exceptionally the last few years, and have pushed over the worth of € 30 billion in 2006. Still Europe and North America are experiencing undersupply in some food categories (Organic Monitor 2006). With the Council Regulation (EEC) 2092/91 on organic production regulations concerning organic seeds became effective. However, these regulations do not include specifications of plant breeding and seed production methods and/or techniques. Today's organic agriculture still relies mainly on varieties derived from conventional breeding programmes. The current growth of organic agriculture poses the risk that due to not available alternatives the sector will turn more and more to conventional methods in order to keep up with the growth of the market. The 'conventionalisation phenomena' already affect almost all actors of organic production (Kratochvil et al. 2005, De Wit & Verhoog 2007). Hence, concerning breeding the calls for more 'organic' in breeding programmes are emerging.

Breeding techniques

A vision on organic plant breeding and its consequences in regard to breeding techniques was elaborated by Lammerts van Bueren et al. (1999). Two years later a dossier on plant breeding techniques was edited by the Research Institute of Organic Agriculture (FiBL) (2001) judging the suitability of the different breeding and multiplication techniques for organic agriculture. One outcome of the discussion were the IFOAM Plant Breeding Draft Standards (Table 1).

Table 1: Plant Breeding Draft Standards suitable and permitted for organic plant breeding (IFOAM 2005)

Variation induction techniques	Selection techniques	Maintenance and multiplication
<ul style="list-style-type: none"> • combination breeding • crossing varieties • bridge crossing • backcrossing • hybrids with fertile F₁ • temperature treating • grafting style • cutting style • untreated mentor pollen 	<ul style="list-style-type: none"> • mass selection • pedigree selection • site-determined selection • change in surroundings • change in sowing time • ear bed method • test crossing • indirect selections • DNA diagnostic methods 	<ul style="list-style-type: none"> • generative propagation • vegetative propagation <ul style="list-style-type: none"> - partitioned tubers - scales, husks, partitioned bulbs, brood bulbs, bulbils - offset bulbs etc. - layer, cut and graft shoots - rhizomes • meristem culture

Not allowed in organic plant breeding are techniques of genetic engineering. The interpretation of genetic engineering, however, is sometimes in a grey zone. Protoplast fusion for example is considered to be not suitable for organic breeding by the IFOAM guidelines, while it is not considered genetic manipulation by the EU regulation on organic farming.

Therefore, varieties derived from protoplast fusion need not to be labelled (Billmann 2008, Lammerts van Bueren 2008). A similar discussion is ongoing with novel breeding techniques, i.e. intra-, fami- and/or cisgenesis (Nielsen 2003, Schouten et al. 2006, Lammerts van Bueren et al. 2007). Contrary to cell fusion, however, these techniques are not excluded by the EU directive (2001/18/EC) on genetically modified organisms and, therefore, they are banned from organic agriculture.

In various European countries some organic actors elaborated and approved additional guidelines besides the IFOAM draft standards for organic varieties. In Austria the ARGE Bio-Landbau generated a negative list of not allowed methods. This list is in accordance with the IFOAM draft standards, but also excludes bread wheat introgressions into varieties of spelt wheat (Surböck et al. 2003). In Switzerland the organic umbrella organization BioSuisse generally banned hybrid varieties from organic cereal production with the exception of maize (Voegeli 2006). The criticism on hybrid varieties was outlined by Arncken & Dierauer (2006). Recently, the Association of Biodynamic Plant Breeders (www.abdp.org) released standards for certified biodynamic plant breeding. In addition to the IFOAM guidelines these standards explicit ban hybrid breeding irrespective of the hybridization method, the production of double haploid varieties or polyploidisation, and protoplast/cytoplasm fusion. The use of hybrid or double haploid varieties as parents for a biodynamically bred variety, however, is allowed.

Breeding goals

Varieties bred by conventional breeding programmes are generally not unsuitable for organic agriculture. Disease resistance, yield, nutrient efficiency, tolerance to abiotic stress and end-use quality are important characteristics for both production systems. For organic varieties, however, the emphasis of some traits is different. While soil-borne and root diseases can be controlled by adequate crop rotation, resistance against some foliar and seed-borne diseases is much in demand. In addition organic varieties demand specific characteristics usually not necessary for conventional breeds, e.g. weed competitiveness is highly beneficial to suppress both undesirable weeds and volunteer plants (Surböck et al. 2003, Wolfe et al. 2008).

Naturally, yield is important in regard to economic revenue. For organic varieties, however, the benefits have to be seen within the whole crop rotation and the closed cycle system. Therefore, taller varieties might be favoured due to better weed suppression, higher yields of straw used for bedding and/or plant remains for organic manure. Due to highly variable conditions in organic farming yield stability is ranked higher. Yield stability is especially important for crops which play vital roles in organic farming, but for which only a few small breeding programmes are existing and the breeding progress is low, e.g. legumes and specialty crops. Combining yield and stability into a single parameter of yield reliability might be meaningful (Eskridge 1990, Annicchiarico 2002).

High end-use quality is an important characteristic for organic varieties. In trading the same quality parameters than for conventional produces are used to determine quality and price. However, limits of certain parameters are sometimes different, e.g. lower protein contents are accepted for organic wheat for bread making. In recent years nutritional quality amended technological end-use quality and breeding programmes for added quality values were initiated. Varieties biofortified with vitamins, minerals and phytochemicals should provide healthier foods. Genotypes with higher concentrations of these health beneficial compounds are often limited in yield. Therefore, such varieties could be more suitable for organic production. Furthermore, it's a general belief that organic farming would produce healthier foods than conventional farming. Whereas breeding for nutritional value is still at the beginning and often influenced by great genotype by environment interaction, the advertising of health benefits of such products is already commonplace.

Breeding strategies

Wolfe et al. (2008) defined organic agriculture by three market types, i.e. global, regional and local market. These market types are served differently by varieties from three different sources: breeding programmes for (i) conventional agriculture, (ii) organic agriculture or (iii) within organic agriculture. Varieties originally bred for conventional agriculture but which perform well under organic management still capture the greatest part of organic seeds. 'Pure' organic varieties, i.e. varieties which were selected and propagated in all breeding steps under organic conditions, are still very rare and often only of local importance. In the last decade several breeding programmes for organic agriculture were established by conventional breeders. Their fate will largely depend on cost recovery. The organic movement would need a tremendous increase of varieties selected under organic conditions. It can be assumed that organic programmes are more expensive, since more breeding goals have to be considered. On the other hand, the market for organic varieties is still relatively small. Various models how to finance organic breeding were discussed in an international workshop (Osman et al. 2007). Breeding strategies have to consider cost effectiveness. Various strategies such as indirect selection, decentralised and participatory approaches, composite crosses/evolutionary bulk breeding including 'older' varieties with valuable 'organic' traits, shifting between organic and conventional programmes (Suneson 1956, Löschenberger et al. 2008, Wolfe et al. 2008) could help to keep the costs for organic programmes low. For wheat it was demonstrated that many characteristics are highly correlated between organic and conventional low-input management. Yield, some quality traits, N use efficiency and weed suppression, however, did not rank satisfactorily consistent (Oberforster et al. 2000, Kempf 2002). Breeding strategies for organic agriculture have also to consider that a high genotype by environment interaction can not be only present between organic and conventional environments but also within organic subsystems. Considering organic traits and/or goals could be advantageous also for conventional breeding programmes. Climate change, fertilizer crisis and increasing costs for energy will adjust conventional agriculture to lower inputs Burger et al. (2008) demonstrated that including organic test sites into conventional programmes can increase the chances of detecting broadly adapted genotypes. Combining various breeding strategies and including 'organic characteristics' at the very beginning of breeding could lead to a greater number of better adapted organic varieties in the nearest future. Besides increasing the number of organic varieties of major crops it of equivalent importance to strengthen breeding programmes of minor, but for organic agriculture important crops, such as legumes and forage crops.

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Direct or Indirect Selection in Breeding for Organic Agriculture

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Interest in plant breeding for organic systems has increased in recent years due to the realization that these systems are not well-served by modern plant breeding for conventional systems, much as marginal environments in developing countries are not well-served by centralized breeding programs either at the national or international level (Desclaux, 2005). More variable environments are found in organic systems and the effects of environmental stress are more critical because farmers cannot standardize environmental conditions similar by using inputs and or remedial solutions such as pesticides in response to problems. This means they must proactively encourage system health, which includes the use of well-adapted and resistant genotypes in the context of a functioning agroecosystem. In addition to the more variable effects of the biophysical environment, the needs of organic farmers are more diverse due to more complex production strategies. (Desclaux et al., 2008; Wolfe et al., 2008). Organic farmers currently have trouble finding adapted varieties, partially because there has been little interest from the private sector in developing varieties for organic agriculture (Desclaux, 2005). This may be due to limited seed markets and regulatory barriers to commercialization of varieties specifically targeted to organic systems.

The first question often asked is: are breeding programs specifically targeted to organic agriculture needed, or is selection in conventional systems sufficient for identifying superior varieties? Specific organic programs are justified if 1) there are traits that are of importance in organic systems that may not be considered in conventional systems, and 2) for traits of importance to both systems, there are significant differences in genotypic performance or trait priority between the systems.

Major reviews have addressed the issue of specific traits for organic and low-external-input agriculture and the need to recover and improve traits that may have been lost through extended breeding for conventional systems. These include competition with and/or tolerance of weeds, durable disease resistance, with different priorities in terms of the most critical diseases, the ability to use organic nutrient sources and the ability to benefit from symbiotic and associative relationships with soil microorganisms, other plants and other beneficial species such as insect predators (Wolfe et al., 2008; Desclaux, 2005; Ceccarelli, 1996a; Lammerts van Bueren et al., 2002; Mason and Spaner, 2006; Murphy et al., 2007).

One common point is that traits of importance are often highly complex and it is difficult to choose appropriate selection criteria. In many cases it may be necessary to select for composite traits in the target environment, for example, selection for yield and protein content in order to increase nitrogen use efficiency in organic systems with low nitrogen status without the need to measure the degree of association with mycorrhizal fungi or the exact dynamics of nutrient uptake and mineralization. However, continued research on the basic mechanisms behind more complex traits is also needed.

In terms of the second criteria justifying targeted organic breeding programs, differences in performance for traits that are important to both systems have been demonstrated in some cases. Murphy et al. (2007) found that the top five genotypes in conventional systems were often ranked much lower in organic systems and vice-versa in four out of the five environments tested. In comparing multiple organic and conventional trials in European countries, Przystalski et al. (2008) found that even with high genetic correlations between

traits in organic and conventional trials, the probability of a variety being in the top 10% in both systems was much lower (0.55 to 0.85 for a genetic correlation of 0.80 to 0.98). In other cases selection in conventional and organic systems resulted in similar rankings, or crossover changes in only a subset of genotypes or for a subset of traits (Osman et al., 2008; Vlachostergios and Roupakias, 2008). In addition to crossover interactions in performance, the priority given to certain traits may differ between conventional and organic systems, for example, quality may be of much higher value in organic systems while yield is of primary importance in conventional ones. So the question of the efficiency of selecting indirectly in conventional systems for performance in organic systems will depend on the degree of difference between the systems. In many cases, comparison studies have been conducted either on research stations recently transitioned to organic production or using varieties selected only in conventional systems, which may not be representative of the true range of potential responses to organic conditions.

The correlated response in environment X to indirect selection in environment Y depends on the heritability of the trait in each environment, and the additive genetic correlation between the environments. Indirect selection can have a greater response than direct selection if the genetic correlation is high enough and the heritability is significantly greater in the environment of selection than in the target environment. The efficiency of indirect selection depends on the degree and type of genotype by environment interactions between the two systems. In practice, if the target and selection environments are very different, the genetic correlation may be very low, and the heritability in marginal environments are not always lower than in the high input environments such as research stations and breeding nurseries (Ceccarelli, 1996a, 1994).

In addition, there is a need to consider not only biophysical G x E between organic and conventional systems, but also the G x E within organic systems. In some cases organic system may be more different among themselves than in comparison to conventional systems in the same region. Even when restricting the considerations of G x E to biophysical stresses, it is not possible to develop single varieties that do well in the majority of organic agricultural systems, for example Baresel et al. (2008) found large variations in nitrogen dynamics between two organic systems with the result that which resulted in significant crossover interactions. When expanding the consideration of G x E to include socio-economic, management and marketing factors, it becomes impossible to generalize as the possible interactions among these domains are very complex. Attempting to define target environments that share common values across all domains most likely results in a separate category for each individual farm. Breeders, farmers and other actors will need to make decisions about which interactions are the most important or determinate in their context and develop breeding strategies that work to take these interactions into account in the selection process. Many different selection strategies may be appropriate for organic systems, and the one chosen will depend on the specific context and actors involved.

If wide adaptation is desired, some degree of indirect selection is necessary. These varieties can be either pure lines or heterogeneous mixtures/multilines. Indirect selection may be most effective if conventional breeding programs are low-input or where the majority of G x E can be explained by environmental factors common to both systems, such as climate or daylength. If genetic correlations between the systems is fairly large, the use of data from trials in both systems may improve selection in either organic or conventional conditions where the addition of environments is important for improving the estimation of performance across the target environment, but where there are not resources to add environments in both agricultural systems (Przystalski et al., 2008).

If the target environments are too different to select a single variety or population that has acceptable performance everywhere, breeders may target very focused regions for different purelines or heterogeneous populations which then evolve specific adaptation. In this case, an analysis of $G \times E$ is a method of choosing the best variety or population for each target environment (Ceccarelli, 1996b). Where direct selection is important, decentralization of the selection process is necessary and this often leads to participatory selection (Sperling et al., 2001; Ceccarelli et al., 2001) because of the need to add many selection and testing sites where farmers have specific knowledge of environmental conditions and plant traits that are adaptive under their conditions. There have been demonstrations of the effectiveness of farmer selection in many cases, for example the program of barley selection at ICARDA, where in a test on research stations and nine farms in Syria, farmer selections were always as good as breeders in increasing yields, with a significant response to selection in almost all cases and with farmer selection significantly better in one case (Ceccarelli et al., 2000). Farmers selected fewer populations in general, meaning they were able to increase selection pressure on their particular farm compared to breeder selection. In many cases of participatory selection, farmers have been extremely competent at selecting superior varieties, and are also able to keep populations separate and deal with segregating populations (Ceccarelli, 1996a; Ceccarelli et al., 2001; Gyawali et al., 2007; Sperling et al., 1993, 2001; Smith and Weltzien, 2000). However, participatory selection requires increased commitment and engagement on the part of the farmers and breeders and may not be appropriate in all cases.

A discussion of indirect and direct selection leads to a discussion of the degree of diversity present in varieties grown across a region, and the value of this diversity for agronomic performance and the future adaptive potential of the crop species. The agronomic benefits of diversity include improved durability of disease resistance and reduction of disease severity (Wolfe, 2000; Finckh, 2008) and greater buffering capacity of heterogeneous populations (Ceccarelli, 1994; Finckh and Wolfe, 2006). This stability arises from individual plant plasticity and from different response norms of individuals within a diverse population, the two mechanisms identified by Allard and Bradshaw (1964), while homogeneous populations rely only on the buffering capacity of the individual plants. In heterogeneous populations, phenotypic stability may arise from genetic diversity that allows the flexible expression of component traits that lead to higher stability for composite traits such as yield and quality.

In terms of the analysis of varietal or population stability, current methods of analysis often do not separate spatial and temporal stability because of averaging trait values over years in each location. While spatial stability is important in selecting varieties with wide adaptation, temporal stability is more important to direct selection in the target environment as farmers are usually concerned with consistent performance on their individual farm. The question remains whether these two types of stability are mutually exclusive. Indirect selection for performance across a wide range of environments may select for generalist phenotypes with a range of reaction norms and higher phenotypic plasticity, without underlying genetic variability, while direct selection in each target environment may select for genetic adaptation to particular conditions with a smaller range of phenotypic plasticity (Kawecki and Ebert, 2004). However, the degree to which selection leads to more generalist or more specialist phenotypes in each case probably depends on whether the variation across location and years is in terms of the same environmental constraints or whether the variability across years is qualitatively different than that across locations.

There is a need for further research on the statistical analysis and modeling of biophysical $G \times E$ in terms of the consideration of different types of stability and the ability to account for

multiple traits with differing priorities depending on the system. There is also a need for greater collaboration across disciplines and especially with organic farmers to address the other components of G x E related to socioeconomic and management variables. Breeding for these systems can benefit from farmer input not only as on-farm trial hosts, but also in defining traits and in identifying and choosing among trade-offs among traits, and in terms of breeding program priorities.

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QTL × E × M: combining crop physiology and genetics

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Introduction

Knowledge on molecular biology and genetics of plants has progressed enormously. However, this knowledge has highlighted in the first place that plant metabolism and its regulation under variable and – especially in organic agriculture – often stressful environmental conditions are extremely complex. Plant and crop scientists still do not fully understand how the plant, as an autonomous organism, or a crop, as a group of mutually interacting plant individuals, is capable of managing its own complexity and how it perceives and interprets all the information it is exposed to in order to survive, propagate its genes and produces useful products for mankind. Modelling can help to bring order in that complexity – at least to some extent – because we can reduce the complex reality to a number of robust algorithms that are capable of catching the dynamics and mechanistics of the most determining processes. Dissecting complex traits with low heritability into relatively simple component traits, which are less sensitive to environmental conditions, will assist breeders in making faster breeding progress.

The new challenge

Modern crop physiology is challenged to bring the information from functional genomics to the crop level, by introducing true biological mechanisms from systems biology into crop models based on a true understanding of the organization of the crop across scales and the crop's response to environmental conditions. Crop physiology is for a greater part developing into the direction of what we call 'crop systems biology', which aims at modelling complex crop-level traits relevant to global food production and energy supply, via building the links between 'omics'-level information, underlying biochemical understanding, and physiological component processes. Essential in crop systems biology is to properly map the organization levels and the communication systems between these levels for the different key processes, from the molecule or gene, all the way up to the crop. Such an approach is clearly needed (and also suitable) when dealing with the interactions between environment (E), management (M) and genetic components (often identified as QTL), further called QTL × E × M interactions, because molecular information (in the form of QTLs for desirable traits) should be evaluated and used at a level where it really matters: where the genotype interacts with E and M. In our framework we distinguish between environment and management. Many researchers consider management as part of the environment but the distinction is useful because it stresses what is manageable (and therefore at least to some extent also predictable in a quantitative way) and what is not.

Where do we stand today?

Many relevant crop traits, such as yield and quality, are quantitative and complex. They are controlled by multiple, interacting genes whose expression may be dependent on environment. The modern molecular marker technologies enable us to dissect the variation in these complex traits into the effects of QTLs. With the progress of QTL mapping new breeding approaches such as marker-assisted selection have become possible and breeding by design has become within reach. However, we should not be too optimistic about these opportunities on the short term, since polygenic control, epistasis and QTL × E × M interactions can impede the use of these approaches. Some of these aspects require continuous

and long-lasting efforts; but for $QTL \times E \times M$ interactions modelling may become handy in the first place.

Whole-crop physiology models are the obvious tools to dissect complex traits into manageable component traits and to describe the effects of environment and management on them in a mechanistic way. This is obviously useful for breeders as they will now have information with much less $QTL \times E \times M$ interaction and therefore QTLs which are more robust, resulting in a more efficient breeding process. But it is also attractive for crop physiologists: until recently models were very poor in capturing the genetic component of these complex traits, let alone the $QTL \times E \times M$ interactions. So if crop physiology and genetics are combined judiciously, crop physiology and modelling research can reinforce the genetic analysis of complex traits, thereby improving breeding efficiency, but at the same time can also create approaches with which crop physiology can use genetic information in crop models.

Because crop models represent causality between component processes and yield, they can predict crop performance beyond the environments for which the model parameters were estimated. This singular property allows the models potentially to resolve $QTL \times E \times M$ into underlying processes on a daily basis and to predict crop performance for any genotype in any environment.

In order to realize these achievements, a model-based approach comprising of five steps is required:

1. Create a crop model that predicts complex traits based on relations between elementary processes and environmental variables.
2. Evaluate the capability of the model to predict the complex trait across a wide range of combinations.
3. Identify QTL for model-input traits using a genetic QTL approach.
4. Develop a QTL-based model whereby the original values of model input traits are replaced by QTL-based inputs.
5. Validate the QTL-based model across environments.

Examples

Examples where the approach described in the previous section has been or is being applied to analyse gene/ $QTL \times E \times M$ interactions and that demonstrate the approach of dissecting complex traits into biologically meaningful component traits in which the environmental effect is already accounted for, are:

1. QTL-based models of time to flowering for rice, barley and rapeseed, based on the response of flowering time to photoperiod and temperature as affected by, for example, sowing time or models of time to flowering in *Arabidopsis* based on gene networks;
2. QTL-based models of the response of elongation rate of maize leaves based on temperature, vapour pressure deficit and soil-plant-water relations as affected by irrigation schedules;
3. QTL-based models of the development over time of soil cover and of tuber formation in potato as affected by fertilizer supply;
4. QTL-based models of use efficiency of nitrogen in barley and of nitrogen and phosphorus in potato as affected by resource input and production environment (conventional versus organic; different types and levels of resource input);
5. QTL-based models of root growth and resource capture in lettuce as affected by transplant management;
6. Gene-based models of fruit growth in tomato based on cell cycling, cell division and cell elongation as affected by temperature regime and fruit load;
7. QTL-based modelling of fruit quality in peaches, based on physical, metabolic and physiological subroutines and affected by fruit load;

8. QTL-based modelling of drought tolerance in rice focusing on complex traits such as photosynthesis, chlorophyll fluorescence, yield components, and yield.

Relevance for organic agriculture

QTL-based modelling and organic agriculture can become a perfect match. In organic farming, the crop eco-physiological principles are not different from those in conventional farming but the systems are more complex, are more difficult to generalize across individual farms, and interactions (including those relating to genotype \times environment \times management) are more significant. Agronomic characteristics of organic production environments are usually much more complex than in the case of conventional agriculture because of the intrinsic variation in process rates, timing and duration (e.g. of mineralization of organic matter in dependence of physical, chemical and biological soil fertility). Moreover, organic agriculture is using an agro-ecological approach taking measures to stimulate the self-regulating ability ('autonomy') of living systems, including (agro-)ecological systems, whereas conventional systems often use external inputs which overrule this ability. Model-based systems analysis for organic agriculture can therefore be a very useful tool in quantifying agro-ecological processes and their consequences for yield, quality and other aspects of system behaviour.

As organic agriculture has fewer management means to adjust the environment to the genotype, it needs varieties that are better adapted to variable low-input (organic) growing conditions. Organic farming aims at optimizing the production system more than the individual crop and thus practices are not aimed at providing optimal amounts of resources to the individual crop but to maintaining system health. Nutrient supply and water supply are therefore less regular, less abundant, and more depending on (variable) environmental conditions, including physical, chemical, and biological soil conditions. For example, mineralization of organic matter and uptake of nutrients depend on availability of soil moisture, thus increasing variation in growth.

This means that by definition organic agriculture seeks holistic approaches and looks for varieties which fit in those approaches. Crop ideotypes for organic systems are more complex (with more trade-offs) than their counterparts in conventional agriculture. Trade-offs should be quantified preferably by modelling approaches. Moreover, organic farmers look for varieties that are robust under their conditions, i.e. show a reliability, an efficiency of functioning, and a persistence of functionality under fluctuating, unpredictable and changing conditions. A good example of robustness might be a large plasticity towards dynamic availability of nutrients and water by maintaining a suitable root architecture throughout the life cycle and a dynamic balance in shoot to root ratio, even when this would mean extra investments in root dry matter that does not contribute to the harvestable yield.

Complex and system-specific characteristics such as robustness might well have a genetic background and are therefore amenable for selection, but this still requires proof by proper research. For that robustness needs to be defined in agronomic terms and specified in crop physiological terms, resulting in those characteristics that can be broken down into component traits for which stable (i.e. environment and management independent) QTLs can be identified. Complex traits can be conceptualized within a modelling framework and tested for a diverse set of organic environments. QTLs can then be identified for these component traits.

The need to break down complex traits into manageable component traits might be against the nature of the holistic thinking in organic agriculture but is a prerequisite for effective breeding on such traits and to allow the use of marker assisted selection techniques for those traits.

In order to let QTL-based modelling contribute to designing robust varieties for organic agriculture the five steps for developing such a model explained above in the section

“Where do we stand today?” would still suffice. However, each of the steps would be extremely laborious and time- and resource-demanding given the complexity of the traits organic agriculture is looking for and in the light of the fact that we have only designed successful QTL-based models for relatively simple growth traits or relatively simple developmental traits.

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Potentials for MAS in general: genetics, crops traits, economy

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Abstract

In the past 20 years the use of molecular markers has gradually expanded from the field of scientific genetic analysis towards the implementation in commercial breeding programs. Currently molecular DNA tools are utilized in plant breeding programs in order to optimize the gain of selection in various ways. In addition, breeding methods are being adapted through the use of molecular DNA tools, in order to develop novel varieties, which without the use of molecular DNA tools, would be very difficult to develop.

DNA technology combined with the laws of inheritance is being used to develop procedures and methods that can elucidate the relationship between phenotypic variation and genotypic variation, thereby generating knowledge of the molecular control of valuable traits. Efficient and effective exploitation of this knowledge is the core business of the modern plant breeder. Many valuable traits have a complex inheritance in plant populations and therefore the molecular control of this type of traits has a complex nature as well. Contrary to the situation of molecular control of simply inherited traits, complex traits will require different molecular breeding strategies (MAS strategies) in order to be effective. This presents serious challenges for the future and requires integration of different knowledge levels (genome, chromosomal regions, genes, gene alleles) as well as breeding strategies and breeding schemes.

Breeding varieties for organic agriculture using molecular DNA tools could greatly benefit from the knowledge available concerning genetics of traits. Organic breeding is faced with the same level of complexity concerning many valuable traits, and thus will require the development of specific MAS strategies in order to be effective. A comprehensive approach towards the variety improvement process, using integration of all knowledge available at different levels of the plant, could be a key to integration of MAS and organic breeding.

Session II: Breeding for disease resistance traits in wheat

Required characteristics for organic wheat varieties with respect to disease resistance

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Organic farming systems differ from conventional systems mainly with respect to soil and disease/pest management and thus with respect to soil properties and to plant protection options. In order to identify the traits necessary for successful organic varieties it is therefore crucial to determine the effects of soil management on plant health and also to focus on the pests and diseases that have a high potential to succeed in organic systems and to concentrate breeding effort onto these.

The differences in plant nutrient supply in comparison to conventional farming systems have generally reduced the importance of aphids and obligate pathogens such as rusts and powdery mildews for which, in addition, a wide range of resistances are available. For *Fusarium* spec. the situation is more complicated. In conventional agriculture *Fusarium* is mainly residue and soil borne due to generally short rotations and to a high amount of maize in conventional systems. In contrast, the wider rotations in organic systems usually reduce the soil borne phase of *Fusarium*. However, due to a lack of effective seed treatment options seed infections can be a real problem. Also, an increasing interest in the production of maize will increase the importance of the soil borne phase of *Fusarium* in organic farming in the future. Therefore, resistance to this pathogen is of great importance.

In general, resistances to seed borne pathogens have to be much more in focus in any organic breeding programme, especially for pathogens that reside in the embryo like loose smut or deep inside the seed like *Fusarium* where heat or other alternative treatments cannot reach.

Overall, any measures that increase microbial activity and diversity in the soil will increase soil health by reducing soil borne pathogens (Weller et al., 2002). Such a systemic approach has been termed biological systems management (Vilich and Sikora, 1996). While appropriate organic amendments such as composts and green manures can be as effective (or even more so) as the traditional ploughing in reducing soil borne pathogens such as *Gaeumannomyces graminis* spec. or *Pseudocercospora herpotrichoides* by increasing resident biocontrol and earthworm activity there are increasingly reports of growers suffering from generalist nematode infestations which can only be reduced by black fallow or very specific antagonistic plants. Nematode problems are notoriously disregarded when assessing plant production problems world wide and organic farming is no exception there.

Resistance breeding has been and probably will remain one of the most successful areas of breeding for sustainability in agriculture and it has been the major driving force for breeding success. At the same time, spectacular resistance break downs again and again have obviated the successes and some have gone so far to claim that plant diseases are “normal agricultural accidents” (Juska et al., 1997) due to a lack of strategies counteracting host-pathogen co-evolutionary processes.

The evolutionary processes in pathogen populations leading to resistance breakdown are mutation, recombination, adaptation, and selection. All these processes, in particular adaptation and selection processes are critically influenced by environmental conditions affecting the pathogen population directly or via the host and the genetic structure of the host population. The beneficial effects of diversity for resistance in space and time, reducing diseases are well known and a multitude of mechanisms has been identified contributing to these effects (see Finckh and Wolfe, 2006 for review) and resistance gene management on the population level will be an important component of future sustainable agricultural systems as

well under organic as conventional conditions. For long-term sustainability it will be crucial to reintroduce diversity for resistance into modern varieties to prevent resistance breakdown using population approaches to breeding such as evolutionary breeding, composite crosses or other approaches (e.g. Finckh, 2008; Murphy et al., 2005; Wolfe et al, 2006, 2008). Modern marker technologies may be of help here, especially when it comes to the registration and identification of varieties within the current legal system.

Besides traditional breeding for resistance there are a number of traits that have been identified that might be of importance to future sustainable disease and pest management. For example, it has been found that certain varieties or species may attract natural enemies of aphids or other insects (Ninkovic and Patterson, 2003; Patterson et al., 2006; Starks, 1972) or that root exudates may have allelopathic effects on pathogens (Friebe et al., 1998; Vilich-Meller, 1992; Vilich, 1993). In addition, genotype specific microbial communities in the rhizosphere apparently also affect plant growth and thus fitness and potentially disease resistance through direct competition, antagonism, or induced resistance (Picard and Bosco, 2006). As inducibility for resistance is also a selectable trait, breeding for inducibility of resistance should be seriously considered in future breeding programmes. This might become especially interesting in combination with diversification strategies.

In conclusion, changing the farming practices will also shift disease and pest problems. Breeding for disease and insect resistance in organic farming must be based on several main goals: disease resistance, diversity for resistance, ability to be induced for resistance, and the ability to attract and sustain beneficial organisms in the system among others. Some of these traits may not be selectable directly and most certainly can only be determined within specific organic growing conditions. This may require more decentralised approaches to breeding to allow for local system specific optimisation and adaptation of plant populations.

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How MAS is included in wheat breeding programmes for disease resistance

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Wheat breeding changed significantly in the past. Beside many technologies in tissue culture, quality analysis, field and nursery technology the emergence of molecular markers is one of the new aspects to be considered by wheat breeders. RFLPs, RAPDs, CAPS/SCARs, Retrotransposon, COS, DArT, SSRs, SNPs and many other marker systems and in the future also the direct use of sequencing machines are/will be under application in wheat breeding research and practical wheat breeding programs. Furthermore, by the development of better DNA extraction technologies, DNA analysis tools as capillary electrophoresis, qPCR machines and chip technologies, the cost per data point are still significantly and fast decreasing, which will make molecular marker strategies possible for budget restricted wheat breeding programs too. Additionally, by the decrease of costs, totally new marker assisted breeding technologies and breeding strategies will be possible.

Therefore, more and more wheat breeders are using DNA marker technologies to identify and to map resistance genes or already specific alleles to select desired genetic variability in accelerated and cost extensive manner. Nevertheless, high precision phenotyping and exact disease screening in connection with sophisticated strategies for the discovery, use and exploitation of genetic variability will be necessary in the future.

In our company we started an R&D program related to molecular markers in wheat in 1998. This process was beginning with joining consortia which developed SSR markers, which were then mapped to their chromosomal location and first used in so called AB-QTL projects. In those projects, SSR markers were used to find correlations to yield QTLs. SSR markers were visualized on ALF Express machines (Pharmacia), the handling of the gels was time consuming and the reliability and reproducibility of SSR allele size was critical.

Meanwhile, we switched to a more sophisticated, more reliable, stable and logistically easier to handle system for the separation of DNA SSR fragments which are now capillary electrophoresis machines (ABI 3100) and a qPCR machine.

SSRs markers are still the markers of choice due to their easy handling, their exact chromosomal location, and easy exchange of scientific data with SSR based experiments worldwide. Often SSRs are multiallelic, which makes them very interesting for plant breeder laboratories. They can also be multiplexed which brings costs down to very interesting levels. Since the start of the marker work in wheat we joined several R&D projects, which were dealing about the mapping of resistance genes for Soil Borne Wheat Mosaic Virus (SbWMV) and Wheat Spindle Streak Mosaic Virus (WSSMV), *Pseudocercospora herpotrichoides* (pch1 gene from *Aegilops ventricosa*), Fusarium Head Blight resistance gene *Fhb1* from Sumai3 and different other FHB QTLs also from adapted wheat material, Drechslera Tan Spot (DTR) QTLs in adapted wheat varieties (Jenga, Solitär) and exotic material (HTRI 1410 and HTRI 3343) and several other resistance genes. Several of our breeders are also working in powdery mildew, rusts (yellow and brown rust) and Septoria.

While it is easy to map single genes as *Sbm1* it becomes more difficult to map QTLs due to their oligo- or polygenic inheritance and the effect of the environment on the phenotype. Nevertheless, by getting more and more markers and dense chromosome maps available and due to good phenotyping technologies (also by the use of doubled haploid lines) it becomes more and more possible to work also with QTL markers and their pyramiding, which is actively done in Fusarium Head Blight, Septoria, DTR etc.

By the use of molecular markers it is therefore possible to use and to exploit genetic diversity in an accelerated manner and therefore new varieties with new superior agronomic, quality

and resistance traits can be offered to the farmers. It will be also necessary to use molecular markers to be able to react faster with new genetic variability for drought tolerance, frost tolerance and other abiotic stresses, not to forget nutrient efficiency.

In the future the knowledge on genetic diversity will cover allele diversity and more exact and specific markers will be developed (also by genome sequencing projects). Haplotypes will become visible in more detail for the breeders and new genetic variability will be introgressed more easily, because the follow up and selection of polygenic traits will become more efficient and more cost effective. Therefore, molecular markers are useful tools for conventional and organic breeding programs. Both philosophies can benefit extremely by this technology, which is still at the beginning.

**Session III: Breeding for qualitative disease resistance in
potatoes to late blight**

Tracing resistance genes in potato by MAS in a professional breeding program ... in perspective of a commercial breeding program

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C. Meijer B.V. is a seedpotato company with an own breeding program. C. Meijer B.V. breeds potatoes for the table, French fry and crisp market and translates the customers' wishes into new potato varieties. Aspects such as quality, crop consistency and resistance are paramount.

Varieties as Lady Rosetta, Lady Claire, Lady Olympia, Melody and Lady Christl are the biggest varieties of its program. The organic variety Bionica recently has been introduced in the organic market. This variety has been bred in co-operation with hobby-breeder Niek Vos.

With that view molecular markers will be used as a tool in the breeding program. For C. Meijer B.V. the strength of markers will be:

- potential to follow and thus combine interesting genes in the genitor development
In the near future this tool will be used to stack resistance genes for late blight (several resistance sources), nematodes as PCN and Meloïdogyne spp. At the time more knowledge has been gained of the genetics behind complex traits, such as dry matter, maturity and quality markers can be used to follow and combine QTL's.
- Expand the breeding program
The use of markers can help to eliminate susceptible candidate varieties in an early stage of the selection; whereas in a traditional selection program some resistance tests can only be finished in a later stage of the selection program.
- Knowledge in-house
Having the DNA techniques in-house will force the company to retrieve all genetic information available to make choices within the program. This knowledge will not only be limited to specific marker use, but also will give more insight in potato genetics which can be used in more aspects of the breeding program.

The use of markers will lead to an improvement of the breeding program. Having resistance traits in good varieties without compromises to other characteristics will become less complex. However, adaptivity of potato towards different soil types, growing area's and climates still will be the next challenge. Drought, salt and climate shocks cause yield and quality loss in many growing area's, s. Genetics behind these characteristics seem complex. More insight and tools could help potato breeders to achieve bigger steps in breeding.

Coming decades most information on total value of a candidate variety will be retrieved from the (well chosen) trial locations. To mirror the complex "phenotypic" wish list of the potato chain on the candidate varieties, DNA-techniques will help more and more but potato breeders better not throw away their boots and field books...

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Organic potato breeding creates added value

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Some 16 years ago I started as a farmer-breeder in potato. The motivation was that organic potato fields yearly turned black because of late blight (*Phytophthora infestans*). Later also in the conventional potato production this devastating potato disease became barely manageable and could only be controlled by using large quantities of pesticides.

The situation in organic potato production struck me, and it appeared to me that such a situation was not positive PR for the organic sector. For me, there was only one answer to such an aggressive disease and that was resistance. Therefore my goal is to select for a potato resistant to phytophthora!

In the Dutch potato breeding company Meijer B.V. and especially in the person of its breeder Guus Heselmans I found the enthusiasm and the trust for a joint effort. What amazed me in the first year was the amount of resistance present in the breeding programme. I could observe this by the number of plants that was left over after first selection through visual negative mass selection, discarding all plants that got infected during the season. These numbers were not in correspondence with the results of Meijer's selection. This difference was caused by the fact that I gave first priority to late blight resistance, whereas the conventional market has first priority for yield (50-60 ton/ha) and can still compensate late blight susceptibility by (relatively cheap) chemical crop protectants.

My focus is thus on phytophthora resistance in the first place. I am able to conduct it in such a way, as I select under organic farming conditions having commercial organic potato production on my farm too. I accept a somewhat lower yield (35-40 ton./ha) and maybe in future less stringent requirements for external (visual) quality of the tubers.

In such a way specific selections get a chance that would not show up in a regular programme due to higher priority to other traits as they are already discarded before the selections can show their resistance. We also lose valuable genitors in such a programme.

In the presentation I will discuss the way I collaborate as an associated farmer-breeder in a commercial potato breeding programme, and how my first 'organic' variety Bionica was registered and marketed since 2007.

Session IV: Breeding for baking quality in wheat

Is heterogeneity an advantage or a disadvantage in breeding for baking quality in wheat?

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Background

A few years ago, in conventional high-input wheat production, particularly in the UK, the question of breeding for baking quality seemed to have come down to a, relatively, simple answer. The key was to ensure that a couple of high molecular weight glutenin sub-units were being expressed in the high yielding (as far as possible) pure line wheat selections, and all was well. More industrial feedstock for the Chorleywood industrial bread-making process (the 40 minute loaf) was then assured. The industrial chemists, producing improvers, extenders and other enzymes, together with industrially-produced yeast and other additives that had been extracted earlier from the flour during milling, could now work with the packaging and advertising experts to ensure that the supermarket shelves were never empty.

However, an increasing number of questions have arisen around this approach, partly as direct questions to do with the process itself and partly as indirect questions concerning the whole context in which wheat is produced.

a) Direct questions

These centre on both the process and the products. Many are concerned with health and nutrition, especially in a time of epidemic obesity and related health problems. Examples of such questions have been raised and collected, for instance, by Whitley (2006; see also <http://www.sustainweb.org/realbread/>). So far as breeding is concerned, they relate to many characters from uptake of minor elements, to protein qualities beyond specific glutenins, including the potential for effective fermentation.

b) Indirect questions

Indirect questions concerning wheat and quality are becoming more numerous largely due to the concerns about a world grappling with the new dimensions of post-Peak Oil and global climate change and destabilisation. There will undoubtedly be an increasingly closer focus on, for example, nutrient and nitrogen use efficiency, the ability to deal, genetically, with pathogens, pests and diseases, and how to approach the problem of unpredictable weather variation. All of these involve interactions with all of the elements of quality, whether defined in the narrow or broad sense.

Dealing with complexity

Before the focus turned on to the critical glutenin sub-units, bread-making quality in wheat had been considered to be a complex character, usually correlated negatively with yield. For the specific end-use of industrial bread-making, the importance of the glutenins allowed the breeders to concentrate on a relatively simple target for breeding, although the negative correlation with yield has persisted, because of the need to try to maximise protein levels. However, given the nature of the newly-raised questions, it seems most unlikely that a simple answer can be found again, even if the same industrial process for bread-making continues to

dominate. The new answers will need to be sought, increasingly, in the introduction of greater diversity together with a greater concern for selection of new varieties in the sites in which they will be grown.

Furthermore, particularly because of climate destabilisation, there will be an increasing need for the availability of different genotypes with opposing characteristics (e.g. the ability to grow well in dry/wet or hot/cold conditions) to be grown close together and to produce acceptable quality under these different conditions. This can be achieved by mixtures and populations.

Some experiences from comparison of pure lines, mixtures and populations

The background to the experimental series with pure lines, mixtures and composite cross populations of wheat will be described briefly in the presentation (Wolfe, 2009).

The twenty parents used in the trials varied for all of the pre- and post-harvest characters that were assessed. The variation was correlated for data obtained under organic and non-organic conditions although the r^2 values were often low. Indeed, there were many significant differences. The general tendency, as expected, was for modern varieties to respond well to non-organic management whereas older varieties did not.

Among the quality characters measured, those that were correlated positively with yield, such as Hagberg Falling Number, were correlated negatively with characters associated with grain protein content. Those characters that were correlated positively with grain protein (e.g. thousand grain weight, specific weight), were correlated negatively with grain yield. In other words, the distributions of variation among the varieties varied considerably among different characters. The problem here is that the characters that we looked at represented an arbitrary selection of a few measurable characteristics: we do not know how representative they are for any definition of bread-making quality in wheat. In this sense it is instructive to note that two independent millers who recently tested the populations for milling quality did so by baking bread from the milled samples, despite the apparent availability of a number of simpler test methods.

The mixtures and populations tended to give intermediate values for each of the measured values, often slightly higher than the mean, but, in the case of populations and Hagberg Falling Number, significantly worse. Interestingly, there was a tendency for the mixtures to perform slightly better than the populations under non-organic conditions, whereas the reverse was true under organic conditions. We believe this could be due to a greater frequency of redundant genotypes in the populations under the restricted conditions of the non-organic system.

Although the mixtures and populations tended to give intermediate values, there was clearly considerable variation within the plots of each. For the populations, visual inspection and some data, including molecular analysis (J Snape, personal communication), indicated that this variation includes transgressive segregation, that is, genotypes expressing characters to levels beyond those found among the parents.

As well as absolute measures of specific characters, we measured the stability (reliability; superiority) of the populations and mixtures compared with the pure stands. Again, for individual characters, the populations and mixtures tended to give intermediate values, although these values were more often better than average when compared with absolute

measures. Individual varieties often showed considerable variation, i.e. they were unreliable or unpredictable, particularly in relation to their performance in non-organic versus organic systems. As a note of caution, some of the apparent unreliability was due to location rather than system; this needs further analysis.

Inspection of a single character, such as yield, for absolute value together with reliability, might suggest that the mixtures and populations performed adequately, but not outstandingly. Most importantly, however, this conclusion requires two qualifications:

a) the performance of the mixtures and populations was predictably adequate, whereas that of each of the pure stands was generally less predictable,

b) if we examine more than one character, the performance of the mixtures and populations was predictable and reliable for each character added, relative to the pure stands. In other words, the overall performance of mixtures and populations under a range of environmental conditions, was notably better, and more predictable, than that of any of the pure varieties.

In this sense, it is important to keep in mind that such an even performance across environments is obtained by having a very large number of interacting genotypes within one space (as noted by Darwin from his own experiments in the *Origin of Species*). Moreover, the frequencies of these genotypes change dynamically in response to environmental conditions.

This approach to uniform performance across many environments is, of course, totally distinct from the current approach of using a single genotype and controlling the surrounding environment through application of a wide range of chemicals. This approach is not sustainable.

The trials described were designed to provide an opportunity to compare pure lines, mixtures and populations. However, in practice, this does not mean that we may not combine some of the advantages of each approach. For example, one or more varieties that are outstanding for particular quality characteristics could be mixed into a population with high potential for both quality and yield. This should provide a better insurance of high quality performance under a range of variable conditions. The composition of such a complex mixture could easily be changed for different locations.

If this approach of using complex mixtures and populations is developed, what useful role could molecular genetics play? There may be several roles – both fundamental and applied. For example, there is a need to develop a better understanding of the variation available in gene bank material – to be able to recognise, for example, a much wider range of genetic contributions to quality. At the more applied level, we need a better understanding of the question of redundancy in complex populations, for example, to help define the number of parents needed to form a population. Molecular analysis could also help in determining response to location – we know very little about the effects of geographical scale on population response: does this occur over a few centimetres, metres or kilometres – does it depend on the characters involved? Indeed, such questions are fundamental to our understanding of evolution as well as to the commercial performance of wheat.

So, despite the current view that purity of crop product is essential for high quality processing of wheat, I would argue that this is neither sustainable nor is it necessarily the best way to accumulate the many characteristics needed for producing different bread and bread products.

However, to make the necessary changes, there is much further work to be done, to which the tools of molecular analysis can provide valuable insights while accelerating progress.

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Application of markers when breeding for baking quality.

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SW Seed breeds winter wheat cultivars for Northern and Central Europe and spring wheat primarily for the Scandinavian market. Several breeding methods are used to bring quality wheat quicker to the market: Doubled haploids, single seed descent, breeding generations in Chile (spring wheat), and early generation selection with molecular - as well as other markers. The baking laboratory plays a key role for selection and quality testing throughout the breeding process.

Baking quality is considered one of the most important and challenging targets for wheat breeding. It is a complex trait composed of several characteristics but a major determinant of quality is protein content together with protein quality. Unfortunately there is a negative correlation between grain yield and protein content and the amount of protein in the flour is largely dependent on environmental conditions.

In the breeding process parental lines with baking quality are crossed with high yielding types. Not only high quality and high kernel yield are combined. Other important traits such as disease resistance and tolerance to abiotic stress are also incorporated. Since most agronomic traits are controlled by Quantitative Trait Loci (QTLs) and are under environmental influence, stability in yield and quality over different environment is extremely important. A better adaptability of a wheat cultivar to e.g. low water, low N, and tolerance to abiotic stress such as drought or cold means broadening the market.

At SW Seed, selection for baking quality is carried out by traditional methods in a baking laboratory in combination with protein marker analysis. A fully equipped baking laboratory in Svalöv performs analysis of grain and flour properties: volume weight and thousand kernel weight, kernel hardness, protein, Zeleny sedimentation, gluten quality and Hagberg falling number (HFN). HFN is an indirect measure of the alpha-amylase content, an unwanted feature for bread quality. The Mixograph mixes water and flour and is a quick and cheap method to measure time of dough development and stability of the dough. This method is used often in earlier generations and for testing wheat from different environments.

Dough strength and extensibility are measured in the Alveograph using fixed volumes of flour and water. In the Farinograph water is added to a fixed volume of flour and water absorption measured. Other technological properties measured by the Farinograph are the time of dough development and dough stability. Often the dough mixed in the Farinograph is further analyzed in the Extensograph which measures the mechanical resistance of the dough. These more detailed rheological analyses are performed in later generations in the breeding programs. In the last stage, the test baking, the texture and volume of the baked bread are measured.

Protein (gluten) concentration and composition are of major importance for baking quality. There is a strong positive relationship between grain gluten concentration and the texture and volume of the baked bread, and an increase in the protein content of flour from 10 to 14% results in an increase in loaf volume of almost 50%. The protein composition is of particular importance for bread-making quality and can be designed for different growing areas and baking processes. With decreased protein content due to low-N conditions, as is the case for

cultivars grown in our part of the world, the ideal protein pattern may differ compared to other parts of Europe. This makes protein (gluten) composition a very important breeding target.

The storage proteins glutenins and gliadins are the main components of gluten. Diversity of allelic composition of these monomeric and polymeric proteins correlates with differences in certain characteristics of dough physical properties – including flour and dough strength and dough extensibility. At SW Seed a half-seed method is used to select for important alleles in the high molecular weight (HMW) glutenins in very early generations as well as whole seed/several seeds methods are used in later generations. Such protein profiles are also useful to distinguish wheat cultivars and for purity assessment, parameters in the ‘Distinctiveness, Uniformity and Stability’ (DUS) test of new cultivars and protein profiles may support morphological DUS traits. Protein profiles are further useful for maintenance breeding to confirm ‘true to type’.

In the SW marker laboratory, a set of microsatellite (SSR) markers distributed over the wheat chromosomes are used to characterize wheat lines and cultivars to produce trees of genetic relatedness or dendrograms. Dendrograms helps breeders to get an overview of the genetic variation in a breeding program and to select parents for future crosses. DNA- and protein markers are used to breed for disease resistance and other traits in wheat. Both microsatellites (SSRs) and isoenzymes are used to select for eyespot disease resistance, and SSRs and other PCR based markers for powdery mildew and *Septoria* resistance. We are part of a consortium with John Innes Centre to develop markers to Hagberg falling number (pre- harvest sprouting and pre-mature alpha-amylase), and a consortium to develop markers to *Septoria* resistance. We use association mapping with Diversity Array Technologies (DArT) markers to improve winterhardiness and EFOS (‘Enzym Fordøjeligt Organisk Stof’, an energy analysis for feed quality) for Scandinavian conditions.

There are several markers for baking quality traits in the public domain. Some successful examples are HMW Glutenins and puroindolines. Genes for puroindoline are completely linked with grain hardness, a major determinant of flour yield. In particular, since these markers are gene-based they can be easily integrated in the breeding programs and when many wheat traits are under simultaneous marker assisted selection, the selection for baking quality can be performed with markers in a cost efficient manner.

Acknowledgement: SLF/Formas is acknowledged for financial support

Session V: Breeding for quality trait (taste) in tomato

Tomato breeding for taste by Oldendorfer Saatzucht

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Prerequisites and aims

As in conventional agriculture, also in organic horticulture tomatoes are of great economic importance. Breeding for organic agriculture aims at different cultivation methods related to different marketing strategies and farming conditions. Small growers approach the market directly, whereas larger growers offer their products to wholesalers' through two to three steps. For such conditions one needs firm tomatoes with a very long shelf life and a high yield, which requires a high wire system and a heated glasshouse. Medium large growers often grow their tomatoes in (unheated) plastic tunnels and in most cases sell their products either on-farm, or at the farmers' market or to a special trader. For this last category the breeding program in Oldendorfer Saatzucht is aiming at. Especially for this type of cultivation a good taste is an essential breeding goal. Further essential preconditions for being adapted to such cultivation system is a sufficient yield, good plant health, good fruit firmness and shelf life and low nutrient requirements.

Breeding program

At the start of the breeding program some real new longlife and semi-longlife types appeared on the market. The growth, yield and taste of a few older varieties propagated under organic conditions were satisfying, but had easy softening fruits and no *Cladosporium* resistance. In 1996 several modern varieties were crossed with some organically maintained varieties. The breeding lines were selected over nine generations for optimal homogeneity to obtain open pollinating varieties.

The genetic resources originated from the first crossings between varieties that have been bio-dynamically propagated over many years, from cocktail tomatoes and some hybrid varieties of the semi-longlife types of 1996. In the subsequent years the lines have partly been improved by single plant selection, and partly by constant new crossings. This included double and three way crossings in order to bring six parents together. The lines were improved through single plant selection. Besides, yearly extensive evaluation, tasting and characterisation have been conducted. Based on those data new single plants were selected. The lines are now partly in the F11 and are therefore homogeneous. The first varieties have been registered.

Selection for taste

The selection on taste is yearly conducted from the F1 onwards. Besides at least 2-3 plants per breeding line are being selected and marked based on the other criteria. From such plants fully ripened fruits are harvested, and vertically sliced and eaten by 3-4 persons. Besides taste, also the inner colour of the fruits, and greening and skin firmness was evaluated. A scale for 1-9 was applied, also for the taste. Next to this also aroma, acid-sugar balance, corny taste, etc. are described. When the fruits of the selected plants are not good enough, one explores further within a breeding line until both plants with the best fruits are found. To be able to choose between the both selected plants for next season's sowing, a good mark for taste has a high priority.

When homogeneity of the lines is achieved, the stage of registration of the variety is reached and the taste is more intensively compared with other varieties and lines.

Experiences with Pedigree Selection

The development of the single plant offspring did not occur linear. The in the F2 selected types were no longer present when the homogeneity became more apparent from the F7 onwards. Especially those lines which were very nice in the F2, developed in a negative way, whereas lines with small fruits and a chaotic growth showed a large potential of different types including beeftypes. Very often one could observe a depression in the F4-F5 for valuable traits. This holds also for taste. Approximately in the F6-F7 the line seems to have found its form and from thereon no more big changes occur. One can then not influence the change of the traits by selection. For instance, when at that time fruits are corny, one is very limited in correcting such trait through selection.

How can one develop a good taste in tomato?

First of all, a good taste has very many aspects and depends on the type of tomato. A cocktail tomato should mainly have a large proportion of sugar with sufficient acid, where a normal fruit type can contain less sugar but should have a 'tomato' aroma. Besides, the firmness (texture) of the flesh is important. Sometimes a 'green' additional flavour appears, related to a not complete ripening. Some lines already tend to taste well in the orange-red stage, whereas others develop the aroma only with full ripening. Slightly overripe fruits can have a slightly dull taste, but this does not always occur. Unripe, flamed fruits often have a better taste.

The ability to develop a good taste will not appear under all circumstances. The development of taste further depends on several conditions. This not only includes the fertilisation level, but also the type of fertilisation, e.g. differing for a rapid soluble organic manure or a ripe and good prepared cow manure compost. Also the soil type and soil conditions have an influence. Further more the climate plays an important role, and thus the tomato's taste differs per year. Phytophthora can spoil the taste, and also the water management is one of the determining factors. Too highly fertilised plants which tend to stay very 'watery', will not taste in later stages of the development, even though the variety has a good potential.

Conclusions

A good taste is a gift one receives when one can realise a balance between outer and inner (genetic) context of the plants. When plants can grow and be selected over generations under varying conditions, a stable good taste will perform that can manifest itself later during the cultivation also under less favourable conditions. From the wide range of phenotypical aspects one can surely state that the characteristics that are related to taste are polygene. Therefore a molecular marker assisted selection does not seem very appropriate. One might determine single aspects of a good taste, such as sugar content by molecular markers, but that is not something which cannot be achieved by conscious selection. A human being can perceive taste in a holistic way and this holds for a breeder as well as for a consumer. My concern is that when at certain stages in the selection process molecular information becomes dominant, the important view on plants as a whole will be blurred.

Application of MAS in tomato breeding programs for taste

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Introduction

Since the early XVI century tomato started spreading in Europe and fruit consumption gradually started in Southern Europe. Nowadays tomatoes are one of the most economically important vegetables, cultivated both for the fresh market and the processing industry. The focus of tomato breeding is on greenhouse tomatoes where the highest profits can be realized. In the 70's and 80's the main breeding aims were high yield and long shelf live but this shifted in the 90's towards improved taste and better nutritional quality.

Flavor is a complex and subjective trait. Flavor traits can be relatively easy to measure (e.g. soluble solids and pH) but can also be the consequence of complex interactions. The use of trained taste panels aims at reducing the subjectivity. Molecular markers have been extensively used to pinpoint those chromosomal regions important for the trait under investigation. After identifying the molecular markers the markers can be used in molecular assisted selection (MAS).

This presentation will focus on the use and developments of molecular markers for taste characteristics in tomato. Special emphasis will be on the marker development within the Centre for Biosystems Genomics initiative.

Outline

One of difficulties in tomato breeding is its limited genepool of tomato; this is caused by the tomato breeding history where several bottlenecks have resulted in the low level of genetic variation. This not only means that there is a low level of variation in traits but also in sequence differences. Several wild crossable wild species have enlarged the breeding genepool of tomato. The most important of these wild species are *S. pimpinellifolium*, *S. peruvianum*, *S. habrochaites*, *S. pennellii*, *S. neorickii* and *S. chmielewskii*. Over 60.000 accessions of cultivated and wild species of tomato are maintained in genebanks all over the world. Until now MAS in tomato has mainly been used for improving simply-inherited traits such as several disease resistances. There is one example of a Quantitative Trait Locus (QTL) for a taste characteristic from a wild species that is actually used in breeding programs. Fridman et al (2004) identified the (QTL) *Brix9-2-5* in an introgression line of *S. pennellii* in a *S. lycopersicum* background. The presence of this QTL increases the sugar yield of tomatoes. In The Netherlands, the Centre of Biosystems Genomics (CBSG: see www.cbbsg.nl for details) has started in 2003 a program for identifying chromosomal regions associated to organoleptic related characters (van Berloo et al. 2007). A set of 94 tomato cultivars was provided by plant breeding companies from their collections of current and historic germplasm. The set was selected to contain a high amount of diversity with regard to many tomato fruit quality aspects. All 94 cultivars were grown in two successive years and scored for metabolic fruit contents using liquid and gas chromatography combined with mass spectrometry (GCMS & LCMS; Tikunov et al. 2005) and other chemical traits such as dry matter content, soluble solids, pH and more). A trained taste panel quantified sensory attributes. Genetic fingerprinting resulted in 1200 polymorphic markers. Association mapping showed for a number of important quality traits clear associations with genomic markers. To further identify marker trait associations a prediction was made which parental lines of four hybrids harbored a maximum of diversity. A half diallel crossing scheme resulted in six F2 populations. These six populations were analyzed for the traits mentioned above and trait-marker associations were calculated. During this phase of the research it became more and

more obvious that the lack of DNA-polymorphisms was hindering the analysis more and more. There is a strong need for good marker coverage of the tomato genome and for good mapping populations. Recent developments have created several possibilities to look for the scarcely present sequence differences in tomato. High throughput genotyping will allow an efficient search for marker-trait associations. Recombinant Inbred Lines (RILs) have been made of a cross between two cherry tomatoes, between a cherry tomato and a round tomato and finally between two round tomatoes. Since RILs are homozygous they are an immortal source of genotypic identical plants which allows experiments with many repeats and in different environments.

The experiments described above will lead to a number of marker trait associations. New technologies will allow the detection of closely linked markers or potentially reducing linkage drag. Whether the possible changes in certain traits are important enough to select for depends of course on the potential added value to the end product (the new improved tomato cultivar). Breeding for taste will always be a difficult task; people have different taste sensations and different moods. The way the tomatoes are grown and their post-harvest handling (refrigerator!) also play a pivotal role in the final quality and taste. The presence of genes and QTLs sets only the bandwidth of some taste characteristics but are not a guarantee for a tasty tomato. Always nicely tasting tomatoes is only possible if besides a potentially good tasting tomato consumers realize that they have to make choices. The fact that only 15% in the decision to buy a specific tomato variety relates to taste.

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Session VI: Organic principles as seen from IFOAM

Principles of Organic Agriculture

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Introduction

The organic movement has been value-based from its very beginning. In the first half of the 20th century all founders of what is now called the organic movement were concerned about the development of agriculture at that time. Different schools of organic agriculture developed. With the expansion of organic agriculture in the 1970s and the development of different standards the need was felt for more co-operation. This led to the foundation in 1972 of the International Federation of Organic Agriculture Movements (IFOAM) by five organic agriculture organizations from South Africa, the USA and Europe.

Since that time the organic business has grown tremendously, and although still a small sector - worldwide 30.4 million hectares, which account for 0.65 percent of the global agricultural land (Willer *et al.*, 2008) – it is growing out of its niche and getting recognized increasingly. Challenges and opportunities accompany the continuous growth.

Organic standards so far prescribe positively the production and processing method; the (limits to) impact on the environment, or the way the produce is packed, transported and marketed are not regulated. Besides the regulation does not go into details e.g. whether specific techniques in breeding are allowed or encouraged. The phenomenon of genetically modified organisms is regulated in both private and public regulation in a negative way: it may not be used for the production and in processing organic products. It can be concluded that the expansion of the organic sector in a world that also develops and brings about new techniques offer challenges to organic agriculture.

Some people in the organic agriculture movement like the pioneers working in the sector over a long time express their unease about its globalized growth (Woodward *et al.*, 1996). They are worried that the values and motives from which the organic movement started are no longer the values of the growing movement today.

IFOAM has taken up this challenge in an attempt to bridge the values from the pioneers to the developments in globalization and harmonization, its extended membership, and also to new philosophical concepts and notions like 'ecological justice' (Alrøe *et al.* 2006). IFOAM came to the conclusion that the basic values, the fundamental underpinning of organic agriculture, needed reflection and discussion.

Approach to articulating and institutionalizing values

From the end of 2003 until September 2005, IFOAM and its members were engaged in the articulation of the principles of organic agriculture. By the very nature of its organization, i.e., a democratic federation, the process within IFOAM was conducted in a participatory manner and not in a top-down way. The process was finalized at the federation's general assembly in September 2005, when the 'Principles of Organic Agriculture' were adopted.

Historically, IFOAM has included a list of principal aims in a preamble to the IFOAM Basic Standards, where they served as an introduction to the Standards. They were written to clarify the aims of organic agriculture and were directly connected with the standards. Over time they were changed as new chapters were introduced, e.g. standards on organic processing. The principal aims pointed at a future perspective, at the goal of organic farming, the horizon, and the reason why one becomes involved in organic farming.

In March 2003 the IFOAM World Board formulated IFOAM's mission as:
"Leading, uniting and assisting the organic movement in its full diversity. IFOAM's goal is the worldwide adoption of ecologically, socially and economically sound systems that are

based on the principles of organic agriculture.” IFOAM’s mission statement and goal refer to the principles of organic agriculture. In order to move on from this general statement to tangible outcomes, it was necessary to enter into details as to what is meant by these principles.

To organize a truly worldwide participatory process, as many as possible different voices from inside and outside the sector must be heard, reflecting different points of view, perspectives and settings. A task force of 8 persons and a consultative group of over 40 persons spearheaded the review of the principles. Participants were recruited for both groups, taking into account diversity in background, region, gender and history in the organic movement.

Method, scope and purpose

To set the scope for the work, in January 2004, IFOAM’s world board formulated terms of reference and a preamble and gave directions to the task force for the final result:

- An independent document, no longer directly connected with the IFOAM basic standards. This direction meant to increase the meaning of the principles. The application should be broader than only for standards. So the principles should be decoupled from the standards and be introduced for all organic agriculture, e.g. in informal settings, in policy making and advocacy.
- A slight change in point of view from ‘principal aims’ to ‘principles’. Where principal aims point at a future perspective, a horizon, the principles should reflect the basis, the ground on which organic agriculture stands. It is the starting point from which organic agriculture can develop. However, in practice the change in perspective is not as huge as theoretically described here. Respondents in the process expressed that the principles not only reflect their personal attitude, but they wish the principles to be a future perspective and vision for the world at large.
- A balance between ‘clear and short’ and ‘complete and holistic’. The principles should be a short and clear description of the values of organic agriculture, easily to convey to outsiders and used for describing in a concise manner what organic agriculture is about. At the same time, the principles should do justice to all different settings, in which organic agriculture is practiced worldwide, where different values are important, depending on the development of the sector and the cultural, social and economic context. The principles should not reflect one dominant view or regional perspective, but be inclusive and mirror the thoughts of the global organic movement.

With these challenging directions given, the work could begin. The task force sent out a first questionnaire to the consultative group about the purpose, function and form of the principles of organic agriculture.

The feedback resulting from the first questionnaire summarized that the principles

- are to be the foundation and framework of organic agriculture;
- will lead and unite the organic movement;
- give guidance (in standards, policies, in general) and inspiration (internally for the movement, externally for change);
- should be universal and are regionally applicable;
- should provide identity;
- should be simple and ethically normative.

Thematic areas and first articulations

A second questionnaire asked the members of the consultative group for input on ‘thematic areas’ on which the principles needed to be developed. The task force considered the input of the consultative group, grouped the input and identified the following overall themes: ‘holistic

health', 'livelihood – equity', 'biodiversity', 'soil', 'cyclical systems', 'animals', 'local markets / accessibility', and 'precautionary principle'.

The third round of consultations elaborated further on this and tested a first rough draft. From the eight themes that were initially identified, 'local markets / accessibility' and 'biodiversity' were not linked to a separate principle, but were considered to be a part of other principles. So six principles were articulated with first wording and were presented for feedback to the consultative group.

The task force on the review of principles of organic agriculture processed the input into a second draft. In order to be more inclusive the draft was translated into French and Spanish. This draft reduced the number of principles to four. 'Animals' and 'livelihood – equity' were both thought to be part of the more overarching principle of fairness. 'Soil' was considered to be a crosscutting theme and 'cyclical systems' was changed into 'ecology'. This fourth round of consultation was sent out to all IFOAM members and provided for a response time of almost two months, giving the respective member organizations the possibility to discuss the draft at their local or regional meetings. Also external stakeholders, like civil servants involved in government regulations, were invited to give feedback.

In the next step, the task force on the review of the principles of organic agriculture studied the comments and took due consideration of the suggestions. All feedback from internal and external stakeholders has been made publicly available, as well as the analysis and response of the task force.

Final draft and approval

The final recommendation for the review of the principles of organic agriculture was submitted to the IFOAM world board, and included a response to the internal and external feedback and a rationale of the task force for its recommendation. In its meeting of June 2005 the world board decided on the motion and wording for the principles of organic agriculture to be put forward to the IFOAM general assembly of September 2005 at Adelaide, Australia.

During an interactive session at the IFOAM general assembly, the so-called motion bazaar, in which more than 50 representatives of member organizations participated, 26 amendments were suggested to the world board for wording. The board considered nine of them to be an improvement of the text. Seventeen amendments that the board did not approve were voted upon, of which the two following were accepted:

To include 'food sovereignty' in the explanation of the 'Principle of Fairness'. The notion of 'food sovereignty' expresses, according to the submitters, the right of peoples to decide on their own food systems and food values, and the right to produce their own food. This notion can be understood as a reaction to the globalized trade in basic food commodities, which often destroys local market dynamics. By amending the proposed principles with 'food sovereignty' the submitters wanted to express that organic agriculture plays a role in stabilizing local markets and positively contributes to local community development.

To include 'indigenous knowledge' in the explanation of the 'Principle of Care'. By including 'indigenous knowledge' agricultural habits from different cultures that often have proven to be sustainable over thousands of years are respected. It adds to 'traditional knowledge' as it points to those cultures that are currently considered to be minorities and are in some cases even under threat of disappearance.

Finally, the IFOAM general assembly of September 2005 approved the amended principles of organic agriculture.

The Principles of Organic Agriculture

Principle of Health. Organic Agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible.

Principle of Ecology. Organic Agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.

Principle of Fairness. Organic Agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities.

Principle of Care. Organic Agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

From Principles to Breeding Standards

The IFOAM Basic Standards contain a section on Draft Standards. Draft Standards are intended to be elevated to full standards. They are also intended to guide standard setting organizations in developing their own regionally adapted standards. Since 2002 Draft Plant Breeding Standards were published as a consequence of initiatives taken by member organizations, (later described by Lammerts van Bueren and Struik, 2004 and Verhoog *et al.*, 2007), supported by the General Assembly.

The General Principle of the Draft Standard is formulated as: ‘Organic plant breeding and variety development is sustainable, enhances genetic diversity and relies on natural reproductive ability. Organic plant breeding is a holistic approach that respects natural crossing barriers and is based on fertile plants that can establish a viable relationship with the living soil. Organic varieties are obtained by an organic plant breeding program. The objectives of organic plant breeding are to maintain and further diversify organic production.’

The Draft Standard holds recommendations: ‘plant breeders should use breeding methods that are suitable for organic farming. All multiplication practices should be under certified organic management. Breeding methods and materials should minimize depletion of natural resources.’

Based on the General Principle and Recommendations, requirements for standards are formulated, containing suitable breeding techniques.

Since the acceptance of the Breeding Standards as Draft in the IFOAM norms, IFOAM revised its norms system, concluding that the existing standards should be brought more to a level of ‘standards for standards’ i.e. a framework for standard setting bodies, rather than direct certification standards. The Draft Breeding Standards however are, in contrast, intended to be direct certifiable standards.

The IFOAM General Assembly in 2008 therefore adopted the following motion: ‘To complete work on the draft plant breeding standards as soon as possible with the view of adopting them as IFOAM (certification) standards.’ With this motion the organization is put to work to finalize the draft plant breeding standards into a full, voluntary, certification standards, minding the code of good practice of standard setting bodies as agreed within the ISEAL alliance, implying due stakeholder involvement.

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Poster presentations

Genetic variation in inducibility of resistance in tomatoes against *Phytophthora infestans* and the influence of biofertilisers and plant strengtheners.

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There are numerous instances in which induced plant resistance responses (IR) have been demonstrated. However, before IR can be made use of in practice it is important to understand as much as possible the ecology and genetics of the inducing agents and their interactions with plants and pathogens. Effects of host genetic background were tested with thirteen tomato varieties and two isolates of *Phytophthora infestans*. Isolate effects on inducibility were tested with six varieties and six pathogen isolates. Leaf disks of plants that had been treated with BABA (DL-3-aminobutyric acid) or water were inoculated seven days later with 20 µl sporangial solutions of 5×10^4 sporangia ml⁻¹. All experiments were repeated three times with six replications each. Disease reductions due to induction ranged from 43 to 100% and were independent of the susceptibility of the variety to the isolates when not induced. The interactions between isolate and variety with respect to inducibility were highly significant.

In a subsequent series of trials, three biofertilizers (BF) and three plant strengtheners (PS) were tested in comparison to chemical fertilizer application and BABA (DL-3-amino-n-butanoic acid), respectively for their effects on the reactions of six different tomato varieties against three isolates of *Phytophthora infestans*. All experiments were repeated twice with six replicates each. Two of the BF (BioIlsa and Biofeed Quality) significantly reduced late blight severity as compared to horn meal and chemical fertilizer with no fertilizer by isolate and fertilizer by variety interactions. All PS significantly reduced susceptibility of all tomato varieties. The combined effects of PS and BF were additive without interactions. However, the interactions among PS, variety and isolates were highly significant suggesting that different resistance mechanisms might be triggered by the PS and BABA. If this is the case then it might be very useful to identify molecular markers associated with the different mechanisms involved in inducibility of resistance for efficient selection and combination of these traits.

SSR based study of grapevine varieties of Carpathian basin and Hungarian origin

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Summary

Grapevine is very rich in varieties, the number of varieties can be estimated at 10,000, many of them are cultivated in the world. During the history of viticulture many varieties have been formed by selecting the naturally occurring genotypes or by deliberate crosses. From the gene centre of *Vitis vinifera* in Trans-Caucasia viticulture dispersed in the Mediterranean Basin, Europe, Asia, Africa and later in America and Australia. Commerce, migrations caused not only the blending of human, but the cultivated plant populations, too. As a consequence grapevine cultivars partly derive from the selection of local or geographically distant crosses. DNA genotyping may result in unexpected facts as for the origin of the cultivars. Not only the proximate, but the second- or third degree relationships can also be determined on the basis of DNA fingerprints. Therefore it is very important, that more and more molecular marker data should be available - beside popular international cultivars - for the local, regional varieties or genebank accessions. One of the objectives of grape genomic researches in the Institute of Viticulture and Enology and in the Institute of Genetics and Biotechnology to characterize with microsatellite fingerprints the grapevine varieties, autochthonous in the Carpathian Basin and the cultivars produced since the 19th century in Hungary.

Mikrosatellite or SSR (Simple Sequence Repeats) fingerprints have become efficient tools for characterizing the grapevine cultivars. In our present study varieties autochthonous in the Carpathian Basin, cultivars bred in Hungary, grapevines (*Vitis vinifera* L.) of various geographic origin were characterized at 12 microsatellite loci. Based on allele size results of 115 varieties the Hungarian Vitis Microsatellite/SSR Database has been established. For sake of comparison international cultivars such as Chardonnay, Pinot noir, Merlot, Heunisch weiss were also included into the analyses. The allele size data obtained can help in determining the genetic distances between the cultivars; tracing down pedigrees of the varieties; discovery of primary and secondary relationships between grapevine cultivars. The database can be reached on the homepage of the Institute of Genetics and Biotechnology, Szent István University, Gödöllő (Address: <http://www.mkk.szie.hu/dep/gent/>).

Research is supported by the Hungarian Scientific Research Fund (OTKA K62535, M36630, M45633) and the GrapeGen06 EU Projekt.

Possible role of the old Hungarian wheat varieties in Fusarium head blight resistance breeding

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Fusarium head blight (FHB) caused by various *Fusarium* species is a widely investigated biotic stress factor throughout the world. Until the 1970s the disease was only reported sporadically in Hungary, but as the old Hungarian varieties were replaced by genotypes suitable for intensive production technologies, nation-wide epidemics began to occur. The aim of the experiments was to detect and analyse the genetic factors responsible for the FHB resistance of old Hungarian wheat varieties. The resistant 'BKT9086-95' line developed from the variety 'Bánkúti 1201' was crossed with the moderately FHB-resistant variety 'Mv Magvas' to create a single seed descent population for the purpose of studying the genetic background of resistance. Based on the results of the artificial inoculation, 15 resistant and 15 susceptible genotypes were selected for the purpose of bulked segregant analysis. The bulk samples and the parents were analysed using the amplified fragment length polymorphism (AFLP) method. The two bulk samples and the parents were tested with a total of 81 primer combinations, and an average of 5.02 deviations per reaction was found between the parents. AFLP patterns similar to that of the resistant parent were found in a further 16 cases. On the basis of the BSA results the testing of the whole population using the AFLP and simple sequence repeat methods has been commenced.

Perspectives of MAS in barley breeding program for organic farming

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Research on breeding for organic farming has been recently started at Priekuli PBS with the aim to investigate the role of growing conditions, the most effective selection criteria and to contribute identification of useful molecular markers. Barley breeding work exclusively in organic growing conditions and partly in conventional and organic conditions is done in small amounts at the institute. Usefulness of molecular methods is currently considered and first steps are made.

In our breeding program marker-assisted selection (MAS) would be useful mostly for traits, determination of which is time consuming and expensive. It could be markers for resistance genes to diseases, which occur naturally not very often or are expected to cause problems in future, and for which artificial infection is complicated to carry out e.g. loose smut and Fusarium Head Blight. Worthwhile could be also the possibility to use molecular markers for complex traits significant for organic farming, like yield stability and nutrient use efficiency, determination of which requires a lot of field trials and measurements, impossible to carry out in regular breeding program.

Barley loose smut (*Ustilago nuda*) can be risky disease for organic farming, especially for seed production. The best way is to grow completely resistant varieties to this disease, which means, that major resistance genes are required and partial resistance could be used only as addition to it. Testing of resistance to loose smut in our breeding program is traditionally carried out by artificial inoculation with local disease spore suspension during flowering. Each flower in 3 spikes per breeding line is inoculated by syringe. The resistant lines can be identified only one year later when plants grown from the infected seeds are flowering. Inoculation of resistant lines is repeated, to approve the resistance. In addition to field tests for loose smut resistance we are currently testing a PCR-based molecular marker for *Un8* gene (Eckstein et al., 2002) for deployment in MAS in our breeding program. The first results were inconsistent and comparison of phenotypic and molecular results will be repeated. Using of MAS would give two advantages: determination of resistant plants will be possible in the same year, even in seedling stage and it will be possible to recognize heterozygous plants or lines. Our research plans in future comprise QTL mapping and identification of useful molecular markers of barley traits for breeding for organic farming: yield stability, traits connected to weed suppression ability, nutrient use efficiency etc.

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Index selection in organic and non-organic trials under various dispersion structures

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Mixed models and index selection theory (Falconer and Mackay 1996) are frequently used as tools for the analysis of plant breeding and variety experiments. Recently, they have been applied in the analysis of a large collection of data resulting from field trials performed in different environments (sites or years) under organic and non-organic cropping systems in several European countries (Przystalski et al., 2008). The main objective of the analysis was to see if the rankings of genotypes tested in the two systems are different and if testing in organic conditions is providing significant information about their performance. For this aim, the estimated variance and covariance components, based on a mixed model, were interpreted in terms of correlation and selection parameters useful for the breeder's decisions. The purpose of the study reported here was to see how to extend the model and the data analysis to the situation where similar questions are asked, but the answer should take into account a more complicated structure of the dispersion matrix of the random effects. The need for such an extension, dictated by different characteristics of the organic and non-organic trials, was suggested by the breeders. We show how the extended covariance structure of the model influences the obtained selection indices and inference.

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Molecular markers to select for natural late blight resistance

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To breed potato cultivars with durable late blight resistance (LBR), a wide genetic base, a pyramid of several resistance genes that employ diverse mechanisms to bring about resistance, and the use of R genes that are not broken down in the area are desirable attributes. Two tetraploid accessions held at the International Potato Center, MF-II (M; group tuberosum) and TPS67 (T; group andigena), were selected for their reportedly high resistance and yield levels across a wide range of production zones, that would facilitate their use in varietal breeding. MT cross progenies were subjected to LBR phenotyping and genotyping via detached leaflet tests with *P. infestans* isolates differing for pathotype composition. Segregation of LBR revealed both accessions possess an individual dominant R gene in simplex (*Rpi-tbrM1* in M and *Rpi-adgT1* in T). These genes are different from all 11 *S. demissum* R genes as represented by the set of R gene differentials. The phenotype of *Rpi-tbrM1* akin that of *R1* as was determined in the detached leaflet tests, but the original *R1* allele as isolated by Ballvora et al. (2002) from chromosome V is absent from the genome of M.

Parental genetic framework maps were constructed using consensus Solanum COSII, CAPS, and SSR markers, and the novel R genes were located on chromosomes XI (M) and IV (T).

The development of PCR markers for high-throughput selection for the two R genes is in progress.

The major results of this research include:

- Two novel R genes conferring resistance to late blight were found,
- The residual resistance effect when these R genes are broken down by local strains of *P. infestans* significantly contributes to the resistance in the field, and
- Pyramiding of these two R genes can increase the crop's health level.

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Marker assisted selection for powdery and downy mildew resistance genes of different origin in grapevine (*Vitis vinifera* L.)

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Abstract

Production of varieties carrying durable resistance is the main goal of resistance breeding. Resistance can be controlled either by one gene or can be the result of several genes. Breeding in viticulture aims at producing cultivars resistant to the most spread fungal pathogens: powdery (PM) and downy mildew (DM). Since no *Vitis vinifera* cultivars carrying PM resistance genes were found till the mid '60-ies, wild *Vitis* species were applied as resistance gene sources. *Muscadinia rotundifolia* is an excellent gene source carrying the *Run1* dominant PM and the *Rpv1* major DM resistance genes. A (*M. rotundifolia* x *V. vinifera*) BC₄ hybrid of French origin has been applied since 1996 in crosses with *V. vinifera* cultivars. However the *V. vinifera* cultivars is classified as susceptible, different cultivars show varying levels of susceptibility. 'Kishmish vatkana' was described as PM resistant *V. vinifera* cultivar, and was involved in breeding (Kozma et al. 2006). Dominant PM resistance gene of 'Kishmish vatkana' was named *Ren1*. For pyramiding the three mildew resistance genes 'Kishmish vatkana' was crossed with the BC₄. Our goal was to apply multiplex PCR for the simultaneous screening for *Run1*+/*Ren1*+/*Rpv1*+ genotypes in segregating BC₅ population deriving from the cross of BC₄ x 'Kishmish vatkana'.

Pyramiding PM and DM resistance genes by the use of molecular markers was investigated in F1 progeny derived from the cross of VHR 3082-1-42 x Kishmish vatkana. 30 sensitive (according to powdery mildew symptoms on leaves) and 808 symptomless genotypes were screened with SSR and BAC-clone derived (CB) primers.

Microsatellite primer pairs were labelled with Cy5 fluorescent dye and fragments were analyzed by ALFExpress (Automatic Laser Fluorometer). PCR of the CB were separated on 1.5 % agarose gel. Multiplex PCR products were separated on 8% polyacrilamide (ALFExpress) and 4% Metaphor gel.

SSR markers were used to select the *Run1*+/*Rpv1*+/*Ren1*+ individuals containing all the three resistance genes. All of the *Ren1* linked SSR primers gave the same results, so we can distinguish the *Ren1*+ and *Ren1*- genotypes. CB and VMC8g9 primers can also distinct the resistant *Run1*+ and *Run1*- samples. VMC1g3.2 is appropriate for selecting the DM resistant BC₅ progeny only in the case of genotypes homozygous for the resistance allele of BC₄ because the same allele sizes were found both in BC₄ and Kishmish vatkana. We need to test more SSR primers linked to *Rpv1*.

According to our results the *Run1*+ genotypes are *Rpv1*+ also, which proves the tight linkage of these two markers, with the exception of two samples which are *Run1*+ and *Rpv1*-. Among the 808 symptomless individuals we found: 246 *Run1*+ and *Ren1*+; 199 *Run1*+/*Ren1*-; 301 *Run1*-/*Ren1*+ and 62 *Run1*-/*Ren1*-.

Products of the multiplex PCR were separated on 4% Metaphor, too. The advantage of this method is that there is no need of expensive fluorescent labelling and ALF machine. Marker assisted selection is unavoidable for selecting *Run1*+/*Ren1*+ genotypes due to the same phenotypic effects.

Research is supported by the Hungarian Scientific Research Fund (OTKA K62535, PD 72424).

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