# Proceedings of the COST SUSVAR/ECO-PB Workshop on Organic Plant Breeding Strategies and the Use of Molecular Markers

17 – 19 January 2005
Driebergen, The Netherlands

Edited by E.T. Lammerts van Bueren, I. Goldringer, H. Østergård

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SUSVAR
COST 860

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European
Consortium for
Organic Plant Breeding

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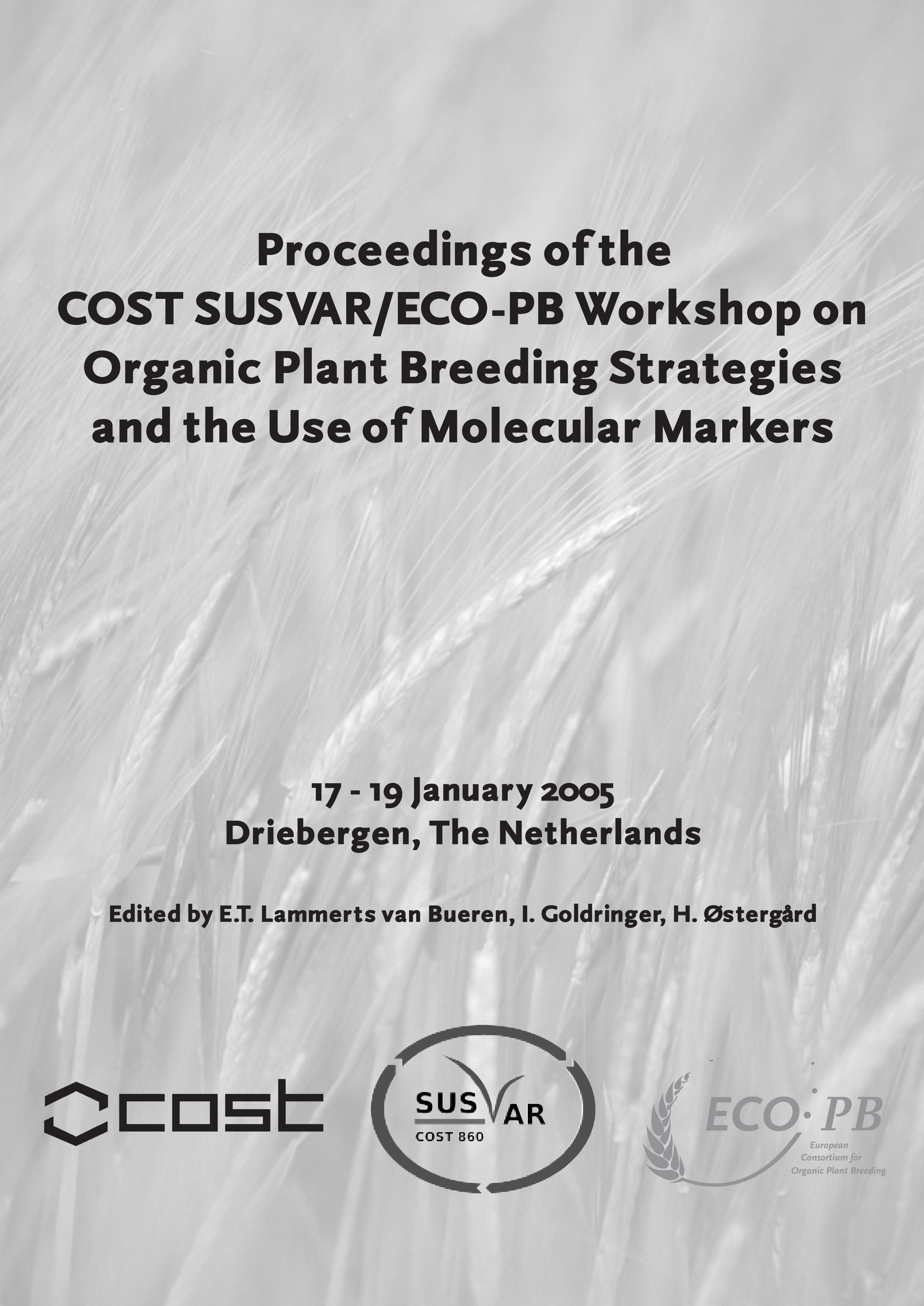

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# Preface

This workshop has been organised as an activity in the SUSVAR (COST 860) network in cooperation with the European Consortium for Organic Plant Breeding (ECO-PB), the Working Group 3 of COST 851. COST has financed these proceedings.
The aim of this workshop is to exchange results and facilitate the discussion on different views on breeding strategies for varieties better adapted to organic cereal production, with special focus on the question whether and how molecular markers can be of benefit for organic breeding programmes. The focus of this workshop is on cereals with a few examples from other crops.

COST is an intergovernmental framework for European co-operation in the field of scientific and technical research, allowing the co-ordination of nationally funded research on a European level. COST Actions cover basic and pre-competitive research as well as activities of public utility.

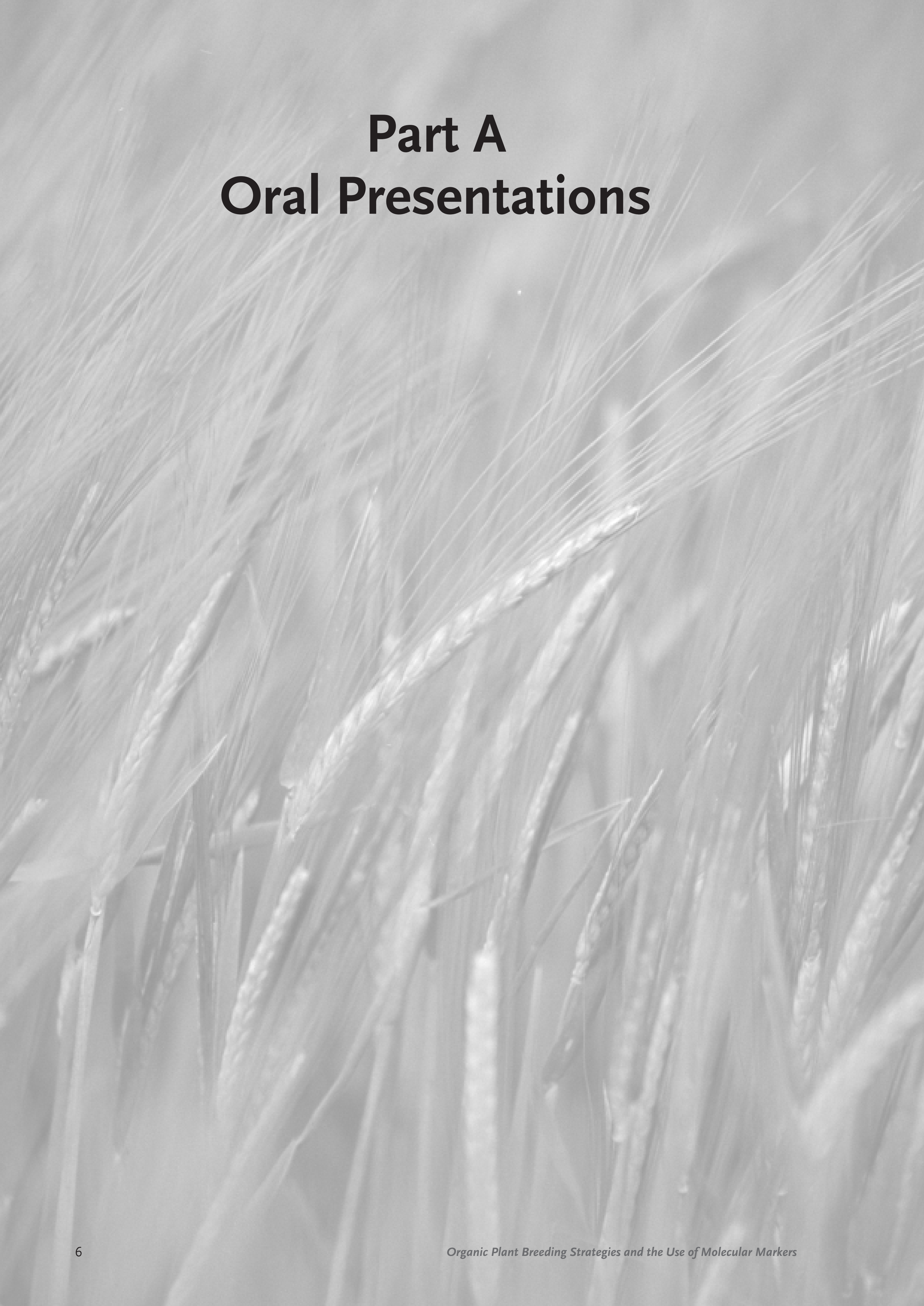
In March 2004, the COST 860 network 'Sustainable low-input cereal production: required varietal characteristics and crop diversity' (SUSVAR) was initiated. By January 2005, 24 European countries had signed the Memorandum of Understanding, the official document defining the network, and researchers from about 100 institutions had started co-operating (SUSVAR homepage: **www.cost860.dk**).

The main aims of the network are to ensure stable and acceptable yields of good quality for low-input, especially organic, cereal production in Europe. This will be achieved by developing ways to increase and make use of crop diversity (e.g. variety mixtures, crop populations or intercropping) and by establishing methods for selecting varieties, lines and populations with special emphasis on the influence of genotype-environment interactions. Finally, the network will also establish common appropriate methodology for variety testing in the context of low-input and/or organic agriculture.

Cereals are an important contribution to food production and the economy in Europe. Reduced inputs of pesticides and chemical fertilisers are universally of great interest, and increasing the area grown under organic conditions receives much public support. For the last 50 years, cereals have been specifically developed to produce high yields under potentially unlimited use of pesticides and synthetic fertilisers. These inputs are therefore necessary to achieve optimal yields independent of the actual conditions in the farmer's field. As a result, the presently available crops and varieties may not be the best to ensure stable and acceptable yields under low-input conditions.

In many countries, national projects are in progress to investigate the sustainable low-input approach. In the present COST network, these projects are coordinated by means of exchange of materials, establishing common methods for assessment and statistical analyses and by combining national experimental results. The common framework is cereal production in low-input sustainable systems with emphasis on crop diversity. The network is organised into six Working Groups, five focusing on specific research areas and one focusing on the practical application of the research results for variety testing: 1) plant genetics and plant breeding, 2) biostatistics, 3) plant nutrition and soil microbiology, 4) weed biology and plant competition, 5) plant pathology and plant disease resistance biology and 6) variety testing and certification. It is essential that scientists from many disciplines work together to investigate the complex interactions between the crop and its environment, in order to be able to exploit the natural regulatory mechanisms of different agricultural systems for stabilising and increasing yield and quality. The results of this cooperation will contribute to commercial plant breeding as well as official variety testing, when participants from these areas disperse the knowledge achieved through the EU COST Action.

Hanne Østergård, Risø National Laboratory, Denmark
Chair of SUSVAR



# Part A
# Oral Presentations

# Organic values and the use of marker technology in organic plant breeding

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## Abstract

Recently there is an upsurge of interest in the values or basic principles underlying organic agriculture. One of the reasons for this upsurge is the fear that, with the extension of organic agriculture, the values of the founders will erode. And values also play an important role in deciding which techniques are acceptable and which not. With respect to the rejection of genetic engineering three values play an important role: the precautionary principle (combined with the holistic principle), the value of naturalness and the integrity principle. Discussion is still going on about the use of marker technology. At the end of the paper the possible implication of these values for the use of marker technology will be discussed.

## Keywords

Organic values, genetic engineering, precaution, naturalness, holism.

## The importance of values

In Europe much discussion is going on at the moment about ‘norms and values’, and the basic ethical values shared in European (western) culture. Behind this is the feeling or fear, that important values get lost, or lose their force in the regulation of individual human behaviour. This decline of the role of values may be due to several factors: the secularisation of modern societies, the rise of multicultural societies, and the increasing emancipation of individuals (individualisation). Because of these developments it looks as if we have to find new (democratic, bottom up) ways of dealing with values; they can no longer be imposed upon from above.

To some extent this also holds for the organic movement. The founders of the organic movement shared a number of basic values (Woodward, 2002) and this was important in the process of establishing an alternative for conventional farming, especially in the pioneering phase. Many older organic farmers still hold on to these values. We now seem to come into a stage in which organic farming comes out of the pioneering phase into a more established phase. It is more or less accepted in society and stimulated by governments as an innovative form of sustainable agriculture. We see many new converters, and organic products are now sold in the supermarket. The production and marketing of organic goods have become subject to the forces of the free market system. This is described by Pollan (2001) as the rise of an ‘organic-industrial complex’. Within the free market system, organic just becomes an economic niche for getting profits.

We hear more and more voices warning that the organic values erode, and that organic farming will be making the same mistakes as did conventional farming in the past. We find this concern underlying a recent paper by Alrøe & Kristensen (2004) from DARCOF, in which they mention several reasons why it is important to establish normative values or principles for organic agriculture:

- To resist unwanted developments
- To support the development and extension of organic agriculture into new areas
- To plan proactive research
- To discuss about the organic rules.

In this list we see that values not only have a limiting effect (to resist unwanted developments, such as genetic engineering). They are also important in giving guidance as to what is ‘really organic’, and this can stimulate producers and others to take next steps in the development of organic agriculture. And to do research for organic agriculture you need some vision as to where we want to go with organic. It makes a difference for the research you do, if you think that organic is the same as conventional agriculture, but without chemical substances and GMOs, or if you think that organic refers to a systemic (holistic) way of production, adapted to the ecosystem at a certain place (using ecological criteria), or to a whole way of life in which respect for (the integrity of) nature and humans is crucial. For the distinction between these three ways of looking at organic agriculture, see Verhoog *et al.* (2003).

The last reason mentioned in the paper by Alrøe & Kristensen refers to the difference between norms (rules) and values. In the context of organic agriculture the rules and norms refer to what is laid down in formal regulations such as laws, usually saying what is not allowed. Laws are imposed from above, after a (democratic) process of political deliberation. We could say that laws are 'frozen ethics', and are usually followed up by all kinds of more specific rules. Ultimately these rules are based on certain ethical values, which always have more content than the rule itself. Take a value such as respect for animals. This value may lead to certain rules as how to deal with animals, but these rules can best be considered as a certain interpretation of the value, which is bound to a specific historical time and space. Very often it is a compromise. Therefore, the last reason for dealing with the issue of values is to renew discussions about certain rules, or to allow more than one interpretation if there are good reasons for doing that.

Discussions about values must involve all stakeholders, must be powerfree, must deal with rules established in the past, must help with the evaluation of practices developing in the present, and must give guidance to future development. Values not only have a cognitive element (such as a holistic theory of life), they also refer to human feelings (such as basic emotive attitudes towards nature) and they have consequences for human action (what to do and not to do) (Verhoog *et al.*, 2003).

## Three basic principles (values) of organic agriculture

We have seen that values can help 'to resist unwanted developments'. One field in which this is applied is the field of modern (bio)technologies. One modern application of biotechnology, the technique of genetic engineering has been rejected in the organic movement from the very beginning. So, it may be good to first look at the reasons (and values) put forward within the movement against genetic engineering, and then see whether these also apply to a development such as marker technology.

The International Federation of Organic Agriculture Movements (IFOAM, 2002) says in a Position Paper that it is opposed to genetic engineering in agriculture, in view of the unprecedented danger it represents for the entire biosphere and the particular economic and environmental risks it poses for organic producers. IFOAM believes that genetic engineering in agriculture causes, or may cause:

- Negative and irreversible environmental impacts
- Release of organisms which have never before existed in nature and which cannot be recalled
- Pollution of the gene-pool of cultivated crops, micro-organisms and animals
- Pollution of off-farm organisms
- Denial of free choice, both for farmers and consumers
- Violation of farmers' fundamental property rights and endangerment of their economic independence
- Practices which are incompatible with the principles of sustainable agriculture
- Unacceptable threats to human health

Therefore, IFOAM calls for a ban on GMOs in all agriculture.

Most of these objections are not formulated in terms of values which are threatened but in terms of the consequences of the technology (emphasis on risks). Exceptions are the references to free choice and to the principles of organic agriculture. But what these principles are, is not made explicit. In this paper an attempt is made to reformulate the IFOAM objections in terms of values and principles. The issue of free choice and other socio-ethical concerns, although extremely important, are left out here. It can be argued that to defend the basic human freedom not to be forced to grow or eat GMOs, which is at the heart of the issue of co-existence, one must rely on the basic principles or values of the organic movement. One can be against GMOs on principle, even if the risks can be controlled in practice. It will be hard for IFOAM to prove scientifically that GMOs *in general* cause 'unacceptable threats to human health' or 'irreversible environmental impacts' (Verhoog, 2004).

### The precautionary principle (the holistic principle)

Objections to the use of genetic engineering in terms of the consequences are sometimes called 'extrinsic' concerns, and concerns in terms of the technology itself 'intrinsic concerns' (Verhoog, 2001). In the literature about ethics and biotechnology in agriculture, intrinsic concerns refer to objections such as that genetic engineering is playing God, is unnatural, is violating the integrity of organisms. Mostly, the intrinsic concerns are more difficult to defend and are taken less seriously by scientists, ethicists, policy makers, than the extrinsic concerns in terms of risks. This is mainly due to the dominance of the thinking of natural science in our society (Verhoog, 2003). With that background, intrinsic concerns are said to be irrational, emotional, based on secular beliefs, etc.

These intrinsic concerns also play an important role in organic farming. Making them explicit can give more insight in what is going on in public debates as well.

In organic agriculture the discussion about risks is transformed from an extrinsic into an intrinsic issue. This can be illustrated with the application of the so-called 'precautionary principle' in a publication by DARCOF (2000). This principle is put forward to explain the ban on pesticides and GMOs in organic farming:

*"The rationale behind the precautionary principle is that in organic farming the interaction between Nature and Man is an important ingredient of the philosophy...Organic farming builds on the concept that Nature is an integrated whole that people have a moral duty to respect, both for its intrinsic value and because, by using its regulatory mechanisms, one can establish a more self-sustaining agro-ecosystem. Nature is a very complex, coherent system, of which Man has often little understanding to appreciate the consequences of specific actions. Damage to Nature and the environment will ultimately damage Man" (p.11)*

In a Position Paper on co-existence the IFOAM EU Group (2003) says that genetic engineering is seen as an inherently risky technology, because it is based on "the reductionist scientific principles that have been shown to be flawed and are increasingly discredited". Verhoog (2004) also suggests that in the discussion about risks the holistic view of organic agriculture should be put forward, as underlying the rejection of GMOs. He relates it to the fundamental distinction between living and non-living nature. It can be concluded that the rejection of GMOs is not based on an actual risk analysis, but on risk perception, with a holistic view of nature (of life) in the background.

## The principle of naturalness

The IFOAM Position Paper says that genetic engineering is incompatible with the principles of a sustainabble agriculture, but the principles are not mentioned in the paper. It is important, however to do this, as sustainablity is interpreted in many ways. Basic ingredients of the organic conception of sustainability are: the carrying capacity of the soil, going along with nature's principles, cyclicity (using renewable resources, creating closed cycles, etc.). It is basically system-ecological thinking applied to the whole agro-ecosystem and beyond. Since man is seen as an integral part of nature, the conception of sustainability also includes social and economic issues, but the basis is the creation of a balanced agro-ecosystem which is healthy in all aspects, and supports life-processes continuously.

What is not mentioned in all these aspects of sustainability is the particular relation between humans and nature, which is related to it. It is here that the value of 'naturalness' comes in (Verhoog *et al.*, 2003). This value is very often used in the organic movement, especially in advertising organic products, but what it means is rarely spelled out. One reason for this is, that it is not an easy concept, and there are some standard objections which have to be overcome. One is, that agriculture is culture and therefore it cannot be natural. The word natural then refers to pristine nature, untouched by human beings. The opposite objection is that man is also part of nature, and therefore all human activities are natural. Both objections put an end to all serious discussions about what is really at stake, namely the relationship between humans and nature.

Every concept of nature is a human construct (product of human thinking), situated in the polarity between culture and nature. Value-neutral concepts of nature do not exist. This applies to the scientific concept of nature as well. Thus, when it is said that organic agriculture is a 'natural' way of farming (compared to conventional farming for instance), we must look at the conception of nature and the human-nature relationship as it is ideally, as it 'ought to be' in organic agriculture. That is why 'naturalness' can be called a value, why it has a normative component.

As can be seen from the DARCOF description of the precautionary principle, man is seen as part of nature (a participant in nature), and therefore Nature is written with a capital to indicate that it is a complex organic whole of which man is a part. This also means that man is interrelated to the other participants (plants, animals, etc.) within this whole. Verhoog *et al.* (2003) say that these other participants should ideally be treated as partners. This means in other words that their relative independence ('autonomy') should be respected. There is a positive attitude towards nature; it is not considered as an enemy, which should be conquered. There is a wisdom in nature, from which we can learn.

The word 'natural' therefore refers to the norm that nature can and should never be fully subordinated to, and controlled by humans, as if it only has an instrumental value. The more this independence is respected, the more natural the agriculture is. Respect for the relative independence of the nature-pole manifests itself at different levels:

- The use of natural substances, instead of chemical (synthetic) substances which often are harmful to the living system as a whole. Also the amount of processing (technical interference, or additives used) can be a factor here.

- Making use of and stimulating the self-regulatory processes of plants, animals, (agro-)ecosystems. Self-regulation is seen as a basic characteristic of life. The organic farmer should make use of the ‘forces of nature’. This sometimes means learning to be patient.
- Respect for the intrinsic value (inherent worth) of nature (natural entities). These values refer to the species-specific, characteristic ‘nature’ of living organisms or ecosystems.

On the basis of respect for the value of naturalness genetic engineering could be rejected as being ‘unnatural’ because it disturbs the harmony or balance of the whole, because the DNA constructs used are not natural substances, they do not stimulate self-regulatory processes, and by crossing species barriers, they do not respect the characteristic way of being (‘nature’) of living organisms. Genetic engineering is based on a mechanistic way of thinking about life, not a holistic way.

### The principle of integrity (Respect for the integrity of life)

The value of integrity is closely related to respect for the intrinsic value of nature and the species-specific characteristic nature of living beings. It can be seen as a further elaboration of a biocentric ethical theory, in which all life is considered to have an ‘inherent worth’ (Taylor), because each living organism has ‘a good of its own’. In animal ethics the concept arose in discussions about the genetic manipulation of animals, to deal with those moral issues which go beyond animal welfare (Verhoog & Visser, 1997). In the Netherlands it has become a criterium used in official bodies to regulate the production of transgenic animals. Rutgers & Heeger (1999) have defined ‘animal integrity’ as follows: “The wholeness and completeness of the animal and the species-specific balance of the creature, as well as the animal’s capacity to maintain itself independently in an environment suitable to the species” (p.45). All the elements in the definition should be satisfied for there to be a state of integrity. Verhoog (1999) has made the concept ‘a good of its own’ operational by distinguishing three levels of the animal’s ‘nature’: the level of animality, the level of species-specificity and the level of individuality. Lammerts van Bueren *et al.* (2003) have further elaborated this concept with respect to plants, who also have ‘a good of their own’. Lammerts van Bueren distinguishes four levels of the plant’s integrity:
- Integrity of life: related to self-regulation
- Plant-specific integrity: related to the ability of the plant to adapt and actively interact with the environment; and with the plant’s potential for natural reproduction.
- Genotypic integrity: related to the amount of genetic variation and respect for reproductive barriers
- Phenotypic integrity: related to the complete life cycle of the plant. Crossing techniques should allow pollination, fertilisation, embryo growth and seed formation on the whole plant.

## Implications for the use of marker technology

Three basic organic values (principles) have been distinguished: the precautionary principle (related to the principle of wholeness), the value of naturalness and the principle of integrity. What do these values imply for the use of genetic markers in organic plant breeding, and the techniques used for achieving that?
In the article about the levels of integrity (Lammerts van Bueren et al, 2003) the authors come to the conclusion that the use of DNA diagnostic techniques does not involve genetic modification and can therefore be used in organic breeding programmes *to supplement trait selection methods in the field*. This is an important addition, especially if it is assumed that all the levels of integrity must be taken into account. Respect for reproductive barriers is not the only element when dealing with the integrity of the plant. Respect for self-regulation and the life cycle of the whole plant (including the plant’s potential for natural reproduction), in interaction with the surroundings, should be taken into account as well.
In another paper (Lammerts van Bueren & Struik, 2004) it is said that DNA merkers can be used according to the non-chemical approach to organic agriculture. The authors do not explicitly say that this implies that it does not fit into the other approaches (agro-ecological and integrity view).

In discussions about the ethical aspects of marker technology we should distinguish between the several uses of genetic markers:
- For fundamental research, to understand underlying genetic mechanisms
- For diagnostic purposes, to do research on the purity of a variety, and to distinguish between varieties
- For selection purposes (MAS)

As to fundamental research there should be no problem as long as the connection to the whole is maintained (holistic principle). It can be compared with research into the nutritional quality of a food product in organic agriculture. The organic quality concept goes beyond nutrient content and also refers to qualities such as how the plant has grown (with or without pesticides, etc.), how it looks (reference to the plant's ideotype) and how it tastes, its contribution to human health, etc. The use of a reductionistic approach to establish the nutrient content of the plant plays a role, but it is subordinated to a holistic view of the plant. The results of reductionistic approaches get their meaning in the context of a view of the whole plant.

One could argue that the same is true when marker technology is used for diagnostic purposes. As a diagnostic technique it could be compared with making a fingerprint, as one of many other ways of distinguishing one organism (variety) from another. It should not be forgotten, however, that genetic mapping always means a selection of certain phenotypic properties which can be quantified, and which differentiate between organisms. It does not automatically imply that it also is the best tool for organic breeding purposes (MAS). It should be remembered here, that it is based on a reductionistic approach in which (some of) the properties of the plant are reduced to genes, or are believed to be determined by genes. Often, the main interest is in genetic markers associated with traits of economic importance, and to speed up the selection process.

Moss (2003) has shown that two very different gene concepts are often conflated in discussions about genetic engineering. One gene concept is related to a comparative approach in which genetic maps within or between populations of organisms are studied to find correlations between genes and phenotypic traits. On the basis of this approach certain (statistical) predictions may be possible, but no conclusions can be drawn about the question whether genes 'determine' or 'cause' certain phenotypic properties (as necessary and sufficient conditions). In the latter case one speaks about a very different gene concept, namely referring to the function of genes in ontogenetic development. In this context there always is an influence from the environment, and also non-coding regions of the genome (junk DNA) play a role. The belief in genetic determinism arises when the two gene concepts are mixed up. Genetic determinism underlies applications in modern biotechnology and the prevalent methods of risk analysis. And it looks as if it also is in the mind of some molecular biologists who, in their enthusiasm for MAS, think that the breeding program can be designed in the lab, on the basis of genetic maps only. Many breeders are more 'realistic'. They will give priority to phenotypic (whole-plant) selection as a necessary precondition for any MAS (Fasoula, 2004). Another reason for giving priority to phenotypic selection is the organic desire to include the farmers themselves in the breeding process from the very beginning (participatory breeding).

The deterministic view of the role of genes (DNA) in living organisms is discutable (Heaf & Wirz, 2001, 2002; Rist, 2000). It is very much the making of artificial (even synthetic) gene constructs, which leads to the belief in genetic determinism. The 'natural' DNA in the genome appears to be much more dynamic than thought before, so much so, that some authors now say that the role of DNA in the organism's development is very much dependent on the state of the organism as a whole, in interaction with the environment. This new view of DNA fits better with the organic agro-ecological view on living organisms. On this basis Haring (2001) rejects MAS for two reasons: by reducing life to the genes we forget the organisation of the plant, and it may have a negative effect on public perception ('The DNA-thinking that we have criticised in the past can not be used now as being necessary for diagnostic purposes').

Often quoted reasons for applying marker technology are to speed up the plant breeding process, and to make it less dependent on environmental conditions. Both reasons are not self-evident in organic agriculture. To speed up the plant breeding process can conflict with the value of naturalness. And dependence on environmental conditions is one of the basic characteristics of the nature (integrity) of the plant, and the ideal of plant varieties which are regionally adapted. Lammerts van Bueren *et al.* (2003) quote Hofmeister (1999) who has developed a 'theology of creation' (Schöpfungstheologie). One of the elements of this theology is the 'Würde der Kreatur' (inherent worth of all creation). Another element is 'Die Eigensinne der Geschöpfe'. All living beings have an inherent meaning ('Sinn'), what has been called a 'good of their own' before, and this includes a specific time dimension ('Zeitlichkeit'). They need a certain time scale to express their own nature.

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# Crop ideotypes for organic cereal cropping systems

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## Abstract

Cultivars for practical use in Organic Agriculture need to fulfil general traits such as high yield potential, baking quality and disease resistance. Breeding programmes should also take into account specific nutrient management strategies, nutrient efficiency, weed suppression ability as well as susceptibility for pathogens and aspects of product quality, which are all related to crop morphology traits that can be easily assessed by visual scoring.

## Keywords

Nutrient efficiency, competitiveness, pathogens, product quality, crop morphology.

## Basic growth conditions (compared with mainstream agriculture)

The general growth conditions in Organic Agriculture can be characterized as follows: limited soil nutrient availability (especially nitrogen) and no split application of nitrogen. Nitrogen availability in Organic Agriculture is a function of the pre-crop effects, the rotation design, and a delayed mineralization and nitrification under the conditions of a temperate climate in early spring, all resulting in delayed early development of cereal stands as well as limited tillering. Furthermore, leaf area index (LAI), leaf area duration (LAD), crop ground cover, water-use efficiency (WUE), light interception and grain yield as well as direct control of pests and pathogens are limited. Need for mechanical weed control (hoeing) results in suboptimal spacing (wide row width).

This contribution considers topics of nutrient management and nutrient efficiency, crop competitiveness as well as pathogens and aspects of product quality, which are all related to crop morphology traits that may be taken into consideration in breeding programmes.

## Nutrient uptake and nitrogen efficiency

Nutrient management in Organic Agriculture can be defined as a systematic target-oriented organisation of nutrient flows. Nutrient management deals with the optimisation of nutrient sources, which are restricted or have to be unlocked by improving utilisation. Nutrient management makes nutrients in the system internally available or keeps nutrients potentially available in the long term.

Consequently, breeding has to make nutrients in the system internally available by increasing rooting density and efficiency of nutrient absorption (see also contribution J. P. Baresel). Selection of an efficient root system, adapted to limited soil nutrient availability, has to consider the following features of the root system: limited competition for assimilates and an extended active root surface. Since the delivering soil volume $V_s$ is proportional to the reciprocal root or root hair diameter $r_0$, optimal geometric conditions for nutrient diffusion to the root or root hair surface are given by the small radius of the root cylinders and root hairs, respectively (Claasen, 1994). High root-length density and a high percentage of active young and fine roots can result from high branching and include *per se* a high number of root hairs that further increase the root surface considerably. High rooting depths enable plants to take up water and (leached) nutrients from deeper soil layers at least in dry periods. As has been demonstrated with the rooting patterns of three winter wheat cultivars in the early 1980s, there is some evidence that the success of cv. *Jubilar,* the leading cultivar of the 1970s, was a result of a high root-length density, realised by small root diameters (Köpke *et al*., 1982). As has been demonstrated by the so-called nitrogen-efficient cultivars, such as the German cultivar *Pegassos* (breeder A. Spanakakis, Strube Company), selection under less favourable soil conditions can result in a better adaptation and nitrogen utilisation, which allows the general conclusion that breeding programmes performed under the typical (specific) growing conditions should *per se* result in well-adapted cultivars.

## Weed suppression

During the tillering phase, competition with weeds is mainly based on crop-shoot growth rate and speed of development. Since the number of crown roots is a function of the number of tillers per plant, these parameters are directly related to root growth. Consequently, breeders should look for high tillering ability as a selection parameter. Rapid early development and high crop coverage (e.g., early prostrate growth under conditions of summer drought) are both beneficial and will result in increased water-use efficiency, because evaporation losses will be reduced. There is some evidence that rapid early development and tall plants in the beginning of the season often lead to higher crop ground cover and higher competitiveness with tall monocots, e.g., *Apera spica venti* or *Bromus tectorum*. Allelopathic exudates may be beneficial, too, but seem to be more important in rye and oats than in wheat or barley.

Cereals are normally not hoed, but problem weeds often need to be hoed. This makes suboptimal spacing (wide row width) necessary, resulting in the breeding target 'adapted morphology'. Under the conditions of Organic Agriculture, optimised leaf area distribution can be realised by using crop types with planophile leaf inclination, especially when spacing is suboptimal (wide row width) (Eisele & Köpke, 1997). Since the measurement of light interception is too time consuming and cannot be performed by breeders routinely on-site, the selection criteria 'crop ground cover' is proposed to be used by breeders when performing visual scoring. A strong negative correlation exists between cereal crop ground cover and weed ground cover, but this depends on the prevailing weed flora (Drews, 2005). The use of cultivars with planophile leaf inclination enhances light interception and concurrently increases shading ability, especially under conditions of low soil fertility or wide row width (prepared for hoeing). With regard to weeds, this leaf inclination type tends to intercept light more efficiently with the same low LAI than erect or higher growth types. Erectophile types are considered as beneficial for LAI > 3.5, whereas planophile leaf inclination is considered as beneficial for LAI < 3.5 (De Wit, 1965).

Besides leaf inclination, the shading ability of planophile cultivars is further enhanced by a higher flag leaf area compared with erectophile types. Since the weed mass produced is a function of the steady influence of the available photosynthetically active radiation (PAR), small differences in crop light interception or shading ability should not be underestimated.

In conclusion it can be said that crop ground cover reflects a combination of characteristics including tiller population, leaf area (leaf size and leaf inclination) and can be easily assessed by visual scoring. Competitiveness is a dynamic trait resulting from a considerable variation of the cultivars' ground cover over the season. Competitiveness during GS 31 to 75 is influenced by shoot parameters, crop ground cover, shoot mass, LAI and crop height, all influencing light interception and all largely negatively correlated with weed parameters. A range of cultivars with early planophile or higher growth and high ground cover can later become more erect with poor ground cover. Other cultivars, showing a more erect early growth habit with poor ground cover produce a higher ground cover in later growth stages. But it is also possible that a cultivar that changes from planophile to erectophile during the growing season will provide continuous high shading as long as the leaf area duration of the tall cultivar is high. Depending on the prevailing weed flora, the use of either an early or a late shader is favourable to efficient weed suppression.

No clear hint is given whether a combination of early and late shaders will result in a more successful weed control. Nevertheless, recommendations on suitable cultivar features, such as crop height, leaf inclination, ground shading, etc. can be given to breeders and publishers of official cultivar lists. Tall plants in the late phase of plant development often realise higher ground cover and higher competitiveness with tall weeds as mentioned before. The effect of increased shading due to tallness on the development of undersown crops, such as clover is considered as being minor. Breeders should keep in mind that straw is needed in organic mixed farms for bedding and that the lower grain yield level in Organic Farming is not necessarily affected by straw length. Consequently, breeding progress need not necessarily be based on an increased harvest index, whereas dwarf types or semi-dwarf types are definitely less competitive for controlling weeds. Further details concerning crop competitiveness have fed a breeders manual developed as a part of our EU funded project 'Strategies of Weed Control in Organic Farming' (WECOF, see contribution S. Hoad).

## Ear-diseases – mycotoxins – product quality

Clear negative correlations between the infection of the flag leaf with *Septoria nodorum* and the insertion height of the flag leaf, the distance of second leaf to flag leaf as well as distance of the third leaf to flag leaf have been determined, indicating that transmittance of spores by rain drops from leaves upwards to the ear can be reduced by increased distances between leaves and between leaves and ear. Grain infestation of *Fusarium spp.* and *Microdochium nivale* was also a function of the ear-to-flag-leaf distance (Engelke, 1992). Although the
Deoxinivalenol (DON) mycotoxin content of winter wheat grains derived from organic compared with conventional cultivation have several times been demonstrated to be lower (Birzele *et al.*, 2002), this issue might play a role under conditions of higher soil fertility esp. nitrogen availability ( Schauder, 2004) and/or pre-crop maize.

Generally, grain yield should be based on a high 1000-seed-weight. Bigger grains result in competitive vigorous seedlings as a function of earlier and homogenous emergence, higher root-length density and root surface and enhanced seed health. A high 1000-seed-weight is indispensable for high flour extraction. Compared to the flour type 550, the effect of protein content on loaf volume of fine coarse meal is only minor. Some cultivars demonstrate that satisfying loaf volume can be realised with low protein contents, and that selecting for high protein content can result in a low yielding cultivar (see also contribution D. Fossati).

The importance of low molecular weight (LMW) glutenins related to rheological properties should be emphasized: Despite typically low crude protein content, minimum conditions or LMW glutenin contents to achieve satisfying rheological properties can be defined for certain cultivars (Kühlsen, 2000). On the other hand there is no doubt that high molecular weight (HMW) glutenins do play a key-role concerning the rheological property of dough (see contributions G. Sharmet, D. Fossati). Furthermore, our colleagues of the DFG researcher group 'Optimising Strategies in Organic Farming' (OSIOL) (Köpke, 2001) have found that in contrast to other studies cultivars with the HMW allel 2 + 12 showed nearly the same high loaf volumes compared with allel 5 + 10 cultivars. The proposition that the baking qualities of cultivars with the allel 5 + 10 in some cases may be superior to cultivars with 2 + 12 seem to depend on the selected cultivars. This was demonstrated by the two selected elite wheat-class cultivars *Carolus* (subunits 1, 7, 2 12) and *Borenos* (7, 9 2, 12). The results of the HMW subunits confirm the importance of the x-type subunits (Pechanek *et al.,* 1997): dividing the HMW subunits into their x- and y-types the x-HMW glutenins attained about 0.1 to 0.2 units higher coefficients than the y-HMW glutenins and these results were quite similar to those for HMW (Kühlsen, 2000; Kühlsen *et al.*, 1999).

## Breeding aim: leaf area duration (LAD)

Since LAD accounts for about half of the variation in grain yields of winter wheat, breeding targets should take plant health (leaf diseases, e.g. DTR), nitrogen efficiency (utilisation) as well as an extended 'post-floral phase' into consideration.

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# Participatory plant breeding methods for organic cereals

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## Abstract

Formal breeding methods were not always suitable to address the very large diversity of both environmental conditions and end-user needs. Both were frequently encountered either in marginal areas of developing countries or in organic farms of EEC. Participatory plant breeding (PPB) methods represent alternatives aimed to improve local adaptation breeding, to promote genetic diversity, to empower farmers and rural communities. The term PPB refers to a set of breeding methods usually distinguished by the objectives (functional or process approach), institutional context (farmer-led or formal-led), forms of interaction between farmers and breeders (consultative, collaborative or collegial), location of breeding (centralized or decentralized), stage of farmers participation in the breeding scheme (participatory varietal selection or participatory plant breeding)...

Among all these methods, the best strategies for organic breeding and their impacts on breeding techniques are discussed. A PPB program actually conducted at INRA – Montpellier (F), involves the whole organic durum wheat interprofessional organization, from farmers to consumers. It is used to assess the interest of a multidisciplinary approach and to discuss the role of each participant in such program. Must participation be seen as a means towards an end or an end in itself?

## Keywords

Participatory plant breeding, organic breeding, genotype x environment interaction, on-farm trials, Durum wheat.

## Introduction: why formal breeding methods are not always suitable?

Organic production in European countries shows great similarities with production in marginal areas in developing countries, such as (i) heterogeneous environment, (ii) large diversity of farmer's needs, (iii) lack of adapted varieties, (iv) disinterest of formal seed sector. Facing such diversity and heterogeneity, conventional plant breeding often fails to meet the needs of farmers and to develop cultivars showing specific or local adaptation.

Professional breeders, often working in relative isolation from farmers, have sometimes been unaware of the multitude of preferences — beyond yield, and resistance to diseases and pests — of their target farmers. Ease of harvest and storage, taste and cooking qualities, crop maturity speed, suitability of crop residues as livestock feed are just a sample of farmers' criteria difficult to grasp in a conventional breeding scheme. In Peru, for example, the PRGA plant breeding working group compiled an inventory of almost 40 different traits interesting farmers for evaluating potatoes (CIAT, 2000). Without close discussions with end-users and observations of their agricultural and social practices, breeders are unable to imagine or anticipate their necessary needs. During their professional training, plant breeders have little exposure to survey/methods needed to elicit structured feedback from farmers (Morris & Bellon, 2004). Moreover organic farmers have to deal with several limiting factors and high heterogeneity that they could not uniform with inputs; therefore they are looking for specific ideotypes according to their own use and cultural practices.

Formal breeding programmes can be briefly described as a centralized sequential process in which breeders collect germplasm, evaluate it under carefully controlled experimental stations, and make crosses among superior materials. The large amount of genetic variability continuously created is then drastically reduced through selection and surviving lines are spread among farmers. The process has been effective for farming systems sufficiently similar to those on experiment stations (Sperling *et al.*, 1993) but not adapted when GxE interactions are large.

Formal breeding tends to focus on "broad adaptability" — the capacity of a plant to produce a high average yield over a wide range of growing environments and years. Therefore, candidate genetic material that yields well in one growing zone, but less in another, is quickly eliminated from the breeder's gene pool (Cecarelli, 1997). Yet, this "specific adaptability" may be exactly what organic farmers require and aims to increase agricultural diversity (Vernooy, 2003).

Facing difficulties to target environment conditions well and register all end-users needs, to translate them into criteria of selection and to build an ideotype, breeders begin to be interested by participatory plant breeding (PPB) defined as end-users' participation in selection process. It appears to be a suitable alternative to match up to organic agriculture expectations.

## PPB: a single term but different approaches - a review

Participatory plant breeding is a relatively recent concept. Indeed, first scientific papers on this subject appeared 10 years ago. But already it refers to a large set of approaches and breeding methods. All these approaches could be integrated into an n-dimension matrix where the following items would be crossed:

### The objectives

PPB mixes usually 2 types of approaches: Functional and Process, which are defined by Thro & Spilane (2000). Functional approach consists of getting better adapted crop varieties i.e. more closely tailored to small-scale farmers' needs, whereas, process approach aims to empower farmers to develop their skills as plant breeders. Belonging to these 2 types, some current PPB objectives are detailed below

- **Getting adapted materials**

This objective is more often mentioned in the literature as: "speeding up the transfer of cultivars and their adoption". Although relatively little empirical work has been done to document the speed of PPB compared to conventional breeding, recently evidence has started to emerge suggesting that PPB can lead to earlier adoption of modern varieties, with no major additional costs (Witcombe *et al.*, 2003). But negative connotation can also be linked to this aim: indeed, it may assume that cultivars are already created by breeders and PPB appears as an opportunity to speed up the adoption by farmers. Setting out clearly the objective permits an assessment of whether the project considers farmers as a simple consumer or as a partner. The first consideration is out of place in PPB projects.

- **Improving local adaptation**

Breeding for specific adaptation is a more sustainable strategy than breeding cultivars that can only express their superiority at high level of inputs (Ceccarelli, 1996). Local adaptation contributes to limit genetic erosion and therefore to avoid major risks due to varietal homogeneity on the territorial scale. Breeding for marginal or organic environments shall include selection of parents and segregating populations in environments similar to farmers' conditions.

- **Promoting genetic diversity**

Breeding for specific adaptation to organic environments implies a re-evaluation of the role of genetic resources such as landraces. In European countries, landraces are unfortunately no longer cultivated. They possess adaptative features and represent a gene cistern that can be really useful for organic environments. Biodiversity which is so important for organic farmers justifies the choice to breed for specific adaptation. Associating end-users with evaluation and management of genetic resources is one important objective. PPB methods, in encouraging the maintenance of diverse locally adapted populations and in-situ conservation of crop genetic resources, enhance genetic diversity.

- **Empowering farmers**

PPB may aim to empower farmers i.e. to bolster their autonomy or to increase their freedom to choose varieties. It allows rural communities to maintain genetic resources they value and enables them to participate in the development of new varieties that suit their needs. PPB methods thus can empower groups that traditionally have been left out of the development process (Mc Guire *et al.*, 1999).

### Institutional context

According to the leader or to the initiator of the project, it is used to differentiate a formal-led PPB program which is initiated by researchers inviting farmers to join breeding research, from a farmer-led PPB program, where scientists seek to support farmer's own systems of breeding, varietal selection, and seed multiplication and dissemination. Based on the work of Franzel *et al.* (2001), a more elaborated differentiation can be proposed by identifying leaders of breeding process designs and those of management.

### Forms of Interaction between actors

The various modes of participation can be thought of as points along a continuum representing different levels of interaction. Each mode of participation can be characterized in terms of how farmers and plant breeders interact to set objectives, take decisions, share responsibility for decision making and implementation, and generate products (Morris & Bellon, 2004). In practice, three kinds of participation are usually distinguished: consultative (information sharing), collaborative (task sharing), and collegial (sharing responsibility, decision making, and accountability) (Sperling *et al.*, 2001).

### Location of selection

Decentralized selection, defined as selection in the target environment, has been used to emphasize favourable GxE interactions. It is a powerful methodology to fit crops to the physical environment and to the crop system. However, crop breeding based on decentralized selection can miss its objectives if it does not utilize the farmers' knowledge of the crop and the environment, because, it may fail to fit crops to the specific needs and uses of farmers communities.

PPB can also be held in centralized research stations. Farmers are therefore invited to visit and practice selection of lines grown at experimental stations.

### Stage of selection

Each plant breeding project includes the following stages:

1. Setting breeding objectives
2. Generating genetic variability (from collection or farmers'fields and/or through crossing)
3. Selecting among variable materials
4. Evaluating experimental varieties
5. Multiplying and disseminating seed

In many cases, farmers' participation is limited to the final steps: evaluating and commenting on few near-finished or advanced varieties just prior to their official release. It is known as participatory varietal selection (PVS), while participatory plant breeding (PPB) concerns participatory selection within unfinished or segregating material i.e. with a high degree of genetic variability (Witcombe *et al.*, 1996). Both terms are included in the participatory crop improvement (PCI) concept.

PVS can be useful before beginning a PPB process because it helps to identify both parents and important target traits. Usually, PPB program used only a few crosses from which large populations were produced (Witcombe & Virk, 2001) and because few parents are employed, their choice is crucial. Very few programs, even in PPB, imply farmers in the first three stages. However, many of the varieties reaching on-farm trials would have been eliminated from testing years earlier if farmers had been given the chance to critically assess them (Toomey, 1999).

This bibliographic review emphasizes the great diversity of PPB approaches. However, all have in common the aim of shifting the locus of plant genetic improvement research towards the local level by directly involving the end user in the breeding process (Morris & Bellon, 2004).

## Interest of participatory plant breeding for organic conditions

Most PPB projects are initiated by international institutes of research and aim to speed up the adoption of cultivars by small farmers in developing countries. Up to now, these projects are essentially built around the implication of farmers in selection processes.

Very few PPB projects are conducted in European countries and they concern essentially organic agriculture (for more details, visit the web site: http://selection-participative.cirad.fr/).

This is not surprising. As mentioned in the introduction, organic production shows great similarities with production in marginal area in developing countries, such as heterogeneous environment, large diversity of farmer's needs, lack of adapted varieties and disinterest of the formal seed sector.

Variability of organic farming systems is so high that developing a variety fitting to fit all situations is not conceivable. Because they are aware of the breeding cost necessary to meet several objectives and also to develop several locally adapted varieties, private breeding companies doesn't want to join in the organic seeds market. But, considering an approach like PPB, we can imagine, without additional costs, developing varieties adapted to an area, a region, a specific environment and why not at the farmer field scale? For these reasons, PPB appears to be a more suitable solution for organic conditions than formal breeding.

Moreover, compared to conventional breeding, PPB seems to be the best alternative to fit the principle aims of organic agriculture for production and processing prescribed by IFOAM, and especially: “(i) to maintain and conserve genetic diversity through attention to on-farm management of genetic resources, (ii) to recognise the importance of, and protect and learn from, indigenous knowledge and traditional farming systems”.

Indeed, because breeding for organic conditions means breeding for sustainability, the process of breeding is as important as the results. Therefore, breeding process must comply with the three following criteria for organic production: closed production cycles, natural self-regulation and agro-biodiversity. According to Lammerts van Bueren *et al.* (1999), equivalent criteria at the socio-economic level are: close interaction between farmers, trade, industry and breeders; regulations geared to organic agriculture and cultural diversity. Yet, PPB can be exactly defined by these words.

## Participatory plant breeding of durum wheat: an INRA pilot project

### Context- Objectives

The French organic durum wheat professional organization is sufficiently small-scale and closely integrated enough to be considered as a model. Indeed, organic durum wheat producers are located in two main territories in the south of France and regrouped into organic farmer’s organizations, traders, seeds collectors and pasta processing industrialists are very few, and no breeding private company is interested by the organic sector. For consumers, durum wheat is a food product profiting from a healthy and environment friendly image.

The PPB program, initiated in 2001 at INRA- Montpellier (F) was based on a demand of organic farmers and pasta industrialists. The quality of durum wheat produced in organic conditions doesn’t meet the requirements of the process industry. Indeed, no less than 15 criteria are required to transform the grain into semolina or pasta. Among them, the most important is the protein level. Under organic conditions and especially when nitrogen is limiting, durum wheat seed becomes un-vitreous like bread wheat seed and prevents the production of semolina. Such unsuitability puts the whole organic durum wheat organization into question, and poses the problem of its durability.

To identify the main causes, a multidisciplinary public research team, associating plant breeders, soil scientists, ecologists, agronomists, economists and sociologists was requested and decided to work in close collaboration with professionals. The action-research program is built around thematic activities in relevant domains and concerns the two main French territories of durum wheat production: Camargue and Pays Cathare (Desclaux *et al.*, 2002). These territories can be mainly differentiated by the existence of animal rearing, the soil salinity, the organic farming systems. First investigations showed rapidly the lack of adapted varieties to limiting nitrogen conditions very frequent in the studied organic crop systems. Indeed, all available durum wheat cultivars came from breeding programs managed under conventional growing systems, with no nitrogen limitations. The need to begin a breeding programme in organic conditions was followed by thoughts about the best way to interact during this programme.

### Different ways of participation and interaction between actors

In this project, which aims to boost interactions between actors, different forms and locations of participation and interactions are sought.

- **Meetings**

Preliminary meetings were organized to define the objectives of breeding and the main criteria. Each actor from farmers to consumers is invited to formulate his ideotype. A multidisciplinary team of researchers leads to a wider identification and understanding of the claims of all professional partners. For example, identification and evaluation of subjective traits as taste, aroma, appearance, texture... requires close collaboration between plant breeders, social scientists, farmers, process industrialists, consumers. Such subjective traits are difficult to measure quantitatively and belong to the register of human perceptions that social scientists help to identify. Formal durum wheat breeding has never focused on these traits and some were “contrary-bred”.

- **Surveys**

A large written survey, containing questions about crop system and farmers’ preferences, was carried out to catch the opinion of a great number of organic farmers in the two territories. Diffusion of such a survey was facilitated by the regional farmer’s organizations that possess an exhaustive file of durum wheat producers in these areas.

Formulation of durum wheat ideotypes was much more different between territories than within. In the Camargue, existence of bull and sheep rearing brings natural nitrogen available for wheat during the vegetative period, but not during the period of seed quality elaboration. Farmers are looking for varieties efficient in the remobilisation of nitrogen from its vegetative parts. In Pays Cathare, nitrogen is limiting even during the first vegetative period and weed infestation is regularly high; the requested variety must have an important root system, and be able to compete with weeds and to draw nutrients efficiently.

- **Informal discussions during field visits**

Regularly and at least during flowering and at physiological maturity, field visits were organized. It's a opportunity for farmers, industrialists and researchers to discuss in concrete terms in front of genetic diversity.

During such visits, all the actors are invited to express orally their opinion and also to write some notations according to a grid drawn up by breeders on the base of preliminary meetings and discussions with other actors. Regularly, this grid is improved. Visits were held both in farmer's fields and in the experimental stations. In the stations, important genetic resources and germplasm collection afford a large diversity of morphologic characters and therefore give rise to new questions leading to ideotypes inconceivable until then.

- **Learning**

Organic farmers are aware of genetic diversity maintenance and are used to grow several species, several varieties and several heterogeneous varietal structures (populations or mixtures). Such heterogeneity aims to maximise adaptability more for temporal scale than for spatial scale. In order to manage this diversity and not only maintain it, the biology (reproduction type, etc.) of cultivated species must be well known. Farmers ask for training on these subjects. On the other hand, the great expertise and observation capacity of farmers are recognized by all the actors. Complementary knowledge leads to dynamic in situ conservation and to the adaptation of a portfolio of varieties.

- **On-farm trials experimentation**

From the beginning of the project, some farmers desired to experiment old varieties, which were the first durum wheat cvs introduce or bred in France 50 years ago. Others farmers asked for populations. We complied with their request and provided them with additional segregating or advanced pure lines and populations resulting from crosses between durum wheat and emmer or wild species. Such tetraploid relative species (T.t. diccocoides, T.t. diccocum, T.t. polonicum, etc.) are expected to bring interesting characters of quality and adaptability. Some pure wild accessions were added in the field.

The main aim of such on-farm and participatory breeding is to approach farmer's preferences and to better target environmental conditions by increasing and managing genetic variability. Due to low available seeds quantity, the farmer's network was limited to 7 locations. In each farmer's field, the experimental design was a randomized complete block with replications. Sowing and harvesting of experimental plots (10m$^2$) require specific experimental materials and are also carried out by the research institute. To pass round these constrains that prevent to approach totally the farmers' management practices, some lines preliminary multiplied, are sown directly by the farmers. A mother-baby design is used for advanced materials.

On-farm selection is conducted not only on farmer's fields but also with farmers. The farmer is implicated in growing and letting evolve plants in his environment. According to the type of materials (genetic resources, segregating pure lines, populations or advanced materials), the farmer can be in a position to innovate, to adapt or to manage dynamically. He gets the possibility to clarify his preferences or reject criteria more freely than in front of a researcher or his peers. He can assume the right to maintain one cultivar and/or create mixtures. Observations of his choices produce much more information than any survey could, about suitable varietal structures and also ideotypes.

The stage of the breeding process at which farmers are involved depend on the type of materials. Agronomic behaviour examination of genetic resources is a preliminary to the early step of the breeding scheme: 'Generating genetic variability'. Owing to their unique knowledge of existing varieties, it is really pertinent to involve farmers in the observation and selection of genetic resources. The following steps, selection and evaluation, are done in close collaboration between farmers and breeders and concern respectively segregating lines or populations and near-finished or finished varieties. Yield and agronomic behaviour data are compiled and analyzed by researchers and diverse criteria of seed quality are measured by industrialists. Results are discussed between all actors and this work of synthesis creates opportunity for feedback and may lead to a re-examination of the first step of the breeding scheme which is 'setting the objectives'.

PPB provides all the actors with the opportunity to assess genotype-by-environment interactions. Most often, environment is only defined by climate and soil data. For example, unfavourable environments are defined by Cecarelli (1996) as those where crop yields are commonly low due to the concomitant effects of several abiotic and biotic stresses. The definition of environments plays a key role in determining breeding strategies. Therefore, we emphasize the consideration of the whole acceptation of environment, including not only physical environment but also socio-economic environment. Both are completely integrated into farmer's management practices that agronomists and social scientists investigate. Strategies of conversion to organic farming and management systems are strongly correlated to farmer motivation. In the project, two extreme types have been identified: (i) pioneers, motivated for ethical reasons and first converted to organic farming, include a high diversity of species in their crop rotations and crop cultivation practices are relatively stabilised, (ii) the newly converted, for whom recent conversion can be seen as a timely strategy to counter difficulties in the formal sector, choose mixed cropping systems (organic and conventional) to limit risks related to a technical and/or economic failure of the organic production system. On these farms, crop cultivation systems are not stabilised, crop rotations little established and cultivation practices, while respecting organic specifications, refer to conventional practices (Mouret *et al.*, 2004). Approaching such a level of knowledge of the broad sense of environment leads to a better mastering of breeding targets.

## Discussion and conclusion

Most often in the literature, PPB methods are presented as the interaction between farmers and breeders.

The organic durum wheat project wants to emphasize the interest of opening the interaction to other professional partners and other researchers from relevant disciplines. Convening the whole of the professional organizations leads to the emergence of new breeding criteria and to a better knowledge and understanding between actors (farmers and industrialists especially).

In organic conditions, diversity of physical location, limiting factors and farming systems is so high, that agronomists are a great help for breeders to better characterize each environment. As the same farming systems are related to social criteria, sociologists may identify them in order to better seize farmers needs and therefore better target suitable varieties.

But participatory plant breeding can not be limited to studies conducted for a limited period of time to document indigenous knowledge and farmers' preferences. To be effective, participation should become a permanent feature of plant breeding programs concerning crops grown in agriculturally difficult and environmentally challenging environments.

The project may be defined as a mix of different objectives: getting adapted materials by improving local adaptation, promoting genetic diversity and empowering farmers. It is neither a farmer-led program nor a formal-led program but really a whole professional organisation and researchers-led program. The form of interaction is collegial. Decentralized design is used and the principle is to conceive farmer's participation during the 3 first steps of the breeding scheme in order to better respond to sustainability stakes of organic agriculture. This represents a major rupture with regard to formal breeding schemes.

Discussion about more participation is interesting. "More participation is not necessarily better. Participation should be seen as a means to an end." (Morris & Bellon, 2003). But, PPB must not be reduced as 'farmer assisted selection'. The farmers involved in our project, assert the right to be considered as true partners of the breeding programme and not only as variety consumers or end-users. For involving researchers the participation of actors can either (i) be a means towards an end or (ii) an end in itself.

Close collaboration between the parties is a must if they are to overcome possible conflicts of interests and agree on a set of breeding goals. An interactive approach to breeding may provide that intensity of collaboration which is so crucial to organic agriculture (Lammerts van Bueren *et al.*, 1999).

For practical and ethical reasons, organic breeding justifies the implication of farmers and end-users in a PPB program.

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# Use of DNA-based genetic markers in plant breeding

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## Abstract

Genetic markers have been used since the beginnings of plant breeding, but the concept of linkage and recently the availability of molecular markers have offered new and powerful tools that can help to perform the traditional tasks of selection or that can change the traditional breeding process. Markers can either be used in a descriptive manner to identify varieties, to study the 'micro-evolution' of composite crosses or variety mixtures or to analyse the breeding progress retrospectively in order to learn from the past. The operative use of markers in plant breeding is connected to the selection of parental lines and progeny lines. The possible implementation of these processes stretches from the introgression of specific chromosome fragments to 'marker-based idiotype breeding'.

## Keywords

Molecular marker, plant breeding, retrospective analysis,,marker-based ideotype breeding, marker-supported selection.

## Genetic markers

Since the beginnings of plant breeding by selection, markers were used. Breeders learned to distinguish their material by certain morphological characteristics and discovered that some of those characteristics appeared obviously very often together with other features of the crop that were more difficult to spot. This was of special interest, when the target traits of interest were inherited quantitatively because it was caused by several genes and influenced by environmental effects Much later, the co-heritance of such traits was theoretically explained by genetic linkage (Sax, 1923). The gene for the marker trait is situated on the chromosome in a position close to a gene influencing the marked trait. Therefore, there is a relatively high probability that the marker gene and the target gene are inherited together to the offspring. Stepwise, as new technologies arose, the morphological markers were supplemented by protein-based markers and historically seen very recently, by DNA-based markers.

Still, genetic markers are used to identify and characterize individual plants or lines or as a tool to facilitate the difficult task of selection of the most promising lines (Jones *et al.*, 1997). Nevertheless, the high potential density and thereby specificity of DNA-based markers together with the still relative high costs of their application implies that changes in the way, breeding is carried out, might be necessary to use the full potential of this tool. Consequently, the decision for the application of DNA-markers in plant breeding, naturally including plant breeding for ecological farming will or would have an impact on the way, breeding is going on. Thus, the plant breeder has to investigate the possible applications of DNA-based markers in order to conclude in which areas he will expect an advantage and what will be the costs for him by adapting his breeding procedure.

In order to characterise the options of application, I tried to categorise them according to the knowledge that is necessary for the application and if the markers are use to describe and analyse material and 'micro-evolution' or as an operative tool for the selection of genotypes. Table 1 gives an overview over the different techniques described in this paper and their categorisation.

## Descriptive use of DNA markers in plant breeding (for organic farming)

The simplest application of DNA markers is to use one or few markers to identify and differentiate different genotypes. In this role their might replace the morphological markers used actually to ensure, that a new variety is distinguishable and to protect the breeders right of ownership of his variety (Soller & Beckmann, 1983). Compared to those morphological markers, DNA markers are more robust, not depending on the development stage of the plant and the differentiation is clearer. Furthermore, as new markers can be added, the number of possible distinct combinations is nearly infinite. As a drawback, it is often mentioned, that this application of markers would lead to higher demands on the uniformity of varieties, as heterogeneity would easily be detected. This could be especially interesting for organic farming, where mostly a certain genetic variance on the field is desired. On the other hand, the potential of detecting heterogeneity does not imply, that complete homogeneity has to be postulated. As the example of out-breeding species shows, thresholds can be defined (Bar-Hen *et al.*, 1995) or – even better – the specific heterogeneity could be specified and could be an important criteria for variety choice, especially for organic farming.

The potential of DNA-markers to identify the genotype from any part and at any developmental stage of the plant – also including kernels – allows further applications: not only the identity of the varieties in variety mixtures but also their relative proportions can be determined already at the kernel level. The availability of inexpensive marker technologies for this purpose, well suited for mass examinations might lead to a real boost in the acceptance of variety mixtures that have shown their advantage showing higher stability not only in field performance but also in environmentally dependent quality traits (Newton *et al.*, 1998). Additionally, this feature of DNA-markers can be used to study the 'micro-evolution' of variety mixtures through several years in several environments. Based on those results, optimized variety mixtures can be defined for specific conditions.

To study the 'micro-evolution' of multi-line varieties and composite crosses efficiently with the help of markers, more information than just polymorphisms of anonymous markers is needed. For this purpose, a linkage map that defines the position of markers on the respective chromosomes is needed. As the order of markers is conserved within a species and even in related species and a growing number of linkage maps are available, this information is available for many markers and especially for SSR-(simple sequence repeat)markers, that are well-suited for the identification of genotypes (Karakousis *et al.* 2003), as they are both robust and highly polymorphic. Including mapping information it is possible to study the fate of distinct parts of the genome in the development of multi-line varieties and composite-crosses (Enjalbert *et al.*, 1999).

A further use of DNA marker profiles together with their mapping information is a retrospective analysis of the breeding history – restricted to the own breeding company or on a larger scale. That way it is possible to see which chromosome fragments were transferred to the successful progeny lines, which fragments are common for progeny lines with a certain trait expression and so on. Using the retrospective analysis, it is possible to use experience about success and pitfalls in the past in a more sophisticated way and to use it thereby more efficiently for future decisions.

Even more detailed information can be obtained from the retrospective analysis, if it is known, where genes of interest for specific traits are localized and how the different alleles for these genes look like in specific lines. That can be achieved either by establishing linkage between marker and a gene of interest or by creating functional marker. For the

*Table 1. Overview over descriptive and operative use of markers in plant breeding in relation to the necessary marker.*

<table>
<tr><th>Marker knowledge prerequisite</th><th>Identification of genotypes</th><th colspan="2">Identification of chromosome fragment (linkage map)</th><th>Identification of gene alleles by linked / functional markers (Establishment of linkage/identification)</th></tr>
<tr><td><b>Descriptive use</b></td><td>Protection of breeders rights</td><td colspan="3"></td></tr>
<tr><td></td><td>Identification of variety components and their proportion in mixtures</td><td colspan="3">Retrospective analysis of breeding process</td></tr>
<tr><td></td><td>Study of the evolution of variety mixtures</td><td colspan="2"></td><td></td></tr>
<tr><td><b>Operative use</b></td><td colspan="3">Study of the evolution of multi-lines and composite-crosses</td><td></td></tr>
<tr><td></td><td></td><td></td><td colspan="2">Introgression of chromosome fragments of interest</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">'Ideotype breeding'</td></tr>
</table>

establishment of linkage, either a population segregating for the specific trait(s) is analysed for the trait(s) and markers or a more general population of lines is analyzed by association mapping. Functional markers are based on the sequence information of the gene itself and directly reflect allelic differences in the gene. Mapping information about linked or functional markers is especially useful, if the respective gene shows a very high influence of the trait of interest.

## Operative use of DNA markers in plant breeding (for organic farming)

The operative use of DNA markers in plant breeding is connected to the difficult task of selection. Selection must be carried out first by picking the right parents for crossings and than by choosing the progeny lines to continue with. Generally, markers-based selection and phenotypic selection have to supplement each other. The breeder has to decide from case to case, where marker-based section is advantageous over phenotypic selection. Marker-based selection also needs to be seen in connection with the descriptive use of markers mentioned above. Particularly the retrospective analysis of the breeding process results in information that is useful for the marker-based selection. Besides the self-generated information, external information from research about linked or functional markers can be used for marker-based selection.

Two main patterns of the usage of marker-based selection in plant breeding have been shown to be successful in the past: the introgression of a gene of interest into an existing line and 'ideotype breeding'. For the introgression of a gene for interest (Toojinda *et al.*, 1998; Gao *et al.*, 2004), the task it to embed a gene of interest found in a line that is often unadapted, 'exotic' or even a wild progenitor (donor line), in the genetic background of another line that should be changed as less as possible (receptor). Here the markers are used to control that a specific chromosome fragment and nothing else is transferred to the receptor line. The procedure is often used for resistance genes. A weakness of this method is that possible other positive gene alleles that even can be found in wild progenitors will be ignored. A method called 'Advanced backcross analysis' (ABA, Tanksley & Nelson, 1996) tries to circumvent this drawback by including a QTL analysis after one or two backcross generations and one selfing generation.

A more radical way of using marker information is 'marker-based ideotype breeding'. Based on information about advantageous chromosome fragments and/or marker alleles, a genotype composed of distinct chromosome fragments from different lines is 'designed'. To reach this aim, different steps of crossing and subsequent marker-based selection are necessary.

## Information and material flow in breeding company using molecular marker

Taking all things together, an information and material flow as shown in Fig 1 could result. Within the breeding company, markers are used as a supporting tool for parental selection, progeny selection and for the retrospective analysis. The information from the retrospective analysis is enhancing the knowledge necessary for the parental selection and the

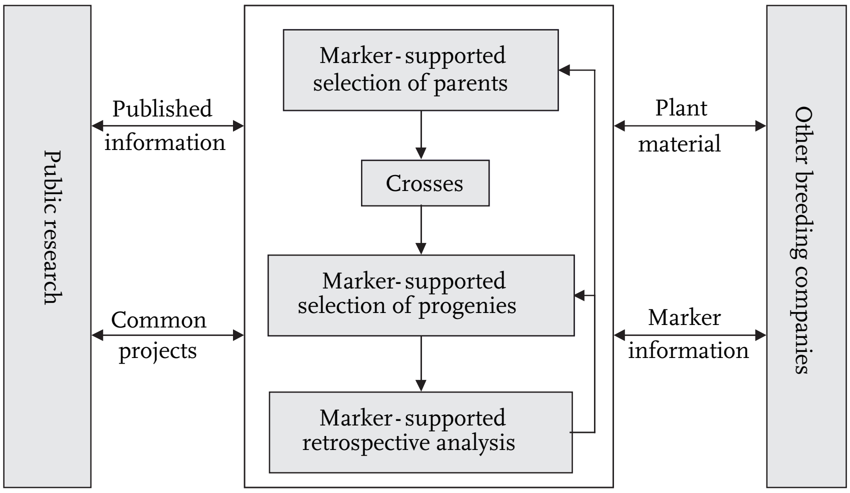

*Figure 1. Information and material flow in a breeding company.*

progeny selection. The company is linked to other breeding companies through the exchange of material and marker information and linked to public research institutions through common projects and the exchange of information, mostly through publications.

## Breeding for organic farming

Principally breeding for organic farming is not completely different from breeding for conventional farming. In organic farming a higher heterogeneity of the material is desired to allow better buffering against harmful and better exploitation of useful environmental influences. Additionally traits with higher priority in organic farming have lower priority in conventional farming and vice versa (Lammerts van Bueren *et al.* 2002). Finally, some techniques like genetic engineering are not accepted in organic farming as they are in contrast with the concept of integrity (Lammerts van Bueren *et al.* 2003). Altogether, this should not interfere with the application of molecular markers as mentioned above. Molecular markers are a tool that can support some of the traditional tasks included in the breeding procedure. To which extent breeder wish to use them and to which extent they want this tool to affect their breeding activities is up to their individual decision and may vary from case to case.

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# Stability of variety mixtures of spring barley

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## Abstract

Six 3-component variety mixtures of spring barley and their component varieties have been grown in 17 different environments (3 years, 3 locations, 3 different growing systems). For three of the six mixtures, the grain yield was significantly higher than the average yield of its components; in none of the mixtures it was significantly lower. The variation in grain yield over environments of all variety mixtures was compared to the variation of all component varieties. The six mixtures were on average more stable than the 14 component varieties grown in pure stands with respect to actual yield as well as to rank values of yield.

## Keywords

Variety mixture, organic growing conditions, yield stability, mixture efficiency.

## Introduction

Modern spring barley varieties have been developed with the aim of combining high productivity and standardized product quality under high-input conditions. The organic growing system is a system where pesticides and synthetic fertilizers are generally not allowed. Hence, biotic and abiotic stresses have to be overcome by growing appropriate varieties and by practicing good farm management. An important question is whether modern spring barley varieties possess the right combinations of characteristics, e.g. weed competitiveness and disease resistance and tolerance, to ensure a stable and acceptable yield of good quality when grown under different organic growing conditions. Despite quite intensive testing of varieties, predictions of performance of varieties are known to be very difficult; this especially within organic growing systems, where pesticides and fertilizers cannot help stabilizing yield. Therefore, using mixtures of appropriate varieties might be a way to obtain more stable and acceptable yields.

In Denmark, official regulations for certification of variety mixtures of spring barley have been practiced for several years requiring 3- or 4-component mixtures with equal proportions of component varieties, medium to high grain yield of each component, little difference between ripening dates and culm lengths of components and high average disease resistance of the mixture.

Variety mixtures have so far been studied mostly under conventional farming conditions and with focus on reducing disease severity by combining varieties with different disease resistance genes. In this study, the performance of variety mixtures in organic as well as conventional growing systems is considered with focus on competitive ability of the component varieties in addition to their disease resistance.

## Materials and methods

In 2002, six 3-component variety mixtures were constructed based on information from official variety testing (Table 1). The mixtures consisted mostly of high yielding varieties. They were made according to official certification requirements with respect to relative yield, disease resistance, and date of ripening. However, larger differences between component varieties than accepted according to the rules for culm length were introduced. The mixtures were combining malting and fodder varieties, as the purpose was to study the competition between different combinations of varieties. These mixtures as well as their components have been included in large variety trials in the years 2002-2004 (Jensen & Deneken 2002, 2003, 2004).

Each year the mixtures were composed of untreated seeds from conventional multiplication of the component varieties in equal proportions according to expected seed germination. The seed for the pure stands were from the same seed batches. Field trials were conducted on experimental research fields at three Danish locations: Jyndevad, Foulum and Flakkebjerg. Three different growing conditions were studied either resembling organic conditions (i.e. no pesticides, weed harrowing or grass-clover undersown, and low input of organic fertiliser (e.g. slurry)) or conventional conditions (use of herbicides and synthetic fertiliser according to local standards, however, without use of fungicides). All together, data were collected in five to six different environments (system x location) in each year 2002 to 2004. The conventional conditions were only applied on two locations in each of the years 2002 and 2003 constituting all together 4 of the 17 environments. Many different disease- and growth characteristics were assessed; here, we will consider only grain yield.

*Table 1. List of component varieties of the six spring barley mixtures studied and of their weed competitiveness.*

| Component variety | Weed competitiveness | Mix1 | Mix2 | Mix3 | Mix4 | Mix5 | Mix6 |
|---|---|---|---|---|---|---|---|
| Alabama | Low | | | x | | | |
| Brazil | *Low* | | x | | x | | |
| Cicero | *Medium* | | x | | | | x |
| Culma | *High* | | x | | | | |
| Danuta | *High* | | | | x | | |
| Fabel | *High* | | | | | x | x |
| Harriot | *High* | | | | | x | |
| Landora | *Medium* | x | | | | | |
| Neruda | *Medium* | | | x | | | |
| Orthega | *High* | x | | | x | | |
| Otira | *High* | x | | | | | |
| Prestige | *Medium* | | | x | | | |
| Punto | *Medium* | | | | | | x |
| Sebastian | *Low* | | | | | x | |

For each mixture and component variety, mean grain yield for each environment as well as variation between environments was calculated. Further, within each environment rank values of grain yield ('1' the highest yield in the environment and '20' the lowest yield) of the 20 mixtures and varieties were considered and mean and variance over environments was calculated. Two measures of stability were applied: environmental variance for grain yield as well as for rank values. Finally, the mixture effect being the difference between the grain yield of a mixture and the average of its components was calculated for each environment.

## Results and discussion

The mixtures performed differently with Mix1 and Mix4 ranking well above the official standard in most environments, in some environments even better than all their component varieties, and Mix1 being among the ten best varieties in all years (data not shown). The grain yield of each mixture was in most cases higher than the average of its components when considering the mean over environments (Table 2). In average, mixtures produced significantly more (0.9 hkg/ha) than the average of their components. Mix1 and Mix4, in addition to Mix5, showed the largest effect. Both Mix1 and Mix4 included two component varieties with high weed competitiveness. The third mixture with this characteristic was Mix5. This mixture showed a significant mixture effect, however, its yield was only medium. One of the component varieties, Fabel, yielded rather low in many environments and this was to some extend compensated by the other components in the mixture. Variety Fabel was also included in Mix6 where the mixture effect was positive but not significant. Based on these results, one may suggest that 3-component mixtures should include more than one good competitor, however, this needs to be confirmed by other studies. For such comparisons of studies, a meta analysis is planned.

*Table 2. Mean yield and mean mixture effects over environments for each mixture.*

| | Mix1 | Mix2 | Mix3 | Mix4 | Mix5 | Mix6 |
|---|---|---|---|---|---|---|
| Mean yield (hkg/ha) | 52.9 | 49.3 | 48.3 | 51.6 | 49.3 | 47.9 |
| Mean mixture effect (hkg/ha) | 1.8* | 0.2 | -0.3 | 1.2* | 1.4* | 0.9 |

In general, mixtures yielded more than component varieties and also their ranking was better than that of components (Table 3). Further, the environmental variance over these very different environments, measured from either the grain yield or from the rank values, was lowest for the mixtures (Table 3). This is interpreted in the way that the mixtures were more stable than the component varieties. This pattern was found despite the mixtures were composed to demonstrate competition between the component varieties.

*Table 3. Comparison between means for 6 mixtures and for 14 component varieties.*

| | Mean mixtures | Mean components |
|---|---|---|
| Mean yield (hkg/ha) | 49.9 | 49.1 |
| Mean environmental variance $(hkg/ha)^2$ | 5.7 | 8.3 |
| Mean ranking[a] | 9.8 | 10.7 |
| Mean environmental variance of ranks[a] | 19.7 | 24.1 |

*[a] highest ranking is 1, lowest is 20*

The final result of natural selection and competition between the components of each mixture will be evaluated from additional data on these six mixtures. Seeds harvested from the mixtures each year have been sown the following year at the same location, resembling farm saved seeds. By means of DNA markers, changes in the proportions of the different components in each mixture will be estimated and related to the characteristics of the different component varieties. These data are waiting to be analysed.

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# Practical breeding for bread quality

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## Abstract

Bread wheat varieties of high-quality are receiving increasing interest in Europe. One reason is the industrialization of bread production that has resulted in rapid and intensive manufacturing processes. On the other hand, the rise of low-input, extensive or organic agricultural production requires wheat varieties that maintain an excellent quality even with a limited nitrogen input. High quality cultivars are characterized by an elevated protein content, high sedimentation values, strong gluten, and dough production that displays elevated water absorption and retention capacity combined with good resistance to extended kneading. The resulting bread ideally shows medium size pores combined with an elevated volume and a pleasant taste. Obviously, the baking quality of wheat is a very complex trait and breeding for high quality varieties is a long and arduous task. In the first breeding generations, protein content might be used as a rather simple indicator. However, protein content is strongly and negatively correlated with yield. Selecting only for high protein content might therefore result in a lower-yielding variety. In practical terms, breeding for high bread quality begins with the choice of the adequate parents lines, selected according to a large number of measured quality parameters and in particular on the composition of their high molecular weight glutenin subunits. Cultivars undergo severe baking tests in laboratory but also by professional bakers. Because the best quality wheat offers particularly good financial returns in Switzerland, breeding for quality remains a very valuable goal.

## Keywords

Wheat, plant breeding, bread making quality.

## Introduction

High quality wheat varieties are drawing increasing interest in Europe. One reason is the industrialization of bread production that has led to rapid and intensive manufacturing processes. On the other hand, the rise of low-input, extensive or organic agricultural production requires wheat varieties that provide excellent quality even under the constraint of limited nitrogen input. The Swiss wheat-breeding program has worked constantly since the beginning of the XXth century with the same basic goals in mind: excellent bread-making quality, resistance to disease, and yield.

## Breeding for high quality wheat in Switzerland

In Switzerland the context, in particular with regard to agricultural policy, offers an explanation for the constant demand for bread wheat of high-quality. Until 1999, the government held a monopoly on the wheat market, thus determining the quality group for each cultivar and fixing the price for each quality group. The harvest was then sold to milling industry at a price comparable to that of imported wheat. The millers wanted to obtain high domestic quality because of the restricted possibility to correct quality with imported wheat, traditionally hard red spring wheat from USA (HRSW) or from Canada (CWRS). Since 1999, "Swissgranum", the inter-professional organization, continues to classifiy the cultivars according to quality and provides indicative prices. The wheat of the best quality offers especially good returns in Switzerland (38 to 41 €/dt). Breeding for quality is, therefore, an important financially-driven objective. The government continues to provide protection against imports; in addition it has established a National List and promotes a more ecological agriculture with support such as "extenso" subsidies: A farmer producing cereals without fungicides, growth regulators nor insecticides qualifies to receive 267 €/ha. At this time 44% of the Swiss wheat is cultivated under this system and 3% under organic practices. Globally 10% of cultivated areas are under organic practice and 86% follows the system of "requested ecological practices." Referred to in French as "PER," (prestations écologiques requises"), these practices consist of conditions regarding crop rotation, controlled and limited use of fertilization and pesticides. As an example, only 120 to 130kg N/ha are used in wheat production, and even with this constrained nitrogen supply high quality level have to be obtained. All the wheat thus produced is used for human consumption, or in case of pre-harvest sprouting or overproduction, for animal feeding. The Swiss wheat price is so high that no exportation is possible. Therefore the quantities and quality of wheat produced in Switzerland have to match as closely as possible the requirements of the domestic market. Swiss consumers are accustomed to having bread with a shelf life of up to 3 days. They prefer, and in particular in the German-speaking part of Switzerland, medium dark bread, or bread with whole-wheat flour. This type of bread requires high quality wheat to obtain good volume and a long shelf life. Industrial bakers produce more than 2/3 of the bread. Industrial-scale activity is less able to adapt their production process to a lower or irregular quality than the craftsman bakers. The demand for high quality wheat is limited in Europe but increasing. One reason is the standardization, intensification and the use of rapid or new processes, such as "frozen dough", requiring higher quality and, in general, stronger gluten.

## Quality measurement during selection

High quality cultivars are characterized by elevated protein content, hard kernels, high sedimentation values, strong gluten, and the production of a dough that displays elevated water absorption and retention capacity combined with good resistance to extensive kneading. The resulting bread ideally shows medium size pores, an elevated volume, a pleasant taste and a long shelf life. Obviously, baking quality of wheat is a very complex trait and breeding for high quality varieties is a long and arduous task.

### Parent selection and crossing

Parent selection is an essential part of a breeding program. In the Swiss wheat breeding program at Agroscope RAC Changins, the parent lines are chosen according to the agronomical value and a large number of measured quality parameters, in particular on the allelic composition of loci encoding some high- (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). The association between the technological values of bread wheat and the presence of alleles coding for HMW-GS, LMW-GS and, in a lesser extend, for gliadin, have been described by many authors (Branlard *et al.*, 2001).

The three main sources of genitors are (1) our own lines; (2) lines exchanged with breeders looking for the same type of quality; and (3) the cultivars on National Lists. With our objective of obtaining high quality wheat in the long run, a large fraction of our lines met this quality standard and were frequently used as the parents in a cross. As the market for high quality wheat is small, and the price not sufficiently attractive, few recent cultivars are available on some Nationals Lists. In France, for example, on the 2004 Arvalis list of 116 cultivars (Bernicot, 2004), only 8 are classified in the best quality (A, "améliorant" or BAF, "blé améliorant ou de force"), and four of theses are from Switzerland, one from Germany, and the rest were already on the list in 1994 (Bernicot, M.-H., 2004). In Germany, more cultivars are available: 19 winter wheat out of 110 are classified in the best quality (E), 12 of them were added on the National List during the last 5 years (Bundessortenamt, 2004). Some of the 250 combinations for winter wheat and 100 combinations for spring wheat are three-way crosses, with one backcross on the best quality parent.

### Nursery

After crossing and up to the F4 generation, no particular quality measurement is carried out since a good estimation of the bread-making quality during these first generations is difficult to conduct on small seed samples. Starting from the F5 generation, grain protein content and grain hardness are analyzed using near-infrared reflectance spectrometry (NIRS) on whole grain. For the flour, the Zeleny sedimentation test (precipitation of proteins in an lactic acid solution, ICC 116/1) is performed.

Protein content is generally considered to be a prime factor for the assessment of the quality of wheat flour. It correlates well with some quality parameters, such as water absorption ability of the flour (Fig. 1), but weaker correlations are observed with other bread quality traits (Figs. 2 and 3). However protein content shows a strong negative correlation with yield, as frequently observed (Stoddard & Marshall, 1990). Protein content and yield of the lines and cultivars tested between 1983 and 2001 in our preliminary and official yield trials (Fig. 4) provide an image of this negative relationship between two important breeding goals. Selecting only for high protein content might therefore result in a low-yielding variety. As an example, Trethowan *et al.* (2001), using a selection intensity of 50%, calculate
the likelihood of selecting among the top 10 lines for loaf volume (LV), strength of the dough (ALW) or yield using flour protein content (FPC) as the unique selection criteria. If the likelihood is 90% and 75% for LV and ALW respectively, it is less than 20% for the yield. Using SDS (an adaptation of the Zeleny test with the use of sodium dodecyl sulphate, ICC 151)

as a selection criterion, the likelihood is 50% for LV, 90% for ALW and less than 50% for yield. They proposed to use the ratio SDS/FPC as a selection criterion, and they thus obtain a likelihood of 56% for LV, 75 for ALW and 60 for yield (Table 1). Following this proposal, we use a similar index: Protein content / Zeleny.

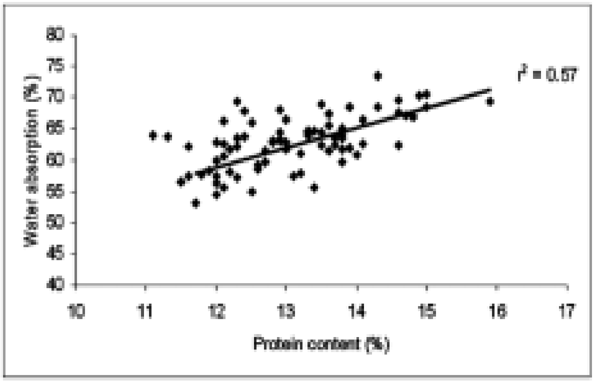

*Figure 1. Protein content and water absorption relationship. Officials trials 1997 to 2001.*

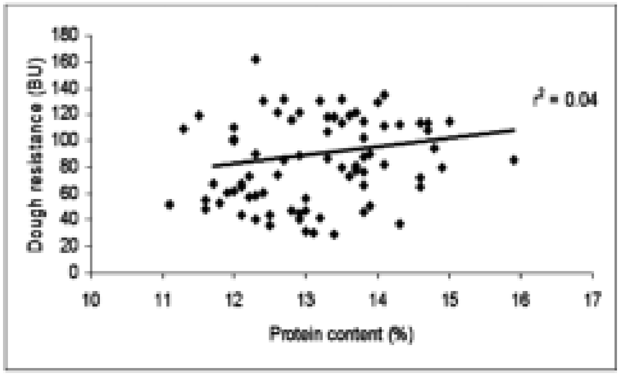

*Figure 2. Protein content and dough resistance.Officials trials 1997 to 2001. (BU=Brabender Units)*

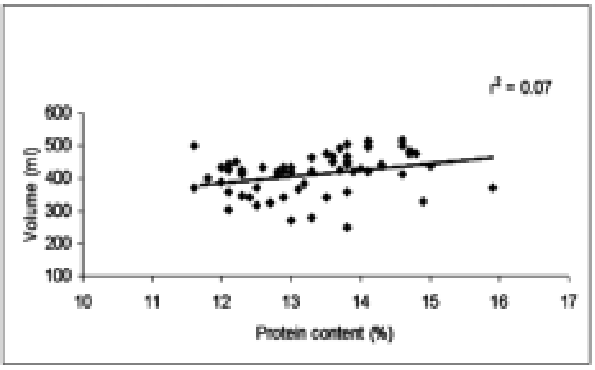

*Figure 3. Protein content and loaf volume. Officials trials 1997 to 2001.*

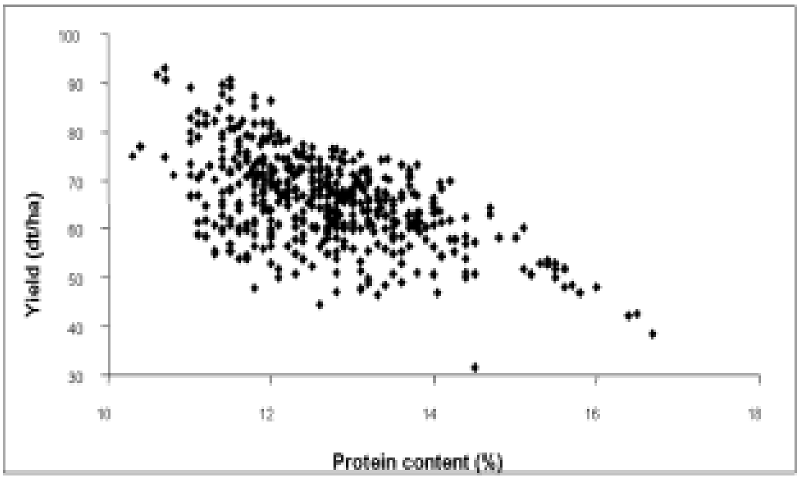

*Figure 4. Protein content and yield relationship. Registration and preliminary yield trials 1983 to 2001.*

*Table 1. Likelihood (%) of selecting lines among the top 10 with a selection intensity of 50% based on flour protein content, SDS, SDS / flour protein content. Based on Trethowan et al. (2001).*

| | **Likelihood (%) of selecting lines among the top 10 with a selection intensity of 50% based on** | | |
|---|---|---|---|
| | flour protein content | SDS | SDS / flour protein content |
| Loaf volume | 90 | 50 | 56 |
| Alveogram strength | 75 | 90 | 75 |
| Yield | <20 | <50 | 60 |

### Yield trials

The yield trials are carried out under "extenso" conditions using low nitrogen fertilization (~120 kg N/ha). As soon as enough grain material is available (F7), the dough quality is also evaluated with the classical methods and apparatus:

- Falling number method (ICC 107/1),
- Gluten Index (ICC 155),
- Brabender Farinograph and
- Brabender Extensograph or Chopin Alveograph.
- Viscosimeter.

Special attention is paid to the bread-making test. The harvests of the preliminary yield trials are used for a "Rapid Mix Test" (RMT). 30 small loaves are prepared with 1kg flour, the volume is measured, and the loaf and crust structure are evaluated. At the end of the breeding procedure, breeding lines are further tested within the official trial network before being registered in the Swiss and EU varieties list. In the evaluation scheme for the final classification, cultivars undergo two final bread-making tests. In the first, the volume is measured along with the porosity on breads baked in pans after 3 different fermentation times. Last but not least, a professional bakery school does a baking test with 5kg flour. The bread volume, crust, color, porosity and taste are observed.

## Results

### Cultivars released

Between 1981 and 2004, 46 cultivars from the Agroscope have been released in Switzerland and 22 in other countries. In 2003, 85% of the domestic wheat production used Agroscope cultivars. In France, the Swiss cultivars represent 1.5% of the multiplication area.

### Use of HMW-GS composition in selection

Branlard *et al.* (1985) have proposed a quality coefficient based on HMW-GS composition. For each quality parameter, the fraction of phenotypic variation explained by the HMW-GS composition varies but can reach 35% (Branlard *et al.*, 2001). In fact, some cultivars can demonstrate good quality even with sub-optimal HMW-GS composition. For example, by looking back at the HMW-GS of leading cultivars between the 1950's and the 1980's in Switzerland (Probus, Zénith and Arina), it is interesting to note that, even with a good quality classification, two of them have a poor HMW-GS quality coefficients. Ignoring the HMW-GS score, they were used for many crosses for many years but, and this is no longer a surprise, only few good-quality lines resulted from those crosses. Nevertheless we also observed that breeding (at that time) without any previous knowledge or assessment on the HMW-GS composition, brought the same favorable subunits alleles 5-10 in all the recent released quality wheat (Table 2). This observation underscores the importance of deep knowledge of the parents. But, as is the case for every selection criterion, the over use of the HWG-GS composition as an indicator would lead to a loss of performing cultivars.

*Table 2. Allelic composition of high-molecular-weight glutenin subunits in some Swiss cultivars.*

| Cultivar | Quality class £ | Year of release | Locus | | | Quality |
|---|---|---|---|---|---|---|
| | | | Glu-A1 | Glu-B1 | Glu-D1 | coefficient $ |
| Probus | 1 | 1948 | 1 | 6-8 | 2-12 | 24 |
| Zénith | 2 | 1969 | Nul | 7-9 | 3-12 | 26 |
| Arina | 1 | 1981 | Nul | 7-8 | 2-12 | 22 |
| Tamaro | "Top" | 1992 | 1 | 7-9 | 5-10 | 65 |
| Runal | "Top" | 1995 | 1 | 7-9 | 5-10 | 65 |
| Zinal | 1 | 2003 | Nul | 7-8 | 5-10 | 45 |
| Siala | 1 or "Top" # | 2005 # | 1 | 7-8 | 5-10 | 60 |
| Cimetta | "Top" # | in test | 2* | 7-8 | 5-10 | 75 |

*£ Swissgranum (Swiss inter-professional organization of cereals) or former the Federal Wheat Administration;*
*$ according Branlard et al., (1992);*
*# to be confirmed.*

## Outlook

Some trends are observed in the demand for wheat quality. From the older and simplified division between bread wheat, biscuit wheat and feed wheat, more segmented quality classes are emerging. New processes in bread making, news kind of bakery products, special quality requirements for specific products (pizza, buns, etc...) are some of the reasons for this change. Some cultivars with special traits (yellow flour, extra strong gluten or taste) could meet a special interest. We are now working with professionals and a "consumer" tasting panel to measure differences between varieties for bread taste. Up to now we have not observed sufficient differences between cultivars to justify discarding or promoting specific cultivars for this trait. Up-to-date knowledge about the needs of the milling industry in terms of cultivar quality would be advantageous to best exploit the qualities of the cultivars.

## Conclusions

Breeding for high quality wheat is a financially attractive goal only in a context where this quality is demanded and its price therefore justified. This is the case in Switzerland, and partially in other countries; breeding for quality remains worthwhile and warranted. By having maintained this goal on the long term we have accumulated good alleles in our gene pool. As bread-making quality is a complex trait, the use of each quality test, or markers, has to be integrated in a selection strategy and used with care. Even if a special trait might offer an advantage, a cultivar will always represent only the "best" compromise between a large numbers of traits.

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# Use of molecular markers to improve wheat quality in organic farming systems

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## Abstract

Organic farming systems for cereal production are characterized by a low level of nitrogen available at grain filling stage. The consequences are a low grain protein content, which leads to poor dough properties. Obviously, organic farming practices should tend to better nitrogen supply, and breeding to the improvement of nitrogen use efficiency. Alternatively, a better balance between the different grain protein fractions could be worked out, so as to provide bread-making suited grain even with low protein content. Particularly, the ratio of high molecular weight (HMW) on low molecular weight (LMW) glutenins, and of glutenins on gliadins are critical for bread making abilities. This relative composition of storage proteins is controlled by both environmental and genetic factors. Genetic factors involved in storage protein composition are the promoter sequences of storage protein genes containing cis-regulating elements, and genes coding for transcriptional factors which interact with these promoters. Although this regulation network is complex and far to be fully elucidated, some breeding tools can be proposed. Most storage protein coding genes have been cloned and sequenced, and specific primers can be used to select the most favourable allele combinations in breeding programmes. Similarly primers have been developed to amplify the four transcription factors known to interact with storage protein genes. The primers have been used to explore allelelic variability in germplasm collections and to develop allele specific markers (SNPs). They could be used for assisting selection for optimized and environmentally more stable protein composition of wheat grain under organic farming practices.

## Keywords

Dough strength, glutenins, gliadins, transcription factors.

## Introduction

The main utilization of bread wheat produced under organic farming systems in Europe is human food, which also gives the farmer a higher income than animal feeding. It is therefore of utmost importance that wheat grain produced in organic farms fits the quality required for the range of bread-making and other cereal product transformation. To be suitable for bread-making, wheat flour must give dough with desirable rheological properties, namely a balance of tenacity, extensibility and “strength”, to allow good bread making. Although these requirements vary from one product to another, they tend to increase when the flour is less “purified”, as usual in organic baking, since bran particles contain more fibre and health-promoting components than white flour. Thus whole flour bread-making usually requires stronger wheat. Under intensive farming practice, i.e. non limiting fertilization, most strong wheat varieties have higher than average protein content, often associated with a slightly lower grain yield. However in organic farming systems, grain protein content is often limited by nitrogen supply from the soil, in the absence of mineral fertilizers, as shown in Figure 1. Therefore improving dough strength at low or moderate (at best) protein content should be viewed as the first objective when breeding wheat for quality in organic farming systems. Other quality traits that must be considered include Fusarium resistance and low mycotoxin production (not considered further here) and health-promoting micronutrient content. However few makers have been developed for health-related traits or micronutrient content, and most published works report markers for major grain components, protein and starch. We will give a review of markers available for genes related to the major and some minor grain components, and some references to the methods that can be used to exploit them in plant (organic) breeding.

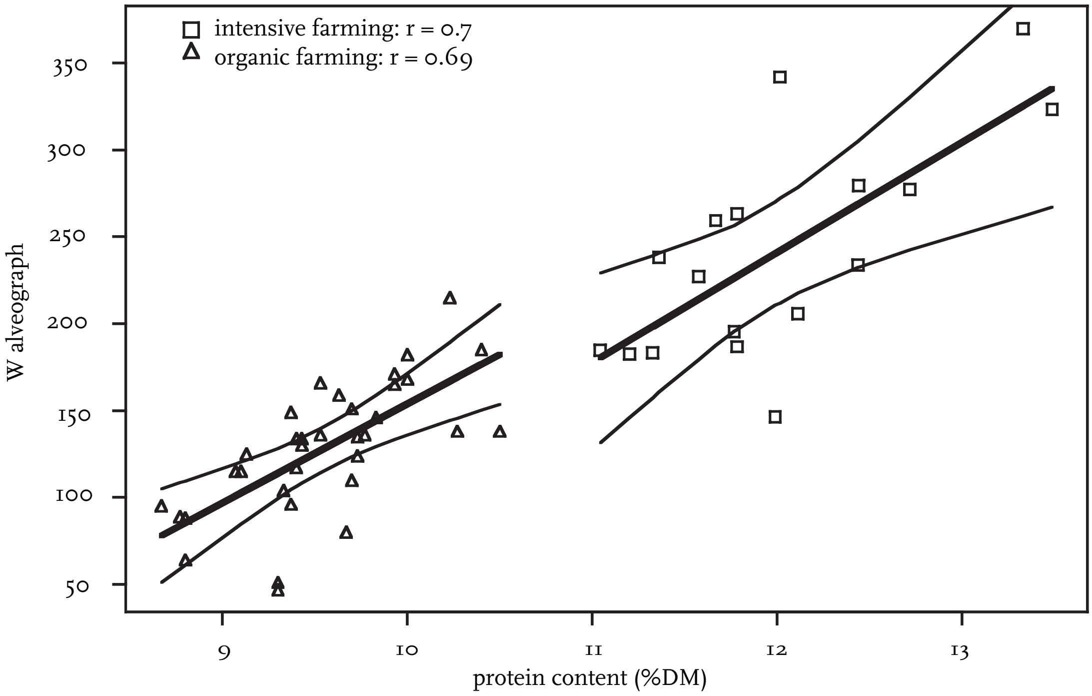

*Figure 1. Comparison of dough strength/protein content in low input systems and organic farming. A similar positive correlation is observed in both systems, but the average protein content is much lower (9.4%) in organic farming than with intensive mineral fertilizer (12.8%). As a consequence, dough strength from organic wheat is often below the threshold required for bread-making (around 150).*

## Molecular markers for storage protein allelic composition

Although grain protein content is the main determinant of dough strength, there are other genetic factors which allow variation in bread-making quality at constant protein content. These factors are associated with storage protein composition, i.e. the relative proportions of the different fractions, namely the gliadins, low molecular weight glutenins (LMW-GS) and high molecular weight glutenins (HMW-GS). These latter were probably the first class of molecular markers to have been proposed for assisting selection. Indeed most alleles are easily distinguished using Poly-Acrylamid Gel Electrophoresis (PAGE), and their effects on dough quality have been estimated through multiple regression on cultivar collections as soon as 1979 (Branlard & Dardevet 1985, Payne 1987, Hamer *et al.* 1992). These methods, nowadays called association genetics, allowed the authors to suggest relative weighting to be given to each allele combination at the three HMW-GS loci (each locus actually comprises two tightly linked genes names x and y) for constructing selection indices, and hence the papers from Branlard and Payne can be considered as historical references for marker assisted selection. Although they explain a lower proportion and quality variation, index weights for LMW-GS alleles and gliadins have also been proposed (Gupta *et al.*, 1994; Fido *et a.,*. 1997; Branlard *et al.*, 2001; Luo *et al.*, 2001). Although PAGE is quite easy to perform from a single (even a half) crushed kernel, molecular markers are now preferred, since the development of semi-automated high throughput genotyping platforms, which enable thousands of markers to be scanned from very little amount of DNA. Therefore a huge effort has been placed to develop molecular markers for the most frequent and useful alleles for HMW-GS (Anderson & Green 1989, D'Ovidio & Anderson 1994, De Bustos *et al.* 2000, 2001), LMW-GS (Cassidy et al 1998, Ikeda et al 2002, Zhang et al 2004, Zhao et al 2004) and gliadins (Zhang et al 2003). Rapid and elegant high throughput methods based on multiplex PCR markers have recently been developed (e.g. Ma et al 2003), and are now routinely used in both public institutes and private companies.

## Molecular markers for quantitative variation of protein fractions and protein reticulation

Dough quality mostly relies on the mechanical properties of gluten, which is a complex macro-polymeric structure which involves the different classes of storage proteins. The skeleton of gluten polymer is made of reticulated HMW-GS, thanks to inter-molecular disulphide bonds, on which LMW-GS and gliadins are linked by either disulphide bonds or other type of molecular interactions. Therefore, beside the number and distribution of cystein residues along the protein, which depends on allelic composition described above, the relative proportion of the different storage protein fractions, and more particularly the ratios of HMW/LMW-GS and of glutenins on gliadins also influence dough properties (Shewry *et al,.* 2001). The relative composition of storage protein fraction, at a given level of nitrogen availability depends on the expression level of the numerous coding genes. The regulation network of storage proteins is only partially understood. So far, three families of transcription factors have been identified to interact with specific sequences located in the promoter region: the bZIP family (SPA: Albani *et al.* 1997, Guillaumie et al 2004, BLZ2: Onate *et al.* 1999), the DOF family (PBF: Mena *et al.* 1998) and the MYB family (GAMYB: Diaz *et al.* 2002). The molecular mechanism of interaction with specific boxes in storage protein promoters has been studied in a few cases (Albani *et al.* 1997, Conlan *et al.* 1999, Carbonero *et al.* 2000). However no quantitative variation of storage protein association has been associated with sequence variability of transcription factors yet. Therefore variability in the promoter region has also been explored (e.g. Anderson *et al.* 1998), and some particular alleles have been found to be more expressed than others (Norre *et al.* 2002), and in a few cases such over-expression has been associated with quality traits (Butow *et al.* 2003). Although more studies are needed to fully understand this complex regulation network, its manipulation through molecular markers seems very promising, particularly to improve the stability of protein composition under extreme environments, such as those encountered in organic farming systems.

In addition to the regulation of storage protein genes, other factors are involved in polymeric gluten formation, particularly oxydo-reduction enzymes which are responsible of disulfide bond formation, such as gluthatione, thioredoxine or protein disulfide isomerase (PDI) or other chaperons which affect protein folding (Carceller & Aussenac 1999). Therefore markers derived from allelic variation of these genes, or even from genes which regulate them, could be also useful to assist quality improvement (e.g. Ciaffi *et al.* 2001).

## Molecular markers for other quality traits

Starch is the main component of cereal grain, and its molecular structure also influences dough properties, although to a lesser extent than proteins. The first genetic factor associated to starch is grain hardness vs softness, which controls the proportion of damaged starch in flour. Damaged starch is more frequent in flour from hard varieties and has a much higher water affinity than non-damaged starch. Therefore flour from hard varieties gives stronger dough, more adapted for bread-making, while flour from soft varieties are preferred for biscuit-making. The causal gene explaining most variation for grain hardness is now well established (Giroux & Morris 1998) and a lot of SNP markers have been designed to identify all known alleles (Morris 2002). The other important aspect is starch composition, namely the ratio between amylase and amylopectin, which is mostly controlled by starch branching enzymes (SBE: Rahman et al 1997). Indeed the branched amylopectin has some desirable properties for end-use product, but also for human nutrition (it has a lower glycemic index, which is protective against diabet). Molecular markers derived from SBE have been used successfully to develop high amylopectin (waxy) wheats (Zhao & Sharp 1998, Yanagisawa *et al.* 2003, Blake *et al.* 2004).

Among the other quality traits that could be looked for more particularly in organic production are the health-promoting components. Among these, we can mention the minerals, and more particularly magnesium, for which whole flour products can be a valuable source. Recently we showed that magnesium content appeared to be heritable and likely to respond to selection (Oury et al 2005), but neither gene nor QTL has been identified so far. Another approach to increase (whole) flour mineral content could be to increase the thickness of aleurone layer. For example we recently identified SNP variation in wheat for the supernumerary aleurone layer gene (Sal1, Shen et al 2003). Other micronutrients such as vitamins or anti-oxidants can be found in wheat. For example QTLs have been found and molecular markers developed for carotenoid content in durum wheat (Elouafi et al 2001) and bread wheat (Mares *et al.* 2001). However their real interest in human nutrition is questionable (much higher content in many fruits or vegetables).

## Methods for efficient use of markers in (organic) breeding

Methods of marker assisted selection can be basically assigned to one of the following class. The first one is (recurrent) population improvement, in which marker "scores" are integrated into selection indices beside phenotypic data, and allow a faster increase of favourable alleles in the population (e.g. Hospital *et al.* 2000). Although there are few applications of such methods in a strict sense, the heuristic method proposed by Ribaut & Hoisington (1998) is closely related. This method consists in a first step of selection using markers only, then in releasing the improved material to classical breeders in the third world, which could be easily extended to participative breeding in organic systems. The other family of marker assisted methods is known as genotype building or gene pyramiding (e.g. Servin *et al.* 2004), depending of the type of crossing scheme employed, the most popular one being the back-cross. Theoretical and practical considerations have been proposed by many authors, and an application to wheat quality was illustrated by Charmet *et al.* (2001). This type of methods proved very useful in many species (e.g. tomato) for exploiting more efficiently the much wider variation found in old landraces and related alien species, such as the diploid progenitors of bread wheat. It is not so clear for me whether such wide crosses could be acceptable in organic breeding practices. Similarly most haploid in wheat are issued from wide pollination by maize to induce gynogenesis. Since haploid and doubled haploid allow gain in selection response efficiency, particularly when combined with the use of molecular markers (e.g. Radovanic & Cloutier 2003), I hope that this technique is not excluded by organic and bio-dynamic breeders as anther culture or male-sterility are, among others. Fortunately DNA marker techniques are accepted if the enzymes used are GMO-free and no radioactivity is used (Muller 2002), as it is the case for markers described in this paper, which can therefore be integrated in future organic breeding programmes.

*Table 1. Summary of quality related genes for which molecular markers (mostly SNPs) are available for marker assisted organic breeding.*

| **Gene** | **Chrom location** | **Target trait** | **Reference for SNPs** |
|---|---|---|---|
| *Sal1* | 7AL0,71-1,00<br>7BL0,48-1,00<br>7DL ( ?) | Aleurone layer (supernumerary) | Shen *et al.* 2003 PNAS 100: 6252-6557 |
| *Ga-Myb* | 3AL3-0,42-0,78<br>3BL10-0,5-0,63 | Transcription factor (storage proteins) | Ravel et al, PAG 2005 |
| *SPA* | 1AL<br>1BL<br>1DL | Transcription factor (storage proteins) | Guillaumie *et al.* 2004. Genome 47: 705-713 |
| *PBF* | 5A<br>5B<br>5D | Transcription factor (storage proteins) | |
| *Glu-A1-1*<br>*Glu-B1-1*<br>*GluD1-1* | 1AL<br>1BL<br>1DL | Storage protein HMW-GS | D'Ovidio & Anderson 1994. Theor. Appl. Genet. 88: 759-763, De Bustos *et al.* 2001 Euphytica 119:69-73 |
| *Glu-A3*<br>*Glu-B3*<br>*Glu-D3* | 1AS<br>1BS<br>1DS | Storage protein LMW-GS | Zhang *et al.* 2004. Theor. Appl. Gent. 108: 1409-1419 |
| *Gli-A1*<br>*Gli-B1*<br>*Gli-D1* | 1AS<br>1BS<br>1DS | Storage protein Gliadins | Zhang *et al.* 2003. Theor. Appl. Gent. 107: 130-138<br>Zhao *et al.* 2004 Zuo Wu Xue Bao 30:126-130 |
| *GSP*<br>*Pina*<br>*Pinb* | 5DS<br>5DS<br>5DS | Grain hardness | Giroux & Morris. 1998. PNAS 95: 6262-6266 |
| *Wx* (waxy) | 7A | Starch branching (amylopectin) | Blake *et al.* 2004. Theor. Appl. Genet. 109: 1295-1302 |
| | 7D | | Yanagisawa *et al.* 2003. Theor. Appl. . Genet 107: 84-88 |
| Agp-L | 1A<br>1B<br>1D | Amylose synthesis | Blake *et al.* 2004. Theor. Appl. Genet. 109: 1295-1302 |
| *SUT* | 4A<br>4B<br>4D | Sucrose transporter | Blake *et al.* 2004. Theor. Appl. Genet. 109: 1295-1302 |

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# Barley seed borne diseases under field selection with natural infection

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## Abstract

Different spring barley collections were tested for susceptibility to barley leaf stripe (Drechslera graminea), covered smut (Ustilago hordei) and loose smut (Ustilago nuda) under organic farming conditions in Northern Germany. Results under simulated natural infection indicate that more factors were important for the degree of infestation than documented for artificial inoculation. Breeding between infected spreader lines and under infection of the seeds was started to increase the background-level of resistance, independent from known and unknown genetic components of resistance, by excluding the medium to high susceptible descendants of each crossing. The described method is presently used as a long term solution to make it possible to multiply seed under organic farming from maintaining up to certified seed beneath the implementation of well known monogene resistance for short term solutions. It shall lead to varieties which can live with diseases on a low and acceptable level of infestation as a part of the ecosystem in organic farming.

## Keywords

Organic resistance breeding, barley leaf stripe, covered smut, loose smut.

## Introduction

Continued breeding and multiplying under organic farming conditions will sooner or later bring all kinds of seed transmitted diseases, which can survive under the environment of the location, where breeding takes place. Any exchange of germplasm introduces more or less diseases, if the wind does not. For this reason during the last 15 years of organic variety testing, seed saving and breeding for hulless spring barley at Cereal Breeding Research Darzau three seed borne diseases of barley appeared. Although they had been trying hard, breeders were not successful to remove all plants with barley leaf stripe, covered smut and loose smut from our collection. Also hot water treatment, which is allowed under organic farming, was not effective enough to get rid of all these diseases during multiplication. Nevertheless breeding for resistance is a breeder's choice and thus searching for resistance and its implementation started step by step.

## Barley leaf stripe

Barley leaf stripe can become a problem under environments with cold temperatures during germination. In Northern Europe spring barley and in Southern Europe winter barley is endangered. For testing susceptibility under natural conditions at Cereal Breeding Research Darzau now barley leaf stripe can infect from spreader plots, which are implemented in the whole breeding area between F1 up to F9 every third plot or plot row every year. These spreader plots contain between 20 to 40 plants with barley leaf stripe per square meter. In F3 barley leaf stripe then is established in and between the descendants for selection under natural infection conditions.

Between 1999 and 2002 about 600 barley gene bank accessions were tested for resistance to barley leaf stripe under natural infection. More than 30% of these accessions were observed to be resistant with less than 1% infection. 1% of all tested accessions got more than 40% infection. In particular varieties with 'Vada'- or 'Betzes'- resistance were observed without infection, but those with 'Lion'- or 'Thibaut'-resistance were found to be susceptible (Mueller, *et al.* 2003). Tests of modern spring barley varieties of the official German list just started in 2004 and first results will be available in 2005. 'Betzes'-resistance is a quantitative resistance and has its origin in Bohemia. It is expected that parts of this quantitative resistance are spread all over the world into many varieties by the use of most famous brewing variety 'Hana' as parent in many crossings and pedigrees (Mueller *et al.* 2003). For this reason an accumulation of partial resistance can be expected during breeding under conditions of natural infection with eliminating the most susceptible descendants.

At present 70% of all descendants in Darzau from F6 to F9 have not more than 3 infected plants per square meter and not more than 5% still have more than 10. Because barley leaf stripe is not checked during field and seed inspection in Germany, it is not necessary to get immunity. For the farmer up to 3 plants/m_ is tolerable under economic aspects, if the number doesn't increase during maintaining. Because always enough plants without leaf stripe can be selected from the susceptible, also prebreeding for other characters than resistance was implemented in the area under infection and not done separately.

## Covered smut

Covered smut becomes visible at the end of ear emergence period. Often the infected ears remain in the leaf sheath or infected plants remain shorter than the healthy plants. In Germany it can be expected that the covered smut spores will take over to the healthy seeds usually only during harvest by threshing and destroying the ears with covered smut. For this reason the disease sometimes can spread during the first generations of multiplying or maintaining unless breeders get aware of the infection.

To simulate a natural infection at Darzau the spores are harvested from smutted ears from well known susceptible checks. They are stored after drying in a refrigerator till one month before sowing. Then the spores are given in a concentration of at least 1g of spores per kg of seed to the ear descendants in the plot magazines from F4 up to F9. For getting a good distribution of spores over the seeds it is necessary to shake the seeds with the spores for at least a few seconds. With this method of inoculation it was not possible to get higher infection rates by using more than 1g of spores per kg seed.

During the years 2002 and 2003 among 55 varieties of spring barley from the official list tested, 13 remained free of covered smut and another 20 got less than 1% infection. Most susceptible to covered smut were 'Tunika' with 13% and hulless 'Taiga' with 15% infected plants (Mueller, 2005b). Because 60% of the varieties got not more than 1% infection, it was risked to start with setting the whole breeding area under infection with covered smut.

At present 70% of all descendants from F6 to F9 have no infected plant per square meter and the descendant with the highest susceptibility had 12. Of course there are still varieties in the maintained barley collection with up to 30. Unfortunately only some of the 70% progenies resistant to covered smut can be found among the 70% resistant to barley leaf stripe.

## Loose smut

Loose smut has to be seen as the most important seed borne disease of barley, because the disease takes over from the infected spikes to the healthy flowers within the day, when they become visible. For selection of resistant varieties loose smut is inoculated artificially as a suspension of 1g of spores to 1 litre of water per injection with a syringe directly into the flowers of one ear per descendant in F3 and/or F4 at Darzau. Seeds from these infected ears are grown in one drilling row beneath the selected ears of the same group of descendants in the following generation. From these infected plants the loose smut can take over naturally into the segregating descendants during the following generations. If necessary and of interest an artificial inoculation is repeated in later generations, for instance to distinguish physiologic resistance from closed flowering or accumulation of natural, unknown partial resistance.

During two test cycles in the years 2002 to 2004 up to 63 modern varieties of the official list were tested under simulated natural and artificial inoculation with loose smut. Relations between artificial and natural testing could only be found related to strong resistance, but there were no relations between degrees of susceptibility in both systems of testing (Mueller, 2005a). Only variety 'Steffi' remained absolutely free from the local loose smut and seems to carry Un6-resistance. Another 12 varieties got less than 1% infected plants under natural conditions. Most susceptible to loose smut were 'Maresi' with an average of 5%, 'Viskosa' with 8% and 'Danuta' with 12% infection. Varieties which remained below 1% infected plants under natural infection could have up to 90% infected plants after artificial inoculation. Closed flowering and escaping of the growing plant related to the hyphi of the fungus seemed to be much more important under natural conditions than expected. Varieties which can keep the infection below 2% will not become an economic risk for the crop producing farmer, but for the farmer, who is going to produce seeds for the market. This points to the necessity of implementing resistance with full immunity to loose smut. But different origins of loose smut from some countries of the European Union tested on a set of spring barley accessions with different sources of resistance showed many interactions. Only a few accessions remained free from infection related to all tested origins of loose smut (Mueller, 2005a).

This indicates a relatively short lifetime for monogene resistance and the need for long term breeding of quantitative loose smut resistance.
In Darzau at present 10% of all descendants from F6 to F9 are resistant to loose smut after artificial infection. There is no information about the natural susceptibility of all descendants up till now, because the natural infection with loose smut is still going to be increased to the whole breeding area step by step.

## Conclusions from evaluations and breeding experience

More than twenty characters have to be looked for in breeding for hulless spring barley for human nutrition and organic farming. Light competitiveness, height, absolutely hulless threshing, spotless grains and viscosity are only some of those characters, which have to be fulfilled by a new variety for this purpose. Resistance to loose and covered smut and barley leaf stripe has to be brought together with all other characters asked for that are still not available in an up to date variety. Parallel to these aims yield and yield stability have to be increased to. All this has to be done in being aware that the market for organic varieties and in particular for hulless spring barley is very small, not allowing high investments. On the other side evaluations showed that there are more or less different sources with partial resistance available in modern varieties and genetic resources.

Perhaps it would be better to think of a susceptible line as a line with a partial resistance, which needs a perfection in coming together with other partial resistance of susceptible varieties. Thus creating very low susceptible varieties during continuous selection and intercrossing under conditions of natural infection. But this long term breeding strategy needs another view to threshold values of seed borne diseases too. It has to be aimed to live with the disease on a low level of infection and not trying to exterminate the disease as a part of the ecological environment, which shows us a lack of adaptation to the present organic growing conditions.

From a practical point of view a short term strategy has to be focused too. The implementation of monogene resistance to loose smut with zero attack is necessary to fulfil the demands of the seed marketing regulations of today. But this kind of “immunity” as an aim of breeding is a non-ecological concept. If the conditions for the disease are given by the environment, but the plant cannot become ill, because the available races of the disease are not virulent related to the resistance, the plant stays vulnerable. This vulnerability is an open door for destruction on other levels of existence, i.e. other diseases. Susceptibility on a low level gives the opportunity to become aware of changes in environment or cultivation, which will bring illness. This gives the chance to change the situation in an ecological way, including varieties related to cultivation and other conditions. For this reason the community of organic plant breeders should also focus on the adaptation of seed regulations to a wise level between ecology and economy.

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More details about the three diseases and results of evaluations are available at www.darzau.de .

# Application of DNA markers in breeding for disease resistance in cereals

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## Abstract

One of the challenges facing breeders during the development of improved crop cultivars for either conventional or organic conditions is the incorporation of resistance to diseases. Since domestication of plants for human use began, diseases have caused major yield losses and have impacted the wellbeing of humans worldwide. The incorporation of disease resistance genes into plants has been successfully achieved using classical methods, which involve selection and evaluation of large progeny populations derived from crosses made between resistant and susceptible parents and subsequent screening under disease conducive conditions. Virtually all agricultural crop cultivars in use today have some form of genetic resistance incorporated, generally against a number of diseases. This may involve single or multiple genes that are characterized as having recessive or dominant effects (Crute & Pink, 1996). Without the incorporation of these resistance genes, crop productivity and yield would be substantially reduced. In the last decades new technologies emerged supporting breeders in selection which takes place on DNA level. However, idea of indirect selection using genetic markers was first reported by Sax (1923) over 80 years ago. Until recently it has been impossible to implement this idea, since not sufficient genetic marker were available. DNA technologies fulfill this requirement and molecular breeding for resistance in cereals became feasible.

## Introduction

Public concerns over pollution, food safety and human and animal health, as well as by the value set on nature and the country-side resulted in recent increase of interest in organic farming systems. Organic farming involves a production management system based on the ecological principles of nutrient cycling, biotic regulation of pests and biodiversity. Synthetic fertilizers and pesticides are generally not allowed (EU-directive 2092/91) and are replaced by sunlight-based inputs, such as plant and animal residues. Therefore, in organic conditions diseases resistance strategy relays on good farm management (crop rotation, soil fertility maintenance, etc.) plant morphology specific and non-specific resistance or disease tolerance mechanisms. Genetically inherited resistance is common target for both conventional and organic breeding programs. Recently, conventional breeding strategies incorporated to some extent method of plant selection on DNA level using molecular markers (marker assisted selection, MAS). There are well-elaborated MAS protocols for manipulation of disease resistance loci mainly against foliar and ear diseases. In the concept of organic plant breeding, DNA diagnostic techniques including selection of individuals on DNA level are allowed (Lammerts van Bueren *et al.*, 2003). Therefore, organic breeding programs could benefit from experiences of conventional breeding in MAS applications. In the next part of the paper, main advantages and limitations of MAS will be presented (mainly based on MAS applications in wheat and barley) and their possible inferences for organic breeding will be provided.

## Mapped resistance loci in wheat and barley

Identification of molecular markers linked to the trait of interest is first fundamental step in their further application in marker assisted selection (MAS). From among various marker systems available, I would like only to recognize marker system specifically related to the resistance loci. Numerous genes that confer resistance to a variety of plant pathogens have been sequenced and characterized (Hammond-Kosack & Jones, 1997; Richter & Ronald, 2000; Hulbert *et al.*, 2001). The genes that seem to be involved in signal transduction and behave in a gene for gene manner (Flor, 1971) have been classified into four groups. The majority resembles intracellular receptors and contains a predicted nucleotide binding site leucine-rich repeat (NBS-LRR) structure. Distinguished conservative motifs have been widely used to design degenerate oligonucleotide primers to isolate R gene analogs (RGAs) by polymerase chain reaction amplification (Kanazin *et al.*, 1996; Yu *et al.*, 1996; Mago *et al.*, 1999; Garcia-Mas *et al.*, 2001; Czembor & Czembor, 2002). Many of these sequences have been located to chromosomal regions containing major R genes as well as quantitative trait loci (QTL) (Leister *et al.*, 1998; Chen *et al.*, 1998; Collins *et al.*, 1998, 2001; Geffroy *et al.*, 2000; Toojinda *et al.*, 2001; Pflieger *et al.*, 1999).

Extensive mapping experiments in bread wheat and relative species allowed identification markers linked to the resistance loci for most diseases (in brackets the casual agent is provided) of wheat reviewed by Gupta *et al.* (1999) and Langridge *et al.* (2001), including powdery mildew (*Blumeria graminis* f.sp. *tritici*), leaf rust (*Puccinia recondita* f.sp. *tritici*), stem rust (*Puccinia graminis* f.sp. *tritici*), stripe rust (*Puccinia striiformis* f.sp. *tritici*), fusarium head blight (*Fusarium* spp.), loose smut (*Ustilago tritici*), karnal bunt (*Tilletia indica*), tan spot (*Pyrenophora tritici-repentis*), eyespot (*Tapesia yallundae*), wheat streak mosaic virus, cereal cyst nematode (*Heterodera avenae*), *Septoria tritici* blotch (Arraiano et al., 2001; Brading *et al.*, 2002; Adhikari *et al.*, 2003, 2004a, b; McCartney *et al.*, 2003), *Stagonspora nodorum* blotch (Czembor *et al.*, 2003; Schnurbusch *et al.*, 2003; Arseniuk *et al.*, 2004). Recent reviews on molecular genetics of disease resistance in barley (Che_kowski *et al.*, 2003; Williams, 2003) provide information about molecular markers closely linked to quantitative and qualitative resistance genes against number of diseases including: powdery mildew (*Blumeria graminis* f.sp. *hordei*), leaf rust (*Puccinia hordei*), stem rust (*Puccinia graminis*), stripe rust (*Puccinia striiformis*), scalad (*Rynchosporium secalis*), barley leaf stripe (*Pyrenephora graminea*), net blotch (*Pyrenephora teres*), barley stripe mosaic virus (BSMV), barley mild mosaic virus (BaMMV), barley yellow mosaic virus (BaYMV), barley yellow dwarf virus (BYDV), scab (*Fusarium* spp.).

## MAS applications

Once new molecular based approach in selection of breeding material has emerged, it starts to compete with classical selection. Apart from attractiveness of molecular markers in many aspects, they should not replace well-adopted and cheap conventional screening systems and philosophical question “to be or not to be” selected with molecular tools should be based on real advantages than just to be trendy. The great advantage of MAS is demonstrated by selection of individuals (on a single plant basis) carrying target genes based on patterns of tightly linked markers rather than on their phenotypes (Koebner & Summers, 2003). Therefore, plant growth stage and influence of various environmental factors can be neglected in screening procedure. MAS can overcome interference from interactions between alleles of a locus or other loci and can increase efficiency of selection for low-heritability diseases (e.g. fusarium head blight), that are difficult to manipulate under classical breeding scheme. In segregating populations, tightly linked markers allow the selection of recessive genes and those difficult in phenotyping. That aspect of MAS is of particular importance in backcross programmes aimed to introgression of resistance alles to elite genotype (the recurrent parent) with the minimum amount of genetic material from the donor (especially in cases where non-adapted germplasm is used) of the resistance; and further in case of pyramiding resistance genes against diseases comprising many physiological races like rusts and powdery mildews. MAS strategies allow to build up more durable resistance under organic farming systems, that is based on several resistance genes (against one disease) including those of quantitative character contributing to more stable yielding and good quality of seed.

Economy issue is the main constraint in wide application of MAS strategy in breeding process. The molecular approach should be economically justified after analysis costs and benefits of the breeding effort. It is largely related to efficiency issue determined by cost per assay and time factor. Molecular approach came to reality for many breeding laboratories after microsatellites (single sequence repeats, SSRs) based markers had been devised in cereals (Ramsay et al., 2000; Röder *et al.*, 1998). This marker system possess several advantages over other markers including: small amount of template DNA required, reveals high level of DNA polymorphism, amenable to high-throughput methods and does not require radioactivity. All these features allowed breeding laboratories to be more self-sufficient in mapping and genotyping, since costs per data point dropped to 1.00 $ (own experiences). However, molecular genotyping technique was recently largely improved in plants by adopting single nucleotide polymorphisms (SNPs) markers established by human genomics research. Number of SNP markers in cereals gradually increase (http://wheat.pw.usda.gov/ITMI/WheatSNP/) and this marker system became more attractive since it does not require PCR (DNA array technology) or a gel based assay, therefore can be easily transferred for high-throughput applications. However, substantial financial resources on genotyping platform development have to be invested initially. There are number of breeding programs for conventional conditions that use successfully and routinely MAS concept in developing wheat resistant elite lines, this include quantitative resistance to fusarium head blight (*Qfhs.ndsu-3BS*), eyespot (*Pch1*), powdery mildew (*Pm1*), leaf rust (*Lr37, Lr47, Lr50*), stem rust (*Sr6, Sr22, Sr38* and *Sr39*), stripe rust (*Yr17*), cereal cyst nematode (*Cre1, Cre3, Cre6*) loose smut and wheat streak mosaic virus (*Wsm1*) (http://maswheat.ucdavis.edu/Index.htm). In Australian breeding programs, MAS strategy is used intensively to develop barley cultivars with resistance to pests and diseases, including: cereal cyst nematode (*Ha2, Ha4*), scalad (*Rrs14*), net blotch (*Rpt4*), powdery mildew (*mlo*), leaf rust (*RphQ*), barley yellow dwarf virus (*Ryd2*) (National Barley Molecular Marker Program). Technical improvements (speed and large number of sample processed simultaneously), advanced marker systems (simplified detection) and further reducing in costs of plant DNA acquire will contribute in wider MAS applications. Finally, financial result of the breeding effort supported with molecular markers will be determined by seed market, food industry and consumer demands. The limited area of organic agriculture will discourage breeding companies to establish MAS breeding programs for organic conditions, especially that wheat and barley seed market value is low. Therefore, initiatives for developing MAS strategies in breeding cereals for organic conditions should look for support in public sector or through internationally funded projects.

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