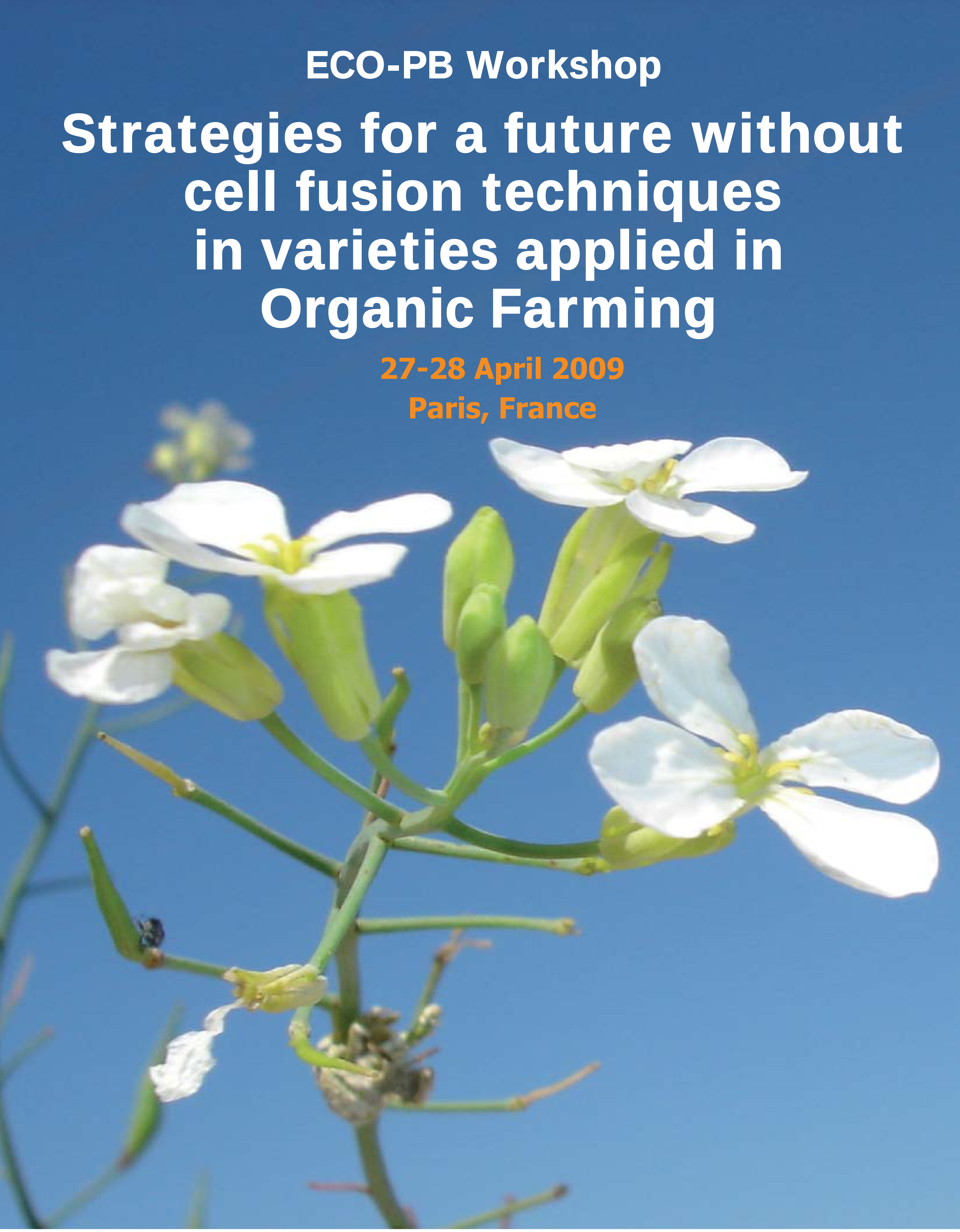
ECO-PB Workshop

Strategies for a future without cell fusion techniques in varieties applied in Organic Farming

27-28 April 2009

Paris, France



ITAB
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ECO·PB
European Consortium for Organic Plant Breeding

Strategies for a future without cell fusion techniques in varieties applied in Organic Farming

PROGRAM

Day 1 (Monday April the 27th):

12.30-14.00	**Registration, coffee/tea time**
14.00-14.15	**Opening and steps to move forward with the IFOAM motion on PPF** *by Edith Lammerts van Bueren, as chair of ECO-PB*
14.15-14.35	**What is PPF and what are the objections against PPF from the organic** principles point of view *by Edith Lammerts van Bueren, Louis Bolk Institute*
14.35-14.55	**Arguments against PPF and advantages for breeders** *by Jan Velema, Vitalis*
14.55-15.15	**Explanation about legal situation - EU regulation** *by Maaike Raaijmakers, Biologica*
15.15-15.30	**Open discussion**
15.30-16.00	**Coffee break**
16.00-16.30	**Presentation of state of the art from :** ➢ France : *by Frederic Rey, ITAB* ➢ Germany : *Klaus Peter Wilbois, FiBL Deutschland BV*
16.30-17.15	**Audience information about their country** *by all participants*
17.15-18.30	**Open discussion on short term and long term policies**

In the evening the organisation team will sit together to incorporate the new information from the discussion into their concept strategies.

Day 2 (Tuesday April the 28th):

8.30-9.00	**Presentation of short and long term strategies** *by Edith Lammerts van Bueren, as chair of ECO-PB*
9.00-10.00	**Discussion**
10.00-10.30	**Coffee break**
10.30-11.30	**Conclusions and following steps: what to do before Santa Fe?**
11.30-12.00	**Introduction on other new GMO techniques** *by Michel Haring, Amsterdam University*
12.00-12.30	**Presentation on Standards for certified Biodynamic plant breeding** *by Karl-Josef Müller*

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Motion on protoplast fusion and on plant breeding standards at IFOAM General Assembly

Edith Lammerts van Bueren, chair of ECO-PB
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From the ECO-PB Newsletter on organic seeds and plant breeding, Issue II/2008, www.eco-pb.org

At the IFOAM General Assembly, motion text on protoplast fusion was put forward for voting and was accepted unanimously (four abstentions, no objections) by the General Assembly of IFOAM at Modena: “The IFOAM GA 2008 confirms that cell fusion, including protoplast and cytoplast fusion, do not comply with the principles of organic agriculture. Therefore we urge the IFOAM World Board to develop clear guidelines on how to deal with varieties derived from cell fusion, including protoplast and cytoplast fusion breeding techniques.” What the consequences are for the use of varieties bred with these techniques in the organic sector in Europe and other parts of the world has to be discussed now. The planned IFOAM World Congress on animal and plant breeding 25-28 August 2009 in Santa Fe, New Mexico will offer a broad platform for proposals for guidelines. Background: The IFOAM definition of genetic engineering (GM) as formulated in the IFOAM Basic standards includes cell fusion. So within the International Federation of Organic Agriculture Movements (IFOAM) there seems consensus on the fact that cell fusion does not comply to the IFOAM principles as GM is banned. However, the problem is how to deal with it in practice since varieties bred with this techniques are not labelled as GM and the use of these varieties is not explicitly forbidden in the EU regulation on organic farming. Some countries have green or red variety lists to show which varieties are bred or not with cell fusion techniques, but most countries have not taken any measures. FiBL-CH has published a report on this issue to enhance the discussion, see www.fibl.ch. Seed companies who want to enter the organic market are seeking clarity on a short term.

ECO-PB had also submitted another motion on plant breeding standards together with the same organisations that were partners in the above mentioned motion to the IFOAM General Assembly with the following text: “Complete the work on the draft plant breeding standards as soon as possible with the view of adopting them as IFOAM (certification) standards”. Also this motion was accepted. The reasoning of the motion was as follows. In the current version of the final draft of the IFOAM Basic Standards (IBS) in chapter 4 “Crop production” it reads “The objective is to ensure that organic practices are implemented along the entire production chain from propagation to final product including the production of seed and propagation materials”. ECO-PB agrees with this objective but wants to ensure that the breeding process is included along with the production of seed and propagation material. Such has already been made explicit in the IFOAM Basic Standards with respect to breeding under animal production and under aqua production by stating that: “Only breeding techniques consistent with organic production methods are used.” Such a statement is missing for crop production! Organic plant breeding has long been an essential part of organic farming. Because of this ECO-PB considers that IFOAM must now give clear guidance regarding the nature of organic plant breeding. It would otherwise be in danger of leaving the door open to all manner of inappropriate claims regarding the term, organic plant breeding. To make a public statement on organic plant breeding methods, it is vital that the current draft standards on plant breeding become full standards as soon as possible.

ECO-PB would like to offer its expertise within the ECO-PB network to assist IFOAM in refining standards for organic plant breeding and adapting them to the new IBS framework (maybe as sector specific certification standards). But regardless of the need for refinement we now demand that in the meanwhile these draft standards stay published to feed the awareness that IFOAM is working on full standards. The World Board agreed to keep the draft standards in place until new standards are accepted and welcomes new proposals to be discussed at the next World Congress on Animal and Plant breeding 25-28 August 2009 in Santa Fe, New Mexico.

What is protoplast fusion and what are the objections against protoplast fusion from an organic point of view?

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Introduction

Plant breeding has changed rapidly during the past decades. The worldwide adoption of F_1-hybrid seed production has stimulated the use of biotechnology to generate parental lines that are cytoplasmic male sterile (CMS). These CMS lines cannot self-pollinate and are thus attractive to use for cross-pollination schemes aimed at the production of F_1-hybrid seeds. Because not all crops exhibit natural variation for natural, spontaneous CMS plant lines, there is a need to transfer certain types of sterility from one crop species to another, for instance from radish (*Raphanus sativa*) to cabbage (*Brassica oleracea*) or sunflower (*Helianthus annuus*) to chicory (*Chicorium endivia*). Because intergeneric crosses cannot be performed naturally, protoplast fusion techniques have been developed. Such an approach is classified as genetic modification according to the definition in the European Directive 2001/18/EC: 'Genetically modified organism means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. As a consequence the products originating from this technique should be banned from organic agriculture.

This paper will explain how cytoplasmic male sterility works and what the procedure of introducing cytoplasmic male sterility by protoplast fusion technique is, and why such a technique is in conflict with the principles of organic agriculture.

Cytoplasmic male sterility

For the production of pure F_1-hybrid seeds the mother line needs to be pollinated only by the selected male parent. Although this can be achieved by manual pollination, plant breeders prefer to use genetic tools to reach this goal. Therefore there are several traits available in some plant species, such as certation[1], dioecy[2], genetic male sterility, self incompatibility and cytoplasmic male sterility. Genetic male sterility and self incompatibility are widely used in cereals and vegetable crops, but have limitations. Genetic male sterility schemes have relied on the use of a selectable seedling or molecular marker to achieve populations that are 100% male sterile. For many male sterile genes, useful linked markers may not be available. In the case of self incompatibility, certain combinations of inbreds will be impossible to make if they share incompatibility alleles. In vegetable crops, such as cabbage, the use of CMS is the preferred choice: it results in 100% pure F_1-hybrid seeds and no fertility restoration genes need to be considered in the breeding program because only the vegetable part is harvested. Unless there are

[1] Competition in growth rate between pollen tubes of different genotypes resulting in unequal chances of accomplishing fertilization.
[2] Having the male and female reproductive organs borne on separate individuals of the same species.

restorer genes present in the father line, CMS is not economically useful in (non-parthenocarpic) fruit crops or seed crops because the F_1 hybrid is male sterile.
CMS is a trait that is inherited maternally and is associated with the heritable material of the mitochondria. If a CMS plant is pollinated, the progeny of this cross will all be sterile because they inherited only the CMS mitochondria of the mother line. Naturally occurring CMS has been found in many plant species: Maize, Bean, Beet, Petunia, Radish, Carrot, Onion, Oilseed rape, Rice and Sunflower (Budar & Pelletier, 2001). In chicory no CMS occurs naturally. To obtain male sterile chicory plants chicory cells (protoplasts) were fused with CMS sunflower cells through protoplast fusion. This resulted in plants with a chicory phenotype. Some of these somatic hybrids were male sterile. In subsequent crossing schemes stable CMS lines were repeatedly crossed to chicory breeding lines to obtain commercial varieties. Analysis of the mitochondrial DNA of these lines revealed that indeed sunflower DNA was present. Up till now the exact piece of DNA that is associated with CMS from sunflower has not been determined.

What is the procedure of protoplast fusion?

Normally plant cells are surrounded by a thick cell wall that not allows two cells to fuse. Protoplasts are plant cells without a cell wall containing only a cell membrane. They are generated by treating plant tissue (e.g. leaves) with enzymes that degrade cell walls. The protoplasts of different plant species can be fused with help of chemical or electric stimuli (somatic hybridisation). During this fusion, the organelles of both plants (chloroplasts and mitochondria) are mixed, while in sexual crosses, only maternal chloroplasts are passed on to the progeny. The resulting tetraploid fusion product has the characteristics of both parent species. During regeneration the chromosomes and the organelles of both parents may be mixed, so that many combinations are produced. Fused protoplasts can grow a cell wall again and divide, resulting in callus from which plants can regenerate *in vitro* with the help of plant growth hormones. To avoid exchange of chromosomes, protoplasts can be treated in such a way that the nucleus is removed or fragmented by radiation. These so-called cytoplasts do contain organelles, but not the (intact) chromosomes of the donor plant. In this way CMS can be transferred to other plant species without mixing the parental chromosomes.

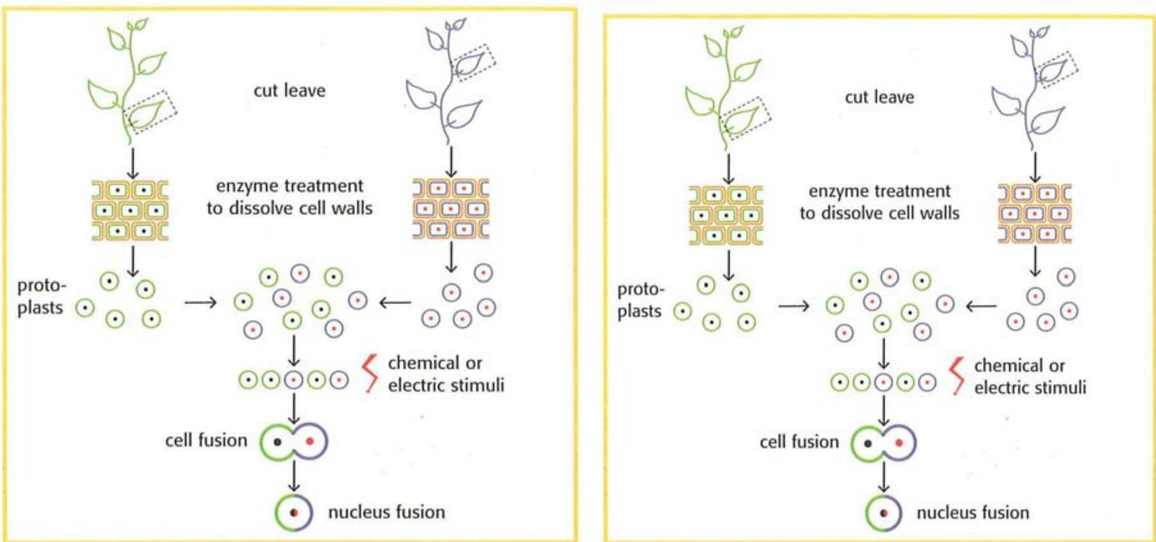

Figure 1. Protoplast fusion (Wyss et al., 2001).

Evaluation of breeding techniques

In organic agriculture the basic attitude towards nature is that of man as a partner of nature and this implies that one ascribes not only extrinsic (instrumental) values of nature for mankind but also intrinsic values out of respect for the autonomy and 'dignity' of living entities as such. This attitude leads to a bioethical frame work of action with a biocentric view, meaning that all living organisms, including plants, are considered ethically relevant[3]. The concept of integrity is the operational dimension of intrinsic value, in which the integrity of (cultivated) plants refers to their inherent nature, wholeness, completeness, species-specific characteristics, and their being in balance with their (organically farmed) environment (Lammerts van Bueren *et al.*, 2003).
Respecting the integrity of life does not mean that one cannot interfere in agriculture, but means that this element of integrity of life will be taken into account when making management decisions in agriculture, e.g. in plant breeding. This requires clear criteria for evaluation of plant breeding techniques.

As organic agriculture is a process orientated and certified agriculture also the (breeding) process to come to new varieties should comply with the principles of organic agriculture. Since 1999 many discussions have been conducted to set criteria to evaluate breeding techniques for the suitability of organic agriculture (Lammerts van Bueren *et al*., 2003), see table 1.

Table 1. The consequences of acknowledging the integrity of plants for the compatibility of breeding techniques with the principles of organic agriculture (adapted from Lammerts van Bueren et al., 2003).

	Variation induction techniques	Selection techniques	Maintenance and propagation techniques
Plant and crop level: Compatible with the principles of Organic Agriculture	Combination breeding Crossing varieties Bridge crossing Repeated backcrossing Hybrids with fertile F1 Temperature treatment Cutting style Grafting style Unradiated mentor pollen	Mass selection Pedigree selection Site-determined selection Change in environments Change in sowing time Test crosses Indirect selection DNA diagnostic methods Marker-assisted breeding	Generative propagation Vegetative propagation: -cut tubers -scales, husks, chipped bulbs -brood buds, bulbils -offset bulbs, etc -layer, cut and graft shoots -rhizomes
Cell level: not compatible with principles of Organic Agriculture, but could be discussed for derogation	Embryo culture Ovary culture *In vitro* pollination Somatic variation	*In vitro* selection	Meristem culture* Anther culture Microspore culture Micropropagation Somatic embryogenesis
DNA level: Not compatible with the principles of Organic Agriculture and should be banned	Induced mutagenesis Protoplast fusion Genetic modification	*In vitro* selection	

* Meristem tip culture is permitted when required by regulation for phytosanitary reasons

As a result in 2002 the first IFOAM draft standards were set for organic plant breeding which stated that only techniques on whole plant level are compatible for organic principles. This

[3] In an anthropocentric framework of action only humans are ethically relevant.

implies that from the process point of view, plant breeding techniques that operate on cell tissue or directly at DNA level violate the integrity of life. As part of appreciating the concept of integrity of life organic agriculture aims at supporting a certain level of autonomy and self-organisation or self-regulative ability of the living farm-ecosystem. It implies that measures are designed in such a way that one supports life processes within the farm-ecosystem and does not try to deconstruct and reconstruct life in a test tube. That is the reason that non-organic, chemical-synthetic compounds are not tolerated in organic farming systems but instead agro-ecological measures are applied.
From a biological point of view cells are the lowest entity of self-organised life, and working beyond that level, such as is the case with manmade protoplasts or cytoplasts, is certainly not in line with the values of organic agriculture. The use of cytoplast delimits the target of transfer even further: the aim is to only transfer a piece of mitochondrial DNA that conveys CMS. In concept this mimics the aims of GM-based gene transfer.
Moreover, somatic hybridisation through protoplast fusion is a way to hybridise the sexually incompatible species and thus enables to cross natural crossing barriers. This is a crucial difference compared to variation inducing techniques at cell level, such as embryo rescue, which can make a cross more effective in cases where natural crossing can in principle occur but not always leads to a useful percentage of fertile seed.

Inbuilt protection
Another concern in the organic agriculture is the increasing need of control of seeds by the seed industry. Natural CMS such as in onion or carrot always occurs in combination with restorer genes and therefore still can be used as parent in breeding programs. In crops in which CMS is introduced from non-crossable species through protoplast fusion no restorer genes are present, and therefore such plants cannot be used in breeding programs and can thus not contribute to further development. However, some breeding companies in vegetable crops prefer CMS due to its 100% inbred free hybrid production, and especially due to its 'inbuilt' protection against use by competitor-breeders, as these varieties have only a limited application in a breeding program. Breeding companies use different plant sources of CMS and have patents on the application of these kinds of CMS by describing the associated DNA changes in mitochondrial genome. They can thus protect their breeding lines. CMS in that sense is primarily about ownership and control of seeds whereas organic production is about stewardship of seeds.
Fortunately, there are still cabbage breeding programmes based on self incompatibility, but for how long? The organic sector needs to be clear in the interpretation of its regulation to safeguard such breeding programs that are more in line with organic principles.

References

Budar F, Pelletier G (2001) Male sterility in plants: occurrence, determinism, significance and use. C R Acad Sci III. 324:543-50.

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Wyss, E., E. Lammerts van Bueren, M. Hulscher and M. Haring (2001). Plant breeding techniques. An Evaluation for organic plant breeding. FiBL Dossier No. 2. Forschungsinstitut für biologischen Landbau, Frick (ISBN 3-906081-13-3): 24pp.

Consequences of the use of Cytoplasmatic Male Sterility and Self Incompatibility in sustainable breeding programs.

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Introduction

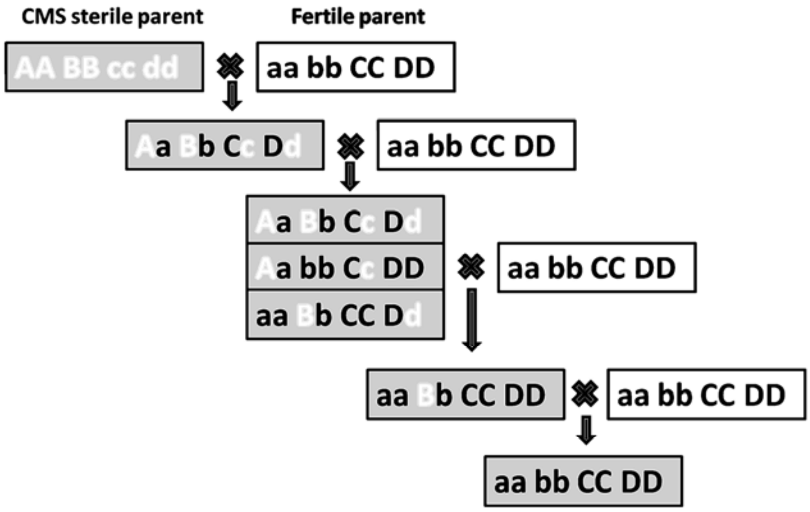
In nature plants have the tendency to maximize their reproduction. Self-pollination would be the easiest way to maximize plant reproduction, but selfing can cause inbreeding depression. This is the reason that in nature many mechanisms exist that prevent inbreeding and to stimulate outbreeding. One of these mechanisms is Cytoplasmatic Male Sterility (CMS). Because absolute sterility would cause the extinction of the plant, so called restorer genes are present in a natural population. This means that a plant with a sterile cytoplast is male sterile, but combined with the presence of a restorer gene the plant is male fertile. In natural populations there will be a balance between fertile and sterile plants resulting in an optimal reproduction without too much risk for inbreds

Cytoplasmatic Male Sterility

Modern plantbreeders have learned to understand these mechanisms and use them to improve varieties. As a result of the increasing significance of the commercial value of seeds, breeders have also used natural mechanisms to protect their company interest. F1 hybrids are a well known example of this strategy but artificially introduced CMS, made with the help of Protoplast Fusion is a much stronger tool to prevent the use of genetic material by others than the breeder of the variety himself. By Protoplast fusion the Sterile Cytoplasma is moved to another species but the restorer genes are not. When I am using "*CMS*" in this report, I mean "*CMS, transferred from a different species by protoplastfusion, without the presence of restorer genes, like in Brassica*". I will demonstrate the consequences by doing an exercise where a plant breeder is trying to use a *CMS* Hybrid in his breeding program.

I would, however, like to stress that it is not my intention to reject all forms of protection for plant breeders' efforts. To prevent reproduction of varieties, plant breeder's rights and (fertile) F1 Hybrids can be of help to commercialize breeder's investments. For social and ecological reasons however, one should not block the passing on of genetic diversity to future generations. Breeding should contribute to a durable and sustainable use of cultivated plants instead of exhausting diversity without leaving anything for our future. By accepting techniques such as Protoplast Fusion in breeding programmes, access to genetic diversity for future breeding programmes becomes limited and the diversity of the food crops grown in our agro-ecosystems more limited.

In the experiment in figure 1 a *CMS* plant is crossed with a fertile parent. The sterile cytoplasma is grey and the genes in this plant are presented in white. The *CMS* parent can only be used as a mother (because it is male sterile). Presume A, B, c and d stand for desirable traits (for example resistance and taste). The breeder wants to select at least some of these genes into new breeding lines. He is faced with the fact that every progeny of a crossing from a *CMS* plant is cytoplasmatic male sterile again because this trait is heritated from the mother parent. That means that every next generation the only thing he can do is crossing with a fertile plant, which, as a consequence, does not have A, B c or d genes. After one or after many generations, the best the breeder gets is a heterozygous genotype, never a homozygous genotype for one of these traits. At the end the breeder has two inevitalble results: 1) Not any gene from the original mother parent is passed on and 2) All offspring is cytoplasmatic male sterile. In other words; 1) all genes in a *CMS* plant are locked up and not available for any future offspring and 2) *CMS* cannot be crossed out.



The conclusion is that it makes no sense to use *CMS* plants in a breeding program (other than making isogenetic *CMS*-lines from lines you already have) and that therefore *CMS* is a very good tool to prevent using genetic material by other (competitor) breeders. The disadvantage is that *CMS* varieties do not contribute to the maintenance of the crop. Every fertile variety of a cultivated species contributes to diversity and the survival of that species; *CMS* varieties do not: they are useless in evolutionary perspective. *CMS* is a short term competitive advantage, but a long term biodiversity destruction.

Self Incompatibility

Self Incompatibility (*SI*) is an alternative mechanism to make F1 hybrids. *SI* is based on a multi-allelic locus, which means that there are more than two different alleles present in a population. Normally a gene has two alleles (e.g. A, a). The *SI* gene has more alleles (S1, S2, S3, ..). S3 is dominant over S2 and S2 is dominant over S1. S2S1 is phenotypic S2, because of the dominance of S2 over S1. Plants with the same *SI* phenotype are incompatible (e.g. S3S3 and S3S3 or S2S2 and S2S1), and plants with different phenotypes are compatible (e.g. S3S3 and S2S2 or S2S1 and S1S1).
Also without a complete understanding of this mechanism in the context of this report it will be clear at least that plants with *SI*-genes are never sterile, only limited in their capacity to cross with similar *SI* phenotypes. The use of *SI* plants in breeding programs is easy; it is even more likely that a cross is compatible when parents are less related. All genetic diversity in *SI* hybrids are available for future breeding programs.

Conclusions

Plantbreeders are not only responsible to provide growers with good seeds. They also are responsible for the sustainable maintenance of our agricultural food plants.
To reject the use of *CMS* hybrids shows good stewardship towards cultivated plants. The use of *Open Pollinated* varieties or *SI* hybrids, on the other hand, contributes to biodiversity and promotes good stewardship.

References

CMS is reported in many publications, but the commercial advantage that genes are “locked up” in *CMS* varieties is never reported.

Priciples of SI is described in most Plantbreeding handbooks. (Look for Homomorfic- and Sporophytic Incompatibility).

What are the rules on cell fusion techniques in the EU public laws and private standards for organic farming?

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Introduction

Cell fusion (including protoplast fusion) is a breeding technique under the (EU and IFOAM) definition of genetic engineering. Therefore it should not be used in organic plant breeding and seed originated from it should not be applied in organic farming. Many organic farmers and breeders agree on that. Furthermore cell fusion techniques are not indispensable for plant breeding.
During the General Assembly from IFOAM in Modena (2008) a motion was accepted which states that "cell fusion, including protoplast and cytoplast fusion, do not comply with the principles of organic agriculture." Therefore the IFOAM World Board was urged to develop clear guidelines on how to deal with varieties derived from those techniques.
Guidelines are needed for this because cell fusion techniques are excluded from the scope of the European regulation on GMO's (2001/18/EC). This means varieties that are made with these techniques are not labelled and therefore not recognizable for farmers as being a GMO. As a consequence those varieties are in fact widely used by organic farmers.

This paper will explain the difference between EU government rules and two major private standards (IFOAM and Demeter) with regard to cell fusion techniques. Secondly it will identify some possibilities to control a ban on these techniques in practice.

EU Regulation on GMO's

According to EC Directive 2001/18: 'Genetically modified organism (GMO) means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'.
The list of techniques of genetic modification (Annex I A) includes 'cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally'.
So according to this Directive cell fusion techniques result in genetic modification. At the same time cell fusion is excluded from the scope of the Directive (Annex I B) when it is a fusion 'of plant cells of organisms which can exchange genetic material through traditional breeding methods'.
For the interpretation of this exemption the European Commission (EC) developed a very broad definition of traditional breeding methods: ' "traditional breeding" means practices which use one or more of a number of methods (e.g. physical and/or chemical means, control of physiological processes), which can lead to successful crosses between plants of the same botanical family". According to this interpretation the fusion of plant cells of organisms within the same botanical family is considered to be "traditional breeding" and therefore not a technique of genetic modification under the Directive. Even if those species, for instance

radish and cabbage, cannot exchange genetic material by natural recombination. With this (political) interpretation the EC has widened the definition of traditional breeding methods and in fact created a gap between the scientific and the legal definition of a GMO.
In the practical implementation this means that all existing forms of cell fusion are excluded from the European GMO regulation. Therefore breeders don't need a license to use this technique and varieties originated from it are not labelled as 'GMO'.

The Organic Regulation

According to the Council Regulation on organic farming (…) (EC 834/2007) it is forbidden to use genetically modified organisms and/or products derived there off. For the definition of a genetically modified organism (GMO) and techniques of genetic modification (resulting in a GMO) the regulation refers to Directive 2001/18: 'The definition of "Genetically modified organism (GMO)" is that given in Directive 2001/18/EC (…) and which is not obtained through the techniques of genetic modifications listed in Annex I.B. of that Directive'
This means that use of "cell fusion of plant cells of organisms which can exchange genetic material through traditional breeding methods" is not considered to be a GMO technique within the scope of this regulation and therefore allowed in the organic production. By using this reference the EC has aimed to attune (and thereby restrict) the definition of a GMO in the organic regulation to the scope of GMO techniques in the GMO regulation. If this also means that the organic sector has to accept the broad interpretation of "traditional breeding methods" as mentioned before is not clear.

IFOAM Basic Standards and Norms

The IFOAM Basic Standards (IBS) are a keystone of the organic movement. Democratically and internationally adopted, they reflect the current state of organic production and processing methods. They provide a framework for certification bodies and standard-setting organizations worldwide to develop their own more detailed certification standards which take into account specific local conditions.

The IFOAM Standards include the following definition of genetic engineering: 'Genetic engineering is a set of techniques from molecular biology (such as recombinant DNA) by which the genetic material of plants, animals, micro-organisms, cells and other biological units are altered in ways or with results that could not be obtained by methods of natural mating and reproduction or natural recombination.'
Techniques of genetic modification include, but are not limited to: 'recombination DNA, **cell fusion,** micro and macro injection, encapsulation, gene deletion and doubling. Genetically engineered organisms do not include organisms resulting from techniques such as conjugation, transduction and natural hybridisation.'
As a general principle 'Genetic engineering is excluded from organic production and processing'. Besides that it is recommended that 'Genetically Modified Organisms (GMOs) and their derivatives should be excluded from organic production processing and handling to the fullest extent possible'.

<u>IFOAM Draft Standards</u>

The IFOAM Draft Standards include a section on *Plant Breeding and Multiplication Draft Standards.* This section contains a list of plant breeding methods that are ‘suitable and permitted for organic plant breeding’. This list excludes cell fusion.

<u>IFOAM Norms</u>
The IFOAM Norms are the IFOAM Basic Standards together with the IFOAM Accreditation Criteria (IAC). IFOAM accreditation is awarded to certification bodies that use certification standards in their certification program that at least meet the IFOAM Basic Standards.
So a ban on the use of cell fusion techniques should be binding for farmers that are certified by a certification body that has an IFOAM accreditation. But as long as there are no special rules or guidelines to implement this ban it may be assumed that varieties made by cell fusion techniques are still used in practice. Therefore a motion was accepted at the general assembly in Modena (2008) which urges the IFOAM World Board to develop clear guidelines on how to deal with varieties derived from cell fusion techniques.

Demeter

Like the IFOAM Norms, the Demeter Norms are private standards. In practice this means they set additional rules to the government (public) regulation. In 2004 Demeter International decided to formerly ban the use of cell fusion techniques from biodynamic agriculture: “…..With resolute specifications for producers the biodynamic community now prescribes in their standards that varieties generated by means of cytoplast fusion techniques are excluded from Demeter production. This amendment will come into force in July 2005….”
To implement this ban Demeter first produced so called ‘green lists’ of varieties that were not made by cell fusion techniques. This was too complicated and did not work in practice. After that they started to develop red lists with hybrids made with the use of cell fusion techniques. Often those varieties can be recognized because they are patented and labelled as CMS or 'super' hybrids.
Demeter certified farmers are not allowed to use the varieties on the red list. According to foundation Demeter in the Netherlands these lists are working well in practice and farmers have enough alternatives.

Discussion and conclusions

Cell fusion is a technique of Genetic Modification. Cell fusion techniques should not be allowed according to the IFOAM standards but so far this ban is not implemented.
As an exemption cell fusion “of plant cells of organisms which can exchange genetic material through traditional breeding methods” is allowed in the organic production according to the European Regulation. The interpretation and broadness of this exemption should be discussed within the organic sector. If the organic sector (in Europe) agrees on when and how they want to ban (certain)varieties made with cell fusion techniques, they could ask the European Commission to regulate this through the (so called) implementing rules of the Organic Regulation (EC 834/2007).
Fact is that because varieties made by cell fusion techniques are not labelled as a GMO they are already widely used by organic farmers. This makes it difficult to implement a ban.
Demeter has seemingly effective banned the use of cell fusion techniques by producing a red list of forbidden varieties. The question is however if it is possible to keep this list updated and complete when the use of cell fusion techniques is increasing and/or seed producers refuse to cooperate.

Another possibility for farmers to avoid varieties made with cell fusion techniques is by using organic seed. According to the EU Regulation on organic farming the use of organic seed is obligatory. Until now almost all seed producers avoid varieties made with cell fusion techniques in their organic seed programme. But since it is not explicitly forbidden to use varieties made by cell fusion techniques for the propagation of organic seed, one can not rely on this too much for the future. Therefore it is necessary that the sector gives a very clear signal to seed companies that they really want to ban cell fusion techniques. The most convincing signal will be made if organic farmers stop using varieties made with cell fusion techniques.

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French situation concerning protoplast fusion-based varieties used in Organic Farming

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History of the French discussions on CMS

80 % of French cabbages are produced in Brittany. At the end of the 1990s, the APFLBB, an organic producers organisation in Brittany, wondered about the consequences of the use of CMS varieties in Organic Farming. Following debates between vegetable growers, scientists, breeders, seed companies, food processors and wholesalers, the administrative council for the regional umbrella organisation for Organic Agriculture (IBB) decided to ask organic growers not to use CMS varieties based on protoplast fusion (PF). This recommendation has been implemented by most organic producers in Brittany.
During the annual national organic fruits and vegetables meeting organised by GRAB and ITAB in 2004, the use of PF-based varieties was discussed, but there was no definitive outcome. Many articles have been published on this subject, especially in Alter-Agri, ITAB's journal. As a consequence, the administrative council for ITAB advises not to use CMS varieties derived from PF.

Most varieties affected

In France, the species most affected by PF issues is the cabbage family, in particular: cauliflower, broccoli, green cabbage, Savoy cabbage and red cabbage. In the near future, Belgian endive *(Cichorium endivia)*, turnip, rapeseed and sunflower will also be affected because of current breeding efforts by seed companies to produce hybrids based on CMS using PF. In the case of Belgian endive for example, more and more CMS-free varieties are being replaced by CMS varieties (ie. *Goldwin* is a CMS variant of *Yellowstar*). The aim is to reduce inbreeding rates with the CMS.
There are currently 2 CMS varieties developed using PF methods listed on the French organic seed database: www.semences-biologiques.org. One is a green cabbage *Sir* (F1, Clause) and the other a Savoy cabbage *Rigoleto* (F1, Clause).
In 2008 alone, there were 815 cauliflower derogations for conventional untreated seeds (cauliflowers are in third position after lettuce (910 derogations) and tomatoes (861)); of this number one out of every four derogations (206) is for a CMS cauliflower. With broccoli, this figure is almost 50% (see table 1 below).

Table 1: derogation number for CMS varieties compared with the total derogation number, for the most effected varieties in France in 2008.

Year 2008 France Species	Derogation number for a CMS variety *(Minimum)*	Total derogation number pro species	%	Seed company concerned
Broccoli	57	125	46%	S&G, Seminis, Sakata
Green cabbage	33	111	30%	S&G, Clause
Brussels sprout	0	58	0%	
Savoy cabbage	22	206	11%	S&G, Clause
Cauliflower	206	815	25%	Clause Tezier, Gautier, Vilmorin, S&G
Kohlrabi	0	35	0%	
Red cabbage	28	63	44%	S&G, Clause
Turnip	0	105	0%	
Belgian Endive	0	inc.	-	
Rapeseed	inc.	inc.	-	

Nb. This table was devised using the 2008 list of derogation combined with the 2008 list of CMS made by APFLBB.

Some statements of French organisations

Unlike other EU countries, the French certification bodies they are not accredited by IFOAM, they only apply the organic regulation (EC 889/2008). Currently the use of CMS varieties based on PF is not explicitly banned in organic agriculture (OA).
However, according to the European directive (2001/18/CE) cell fusion techniques are defined as producing GMOs[4]. Consequently, the implementation of the European regulation of organic farming, that bans the use of GMOs, should include the banning of varieties based on protoplast fusion in organic agriculture.

Organic sector of CERAFEL : these organic producers consider that: "because CMS varieties are not specifically banned in organic farming and are required to meet the market's needs, they should be continued to be used according to producers choice, who have access to breeding successes".

APLFBB : This organic producers organisation has built a trademark "*Bio Breizh*" with standards that include the ban of PF varieties. For each seed order, they check that CMS varieties are not used. Each year, they build a CMS variety list (see the 2008 list in the annex 1). This organisation specifically promotes cabbage breeding without CMS.
BIOMAS and producers from Armorique Maraîchère grouping comply with the IBB's recommendation and ensure that each seed they buy or advise is not based on PF.
PRONATURA, the biggest French organic fruit and vegetable distributor, does not sell vegetables based on PF.
These initiatives demonstrate that it's possible to supply the European market with a full range of cabbage varieties from the existing CMS-free cabbages currently available.

CIRAB: this association coordinates research and experimentation for OA in Brittany. It only validates organic variety tests if varieties are CMS-free.

[4]Cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods is being excluded from the Directive, which concern the release of GMOs, but not from his GMOs definition.

ITAB (The French Technical Institute for Organic Farming): supported the IFOAM Motion: "IFOAM GA confirms that cell fusion, including protoplast and/or cytoplast fusion breeding techniques, do not comply with the principles of Organic Agriculture" proposed by ECO-PB in June 2008 in Modena.
We must now put in place a framework to manage the transition period. Decisions should not penalize producers nor significantly reduce the varieties available for OA.

Conclusion

According to the GMOs definition in the European directive 2001/18/CE, the use in OA of varieties derived from protoplast fusion or using mutagenesis should not be used.
With regards to protoplast fusion, because it is not realistic to debate each breeding technique in detail, it is necessary to first define a few criteria that will determine if a breeding technique complies with OA principles. For example, the choice of the cell as limit of the living integrity could be one of these criteria.
To achieve this work, it is necessary to have more transparency on techniques used to breed a new variety; to enable this, a modification of the European regulation is necessary.
The questions raised under this forum and the positions presented form a good basis to move forward the discussion and produce a framework to manage the changes proposed; whilst sympathetically encourage innovative research that could better comply with OA principles without reducing quality, crop yields and bio-diversity.

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Annex 1: List of CMS varieties derived from protoplast fusion in 2008, from APFLBB

Association des Producteurs
de Fruits & Légumes
Biologiques de Bretagne

LIST OF CMS VARIETIES DERIVED FROM PROTOPLAST FUSION.(LAST UPDATES : 12/9/08)

CAULYFLOWER

-ALINGHI F1 (CLAUSE)
-AMERIGO F1 (S&G)
-AMSTERDAM F1 (Vilmorin)
-ANIQUE F1 (ENZA)
-ARMADELLO F1 (Seminis)
-ARMADO F1 (Gauthier)
-ASTROLABE F1 (CLAUSE)
-AVIRON F1 (CLAUSE)
-BALTIMORE F1 (CLAUSE)
-BARCELONA F1 (CLAUSE)
-BODILIS F1 (Vilmorin)
-BORIS F1 (Vilmorin)
-BRICK F1 (CLAUSE)
-BRIGANTINE F1 (CLAUSE)
-CADILLAC F1 (S&G)
-CAPVERT F1 (CLAUSE)
-CENDIS F1 (Vilmorin)
-CFL 098 F1 (S&G)
-CFL 373 F1 (S&G)
-CFL 395 F1 (S&G)
-CFL 4701 F1 (S&G)
-CFL 4716 F1 (S&G)
-CFL 4722 F1 (S&G)
-CFL 703 F1 (S&G)
-CFL 705 F1 (S&G)
-CFX 149 F1 (Sakata)
-CHAMBOR F1 (Seminis)
-CHARIF F1(S&G)
-CHF 0541 F1 (Clause)
-CHF 0601 F1 (Clause)
-CL 07140 F1 Clause)
-CL 4470 F1 (CLAUSE)
-CLAPTON F1 (S&G)
-CLARIFY F1 (S&G)
-CLOVIS F1 (Vilmorin)
-CLX 33 101 F1 (CLAUSE)
-CLX 33 203 F1 (CLAUSE)
-CLX 33 304 F1 (CLAUSE)
-CLX 33 321 F1 (CLAUSE)
-CLX 33 322 F1 (CLAUSE)
-CLX 33 404 F1 (CLAUSE)
-CLX 33 406 F1 (CLAUSE)
-CLX 33 411 F1 (CLAUSE)
-CLX 33 502 F1 (CLAUSE)
-CLX 33 506 F1 (CLAUSE)
-CLX 33 508 F1 (CLAUSE)
-CLX 33 509 F1 (CLAUSE)
-CLX 33 511 F1 (CLAUSE)
-CLX 33 514 F1 (CLAUSE)
-CLX 33 515 F1 (CLAUSE)
-CLX 33 516 F1 (CLAUSE)
-CLX 33 604 F1 (CLAUSE)
-CLX 33 609 F1 (CLAUSE)
-CLX 33 812 F1 (CLAUSE)
-CLX 33 902 F1 (CLAUSE)
-COLDEN F1 (S&G)
-COLOSSEO F1 (CLAUSE)
-CORNELL F1 (Seminis)
-DALMAR F1 (S&G)
-DELFINO F1 (CLAUSE)
-DELSOL F1 (CLAUSE)
-DEXTER F1 (RZ)
-DRAKAR F1 (Gauthier)
-E 3195 F1 (ENZA)
-E 51156 F1 (ENZA)
-EAGLE F1 (S&G)
-EASYTOP F1 (S&G)
-ECRIN F1 (Orsetti Seeds)
-ESCALE F1 (CLAUSE)
-EZ 74 713 F1 (
-FANGIO F1(CLAUSE)
-FARMAN F1 (CLAUSE)
-FASTNET F1 (CLAUSE)
-FLAMINIO F1 (CLAUSE)
-FREEDOM F1(Seminis)
-GALICIA F1 (CLAUSE)
-GALILEO FA (CLAUSE)
-GALIOTE F1 (CLAUSE)
-GIPSY F1 (CLAUSE)
-HERMINE F1 (CLAUSE)
-HILARY F1 (S&G)
-HISPALIS F1 (Vilmorin)
-ICEBERG F1 (CLAUSE)
-INTREPID F1 (CLAUSE)
-ISATIS F1 (CLAUSE)
-KAMIS F1 (Vilmorin)
-KORLANU F1 (S&G)
-LIBERTY F1 (CLAUSE)
-LIVINGSTONE F1 (S&G)
-LOCRIS F1 (Vilmorin)
-MAGELAN F1 (S&G)
-MAORENO F1 (CLAUSE)
-MARCHER F1 (S&G)
-MARSOUIN F1 (CLAUSE)
-MASCARET F1 (CLAUSE)
-MATELOT F1 (CLAUSE)
-MAYBEACH F1 (CLAUSE)
-MENPHIS F1(Vilmorin)
-MERIDIEN F1 (CLAUSE)
-MEXICO F1 (CLAUSE)
-MILDIS F1 (Vilmorin)
-MINEAPOLIS F1 (Vilmorin)
-MOBY DICK F1 (CLAUSE)
-NAVIGATOR F1 (CLAUSE)
-NEMO F1 (CLAUSE)
-NESSIE F1 (CLAUSE)
-OBS 812 F1(OBS)
-OBS 814 F1(OBS)
-OBS 825 F1 (OBS)
-OBS 831 F1(OBS)
-OBS 836 F1 (OBS)
-OBS 844 F1 (OBS)
-OBS 846 F1 (OBS)
-OCEANO F1 (CLAUSE)
-OPTIMIST F1 (CLAUSE)
-OSLO F1 (CLAUSE)
-OURASIS F1(Vilmorin)
-PERICLES F1 (Gautier)
-PIZARRO F1 (CLAUSE)
-PLESSI F1 (Gauthier)
-RAFALE F1 (CLAUSE)
-RAFT F1 (CLAUSE)
-REDOUTABLE F1(CLAUSE)
-REGATA F1 (CLAUSE)
-RS 5278 F1 (Seminis)
-RS 5353 F1 (Seminis)
-RS 5625 F1 (Seminis)
-RS 5672 F1 (Seminis)
-RS 5680 F1 (Seminis)
-RX 5716 F1 (Seminis)
-RX 5951 F1 (Seminis)
-RX 5978 F1 (Seminis)
-RX 5984 F1 (Seminis)
-SCUDO F1 (CLAUSE)
-SERAC F1(Gautier)
-SG 4397 **F1** (S&G)
-SG 4404 **F1** (S&G)
-SG 4716 **F1** (S&G)
-SG 5772 F1 (S&G)
-SLOOP F1 (CLAUSE)
-SOLIS F1 (S&G)
-SUBITO F1 (CLAUSE)
-T 2144 F1 (Gauthier)
-TARANIS **F1** (Vilmorin)
-TERTES F1 (S&G)
-TETRIS F1 (S&G)
-THALASSA F1 (CLAUSE)
-TIROL F1 (Gautier)
-TORENS F1 (Gauthier)
-TRENT F1 (CLAUSE)
-TRIOMPHANT F1 (CLAUSE)
-TROFEO F1 (Sakata)
-V 1209 F1 (Vilmorin)
-V 1236 F1 (Vilmorin)
-V 1267F1 (Vilmorin)
-V 1346 F1 (Vilmorin)
-V 5007 F1 (Vilmorin)
-V 5102 F1 (Vilmorin)
-V 5140 F1 (Vilmorin)
-V 6322 F1 (Vilmorin)
-V 7670 F1 (Vilmorin)
-VEDIS F1 (Vilmorin)
-VERONIE F1 (ENZA)
-VITAL F1 (ENZA)
-VIVIANE F1 (ENZA)

GREEN AND ORANGE CAULYFLOWER

-API F1 (CLAUSE)
-CHEDDAR F1 (Seminis)
-CLX 33301** F1 **(CLAUSE)
-EMERAUDE F1 (CLAUSE)
-MAGNIFICO F1 (CLAUSE)
-SUNSET F1 (CLAUSE)
-TREVI F1 (CLAUSE)
-VITAVERDE F1 (Seminis)

BROCCOLI

-CHEVALIER F1 (Seminis)
-CUMBAL F1 (CLAUSE)
-IRONMAN F1(Seminis)
-MONACO **F1** (SG)
-MONOPOLY **F1** (SG)
-MONTFORT **F1** (SG)
-RUMBA F1 (CLAUSE)
-TAMBORA F1 (CLAUSE)
-AQUILES F1 (Sakata)
-NAXOS F1 (Sakata)
-PARTHENON F1 (Sakata)

GREEN CABBAGE

-ALTESS F1 (CLAUSE)
-BLOKTOR F1 (S&G)
-BOURBON F1 (CLAUSE)
-BRADY F1 (CLAUSE)
-BRIGADIER F1 (CLAUSE)
-CABRI F1 (CLAUSE)
-CAID F1 (CLAUSE)
-CAMPRA F1 (CLAUSE)
-CENTURION F1 (CLAUSE)
-CHANCELOR F1 (CLAUSE)
-CILION F1 (S&G)
-CLASSIC F1 (CLAUSE)
-CLX 3962** F1 **(CLAUSE)
-CLX 3973 F1 (CLAUSE)
-CLX3903ms F1 (CLAUSE)
-CLX3983 F1 (CLAUSE)
-COMPANION F1 (CLAUSE)
-CONSUL F1 (CLAUSE)
-COUNT F1 (CLAUSE)
-COUNT F1 (CLAUSE)
-DUCHESS F1 (CLAUSE)
-EMINENCE F1(CLAUSE)
-GUARD F1 (CLAUSE)
-GUARDIAN F1 (CLAUSE)
-KAPORAL F1(CLAUSE)
-KILATON F1 (S&G)
-KILAXY F1 (S&G)
-KILAZOL ***F1*** (S&G)
-LADY F1 (CLAUSE)
-LEGION F1 (CLAUSE)
-LIBERATOR F1 (S&G)
-MOZART F1 (CLAUSE)
-NIZ 17 669 F1 (CLAUSE)
-NOBILIS F1 (CLAUSE)
-ORACLE F1 (CLAUSE)
-ORENOQUE F1 (CLAUSE)
-PICADOR F1 (CLAUSE)
-POET F1 (CLAUSE)
-POTOMAK F1 (CLAUSE)
-QUALITOR F1 (S&G)
-QUISOR F1 (S&G)
-RAMKILA ***F1*** (S&G)
-REGENT F1(CLAUSE)
-SHERPA F1 (CLAUSE)
-SIR F1 (CLAUSE)
-SQUADRON F1 ? (CLAUSE)
-STENTOF 1F (CLAUSE)
-TEKILA F1 (S&G)
-TOLERATOR F1 (S&G)
-TROUBADOUR F1 (CLAUSE)
-UNIFOR F1 (S&G)

RED CABBAGE

-CLX 4015 F1 (CLAUSE)
-FUEGO F1(CLAUSE)
-NIZ 13 206 F1 (CLAUSE)
-REBALL *F1* (S&G)
-REBBECA *F1* (S&G)
-REDCAP F1 (CLAUSE)
-REDGUARD F1 (CLAUSE)*
-REDSKY F1 (CLAUSE)
-REGILIUS *F1* (S&G)
-ROCOCO F1 (CLAUSE)
-RODERICK F1 (CLAUSE)

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SAVOY CABBAGE
-BOHEME F1 (CLAUSE)
-CAPRICCIO F1 (CLAUSE)
-DAPHNE *F1* (CLAUSE)
-EMERALD F1 (CLAUSE)
-EXELVOY F1 (CLAUSE)
-FIONA F1 (S&G)
-ISOLDE F1(CLAUSE)
-KAMCHATKA F1 (S&G)
-MANON F1 (CLAUSE)
-MEDEE F1 (CLAUSE)
-NORMA F1 (CLAUSE)
-ORFEE F1 (CLAUSE)
-OTHELLO F1 (CLAUSE)
-OTTAWA F1 (S&G)
-PENELOPE F1 (CLAUSE)
-RIGOLETO F1 (CLAUSE)
-SALOME F1 (CLAUSE)
-SUPERVOY F1 (CLAUSE)
-TRAVIATA F1 (CLAUSE)
-TURMALINE F1 (CLAUSE)
-VERTO F1 (S&G)
-ZIRCON F1 (CLAUSE)

ROMANESCO
-CELIO F1 (CLAUSE)
-CLX 33 221 ***F1*** (Clause)
-CLX 33 316 ***F1*** (CLAUSE)
-COLOSSEO ***F1*** (CLAUSE)
-FLAMINIO ***F1*** (CLAUSE)
-GITANO F1 (CLAUSE)
-LAZIO F1 (CLAUSE)
-NAVONA F1(CLAUSE)
-PINCIO F1 (Seminis)

KOHLRABI
-E545991 F1 (ENZA)
-ERIKA F1 (CLAUSE)
-OASIS F1 (ENZA)
-OCTAVE F1 (ENZA)
-ORCADES F1 (ENZA)
-SEGURA F1 (Seminis)

BRUSSELS SPROUT
-ABACUS F1 (S&G)
-BRETON F1 (CLAUSE
-COBUS F1 (S&G)
-CRONUS F1 (S&G)
-CUMULUS F1 (S&G)
-GUSTUS F1 (S&G)
-MERCURIUS F1 (S&G)

PONTED CABBAGE
-CLARENCE F1 (S&G)
-REGENCY F1 (CLAUSE)
-RUBENY F1 (CLAUSE)

***to avoid, waiting for confirmation**

BELGIUM ENDIVE

-CRENOLINE F1 ? (Vilmorin)
-ECRINE F1 ?(Vilmorin)
-Elegance F1 (Momont)
-MH 9593 F1 (Momont)
-MH 9724 F1(Momont)
-MH95-09 F1 (Momont)
-MH96-96 F1 (Momont)
-RUBISCO F1 (Vilmorin)

TURNIT
-PLESSIS F1 (CLAUSE) ?

Strategies for a future without cell fusion techniques in varieties applied in Organic Farming – State of the art in Germany

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The IFOAM definition of genetic engineering (GM) as formulated in the IFOAM Basic standards includes cell fusion. So within the International Federation of Organic Agriculture Movements (IFOAM) there seems consensus on the fact that cell fusion does not comply to the IFOAM principles as Genetic Engineering (GE) is banned. On the other hand, varieties bred with these techniques are not labelled as genetically modified organisms (GMO) and the use of these varieties is not explicitly forbidden in the EU legislation on organic farming. But due to the fact that cell fusion techniques are nowadays deployed especially to transfer cytoplasmatic male sterility (CMS) in cabbage breeding (cauliflower, broccoli, kohlrabi etc.) and that those varieties may also be used in organic farming there was a need for clarification.

Therefore, in 2008 during the General Assembly a motion (motion 26.1) was put forward that the IFOAM General Assembly may confirm that proto- and cytoplast fusion techniques do not comply with the principles of organic agriculture. The motion passed and the General Assembly confirmed that cell fusion in general, and protoplast, and/or cytoplast fusion breeding techniques in particular, do not comply with the principles of organic agriculture. Therefore, the General Assembly urged the World Board to develop clear guidelines on how to deal with varieties derived from cell fusion including protoplast and/or cytoplast fusion breeding techniques.

In 2005 the German organic farmers organisation Demeter passed a resolution to exclude varieties which haven been bred using protoplast or cytoplast fusion for Demeter vegetable grower (Wilbois 2005). Since such “CMS-varieties” are usually not easy to recognize for the grower, Demeter has published a negative list (cf. Table 1) listing known “CMS-varieties” which were no longer appropriate for growers using the Demeter label. Besides Demeter two further organic farmers organisation (Naturland and Gäa) followed and excluded “CMS-Varieties” from the production under their labels. Within the largest German organic farmers organisation “Bioland” the issue is under discussion and exclusion might follow very soon.

Table 1: Number of “CMS-Varieties” from eight different Companies listed in the German negativ list dated December 2008 (Regnat 2008)

#	Crop	Number of different CMS-Varietie
1	Cauliflower (white and coloured)	85
2	White cabbage	44
3	Savoy cabbage	20
4	Kohlrabi	13
5	Broccoli	12
6	Red cabbage	9
7	Brussels sprouts	7
8	Romanesco	4
9	Chinese cabbage	1

Organically produced seed of varieties as listed in the official German organic seed data base OrganicXseeds (www.organicXseeds.de) are bred without using cell fusion techniques. Therefore, using organic seed is a way to safeguard the renunciation of “CMS-Varieties” and hence techniques that do not comply with the principles of organic farming.

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Novel Breeding techniques : molecular biology combined with plant tissue culture

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Key words: genetic modification, mutagenesis, reverse breeding, cisgenesis

Abstract

Plant breeding has experienced an enormous increase in several applications of molecular biology over the past few decades. The most pronounced technology is genetic modification (GM), which organic agriculture has decided to refrain from. Drawing the line to keep GM crops out is mainly based on the evaluation of the process of genetic modification. Because more and more novel breeding methods that rely on molecular biology techniques are being introduced to develop new varieties, organic agriculture is challenged to evaluate these methods. In this paper we will describe the essentials of four breeding methods: next to new techniques such as reverse dihaploid breeding and cisgenesis, also marker assisted breeding and induced mutagenesis will be discussed.

Introduction

In the twentieth century plant breeding has undergone revolutionary changes. With the advent of induced mutagenesis, cell culture techniques and DNA- analysis / modification techniques the emphasis has shifted from field work to laboratory analyses. The major drive behind these developments is the urge to shorten the developmental cycle of novel crops. Most, if not all breeding is aimed at producing F1 hybrid seeds. Parents used in these crosses have to have a high degree of inbreeding to ensure a predictable outcome of the F1 hybrid. More and more biotechnology tools are being applied to quickly generate new inbred lines. In some cases genetic modification is part of the new developments, either directly visible (in the case of ‘cisgenesis’) or hidden in de process or the concept behind it (in the case of Marker-assisted selection and reverse breeding). In addition to these approaches breeders aim to apply our knowledge of gene function in creating targeted mutants. This paper aims to make organic farmers and policy makers aware of the new biotechnological developments and wants to stimulate the discussion with regard to the ethical assessment of the applications with regard to organic values.

Description of novel breeding technologies

DNA marker assisted selection as a tool in plant breeding

Crossing plant lines with different desired traits can result in progeny lines that combine the parental properties. Traditional breeding relied on visual markers for the desired traits in large-scale field trials. This always required a vast number of crosses and testcrosses that had to be evaluated in the field. For many traits bioassays have been developed that can be done in the laboratory rather than the field (i.e. disease resistance). With the discovery of polymorphic regions in the DNA of parental lines it has become possible to link phenotypes to certain polymorphic parts of the plant’s genome. The DNA fragment has become a “marker” for a phenotype. For many crop species thousand of such DNA markers have been associated with phenotypes or chromosome regions. It is now standard practice in plant breeding to pre-select plant material for specific traits using DNA from seedlings (Peleman & Van der Voort, 2003). Only those lines that harbor the desired DNA fragments are selected for field trails and evaluated for their agronomical performance.

Dihaploid plants from tissue culture and their application in "reverse breeding"

To speed up the time consuming process of repeatedly backcrossing with either parent to obtain "pure" breeding lines, which can then be combined into an F1-hybrid, a tissue culture technique has been developed: anther culture or microspore regeneration. The rationale of this approach is simple. From a developing pollen grain a complete plant can be regenerated in tissue culture. Because the pollen grain is haploid, the plant that is obtained is haploid as well. Through treatment with the synthetically produced plant compound colchicine, such a plant can become diploid again. It now has two copies of the original chromosomes it contained as a pollen grain (dihaploid): it is homozygous for practically al traits!! Using DNA markers those dihaploid plants can be selected that combine all traits that were present in the "elite" parents. In "reverse breeding", dihaploid technology is combined with directed gene technology. The aim is to generate breeding lines from an excellent hybrid that originates from a complicated crossing scheme. This excellent line contains all the desired traits, but cannot be used as the origin for seed production because not all traits are homozygous present. Upon self-pollination the progeny will receive only part of traits and the seed batch will not be uniform. In "reverse breeding" this hybrid is used to generate a transgenic plant that will be impaired in genetic recombination during pollen production. Pollen from this transgenic plant will only contain "complete" chromosomes and no recombinants. Dihaploid plants originating from this pollen will thus contain only a limited number of chromosome combinations. Sorting these dihaploid lines out with DNA markers will allow you to find these dihaploid lines that together would re-establish the original hybrid. By carefully combining dihaploid lines from different transgenic plants a non-GM F1-hybrid product is obtained from this process.

Gene transfer from related plant species: cisgenesis.

The essential characteristic of a genetically modified organism (GMO) is that it contains 'genetic material that has been altered in a way that does not occur naturally by mating and/or natural recombination'. Because consumers have explicitly expressed their concern about using "foreign" genes in crop plants, scientists have turned towards alternatives that rely completely on genes from related plant species. They have coined the term "cisgenesis" to distinguish this approach from the current GM products that result from "transgenesis". Cisgenesis is especially focused on pathogen resistance genes from wild relatives to avoid the tedious crossing process, which can certainly be difficult with crops that are normally propagated vegetatively (potato) or have long life cycles (fruit trees like apple). Because the plant gene that is introduced through genetic modification could theoretically also be crossed into the crop plant, molecular scientists argue that it no longer should be called a GMO. Moreover they argue, because the gene that is introduced through genetic modification is a "natural" plant gene it will hold less risk for the environment and human health and therefore the cisgenic product need not be scrutinized as thoroughly as GM-crops (Schouten et al., 2006). However, the DNA insertion position on the genome is unpredictable (Cellini et al., 2004). Even when a gene originates from a crossable species, genetic engineering will per definition cause it to land on a different and thus new position in the genome compared to the situation where the same gene was introduced by traditional crossing. The expression of a gene in a GMO product is therefore always uncertain and may unpredictably influence the expression of other genes. In case of traditional breeding the desired gene is embedded in the region of a chromosome that has been assigned to it through natural evolutionary processes.

Induced and targeted mutagenesis

Upon the discovery of agents that can induce genome alterations plant breeders have started using chemical and ionizing radiation to induce new variation in crop plants. Traditionally this meant that seeds were treated with chemicals (for instance EMS) of radiation. The dose of these treatments was set to such a level that approximately twenty percent of the seedlings would not survive. The remaining seedlings were grown to maturity and allowed to self-pollinate. The resulting seeds were sown and plants were tested for altered phenotypes. Interesting plant lines were backcrossed to remove other mutations from the background and finally an inbred line was created that held the new trait. Many dwarf varieties have been obtained by mutagenesis. With the advent of molecular biology and knowledge of the DNA code of complete genomes targeted mutagenesis has become possible. A short piece of synthetic DNA or a synthetic DNA-RNA hybrid can be introduced into a cell. The presence of this short piece of DNA triggers a repair mechanism that can replace the DNA sequence in the genome by the DNA sequence that has been synthetically created. In this way minute changes in the genome can be created. A single DNA base change in the code of a gene can lead to a completely different phenotype. For instance, in this way crop plants (tobacco and rice) have been created that are resistant against a sulfonylurea herbicide like chlorsulfuron (Kochevenko and Willmitzer, 2003). Plant protoplasts were bombarded with gold particles coated with a synthetic DNA-RNA hybrid, targeting the ALS (acetolactate synthase) gene. The regenerating protoplasts were then exposed to the herbicide in low doses. Plants that we highly tolerant to the herbicide were thus selected and could be shown to have a single base change in the targeted ALS gene. Although in principle this technique could be applied to any given gene the efficiency is very low: millions of individual plants would have to be screened to find a single mutant. Applying this DNA based mutagenesis to GM plants that have extra enzyme that stimulate the exchange of synthetic DNA with native DNA may enhance the success rate to a level that may make it applicable to other genes.

Discussion and conclusions

Should varieties obtained through cisgenesis, reverse breeding or mutagenesis be allowed in organic agriculture? Organic agriculture has banned all products originating from genetically modified plants. A logic conclusion would be that products of cisgenesis and reverse breeding both should be subject to the current GMO regulations in organic agriculture and should thus be banned from organic agriculture (Lammerts van Bueren et al., 2007). This also holds true for the future application of targeted mutagenesis using GM plants. Because the products from reverse breeding and targeted mutagenesis do not carry any traces of the genetic modification involved in the process, one will be unable to detect them if the breeding companies do not label them. Evaluating the application of DNA marker assisted selection in organic plant breeding we have to consider two aspects: the way such markers are produced and the concept behind their application. DNA markers are typically generated using a PCR approach. This involves enzymes originating from GM bacteria, so strictly spoken these DNA markers are inappropriate for organic plant breeding. In addition, the reductionist gene-centered view behind DNA markers can be challenged in the light of the holistic view organic agriculture. Finally, chemical and radiation induced mutagenesis should be evaluated; this process requires synthetic compounds and non-natural radiation sources. In my opinion this approach would not fit the non-chemical, all natural approach intrinsic to organic agriculture.

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Standards for certified biodynamic plant breeding

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Abstract

Based on mission-statement of ABDP (Association of biodynamic plant breeders eV), which can be found on its website, Demeter eV developed standards for biodynamic plant breeding and put them into action in January 2009. The standards focus on transparency of the breeding process latest published at the beginning of marketing a new variety, the necessary documentation and clear borders to prohibited methods. The standards are open to new developments and new knowledge about new and old breeding techniques and they take into account the decicisons of the international organic agricultural movements represented by IFOAM.

Introduction

Plant breeding is not only crop production, but also a permanent research and development. Those methods, which are not accepted by the community of organic and biodynamic farmers, traders, processors etc. should be described and excluded. But to dictate the presently well-known allowed techniques on a positive list with the consequence that any new method, which corresponds with the idea of biodynamic farming or spiritual developement of mankind, but still not allowed, cannot be used for breeding, is an obstacle for any development in breeding. Nevertheless any new method contains risks and chances. As far as breeding is a business with strong competetiveness many breeders don't want to publish their ideas before marketing the variety. But from the moment a variety becomes available for market and consumption any consumer should be able to make his own decisions about the use of a variety also with respect to the breeding method. For this reason the methods used for breeding a new variety have to be published latest from the beginning of use. It gives room and time for developing new methods and enables consumers to decide themselves. Even the biodynamic community can start a discussion and decide, whether varieties out of these procedures or a new methods should be prohibited or not. This idea was crucial for developing standards for certified biodynamic plant breeding with a focus on transparency.

At present the aim of the biodynamic plant breeding standard is aims to describe varieties that arise from biodynamic plant breeding using defined criteria in order to differentiate these varieties from others, which are not allowed to use this descriptor. The labelling of varieties in the way this standard foresees as "From Biodynamic Plant Breeding" is intended to make the breeding methods standing behind clearly visible.

Any variety that is offered for sale and which originates from biodynamic breeding must have a full biography of its development published on the internet and freely available.

General requirements for breeding new varieties biodynamically

Breeding should take place on at least organically certified fields and it must be guaranteed that the biodynamic preparations are used in such a fashion that their influence can be expected to reach the plants and the soil. This shall be regulated in writing with the certified organic farm in question using for example a contract which ensures the required production parameters. The breeding enterprise, as well as the all aspects of the breeding activity itself, must be accessible for Demeter inspection at any time.

Breeding a new variety begins with accidental or intentionally initiated cross pollination or a mutation in the sense of an inheritable alteration, with a subsequent selection procedure. A minimum of four years subject to biodynamic conditions is required.
The following breeding methods are prohibited:
- All plant breeding methods prohibited by IFOAM
- Hybrid breeding irrespective of the hybridization method
- Production of double haploid varieties or polyploidisation
- Varieties bred using protoplasm or cytoplasm fusion techniques.

The use of hybrid or double haploid varieties as parents for a Biodynamically bred variety is allowed.

New Biodynamic varieties must be recognized as such (for example by registration at the respective plant variety registration office) if the seed is to be sold to third parties in an area with a valid seed marketing law.

If official registration of a new variety is not required due to its production and use within a closed system, application can be made to Demeter e.V. for recognition of the variety as "From Biodynamic Breeding". This is only possible if the varietal descriptor leads to the expectation that the seed meets the legal seed variety requirements of distinctiveness.

Regulations applying to the standards for certified Biodynamic plant breeding

For certification the initial entry of a seed sample into the breeding enterprise has to be documented (delivery note, entry voucher, supplier, quantity, state of treatment, genetic engineering risk). Every seed sowing must be recorded on a field plan, which shows the area from which the harvest was taken for further selection. It must be possible to trace plants growing on this area back to the generation before from documentation on file. Every plant or plot number must be used only once in each year. Handing over of seed has to be documented according to variety, seed lot, quantity, treatment, recipient, with a copy of the delivery note as officially required for organic certification. These vouchers provide a method for following the biography through generations or over successive vegetation periods.

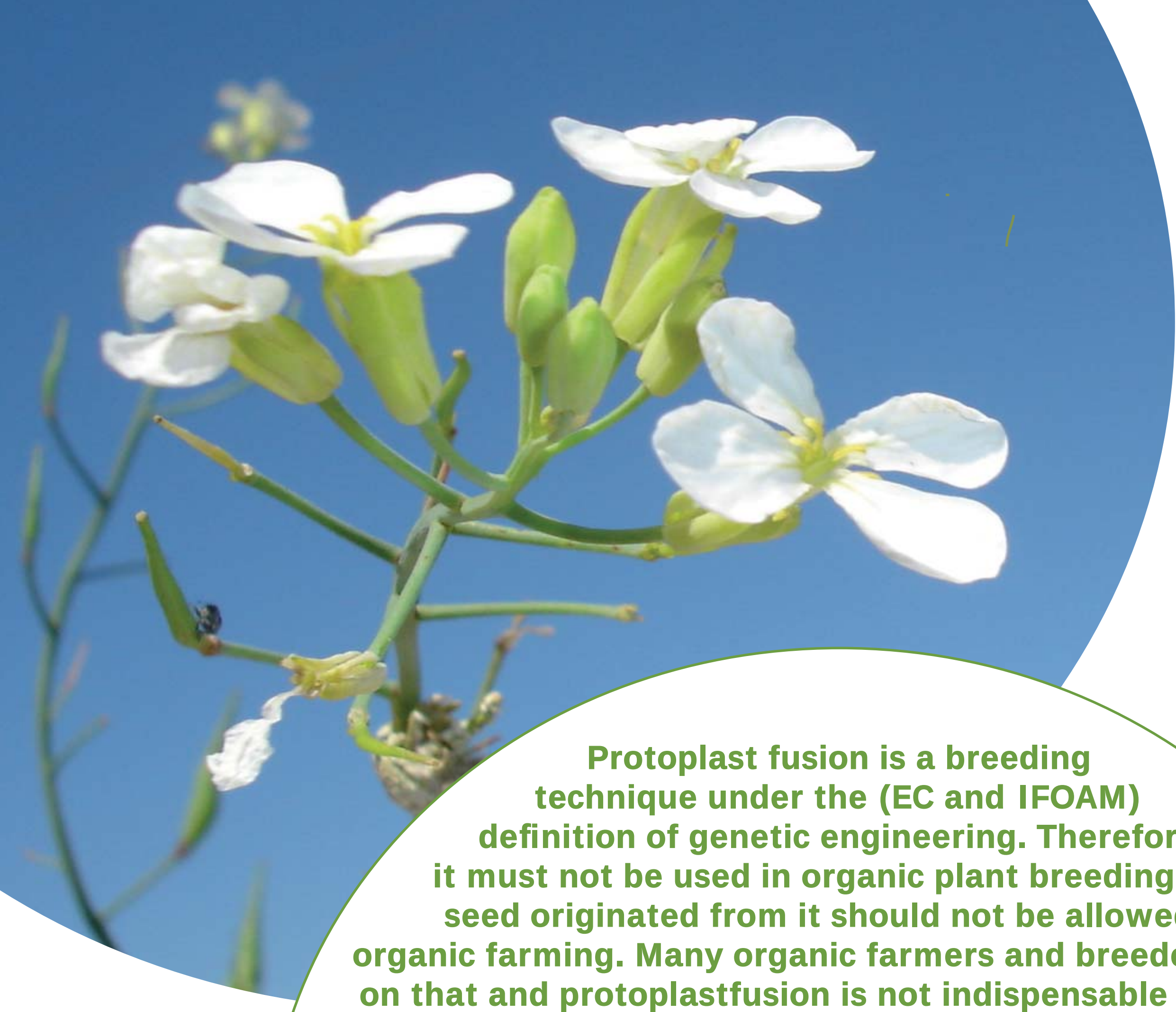
Steps towards transparency in the development of varieties

The declarations made in the description of a variety's biography will be checked by an inspection body appointed by the respective organisation. The respective Demeter certification body will grant certification of a variety as "From Biodynamic Breeding". Outside specialists may be consulted if necessary. In principle, the clearer the declarations are about a variety's biography of development, the trustworthier they are!
A biography of development needs declarations relating to the following questions:
1. What plant it is, and who has prepared the biographical text on which date?
For example: Species, culture, name of variety, name of breeder, date of declaration.
2. Where the starting material originates?

Name, supplier, year of first cultivation, what was crossed with whom.
3. Under which circumstances the breeding line was cultivated and selected?
Location, special methods of cultivation, particular aspects (people, enterprise structure, fertilisers used). Was there a personal concern/request (philosophy, intention, motivation, purpose)?
4. Which method of selection was used?
- Mass selection (positive/negative), how many individuals were chosen out of a population of how many?
- In case of single plant selection: Was cultivation and testing done with separated single plant descendants as pedigree selection or with bulked populations?
- Was the method of selection changed or varied over the generations or were special selection criteria used only in specified selection periods?
- Were special test methods used with the results then influencing decisions?
- Under which circumstances were additional tests carried out?
- Were there particular criteria, which had to be fulfilled before introduction into usage?
5. When was the variety released by the relevant authorities?
6. How was the seed multiplied through to its delivery quantities?
7. How can the variety be described today? Typical varietal characteristics, recommendations for cultivation, practical results from the field, results from quality tests and if a description of the formative forces or the result from a picture forming quality assessment method (e.g. copper-chloride-crystallisation) is available, its publication is desirable.
These steps to transparency are written to give an idea about the meaning. It is not an imperative to fulfil each. They can be reduced, precised and extended by the breeders and the certification bodies. At the end they should be satisfactory from consumers point of view.

Perspective

Many biodynamic plant breeders and people from Demeter movement had been involved during development of the plant breeding standards and accepted it as a standard to start with. It is a standard to develop consciousness about plant breeding and all the methods used for it. Clear borders were drawn, but also a door opened for further developments. Biodynamic plant breeders hope to get a feedback about these breeding standards from the organic community world wide to become aware of deficiencies, to fill them with life and to discuss new items.



Protoplast fusion is a breeding technique under the (EC and IFOAM) definition of genetic engineering. Therefore it must not be used in organic plant breeding and seed originated from it should not be allowed in organic farming. Many organic farmers and breeders agree on that and protoplastfusion is not indispensable for plant breeding. But in reality its products might be widely used by organic farmers. Therefore the question is how do we ban proto-plastfusion from organic farming?

This Workshop takes place between Ifoam congress 2008 in Modena (in particularly the workshop on GMOs and the motion passed "cell fusion, including protoplast and cytoplast fusion, do not comply with the principles of organic agriculture"), and the 1st IFOAM Conference on Organic Animal and Plant Breeding in Santa Fé (New Mexico, USA) in August 2009. Based on IFOAM's motion on cell fusion, this meeting aims to define short and long term strategies to ban there use in Organic Farming. It should also give a clear signal to seed companies who choose not using this technique.

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