DOSSIER

FiBL

Plant Breeding Techniques

An assessment for organic farming

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Contents

Growing organic food depends on suitable varieties and growing methods. The establishment of breeding programmes based on the specific objectives of organic agriculture is an important prerequisite to further increase the efficiency and yield stability of organic food production. Further, the increase in genetically modified varieties, greater consolidation within the seed market and limitations in the use of genetic resources due to the patenting of living organisms mean that alternative approaches in plant breeding are required.

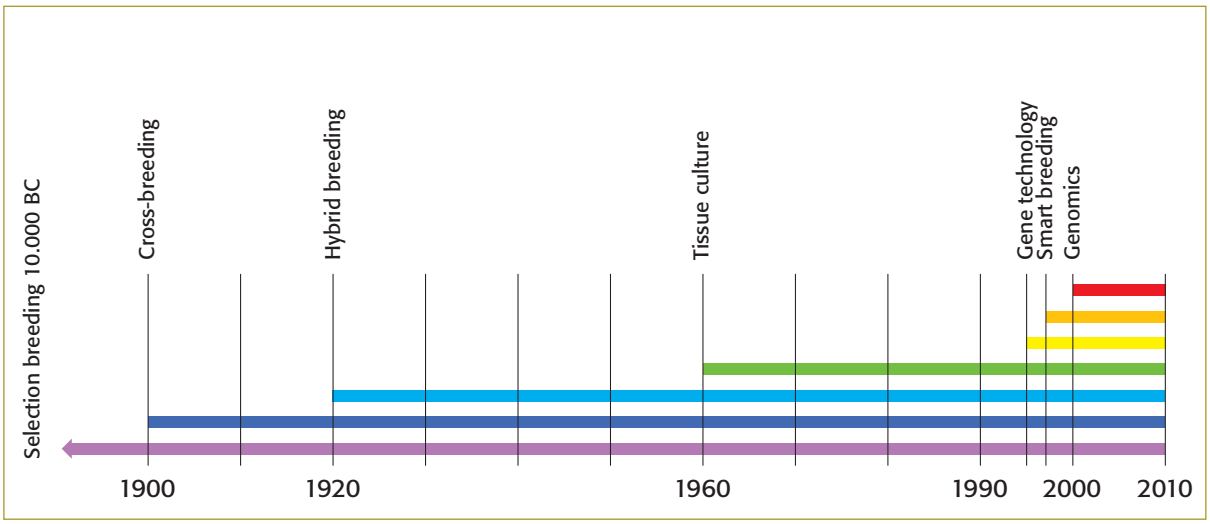
This dossier describes traditional, modern and nearly practice-ready techniques for plant breeding along with assessments of their suitability for organic farming. The overview of techniques is complemented by a position paper from an expert workshop on ecological plant breeding according to organic farming criteria.

The dossier will provide decision makers and practitioners of organic farming with the requisite information and criteria for an objective and transparent assessment of plant breeding methods.

Introduction

Organic farming looks at the breeding of new varieties in a holistic way. Thus, not only the variety itself but also the process of varietal development must comply with the guiding principles of organic agriculture. Important criteria are taken into account such as preserving the integrity of the plant, increasing genetic diversity, respect for crossing barriers, and interactions of the plant with the living soil and climate. Breeding techniques that are used to produce genetic variation for selection and propagation must be evaluated for compatibility with organic farming. Genetically modified organisms (GMOs) are excluded from organic farming, as isolated DNA is exchanged within or across species, which would not occur under natural conditions. Other methods that do not currently need to be declared, such as protoplast fusion, *in vitro* propagation, induced mutations and hybrid breeding are critically discussed in the sector.

Milestones in plant breeding



Source: modified from www.die-pflanzenzuechter.de/innovationen.html

The discussion about the compatibility of modern breeding techniques with organic farming has been central to the organic sector for several years as part of national and international workshops and conferences (Wyss et al., 2001; Arncken, 2002; Wilbois, 2002; Arncken and Thommen,2002; Arncken and Dierauer, 2005; Lammerts van Bueren and Wilbois, 2003; Lammerts et al., 2007; Billmann et al., 2008; Oehen and Thommen, 2009). Until now, a comprehensive assessment of modern breeding techniques has not been realised due to the complexity of the issue which is emotionally charged by the GMO debate. New techniques (i.e. smart breeding) are constantly emerging out of research and development activities that are rapidly implemented in conventional breeding programmes raising new questions.

The complexity of these new technologies can cause great scepticism by consumers and stakeholders of organic farming. However, if modern breeding techniques are rejected a priori, the organic farming sector risks to be left out of the breeding progress unable to compete with growing challenges of productive and sustainable food production (BMVEL 2002). Therefore, it is important to gain an overview of current breeding techniques, along with those still under development, and to define criteria that ensure an objective and transparent assessment of each technique in light of the principles of organic farming. An adapted organic plant breeding approach must include the development of improved varieties for organic farming without violating its ethical and ecological principles.

The use of genetically modified varieties is prohibited in organic farming according to EC Regulation No 834/2007. The same regulation requires to use only organically produced seeds and propagating material. For this, the mother plant (in the case of seeds) or the parent plant (in the case of vegetative propagating material) must have been produced under the provisions of this regulation for at least one generation (for annual crops) or for a period of two growing seasons (for perennial crops).

Why do we need organic plant breeding?

Requirements for organic farming

For an efficient and sustainable production of food under organic conditions, both varieties and cultivation methods must be optimised for the site. Since currently available crop varieties mainly originate from conventional breeding programmes (Lammerts van Bueren et al., 2011), the genetic potential for organic agriculture is far from being fully exploited. Traits relevant to organic farming, such as resistance to seed-borne diseases, weed suppression and nutrient use efficiency, are not considered in the selection of plants grown from dressed seeds, treated with herbicides and receiving high levels of fertilisation. Thus, breeding programmes that focus on the specific breeding objectives and cultivation methods of organic agriculture are urgently needed to increase efficiency and yield stability in the production of organic food.

Organic agriculture stands for a high genetic diversity at the farm level. It is necessary to grow a **wide range of crops** in order to deal with the heterogeneous environment found in organic farming systems in terms of field conditions, stocking densities, crop rotations and marketing options. For this, numerous **regionally-adapted cultivars** should be made available.

These varieties must deliver sufficiently high and, above all, **stable yields** with minimal use of external resources and be of **high quality** with regard to technical and nutritional requirements. Organic farming differs from conventional farming in many aspects, particularly regarding the type and amount of fertiliser and the approaches to weed and pest control. Thus, organic farming strives for closed-loop nutrient cycles in which organic plant and animal fertilisers generated on-farm are used instead of quickly soluble mineral fertilisers. Natural resources can be utilised optimally by cultivating legumes and green manures for biological nitrogen fixation and choosing nutrient efficient crop varieties. Weed control is achieved through optimised crop rotations, mechanical methods and fast-growing competitive crop varieties rather than through the application of herbicides. Disease and pest control can be managed through targeted support of predators, parasitoids and symbionts (i.e. functional biodiversity) along with the cultivation of resistant varieties instead of using pesticides.

In addition to the many crop traits that are also important in conventional farming, varieties for organic farming must have additional features. These include:

- Resistance to soil- and seed-borne diseases (this is no longer considered in conventional breeding programmes, since efficient synthetic chemical treatments are available)
- Rapid youth development
- High weed suppression and tolerance
- Good lodging resistance at greater plant heights
- Increased nutrient use efficiency through extensive root systems and the promotion of symbioses with soil organisms
- Quality-related traits

The objectives of organic plant breeding should be tailored to the individual crop and market, taking into account the needs of farmers, breeders, traders and consumers.

Soybean flowering.

Varieties for organic farming

Breeding system	Testing system	Multiplication system
Conventional breeding (Cat. I)	Conventional	Organic
Conventional breeding (Cat. I)	Organic	Organic
Breeding for organic farming (Cat. II)	Organic	Organic
Organic Plant Breeding (Cat. III)	Organic	Organic

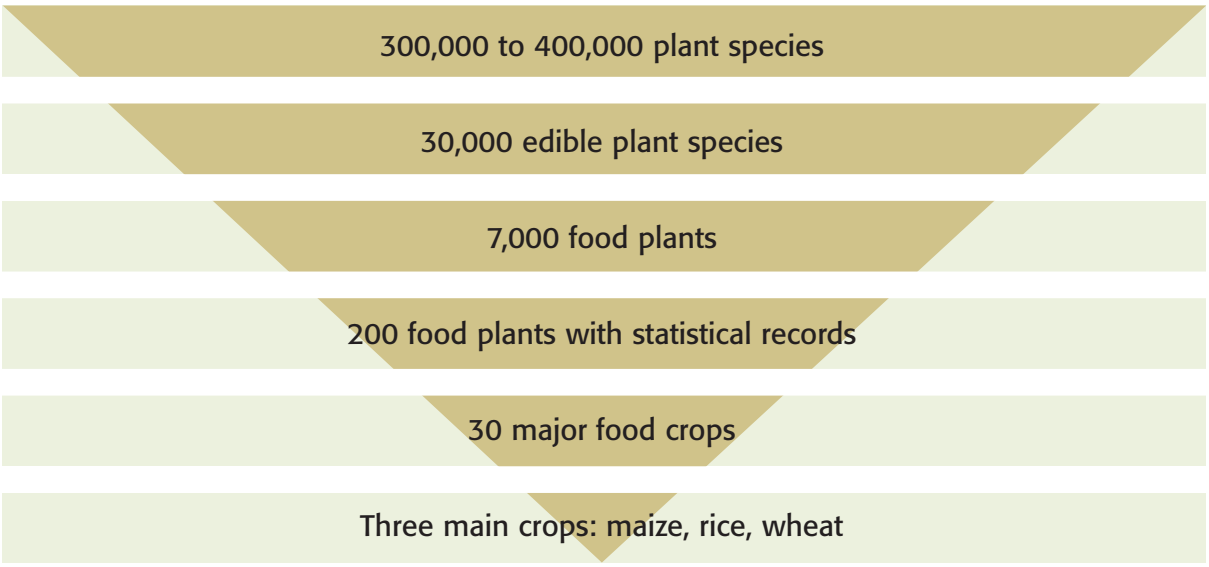
Consolidation of the seed market

Plant breeding is dominated by commercial breeding companies which refinance their breeding activities through issuing licenses. Breeding activities outside of the commercial sector are generally limited to the development of precursor material, which is then further developed into varieties by private breeders. Breeding efforts focus on a relatively **small number of plant species** (e. g. maize, rapeseed, rice, soy) that are economically important and provide a rapid return on financial investment. This increases the yield gap for minor crops and the cross margin falls progressively behind that of major crops (e. g. for N-fixing legumes, irreplaceable in organic agriculture). The consequences are increasingly simplified crop rotations and the loss of expertise in cultivation, as is seen in the example of organic faba beans.

The **commercial seed industry** has undergone an extraordinary consolidation in the past 40 years. The development away from small, family-owned companies to large multinational corporations began with the breeding of hybrids. Large agrochemical companies that were mainly active in biotechnology research began to buy up breeding and seed companies in the 1980s. In the 1990s, large, global multinationals were built up through acquisitions and mergers of competitors. The merging of agrochemical companies, pharmaceuticals and seed producers was enabled by exploiting important synergies and the use of a common technology platform. Genetic engineering methods had just been developed, but the capital investment costs remained very high. It therefore made sense to use the acquired methodological knowledge for both the development of pesticides and pharmaceutical agents and new properties into crop plants. New properties were patented which ruled out the use of this plant material by other breeders and also precluded farmers from resowing their farm-saved seeds. At the same time, the idea was developed to sell seed and crop protection products together as a package in order to retain customers (Harl, 2000).

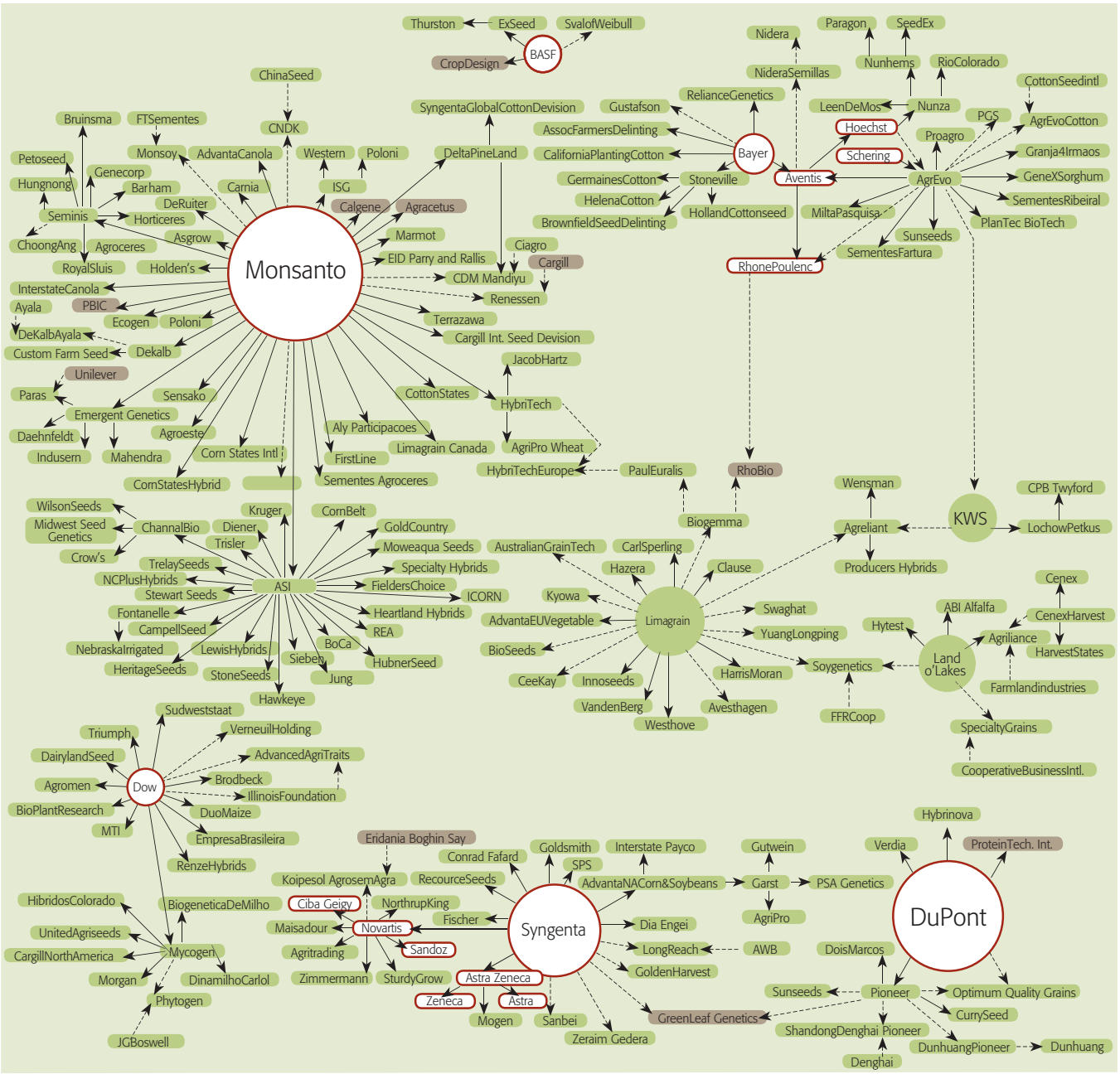
Today, seed sales are dominated globally by a small number of companies. The three largest companies Monsanto, DuPont and Syngenta control 53 % of the global proprietary seed market (ETC Group, 2011). There has been a strong consolidation with respect to the ownership of variety rights for the most important crops (e. g. wheat, maize, soy, potato, ryegrass and canola oil), especially in developed countries. Worldwide, the top 10 companies share the Plant Variety Protection certificates of over 40 % of wheat and up to 70 % of oilseed rape and maize varieties. As a consequence, there has been a significant loss of varieties. Furthermore, the market-leading seed companies are increasingly working with genetic engineering techniques. The concentration of power, the structures and the techniques contradict the fundamental principles of sustainability which are central to organic farming.

Loss of diversity in plant breeding



Source: modified from Haußmann and Parzies (2009)

Structure of the seed industry in 2008



Graphic from AGROPOLY, Berne Declaration, April 2011, modified according to Howard (2009), www.mdpi.com/journal/sustainability

Legal framework

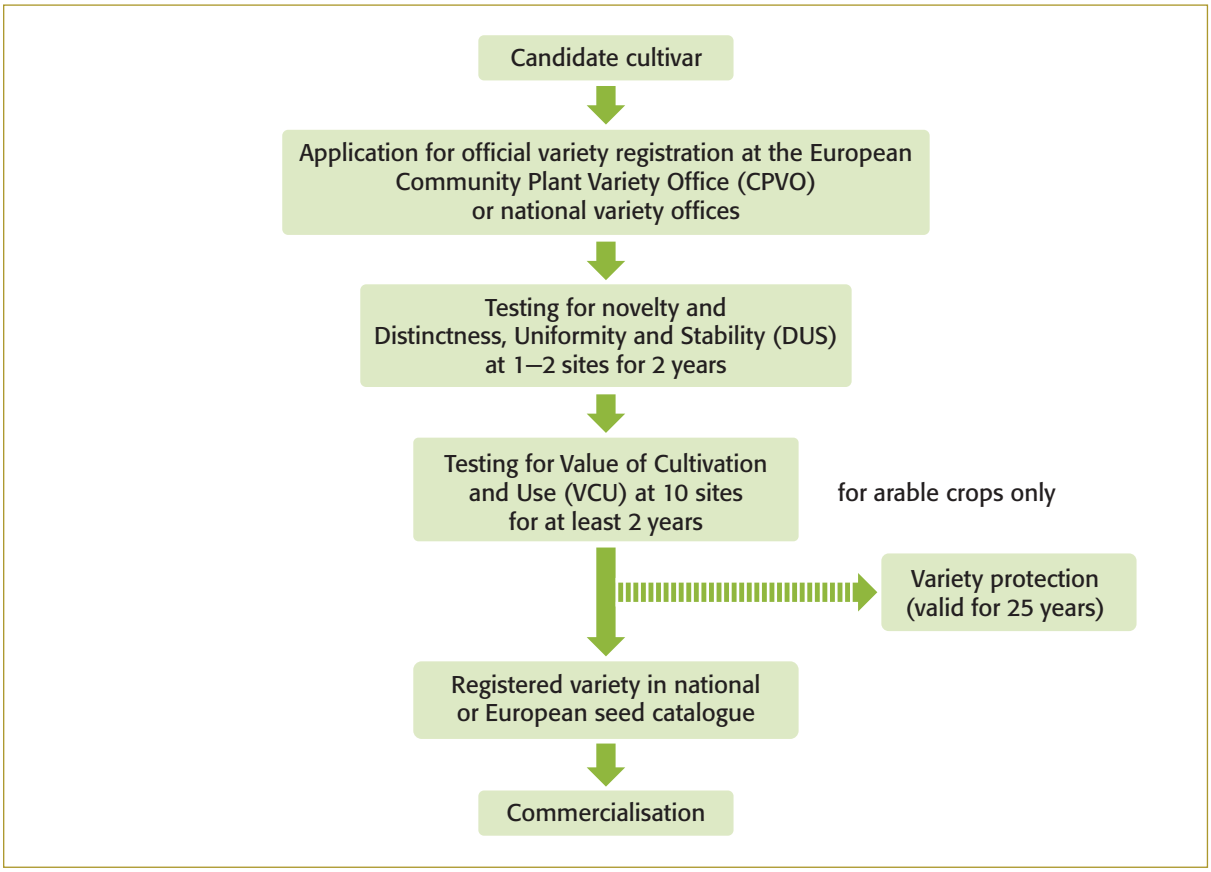
Plant variety protection

The current legal framework for plant breeding, seed propagation and distribution consists of a large number of national and international guidelines, conventions, directives and regulations. A crucial step on the way to legal protection for plant breeding was the International Convention for the Protection of New Varieties of Plants (UPOV) on 2 December 1961 (www.upov.int/upovlex/en/upov_convention.html), which established uniform international rules for the protection of plant varieties. The UPOV Convention provides a form of intellectual property protection *sui generis*, which has been adapted and developed for the process of plant breeding and which recognises the right of the breeder to the exclusive use of the variety. The breeder may request royalties for all seed multiplications of his or her variety as a form of payment for the breeding efforts. The consent of the breeder, however, is not required if other breeders want to use this variety for further breeding (breeders' exemption) or if the farmer wants to save and propagate their own seed (farmers' privilege). A fee for farm-saved seeds may be charged for such usage depending on the country and area of cultivation. These exceptions are a central part of the UPOV Convention (Le Buanec, 2006) and are very different from patents.

In order for plant variety rights to be granted, a variety must be distinct, sufficiently uniform, stable (i.e. genetically stable), and new. It must also have a new variety name registered (Miedaner, 2010). The first three criteria are evaluated in registration checks (DUS: assess Distinctness, Uniformity and Stability). These registration checks focus primarily on morphological traits, which are not necessarily agronomically relevant.

National seed trade laws regulate the registration process and determine whether a variety is approved for marketing. For an agricultural crop, this variety approval depends on the so-called 'value of cultivation and use' (VCU) which needs to be different from existing approved varieties. The traits that are taken into account to determine the VCU are regulated for each crop by the national or European plant variety offices. Until now, this evaluation has been carried out in most countries under conventional conditions and is strongly focused on yield potential. This system is efficient in that it protects good varieties and thereby ensures that new varieties are developed. However, it only partially provides for the needs of organic farming, since it is focused on varieties that will serve the widest possible geographical distribution and a large market (Borgen, 2009; Lammerts van Bueren et al., 1999; Rajaram and van Ginkel, 2001).

Procedures for plant variety approval and protection



If species homogeneity is too low, a variety might not be protectable or marketable. Therefore, much effort is currently underway to change the approval process so that genetically heterogeneous, yet adaptable, varieties can come to market. Since 1 July 2010, in Switzerland it has been possible to admit such so-called 'niche varieties' to the register without DUS examination.

The breeders' exemption and the farmers' privilege were clearly embedded in the older UPOV agreement (1978), whereas in the new UPOV Convention of 1991, the rights of other breeders to use protected varieties for research purposes are limited and the privileges of farmers are restricted. Farmers are allowed to multiply protected varieties for their own use, but they may not exchange or sell seeds to one another. Prior to the introduction of the UPOV (1991), plant breeders had to choose whether to protect their varieties by breeders' rights, or by a patent. UPOV (1991), however, has made it possible for plant varieties to be protected by breeders' rights, as well as by a patent. In addition, the variety rights were extended to all plant genera and species and the commercialisation of seeds can only be granted for protected varieties. Critics of the amended regulations are concerned that they put small producers at a disadvantage because it is not legal to sell their own landraces. In general, this could lead to genetic depletion and limitation of crop diversity.

Patents

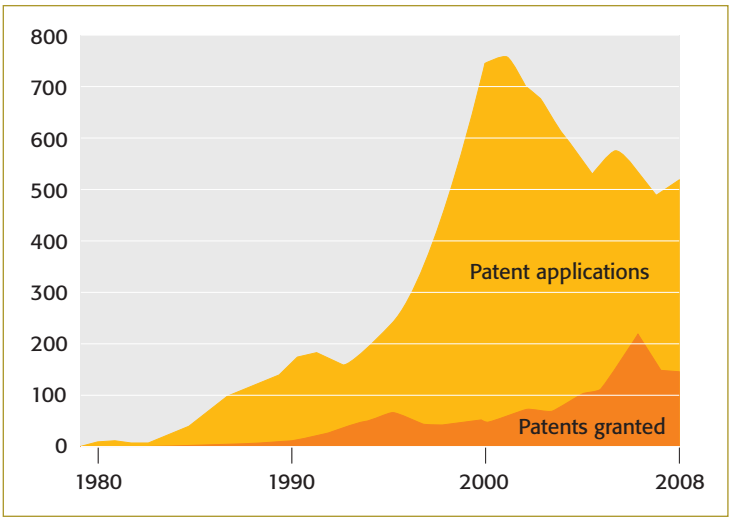
In the United States, in addition to Plant Variety Protection (Plant Breeders' Rights), utility and plant patents are used. In the EU, however, the patenting of plants and seeds is still highly controversial. Although limited patenting was allowed in Europe in the early stages of developing genetic modification techniques, recently, various patent applications have been made for non-genetically modified broccoli, melons and tomatoes.

According to Article 4, paragraph 1 (a) of the EU Organic Patent Directive 98/44/EC, plant varieties and animal breeds are prohibited from being patented. A variety or breed is shaped by its whole genome and defined by the expression of certain attributes that result from a given genotype or combination of genotypes. In contrast, plants or animals that are below or above the level of variety or breed can be patented. Furthermore, methodologies are patentable that relate to more than one variety or breed.

Excluded from patentability under Article 4 of Biotechnology Patents are 'essential biological processes for the production of plants or animals'. The same applies under Article 53 of the European Patent Convention (EPC) which states that "European patents shall not be granted ... for plant or animal varieties, or essential biological processes for the production of plants or animals. This does not apply to microbiological processes or the outputs produced with the aid of these processes".

In April 2011, the European Patent Office (EPO), however, granted a patent on melons resistant to certain viruses (EP1962578). Originally appearing in an Indian melon variety, the resistance trait can be transmitted both by purely conventional breeding and by genetic modification to other melon varieties. In this case, a product claim is made which is partially described by a process. Therefore, in some patents relating to broccoli and tomato, the plants are defined as products according to the underlying breeding procedures (in the case of broccoli a so-called smart breeding process).

Number of patent applications on cultivated plants and number granted by the European Patent Office



Source: modified from Then and Tippe (2009), www.no-patents-on-seeds.org

The current approach of the EPO seems to assume the patentability of plants, even when these come from conventional breeding. Consequently, the EPO has, in principle, no objection to the patenting of a seedless tomato that is described essentially by the use of a conventional breeding process (EP1026942) (www.bmelv.de search for 'Biopatente-Product-by-process'). Indeed, several hundred patents have already been granted on plants (www.no-patents-on-seeds.org/de).

The application of patent law to plant breeding has led to far-reaching restrictions on the use of genetic diversity, does not allow any breeders' exemption or any multiplication of seeds. In addition, patents often cover all stages of the value chain – from field to food – and thus reduce the independence of producers. In organic farming, and also by many consumers, patents on living organisms and food are rejected.

Genetically modified organisms

According to the International Federation of Organic Agriculture movements (IFOAM) and the EC ECO-base directory Regulation No 834/2007 of 28 June 2007, only those varieties that are not genetically modified are approved for use in organic production. EU Directive 2001/18/EC of the European parliament and the Swiss Gene Technology Act require genetically modified plants to be legally subject to labelling requirements.

To date, however, the problem is that the legal definitions differ from the definitions of IFOAM. While plants that have arisen from cell fusions are not considered genetically modified organisms (GMOs) under Swiss law, they are classified according to European law as GM when the plants involved cannot be crossed by means of conventional breeding techniques.

According to IFOAM, however, cell fusions are classified as genetic engineering and any varieties derived from them cannot be used in organic farming. The products of cell fusions, such as many hybrids of cauliflower, broccoli and other vegetables, are not subject to labelling which causes considerable uncertainty in organic farming.

The European Consortium for Organic Plant Breeding (ECO-PB) is making a big effort to find a pan-European solution in order to exclude these varieties from organic cultivation. In the meantime, various organisations have adopted a ban within their guidelines against the use of plants developed by cell fusion, although, this has temporarily led to a significant limitation in varietal choice for the producer. The different definitions of genetic modification have caused much resentment among conventional breeders and breeding researchers as they were often not aware that the breeding techniques they used are rejected by organic agriculture.

Strategies for an optimal selection of varieties

EC Regulation No 834/2007 requires that organic producers exclusively use seed and propagating material that is organically grown. To this end, the mother plant, in the case of seeds, or the parental plant, in the case of vegetative propagating material, must have been cultivated for at least one generation under organic conditions. For perennial crops, the stipulation is a period of two growing seasons. Seed produced in such a way is referred to as 'organic seed'; however, this says nothing about the way in which the variety was actually bred or whether it is suitable for organic farming (see below). As an exception, untreated non-organically propagated varieties may be approved if no suitable varieties from organic propagation are available. Thus, any varieties whose seeds or vegetative propagation material were grown under organic conditions are allowed in organic farming, as long as they are not genetically modified or excluded by organisation guidelines.

The following categories of varieties can be distinguished (Wolfe et al., 2008):

I Conventional breeding programmes

In conventional breeding programmes, selection and propagation take place under conventional management using synthetic seed dressings, herbicides etc., and an optimal supply of nutrients is provided. Variety development is oriented to the needs of the large conventional sector markets. Under the assumption that varieties perform equally well under both conventional and organic systems, the best varieties from conventional breeding programmes are often also cultivated in organic farming.

Examples:	
Cereals:	Agroscope, Changins-Wädenswil (CH); INRA (F); KWS-Lochow (D)
Fruit:	Agroscope, Changins-Wädenswil (CH); Institute for Plant Breeding Research, Dresden-Pillnitz (D)

II Breeding programmes for organic farming: product-oriented

In breeding programmes with a special focus on organic farming, special breeding objectives of organic agriculture are included into ongoing conventional breeding programmes. Methods of genetic engineering (including cell fusion) are not used. For example, to breed for organic production, typically, crossing and early selection occurs under conventional conditions, whereas, later generations are tested under both conventional and organic approaches. The maintenance and production of precursor and basic seed can also take place conventionally; however, the propagation of certified seed must always be carried out exclusively under organic conditions.

Examples:	
Cereals:	Saatzucht Donau (A)
Vegetables:	Bejo (USA); Vitalis (D)
Maize	KWS Saat AG (D)

III Organic breeding programmes: process-oriented

Organic plant breeding programmes focus exclusively on the specific requirements of organic farming. All steps in the programme, including crossing, selection, propagation and conservation of the varieties, are carried out under organic conditions. The applied breeding techniques are in accordance with the basic concept of organic agriculture. Varieties bred in this way are referred to as 'organic varieties' and may be advertised as such (e.g. www.bioverita.ch).

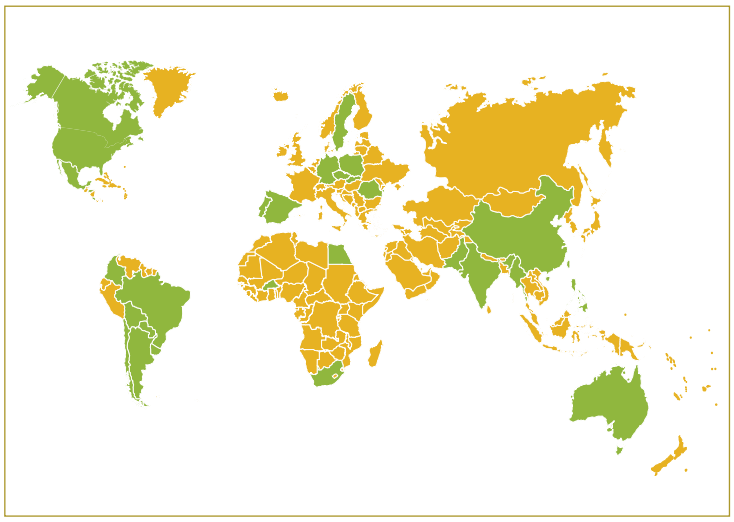
Examples:	
Cereals:	Cereal breeding Peter Kunz e.V. (CH); Cereal breeding research, Darzau (D); Keyserlick Institute (D); Dottenfelderhof Association (D); The Organic Research Centre, Elm Farm (GB)
Vegetables:	Sativa Rheinau AG (CH); Kultursaat e.V. (D); Verein Saat: Gut (D)
Fruit:	PomaCulta (CH)

For many organisations, developing specific breeding programmes for organic agriculture has come second to the propagation of seed based on ecological principles; however, an exception is the biodynamic movement who have focused on breeding questions at a very early stage. Decades ago, they initiated their own breeding projects, which are now bearing fruits (Association of Biodynamic Plant Breeders; www.abdp.org). The reasons why such breeding initiatives rarely arise is due to the relatively small acreage of organically farmed land as well as the high costs associated with development and approval of new varieties.

During a meeting of the European Consortium for Organic Plant Breeding (http://www.eco-pb.org/fileadmin/ecopb/documents/proceedings_070227.pdf) a variety of **strategic funding models** were discussed for a successful establishment of organic breeding programmes (ECO-PB, 2007). If one takes into consideration the approaches that are currently successful, future funding models will be based on a combination of public funds, private donors (foundations), project-based research funding and partnerships between organic growers and businesses.

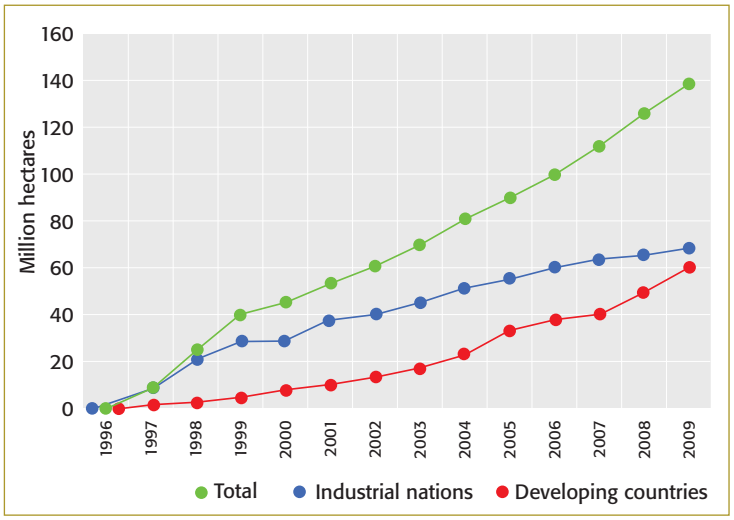
Depending on the assessment criteria and the evaluation of the breeding techniques applied to develop current and future varieties, species and variety diversity could be further restricted and an increasing detachment from the conventional gene pool could take place. To prevent this, the course must be changed promptly. This means, more ecological breeding projects should be initiated and financed over the long term, and collaborations and synergies should be sought with conventional breeding organisations. In order to support this, it is important that the organic community appears as unified as possible, communicates its concerns to the broader sector and holds an open dialogue between breeders, researchers, farmers, retailers and consumers.

Countries growing genetically modified varieties (green) until 2010



Source: Clive James, ISAAA 2011 www.isaaa.org

Global acreage of genetically modified varieties

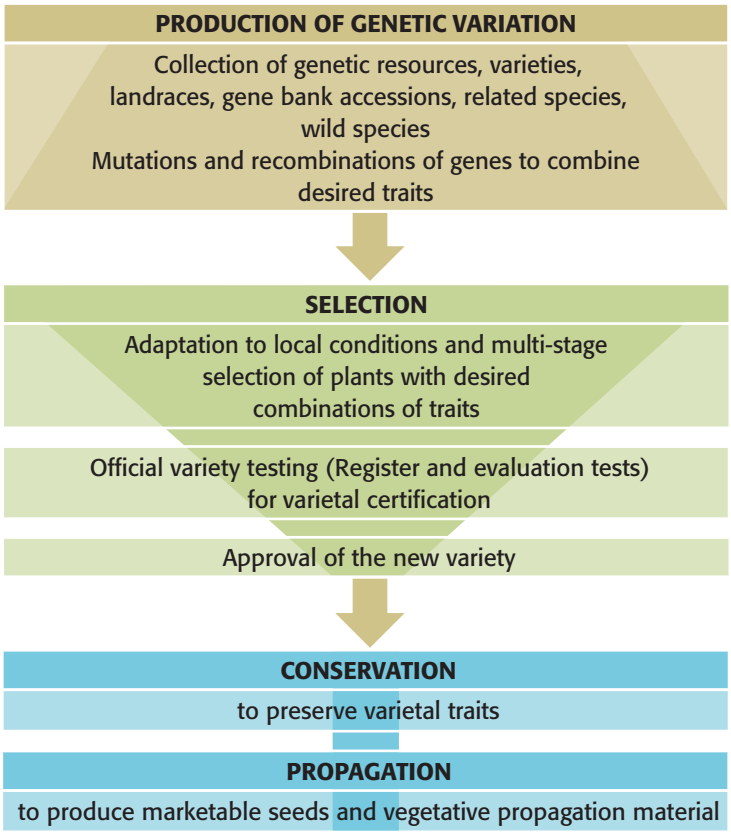


Source: Clive James, ISAAA 2011 www.isaaa.org

Techniques for the breeding and propagation of plants

How are plants bred and propagated?
Plant breeding describes all activities that aim to improve the genetic characteristics of a crop. The art of the process is to find new positive traits that are heritable, and to combine these with other desirable traits. In each case, the best compromise should be sought from the large number of possible target traits.

Breeding steps and their influence on genetic diversity



For this purpose, parental plants with the desired traits must be collected, often from other varieties, from gene banks or related wild species. These are then crossed to obtain the desired combinations of traits in the progeny. The result of such crosses is a large number of seeds with different genetic constitutions (i. e. a segregating population). Within the next few generations, those plants with the best combination of desired traits must be selected. To assist with this, a number of techniques are available from which the breeder chooses the most appropriate depending on the crop, mode of reproduction (e. g. self-pollinators, cross-pollinators or those which require vegetative propagation) and the desired traits. It must be ensured that the desired traits are stably transmitted to the progeny, which may require 6 to 10 generations.

In official variety testing, new varieties are grown in parallel with standard varieties and traits are determined and compared. If the variety is new, different from all existing varieties, the traits are homogeneous and stable over time, and it has a name, it can be awarded plant variety protection. To be accepted as a variety under the Seed Act, most arable varieties must also have a value for cultivation, i.e. have an advantage in one or more traits over existing approved varieties.

In general, plant breeding techniques can be categorised according to the following three areas:

- **Generation of genetic variation** through collection of genetic resources, mutation and recombination of genes
- **Selection** and narrowing of genetic variation for genotypes which best exhibit the desired combination of traits
- Maintenance and **propagation** of the best varieties

In each of the three steps, various techniques can be applied to different anatomical levels of a plant:

- **Whole plant level,** i.e. the single plant, its progeny or a population
- **Tissue level,** i.e. the plant parts, organs or cell cultures
- **Cell level,** i.e. an isolated single cell, protoplasts, pollen or egg cell
- **DNA level,** i.e. the nuclear DNA or extra-chromosomal DNA

The techniques can also be differentiated by the environment in which they are applied:

- Field experiments in interaction with soil and climate (e. g. under organic growing conditions)
- Pot experiments using an artificial substrate under standardised conditions (e. g. cultivation in the greenhouse)
- *In vitro* experiments using artificial nutrient medium under sterile conditions (e. g. meristem cultures, leaf cell cultures)
- Cell suspensions (e. g. protoplast cultures)

On the following pages, the most important breeding and propagation techniques are explained, examples of their application are given and critical issues from the perspective of organic farming are addressed.

Breeding steps using the example of tomato – simplified illustration

1

The flower of a selected tomato plant is emasculated…

2

… while the pollen of another selected tomato plant is collected

3

For fertilisation, the pollen is transferred to the stigma. This crossing creates variability in the progeny.

4

The progeny is selected according to specific criteria, e.g. disease resistance, taste or yield.

5

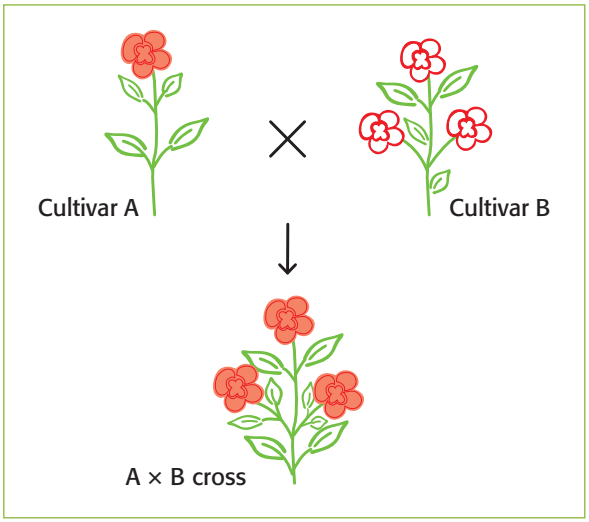
After a long testing period, the new variety is propagated…

6

… to obtain marketable seed.

Production of genetic variation

Targeted crossing within a species



Method:
To control the pollination of plants and produce targeted crosses, the flower buds of mother plants are emasculated, isolated and hand pollinated at the time of female flowering with pollen from the desired father plant. This is done either with a dusting brush or by distributing previously collected pollen over the emasculated female flowers so that it reaches the stigma. For this technique, synchronisation of the flowers is very important because the stigma is usually only receptive for a short time, and the pollen is also only viable for a short time. Staggered sowing dates are often used to achieve this synchronisation, or alternatively, pollen can be dried and frozen until needed.

If crosses are made with unselected material or landraces, the progeny is often not adapted and back-crossing must be carried out in which the progeny is crossed several times with the original breeding stock. In this way, varieties arise that are very similar to the starting material (parents), but also possess the desired newly-introduced traits.

Application:
Targeted crosses are common practice in plant breeding to increase genetic diversity through new combinations of genes and to combine the traits of the chosen father and mother plants.

Through this method, an infinite number of new genetic combinations can be created, some of which may result in plants that are better adapted to the environment and needs of consumers.

Critical issues from the perspective of organic farming:
None

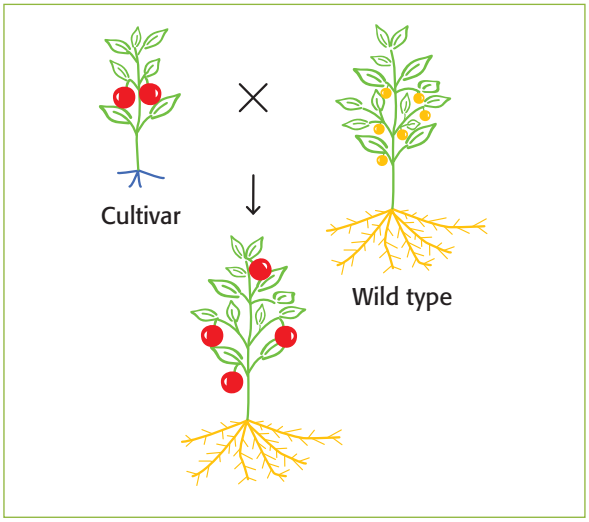
Example: Crossing wheat

Preparation of a flower (emasculation) …

… pollination by hand …

… and protection of the ears against foreign pollen using small bags after pollination.

Inter-specific hybrids



Method:
If genetic variation within a crop is insufficient to achieve improvements in breeding, crosses between two different species can lead to improvements.

Related crops or wild species can be crossed more or less laboriously. For example, while very closely related species (e. g. wheat and spelt) can be crossed without problems, the endosperm is often poorly developed when it comes to crossing more distant species. Thus, there is a reduced nutrient supply to the embryo. To increase the success rate of viable embryos, different *in vitro* methods can be used (see chapter '*In vitro* propagation / cell and tissue culture', page 38).

Where there are differences between the species in their chromosome number, several backcrosses must usually be carried out until it is possible to produce fertile and genetically stable progeny. During this process, several chromosomes are often eliminated. With inter-specific crosses, the genomes can partially sum up spontaneously, forming allopolyploid species (e. g. wheat, oilseed rape).

The mentor pollen technique involves mixing pollen from the desired father plant with radiation-sterilised yet viable pollen from the mother plant species (mentor pollen). A pollen tube develops from the mentor pollen, transporting the intact pollen of the desired father plant to the ovary for fertilisation.

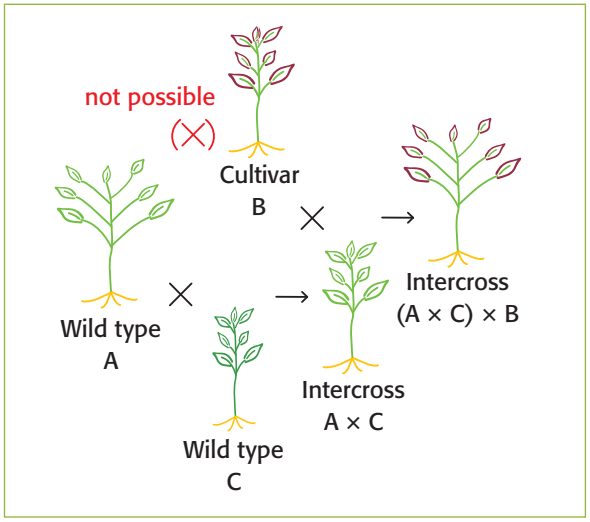
In the pistil technique, the pistil of the mother is partially removed so that the pollen tube of the father pollen must cover only a short distance in order to fertilise the egg cell.

Application:
Inter-specific crossings are common practice and increase the gene pool available to breeders. Many resistance genes have been cross-bred from wild species into cultivated species, for example, introgression of scab resistance genes from the wild apple into the dessert apple, or leaf rust resistance from wild grasses into wheat. This technique has also allowed new crops to be developed, e. g. canola and triticale, as well as many ornamental plants.

Critical issues from the perspective of organic farming:

- Crossing barriers between species are not clearly defined boundaries, but become stronger with increasing differentiation of the species, i.e. the chance of successful fertilisation and seed formation decreases correspondingly.
- Due to technical interventions, such as *in vitro* fertilisation of the egg cell and pollen or *in vitro* cultivation of the embryo shortly after fertilisation, crossing barriers may be further reduced.

Bridge crossing

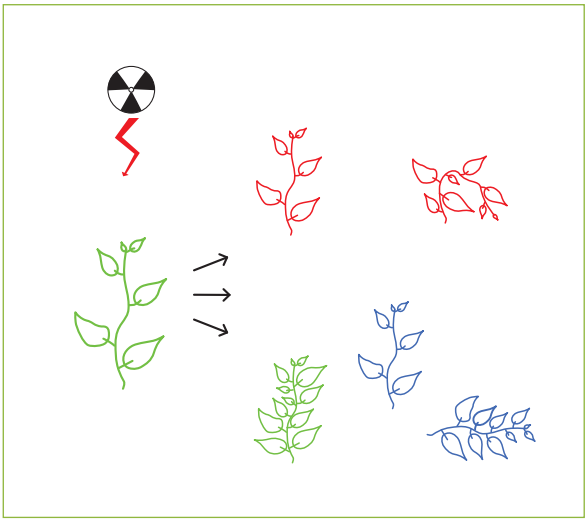


Method:
To overcome crossing barriers between two incompatible plant species, such as wild type A and cultivar B, the introgression of desirable traits from the wild type can occur via a third wild type C which is compatible with both plant species A and B. The wild type A is first crossed with the wild type C. The plants with the desired traits are then selected and crossed with the target cultivar B.

Application:
Through bridge crossing, incompatible plant species, e. g. certain brassicas, can be crossed with each other. This method can be applied when the desired features are easy to select, however, it is very time consuming. Once the desired feature has been introgressed, any undesirable traits must be eliminated again by multiple back-crossings.

Critical issues from the perspective of organic farming:
None

Induced mutagenesis



Method:
New traits in plants often arise through mutations, i.e. changes in the DNA. Mutations can occur spontaneously during cell division (e. g. by mismatches in DNA replication) or can be artificially induced by physical stimuli (e. g. UV radiation, cold or heat shock, x-rays, neutron radiation) or chemical agents (e. g. ethyl methanesulfonate (EMS).

Mutation can be induced by exposing either individual plant parts (e. g. seeds, pollen, tubers or buds) or whole plants to the mutagenic influence to increase the mutation rate. While chemical mutagenesis mainly causes point mutations (changes of individual DNA bases), ionising radiation usually results in chromosome breakage. This favours so-called chromosome mutations, such as chromosome loss (deletion), incorrect structural arrangement (translocation, inversion) or the doubling of chromosome units (duplication). In chemical treatment with colchicine, the entire set of chromosomes is doubled (genome mutation).

When mutation occurs in the germ line (pollen, egg cell or embryo), it is inherited by the progeny which must then be tested to determine whether the mutation is stable. Only a small proportion of mutant plants show promise for further breeding, since most mutations have negative effects.

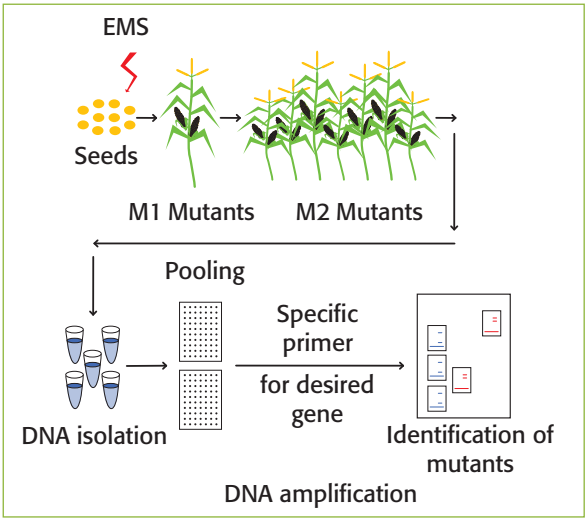
Application:
Induced mutation is primarily used when it is only individual traits that need to be improved. By increasing the mutation rate, the probability of finding new traits is increased and in many instances mutation breeding has been used to generate novel resistances. During the 1960s, countless experiments with gamma rays (60Cobalt) or fast neutrons were carried out, but today

it is mainly chemical mutagenesis that is used. This has resulted in over 1,800 new varieties, such as mildew-resistant barley, malting barley with improved malting quality, short-strawed grain, oilseed rape with an altered fatty acid profile and several ornamental plants. Often, induced mutation is combined with *in vitro* selection for resistance to salt, heavy metals or other chemical compounds toxic to the plant.

Critical issues from the perspective of organic farming:

- Ionising radiation and most synthetic chemical mutagens are currently not allowed in organic farming and should not be applied to the germline of plants (egg cell, pollen or embryo).
- Induced chromosome breakages violate the integrity of the genome.

Tilling



Method:
TILLING (Targeted Induced Local Lesions IN Genomes) is a further development of classical mutagenesis. In the process of TILLING, mutagenesis (usually by means of ethyl methanesulfonate, EMS) is combined with a new screening procedure that identifies point mutations in a specific segment of a particular gene. The method allows high-throughput testing of a very large number of potential mutants. However, mutations can only be detected in those parts of DNA that have been previously sequenced.

Treatment with mutagenic chemicals not only results in the desired point mutation, but also causes mutations in the entire rest of the genome. Consequently, the desired trait in the mutant must then be transferred by back-crossing into stable varieties.

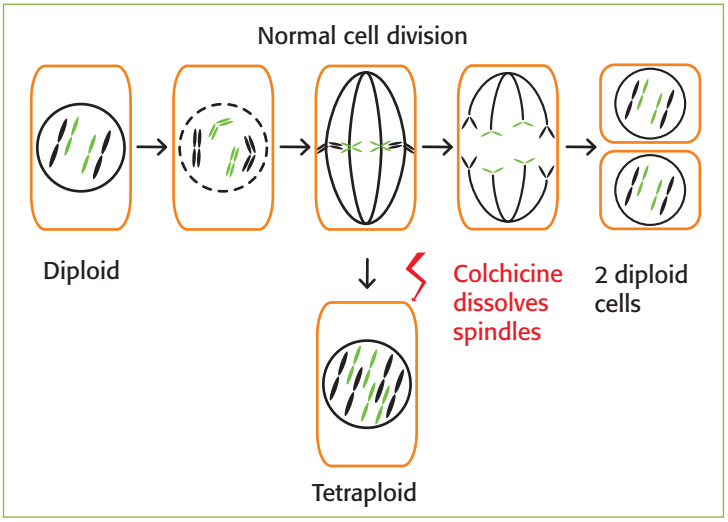
A variant of TILLING is Eco-TILLING, which avoids mutagenic chemicals. Instead, the desired mutation is identified in germplasm collections and gene bank accessions.

Application:
Increasing knowledge of gene functions makes TILLING a very efficient mean to identify alleles or new traits which can then be crossed into breeding material. Examples of breeding achievements which have used this technology include a potato variety that produces only amylopectin, tomatoes with improved salt resistance, gluten-free wheat varieties and drought-tolerant cereals and soybean.

Critical issues from the perspective of organic farming:

- Most chemical mutagens are currently not allowed in organic farming and should not be applied to the germline of plants (egg cell, pollen or embryo).

Polyploidisation

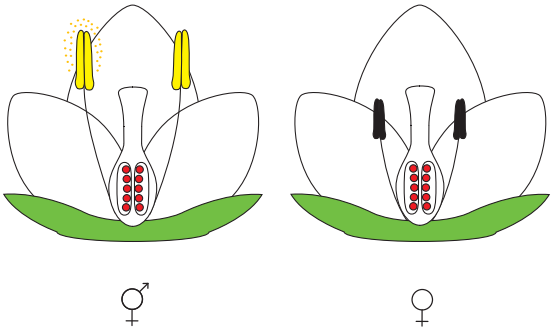


Method:
In polyploidisation, the chromosome number of a plant species is multiplied. Whereas, most species are diploid and have two copies of each chromosome, the doubling of chromosomes, for example, gives rise to tetraploid plants with four copies each. The doubling of chromosomes affects all genes of a genome and is also referred to as genome mutation. In nature, there are various types of polyploidy. A distinction is made between autopolyploids, which have two or more sets of homologous chromosomes derived from a single species (e. g. in the tetraploid potato AAAA), and allopolyploids, which are polyploids produced from several different genomes (e. g. hexaploid wheat, which has three different genomes, AABBDD). Polyploidy may occur spontaneously or can be induced by chemicals (e. g. colchicine). The resulting autopolyploid plants are usually more vigorous and robust, and have larger fruits than the original diploid plants.

Application:
Polyploidy is used to grow more robust and higher yielding crops (e. g. autopolyploid rye and red clover), to restore the fertility of inter-specific crosses (e. g. allopolyploid triticale, canola and cotton) and to develop double haploid plants (see chapter 'Double haploid (DH) plants', page 20). Furthermore, tetraploid cross-breeding plants are required to produce seedless fruit.

Critical issues from the perspective of organic farming:
- If tetraploid and diploid plants cross, the resultant triploid progeny is sterile.
- The most commonly used anti-mitotic drugs, e. g. colchicine and the herbicide oryzalin, are not permitted in organic farming.

Cytoplasmic male sterility (CMS)

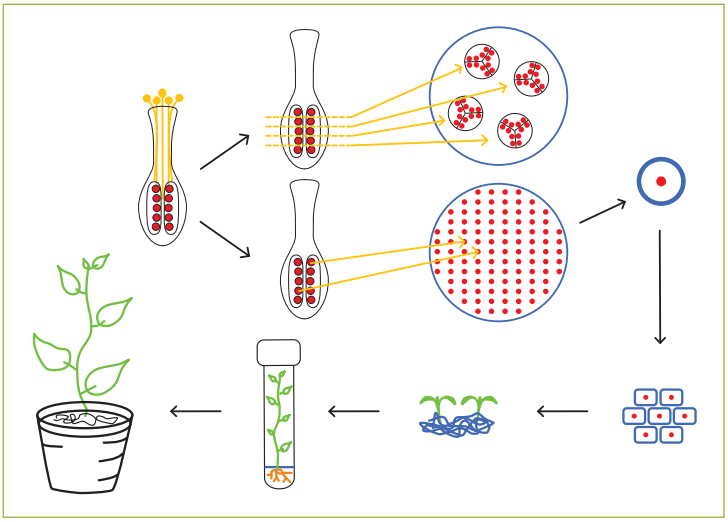


Method:
Cytoplasmic male sterility (CMS) occurs when the male flowering organs (anthers, pollen or male gametes) develop incompletely due to a malfunction of the mitochondria. This malfunction occurs when the interaction between the nuclear genome and mitochondrial DNA is disturbed.

CMS is initiated in nature by spontaneous mutations in mitochondrial DNA. Depending on the mutation, it may be that no pollen or only malformed pollen is produced. It may also be that pollen is produced, but it is incapable of germinating and thus sterile. Since the mitochondria are passed to the progeny almost exclusively via the egg cell, this is referred to as maternal inheritance. In most cases, it is possible to restore male fertility by crossing with chromosomal 'restorer genes'. To produce a hybrid, line A is crossed with a male sterile plant that possesses the mutated mitochondria. The seeds harvested from the sterile mother plant will also develop into male sterile plants. In this way, through repeated backcrossing, line A can be produced in a male sterile version (CMS line). The sterile line A is then grown together with the fertile line B and both flower together. The seed harvested from the mother plant is hybrid seed (AxB).

To ensure that these hybrid seeds produce fertile progeny and can produce yield via generative organs (seeds), line B must have a chromosomally inherited restorer gene that can induce male fertility again. With vegetables like cauliflower, however, this is not strictly necessary as it is the flower heads that are harvested and not the seeds.

Ovary and embryo culture



Application:
In many cases, CMS is necessary for large-scale hybrid seed production, for example in canola, rye, maize and many vegetables.

Critical issues from the perspective of organic farming:
For hybrid varieties whose fertility is not restored by restorer genes, no progeny can be produced, i.e. seed saving is not possible. These individuals may only be used as mother plants for further breeding. Male sterility is passed on to the progeny.

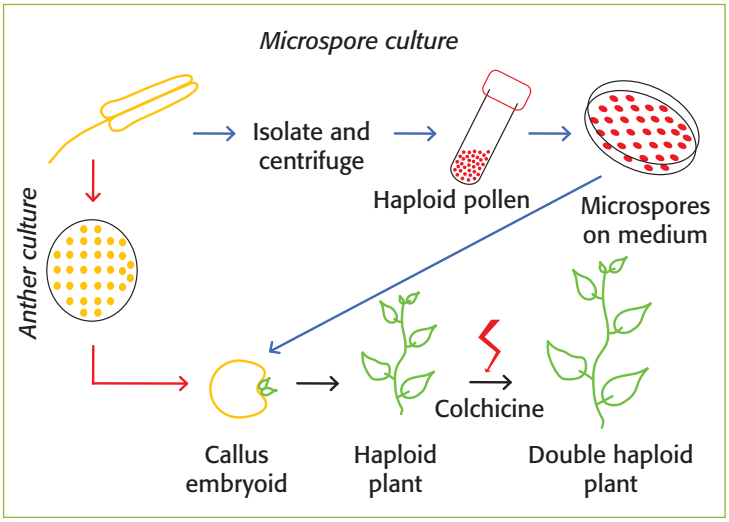
Method:
In inter-specific crosses (see page 15), the endosperm is often poorly developed resulting in a reduced nutrient supply to the embryo. In order to increase the success rate of viable embryos, a method called embryo culture (also known as embryo rescue) may be used. In this technique, after inter-specific fertilisation has occurred, the embryo is isolated from the flower and placed onto nutrient medium for germination, thereby increasing the number of progeny.

With ovary culture, whole or dissected ovaries containing fertilised egg cells are transferred to a substrate. The ovules swell and, at an appropriate stage, viable seeds can be removed and cultivated separately.

Application:
Ovary and embryo culture are techniques which are frequently used to introgress resistance genes of closely related species. The approach has been used for tomato, cucumber, pepper, lettuce, wheat, triticale and many other crops.

Critical issues from the perspective of organic farming:
- By using *in vitro* cultivation of the embryo after fertilisation, crossing barriers may be over-ridden.
- Development of the embryo takes place under artificial, sterile conditions on synthetically prepared nutrient medium.

Double haploid (DH) plants



Method:
The aim of producing double haploid (DH) lines is to generate homozygous inbred lines from heterozygous progenies of crosses, which is normally only possible through continued selfing for 5–6 generations. Since a diploid plant contains two sets of chromosomes, each gene locus can have two characteristic forms (alleles). When the number of chromosomes is halved (haploid), only one allele per locus is still present. When doubling this haploid chromosome set, the alleles at each locus will be homozygous. Diploid inbred lines, generated in this way, can be selfed to give genetically identical progeny, or be used as crossing partners to produce hybrids.

Whole plants can be produced by regeneration from haploid pollen (microspores) or haploid egg cells. In the case of anther culture, the immature anthers are cultured *in vitro*. Phytohormones are then applied to promote cell division in the immature pollen. This results either in an undifferentiated mass of cells (callus) or so-called embryoids, from which haploid plants can be regenerated. Microspore culture is a further development of this technique, whereby only the immature pollen (microspore) is cultured in liquid medium instead of whole anthers. Ovary culture works in a similar way and attempts to regenerate haploid plants from the enclosed haploid egg cells.

The haploid plants are viable, but fragile and sterile. By chromosome doubling, fertility can be restored. In some cases, this happens spontaneously during the *in vitro* phase, but in other cases it needs to be induced using colchicine (see chapter 'Polyploidisation', page 18). The resulting plants are called double haploids or DH lines, and they are completely homozygous inbred lines with a diploid set of chromosomes. As an alternative to *in vitro* culture, the production of haploid plants can also be initiated by pollination with so-called inducer lines.

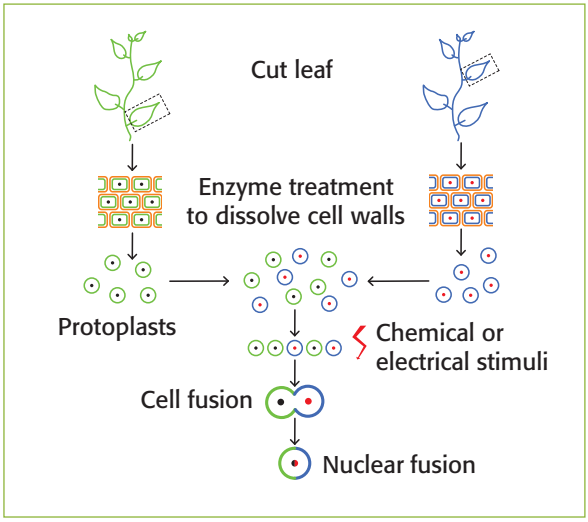
Applications:
DH lines are used to speed up the breeding process (e.g. in barley, maize and potatoes). The generation of homozygous inbred lines from cross-bred progeny in a single generation is of great advantage for self-pollinators, because you can directly select for homozygotes which exhibit all the desired traits.

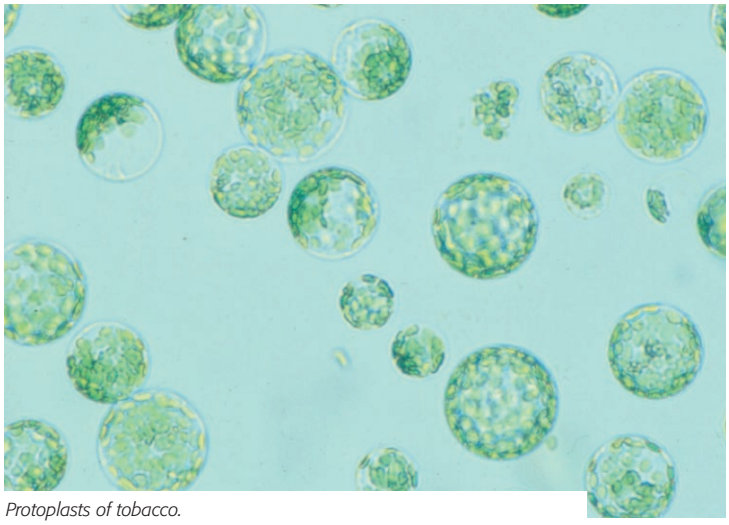
In hybrid breeding, DH lines can be used to quickly create experimental hybrids and to select for their crossing potential for hybrid varieties.

Critical issues from the perspective of organic farming:

- Egg cells and pollen can be reprogrammed by phytohormones into somatic cells, in which case, there is no fusion of the egg cell and pollen and, thus, no recombination of genes.
- Synthetically produced colchicine is not permitted in organic farming.

Protoplast fusion





Protoplasts of tobacco.

Method:
Protoplasts are cells without a cell wall. They are usually obtained by treating fragments of leaves with appropriate enzymes to dissolve the cell wall and release the protoplasts in a suspension. In cell fusion, somatic cells are fused. Fusion of protoplasts can be achieved by adding certain chemicals (e.g. polyethylene glycol, PEG) or by applying short electric pulses (electrofusion). Hereby, two cells, with the complete, usually diploid chromosome set complement, are fused without previous meiosis and gamete formation. In protoplast fusion, the cytoplasts including the mitochondrial and plastid DNA of both partners are fused. If the cell nuclei fuse simultaneously, it is termed protoplast fusion or somatic hybridisation.

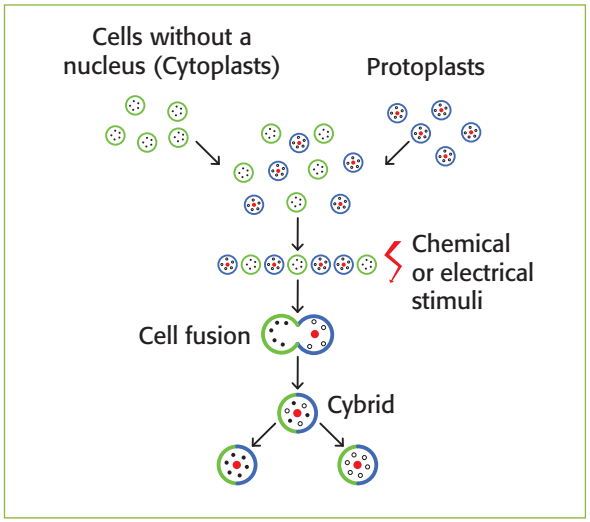
In contrast to gamete hybridisation (i.e. fusion of the egg cell and pollen), the fusion of protoplasts is not preceded by any reduction in the number of chromosome sets. Therefore, the fusion product is usually tetraploid and combines the organelles of both plant cells (chloroplasts and mitochondria, including their chromosomal DNA). In conventional crossing, only the maternal chloroplasts and mitochondria are transmitted to the progeny. During the regeneration and proliferation of somatic hybrids, the chromosomes and organelles of both parents may be mixed, so many new combinations arise.

Application:
Protoplast fusion allows the rapid creation of inter-specific hybrids that would otherwise occur only very rarely, or would need to be generated using embryo culture or bridge crossing. In intra-specific protoplast fusions the genetic information of two plants can be selectively combined. This is of particular interest for monogenic traits, e.g. resistance genes, or if inherited traits, arising from the nucleus are to be combined with extra-chromosomally inherited traits.

Critical issues from the perspective of organic farming:

- Protoplast fusion can override crossing barriers.
- The integrity of the cell is compromised by the forced fusion of two protoplasts. Organelles of different individual plants come together, which would be extremely rare under natural conditions. Thus, the gene regulation between the nuclear genome and extra-chromosomal DNA can be impacted.
- If tetraploid fusion products out-cross with diploid plants, triploids are produced, which are sterile.

Cytoplast fusion

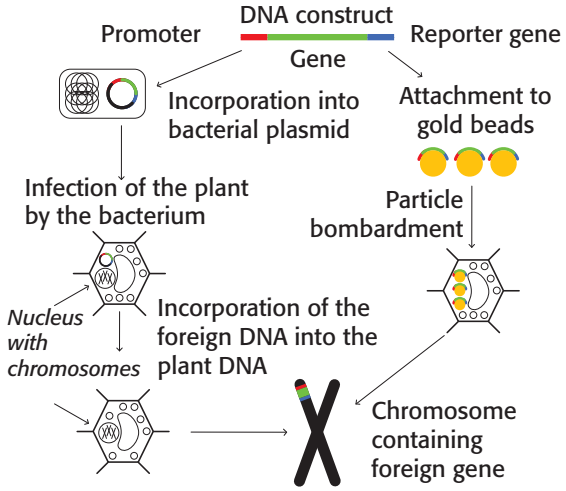


Method:
In cytoplast fusion, cell fusion takes place as in protoplast fusion. In contrast to protoplast fusion, however, the nucleus of the protoplast of one plant is destroyed, for example with X-rays. The resulting cytoplasts contain all cellular constituents such as mitochondria and chloroplasts, but no intact chromosomes. Subsequently, the cytoplasts lacking functional nuclei are fused with complete protoplasts to create so-called cybrids. This is also referred to as asymmetric cell fusion. The goal is to transmit extra-chromosomal plastids and mitochondrial DNA from one species to another without changing the nuclear genes. If, for example protoplasts of broccoli are fused with cytoplasts of radish, the interaction between the radish mitochondrial DNA and nuclear genes of broccoli can produce male sterile broccoli plants for use in hybrid breeding.

Application:
With cytoplast fusion, it is possible to combine new plastid DNA with nuclear genes. Thus, traits that are controlled by the plastids can be selectively transmitted. The cytoplast is used in order to induce cytoplasmic male sterility (CMS) (e. g. in cauliflower and broccoli) or integrate individual resistance from wild relatives into crop species (e. g. in potato and rice).

Critical issues from the perspective of organic farming:
- The integrity of the cell is compromised by the forced fusion of cells from different species. Organelles of different individual plants come together which would be extremely rare under natural conditions and this affects gene regulation between the nuclear genome and extra-chromosomal DNA.
- Natural crossing barriers are overcome.

Gene transfer for the production of transgenic species



Method:
The aim of gene transfer is to introduce a new trait into a variety that does not occur in the breeding material. An essential prerequisite for this is the identification and isolation of the corresponding genes. In a DNA construct, the DNA sequence of the target gene is linked to a promoter that controls the expression of the gene as well as a reporter gene that indicates whether the gene transfer was successful. Initially, herbicide or antibiotic resistance genes were often used as reporters: successfully transformed cells express this gene and can develop normally on a medium added with an herbicide or antibiotic, while the non-transformed cells die. Gene transfer, i. e. the genetic construct is transferred to the nucleus and incorporated into the DNA of the plant, can take place by direct or indirect methods. In direct methods, the gene construct is directly introduced into the cell. Indirect methods very often use *Agrobacterium tumefaciens* to transfer the gene construct. Here, the gene construct is incorporated into the bacterial DNA, and then the plant cells are infected with the bacterium.

When gene transfer takes place via particle bombardment, tiny gold or tungsten beads coated with DNA are propelled into a cell suspension or onto plant calluses by means of compressed air; however, the success rate of stable incorporation of foreign DNA using this method is very low.

In gene transfer via endocytosis, a suspension of protoplasts is created from cells without nuclei and the desired gene construct is added. Subsequently, the membrane is rendered temporarily permeable using chemical agents, electrical means or continuous temperature shocks, so that the foreign DNA can pass from the solution into the cell nucleus. From these protoplasts, whole plants are regenerated subsequently.

Integration of the gene construct occurs at a random position within the genome, and it is possible for several copies to be incorporated. Integration into the genome can cause so-called position effects, such as knock-out of a gene or alteration of gene expression in neighbouring genes. Depending on the gene construct, the newly transferred genes may be expressed constitutively (in each plant cell) or they can be specific to a particular organ or developmental process. This is achieved by coupling the construct with respective promoters. Despite successful transformation, the gene construct is not always expressed. In this case, RNA interference has been identified as a possible cause (see chapter 'Gene silencing – RNA interference', page 26). Therefore, the transgenic plants must be laboriously tested for their agronomic traits. Often, the current breeding material must be backcrossed several times before transgenic plants are marketable. There may be single or multiple genes transferred simultaneously (gene stacking) so that, for example, transgenic maize can have an herbicide and several Bt genes against a range of pests.

Application:
Monogenic traits can be transferred across species using gene transfer. An example is the Bt gene which has been transferred from the bacterium *Bacillus thuringiensis* to maize, cotton, soybeans, etc., to protect the plants against insect damage.

Gene transfer via Agrobacterium works very well in dicotyledonous plants such as tobacco, rapeseed, soybean and cotton.

Gene transfer via particle bombardment is mainly used in monocot cereal species such as maize, wheat and rice, which cannot be transformed by Agrobacterium (www.transgen.de/daten-bench/plant).

Critical issues from the perspective of organic farming:

- Genetically modified organisms are not allowed in organic farming.
- The integrity of the plant genome is destroyed and crossing barriers are overcome.
- There is a potential risk for outcrossing to other organisms, which creates a problem for co-existence within small areas.
- The plant is reduced to DNA building blocks which are almost always patented, preventing seed saving and continuation of breeding. Thus, monopoly in the seed market is supported and biological diversity decreases.

Cisgenesis

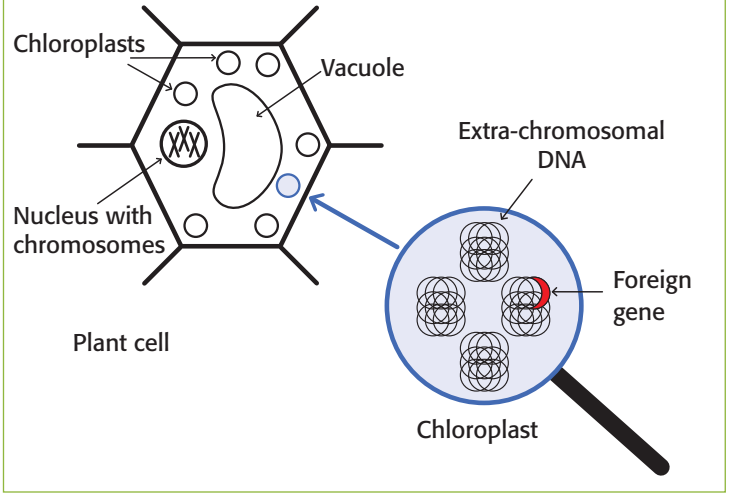
Method:
Cisgenesis uses the same methods as described in chapter 'Gene transfer for the production of transgenic species'. The difference, however, is that the isolated gene and its promoter and reporter genes originate from the same plant species and, therefore, do not violate any natural crossing barriers. In gene transfer using Agrobacterium, the bacterium's own T-DNA sequences may not be transferred.

Application:
Cisgenesis is mainly used in crops propagated vegetatively when monogenic traits (e.g. resistance genes) are to be transferred from a wild type or a species with poor agronomic traits to a desired variety without changing the other traits of this variety. Through the transfer of individual genes, a single feature of an existing species can be improved without introducing other genes as it occurs in conventional cross-breeding. Thus, the transfer of unfavourable genes located on the same chromosome (linkage drag) can also be prevented. This is used to big advantage in crops such as apple and potato.

Critical issues from the perspective of organic farming:

- Genetically modified organisms are not allowed in organic farming.
- In cisgenic plants, as well, the intact DNA of a plant is directly modified through gene transfer and the integrity of the nuclear genome is disturbed.

Plastid transformation

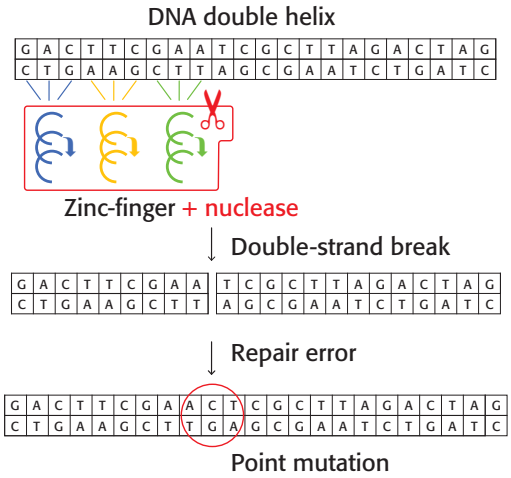


Method:
In plastid transformation, the gene construct is not integrated into the nuclear genome (nuclear DNA), but instead into the extra-chromosomal DNA of the plastids (chloroplastal or mitochondrial DNA). An advantage of transferring foreign DNA into plastids is the high level of gene expression due to the large number of gene copies. In addition, plastids are controlled differently to chromosomal genes and, as a consequence, there is no risk of gene silencing by RNA interference (see chapter 'Gene silencing – RNA interference', page 26). Multiple genes can be transmitted simultaneously and the plastids are almost exclusively maternal in origin, i. e. inherited via the egg cell. Therefore, the risk of outcrossing via pollen is substantially lower compared with plants that have foreign DNA in the nucleus.

Application:
To date, this method has only been successfully applied in tobacco to produce bioplastics.

Critical issues from the perspective of organic farming:
- Genetically modified organisms are not allowed in organic farming.
- The integrity of the nuclear DNA is retained, but the extra-chromosomal DNA is altered which violates cell integrity.

Site-directed mutagenesis triggered by zinc-finger nucleases

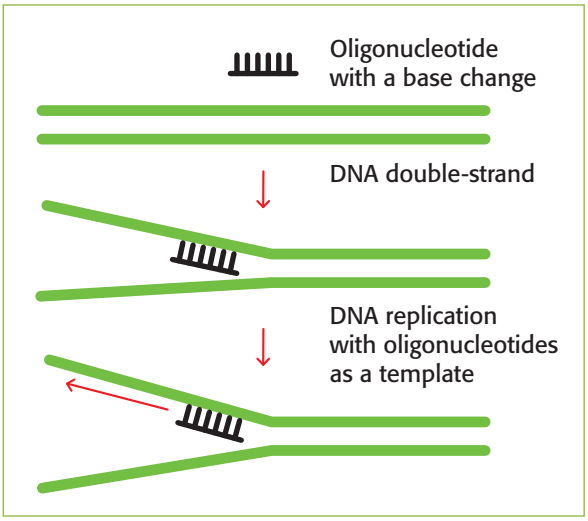


Method:
Zinc-finger nucleases are synthetic proteins containing zinc-finger domains which bind to a specific DNA triplet and nucleases that can cleave the DNA double helix. By coupling several zinc-finger domains, the DNA double-strand can be cut at a specific point. Via the plant's own subsequent repair mechanisms, base substitution or base shift (frameshift) may occur which can create a mutation or loss of gene function.

In this method, no recombinant DNA is introduced into the cell, only the synthetic zinc-finger nucleases. This is usually achieved by transfection, electroporation or via Agrobacterium. Zinc-finger nucleases may, in addition, be coupled with short, isolated DNA sequences (oligonucleotides) that are used as a template after the double-strand break and can thus be used for targeted alteration of the gene sequence (also known as targeted mutagenesis via oligonucleotides). If zinc-finger nucleases are coupled with larger functional gene constructs (foreign genes), the foreign gene is inserted at the exact location in the genome where the zinc-finger domain attaches to the DNA. This increases the efficiency of gene transfer and simultaneously prevents undesired position effects (also known as gene transfer with targeted integration).

Application:
There have already been some applications in plant breeding. Apart from mere mutation using the plant's own repair mechanisms, zinc-finger nucleases combined with isolated gene constructs are said to have great potential for use in targeted gene transfer. Due to the high sequence specificity of the zinc-finger, it can be assumed that all copies of the genes have mutations. This is particularly advantageous in polyploid species.

Site-directed mutagenesis via oligonucleotides



Critical issues from the perspective of organic farming:

- Zinc-finger nucleases are synthetic proteins, which do not occur in nature.
- The technological use to transfer zinc-finger nucleases into the nucleus of the plant cell compromises the integrity of the cell.
- When zinc-finger nucleases are transferred into the nucleus of the plant cell, the integrity of the cell is compromised.

Method:

Site-directed or targeted mutagenesis is the transfer of a specific DNA sequence to enable selective modification of DNA in a particular gene segment. A short synthetic DNA or RNA sequence of 20 to 100 bases (oligonucleotide) is transferred into a cell (for example, by electroporation, polyethylene glycol (PEG) treatment of protoplasts or particle bombardment). This oligonucleotide contains the desired point mutation and acts as a template to generate this point mutation in the plant. The template is later degraded in the cell and no longer detectable.

The success rate of this mutagenesis is increased due to the short DNA sequence and the targeted transfer. In addition, there are significantly less side-effects compared with conventionally induced mutagenesis.

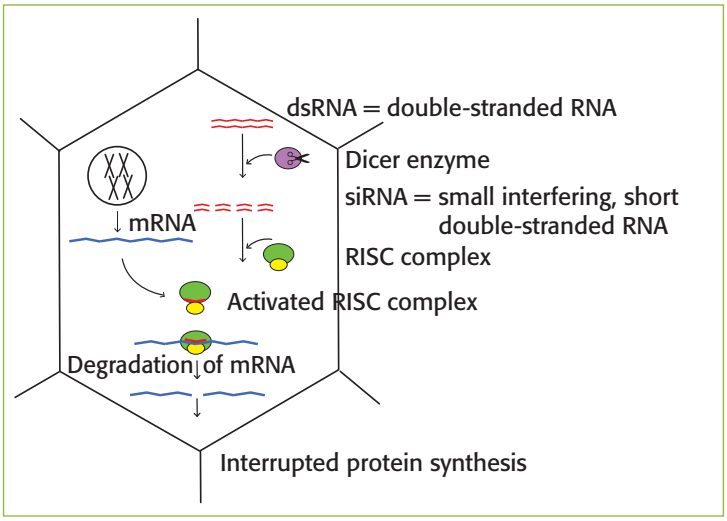
Application:

Oligonucleotide triggered mutation is used when a specific change in known gene sequences is desired to improve a particular feature. The success rate is much higher compared with non-directed mutagenesis as only the target gene is affected. The DNA sequence of a gene can be altered via the introduced oligonucleotide. Thus, far fewer side effects are to be expected compared with traditional mutagenesis. The generation of a new barley line (mlo mutant) resistant against a broad spectrum of mildew races is currently in the research phase.

Critical issues from the perspective of organic farming:

- Genetically modified organisms are not allowed in organic farming.
- The isolated DNA sequences are introduced into the nucleus via technical intervention and, thus, violating the integrity of the cell as a functional unit.

Gene silencing – RNA interference (RNAi)



Method:
RNA interference (RNAi) is a natural mechanism of gene regulation in plants, animals and humans, which leads to the deactivation of genes in cells. According to the current state of research, RNAi is triggered by small, double-stranded RNA (dsRNA), which can lead either to methylation of the promoter (i.e. inhibition of transcription) or degradation of the mRNA which prevents the formation of the protein (i.e. inhibition of translation). The DNA sequence of the gene remains unchanged – it is the gene expression that is down-regulated. Therefore, it is also described as an epigenetic effect.

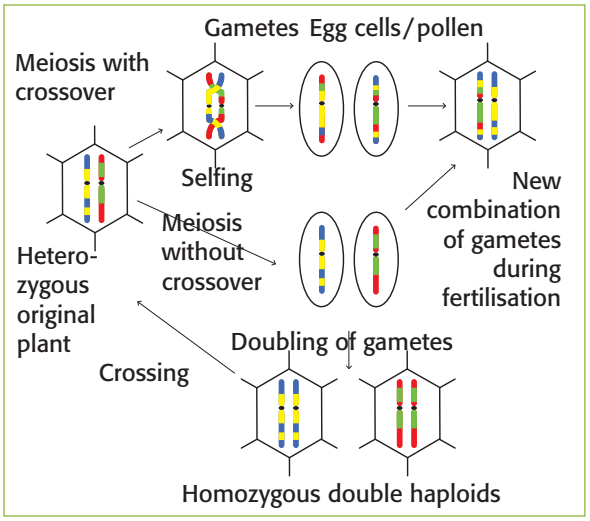
To suppress the expression of target genes, short gene constructs with the corresponding sense and antisense DNA or RNA can be transferred into the genome (e.g. via particle bombardment or electroporation). If the gene construct is transcribed, mRNA strands fold into a hairpin shape and form double-stranded RNA molecules. This dsRNA triggers the RNA interference described above, irrespective of the gene copy number present in the plant. The DNA/RNA constructs are not incorporated into the genome, and the effect of RNAi may decrease in future generations.

Application:
For research purposes, short RNA constructs are transferred directly into cells to determine the effect on gene regulation. The RNAi technique can be used to influence the expression of gene products when synthesis pathways are well known and when the trait characteristic can be achieved by switching off individual key enzymes. The big advantage of the RNAi method is that this post-transcriptional effect is inherited dominantly, i. e. no matter how many copies of a functional gene are present in the plant, their translation into functional proteins is completely suppressed.

Critical issues from the perspective of organic farming:

- Genetically modified organisms are not allowed in organic farming.
- Isolated DNA or RNA sequences are brought into the nucleus via technical interventions, thus, violating the integrity of the cell as a functional unit.
- It has been observed that gene expression can also be boosted by RNAi. Since the RNA interference is involved in control pathways, the gene expression balance of other traits could also be interfered with.
- Up to date, there is little empirical data on possible risks.

Reverse breeding



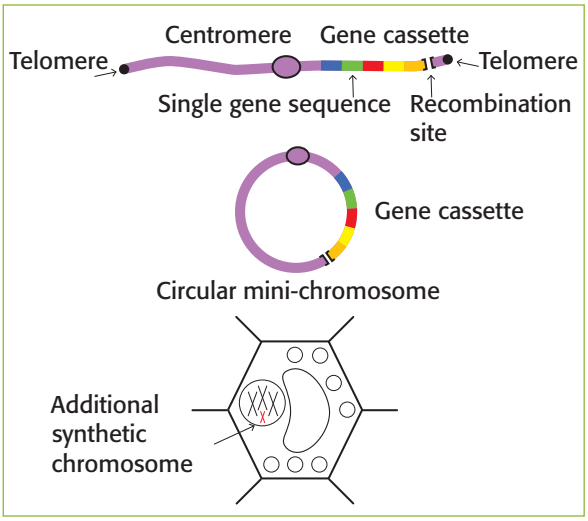
Method:
In reverse breeding, the process of hybrid creation is reversed. The technique attempts to reproduce genetically identical progeny from a selected, heterozygous plant with all desired positive features. This would usually not be possible because genes are recombined during meiosis when selfing heterozygous plants. Thus, a hybrid can be selfed, but the traits of the progeny segregate widely due to novel combinations.

Reverse breeding attempts to prevent recombination of genes by suppressing crossover events and, thus, divide hybrids in reproducible hereditary components. Recombination is suppressed using RNA interference (see previous chapter) and double haploid technology is used to create homozygous inbred lines in a single step. These can be propagated as often as required. The homozygous inbred lines are subsequently crossed with each other to reproduce the original heterozygous genotype.

Application:
Reverse breeding can be used in heterozygous allogamous plants for generative propagation. Until recently, this was only possible for vegetatively propagated genotypes.

Critical issues from the perspective of organic farming:
- Isolated DNA or RNA sequences are brought into the nucleus via technical interventions, thus, violating the integrity of the cell as a functional unit.
- Reverse breeding interferes in the overall control of gene expression, and the self-organisation of the cell is disturbed.
- The variety must be recreated from each of the hereditary components. Seed saving is not possible without a decline in performance.

Transformation via mini-chromosomes



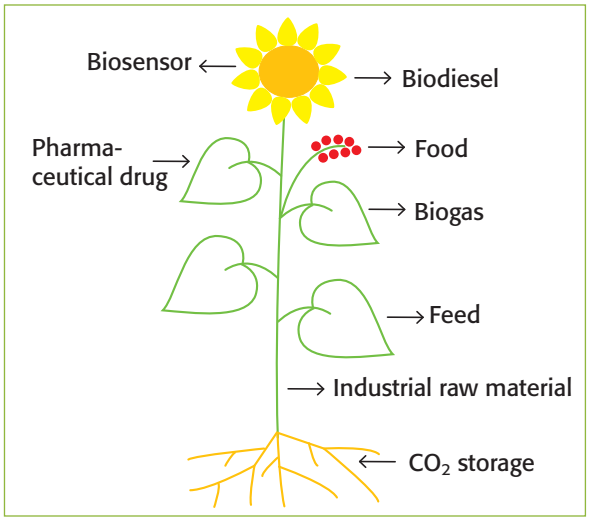
Method:
To incorporate a large number of new genes into the plant genome, a whole group of gene constructs can be transferred by means of artificial mini-chromosomes. These mini-chromosomes can be prepared in two different ways: by shortening the natural chromosomes, or by de-novo synthesis of all the functional elements of a chromosome. Subsequently, the desired gene is inserted into the mini-chromosomes and introduced into the cell nucleus. Mostly, recombination sites are transferred at the same time to integrate further gene constructs at a later stage.

Application:
This method is useful when many existing gene constructs are to be transferred into a variety/species, or if entirely new metabolic processes are to be induced for industrial or pharmaceutical purposes. The mini-chromosomes are not incorporated into the existing plant chromosomes avoiding undesired position effects. The localised foreign genes on the mini-chromosomes show stable expression and heredity.

So far, the method has mainly been used to increase biomass production.

Critical issues from the perspective of organic farming:
- Genetically modified organisms are not allowed in organic farming.
- The integrity of the plant genome is disrupted.
- The plant is degraded to purely function as a metabolic producer.
- There is a ready transition of this technique to synthetic biology (see next chapter).

Synthetic biology



Method:
With the help of synthetic biology, new DNA blueprints can be created. Individual components, such as nucleotides or amino acids, are reassembled or synthesised without a natural precursor. Current research mainly deals with the synthesis of bacteria to produce new enzymes in large quantity and high purity. In this case, the original DNA of the bacterial cell is replaced by a synthetic genome. In fundamental plant research, it is already possible to create artificial mini-chromosomes (see previous chapter) and synthesise novel chloroplasts.

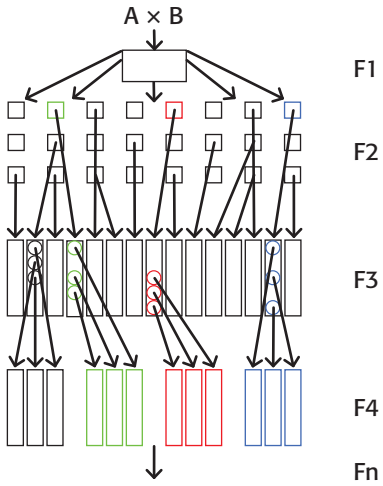
Application:
Early applications of synthetic biology are tested on simple bacteria. In comparison to standard methods of genetic engineering, rather than constructing new individual gene segments (e.g. integrating a Bt gene from a bacterium into the maize genome), synthetic biology can produce new genes and genomes from chemicals based on computerised DNA layouts. In this way, DNA sequences can be synthesised without a natural template, e.g. to create new proteins. It is being attempted, for example, to obtain novel substances in pure form via synthetic bacterial DNA templates.

Critical issues from the perspective of organic farming:

- Genetically modified organisms are not allowed in organic farming.
- The method raises ethical concerns about illusions of omnipotence and the reduction of organisms to the sum of their individual components. Synthetic biology represents a strong intervention in creation.

Selection

Phenotypic selection in the field



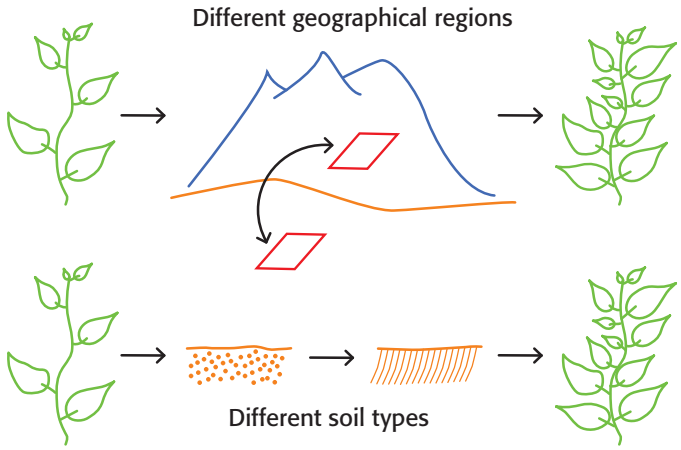
Method:
Breeding progress is achieved by selecting the best performing individual plants or their progeny. These are then grown in the field and evaluated on the basis of previously defined breeding objectives.

Phenotypic selection on single plants is subject to large estimation errors, since genotypic effects are masked by environmental effects. In later generations, the progeny is sufficiently homogeneous that phenotypic selection can be aided by replication in multiple test plots. Therefore, phenotypic selection in early generations is less efficient than in later generations. The further a breeding line has progressed, the more information is available to the breeder for selection decisions. As the demands on an optimal variety are very high, it is unlikely to identify a single plant that combines all the beneficial traits. Rather, it is the skill of the breeder to find the best compromise out of the existing variation.

Application:
Phenotypic selection is essential for all breeding programmes.

Critical issues from the perspective of organic farming:
- From the perspective of organic farming, the interaction of a plant with soil and climatic conditions is a prerequisite for the development of locally adapted crops.
- All selection steps should take place under organic growing conditions.

Shuttle breeding



Method:
Changing the environment during the selection process is an attempt to increase the adaptability of varieties, by alternately testing the breeding material at two or more very different locations. For instance, if the first selection of progeny occurs under dry conditions, the next generation of plants is grown and selected in a damp location. The third generation will then be selected again under drought stress, and so on.

Application:
Besides promoting adaptation to abiotic stress factors such as heat, frost, drought, waterlogging, salinisation, acidification, etc., this method is also used to improve resistance against various pests and diseases. Shuttle breeding is mainly used in early generations. In later generations, sufficient seed is available to simultaneously test at various locations. The more the selection locations resemble later production sites, the greater the selection success.

When testing at several sites with very different soil and climate conditions and varied disease and pest pressures, varieties are bred that can adapt to a range of environmental conditions (stable yielding multi-regional varieties). Selecting under regionally delimited conditions on organic land increases the chance to develop varieties that are best adapted to a specific site and the farming system, but less suitable for other growing conditions (i.e. locally adapted varieties).

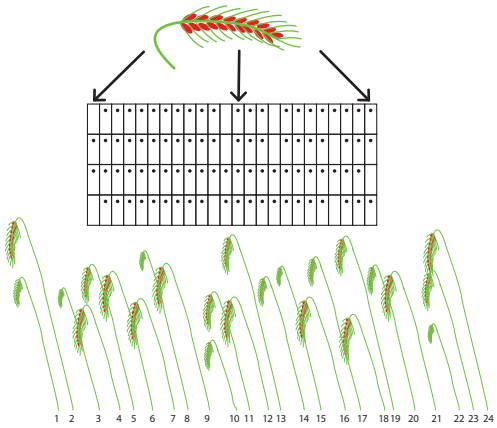
Change of sowing time

Method:
Changing the sowing time (early or late spring, early or late autumn) is usually carried out in order to select for criteria such as day length insensitivity, lower demands for flower formation or yield and quality stability at varying growing periods.

Application:
This method is mainly used for cereals, for example, to develop wheat varieties that can be grown as spring or winter crop.

Critical issues from the perspective of organic farming:
None

Ear-bed method

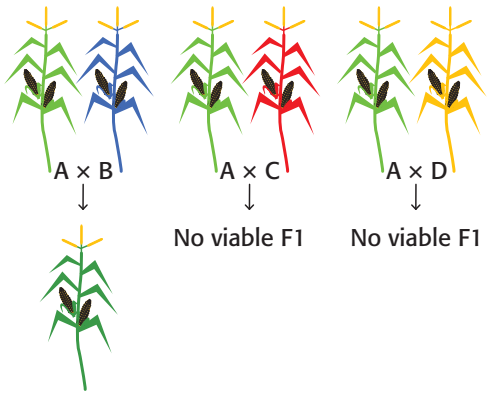


Method:
In the ear-bed method, the grains are sown in the same order as they appeared on the ear. Thus, the position of the plants in the seedbed (ear-bed) reflects the original arrangement of the spikelets on the ear, which developed at different rates.

Application:
This method was developed by biodynamic growers especially for cereals, to increase selection efficiency.

Critical issues from the perspective of organic farming:
None

Test crosses

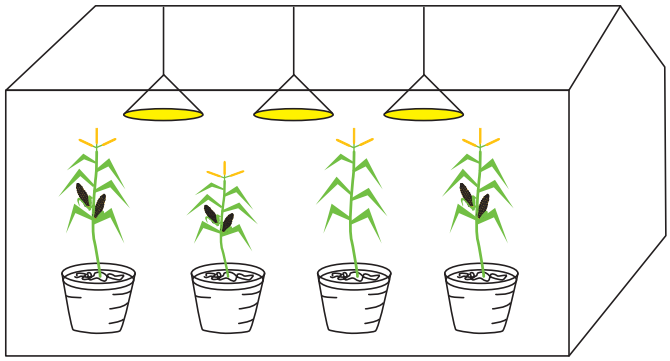


Method:
For breeding hybrids and crossing varieties, it is not enough to select parents purely on the basis of good individual performance; parents must have a good combining ability. To test this, promising parent plants undergo test crosses and the performance of the progeny is assessed in the field in the following season. The assessments are used to determine the general combining ability (GCA) and specific combining ability (SCA) of the parent plants.

Application:
Test crosses play an especially important role in cross-pollinated crops such as maize, rye and forage grasses, and in hybrid breeding.

Critical issues from the perspective of organic farming:
See chapter 'Hybrids', page 42.

Phenotypic selection under controlled conditions



Method:
Individual traits, for which only one gene is usually responsible such as brown rust resistance in wheat, can be assessed at the seedling stage in the greenhouse or climate chamber. The advantage here is that the environmental conditions can be controlled and the tests are independent of the season.

With quantitatively inherited features, i.e. influenced by several genes, there is usually only a weak correlation between the trait observed under controlled conditions in the greenhouse and the trait of adult plants observed in the field. In this case, tests under controlled conditions only provide a first indication which must be verified under field conditions.

Application:
Phenotypic selection under controlled conditions is used to select for traits that are inherited by one or a few genes and that can also be detected with confidence at a young stage or on single plants. It is a type of preselection that can also be carried out in the winter months. Furthermore, artificial infections with pathogens are easier and safer to perform in a climate chamber than under field conditions. In pathogen containment greenhouses, it is even possible to select for organisms that would be subject to quarantine requirements.

Critical issues from the perspective of organic farming:
- Under controlled conditions there is no natural selection or interaction with the target environment.

Analytical / technical selection

Method:
Many quality or technological traits cannot be detected on the basis of phenotype. To identify these features, various tests have to be performed in the laboratory. For example, wheat quality is examined through several rapid assessments in order to make predictions for baking performance. Another test looks at the glucosinolate content of broccoli that is determined to evaluate its anti-carcinogenic potential.

Application:
Targeted improvement of quality traits to meet the market needs.

Critical issues from the perspective of organic farming:
None

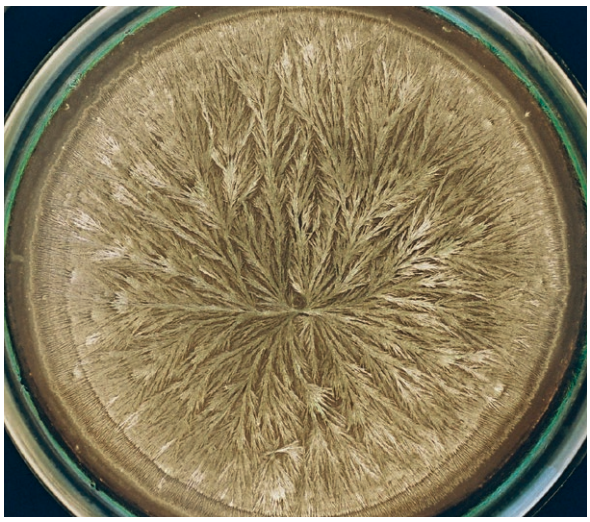
Organoleptic selection

Method:
Organoleptic examination of food or other edible products involves assessing the look, smell and taste perception by the consumer. For this purpose, blind tests are performed with new varieties of interest according to a particular experimental design.

Application:
Organoleptic selection based on the sampling of harvested products is routinely applied mainly in vegetables, fruits and herbs. Organoleptic tests allow selection according to consumer preferences.

Critical issues from the perspective of organic farming:
None

Selection using picture forming methods

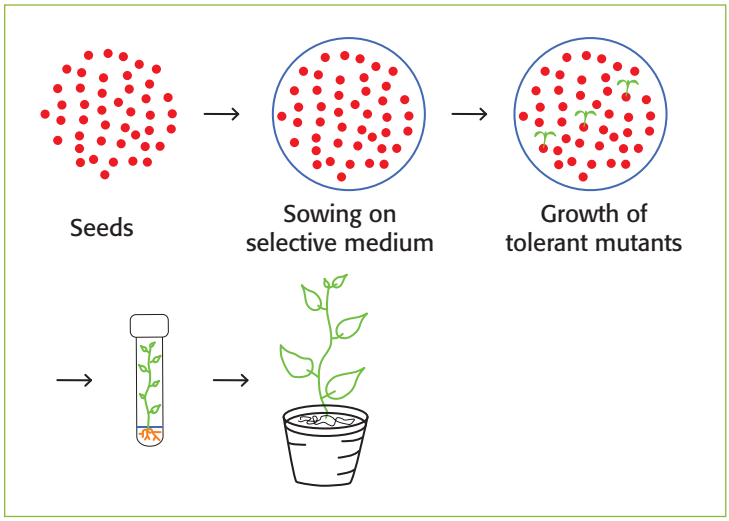


Method:
There are several methods of selection based on image formation, for example bio-crystallisation (copper chloride crystallisation) and circular chromatography (Chromatest) according to Pfeiffer, and capillary dynamolysis method according to Wala. The basic principle of these methods is that a shape structure or colour spectrum can be derived for the sample, e. g. a crop-pattern. The results are sample specific images that can be compared with reference images in order to make statements about the quality and vitality of the samples.

Application:
These methods are primarily used in biodynamic cultivation to increase the vital forces of food. However, they can also be used to differentiate conventional and organic produce.

Critical issues from the perspective of organic farming:
None

In vitro selection

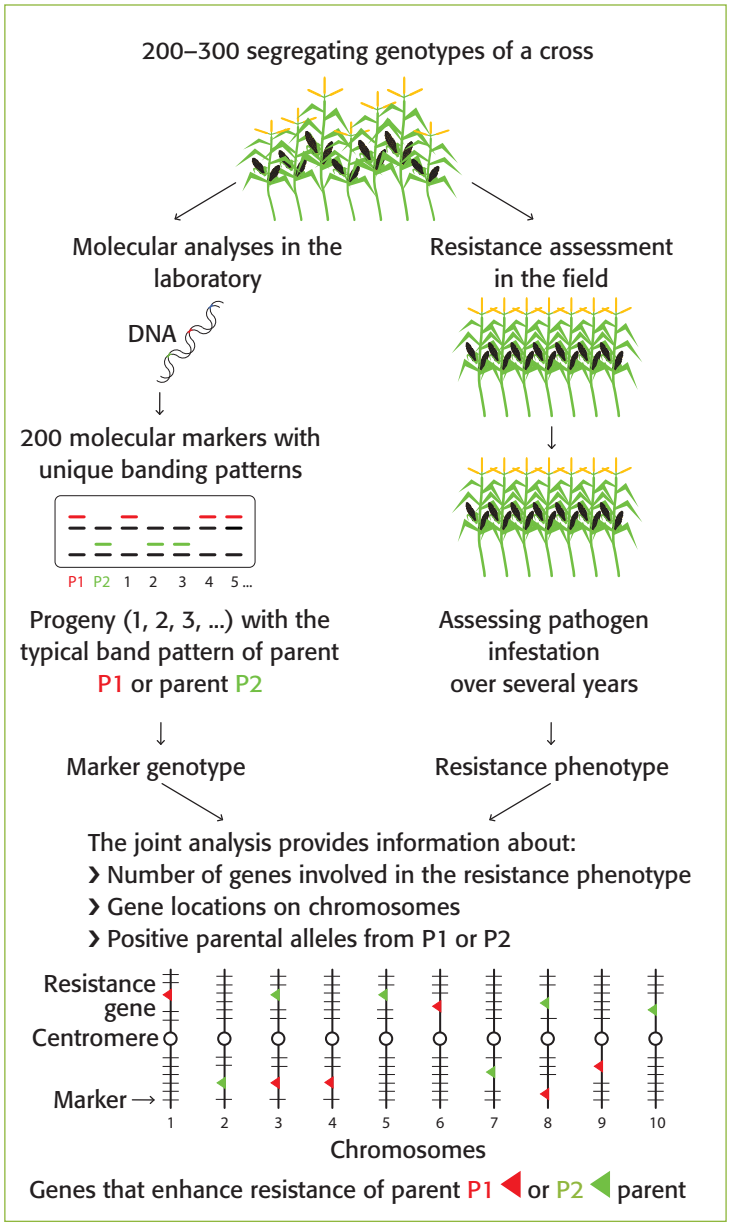


Method:
In *in vitro* selection, sterile plants, seeds, individual plant organs or single cells (protoplasts) are grown on artificial nutrient medium. By adjusting the composition of the growth medium, the plants can be tested for stress tolerance to biotic and abiotic influences. Apart from the selection, *in vitro* culture conditions can be used to induce new mutations (somaclonal variation). For the selection of fungal resistance, seeds or isolated embryos are transferred to a medium containing the fungal pathogen or toxin. Germinating seeds exhibit resistance. *In vitro* selection can be used to test many genotypes for a specific trait. It is a type of pre-selection technique which can greatly reduce the number of genotypes that need to be grown and selected in the field.

Application:
In vitro selection is a very efficient and cost-effective method for the selection of traits that can be detected at the cellular level. In particular, this includes tolerance to abiotic and biotic stress factors that can be simulated by changing the culture medium, e. g. high salt content for the selection of salt-tolerant varieties. Depending on the magnitude of the stress applied, selection can be made for complete or partial resistance.

Critical issues from the perspective of organic farming:
- Selection takes place in an artificial environment, and cultivation on artificial nutrient medium usually involves the addition of synthetic phytohormones.
- Interaction of the plant with the soil and the climate is not possible.

Marker assisted selection (MAS)



Method:
Molecular markers are diagnostic aids to determine differences in the DNA sequence. They are inherited in a Mendelian fashion. Molecular markers covering the entire genome allow kinship determinations and can be used for to select potential crossing parent. They can also be used for the selection of desired traits but, in this case, linkage analyses must first be carried out in order to identify markers that correlate with differences in the corresponding phenotypic traits in the field.

For a monogenic (qualitative) trait, two flanking markers are sufficient to identify the gene of interest. However, for polygenic (quantitative) traits, flanking markers are needed for each gene involved (quantitative trait loci = QTL). For the practical implementation of marker-assisted selection (MAS), leaf tissue DNA is extracted from the progeny of a cross to carry out sequence analysis. Plants that exhibit the desired pattern of bands are selected. The markers are only used for diagnostic purposes and do not change the DNA of living plants.

Application:
Molecular marker analysis is a diagnostic procedure which allows the selection of monogenic and polygenic traits at the DNA level, regardless of the environment in which plants are grown.

Marker assisted selection has been successfully applied in backcrossing and for introgression of a particular gene into an existing variety. It has also been used for stacking various Mendelian inherited resistance genes that are phenotypically indistinguishable, and for selecting quantitative traits (QTL) which are difficult to identify phenotypically. Markers can be used to analyse the genetic diversity of a crop or its pathogens very accurately. This provides important information to select crossing partners, identify a range of germplasms to keep in main gene banks, determine pathogen populations and develop strategies to avoid the breakdown of resistances.

Critical issues from the perspective of organic farming:

- In the development and application of molecular markers, enzymes are used which are usually produced from genetically modified bacteria.
- Plants are evaluated merely based on their DNA sequence. Genotype-environment interactions and epigenetic effects are neglected.

Proteomics / Metabolomics

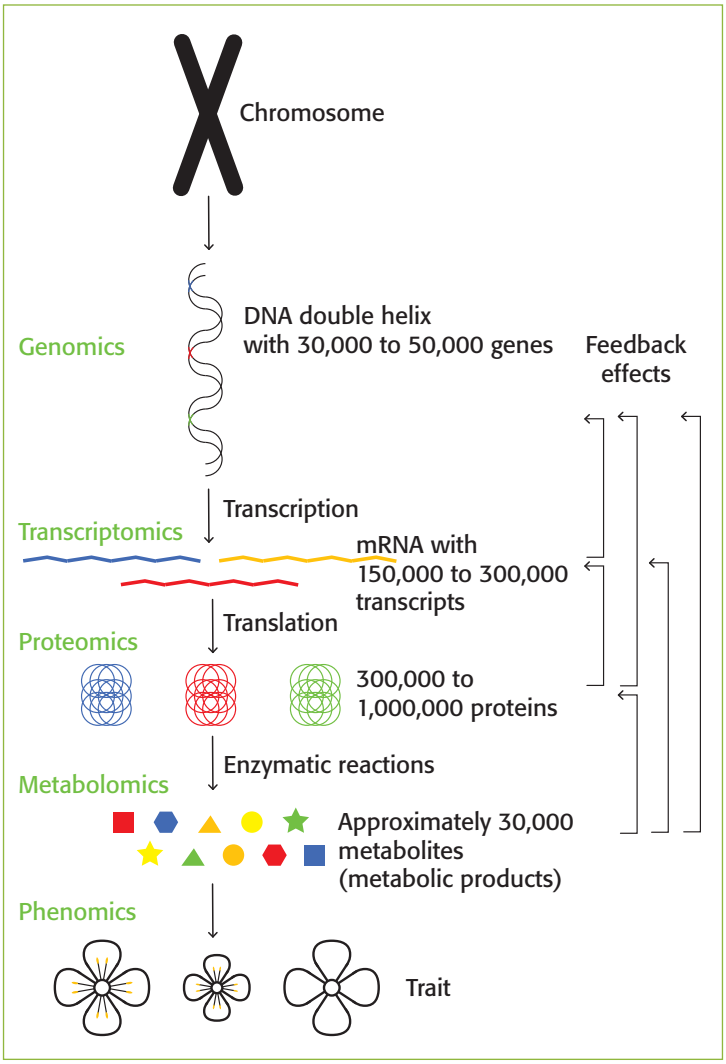
Method:
Proteomics is the study of the proteome, i.e. the sum total of all known proteins in a cell or a plant under defined conditions and at a defined time. In contrast to the DNA sequence, which is identical in all cells and at all times, the quality and quantity of protein composition may change depending on environmental conditions and growing stage.

Similarly, metabolomics (metabolite profiling) refers to the quantitative analysis of various metabolic products (metabolites). While DNA marker analysis is used to indicate the presence of a gene responsible for a given trait, proteomics can be used to determine which genes are actually expressed. The study of the protein and metabolite composition in a wide range of genotypes can give important information on which metabolites play a role in the expression of certain trait (e.g. heat tolerance). If the function of individual proteins or metabolites is known, selection can be carried out directly at the protein or metabolic level.

Application:
Selection on the basis of metabolites is currently used mainly for fruits, vegetables, medicinal plants and renewable resources in order to improve the organoleptic, nutritional and technological quality. Such new technologies enable a systematic and accurate analysis of plant structures, functions and interactions with a dynamically changing environment. Results obtained from model plants indicate that the expression of complex traits can be predicted on the basis of molecular and biochemical analyses of hundreds or thousands of measured values. These techniques are currently implemented in breeding research to better understand the responses of plants to environmental factors and, from this, efficient selection strategies can be developed.

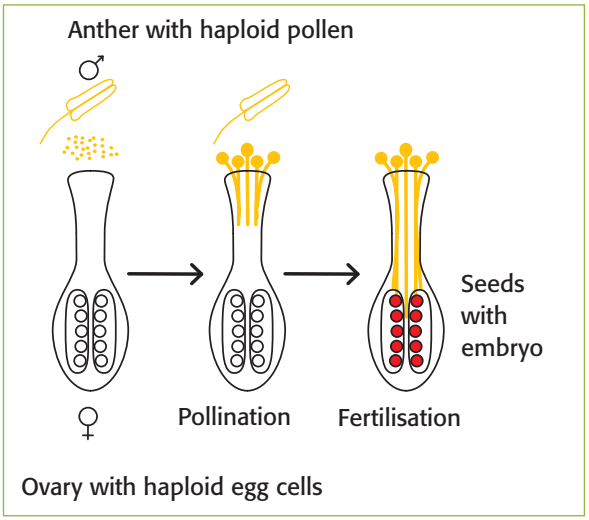
Critical issues from the perspective of organic farming:

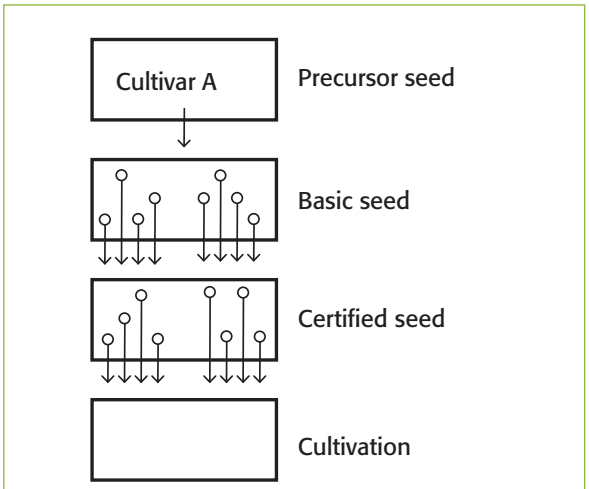
- It is a very technological method. The functions of the plant and its interactions with the environment are broken down to their individual components and correspond to a reductionist approach.



Propagation

Generative propagation



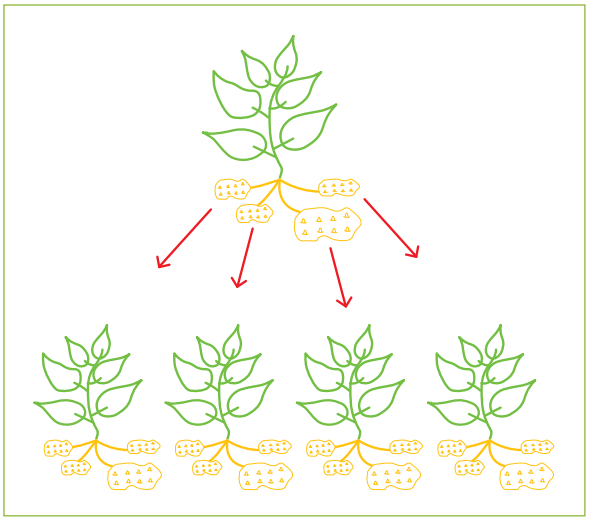


Method:
If plants or populations are propagated by seed, it is called generative propagation. Via the fusion of egg cell and pollen, the progeny receives one set of chromosomes from the mother and one from the father plant. When this involves fully homozygous inbred lines, the F1 progeny is genetically identical to the parent plant as pollen and egg cell have the same set of chromosomes. In heterozygous plants, during meiosis and subsequent fertilisation, the parental genes are recombined so that the progeny shows a range of different traits. Since pollen mainly consists of the cell nucleus, cell organelles and their extrachromosomal DNA are inherited from the egg cell, i.e. are subject to maternal inheritance. Propagation can be carried out in a field, greenhouse or climate chamber.

Application:
Most cultivars are propagated by seed.

Critical issues from the perspective of organic farming:
None

Vegetative propagation



Method:
In vegetative (or asexual) propagation, plants are increased by cuttings, division, runners, bulbs, tubers, etc. These methods rely on the ability of a plant to regenerate from an organ or single cell back to a whole plant. The set of chromosomes remains unchanged, although the organelles can segregate. Vegetative propagation is used to increase heterozygous plants in a genetically stable manner (along with apomixis and hybrid breeding).

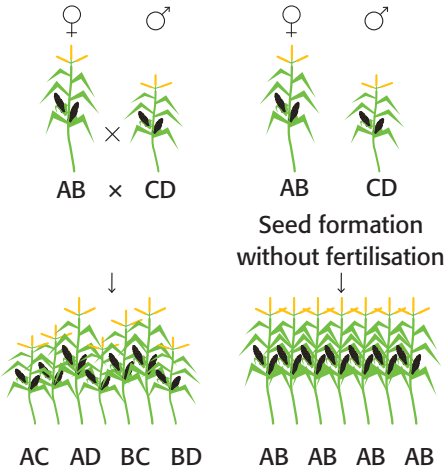
Vegetative propagation takes place in the field (e. g. potato), cold frame (e. g. fruit), greenhouse or climate chamber, depending on the crop. The techniques are diverse and specific to each crop. For example, in fruit cultivation, cuttings (scion) are often grafted onto rootstocks. Otherwise, rooting hormones are used to promote the rooting of cuttings. Synthetic pesticides are often used to prevent the spread of bacterial and fungal diseases. In contrast to seed, the storage period is limited for vegetatively propagated plant material.

Application:
Vegetative propagation is used for cloned varieties of potato, apple, grape and many ornamental plants. In addition, parental components for hybrid or polycross varieties are sometimes propagated vegetatively.

Critical issues from the perspective of organic farming:

› Application of synthetic rooting hormones and pesticides.

Apomixis



Method:
Some plants can reproduce asexually via seeds. This phenomenon is called apomixis. During seed formation, meiosis (which is essential for sexual reproduction) is either suppressed or circumvented so that the embryo is genetically identical to the mother plant. For some plants, apomixis is obligatory and their seeds contain only apomictic embryos. However, there are also facultative apomictic plants which can form either sexual or apomictic embryos in their seeds. Despite the ability for asexual propagation, pollination is usually necessary for seed to set. Many apomictic species are polyploid.

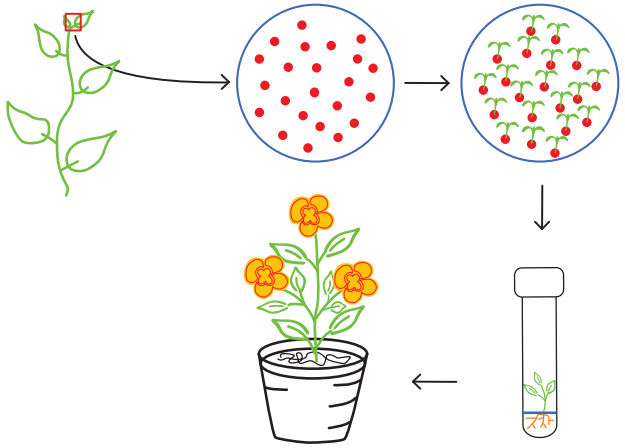
Application:
Apomixis occurs naturally in some crops (e. g. meadow bluegrass) and wild plants (e. g. St John's wort, dandelion) and is of interest to breeders because it combines the advantages of seed propagation, such as health and quality, with identical reproduction of the maternal genotype. The main advantages of apomixis over vegetative propagation are higher propagation rates and less plant health problems. Apomictic reproduction is considered a promising method to multiply heterozygous varieties and to preserve heterosis in hybrids.

Critical issues from the perspective of organic farming:

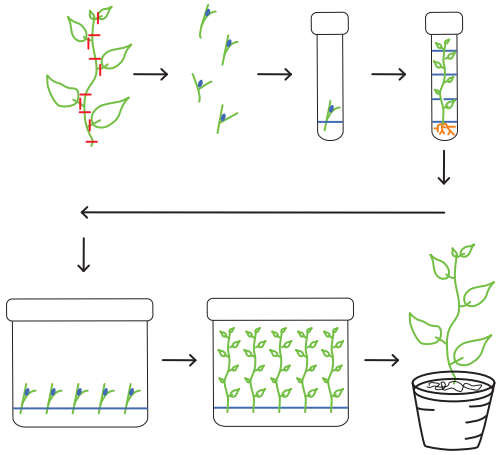
› Apomictically propagated plants cannot be used for further breeding, because the progeny is genetically identical to the parent plant.

In vitro propagation/cell and tissue culture

Meristem culture



Node culture



Method:
For *in vitro* propagation, parts of plants, tissues or single cells are grown on a sterile nutrient medium and reproduced vegetatively. Depending on the plant species, different parts of the plant are used, e.g. stem part with an axillary bud, leaf part or an onion scale. These plant parts grow into shoots, which can be propagated again. This process is repeated until enough plants are produced. If root formation takes place, plants are hardened off *ex vitro* and then planted in greenhouses and/or the field.

When *in vitro* methods are used for vegetative propagation via the meristem, or shoot tip, the process is known as meristem culture. The meristem consists of undifferentiated cells that are able to divide. Due to the speed of cell division, the material generated is often free from diseases and viruses. Therefore, meristem culture is often used to produce virus-free plant material.

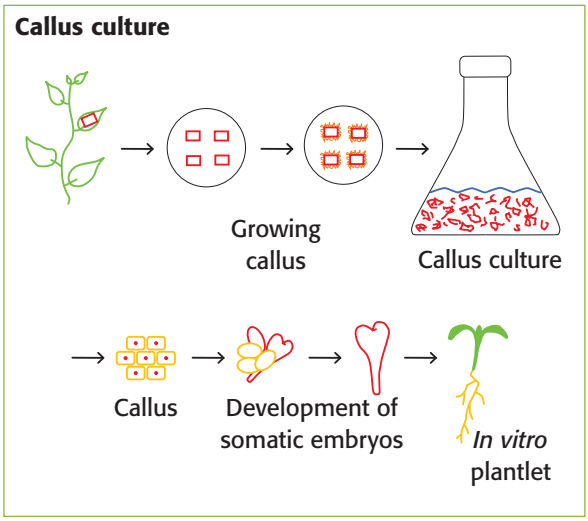
Leaf tissue, flower heads or nodes can also be taken and cultured on nutrient media to increase the rate of proliferation. The differentiated cells of each tissue are first dedifferentiated and then stimulated to divide. This results in so-called calluses (undifferentiated cell clusters), which can be stimulated to form shoots and roots, or directly form somatic embryos which germinate subsequently. These shoots can be induced to form numerous shoots resulting in a very high propagation rate.

In extreme cases, whole-plant regeneration can be achieved from a single cell. For example, if leaf tissue is digested enzymatically, the cell wall dissolves and individual protoplasts (cells without a cell wall) are released. These protoplasts can be cultured in liquid medium to regenerate a whole plant.

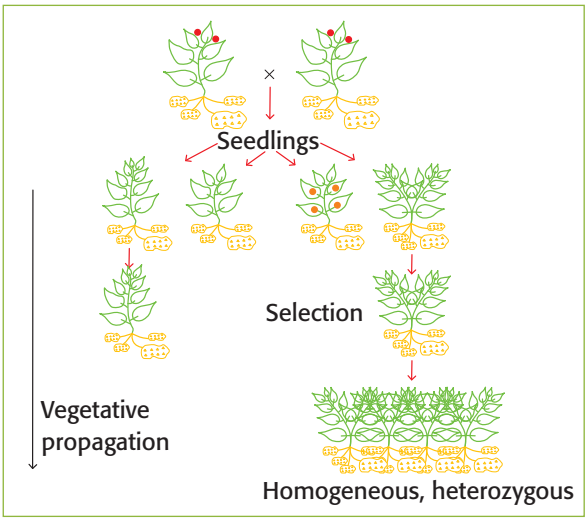
Application:
In vitro propagation is used for the propagation of virus-free plant material (e.g. potato) and for rapid propagation of clones and parental components for crosses and hybrid varieties. The method provides a way to multiply genetically identical plants in a very short time. Within a year, over a million genetically identical progeny can be produced from a single plant. The spread of disease is prevented due to the sterile culture conditions. Otherwise, disease contaminations can bring vegetative propagation to a halt. The method is very space-saving and efficient, and can be partially automated. It is possible to store *in vitro* cultures at low temperatures for long periods of time, which is increasingly used for conservation and preservation of valuable genetic accessions (cryo-conservation).

Critical issues from the perspective of organic farming:

› The cultivation is carried out on artificial nutrient medium, usually with the addition of synthetic phytohormones.



Clonal varieties



Description:
Plant species that are mainly propagated vegetatively pass on their genetic composition to the progeny unchanged. Such species are usually used to create cloned varieties. The basic process of clone breeding takes place in three stages:

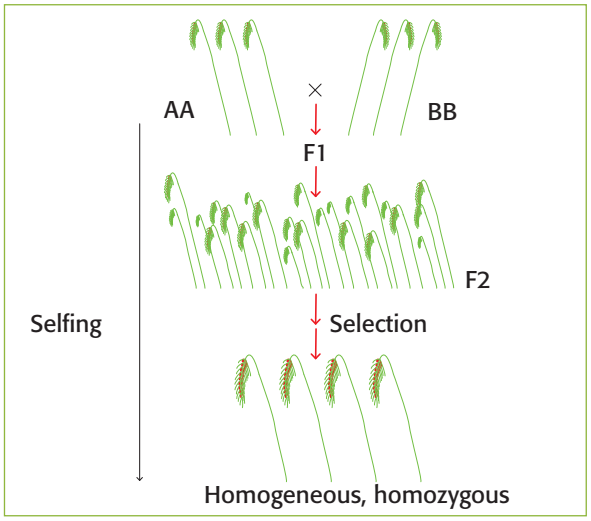
i. Creation of genetic variability through sexual propagation
ii. Selection of the progeny
iii. Vegetative propagation of the best single plants followed by variety registration

Cloned species are generally highly heterozygous and homogeneous due to the vegetative propagation. Care must be taken to avoid phytosanitary problems (e. g. infection by bacteria, fungi or viruses). In the case of seed propagation, there is a wide phenotypic segregation.

Application:
Clone varieties can be bred in less than 5 years because selection can occur with the first filial generation (F1). Unique phenotypes can be maintained, propagated and used for maximum heterosis, depending on the divergence of the parents. Clone varieties are common for perennial crops and those with a high vegetative reproduction rate (e. g. potatoes, fruit, vine, ornamental plants).

Critical issues from the perspective of organic farming:
None

Inbred lines



Description:
Inbred lines are usually developed for self-pollinating plant species. The basic process of line or pedigree breeding involves several steps:

i. Creation of genetic variability via targeted crossing of two parents
ii. Propagation of the homogeneous F1 progeny via continued self-pollination
iii. Mass selection in the diverse F2 to F4 generations
iv. Single plant selection in the F5 to F8 generations
v. Generative propagation of the best inbred lines for cultivar registration

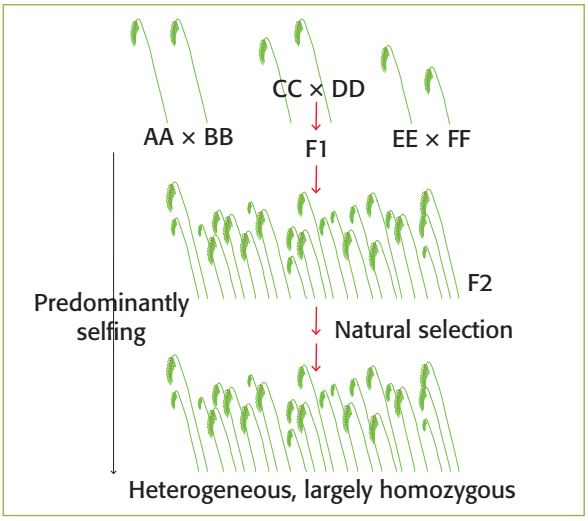
The inbred lines are highly homozygous and homogeneous. The progeny of an inbred is genetically unchanged and can be reproduced easily.

Application:
Targeted crossing of the inbred lines can enforce cross-pollination that rarely occurs in self-pollinators. In this way, the parent genes are recombined creating high genetic diversity that can be used to select new varieties. Inbred line breeding is common practice in self-pollinating crops such as wheat, barley, pea or soybean.

Critical issues from the perspective of organic farming:

› Inbred lines are genetically very similar and, thus, more susceptible to pests and diseases.

Composite cross populations (Evolutionary populations)



Description:
Composite cross populations (CCPs) are generally developed to achieve a higher genetic diversity within self-pollinating species:

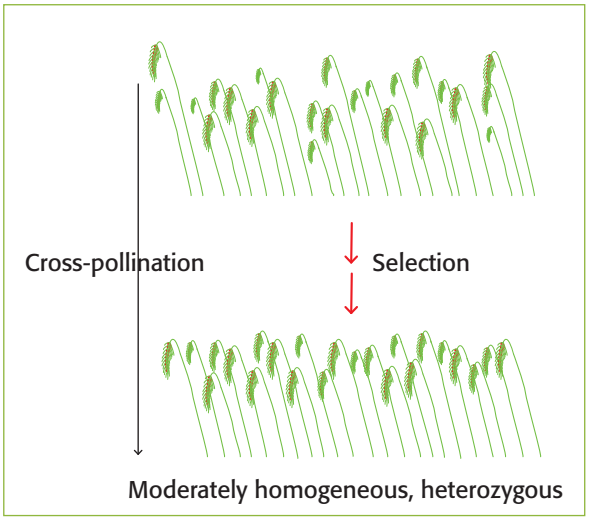
i. Many crosses between elite varieties
ii. Combined propagation of the entire progeny in the target environment

Propagation takes place over several generations at the location in which the variety will be grown. This ensures that the best adapted genotypes proliferate faster under natural selection. In addition, higher heterozygosity is achieved compared with pure lines or varietal mixtures due to the high allele diversity.

Application:
CCPs allow local selection and adaptation of varieties to the target environment. The CCPs are more heterozygous and heterogeneous than inbred varieties and should therefore be able to respond more flexibly to fluctuations in environmental conditions. Currently, first pilot trials are carried out for self-pollinating species (composite cross populations of winter wheat).

Critical issues from the perspective of organic farming:
None

Open-pollinated varieties

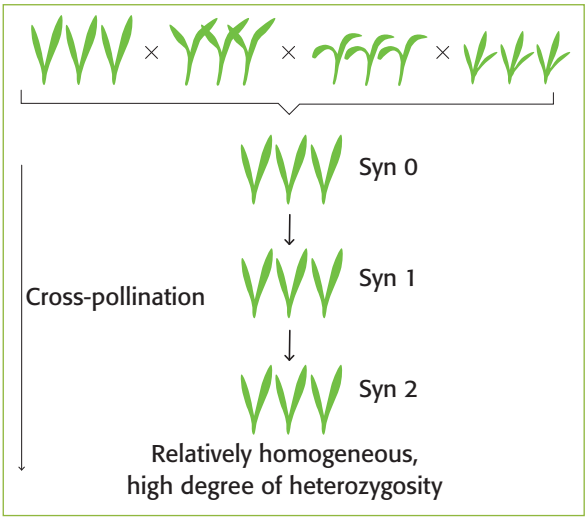


Description:
Open-pollinated varieties are traditionally developed from cross-pollinated plant species. Mass selection is the simplest selection method to improve the initial population. Instead of simple mass selection, pairwise crosses of the best individual plants and recurrent selection can be performed to increase the selection success. Relatively labour-intensive maintenance breeding procedures must be carried out to maintain a variety. For instance, open-pollinated varieties must be separated sufficiently from other populations to retain their varietal traits. The open-pollinated varieties possess medium to high heterozygosity and are moderately homogeneous.

Application:
Open-pollinated varieties are traditionally used for cross-pollinated species. They are genetically heterogeneous and heterozygous (e. g. rye, maize). About half the maximum heterosis effect can be exploited. Due to their genetic variability, open-pollinated varieties should be able to better adapt to new environmental conditions compared with inbred lines or hybrids.

Critical issues from the perspective of organic farming:
None

Polycross varieties

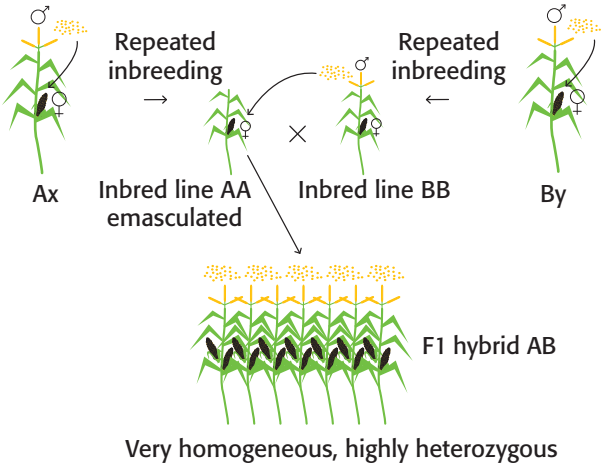


Description:
The aim of polycross varieties is to improve the homogeneity of an open-pollinated variety, without losing the high level of heterozygosity. A limited number of parents (4–20) are intentionally crossed or bloom together. The progeny possesses a high degree of heterozygosity, but are more homogeneous than a population variety. They can be propagated via open pollination over several generations. At some stage, new seed must be recreated from the parent components. It is important that the parents are previously tested for their combing ability. Polycross varieties are an intermediate stage between open-pollinated varieties and hybrid varieties that must be created anew from the original parental lines to maintain their maximum yield potential.

Application:
Polycross varieties are common practice in forage grass breeding and for partial cross-pollinators (e. g. faba bean). They utilise about three quarters of the maximum heterosis effect. They are heterozygous, but more homogeneous than open-pollinated varieties. Unlike hybrids, farmers can resow saved seeds for several generations without a substantial drop in performance.

Critical issues from the perspective of organic farming:
None

Hybrids



Description:
Hybrid breeding makes use of the so-called heterosis effect. It is the term used to describe the superiority of a hybrid cross compared with its parental lines. The advantage of hybrids is positive effect on plant performance due to high heterozygosity at many loci. This effect can be fully exploited when genetically distant inbred lines are crossed. These hybrids have maximum heterozygosity and are also very homogeneous. However, the downside is that the traits of the F2 progeny vary widely, so that only a part of the progeny has an equivalent level of performance compared with the hybrid parents. Therefore, new hybrid seed must be created each season.

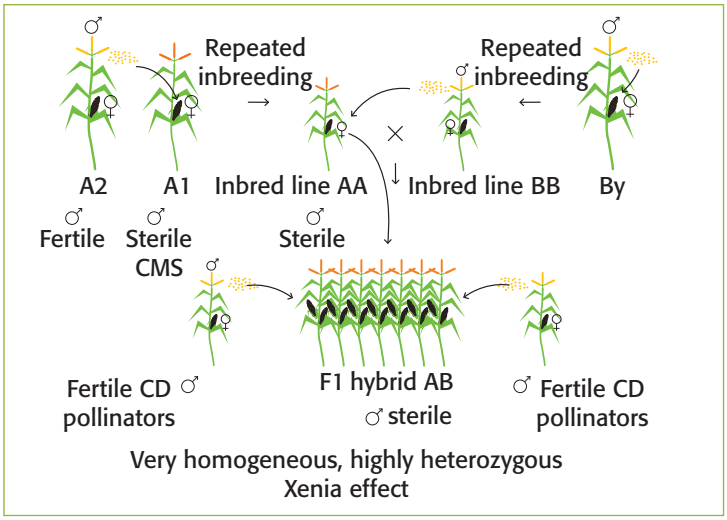
The maternal line must either be male sterile or emasculated for hybrid seed to be produced at a reasonable cost. The most commonly used technique is cytoplasmic male sterility (CMS). The maternal line is incorporated into the CMS plasma by repeated backcrossing. This results in two nearly identical lines: the sterile mother line in the CMS plasma and the corresponding fertile line in normal plasma. The fertile line is necessary for the propagation of the sterile mother line and is referred to as the maintainer line. In order to produce hybrid seed in cultivation, the CMS plasma must be made male fertile. Therefore, father lines are selected which have the so-called restorer gene. Restorer genes are chromosomally inherited and can restore the male fertility in the CMS plasma again.

Application:
The use of hybrids is common practise in cross-pollinating species such as maize, rye, rapeseed, sunflowers, carrots and cabbage. However, they are also increasingly used in self-fertilising crops (e. g. cotton, wheat) due to high homogeneity and very good performance.

Critical issues from the perspective of organic farming:

- Hybrids cannot be reproduced without a decline in performance. This limits the autonomy of the farmer and promotes dependence on seed companies.
- CMS hybrids cannot be used to breed of new fertile varieties without a restorer genes to reestablish male fertility (e. g. broccoli and cauliflower). Thus, the breeders' exemption is nullified restricting breeding progress.

Plus-hybrids with xenia effect



Description:
In the plus-hybrid system, cytoplasmic male sterile (CMS) hybrids (80 %) are pollinated by a genetically unrelated male fertile hybrid (20 %) in order to achieve additional yield increases. In addition to heterosis, the CMS hybrids also benefit from the direct influence of male sterility (no energy for pollen production needed) and from the xenia effect. The xenia effect appears when an egg cell is fertilised by pollen that does not correspond to the maternal ovarian tissue causing the formation of larger seeds.

Application:
Plus-hybrids with xenia effect are mainly used in maize after seed production became increasingly based on CMS hybrids. Xenia effects increase grain yield by increasing seed weight. The incorporation of 20 % unrelated fertile hybrids increases genetic diversity in the field.

Critical issues from the perspective of organic farming:

- CMS hybrids without restorer genes are male sterile and are therefore limited in their ability to reproduce. They cannot be used as pollen donors for further breeding, but only as a seed parent with the progeny inheriting the male sterility. Thus, the breeders' exemption is nullified and breeding progress restricted.

Overview of different types of varieties

	Clonal varieties	Inbred varieties	Composite cross population	Open-pollinated variety	Polycross varieties	Hybrids	Plus-hybrids
Propagation method	Vegetative	Generative	Generative	Generative	Vegetative / generative	Generative	Generative
Heterozygosity	Medium – high	<5 %	Low	High	Very high	Fully heterozygous	Fully heterozygous
Homogeneity	Very high	Very high	Low	Low	High	Very high	Very high
Opportunity for further breeding developments?	Yes	Yes	Yes	Yes	Yes	Yes, except for CMS without restorer gene	Only if restorer gene present
Seed-saving and replanting?	Yes	Yes	Yes	Yes	Yes	No	No

Criteria for the evaluation of breeding techniques

On 2 March 2011, an expert workshop was held in Frankfurt with 30 representatives from breeding companies, research organisations, producer associations and ECO-PB. The aim was to identify criteria for the assessment and prioritisation of breeding techniques for organic farming.

The workshop led to the consensus that in organic plant breeding not only the integrity of the genome, but also the integrity of the cell - as the smallest unit of life from which a plant can grow – should be preserved. Respect for the cell as an inseparable unit includes the interaction of all cellular components that are required for a living cell to be a functional entity, such as chromosomal and extra-chromosomal DNA, organelles, cytoplasm, the cell membrane and their associated regulatory mechanisms. These regulatory processes (i.e. epigenetics) determine why an undifferentiated cell with the same genome can develop into a flower, leaf or root cell. According to organic farming, the cell is to be respected as a living unit and protected from any technical and physical interventions.

Based on the workshop and subsequent discussions, a position paper on organic plant breeding was produced, which is supported by experts and stakeholders from the organic sector. This position paper strengthens breeding for organic farming, provides certainty for breeders and farmers, and raises public awareness about the importance of seeds and breeding for a sustainable food production. In addition, the position paper creates transparency and conveys the principles of organic plant breeding, and serves as a basis for organic farming associations to develop breeding guidelines.

Position paper on organic plant breeding

(Based on the results of an expert workshop held on 2nd March 2011, Frankfurt am Main, and approved by the ECO-PB General Assembly on 6th Novmber 2012)

Organic plant breeding is embedded into the general principles of organic farming. According to the International Federation of Organic Agricultural Movements (IFOAM), the persons acting in organic farming take care of the preservation and improvement of soil fertility, promote the genetic diversity of plants, animals and other organisms of the agro-ecosystem, conserve natural resources and strive for a stable ecological equilibrium. They take social responsibility and stand up for justice and equality. In organic farming, special responsibility is taken for the protection of the environment and for safeguarding the livelihood for present and future generations (www.ifoam.org).

Cultivated plants are the basis for our food. For thousands of years, plant breeding has been intrinsically tied to our culture. It is therefore of vital importance for our future that farmers have access to seeds and vegetative propagation material of a wide range of locally adapted crop species and varieties. They should be allowed to adapt and improve them (crop species and varieties) by cultivation in their local and on farm conditions. Genetic diversity within and between species allows plants to adapt to changing environmental conditions, and it enables us to improve our crops through breeding according to our needs.

Hereby the dignity of creatures has to be taken into account. Like all living organisms, plants have an intrinsic value independent of human interests. Organic plant breeding promotes genetic diversity and takes into account the ability of natural reproduction. It also respects the genetic integrity of a plant, its crossing barriers and regulatory principles and is committed to safeguard the fertility, the autonomy and the evolutionary adaptation of our crop plants. This means that when varieties are chosen for organic farming, not only their suitability for cultivation but also their breeding history has to be considered. Given the multitude of breeding methods and techniques presently applied to develop future varieties, this is not an easy task. To meet this claim and to send appropriate social and political signals, specific criteria were defined and ranked in order to allow a transparent evaluation of breeding methods and derived varieties.

Aims of organic plant breeding

- The breeding goals match the respective crop species and the needs of the complete value chain of the organic sector (producers, processers, traders and consumers). The breeding goals aiming at the sustainable use of natural resources and at the same time account for the dynamic equilibrium of the entire agro-ecosystem.
- Organic plant breeding supports sustainable food security, food sovereignty, secure supply of plant products (e.g. fibre, medicine, timber), and the common welfare of society by satisfying nutritional and quality needs of animal and human beings.
- Organic plant breeding sustains and improves the genetic diversity of our crops, and thus contributes to the promotion of agro-biodiversity.
- Organic plant breeding makes an important contribution to the development of our crops and their adaptation to future growing conditions (e.g. climate change).

Ethical criteria

1. The genome is respected as an indivisible entity and technical / physical invasion into the plant genome is refrained from (e. g. through transmission of isolated DNA, RNA, or proteins, or through artificial mutagenesis).
2. The cell is respected as an indivisible functional entity and technical/physical invasion into an isolated cell on growth media is refrained from (e. g. digestion of the cell wall, destruction of the cell nucleus through cytoplast fusions).
3. The ability of a variety to reproduce in species-specific manner has to be maintained and technologies that restrict the germination capacity of seed-propagated crops are refrained from (e. g. Terminator technology).
4. A variety must be usable for further crop improvement and seed propagation. This means that the breeders' exemption and the farmers' right are legally granted and patenting is refrained from, and that the crossing ability is not restricted by technical means (e. g. by using male sterility without the possibility of restoration).
5. The creation of genetic diversity takes place within the plant specific crossing barriers through fusion of egg cell and pollen. Forced hybridisation of somatic cells (e. g. through cell fusions) is refrained from.
6. In complementation to the presently widely used hybrids, non-hybrid varieties shall be bred in order to give farmers the choice to produce their own seeds (farmers' privilege).
7. The principles of organic farming (the principles of health, ecology, justice and care) form the guidelines for breeding activities.

Criteria concerning breeding strategies

8. The environment in which selection takes place is in accordance with organic cultivation methods in order to account for the plant-environment interaction, to accelerate the selection gain, and to benefit from possible epigenetic effects. This means, that selection takes place under organic farming conditions.
9. The phenotypic selection in the field can be supplemented by additional selection methods (e. g. analysis of natural compounds or molecular markers for diagnostic purposes).
10. Organic plant breeders shall develop organic varieties only on the basis of genetic material that has not been contaminated by products of genetic engineering.

Socio-economic criteria

11. The exchange of genetic resources is encouraged and any patenting of living organisms, their metabolites, gene sequences or breeding processes are refrained from.
12. The breeding process, the starting material (e. g. used crossing parents, starting populations), and the applied breeding techniques will be disclosed to enable producers and consumers to choose varieties according to their values (e. g. clear declaration of varieties derived from mutation breeding).
13. Participatory breeding programmes involving all stakeholders (producers, processors, retailers and consumers) are promoted.
14. A plurality of independent breeding programmes and breeders with different types of crops to increase agricultural biodiversity is aspired.

Choice of varieties in organic farming

All varieties of which seeds or propagation material have been propagated under organic growing conditions are currently allowed in organic agriculture, provided they are not declared as genetically modified varieties (Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products). According to a derogation rule, untreated, non-organically propagated varieties are only permitted, if no suitable varieties from organic propagation are available. Among the varieties the following categories can be distinguished:

I. Varieties derived from conventional plant breeding that are suitable for organic farming with the exception of genetically modified varieties (conventional breeding, organically propagated, or, if necessary, conventionally propagated but untreated),
II. Varieties derived from plant breeding programmes with a special focus on the breeding goals or selection environments for organic farming, and organic seed propagation (product-oriented breeding for organic farming, organically propagated), and
III. Varieties derived from organic breeding programmes or organic on farm breeding, which have been bred under organic farming conditions considering to the above mentioned criteria (process-oriented organic plant breeding, organically bred and propagated).

According to the achieved minimal consensus, varieties, which were bred using techniques that violate the integrity of the genome (e. g. transgenic plants) or the integrity of the cell (e. g. cytoplast fusion), have to be excluded from the choice of varieties for organic agriculture. For future acceptance of varieties of Category I and II in organic farming, the above criteria (especially criteria 1–5) have to be taken into account. Thus, the above mentioned criteria provide also guidelines for breeding programmes for organic farming.

Varieties that are currently available for organic farming are mainly derived from conventional plant breeding programmes. This spectrum needs to be urgently supplemented or replaced, as for certain crops, such as cotton, soybeans, and corn, genetic engineering (violation of the first criterion) is frequently applied, while in other crops, like e. g. in broccoli or cauliflower, breeding is exclusively focused on male sterile hybrids originating from cytoplast fusion (violation of the second criterion). In these cases, the choice of varieties for organic farming is already today severely limited. In addition, the strong monopolisation on the seed market, the concentration of breeding efforts on a few major crops, and the dominance of conventionally propagated seeds lead to further restriction of the range of varieties for organic farming. Seeds and vegetative propagation material are one of our most important resources. It is therefore essential that varieties of Category II and III are promoted actively.

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