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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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**).

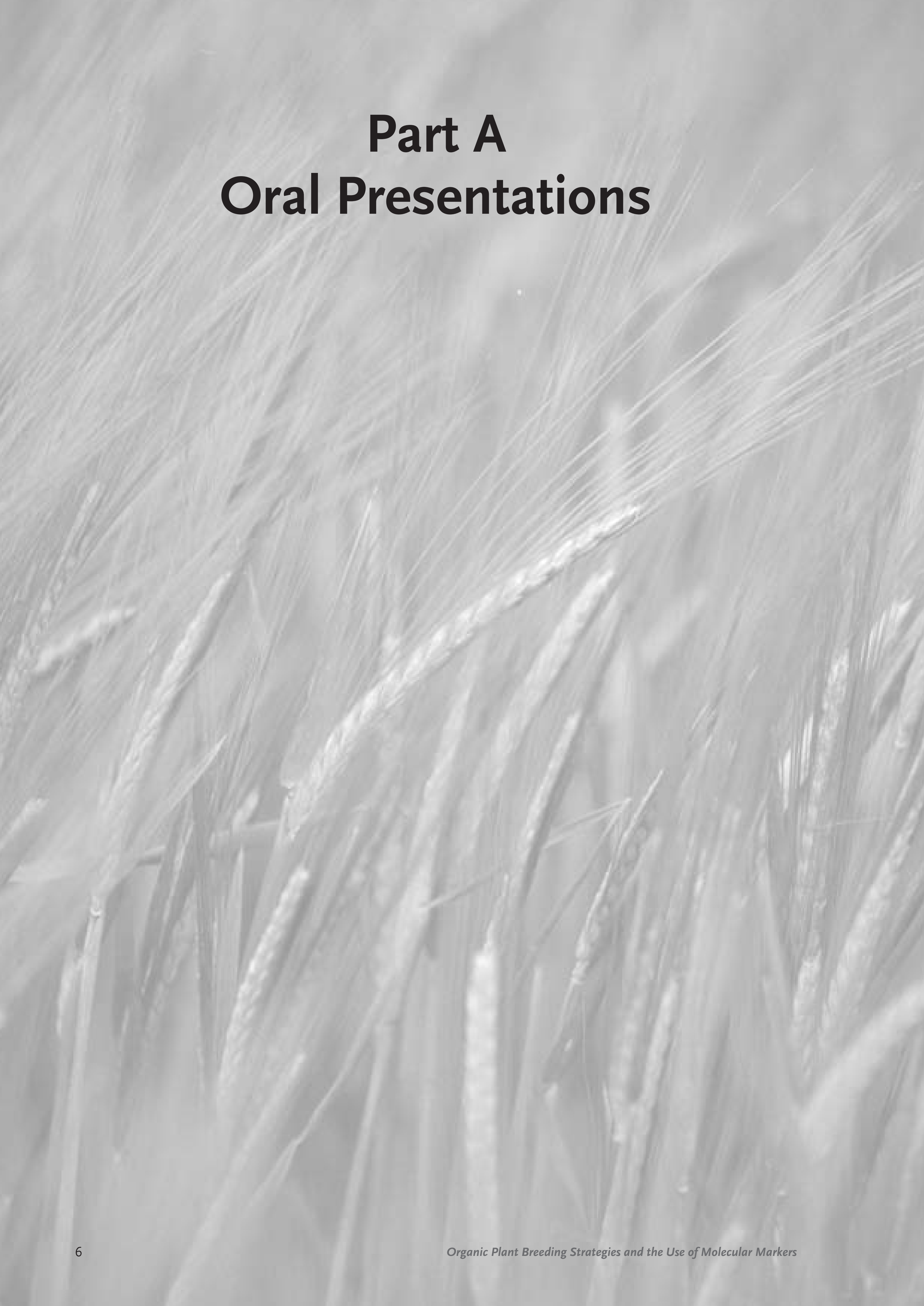
The main aims of the network are to ensure stable and acceptable yields of good quality for low-input, especially organic, cereal production in Europe. This will be achieved by developing ways to increase and make use of crop diversity (e.g. variety mixtures, crop populations or intercropping) and by 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.

Marker knowledge prerequisite	**Identification of genotypes**	**Identification of chromosome fragment (linkage map)**	**Identification of gene alleles by linked / functional markers (Establishment of linkage/identification)**
Descriptive use	Protection of breeders rights		
	Identification of variety components and their proportion in mixtures	Retrospective analysis of breeding process	
	Study of the evolution of variety mixtures		
Operative use	Study of the evolution of multi-lines and composite-crosses		
			Introgression of chromosome fragments of interest
			'Ideotype breeding'

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

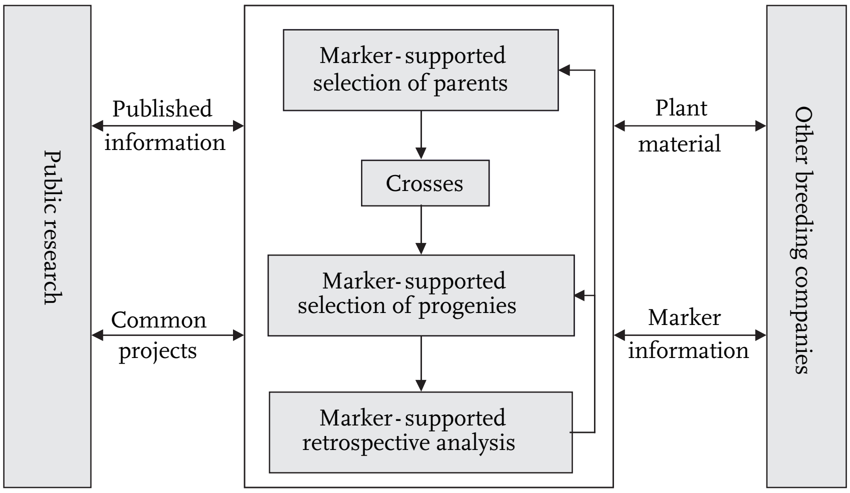

Figure 1. Information and material flow in a breeding company.

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.

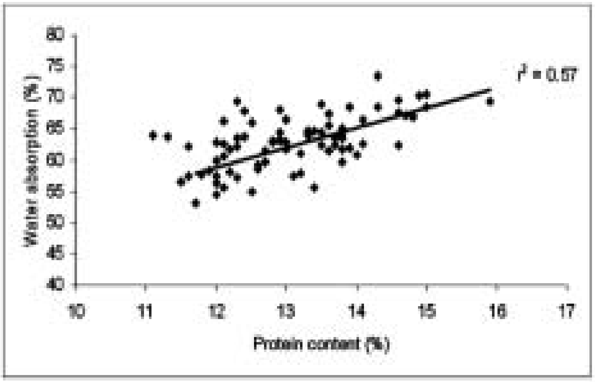

Figure 1. Protein content and water absorption relationship. Officials trials 1997 to 2001.

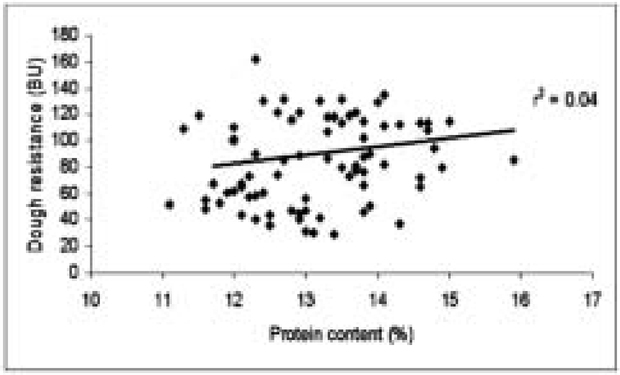

Figure 2. Protein content and dough resistance.Officials trials 1997 to 2001. (BU=Brabender Units)

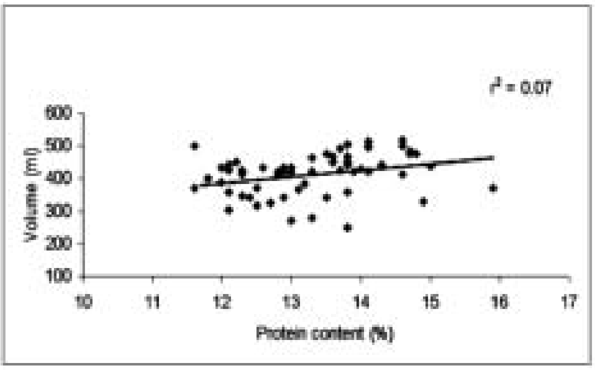

Figure 3. Protein content and loaf volume. Officials trials 1997 to 2001.

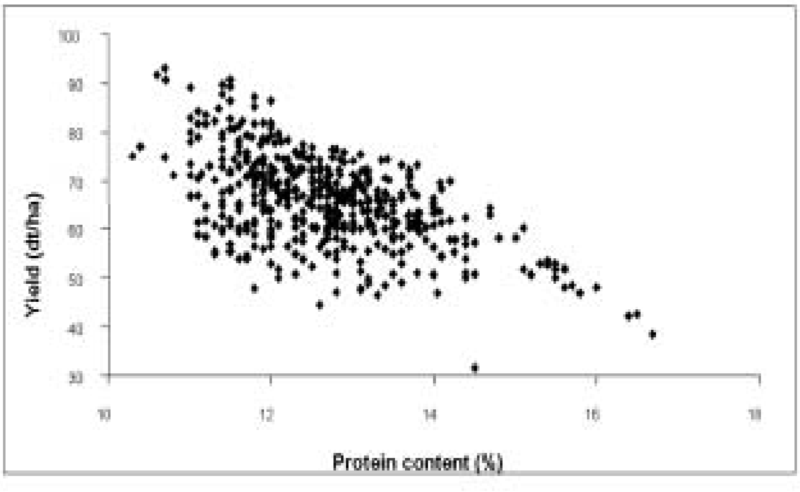

Figure 4. Protein content and yield relationship. Registration and preliminary yield trials 1983 to 2001.

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 coefficient $
			Glu-A1	Glu-B1	Glu-D1	
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.

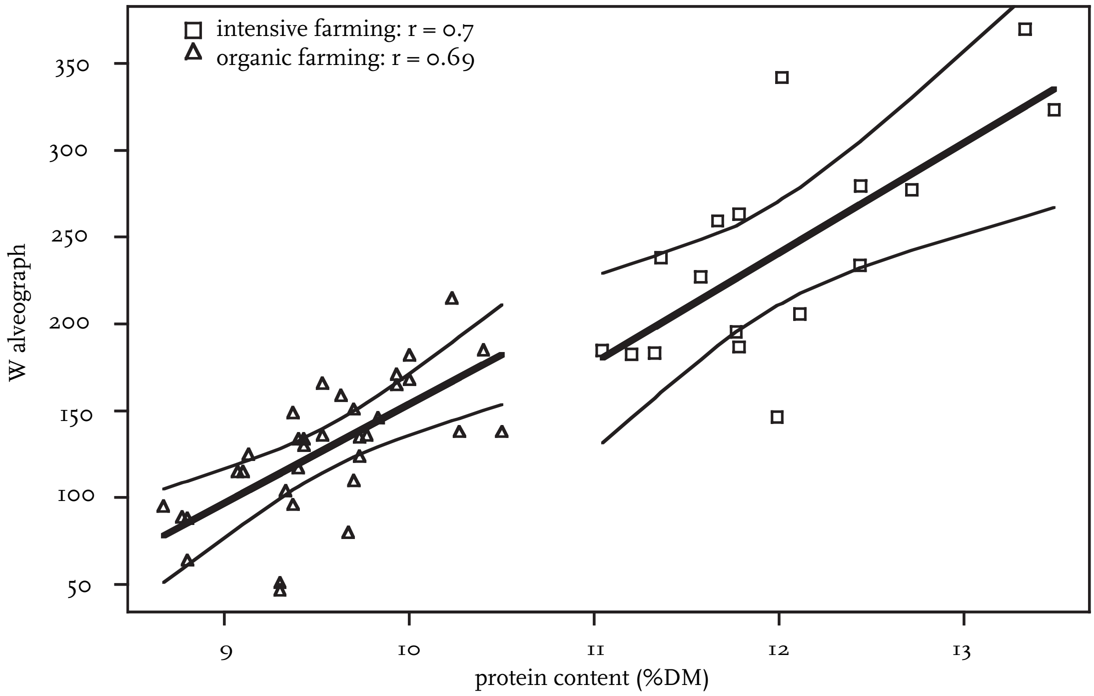

Figure 1. Comparison of dough strength/protein content in low input systems and organic farming. A similar positive correlation is observed in both systems, but the average protein content is much lower (9.4%) in organic farming than with intensive mineral fertilizer (12.8%). As a consequence, dough 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 7BL0,48-1,00 7DL (?)	Aleurone layer (supernumerary)	Shen *et al.* 2003 PNAS 100: 6252-6557
Ga-Myb	3AL3-0,42-0,78 3BL10-0,5-0,63	Transcription factor (storage proteins)	Ravel et al, PAG 2005
SPA	1AL 1BL 1DL	Transcription factor (storage proteins)	Guillaumie *et al.* 2004. Genome 47: 705-713
PBF	5A 5B 5D	Transcription factor (storage proteins)	
Glu-A1-1 *Glu-B1-1* *GluD1-1*	1AL 1BL 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 *Glu-B3* *Glu-D3*	1AS 1BS 1DS	Storage protein LMW-GS	Zhang *et al.* 2004. Theor. Appl. Gent. 108: 1409-1419
Gli-A1 *Gli-B1* *Gli-D1*	1AS 1BS 1DS	Storage protein Gliadins	Zhang *et al.* 2003. Theor. Appl. Gent. 107: 130-138 Zhao *et al.* 2004 Zuo Wu Xue Bao 30:126-130
GSP *Pina* *Pinb*	5DS 5DS 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 1B 1D	Amylose synthesis	Blake *et al.* 2004. Theor. Appl. Genet. 109: 1295-1302
SUT	4A 4B 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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Field evaluation criteria for nitrogen uptake and nitrogen use efficiency

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Abstract

Objectives of this contribution are N-uptake and N-efficiency (NE) of wheat with special regard to its rule in plant breeding for organic farming (OF) systems. After general considerations on factors determining NE, results of two series of trials are presented. The first trial was conducted over 2 years, on a genetically large assortment of about 70 genotypes in 10 different environments. Aim of these trials was, to check, to which extent differentiation of environments in breeding and enlargement of the gene-pool is necessary to enhance N-uptake capability of wheat varieties under the conditions of organic farming. Subjects of the second trial were course of N-uptake in time, N-Partitioning and N-Translocation. This trial was conducted on a limited number of varieties in 12 environments. The most important conclusions from the results were: (1) Environments within OF in Germany may differ widely; thus, differentiation of target and selection environments is necessary even within OF. (2) Selection of varieties with particular aptitude to OF within conventional breeding programs is unlikely to be successful, especially in environments with very low productivity. (3) Selection of varieties with very high protein yields and thus, better baking quality under the conditions of OF, is possible in special breeding programs using a larger gene-pool. (4) Under OF conditions, late N uptake capability is less valuable than under conventional conditions; in these environments, genotypes with high uptake capability in the earlier growth stages and translocation efficiency will be more adapted. (5) Marker assisted selection appears to be not suitable for NE, because this trait depends on many factors, whose importance vary according to environment characteristics.

Keywords

Genotype-environment-interactions, wheat, organic farming, molecular markers.

Introduction

The objectives of this contribution are genotype-environment-interactions (GEI) and nitrogen efficiency. The contribution is articulated in two parts, the first deals with GEI, identification of target environments, choice of selection environments and breeding material. The second part concerns possible reasons for genetic differences of N-uptake and N-use efficiency.

Baking quality of wheat grown under organic management practises often does not match the requirements of the milling and baking industry. It has therefore often been proposed, to breed special varieties adapted to the conditions of organic farming. There are two ways to improve baking quality; one is improvement of protein quality, the other improvement of nitrogen efficiency (NE) and thus, grain protein content. Subject of this contribution is nitrogen efficiency. There are various definitions of NE; in this context NE is defined as the ratio of N in the grain to total N-supply to the plant. N use efficiency (NUE) is the ratio of grain N uptake to total N-uptake.

The first condition for breeding varieties adapted to special environments is the existence of GEI. This depends largely on the gene-pool used as basic material for selection. However, in current breeders' material, genetic variability is generally limited; it has therefore to be checked, if enlargement of the gene-pool and special breeding programmes are necessary, and which type of material should be included. The second step will be, to define target environments, where the future varieties will be grown. We want to select varieties for OF, but organic farming systems, even if restricted to Germany, cover a large span of yield levels, ranging from ca. 2.5 to 6 t/ha (BMVEL, 2002) . We can therefore expect extremely different growth conditions within organic farming systems, which probably are more diverging than between organic and conventional farming in general. Consequently it should be considered, if differently adapted varieties (for example to different levels of input or different regions) are needed for organic farming.
Selection environments should be ideally similar to target environments, but breeders want to minimize their trials and,

generally, prefer environments with higher productivity, where control of growing conditions is easier and thus, growth conditions are more homogeneous and heritability will be consequently higher. The possibility to apply information obtained under certain (generally high-input-) conditions to environments with lower productivity is therefore another item of our considerations. Understanding the reasons for GEI helps to develop ideotypes and indirect selection criteria.
The amount of N finally present in the grain depends on uptake, distribution in the plant and losses, occurring after uptake. Uptake ability may be affected by root development, physiological factors like nitrate reductase activity (NRA) or duration of root hair life, relations to soil microorganisms and symbiosis. The physiological factor, which has been most extensively investigated, is NRA. Genetic differences regarding NRA could be proven, but often it was not correlated with N-uptake (Deckard & Bush, 1979).

Both, genetic differences and correlations with N-uptake could be found for root growth. Since formation of new roots is necessary to make accessible unexploited soil areas and duration of root hair activity is limited, the course of root formation may also be important. Source-sink-relationships change during development; the crucial point is flowering. After flowering, the developing grain becomes the major sink; N absorbed by the roots is transferred directly to the grain and N-remobilisation processes and transfer to the grain begin (Simpson *et al.*, 1983) N-partitioning is the result of uptake before flowering (into vegetative tissues), direct uptake after flowering and translocation processes. Losses may occur in the solid (leaf fall, stamina and pollen, spores of fungi), liquid (root exudation, leaching from leaves) and in the gaseous (NH_3-losses) state (see Wetselaar & Farquhar, 1980).

The main source of nitrogen in OF systems is mineralization of organic matter deriving from previously planted crops and organic fertilisers supplied before crop planting. In OF systems, wheat generally follows grass-clover leys, which contain amounts of nitrogen up to 300 kg (Loges *et al.*, 1999). The consequence are extremely high levels of soil mineral N in autumn (80-140 kg/ha), when N-uptake by wheat plants is still very low. In late spring and summer, N-uptake by wheat typically exceeds further mineralization, so that (generally during heading or flowering) levels of mineral N in the soil may decrease to very low levels (Scheller, 1992). N-supply in OF systems is therefore often characterized by excess in the early development and by deficiency in the late growth stages. It can be concluded, that low protein levels are not only due to low amounts of N mineralized during vegetation, but also to the course of N-mineralization, which does not always match plant needs.

Subsequently, two series of trials are described; the first concerns GEI of N-uptake, the second course of N-uptake in time and N-partitioning, which may be of particular importance in OF.

Materials and Methods

GEI and adaptation: the Plant Material consisted of 70 Genotypes, which can be subdivided into four Groups:

1) Standard commercial varieties, which are included as reference
2) Breeding lines of German wheat breeders, which, according to previous results, were potentially adapted to the conditions of OF. These genotypes were included, to represent the potential of current breeders' material in Germany.
3) Old varieties as example for the potential (and problems) of genetic resources
4) Varieties bred for OF, representing the potential of special breeding programs including genetic resources

The Trials were conducted in 5 locations over 2 Years. Three sites were managed according to the principles of organic farming, two sites conventionally, but with reduced N fertilization (120 kg/ha) and without use of pesticides. Yield levels ranged from 2 to 8 t/ha. Yield, Grain protein content and quality parameters were assessed.

Nitrogen uptake and partitioning: objectives of these trials were N-Uptake and N-partitioning and translocation processes. Six varieties (*Altos, Batis, Bussard, Capo, Dream and Ökostar*) were grown in replicated plots over three years in four locations. All trials were conducted on organic farms, management practices followed the guidelines of OF. Mean yields ranged from 2.5 to 7 t/ha. Total aboveground N-uptake and soil mineral N were assessed in the development stages 32, 62 and 95, grain and straw N content were determined separately.

Root growth of the same six varieties was observed in cylindrical vessels of transparent polyethylene. The height of the vessels was 1.4 m, diameter 25 cm. A grid was designed onto the walls (distance: 10 cm), root growth was assessed counting grid intersections with visible roots weekly. A sandy loam with high content of organic matter was used as substrate. The vessels were watered equally to ensure optimum water availability during the whole growth cycle. The trial was conducted outdoors; the vessels were insulated and darkened using polyurethane foam sheets.

Results and Discussion

GEI and Adaptation

The data were evaluated using linear regression of individual yields onto environmental means according to Finlay (1963). Numerous genotypes with significant GEI could be identified. Figure 1 shows two examples of varieties with positive (Zentos) and negative (Diplomat) GEI. The expected values PY_{min} and PY_{max} for the highest and lowest environmental mean is used to characterize aptitude to HI or LI (high- and low-input) environments, as shown in fig. 1. Similar, but less pronounced relationships can be found in less extreme environments. In figure 2, PY_{min} is plotted against PY_{max} as a scatter plot. Each mark corresponds to one genotype. Marks near the dotted line correspond to genotypes with little or no GE-Interaction. Marks above the dotted Line represent varieties with positive GEI; those with negative GEI are situated below the line. Varieties with HI-, LI- and combined aptitude are represented by marks situated in the partial areas labelled ”HI’”, “LI” and “Combined’’, respectively.

Most commercial varieties and breeding lines turned out to be HI-varieties or varieties with no GEI. Under conditions of OF, N-uptake of these genotypes were not high enough to approach the limit of 11.5% crude protein which is requested by the milling and baking industry. Landraces and old varieties showed pronounced aptitude to LI-environments. The Varieties bred for organic farming behaved differently: Protein yields of cv. Atar, Pollux and Asita were extremely high, in the case of Atar only under HI-Conditions, in the case of Pollux and Asita both under HI and LI-conditions. Arus and Wega behaved tendentially as LI-varieties while Ökostar did not show any particular aptitude. The high levels of N-uptake were not achieved exploiting GEI, but introducing characteristics (extremely high crude protein contents and relatively low yields), which are not desired under HI-Conditions.

The range, in which the genotype is superior or inferior to the mean is marked by the point of intersection of the regression line with the diagonal (G, see fig. 1). Figure 4 shows the distribution of these points, which are, both in genotypes with positive and negative GEI, concentrated around a protein yield level of 400 kg/ha. No difference could be found in the distribution of crossover points between the four groups. We can conclude, that there is a need for breeding varieties adapted to the environments with these low protein yield levels (because many commercial varieties will per form less than the average) and because crude protein contents are too low in this range. On the other hand, selection will be more successful for these environments (because many genotypes will perform better than the average).

Selection environments may differ from target environments, if the range of varieties is similar in both environments. This can be measured by correlation coefficients, which may be calculated using the measured or the expected individual protein yields. Figure 3 shows correlation coefficients of expected (curves) and measured (dots) Yield level with PY_{min} and PY_{max}. While results obtained at very low yield levels, are of limited significance for higher yielding environments, results obtained in HI-environments may be transferred to lower-yielding environments over a certain range, which corresponds approximately to protein yield levels of conventional agriculture in Germany (650-1100 kg/ha).

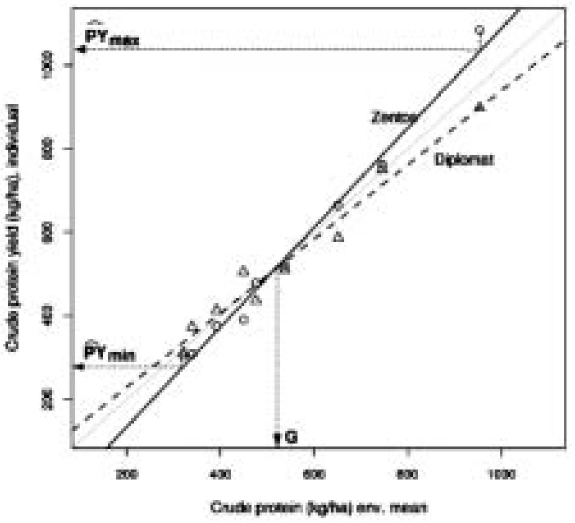


Figure 1. Protein yields under LI and HI conditions (PY_{min} and PY_{max}).

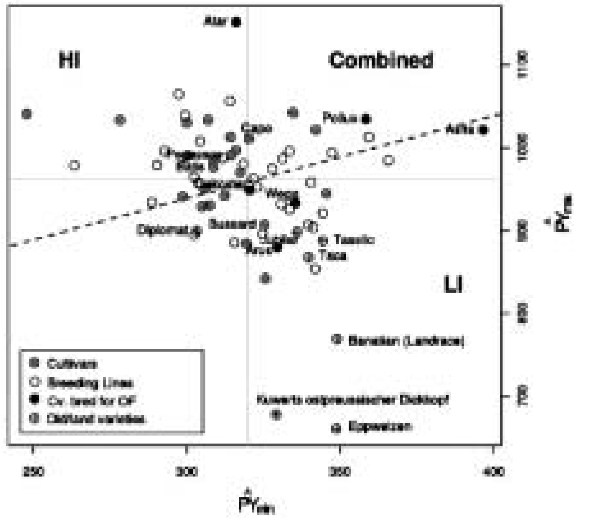


Figure 2. Protein yields under LI and HI conditions (PE_{min} and PE_{max}).

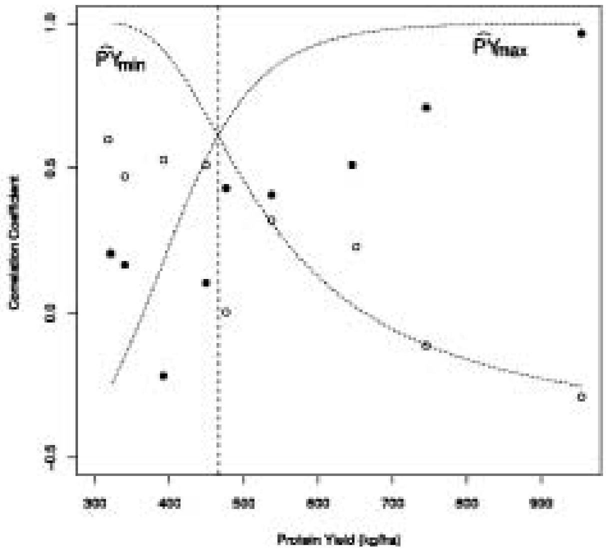


Figure 3. Coefficients of correlation of estimated (lines) and measured (points) protein yields and (PY_{min} and PY_{max}).

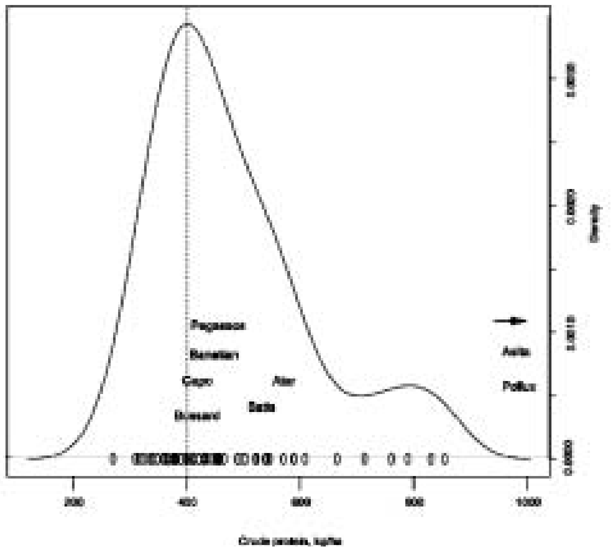


Figure 4. Distribution of intersections (G, see fig. 1).

In environments with very low yield levels, selection under LI-conditions is necessary, in the intermediate range, appropriate selection environments will be more effective.

N-uptake and N-portioning

In fig. 5, N-uptake of the most diverging varieties in the growth stages (tillering [EC<32], stem elongation and heading [EC 32-65] and Grain filling [EC > 65]) is represented in high and low yielding environments. In the high-yielding environments, significant differences could be found, both in total N-uptake and in N-uptake in the single growth stages. While cv. *Batis* and *Ökostar* absorbed more N during grain filling, cv. *Capo* absorbed tendentially more N during heading and shooting, but very small amounts during grain filling, so that net losses occurred in this stage. In the low yielding environments (where we can expect N-supply in the later growth stages to be very limited) , these differences disappeared.

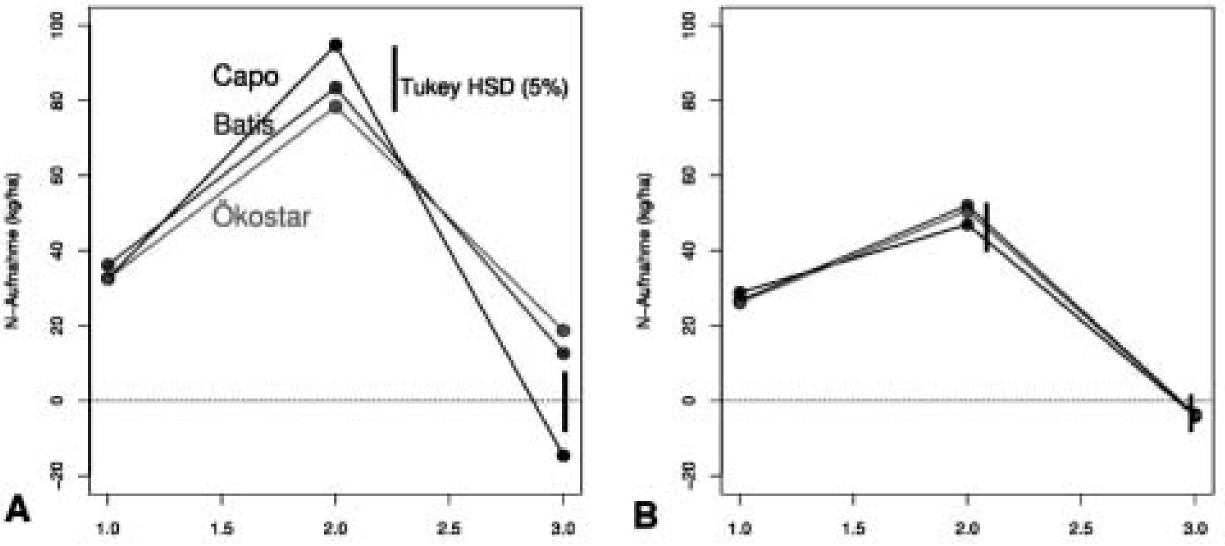
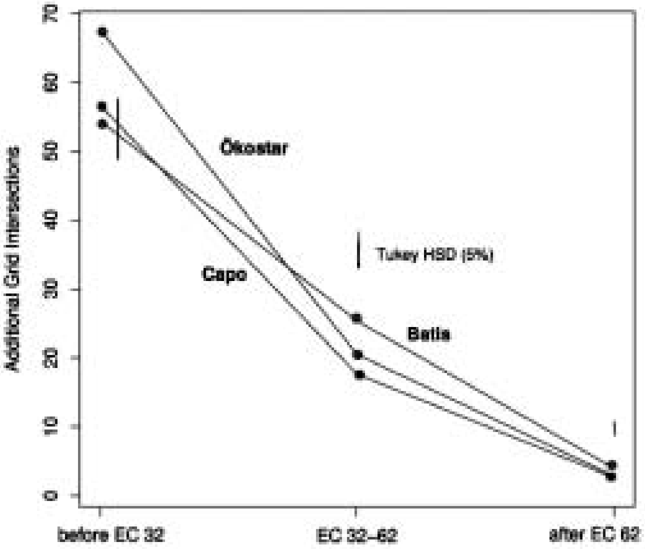


Figure 5. N-uptake in three growth stages in environments yielding more (A) or less (B) than 80 kg N/ha (resp. 14 and 6 environments).



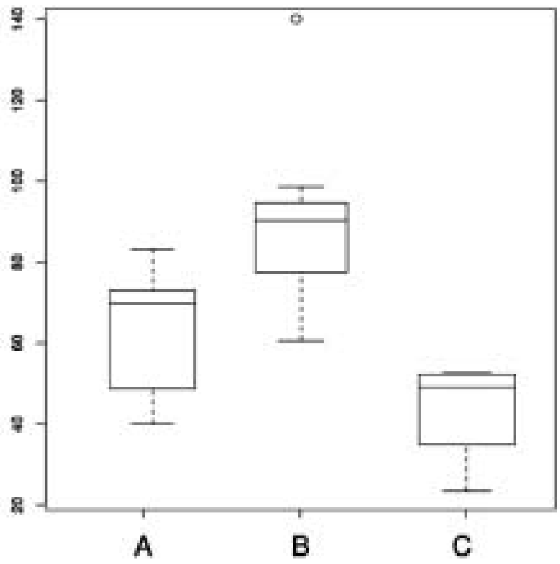


Figure 6. Root formation in three growth stages

Figure 7. Root formation in spring wheat varieties and landraces: A: German cultivars (6), B: Mediterranean landraces (14), C: Siberian landraces and cultivars (8)

Root growth

In figure 6, root formation in three growth stages is presented. Significant differences in total root formation and course of root formation could be found in the three cultivars mentioned above. Root growth coincided in part with N-uptake: cv. *Batis*, whose N-uptake in the later growth stages was particularly high, developed more roots during stem elongation and heading, in cv. *Capo* root formation and N-Uptake in the later stages was low. Cv. Ökostar behaved differently: Root formation was particularly high in the earlier development and relatively low in the subsequent growth stages. In fig. 7, total root formation, observed under the same conditions of cultivars and landraces of spring wheat are shown (unpublished data). Although root growth of modern cultivars is not particularly low, that of the Mediterranean landraces is higher and thus, root growth may be improved in future breeding programs.

Conclusions

- In Germany, yield levels of wheat in OF are ranging from 2.5 to 7 t/ha. Thus, selection of varieties covering the whole range of OF is hardly possible and differentiation is necessary even within OF.
- Differentiation of selection and target environments is necessary for LI environments, yielding less than 400 kg/ha.
- Commercial varieties are adapted to HI-environments, their total N-uptake under OF conditions was too low to meet the requirements of 11.5% crude protein in the grain.
- Breeder's material did not reveal to be better adapted; consequently, separate breeding programs and larger a gene-pool are needed for OF, especially for low-yielding environments
- The performance of old varieties and landraces in these trials show, those genetic resources may be used for this purpose without decreasing performance too much.
- Some of the cultivars for organic farming show, that improving N-uptake is possible in special breeding programs using genetic resources;
- High N-uptake capability is an advantage in HI environments, where N-supply is high also in the later growth stages, but less valuable in LI-environments, where N-supply in the later growth stages is often limiting. In these environments, genotypes with high uptake capability in the earlier growth stages and translocation efficiency will be more adapted.

For marker-assisted selection, traits should not be too complex; however, N-uptake and N-use efficiency is a very complex trait depending on many single factors, whose importance vary according to environmental characteristics. Table 1 gives an overview over the most important factors for N-use-efficiency and their supposed importance in HI and LI-systems. Many details are not well known and have to be still investigated. Hence, marker assisted selection for N-uptake and N-use efficiency will be difficult and it will be more advisable to search for markers for the single factors contributing to NE. In this way, selection for N-efficiency under defined conditions might be possible, because some factors are more important under HI- other under LI- conditions. However, most of the single factors are complex, too. Thus, we can conclude, that marker assisted selection is not very suitable for N-efficiency and should be preferably applicated to other, genetically more simple traits.

Table 1. Factors affecting N-uptake and use efficiency and their relative importance for HI and LI systems.

Factor	HI-conditions	LI-conditions
Root formation	-	+
- Early	+	-
- Late	-	+
Physiological factors (NRA)	-	-
Symbiosis	-	+
Early uptake	-	+
Late uptake	+	-
Translocation	-	+
Losses	-	+
Biomass	-	+
Harvest Index	+	-

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Prospects for marker assisted selection for nitrogen accumulation characters

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Current Trends

Commercial plant breeding has historically sustained an increase in the genetic yield potential of recommended cultivars in the UK of 1% per annum {Silvey, 1986 2286 /id} and this increase has been maintained in recently recommended cultivars where fungicide treated yield has increased by 0.6 and 0.7 t/ha in spring and winter barley respectively (Figure 1). A similar trend can be seen in the untreated yield of both crops but overall potential is over 1 t/ha less for both crops. Since 1991, the average yield of the UK barley crop has not, however, significantly increased. Whilst this could be explained by a decline in the proportion of winter barley over the period, the average yield of each crop has not significantly increased so it would appear that UK farmers are not able to realize some of the potential found in new cultivars. This trend is not apparent in winter wheat and probably reflects a concentration upon wheat in UK arable agriculture.

Using further Grain Nitrogen (GN) and yield data gathered on the Home Grown Cereals Authority and Crop Evaluation Limited funded UK recommended list trials, we can derive Nitrogen Yield (NY) and estimate the mean NY for each cultivar recommended since 1992. Regressing these data against the year of introduction of each cultivar shows that there has been a significant increase in NY for each crop (Figure 2). This could be due to the significant progress in grain yield over the same period but could also reflect changes in nitrogen usage and/or utilization. The regression for GN against year of introduction shows that there has been a significant decrease in the mean GN of recommended list spring barley cultivars over the period since 1992 but a non-significant relationship for winter barley. The above observations illustrate trends but need not necessarily imply changes in the genetic potential in nitrogen usage parameters as the decrease may be dependant upon the increased sink capacity.

Whilst the yield potential of barley cultivars has clearly increased since 1992, average on-farm barley yield has not significantly increased over the period (Figure 3). There is an increasing trend but the considerable variation from year to

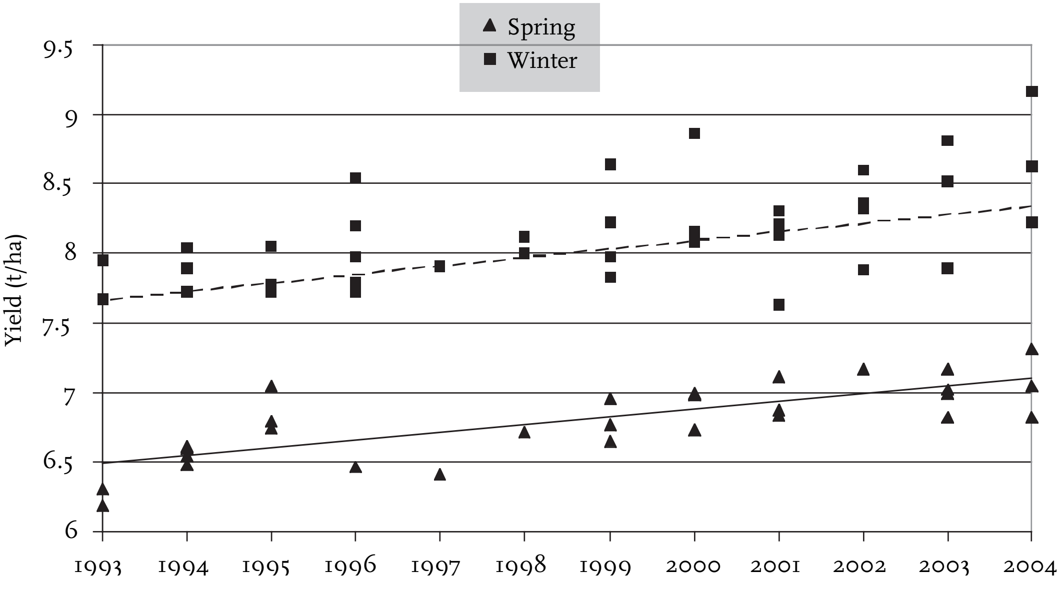

Figure 1. Regression of recommended spring and winter barley cultivar mean treated yields against year of first recommendation.

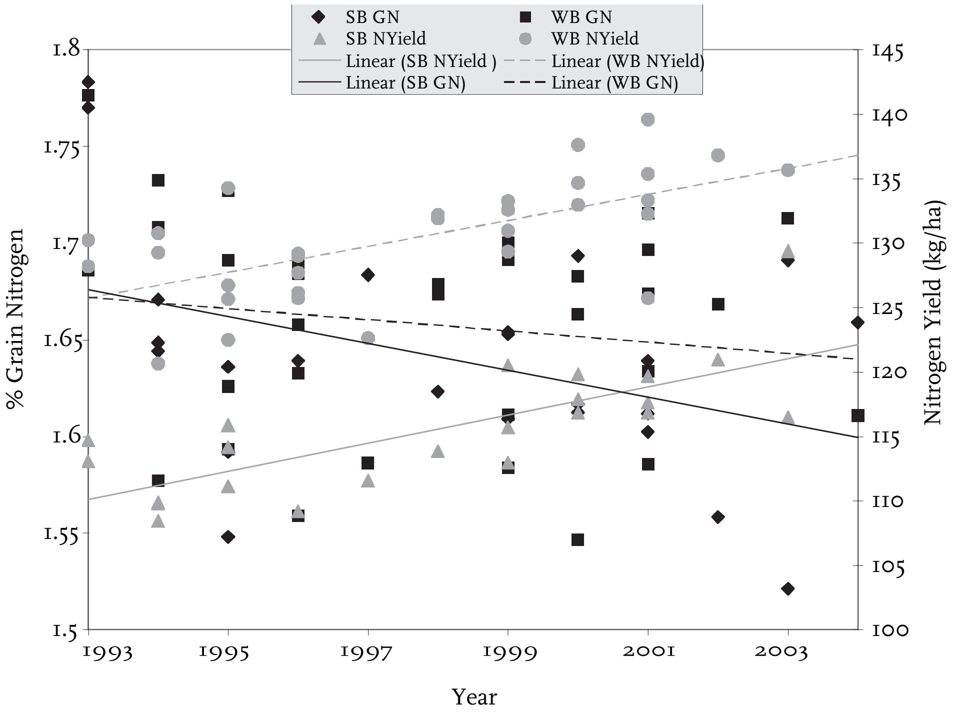

Figure 2. Regression of spring and winter barley recommended list cultivar mean grain nitrogen contents and nitrogen yields against year of first recommendation.

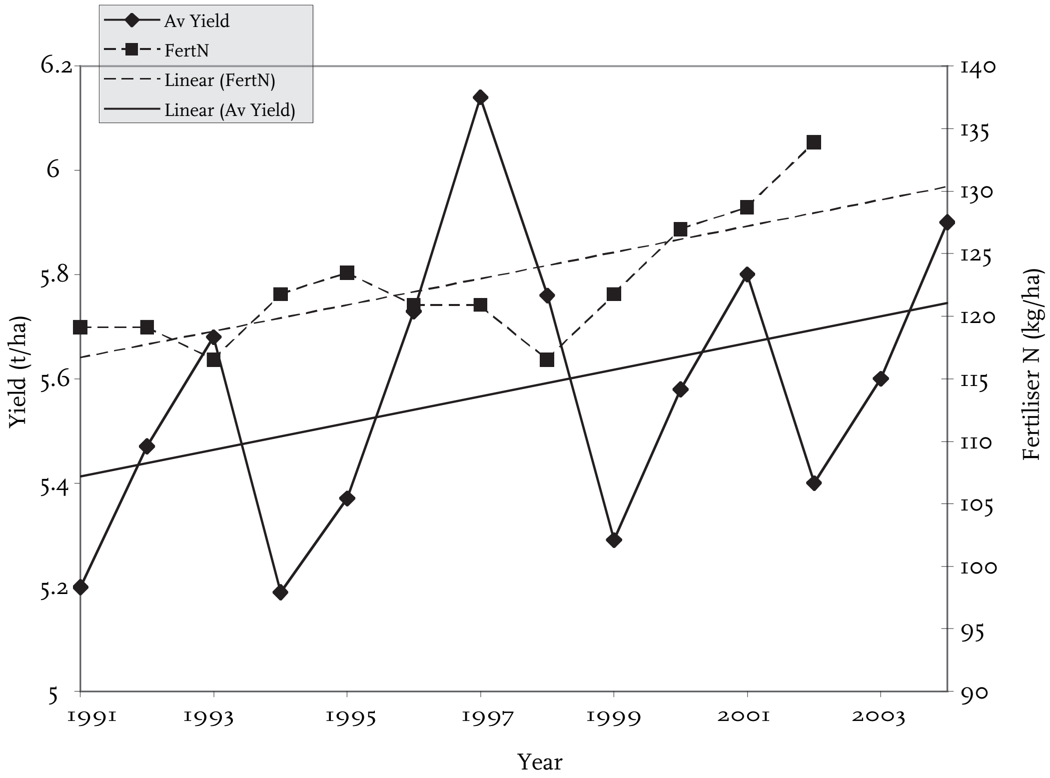

Figure 3. Average UK barley yields and fertiliser nitrogen inputs regressed against harvest year from 1991.

year, plus the confounding factor of a decrease in the area of the higher yielding winter barley crop over the same period, renders the trend non-significant. Over the same period, the average amount of nitrogenous fertilizer applied to the UK barley crop has increased, particularly since 1998, and this trend is significant over time. Taking the two trends together might suggest that overall nitrogen use efficiency in barley in UK agriculture has decreased but the barley market is heavily influenced by the grain nitrogen requirements of the malting industry and that has also increased slightly over recent years. Berry *et al.* studied average wheat yield and fertilizer use over a longer period and showed that yield had increased markedly but fertilizer inputs had remained relatively static since the early 1980's.

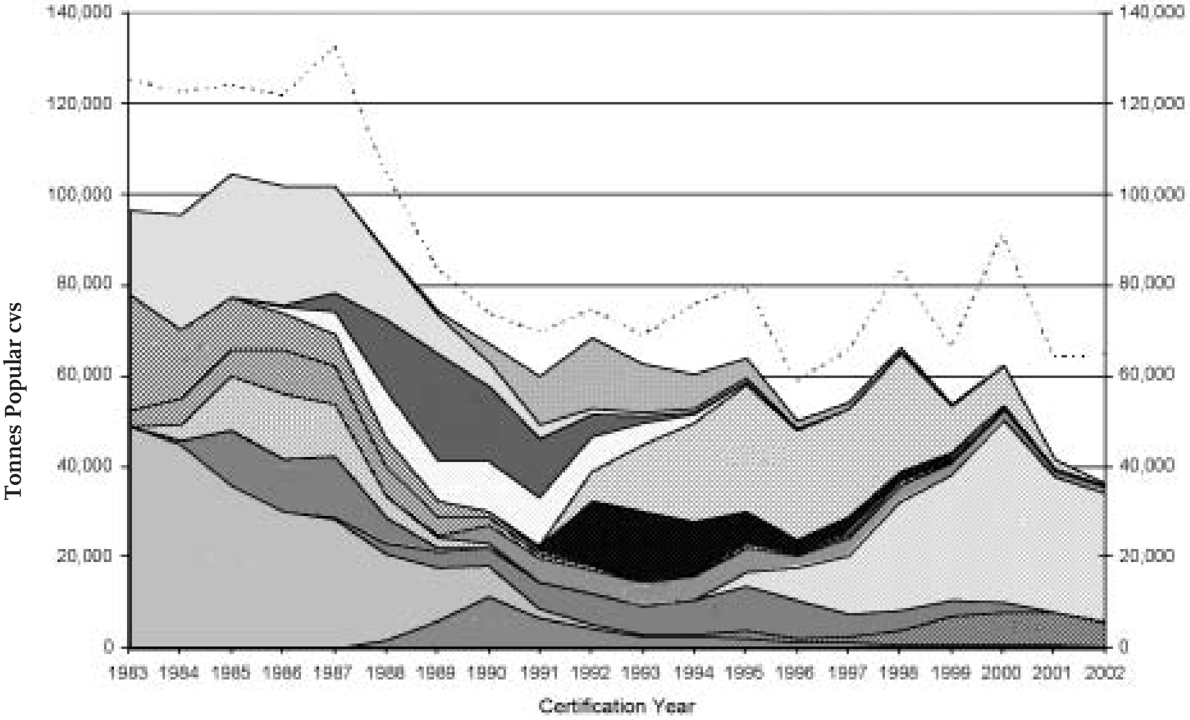

Figure 4. Production of certified seed of 16 popular spring barley cultivars by year (shaded portions) compared to overall production of certified seed (broken line).

From 1983 to 2003, 60 spring barley cultivars have been recommended but certified seed sales have exceeded 40,000 tonnes for just 16 of these cultivars (Figure 4). These 16 can be viewed as commercial successes but the remainder could be viewed as commercial failures. Figure 2 also shows that the volume of certified spring barley sales has halved over the 20 year period and this represents a real decline in income to commercial plant breeders. Barley is viewed as a minority crop by many of the large commercial breeders as the returns are declining and few companies can be making a profit. This raises the real question of whether commercial breeding can sustain niche opportunities such as those offered by organic or low-input agriculture.

Marker assisted selection (MAS)

The advent of molecular markers has led to the development of a number of genetic maps for crop plants and these maps have been used to search for Quantitative Trait Loci (QTL) for a range of traits. Over 40 different crosses were reported by {Thomas, 2003 2322 /id} to have been mapped in barley and a considerable number of additional barley mapping populations have been added to the scientific literature since then, notably by the efforts of the Australian Barley Marker programme {Langridge, 2003 2347 /id}, making barley the most comprehensively mapped crop plant species in the public domain.

With a comprehensive genetic map and a means of measuring the phenotype that one is interested in, it is possible to detect regions of the barley genome that are associated with the genetic control of a character and its interaction with the environment. This process is heavily dependent upon having good phenotypic data and is this most readily obtained from characters with a high heritability or those that are determined by genes that produce qualitative phenotypic differences. Such characters tend to be disease resistance and short straw genes but, as these are easily observed phenotypically, it is questionable whether it is worth deploying markers as a surrogate in selection for desired expression. There are a few instances where it is difficult to carry out efficient phenotypic screens for a particular disease. The Barley Yellow Mosaic Virus complex is one such instance and has been the subject of considerable effort in developing molecular markers for use in Marker Assisted Selection (MAS). It is now an example where many breeders use MAS instead of Conventional Phenotypic Selection (CPS) {Thomas, 2003 2322 /id}.

Quantitative characters tend to have a lower heritability than qualitative characters but many of the important performance characters are quantitative. If one had robust marker character associations for such traits, then the use of MAS to provide an elite gene pool upon which CPS could be carried out would be more efficient. MAS has, however, yet to impact upon quantitative characters in elite commercial breeding programmes because few, if any, relevant associations have been detected {Thomas, 2003 2322 /id}.

Grain nitrogen content is one of the parameters involved in estimating nitrogen use efficiency and has been the subject of QTL mapping in a number of crosses. We have used PLABQTL {Utz, 1996 1957 /id} to detect QTL for grain nitrogen content in three SCRI crosses (Blenheim x E224/3 (BxE), Derkado x B83-12/21/5 (DxB) and Tankard x Livet (TxL)). Using marker and phenotypic data available from graingenes (www.pw.usda,gov), we derived grain nitrogen contents for the two North American barley populations, Steptoe x Morex (SxM) and Harrington x TR306 (HxT) and used the same software to detect QTLs for the character. The North American variety Karl has been shown to have a large effect QTL for grain protein content {See, 2002 2357 /id;Utz, 1996 1957 /id} on chromosome 6H together with a minor QTL on chromosome 2H. We then aligned the QTL with the SxM Bin Map {Kleinhofs, 1998 1955 /id} to determine how many loci were detected in the same regions of the genome (Table 1).

Table 1. Location of QTL for grain nitrogen content and nitrogen yield from six spring barley mapping populations on the Steptoe x Morex Bin Map. Populations are Blenheim x E224/3 (BxE), Derkado x B83-12/21/5 (DxB), Harrington x TR306 (HxT), Lewis x Karl (LxK), Steptoe x Morex (SxM) and Tankard x Livet (TxL).

Chromosome		**Grain Nitrogen**		**Nitrogen Yield**	
2H	Bin	1	5	4	8
	Cross	HxT	BxE	SxM	SxM
3H	Bin	2	5	6	7
	Cross	SxM	SxM	SxM	SxM
4H	Bin	1	6	6	
	Cross	HxT	HxT BxE	BxE	
5H	Bin	1	5	1	6
	Cross	HxT BxE	SxM BxE	HxT	SxM
6H	Bin	1	6	6	
	Cross	SxM	LxK	SxM DxB	
7H	Bin	1	5	1	
	Cross	SxM	DxB	DxB	

Despite the presence of storage protein loci on chromosome 1H, no QTL were detected on that chromosome. QTL were detected in between 3 (6H) and 5 (2H and 3H) on the remaining 6 chromosomes but the same bin was not often detected in more than one population (Table 1). The low protein QTL from Karl was not found in any of the other 5 crosses, although a QTL was found in a neighbouring bin in HxT.

Nitrogen Use Efficiency (NUE), however it is defined, is a derived character and is likely to be subject to more error variation than grain nitrogen content. Nevertheless, {Mickelson, 2003 2366 /id} detected 3 QTL for remobilization of leaf nitrogen, two of which appear to be located in Bin 1 of chromosome 5H. The remaining QTL is putatively located on chromosome 6H but the nearest known marker is also located in Bin 1 of chromosome 5H on composite barley maps so all three QTL may all be located in the same region of the barley genome. Wherever they are located, none of the QTL were co-located with yield or grain nitrogen QTL {Mickelson, 2003 2366 /id}.

With the exception of the Lewis x Karl cross, we can use grain nitrogen content and grain yield to derive nitrogen yield. Whilst this does not take any account of differences in uptake and remobilization, it does illustrate represent one of the main offtakes of nitrogen from the system. Using the same approach as for grain nitrogen content, we again did not detect any QTL on chromosome 1H. For the remaining chromosomes, QTL were detected in between one (4H and 6H) and four (3H and 5H) bins. For this character, the same bin was detected in more than one population just once – Bin 6 on chromosome 6H. Establishing NUE phenotypes requires considerable investment in resources and one must question whether adopting an un-informed QTL mapping approach will lead to any exploitable results.

As part of a larger BBSRC funded project, we genotyped ?? spring and winter barleys that had been placed on the UK recommended list from 1992 to 2002 with 48 previously mapped Simple Sequence Repeat (SSR) loci (Macaulay et al 2001). Using grain nitrogen and yield data derived from the HGCA/CEL funded Recommended List trials (see above), we calculated the mean nitrogen yield of each of the entries. We then used ANOVA to identify those SSR markers that were significantly associated with nitrogen yield. These loci were then aligned with the Steptoe x Morex Bin map {Kleinhofs, 1998 1955 /id}.

For spring barley, no QTL were detected on chromosome 1H, reinforcing results from the mapping population. In contrast, 2 QTL were detected on 1H for winter barley but neither was in the region of the hordein loci (Table 2). For the remaining chromosomes, between one (4H and 5H) and four (6H) bins were significant for spring barley and between one (2H and 5H) and three (3H and 6H) bins for winter barley. It is, however, quite striking that 8 of the 13 significant bins detected in the spring barley were also detected in the winter barley recommended list cultivars. As these QTL are detected in the elite gene pool that commercial breeders focus upon, these results suggest that QTL detected in this way could be robust enough to be of value in MAS schemes for NUE.

Table 3. Location on the Steptoe x Morex bin map of markers significantly associated with nitrogen yield in spring and winter barley recommended list cultivars.

	Nitrogen Yield QTL Bins			
Chromosome	**Spring Barley**		**Winter Barley**	
1H			7	12
2H	2	10	2	
3H	6	13	6	13
4H	6		6	10
5H	9		6	
6H	1	6	1	7
7H	6	7	6	

Prospects

With the public availability of the rice genome sequence and the large number of wheat and barley Expressed Sequence Tags in public databases, we can use syntenic relationships to align barley maps with the rice genome. There is also considerable effort in the barley and wheat research community to move from relatively anonymous markers to functional markers through the production of extensive Single Nucleotide Polymorphism (SNP) marker libraries. The ability to associate a QTL with one or more functional markers representing genes known to be implicated in the particular phenotype is a major improvement in the search for robust QTL for deployment in MAS schemes. A recent review of NUE suggested that levels of nitrogen assimilation enzymes do not limit primary N assimilation and hence yield but that there was strong evidence that cytolsolic glutamine synthetase (GS1) was a key enzyme in mobilization of nitrogen from senescing leaves and hence was positively correlated with yield {Andrews, 2004 2365 /id}. In a study of maize, QTL for both yield and GS1 activity co-located with loci for GS1 structural genes {Hirel, 2001 2367 /id}. Searching the public databases reveals several barley cytosolic GS1 homologues and, using rice barley synteny, two of these appear to be in regions detected by the survey of the recommended list, namely those in bin 7-8 and bin 6 on chromosomes 6H and 4H respectively.

The availability of the Affymetrix Barley1 Gene Chip that samples some 22,000 barley genes represents a valuable tool for future investigation of traits such as NUE. It is a valuable filter to select potential gene targets for manipulation in complex characters. Having identified the targets, one can then survey the adapted and, if necessary, the unadapted gene-pools for variants, assess their potential value, and use MAS to introgress the best into an appropriate genetic background.
For the purposes of this paper, we have refrained from considering the genetics of rooting characters. Recent studies have shown that there is variation in barley in both seedling root architecture {Bengough, 2004 2368 /id} and root system size (Chloupek, Pers comm.) and it is likely that these differences coupled with variation in root exudates will affect nitrogen acquisition and therefore impact upon NUE. To conclude, NUE is essentially a term understood by plant physiologists and has largely been ignored in genetical studies. With the advent of new genetical tools, there is huge potential to ally these with physiological insights into experimental design and interpretation to arrive at a better understanding of a key character for sustainable agriculture.

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Field evaluation and selection of winter wheat for competitiveness against weeds

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Abstract

The evaluation of new varieties in relation weed suppression should consider characteristics such as growth habit, speed of early development and tillering ability. Weed suppression cannot be attributed to a single characteristic. Instead the interaction between a series of desirable characteristics has been shown to be important, with varieties compensating for weakness in certain characteristics with strengths in others. Generally, a high season-long crop ground cover is important. The balance between different characteristics for weed suppression will determine the value of the variety for early, late and season-long weed control, and for the climatic zone. Selection for general growth habits is based on understanding the role of different characteristics in weed competition. These habits will be of value under different soil and cropping conditions or locations in Organic Farming. Defined growth habits are: (1) The continuous planophile habit which has a clear advantage for weed suppression over the erectophile type at a given plant or shoot population density. (2) The early season erectophile to late season planophile habit is a good model when crop establishment is high. (3) The early planophile to late erectophile habit can compensate more for lower crop establishment than the early erectophiles. (4) A continuous erectophile habit is only beneficial when weed levels are low, crop establishment is sufficiently high or crop row widths are very narrow.

Background

Selection of new cereal varieties for competitiveness of against weeds under organic conditions requires identification of crop characteristics (or traits) and development of routine methodologies to indicate their potential usefulness. The EU funded project on Strategies of Weed Control in Organic Farming (WECOF) has examined the key morphological characteristics which are involved in the ability of winter wheat to reduce weed growth through light interception and shading so as to determine their relative importance. Particular emphasis was on the determination of the wheat ideotypes to optimise weed suppression. This paper focuses on variety trials carried out in south east Scotland that consisted of a wide range of genotypes considered as having potential for weed suppression in Organic Farming. The following discussion is supported by information drawn from the WECOF Core Trials carried over three years in Germany, Poland, Spain and Scotland, covering Mediterranean, Continental and cool, temperate climates. Evaluation and selection for weed suppression will be of advantage for organic farmers, and farmers practicing integrated methods of farming, as well as benefiting breeders of suitable varieties.

Plant characteristics for weed suppression

Although some varieties have higher weed suppression than others, this is usually not attributed to a single characteristic, either within or between varieties. The interaction between a series of desirable characteristics is important is weed competition (Eisele & Köpke, 1997) and this will include strengths in some characteristics compensating for weaknesses in others. Certain key characteristics are indicated as generically desirable for organic wheat varieties to improve weed suppression: (1) good establishment ability, (2) high tillering ability, (3) increasing plant height (Wicks *et al.*, 1986; Korr *et al.*, 1996; Didon & Hansson, 2002), (4) planophile leaf habit and high leaf area index through production of large leaves (Niemann, 1992; Hucl & Huel, 1996; Seavers & Wright, 1999), (5) plant growth habit and leaf inclination (Eisele, 1992;

Niemann, 1992; Lemerle *et al.*, 1996) and (6) high yield potential. Many of the individual plant traits in (1) to (5) can be used to define plant growth habits (which are discussed later in this paper). These characteristics also determine whole crop measures such as leaf canopy size and light interception. Thus, crop ground cover also comprises a broad range of plant characteristics (e.g. Huel & Hucl, 1996; Ogg & Seafield; Didon & Hansson, 2002).

Results from our variety trials strongly suggest that crop ground cover is the most important crop feature for competing against weeds (Davies *et al.*, 2004). This view is supported by results from WECOF Core Trials (Drews, 2005) and other research (Richards and Whytock, 1993; Lemerle *et al.*, 1996). Figure 1 demonstrates how crop ground cover is inversely (and significantly) related to weed ground cover and crop cover. The affect of the crop on later weed growth was evident in much of our work (Figure 1). When a variety competed well against weeds this was associated with a relatively high fractional light interception. For example light interception at ear emergence was significantly and negatively correlated with subsequent weed growth ($R^2 = 0.45$; data not shown). Other work has shown that higher weed suppression was associated with relatively high light interception in the upper leaf canopy of tall, planophile cultivars (Verschwele, 1994; Christensen, 1995; Amesbaur & Hartl, 1999). Seasonal variations in plant establishment and individual genotype responses in tiller production and/or tiller retention can make it difficult to group varieties in field trials into consistently good or poor for ground cover and light interception. However, it is possible to describe general growth habits of current varieties in such a way that will benefit the selection of weed suppression ability in new genotypes.

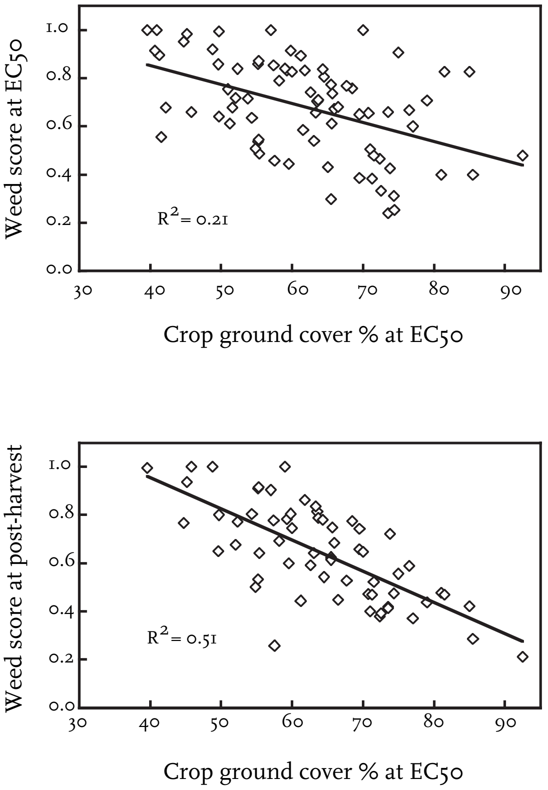

Figure 1. Relationship between crop ground cover (%) at EC50 and weed ground cover at EC50 (upper figure) and post-harvest (lower figure). Data represent all varieties across four seasons. Weed ground cover is scored as the percentage weed cover for each variety in each season expressed as a fraction of maximum weed cover at the relevant growth stage, thus enabling each season's data to be placed on the same scale.

Working towards defining growth habits and cereal ideotypes

Use of a wide range of genotypes have enabled us to develop guidelines for the types of variety that are best suited to weed suppression in Organic Farming. The balance between the characteristics will determine the value of the variety for early, late and season long weed suppression, and for the climatic zone. For example, in very dry zones, highly erectophile habits (especially after stem extension) may be preferred for later moisture conservation; in such situations early ground cover, height and yield may be of greater consequence for weed suppression, along with leaf size, and possibly tillering ability. In such zones, early weed suppression is generally the requirement anyway and the characteristics indicated would provide that benefit. In cool, moist climates, season long weed suppression may be required and early prostrate/ later planophile habit with large leaves, and high tillering ability because of greater establishment risks, are perhaps more important, with some compensation possible with height increase. High yielding potential also appears to be important.

However, a key factor for all these characteristics is a robust consistency in weed suppression and yield required for organic varieties. It is clear that some varieties are more robust than others, though it is not always clear why this is so. Our own results suggests that about 40% of the variation in ability to suppress weeds appears not to be linked to the measured characteristics. This leads to hypothesise what characteristics should also be assessed after suitable research. Root competition was not evaluated and is relatively poorly understood; particularly genotypic variation between varieties. The potential for allelopathic differences between varieties has been alluded to in the literature (Didon, 2002; Didon & Hansson, 2002) derived from in-vitro testing, but little is known about in-vivo behaviour.

For plant breeders, targets for plant and crop characteristics should be considered in relation to generalised growth habits that are of value under different circumstances or locations in Organic Farming. Examples of current varieties that provide a basis for developing future ideotypes are highlighted in Table 1. The types described below represent a wide range that is suited to different situations of climate and weed development. All types benefit from good crop emergence. An early planophile type can provide earlier canopy closure and a degree of buffering against poor to moderate crop establishment.

Table 1. Leaf characteristics and general plant growth habit of selected wheat varieties from WECOF.

Variety	**Angle of flag leaf (°)**	**Mean angle of all leaves (°)**	**Mean leaf width**	**Mean Leaf length**	**Plant growth habit**
Chablis	76	52	N	S	Continuous planophile
Maris Widgeon	55	44	M	M	Erectophile to planophile
Rialto	31	35	W	M	Planophile to erectophile
Zyta	23	26	M	S	Continuous erectophile

Leaf width: Narrow < 14 mm; Medium = 14-15 mm; Wide > 15 mm

Leaf length: Short < 22 mm; Medium = 22-25 mm (Long > 25 mm, e.g. oat leaves, not shown above).

The leaf angles were measured from the stem at anthesis (i.e. a more erect leaf has a low angle). Mean leaf width and length were measured at ear emergence and are defined below. Plant growth habit refers to the change in leaf habit from pre-tillering to post-anthesis.

A continuous planophile habit has a clear advantage over the erectophile habit at a given plant or shoot population density. This habit appears to be particularly beneficial in shorter varieties and under circumstances where a crop requires sustained weed suppression, especially during the autumn and stem extension. An early planophile to later erectophile habit can compensate better for lower crop establishment than early erectophiles, though rapid leaf development or large leaves would enable varieties of this type to take full advantage of their leaf habit. The early erectophile to later planophile habit is a good model when crop establishment is high and if crops are sown in narrow rows. This structure can provide high fractional light interception throughout the season. The later planophile habit is the most beneficial habit where there is late weed growth i.e. from stem extension onwards. The erectophile habit has been the long established ideotype for high yields in cereals for high-input agriculture and unlimited nutient supply. This habit can be an advantage when weed levels are low, but it is a risky strategy when competition from weeds is high, especially early in the growing season. If an erectophile is desired then increased height may be of value. Shorter vartieties would benefit from an ability to produce and retain a high number of shoots per plant.

Genotype selection in plant breeding programmes

One of the outcomes of the WECOF project was a guide to genotype selection for plant breeders. The following descriptors are derived from this study plus literature reviews and field observations. We list the key characteristics, and how important they are in weed suppression for different climatic regions.

Early growth habit

An early prostrate habit (at the start of tillering) combined with a moderate to high leaf area index (either through rapid leaf development or good crop establishment) has been determined as being closely linked with reduced weed growth, despite the fact that shading is at a low level at this stage. Other competitive characteristics such as nutrient and water competition are suspected to play an important part, as may allelopathy, at this early stage. However, an early prostrate habit appears to be a good indicator of such competition. Ground cover at early tillering is strongly correlated with weed suppression throughout the season Richards & Davies, 1991). An erectophile habit at early tillering tends to require a higher crop establishment to be as equally competitive as an early planophile.

Although plant characteristics such as the amount of ground cover per plant and the amount of leaf area per unit of ground cover are only weakly correlated with weed ground cover, our results suggest that these measures are useful indicators of competitive ability for crops at equivalent plant population densities. This would be a particularly useful feature for plants to retain at moderate to high plant population densities, rather than simply used to compensate for poor establishment. Optimising the early growth is likely to be very important in reducing autumn and winter weed emergence in cool temperate climates.

High tillering capacity

Shoot population density is a function of plant number and the ability of a plant to produce and maintain tillers. Consequently, some varieties have a relatively high shoot population because of good establishment, whilst others produce a higher than average number of shoots per plant: some varieties may have both characteristics. High tillering ability is likely to be most important at low plant populations i.e. 150-160 plants per square metre or less. As organic seed is not treated for disease and pest control, establishment in adverse conditions can be reduced significantly. Data from our own trials and from farm crops indicates that the percentage of plants established is less under organic than under conventional conditions. Therefore high and consistent establishment across a range of soil conditions is a particular requirement for organic varieties. The selected varieties should be high tillering types to cope with such situations. High tillering capacity is essential in cool temperate conditions, but perhaps less important in Mediterranean regions where establishment conditions are usually more favorable.

Rapid early growth to stem extension

Rapid early growth allows the crop to maintain a light interception lead over the rapidly growing weeds, and with the right habit, shade newly emerging weeds. Ground cover by the crop at the end of tillering is strongly correlated with weed suppression up to full canopy cover and up to harvest. In Mediterranean regions, rapid autumn and early spring growth is required to cope with weed emergence with the crop and a further emergence in early spring. In continental regions or in late sown crops in cool temperate regions a rapid early spring growth is particularly required to shade a largely spring emerging weed flora. In early sown crops rapid autumn tillering is required, as well as rapid early spring growth.

Plant habit

We have determined that the plant habit is considered planophile when leaf angles from the main stem and tillers exceed 30° to 45° from the vertical. A leaf may be relatively straight or slightly curved. A highly curved or extended leaf in which the leaf tip exceeds an angle of 60° from the stem can also be described as planophile. In general, highly planophile morphologies increase light interception and shading of weeds, and this compensates significantly for lack of height. Some varieties change from planophile to erectophile (leaf angle is less than 30° from the vertical, straight or curved) over the season. The WECOF project has devised several scoring systems for assessing plant growth habit in the field. Typically, these scoring systems are based on a five-point scale for ease of use. They take into account plant structure based on leaf inclination and features such as plant height to width ratio. Our aim is to incorporate protocols for assessing plant habit into plant breeding programmes and variety testing systems. This would enable plant breeders to identify potentially useful material and for testing organisations to identify candidates for selection.

The four varieties highlighted in Table 1 provide examples of the growth habits identified in the WECOF project. Although each of these types can provide good suppression against weeds under different conditions for crop establishment, or timing and amount of weed emergence and growth, it appears that the competiveness of the continuous erectophiles and late erectophiles is compromised when plant populations are poor. Where breeding lines are exclusively of erectophile types, then it should be possible to improve weed suppression through shading by increasing LAI with increased height and leaf size. Therefore a continuous planophile or a more rapid growing early-erectophile with a later planophile habit is more robust. Types that maintain either a planophile or erectophile habit for the whole season appear to best suit conditions of poor or good crop establishment, respectively. Organic farmers may sow in narrow or wide rows. In general wide rows are used to aid hoeing for control of difficult perennial weeds, such as docks, thistles, common couch and scrambling weeds such as cleavers. Narrow rows are preferred for general weed suppression as shading is increased. Where narrow (10-16cm) rows are used both planophiles (short or tall) and tall erectophiles with large leaves are successful. Where rows are over 20 cm then erectophiles have to be very tall with large leaves to give a comparable shading ability as planophile varieties.

Plant height

Although there was no clear indication of height alone being useful in competition against weeds, very tall varieties would appear to be competitive at moderate to good plant population densities. Height can compensate for an erectophile leaf habit, but a relatively short planophile can give the same shading ability and weed suppression of shorter weeds. Tall varieties may have an advantage for some very tall grasses and scrambling weeds (Baylan *et al.*, 1991; Blackshaw, 1994; Ogg & Seefeldt, 1999; Cousens *et al.*, 2003). Leaf size is an important factor in shading, with larger leaves of particular assistance in erectophile varieties. The varieties that have the poorest weed suppression are short, erectophile varieties or planophile varieties with small or narrow leaves.

Considering crop ground cover in plant selection

As stated earlier, crop ground cover integrates several plant and crop characteristics. Cover measured from directly above the crop is a good indicator of shading characteristics and can be used as a guide. However, total leaf area index (LAI) or green area index (GAI) are also good correlants with shading and weed suppression. So, a tall, large-leaved erectophile may have similar shading ability, or possibly even greater ability later in the season, than a short planophile variety. So a variety that changes from planophile to erectophile over the season will give continuous good shading so long as it is tall later on. Some varieties have a consistently higher leaf area index than others. This is influenced by plant and crop factors such as leaf size and percent establishment. When a variety competes well against weeds this tends to be associated with a relatively high fractional light interception (Verschwele, 1994; Christensen, 1995). In the Mediterranean region a planophile habit in autumn and early spring would be useful. Later weed growth is reduced by the hot, dry conditions, so later wheat growth may be erectophile. Most varieties in this region are erectophile at present, so large leaves and height becomes more important characters in maintaining shading. In continental regions a planophile habit in the spring is useful; dry and warm conditions in summer reduce weed growth, so later growth may be erectophile. A tall early erectophile with large leaves may be suitable. In cool, temperate regions a planophile habit is useful throughout the season as weed growth may continue through to canopy closure and beyond in some cooler, wetter summers. If the variety is or becomes erectophile, they must be tall with large leaves.

Selection for yield

From the WECOF trials and trials elsewhere, there is an indication that the best weed suppressors tend to be amongst the better yielding varieties. Our own work indicates a significant negative correlation between yield and weed cover immediately post-harvest ($R^2 = 0.46$, data not shown). It is clear that yield benefits are not lost in selecting for weed suppression in winter wheat. This has implications for plant breeding programmes, because the development of competitiveness against weeds does not exclude development of high yielding varieties.

Concluding remarks

The information we have leads us to conclude that new varieties for organic agriculture need to be more robust in both their percentage establishment under contrasting conditions and in their ability to produce as high as possible number of shoots per plant: either through tiller production or tiller retention. The balance between different characteristics for weed suppression will determine the value of the variety for early, late and season-long weed control. The continuous planophile habit has a clear advantage for weed suppression over the erectophile type at a given plant or shoot population density, but there are also benefits of early and late planophile habits depending on the relative establishment of crop and weeds during the season. It is clear that selection for variety types should be considered in relation to climatic factors that affect both crop and weed growth.

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Possibilities to use marker assisted selection to improve allelopathic activity in cereals

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Abstract

Most breeding criteria are common for both conventional and organic cultivars, but there are some differences. Weed competitiveness is a trait that is very important in organic farming but until now mostly ignored in conventional breeding. This is slowly changing with increasing herbicide restrictions and problems with herbicide resistant weeds. Along with early vigour and an increased vegetative growth, allelopathy traits could be used to improve the weed competitive ability in cereals. There are more and more evidences that allelopathy are and could contribute even more to the weed competitiveness of cereals. The changes need not be very drastic. In the allelopathic rye the weed problems are much lower than in wheat and making wheat as allelopathic as rye could probably be done without any negative influences on the environment. New gene sources have to be identified as well as efficient selection tools. Studies in rice and wheat have shown that the allelopathic traits are quantitatively inherited and marker-assisted selection procedures could therefore facilitate the introgression of useful alleles and their pyramiding into elite cultivars. However, QTLs controlling allelopathic effects must first be identified either using already existing genetic linkage maps or creating new from segregating populations. Different bioassays are used to identify mapping parents, as well as phenotyping of populations. In this paper examples from the literature where QTLs have been identified will be presented, as well as the work and intentions in this area at Svalöf Weibull AB.

Keywords

Allelopathy, weed competition, breeding, genetic markers.

Introduction

Weeds are a major constraint limiting crop yield in most agricultural systems and organic systems in particular. In conventional farming systems, weeds are routinely controlled by herbicides, but this practice has now been questioned due to concerns about the environment and human health. Widespread use of herbicides has also created new weed problems, in terms of a shift in the weed population and herbicide resistant weeds (Lemerle *et al.*, 2001). As a result, there is an increasing interest in an integrated weed management based on a wide range of control options (Grundy & Froud-Williams, 1997; Mortensen *et al.*, 2000). One of these options is the inherited weed suppressive ability of the crop. In contrast to the control of pest and diseases, very little plant breeding has been carried out to improve weed suppressive ability. Likely reasons for this are the availability of effective herbicides but also a poor understanding of competitive ability, its mechanisms, components and relative importance. Morphological or physiological traits of importance linked with plant canopy establishment are leaf inclination, early vigour, plant height, tillering capacity, seed size, initial shoot and root growth rates and nutrient and water uptake. Most of these morphological traits are negatively correlated with yield. Therefore there is a risk that new high yielding cultivars will be less and less competitive. Not acknowledge allelopathy as an important factor could also explain the limited success in previous breeding programmes for weed competition (Olofdotter *et al.*, 2002). Allelopathy is now receiving increased attention, although the importance of this phenomenon is still questioned of some since it is difficult to separate from competitive traits. With increased knowledge it should be possible to design new cultivars which combine the suppression of weed growth with a good overall agronomic performance. The use of a single trait may not be sufficient but integrating a number of traits, such as competition for light and nutrients and allelopathy would offer a potential to make use of an inherited competitive ability by plant breeding. The changes need not be very drastic. In the allelopathic rye the weed problems are much lower than in wheat and making wheat as allelopathic as rye could probably be done without any negative influences on the environment. In this work identification of QTLs and using PCR-markers linked to these could be a useful tool. Examples of breeding efforts from the literature as well as studies and intentions in this area at Svalöf Weibull AB will be presented here.

Does allelopathy play a significant role also in the field?

There are plenty of field observations indicating an allelopathic interference with weeds. It is e.g. known that the weed problems are smaller in rye and Triticale than in wheat or barley. This could be referred to a more early and intense vegetative growth but also to allelopathy. Rye is known to be very allelopathic and straw residues are today used to control weeds in the next crop. Also barley and wheat are known to be more or less allelopathic and cultivar differences have been found. The question is if the results from the laboratory tests do have any field relevance? In rice Olofsdotter and Navarez (1996) found that the result from the bio-assay was well correlated with their weed suppressing activity under field condition and up to 34 % of the reduction in total weed biomass could be referred to allelopathy (Olofsdotter *et al.*, 1999). In a 4-year study with barley Bertholdsson (2005) also found similar correlations, although the correlations were insignificant some years. Since it is impossible, unless using near isogenic lines, to separate the effects of competitive and allelopathic traits, multiple regression models were used to calculate the various effects. In these studies 22-57 % of the variance of the weed biomass was explained by differences in early vigour of the barley cultivars. By adding an allopathic trait, measured with a bioassay, this figure was increased to 44-69 %, i.e. the contribution from allelopathy was 12-26 %. In wheat the figures were 14-21 % for early vigour and in combination 27-37 %. Hence the overall explained variance was lower in wheat than in barley as also the allelopathic contribution. The relative low allelopathic activity in wheat opens a possibility to improve the weed competitive ability of spring wheat. Predictions, with the assumption that an increased allelopathic activity will not have negative influences upon plant growth, show that an increased allelopathic activity to the level of the most allelopathic barley could result in a reduction of the weed biomass with about 60 % (Bertholdsson, 2005).

Evaluation of allelopathic activity

The allelopathic activity is usually determined with a bio-assay with a weed or another crop as a receiver plant. In our work we are using an agar-gel based method with ryegrass as receiver plants adapted from Wu *et al.* (2000). Number of test plant, days and gel concentration and quantity has been adjusted to give the highest possible correlation with observed cultivar differences in weed competitive ability in organic field trials. It is therefore hypotheses that the bioassay could be used as a non-specific method and not only for the allelopathic interferences against ryegrass. The hypothesis is supported by the fact that similar correlations also were obtained using rape seeds (*Brassica napus v. oleifera* L.) and studies in rice showing that allelopathic rice could suppress both mono-and dicot weed species (Olofsdotter *et al.*, 2002).

In the bioassay used at Svalöf Weibull AB, plastic tissue culture vials (Phytotech, 400 mL) are used with 30 mL 0.3 % water agar, six pre-germinated barley or wheat seedlings planted circular along the vial wall, and 10 pre-germinated perennial ryegrass (*Lolium perenne* cv. Helmer) seedlings, planted in the centre of the vial. The cereal and ryegrass seed are pre-germinated on filter paper in darkness for two days at 25°C. The vials are loosely sealed with a lid and placed in a growth chamber with a light/dark cycle of 16/8 hr, at a temperature of 20°C and inflorescent light of 52 μmol x m^{-2} s^{-1}. After seven days, the root area of ryegrass is measured using an image analyser (DIAS, Delta-T Devices, Cambridge, England). Vials with only ryegrass are used as controls. In the initial studies the barley and wheat roots were dried at 80°C for 48hr and dry weight was measured after removal of the root crown. Now the fresh weight is used instead. Potential allelopathic activity (PAA) is calculated according to $PAA = (1-A1/A2)*100$ with $A1$= ryegrass root area in presence of barley or wheat and $A2$= ryegrass root area without barley or wheat. Based on PAA, specific potential allelopathic activity (SPAA) is calculated as SPAA=PAA/root weight. All tests are done with 4-6 replicates (Bertholdsson, 2004, 2005). Using SPAA instead of PAA may reduce the effect of differences in root growth. In practice this is of less interest since it is the total allelopathic activity that should be selected for. In genetical studies, however, the allelopathic proprieties should be separated from any root morphology. On the other hand, Jensen *et al.* (2001) and Bertholdsson (2004) did not find any significant correlation between root morphology of the lines and their allelopathic potential.

Genetic variations of allelopathic activity

The success of introgression of useful alleles and their pyramiding into elite cultivars is dependent on the available genetic variation. In Nordic spring barley there have been a loss of allelopathic properties during the breeding process and it may be necessary to re-introduce some alleles from landraces or old cultivars (Bertholdsson, 2004). Ongoing studies will show if the allelochemicals differ in various landraces. If so it should be possible to introduce alleles from different landraces to improve the allelopathic potentials of barley. Genetic markers could facilitate this work. However, a screening of modern cultivars revealed that it is possible to find high activity even among some newly released cultivars. It is possible that they already have such genes from different landraces according to the pedigree. One draw back with these is that they in general have a shorter straw and lower vegetative growth than older cultivars. High allelopathy on its own may not be enough to be able to compete well with the weeds. Hopefully, it will be possible to combine alleles coding for various allelochemicals and early vigour and in this way improve the weed competitive ability.

In Swedish spring wheat the allelopathic activity is low even in landraces and old cultivars. Fortunately, wheat from central Europe and other parts of the word is more allelopathic. A screening of 813 cultivars revealed cultivars with allelopathic proprieties close to the most allelopathic barley. Some of those were Triticale but a few seem to be wheat or maybe rye-substituted wheat lines (Figure 1). Wu *et al.* (2000) also found a considerable genetic variation of allelopathic activity in wheat germplasm. Furthermore, studies of near isogenic lines indicated that the allelopathic activity might be controlled by major genes and they concluded that it should be possible to breed for cultivars with enhanced allelopathic activity. There is, however, strong evidence that allelopathy is due to a complex of chemicals and thus quantitatively inherited. Therefore, manipulation of allelopathy based on phenotypic selection is time-consuming and labor-intensive as well as influenced by environmental factors. DNA markers may therefore provide a powerful tool to dissect and manipulate quantitative trait variation through QTL mapping experiments and marker-assisted selection.

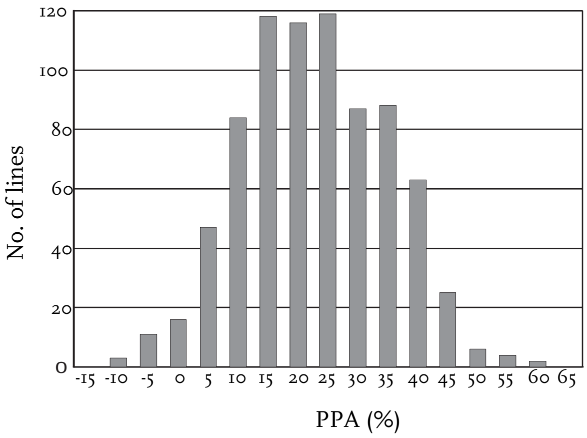

Figure 1. Frequency distribution of the potential allelopathic activity (PAA) of 813 spring wheat cultivars measured as the root growth inhibition of ryegrass in presence of wheat plants. Most Swedish cultivars have a PAA value below 20.

Allelopathy QTLs in rice and wheat

In cereals, most work has so far been done in rice and wheat. Jensen *et al.* (2001) studied the allelopathic activity against barnyardgrass (*Echinochloa crus-gali*, L) of a population of 142 recombinant lines derived from a cross between two cultivars with low and high allelopathic activity. They found transgressive segregation for allelopathic activity in both direction and four main-effect QTLs located on three chromosomes were identified. The four QTLs explained 35 % of the total phenotypic variation of the allelopathic activity. The mapping results indicated that allelopathy in rice is a quantitative trait involving several loci and possibly some degree of epistasis i.e. interactions between genes. These initial results are now verified using another population and genotyping of near-isogenic lines is underway using microsatellite markers (Olofsdotter *et al.* 2002). Okuno & Ebana (2003) used a F_2-population of a cross between a highly suppressive and less suppressive rice cultivar. The allelopathic effects were assessed using water-soluble leaf extracts on root growth of lettuce and duck salad. Seven QTLs on different chromosomes were identified that explained 9.4 – 16.1 % of the total phenotypic variation in weed biomass. With one exception, the QTLs were found on different chromosomes in the two studies.

A study of a DH population of 271 winter wheat lines showed that wheat allelopathic activity was normal distributed with a substantial transgressive segregation as in rice. Contrary to rice two major QTLs were identified on chromosome 2B (Wu *et al.*, 2003). Each of the two QTLs on 2B came from different parents and had similar genetic scopes. However, the QTLs detected were not able to account for the phenotypic differences in allelopathic activity between the two parents. In was concluded that the genetic control was complicated since the QTLs were on the same chromosome. The QTLs found had a relative high significance and therefore should be further validated over a range of genetic backgrounds before robust PCR markers can be selected and implemented in wheat breeding programs. A step forward would be to identify the allelochemicals responsible for the weed suppressive effects and find the genes involved in the biochemical pathways and check whether they co-segregate with the QTLs identified. Allelochemicals have been identified not only in rice but also in wheat (Wu *et al.* 2001) and in wheat, barley and oat (Baghastani *et al.* 1999), but linking these to special genes and QTLs remains. It is also important to study if the identified QTLs using a bioassay are correlated with actual allelopathic activity under field conditions (Olofsdotter *et al.*, 2002)

Phenotyping of mapping populations

At Svalöf Weibull AB an already mapped DH-population (Lina x *H.spontaneum* L.) has been phenotyped with the agar-gel method. This is one of the most polymorphic mapping population in barley with a detailed linkage maps suitable for QTL mapping. The map that will be used to detect QTLs for allelopathy consist of 1061 markers (334 RFLPs, and 727 SSRs, SNPs and other PCR based markers) (DNALandMarks, BPS, Canada). In the allelopathy test Lina showed high and the *H. spontaneum* line low activity (Figure 2). Contrarily to the studies in rice and wheat there seems to be no transgression if the standard error is considered.

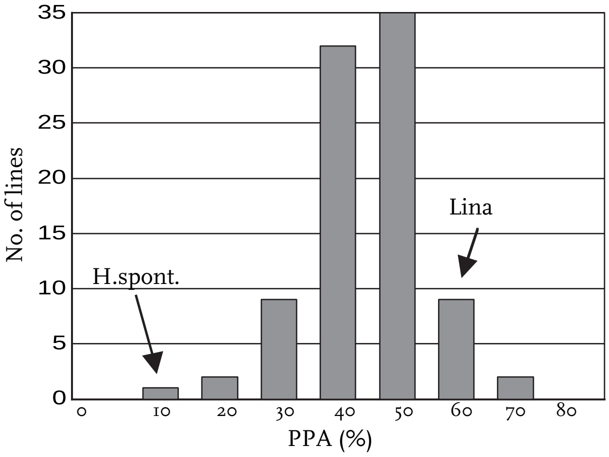

Figure 2. Frequency distribution of the potential allelopathic activity (PAA) measured as the root growth inhibition of ryegrass in presence of barley plants of 97 DH-lines from the population Lina x H.spontaneum.

In wheat a high quality Swedish cultivar Zebra (SW) with rather good competitiveness against weeds but low allelopathic activity (PAA=13) has been crossed with two non-adapted foreign cultivars with high allelopathic activity (PAA=5-58). Parallel to a standard backcrossing and selection program, DH-lines or inbreed lines will be produced and phenotyped for allelopathic activity.

Marker-assisted selection (MAS)

When data from phenotyping and mapping are available QTLs can be identified. After verifying these in other gene backgrounds, closely linked PCR markers on both sides of the QTL could be used in several ways to enhance breeding either by control of the target genes (foreground selection) or control the genetic background (background selection). Marker assistance is expected to provide higher efficiency, reduced cost and / or shorter duration of a backcross breeding scheme, compared with conventional methods (Hospital, 2003). Especially markers for the background selection could be useful if non-adapted cultivars and landraces are used as parents. The use of markers could hasten the recovery of recipient parent genotype. This must be done in combination with a foreground selection either using markers or phenotype.

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Field selection and marker-assisted breeding, to do or not to do in organic breeding programmes?

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Abstract

Organic agriculture has realized in the past decade that it will have to organize its own plant breeding programme in order to develop varieties that fit their culturing methods and quality standards and to maintain open pollinated varieties. Large-scale field trails across Europe have resulted from these efforts and new varieties have been developed successfully. Still, progress can sometimes be tantalizingly slow. In the mean time the large plant breeding companies have turned their attention to molecular markers as tools to enhance development of new varieties. This has led to a successful stacking of traits and a continuous flow of new products. Can this "success" be transferred to organic plant breeding, in other words would it benefit from a marker-assisted selection approach?

Keywords

Organic plant breeding, molecular markers, holism, reductionism.

Introduction

To be able to decide upon the above question the concepts of an organic plant breeding program should be defined and portrayed against the background of conventional plant breeding. In Table 1, I have tried to summarize these differences in breeding concepts. Organic plant breeding starts to consider the whole agro-ecosystem it is part of and tries to define bottlenecks and opportunities from within. Conventional or maybe more precisely, commercial plant breeding starts to consider traits in the germplasm that is proprietary or can be obtained commercially. Most commercial products will be F1 hybrids, contrasting with the efforts of organic plant breeders, who aim to produce open pollinated varieties that can be further improved by population selection. Commercial plant breeding practices a gene stacking approach in which breeders try to combine mono- or oligogenic traits into their parental lines. These traits have to be attractive for a worldwide market and are developed for high-input (mineral fertilizer, crop protection chemicals and greenhouse) agriculture. Organic breeding envisions a more local and regional marketing and breeding system, which involves the expertise of local farmers and the possibility to breed for varieties that are adapted for certain regions. In general, organic plant breeding envisions a strategy that is aimed primarily at plant quality rather than yield and homogeneity.

Table 1. Breeding concepts in the two extreme plant breeding "styles".

Organic plant breeding	Conventional plant breeding
Agro-ecosystem driven	Germplasm driven
Open pollinated varieties	F1: hybrid
Population selection	Concept of gene stacking
Regional adaptation	World-wide market
Low-input traits	High-input traits
Quality	Yield and homogeneity

In summary one can conclude that the basic concepts of organic plant breeding can differ enormously from that of the current commercial plant breeding approaches, although one should consider that both part of their breeding efforts are not necessarily so far apart.

Marker assisted breeding

Marker assisted breeding builds on a vast history of scientific knowledge of genetic components that are proposed to determine desired traits. These traits can be as simple as monogenic pathogen resistance traits or as complex as quantative trait loci (QTLs). These traits are all within the "germplasm space" that a breeding company or research groups have defined (or claimed). It is a knowledge-intensive approach that aims to predict the outcome of a selection process as a designed combination of genome fragments, 'breeding by design"(see ref. Peleman and van der Voort). The technology as such is dependent on laboratory assays, which describe the genetic make-up of parental lines. For every trait a reliable genetic marker has to be developed or copied from scientific literature. Every breeding population has to be profiled with this set of "markers" in order to obtain material that at least contains this part of the genetic information. Although automation of DNA profiling techniques has reduced the cost and labour enormously, it still requires high-tech lab facilities. In the extreme form of marker assisted breeding seedlings that have been obtained from a cross to introduce new traits will be selected only on the basis of their genetic make-up and then tested in the laboratory, greenhouse or field to investigate their performance. Commercial breeders more and more rely on the genetic maps of their favourite inbred lines to design new varieties. Breeding activity is thus focussed on finding markers that correlate with new traits and to stack this knowledge in new inbred lines.

Organic plant breeding tries to use the full potential of a crop to generate open pollinating varieties. To achieve this in their local agro-ecosystems, field selection in large and diverse populations has been the logical approach (see Table 2). Because field trails are carried out on organic farms, plants are selected under the conditions of the actual agro-ecosystem. In addition, this allows regional variation to be part of the selection methods. Organic plant breeding is still building its own, specific set of breeding criteria to be able to design strategies that will result in a crop with the desired performance. Instead of "trait-stacking", organic plant breeding practices whole plant selection in field situations. Seed batches of interesting varieties are shared between participating breeders/growers to enhance the progress.

Table 2. Characteristics of contrasting selection approaches.

Field selection	Marker-assisted selection
Man power demanding	Lab power demanding
Generation population	Genetic maps, QTLs
Field evaluation	Lab evaluation
Regional evaluation	Genetic maps, QTLs
Whole plant/ecosystem	Correlation traits with markers
Shared pool germplasm	Protected inbred lines

In summary, commercial conventional plant breeding can benefit from marker assisted breeding because its infrastructure is aimed at the continuous generation of new breeding lines. Time to market is a serious economical factor for these companies. If one can stack traits more rapidly, this will be a positive result. Organic breeding is not yet a large-scale commercial activity, which puts different constraints on its development. Not the number of new breeding products but the quality of product is at the moment an important issue. Can an organic plant breeding product distinguish itself from conventional products?

DNA Markers in research

In this EU-COST meeting several research institutes presented their results with regard to the use of DNA markers in research projects aimed at describing traits that are interesting for organic plant breeding. It clearly illustrated the approach that research takes to solve an agronomical or product-related problem (Table 3). The aim is rather to generate a model that fits the experimental data and addresses the hypothesis formulated. Not necessarily does this have to result in a product with market value. Research results show that the description of complex traits such as nitrogen efficiency, baking quality, yield, weed suppression and taste with the help of DNA markers is not an easy task. In general this leads to the

formulation of QTLs that only explain parts of the effect. Moreover, these QTLs can vary in different crosses illustrating the complex nature of the traits. Only monogenic disease resistance traits can be easily converted into reliable DNA markers. But even then monogenic resistance can vary in its effectiveness in different genetic backgrounds.
In contrast to the research approach, commercial breeding will build on research knowledge and convert this into a new product with market value. There is little "discovering" activity in this process. The solid believe in DNA based trait inheritance has stimulated the conversion of trait-based thinking to gene-based breeding. To my opinion organic plant breeding aims to breed by discovery rather than by solely copying research results. In field situations under organic conditions selection in large populations, with newly developed quality criteria, this will lead to the discovery of new varieties.

Table 3. Differences in aims of plant research and commercial breeding.

Research	Commercial Breeding
Hypothesis driven	Customer demand driven
Experimental populations (<5)	Breeding populations (>5)
Laboratory evaluation:	
DNA, RNA, Protein, Metabolite	Field and product evaluation
Genome organisation	Germplasm evaluation
Biodiversity	Market value
Physiological model	Protected (inbred) lines

In summary, when considering DNA markers and their value in breeding programmes one should keep the distinctions between research-aimed and product-oriented breeding in mind. Research formulates new hypotheses that help developing new concepts. These new hypotheses do not necessarily have to allow convertsion into actual products.

How would consumer perceive of the application of DNA markers in organic plant breeding?

Organic agriculture can attribute its success to a large extent to its image as the "natural " way for the production of vegetables and cereals. Its so-called holistic view on agriculture and its man-made ecosystem sets it apart from industrial/chemical agriculture that preaches the reductionistic view. Already organic agriculture has positioned itself against biotechnological developments such genetic modification. Consumers have appreciated this clear statement. Introduction of a technology that again focuses on genes and DNA only, could confuse consumers and suggest that organic agriculture has converted itself to this technological track. The full potential of marker assisted breeding is associated with tissue culture technologies such as anther or pollen culture and protoplast fusion. Organic agriculture can not risk damage to its "natural" image, which is likely to occur when more molecular tools are introduced into its breeding program.

Summary

In this paper I have tried to picture the position of organic agriculture with regard to marker assisted breeding. It is a personal view describing the attitudes of organic agriculture and conventional breeding. Where necessary the differences have been formulated more black and white than will be perceived by either party. My experience with genomics and DNA-based plant research has convinced me that not everything in a living organism can be explained by the linear genetic dogma. Only in the actual agro-environment will one be able to discover the full potential of a crop plant. Furthermore, I propose that plant nutritional quality should be emphasized more in breeding programs, which mean that we will have to start measuring traits that are determined by both plant species and human consumer. Given these different attitudes towards plants as a collection of genome fragments and plants as organisms in a man-made biosphere I will finish with the statement that it may be a waste of time to apply marker-assisted selection to an organic plant breeding programme. This will limit the discovery range of a plant breeder and transfer money needed for actual product development to laboratory exercises.

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Reflection on the workshop

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Nowadays in breeding research a lot of attention is being paid to the development and use of molecular markers. In Europe genetic engineering is highly criticised by consumers and banned by organic agriculture. Nevertheless, molecular scientists keep their trust in the potential of the knowledge and techniques at DNA-level. Genomics research has progressed and now seems to be the new keyword to monopolise the research resources. Some molecular scientists would like to have the organic sector embrace the DNA markers as diagnostic tool in breeding programmes and promote genomics as a green tool, but this is not self-evident for the organic sector.

Markers can be applied as a diagnostic tool in breeding programmes to supplement phenotypic selection methods in the field. Their potential for organic agriculture has yet to be proven. The organic sector is sceptical about DNA markers because the field performance and genotype-environment interaction are of primary importance in organic plant breeding. This workshop was organised to discuss the question: Does the DNA marker technology contribute to the practical and specific needs in organic plant breeding?

Principles and Values of organic agriculture

We started this workshop with several introductions to give some background on the principles organic breeding. The first contribution was on the ecological and ethical framework in which organic agriculture operates. It was not easy to put these ethical points in perspective in the following technical discussion. Then an overview of the characteristics of organic cereal varieties was presented. As farmers have largely developed organic agriculture, and only recently plant breeding research has become involved, it is interesting to see the importance of organic farmers participating in the plant breeding process. The possibilities of participatory selection methods in plant breeding and the experience INRA has gained with organic farmers in this matter were presented. It was discussed whether marker assisted selection (MAS) could be included in these participatory breeding approaches. Serious doubts were expressed because experiences of participatory plant breeding/selection programmes showed that farmers often can better assess whether varieties are suitable for their needs than breeders.

These introductory lectures were completed with general background information on the use of DNA-based genetic markers in plant breeding. In the discussion that followed the question was raised by practical breeders whether phenotypic traits are the most ‘holistic’ and the more reliable markers compared to DNA-markers.

During the this part of the workshop the focus was mostly on the ecological issues, starting from the question of how to produce varieties that are better adapted to the organic farming systems. In the discussion on the possible value of molecular markers for organic agriculture, the question arose how to look in a holistic way at plant traits and how to incorporate a reductionistic tool in a holistic method for organic plant breeding. It was concluded that such a holistic method is not yet available (“How to create happy homes for happy genes”).

Application

After the framework of organic farming was set, the presentations focussed on traits that are important and most typical for organic cereal varieties, such as yield stability, baking quality, broad disease resistance, nutrient uptake/utilisation and weed competitiveness. These characteristics are determined by quantative trait loci (QTLs) and therefore not easy to select for, as many genes on different chromosomes are involved. For each above-mentioned characteristic we compared the state of the art on the level of field selection and of molecular marker assisted selection.

There appeared to be quite some uncertainties about the correct understanding of quality. It was made clear that one must be absolutely certain about the correlation between trait and DNA marker, when using MAS or else the wrong allele might be selected for. The trend was observed that protein markers give more information about cereal quality than DNA markers. In the discussion on yield stability and genotype x environment interactions it was stated that the transfer of a QTL from one population to another population is not always successful. Furthermore comparative breeding experiments revealed that breeding for disease resistance in cereals can be achieved as effectively with traditional methods as with MAS.

In the last plenary discussion a lively discussion developed, part of which evolved around the issue of the genetic dogma. Michel Haring's statement that DNA marker-based breeding results from a reductionistic view, which cannot be applied to complex traits, was challenged. The example of selecting monogenic resistance genes against BaYM virus by marker-assisted breeding was put forward to illustrate the power and necessity of the technique. Others claimed that both research breeding and commercial breeding do not fully rely on DNA markers, field evaluation is equally important! Some molecular scientists stated that visual (morphological) markers have always been used to help selecting for complex traits, and that DNA markers are nothing more than complex visual markers. Breeders realize that field performance is the key issue in breeding line selection.
Again the question was addressed to what extent a phenotype can be predicted by genotype. Is this merely a matter of probability, which decreases with the number of genes involved? Or do we underestimate the complexity of a phenotype by thinking that one can predict each phenotype by looking for QTLs? The practical breeders were convinced that there is a self-regulating principle for the application of MAS: "if it doesn't work we will not use it, as for breeders, the first thing is phenotype, and we can only spend our money once". The organic sector should evaluate the application of MAS on a case by case basis to see whether the problem can to be solved on an agronomic or genetic level. Finally, it should be considered that consumers can easily confuse the concept of DNA markers with genetic modification which would lead to an unwanted negative image of organic plant breeding

Some scientists stated that the desired traits in organic plant breeding are extremely complex, such as yield stability, efficient nutrient uptake/utilisation, broad disease resistance, weed competitiveness, which in the short run would make molecular markers inefficient in practical breeding programmes for organic agriculture. In conventional agriculture one is able to control the environment to a greater extent than in organic agriculture, and thus one could believe that in conventional breeding one can more easily pinpoint certain traits. These kinds of markers give support to selection at a level that is not relevant in terms of practical handling in organic agriculture.

One could imagine that in fundamental research molecular markers can be of importance to understand underlying mechanisms. A possible area for application is in backcross programmes with monogenetic disease resistances introgressed from wild relatives to avoid linkage drag. As an example loose smut in wheat was mentioned, a disease, which appears at flowering stage, and for which no organic measurements are available.
Another aspect of applying DNA markers was discussed. For the robustness of the organic farming systems, diversity at different levels (farm, crop, and variety) is very important. With molecular markers one can monitor diversity in breeding stocks in order to maintain diversity among varieties.

Conclusions

By presenting the state of the art and the possibilities of both phenotypic and genotypic selection this workshop contributed to a better understanding of the application of molecular markers in breeding programmes for organic agriculture. MAS can be a useful tool for breeding research, especially when monogenic traits are involved. Nevertheless, most of the participants agreed that on the short term molecular markers will not be available for the quantative traits that are of specific interest for the practical breeding programmes for organic varieties. Phenotypic selection in the field is still the best way to select for organic agriculture.
But there are also others questions to be answered that were only shortly addressed during this workshop, such as a cost-benefit analysis. How efficient can MAS be for organic plant breeding programmes? The research to develop molecular markers is very costly and the organic sector doubts if such research should have such high priority over other urgent research required for the optimisation of the organic farming systems. In one of the presentations (Pawell) an estimation of the possible reduction in costs for breeding programmes was presented. The conclusion was that, although it could bring breeding costs back to 1/10 of field selection (when combined with dihaploid selection in tissue culture), molecular markers will never be payable for farmers, even taking the lower costs in future into account.
The workshop did not address the question whether the current generation of molecular markers complies with the principles of organic agriculture (non-chemical, agro-ecological and integrity of life), and which alternative procedures are available or to be expected in future. Molecular markers are generated using products of genetically modified bacteria, radioactive isotopes and carcinogenic chemicals, components that are not permitted within the organic standards. In addition, to confirm the link between a trait and a genetic marker, genetically modified plants sometimes have to be generated. This is not compatible with the principles for organic plant breeding. For the QTL analysis, in some case

dihaploid plants are produced with the help of in-vitro techniques, which are under discussion in organic agriculture respecting integrity of plants. All these technical aspects could already raise controversial arguments about MAS.
Further investigation and discussion is necessary to be able to decide from case to case whether molecular markers can be of added value for organic breeding programmes or for fundamental research in understanding desired complex traits.

Part B
Posters

(alphabetical order of first authors)

BIO SUISSE considers hybrid rye ban

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Hybrid varieties are increasingly and widely used in organic farming, although there has never been a conscious decision to do so. This is especially true for vegetables and maize. For bread cereals, especially rye, the decision can still be taken in Switzerland. Organic rye is grown on only 250 ha, about 20% of which are hybrid varieties. About 60% of the total organic demand is imported.

Why use hybrid varieties?

- higher yield, which is able to overcompensate
- higher price for seed (ca. 10 % more yield in simple organic
- variety trials in 2003/2004) (Dierauer, 2004.)
- greater homogeneity
- better lodging resistance
- better sprouting resistance

Why ban hybrid varieties?

Ecological aspects

- Lower genetic diversity on the field
- Open-pollinated (OP) varieties contribute to in situ conservation of rye germplasm
- high genetic vulnerability of hybrid varieties due to uniform cytoplasm (source of male sterility)
- Loss of recessive traits during inbreeding of parent lines (such traits may become interesting in future) (Müller, 1996)

Quality aspects

- Male sterility in the mother line: is that the progeny for bread cereals the consumer wants?[3]
- The consumer receives the harvest from which the F2 would grow. From many of these grains unsatisfactory, weak or extreme plant types would grow. The consumer does not know that.
- Hybrid varieties support the tendency of present conventional farming to enhance mainly growth, biomass accumulation and nutrient uptake (Mueller, 1996).
- Reduced pollen production due to only partial restoration of male fertility. For biodynamic understanding, this means a loss of warmth quality, leading to lower inner quality. This is one reason why Demeter decided to ban hybrid cereals (except maize).

Socio-economic aspects

- Higher yield may contribute to decreasing producer prices and therefore lower returns to the farmer.
- Population varieties can be multiplied by the farmer whereas hybrids create dependency on the breeder (ABDP, 2003).
- Maintenance of demand for OP varieties (otherwise they may be lost soon).

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Barley landraces as source of resistance to powdery mildew for breeding for organic farming

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Introduction

Organic farming is gaining social, political and scientific importance and recognition. The proportion of the organic area in total agricultural land in EU countries is about 3.0% and is steadily growing. Because of this it is growing need for breeding varieties for organic farming with effective resistance to abiotic and biotic stresses (1).

Powdery mildew caused by the pathogen *Blumeria graminis* f.sp. *hordei* (synamorph *Erysiphe graminis* f.sp. *hordei*) is very destructive foliar disease of barley in many regions of Europe (2, 3).

Barley landraces constitutes a rich genetic resource, and many examples of their successful use have been reported. However only for less than 2 percent of barley landraces the attempts were made to identify powdery mildew resistance genes using differential lines and isolates. These studies were mostly conducted in Germany, Denmark and Sweden and on smaller scale in other countries such as The Netherlands, Czech Republic and Poland (3, 4, 5, 6).

The objective of this study was to determine the powdery mildew resistance in barley landraces collected from West Asia, North Africa and Southern Europe and to select resistant lines for breeding for organic farming.

Materials and methods

Seed samples of 385 barley landraces were used for screening for resistance to powdery mildew. These landraces were collected in 20 countries (Albania - 3, Algeria - 22, Croatia - 8, Cyprus - 4, Egypt - 37, Greece - 29, Iran - 35, Iraq - 21, Israel - 6, Jordan - 21, Lebanon - 4, Libya - 6, Macedonia - 35, Morocco - 25, Portugal - 17, Spain - 13, Syria - 22, Tunisia - 30, Turkey - 42 and Yugoslavia - 5) and originated from National Small Grains Collection, U.S. Department of Agriculture, W. Aberdeen, Idaho, USA. About 9.4 % (36 landraces) showed resistance to powdery mildew. From these landraces 43 single plant lines were selected. These lines were tested in series of experiments with 20 differential isolates of powdery mildew. The isolates were chosen according to their virulence spectra that were observed on the Pallas isolines differential set and 7 additional differential cultivars.

Results

Eight lines selected from 7 landraces (1 from Jordan, 1 from Israel, 1 from Croatia, 2 from Greece, 1 from Turkey and 1 from Macedonia) showed resistance to all isolates used. The most common resistance reaction observed was reaction 2 (47%). In 18 tested lines (41.9%) the presence of 7 genes for resistance were postulated. The most frequently postulated resistance gene was *Mlat* (10 lines). For 25 (58.1%) tested lines it was not possible to postulate the presence of specific resistance gene.

Discussion

The results presented here come from the tests performed on seedlings, which may not always predict adult plant resistance. However, determination of powdery mildew resistance genes based on tests performed on seedlings using isolates with different virulence spectra is effective and sufficient for breeders and pathologist needs. Confirmation of putative resistance genes or alleles can only be established through evaluation of progeny from crosses and backcrosses among appropriate genotypes (2, 3, 5).

In general, results of this study confirmed that barley landraces possess mildew resistance genes different from genes present in cultivated varieties. As isolates used in this experiment had virulences corresponding to all major resistance genes used in Europe to date, it may be concluded that these lines had resistance to all powdery mildew virulence genes prevalent in Europe. These highly resistant lines should be used as new sources of effective resistance to powdery mildew of barley in breeding programmes for organic farming.

Selection index for winter wheat cultivars suitable in organic farming

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Weed competitiveness, productivity and baking quality are specific problems in organic farming. Productivity and baking quality are linked to nutrient acquisition ability: nitrogen uptake and nitrogen-use efficiency. For those characters, the selection of new lines is necessary. Thus, we proposed an index selection method. Fifteen years after registration by INRA of the cultivar Renan by G. Doussinault (INRA), winter wheat cultivars, originating from public and private breeding programs combine, as never before, resistance to diseases with satisfactory yield potential in integrated farming (Rolland *et al.*, 2002; Rolland *et al.*, 2004). One of the objectives of INRA cereals programmes is to evaluate genetic material, originating from breeding programmes for low input systems (high disease and lodging resistance, low seeding rate, standard quality), under organic farming crop system. The aim of the study is to define important agronomic characteristics of cultivar types adapted to organic farming in collaboration with ITAB[1].
The study was conducted in a multiple-site (field experiments in Bretagne (B), Ile de France (IdF), Midi Toulousain (MT) and Poitou (P)), multiple-year (2002, 2003 and 2004) trial network with 25 winter wheat varieties in organic farms. Seeds were sown in a plot trial with four replicates. Plots were assessed for weeds, diseases, nitrogen nutrition index, yield and grain quality.

Crop yield: In 2004 in Rennes (B) and Sermaise (IdF), soil fertility was favourable for high crop yield in organic crop management plans (table 1). Diseases, such as eyespot, yellow rust or *Septoria tritici*, were adequately controlled by resistant varieties. Winter wheat provided average yields of more than 6 t/ha in Rennes and Sermaise. This must be compared to 7.5 t/ha for wheat in low input crop management plans. In Poitou, natural fertility was very low so crop yield was about 3 t/ha. Toulouse (MT) gave intermediary results with 5 t/ha.

Weed competitiveness: Important wheat traits influencing shading ability and thus weed growth are plant height and ground cover. In Rennes, we proposed, as Hansen (2000) did, to approach the weed competitiveness (IC) with two parameters: crop canopy height (H) and wheat ground cover at GS34 (PC). Pegassos is the cultivar with highest IC, with planophile leaf inclination (Drews, 2002).

$$IC = 2 \times PC + H$$

Quality index: Putting together information on French bread-baking drawn from 2 years of multi-local experimentation (16 cultivars averaged on three locations, years 2002 and 2003) and results of various technological tests, it appeared a relatively close connection between French bread-baking, protein content and Zeleny.

$$IQ = \text{Protein content} + \text{Zeleny}$$

Global Index: To select cultivars adapted to organic farming using most important agronomic traits, we proposed a global selection index (IGS) which will take into account yield (Y), quality and weed competition.

$$IGS = Y + 2IQ + IC$$

Varieties showed significant differences: Saturnus, Aristos, Renan, Capo were most valuable (Goyer, 2004). The future effort in breeding programmes will thus contribute, with agronomy, to bring answers to the specific requests of organic farming and sustainable agriculture.

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[1] ITAB Institut technique de l'agriculture biologique

The breeding strategies for low-input conditions in Romania

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Production of field crops is a significant component of the Romanian agriculture. More than 60% from the total of arable (about 10 000 thousand hectares) is cultivated in various environments with cereals: maize (31.6 %), wheat and rye (27.2 %), barley (5.6 %) and others. Among these, wheat is one of the most important crops for human and animal consumption in Romania. That's why the breeding activity is generally very dynamic not only in traditional cereals, but as well as for the newest introductions, as triticale, which registered in the past decade an ascending trend in the preferences of farmers. The most part of the current bread wheat cultivars and an important percentage of the maize hybrids is the result of national efforts in this field.

After the political change occurred in 1989, the breeding activity has to face with many new challenges. A top priority became the appearance of many small private farms (2-2.5 hectares). As a consequence, the farmers generally are not yet able to apply improved crop technology that demonstrated over years, to be quite detrimental for the amount and quality of yield.
In these circumstances, there is great necessity to change the Romanian crop system, also, toward low-inputs conditions.

The breeding of new cultivars suited for low-input system is currently the most important target in this direction, not only for the Romanian cereal market, but as well as for those from Europe and the others.

In consequence in our Institute were adopted new research directions based mainly on breeding strategies for low-input systems utilized in various environmental conditions of Romania. Some of such directions are:
-the simultaneously routine screening of advanced breeding genotypes in conventional and low-input field conditions, including also organic farming system, for wheat, barley and maize;
-breeding for resistance to diseases, with emphasize on breeding for resistance to *Fusarium, Tilletia* and associated mycotoxins;
-the initiation of molecular approach of selection for such traits (also in cooperation);

The interest of the national authorities for these very important and current topics is materialized by funding of three new research projects that are in run:

1) Identification of molecular markers associated with resistance genes against bunt *(Tilletia caries, T. laevis, T. controversa*), in order to obtain new wheat cultivars. National Research Project BIOTECH 613/2003 (2003-2005);
2) Selection assisted by molecular markers for improvement of resistance to *Fusarium graminearum* and associated mycotoxins in wheat. National Research Project BIOTECH 4545/2004 (2004-2006).
3) Evaluation of resistance to low temperatures and soil pathogens in corn. National Research Project AGRAL 2469 /2004 (2004-2006).

Evolutionary breeding of wheat for low input systems

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A major limit to progress in low input systems is the lack of appropriate crop genotypes selected for productivity in low input agriculture. Modern wheat varieties selected for high input production can often produce yields that are tolerable in the sense that they are often greater than yields achieved by older varieties bred under moderate inputs. However, as Weiner (2003) has pointed out, substantial reductions in inputs exposes crop plants to major increases in the diversity and variability of production environments. This means that genetically variable host populations are more likely to be both higher yielding and more stable in output, assuming that they derive from appropriate parents.

Winter wheat populations

To produce such populations, Elm Farm Research Centre with the John Innes Centre, developed 6 populations based on wheat varieties that have been successful on a large scale for a long period during the last 50 years either as varieties or as parents. The populations were based on all possible combinations of 10 high yield parents, or 12 high quality parents, or intercrosses from all 22 parents. Each of these bulk populations was subdivided further into populations that did, or did not, contain a similar range of male sterile crosses developed from naturally occurring male sterile parents.

Field trials

Field trials were established at four sites, Metfield Hall Farm (continuous wheat in Suffolk), Wakelyns Agroforestry (organic farm adjacent to Metfield Hall), Morley Research Station (Integrated Crop Management farm in Norfolk) and Sheepdrove Organic Farm (organic farm in Berkshire). The first field exposure of the populations was in 2003-4 in the F3 generation.

Field performance

The poster will summarise some of the field observations from the first year of selection, showing the major differences between selection under conventional and organic conditions and the dynamics of selection for plant height. Such changes will be tracked by molecular markers to estimate the contributions of different parents to population performance.

Practical development

Dominance of pedigree line breeding has led to a legal and administrative framework for the introduction, use and protection of varieties, which limits the genetic response to variable environments. Analysis of the population approach can provide a fundamental contribution to debates about wider sustainability issues, including aspects of the ownership and development of future genetic resources. Against the perceived problems of utilising wheat populations, it is important to note that procedures are well defined and accepted for dealing with rye where populations account for probably more than half of European production (Miedaner, pers. comm.).

Acknowledgement

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Broadening the genetic base for better-adapted varieties for organic farming systems: participatory characterisation, evaluation and selection of onion accessions for new base populations

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Until now, organic farmers have been dependent on varieties bred for conventional high-input farming systems. Although organic farmers benefit from the improvements of modern breeding activities, the fact that most organic farmers in northwestern Europe use modern varieties does not imply that those are the best varieties for optimising organic agriculture. As organic farming refrains from high and chemical inputs it needs varieties better adapted to organic conditions to improve the yield stability and quality of organic crops. For such type of varieties additional characteristics are required such as mineral efficiency and weed-suppression. Exploiting gene bank material can be helpful as such additional properties might have disappeared by selection under modern, high input conditions. Establishing base populations with a broad genetic diversity can offer new genepools for specialised breeding programmes focused on organic farming systems.

In a recent co-operation between the Centre for Genetic Resources, The Netherlands (CGN), Louis Bolk Institute (specialised in organic farming) and organic farmers, 37 onion accessions were characterised and evaluated under organic growing conditions. The accessions were divided in 5 different groups according to their market use. Farmers have assessed these accessions using criteria important for organic farming systems such as leafiness, field tolerance for pest and diseases, yield capacity and storage ability. To establish several base populations the farmers, in collaboration with the researchers selected the best plants within the five groups of onion accessions. The selection process was documented by the researchers and performed during three phases of the crop: a) during full leaf phase for field characteristics, b) after storage for characteristics on yield and storage ability, and c) before flowering for generative traits.

The collaboration between farmers and researchers had the following results:

- For the first time in the Netherlands gene bank material has been characterised under organic farming conditions. This makes these accessions more accessible for the utilisation in organic breeding programmes.
- Farmer participation resulted in including additional plant traits for genebank characterisation as well as new selection criteria for breeding programmes
- The joint characterisation and evaluation also gave researchers insight on how farmers evaluate and value certain plant traits
- It was noticed that these open pollinated varieties showed a substantial diversity, which can be further exploited for organic farming systems. Variation for important properties was found within and between the 5 groups.
- The group defined also 6 high performing accessions which may also be exploited on its own in order to achieve better material for organic farming systems.

Seeds of six new base onion populations are now available for organic breeding programmes, and will be used in further selection.

Performance of Latvian spring barley (*Hordeum vulgare* L.) varieties and breeding lines in conditions of organic farming

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Organic farming is a comparatively new and growing direction of agriculture in Latvia. There are plans to expand the area of agricultural land, which is certified for organic farming from 1 % to more than 3 % in the year 2010. The choice of varieties is essential for farmers. In order to recommend the most appropriate spring barley varieties, trials in certified fields for organic farming were carried out in several Latvian research institutions. The poster summarises the data about yield, grain quality, plant height, the length of vegetation and infection with diseases of six registered Latvian barley varieties recommended by breeders as suitable for growing in conditions of organic farming, which were tested in 2003 (2 test sites) and 2004 (4 test sites). There were no significant differences between mean grain yield of tested varieties observed (P-value= 0.67 and 0.12 in 2003 and 2004 respectively). The influence of test site on grain yield was not significant in 2003, but it was significant in 2004. The highest seedling emergence was registered for variety 'Sencis', but it was lower for 'Idumeja'. Useful trait for organic farming can be earliness because of rapid development of plants, which can be related to better ability to compete with weeds and use the nutrients from soil, as well as a possibility to harvest the grain earlier and have a longer growing period for following crop (e.g. green manure). The duration of vegetation of variety 'Idumeja' was on average 7 days shorter than that of check variety 'Abava'. The highest mean TGW was estimated for varieties 'Idumeja' (47.0 g) and 'Ruja' (46.7 g). None of the varieties exceeded the mean grain volume weight of check variety 'Abava' (679 g l^{-1}). There were no significant differences between the crude protein content among the varieties detected. Serious problems for seed certification in organic farming can be caused by loose smut (*Ustilago nuda*). None of the varieties showed complete resistance to this seed-born disease, but the lowest infection level was detected for varieties 'Malva' and 'Idumeja', which can be recommended for organic farming from this point of view. Testing of advanced barley breeding lines for organic farming was started at Priekuli Plant Breeding Station in 2004. None of the tested 11 lines had lower yield if compared to check variety 'Abava', but 5 lines had significantly higher yield. The yield of the tested breeding lines did not significantly surpass the variety 'Ruja'. Two lines with resistance and one line with low infection level with loose smut were selected for further testing. Although a special breeding program for organic farming will apparently not be started in Latvia in the nearest future, several registered varieties can be recommended to organic farmers and testing of appropriate breeding lines in organic conditions may be a useful tool for selecting a new varieties. A new variety with resistance to loose smut could be applied for official testing in the nearest years.

Breeding Scheme for ORGANIC WINTER WHEAT

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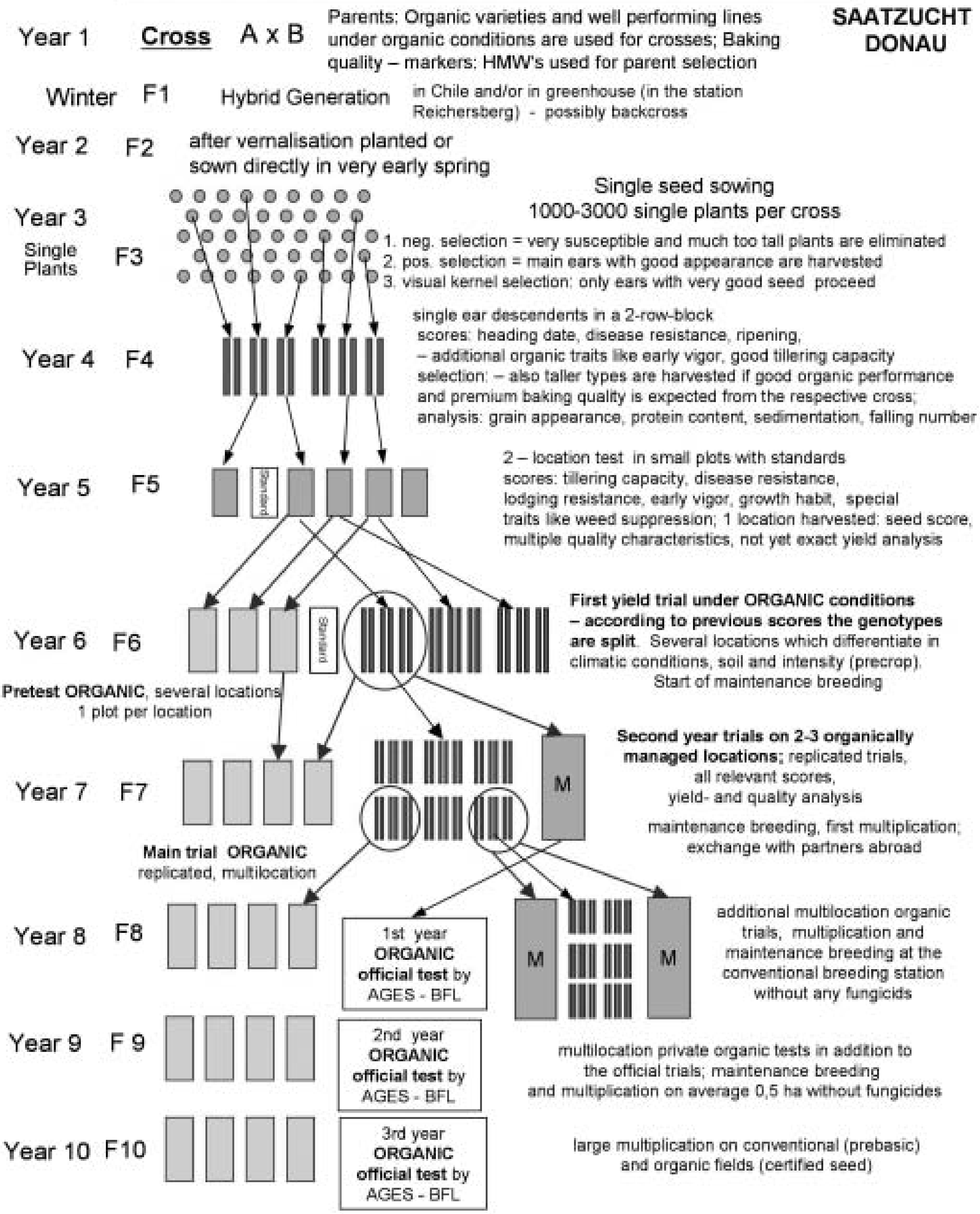
SAATZUCHT DONAU



Year 1 Cross A x B
Parents: Organic varieties and well performing lines under organic conditions are used for crosses; Baking quality – markers: HMW's used for parent selection

Winter F1 Hybrid Generation
in Chile and/or in greenhouse (in the station Reichersberg) - possibly backcross

Year 2 F2 after vernalisation planted or sown directly in very early spring

Year 3 Single Plants F3
Single seed sowing
1000-3000 single plants per cross
1. neg. selection = very susceptible and much too tall plants are eliminated
2. pos. selection = main ears with good appearance are harvested
3. visual kernel selection: only ears with very good seed proceed

Year 4 F4
single ear descendents in a 2-row-block
scores: heading date, disease resistance, ripening,
– additional organic traits like early vigor, good tillering capacity
selection: – also taller types are harvested if good organic performance and premium baking quality is expected from the respective cross;
analysis: grain appearance, protein content, sedimentation, falling number

Year 5 F5
2 – location test in small plots with standards
scores: tillering capacity, disease resistance, lodging resistance, early vigor, growth habit, special traits like weed suppression; 1 location harvested: seed score, multiple quality characteristics, not yet exact yield analysis

Year 6 F6
First yield trial under ORGANIC conditions – according to previous scores the genotypes are split. Several locations which differentiate in climatic conditions, soil and intensity (precrop).
Start of maintenance breeding

Pretest ORGANIC, several locations
1 plot per location

Year 7 F7
Second year trials on 2-3 organically managed locations; replicated trials, all relevant scores, yield- and quality analysis
maintenance breeding, first multiplication; exchange with partners abroad

Main trial ORGANIC
replicated, multilocation

Year 8 F8
1st year **ORGANIC official test** by AGES - BFL
additional multilocation organic trials, multiplication and maintenance breeding at the conventional breeding station without any fungicids

Year 9 F 9
2nd year **ORGANIC official test** by AGES - BFL
multilocation private organic tests in addition to the official trials; maintenance breeding and multiplication on average 0,5 ha without fungicides

Year 10 F10
3rd year **ORGANIC official test** by AGES - BFL
large multiplication on conventional (prebasic) and organic fields (certified seed)

December 2004: release of premium baking Quality Winter wheats AUROLUS and PIRENEO after 3 years organic VCU test in Austria (www.saatzucht-donau.at; www.ages.at)

Phenotypic and molecular evaluation of Durum wheat varieties for organic farming in water stressed environments

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Low input cropping systems are characterised by reduced use of pesticides and chemical fertilizers in order to realize sustainable crop production which can easily integrate in the common agricultural policy. The most restricted low-input system is the organic farming system as defined by EU-directive 2092/91 where pesticides and chemical fertilizers are generally not allowed.

Modern cereal varieties have been developed with the aim of combining high productivity and stability in both production and quality. In order to reduce the effect of variation due to the environmental conditions, pesticides and chemical fertilizers are being used in conventional agriculture. Under organic conditions, crops, especially modern varieties selected in a logic of high-input agricultural practice, became more susceptible to a number of factors limiting their production. So characteristics like tolerance to stresses, both environmental and biotic, and capacity to assimilate and relocate nitrogen with high efficiency became determinant for the choice of varieties for sustainable agriculture.

Our research program involves the evaluation of the wheat diversity for yield capacity in organic conditions and for water use efficiency (WUE). Besides the evaluation for WUE, the dissection of the genetic bases of drought tolerance in durum wheat is realised also *via* the construction of molecular marker maps.

The first approach is based on the phenotypic characterisation of a large collection of durum wheat genotypes for WUE. About one hundred durum wheat genotypes (modern varieties with high productivity value, local populations, old cultivars and genotypes selected in arid environments) have been chosen and grown in Foggia, an environment characterised by a mild water stress, under rainfed conditions or with supplementary irrigation. Among genotypes tested in these conditions, twelve durum wheat varieties have been selected to be evaluated in organic and conventional cropping systems.

The diversity for yield performance under the different tested conditions has been evaluated in order to identify genotypes with high aptitude to be grown in organic systems and with a minimal G x E interaction, so as genotypes with both high yield potential and high yield stability.

The second approach aims to the construction of durum wheat functional maps containing information on QTL for drought tolerance as well as data regarding the expression of stress-related genes. Six cultivars characterized by different behaviour in terms of yield, grain quality and drought tolerance have been selected and used as parental lines of three segregating populations: Ofanto x Cappelli, Trinakria x Creso and Cirillo x Neodur. F5 seeds are now available from each cross and more than sixty microsatellite and biochemical markers with known map position have been found polymorphic in each population. These markers are distributed on all chromosomes representing a set of anchor probes that will allow the mapping of other markers, in particular SNPs.

Water soluble carbohydrate contents of a perennial ryegrass collection – a valuable source for the improvement of future varieties

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Introduction

The objective of this study was to identify perennial ryegrass accessions displaying high fructose and glucose contents and an improved ratio between fructose and glucose fractions across different time points throughout the year. Fructose and glucose are the main constituents of the water soluble carbohydrate (WSC) fraction in perennial ryegrass. For animal nutrition the amount of WSC is crucial as the primary energy source to metabolise the intake of plant protein. The ratio between fructose and glucose fractions is important since fructosan chains, which are an excellent energy source for ruminants, are built from fructose. Furthermore the seasonal variability of WSC content in feed reflects the changing balance between protein and carbohydrates. Ecotypes could be a good source for breeding towards improved quality components and other characteristics which are interesting for organic farming. Possibly less additional feed supplements could be required if the feed quality is better balanced.

Materials and Methods

In the summer of 2003, 33 perennial ryegrass entries were grown from true seed and planted as spaced plants in the field. 40 plants per accession were divided into 4 pools for analysis. The plant material was selected from a collection of historic indigenous Irish accessions held at Oak Park (23 entries) and current commercially grown varieties (10 entries). In 2004, at four time points during the growing season, samples were taken and processed for WSC analysis via HPLC as described by Jafari *et al.* (2003). Means and standard deviations were calculated and entries were assigned to one of four classes (1 = very good to 4 = poor) based on percentage of dryweight attributed to carbohydrates.

Results

At the four time points across ecotypes and varieties a high variability was found for both the WSC content and the ratio of fructose/glucose (Table 1), *e.g.* contents of fructose ranging between 1.25 and 18.99%. Generally the material displayed wide genetic variation across the traits investigated. Among the ecotypes, several entries were superior to the commercial varieties at the third cutting time point (Table 2).

Conclusions

Perennial ryegrass genetic resource collections such as that held at Teagasc Oak Park, hold a great potential for improving the quality of ryegrass varieties. Further ryegrass traits should also be examined, *e.g.* digestibility and fatty acids. The high quality ecotypes identified in this study will be investigated further in 2005-2006. To exploit the natural variation of perennial ryegrass ecotypes will be usefull as well for the development of new varieties especially suitable for organic farming.

Acknowledgements

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Reference

Jafari, A., Connolly, V., Frohlich, A., & Walsh, E.J. (2003) A note on estimation of quality parameters in perennial ryegrass by near infrared reflectance spectroscopy. *Irish Journal of Agricultural & Food Research* 42: 293-300.

Phenotypic vs. marker-based selection to fusarium head blight resistance in spring wheat

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Fusarium head blight (FHB) results in yield and quality losses and contamination of grain by several mycotoxins with deoxynivalenol (DON) as the most prominent. An advisory level of 0.5 mg kg^{-1} for cleaned cereals has been passed by the German Government in 2004. In Organic Farming FHB should be of lower importance as long as maize is avoided in the crop rotation, but still exists as problem when moist weather at flowering time prevails. FHB resistance is a complex trait quantitatively inherited by several loci and high genotype-environment interaction variance that makes phenotypic selection of resistant cultivars complex. Markers for several QTL have been published that may be useful for the development of FHB resistant cultivars in the future. The aim of this study is to compare the realized selection gain from phenotypic and marker-based selection for FHB resistance in spring wheat.

Resistance sources were taken from two recent mapping populations. Derivatives of the resistant cultivars CM82036 (Sumai3/Thornbird) and Frontana were crossed with the two susceptible German spring wheat cultivars Munk and Nandu to establish the source population in the year 2000. Phenotypic evaluation and selection of the experimental lines was performed in field experiments spray-inoculated by *Fusarium culmorum*. Within a selection program starting with evaluation of 1,075 $F_{1:2}$ lines, the 300 best genotypes were selected for low FHB severity at two locations in 2001. In the following year, these genotypes were re-tested at four locations and the 20 best lines were selected and recombined in a factorial design. The resulting 1,100 F_1 plants were selfed and tested as $F_{1:2}$ lines in 2003 at two locations. Thereof, 135 $F_{1:3}$ genotypes were selected and re-tested in 2004. Marker-based selection was done for QTLs on chromosomes 3B and 5A (CM82036) and 3A (Frontana) by flanking SSR markers in three subsequent selfing generations. In the original mapping populations these QTL accounted for 32, 23, and 16% of phenotypic variation, respectively. Finally, 30 $F_{3:4}$ lines were selected that harboured the three QTL for the respective resistance alleles homozygously and selfed. All plants per line were bulked at harvest to produce enough seed for testing. The resulting $F_{3:5}$ bulks were tested along with the phenotypically selected $F_{1:3}$ bulks at four locations in 2004.

Mean disease severity (% infected spikelets per plot) of the unselected source population was 22.7%. Phenotypically selected progeny had a mean FHB severity of 9 and 12.1%, depending on the procedure, marker-selected genotypes of 12.4%. Phenotypic selection, however, took twice as long as marker-based selection, but has the advantage of selecting towards several traits simultaneously. In conclusion, use of molecular markers for FHB resistance is valuable for the breeder, especially when the time frame is narrow. A combination of both selection methods seems to be most favorable: (1) marker-based selection to include genotypes with the relevant QTL and (2) phenotypic evaluation in the field to exploit the full selection gain and to consider additional agronomic traits, like flowering date, plant height, and other disease resistances.

Molecular markers for disease resistance in various crops obtained by NBS profiling

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Breeding for disease resistance is one of the major efforts in many breeding programmes. To select for resistant plant materials, greenhouse or field assays have to be developed that often are laborious and require extensive knowledge on plant-pathogen interactions. Results of resistance tests may sometimes differ from test-to-test or from year-to-year due to environmental variation, making the interpretation of results difficult.

Molecular markers are a powerful tool in breeding programmes that facilitate the introgression of genes of interest into well adapted breeding material. With the use of molecular markers plants with interesting genes can be selected in an early stage without the need of testing such plants in the field or greenhouse. The types of markers that are currently being used extensively are neutral markers such as AFLP and SSR markers. A disadvantage of these markers is that these are not consistently linked to resistance when looked at in other populations. To improve the application of molecular markers for disease resistance NBS profiling has been developed at Plant Research International.

NBS profiling is a technique for efficient tagging of several disease resistance genes and their analogues (RGAs) simultaneously. The technique is based on the knowledge that the most prevalent class of disease resistance genes (approx. 80%) contains a so called NBS domain that is highly conserved between genes and even between plant species. The presence of conserved NBS structural motifs in different resistance proteins implies their involvement in protein complexes that recognize pathogens and trigger signal transduction leading to induction of defence responses. With NBS profiling, markers are produced in, or in the close proximity of the gene conferring resistance. In general, NBS profiling results in a much closer linkage between the resistance locus and the marker than would be the case with neutral markers like AFLP or SSR. By combining precise phenotyping with NBS profiling we have proven that we can find molecular markers tightly linked to disease resistance in several crops.

NBS profiling is a technique that is also very useful in organic plant breeding where resistance to diseases is of high importance. Applications useful for breeders include development of markers tightly linked to resistance genes of interest and screening of germplasm to find new resistance genes and resistance sources. In this poster the use of NBS profiling and the possible benefits for sugar beet breeding will be discussed on the basis of a few examples.

Leaf stripe resistance in barley: marker assisted selection and fine mapping of the resistance gene *Rdg2a*.

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Barley leaf stripe is a seed-borne disease caused by the fungus *Pyrenophora graminea* Ito and Kuribayashi . In susceptible cultivars, the disease causes brown stripes and severe yield reductions. The use of resistant cultivars is one of the most sustainable and economic method of controlling barley leaf stripe. A dominant gene, called as Rdg2a, confers complete resistance to the highly virulent isolate Dg2 (I2) of *P. graminea,* the most virulent isolate among 12 tested.
The colonization of the Dg2-GUS strain is stopped at the level of the scutellar node in the NILs Mirco-Dg2 and the plant response is apparently not associated with a whole cell hypersensitive response, as shown under UV light excitation autofluorescence.
Rdg2a was mapped to chromosome 7H in a population derived from a cross between Thibaut (*Rdg2a*) and Mirco (*rdg2a*) and the STS marker MWG2018 was found to be associated to the disease resistance gene locus (MWG2018 was mapped at 0,2 cM in proximal position with respect to *Rdg2a*). Therefore the reliability of this marker to assess the leaf stripe resistant phenotype in barley breeding programs has been evaluated. A large number (150) of barley cultivars and accessions were screened for their reaction to a highly virulent monoconidial isolate (Dg2) of the pathogen and genotyped for the allele of the molecular marker. About fourty resistant genotypes were identified and four were shown to possess the same allele as the cultivar Thibaut at the marker locus. One of them, cv. Rebelle, is being used as a source of leaf stripe resistance in marker assisted barley breeding programs.

Handling faba bean (*Vicia faba* L.) landraces using morphological and RAPD data

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The inclusion of faba bean into a sustainable scheme of agriculture requires the use of landraces and new varieties with appropriate characteristics. In order to breed these new varieties, the unexpensive maintenance of sufficient genetic variability within the species is necessary. The development, therefore, of an applicable handling model for the above purpose would be useful.

Fifty-five faba bean landraces were collected from diverse areas in Greece and planted at two sites, without irrigation or agrochemical application. Observations were taken on fifteen morphological plus seven yield-associated traits for placement of the landraces under study into well-defined groups. Four dissimilarity coefficients (Manhattan, Average Taxonomic Distance, Euclidean Distance and Squared Euclidean Distance) and four multivariate methods (Principal Component Analysis, UPGMA, Neighbor-joining and Principal Coordinate Analysis) were evaluated.

Neighbor-joining was chosen as the most suitable method for its capacity to classify the populations which belong to the botanical variety *Vicia faba* var. *minor* into one group. This method, combined with Principal Component Analysis using the yield-associated traits, placed the populations into six groups. Based on the above results, a model was developed for conservation purposes for faba bean and other species of similar pollination habits. The main steps of the model include: i) the classification of the accessions into a number of groups, ii) populations mixing in each group, iii) development of "new" populations and iv) adaptation of the new "populations" to various environments through cultivation at different sites.

According to this model, the new "populations" could satisfactorily represent the entire genetic material. The advantages of this model for the breeders are i) the use of the new populations in addition to the accessions kept in the gene bank and ii) the derived populations could also be used as well in mass selection breeding schemes. Farmers can grow these locally adapted "populations" with reduced inputs and perhaps organically.

Additionally, in order to find the most suitable method for classification purposes within *Vicia faba* L. using RAPD (Random Amplified Polymorphic DNA) data, three multivariate statistical methods (UPGMA, Neighbor-joining, Principal Coordinate Analysis) were compared with regard to the value of heterogeneity of the resulting groups (or subgroups). The test showed that the last two methods gave the most satisfactory results.

Triticale with improved nitrogen-use efficiency for organic farming

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Organic farming systems are characterized by reduced nitrogen supply. Nitrogen (N) is one of the most important yield limiting factors and an essential component for proteins. For organic farming cereal varieties are required that unite efficient nutrient uptake and utilization traits, and the stability of yield under different environmental conditions. Triticale combines high protein content with high protein quality. Due to its tolerance to marginal conditions and high competition against weeds it is very suitable for organic farming. Breeding triticale varieties with improved N-use efficiency can help to increase productivity of organic farming and improve the protein supply in animal feed. N-use efficiency is defined as the ability of a genotype to produce superior grain yields under low soil N conditions in comparison with other genotypes. The objectives of the project presented are to (1) estimate quantitative genetic parameters for N-use efficiency, (2) analyse the contribution of N-uptake and N-utilization for improved N-use efficiency, and (3) investigate breeding strategies for improved N-use efficiency that meet the special demands of organic farming systems.

All materials are tested at two locations with two N-regimes (fertilized *vs*. non-fertilized) at organic farms. N fertiliser are used according to the rules for organic farming. In 2004 elite germplasm ($n = 64$) was tested at two locations with two different N levels. At both N levels significant genotypic variation ($P<0.01$) was found for grain yield. At the low N level genotypic variation, but also error variation, was higher than at the high N level. Coefficients of phenotypic correlation between grain yield at high N and low N level was moderate ($r_p = 0.66$; $P<0.01$). Results for further agronomic traits at both N levels will be presented. N-use efficiency can be divided into two main components, N-uptake and N-utilization efficiency. N-uptake refers to the quantity of N absorbed by the plant whereas N-utilization efficiency quantifies the amount of grain produced per unit of N uptake. In 2005 a reduced set of genotypes will be investigated in order to estimate the contribution of N-uptake and N-utilization for improved N-use efficiency. Segregating populations of parents differing in N-use efficiency will be tested in 2006.

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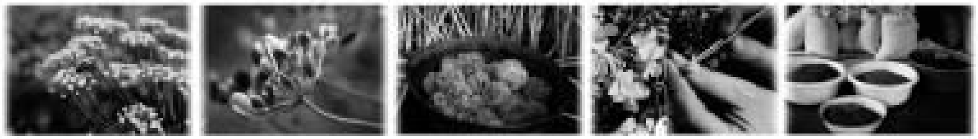

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The European Consortium for Organic Plant Breeding (ECO-PB), founded 20th April 2001 in Driebergen (NL), pursues ...

- providing a platform for discussion and exchange of knowledge and experiences
- the initiation, support of organic plant breeding programmes
- the development of scientific concepts of an organic plant breeding
- the provision of independent, competent expertise to develop standard setting with respect to organic plant breeding

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The European Consortium for Organic plant breeding (ECO-PB) ...

- contributes to the optimisation of organic agriculture by stimulating breeding programmes for varieties better adapted to the organic farming systems
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- stresses the role of biodiversity through a sustainable maintenance of cultivated plants as part of our cultural heritage

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- website www.eco-pb.org
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- 1rst European Symposium Berlin, Nov 2002
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- Round Table Meeting on Organic Seed Regulation 2004, Frankfurt, April 2003
- Round Table Meeting Brussels on Organic Seed Regulation 2004, 8-9 Dec. 2003
- Partner organisation of the 1rst World Organic Seed Conference with IFOAM/FAO/ISF, Rome, July 2004, see www.organicseedconf.org
- Support to the realisation of the COST 860 SUSVAR programme, see www.cost860.dk

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