Annex 2: Permitted methods for the production of genetic variation for the breeding of bioverita varieties

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	Brief explanations
Use of spontaneous mutations	Changes in the genetic material occur naturally, so-called mutations. This genetic diversity can be used in breeding. May occur during cultivation but can also be targeted, eg by growing under stress conditions.
Use of natural high-altitude irradiation	The mutation rate can be increased by targeted cultivation at high altitudes, provided that the ground on which the plants are grown is biologically certified.
Castration	Manual removal of male flower buds
Selfing	Natural or manual pollination control
Crossing within the species	
Crossing across species	For example, crosses of dessert apples with wild apples in order to insert scab resistance genes in the cultivar
Bridge crossings	Applied, for example, for crossing in of resistances from related wild species if the cultivar and the species concerned do not cross. Initially a species closely related to the wild species is crossed, and the resulting crossing is crossed with the cultivar in a second step.
Mentor pollen techniques	Mixing pollen of various species to achieve fertilization in crossbreeds which would otherwise not be possible. Pumpkin, for example
Grafting	
Eurythmy	
Tone frequencies	
Naturally occurring, genetic or cytoplasmic male sterility with restorer genes. For deliberate use of this technique the breeder must first submit and justify an application to bioverita. This request must be approved before proceeding.	Male sterility, which is inherited via cell nucleus or via cytoplasm, occurs naturally in many species (carrots, onions, gentian, etc.). This can be used in breeding if it is ensured that the male fertility can be restored by appropriate core genes (restorer genes). This male sterility can be used to simplify crosses and create a polycross. It is shown here to distinguish it from artificial cms sterility, which is based on cytoplast fusion, which may not be used.

Permitted selection methods for growing recognized bioverita varieties

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	Brief explanations
Phenotypic selection under ecological conditions	Phenotypic selection on certified organic sites is a prerequisite for the genetic and epi-genetic adaptation to the organic cultivation system and a core component of organic plant breeding.
Additional selection under controlled conditions	For example in greenhouse cultivation, under polytunnel etc.
Artificial selection stress (for example increased disease pressure)	For example selection after artificial septic infection in the field or selection for fire blight *(Erwina Amylovora)* -resistance in the greenhouse.
Indirect selection	For example selection for increased wax layer of the spike to increase the resistance to glume blotch (*Phaeosphaeria nodorum*) by wheat
Imaging techniques	For example copper chloride crystallization, paper chromatography, chroma-test
Organoleptic selection	
Analytical / Technological methods	For example brix content in the case of carrots or onions, amino acid content in maize, selection on the basis of baking properties determined in the laboratory for cereals or oil properties in sunflowers
Marker-assisted selection. This method can be used if the breeder submits and justifies an application to bioverita before starting a breeding program. This request must be approved before proceeding.	Genetic markers are used only for diagnosis, as a complement to phenotypic selection. Pure genomic selection only on the basis of DNA analysis is excluded
Eco-Tilling. This method can be used if the breeder submits and justifies an application to bioverita before starting a breeding program. This request must be approved before proceeding.	Search for naturally occurring mutations for a defined gene, using diagnostic DNA methods as a complement to phenotypic selection. This method is listed here to contrast with the impermissible tilling method which induces artificial mutations.
Proteomics This method can be used if the breeder submits and justifies an application to bioverita before starting a breeding program. This request must be approved before proceeding.	Comprehensive analyses of the protein composition of a plant (resulting from the expression of the genes at a particular development time) are used only as a supplement to the phenotypic selection. Pure proteomic selection is excluded
Metabolomics This method can be used if the breeder submits and justifies an application to bioverita before starting a breeding program. This request must be approved before proceeding.	Comprehensive analyses of the composition of all metabolic products of a plant (resulting from the expression of the genes at a particular development time) are used only as a supplement to the phenotypic selection. Pure metabolomic selection is excluded

Permitted methods for the propagation of recognized bioverita varieties

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	Brief explanations
Seed multiplication	
Vegetative propagation	
Apomictic multiplication, if this naturally occurs in the species in question.	In some plants, apomixia occurs naturally. This produces seeds that develop from the female ovum without true fertilization and are thus genetically identical to the mother plant. Examples: dandelion, citrus, St. John's Wort
Stratification	Cold treatment of seeds simulating winter rest to induce germination
Vernalization	Cold treatment of seeds with a winter phase in order to induce flowering

Recognized Bioverita varieties can have the following types of varieties

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	Brief explanations
Clones	Vegetatively propagated varieties, for example potatoes, apple
Line varieties	Homogeneous variety that has been produced by self-pollination, for example barley, wheat, soy, pea, tomato
Bulk evolution (Composite Cross Population CCP)	Genetically broad population resulting from multiple targeted crossings, for example CCP of winter wheat, which adapts to the environment through natural selection.
Population varieties	An open-pollinated population, which is in a genetic equilibrium and is therefore stable over generations
Multicomponent varieties (Polycross varieties, Synthetics, FIC = Family Intercross)	Varieties that are made from several components and are reproducible , for example polycross varieties of fodder crops, synthetic varieties of broad beans or FIC varieties of pumpkins
Population crossings	An open-pollinated population resulting from the crossing of at least two open-flowering population varieties.

Methods NOT permitted for producing genetic variation for breeding varieties approved by bioverita

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	Brief explanations
Direct and indirect gene transfer	Transfer of isolated foreign (transgenic) or genotypic (cisgen) genes in the cell nucleus of the plant through agrobacteria, particle bombardment, injection methods, endocytosis, infiltration, etc., for examples Bt maize.
Cisgenesis	Transfer of isolated heterologous or species-specific genes into the plant using agrobacteria, particle bombardment, injection methods, infiltration, endocytosis etc, for example cisgenic apple varieties with scab resistence.
Grafting of a scion onto a genetically modified rootstock	Transfer of isolated heterologous (transgenic) or species-specific (cisgenic) genes into the nucleus of the plant by agrobacteria, particle bombardment, injection methods, endocytosis, infiltration into the root stock. For example, phylloxera resistance in vine rootstock, fire blight resistance in the rootstock of apples.

Plastid transformation	Transfer of isolated heterologous (transgenic) or species-specific (cisgenic) genes into the mitochondria or chloroplasts of the plant by agrobacteria, particle bombardment, injection methods, infiltration, etc.
Artificial minichromosomes	Inserting additional artificial chromosomes that contain a variety of new genes.
Synthetic biology	Creation of organisms by creating new DNA blueprints from the basic building blocks of DNA.
Site-specific mutation triggering using zinc finger nuclease, oligonucleotides, TALEN, CRISPR/Cas9, etc.	In targeted mutation triggering, individual building blocks of a certain gene are altered. For this purpose, synthetically produced DNA segments are introduced into the cell temporarily or permanently. For example Clearfield rape with broad herbicide resistance.
RNA interference, DNA methylation	Change in the expression of individual genes by short RNA pieces that are temporarily or permanently introduced into the cell and specifically influence the reading of genes.
Artificially induced mutation release	Increase in the mutation rate for example through γ-radiation, chemical mutagens.
Tilling	Inducing mutations and subsequent selection of mutants of a certain gene
Cell fusion (protoplast fusion and cytoplast fusion)	Forced fusion of two cells (protoplasts or cytoplasts), which are neither ova nor pollen. This somatic hybridization can be induced between member of the same or different species by means of chemical or electrical stimuli. For example cytoplast fusion between radish and cauliflower to produce CMS cauliflower.
Reverse Breeding	Elimination of the naturally occurring recombination of genes during meiosis in order to reproduce a heterozygous single plant via seeds. To achieve this, short RNA fragments are (for example) introduced temporarily or permanently into the cell, which prevent this.
"Early Flowering"	In apple cultivation, this means gene transfer of a gene from a poplar into the apple, so that a flower induction takes place during first growth already. This can result in accelerated generation cycles. This transgene is eliminated later on, so that it is no longer present in the final product.
CMS Sterility without restoration	Use of CMS without the restoration of the pollen fertility prevents further breeding and is therefore not allowed.
In vitro selection	Selection of individual cells, plant lines or seeds on artificial nutrient medium for a specific property, for example salt tolerance

For further information on the individual techniques, see the FiBL dossier "Techniken der Pflanzenzüchtung" 2012 in the FiBL online shop or download from: https://www.fibl.org/fileadmin/documents/shop/1200-pflanzenzuechtung.pdf