University of South Bohemia in České Budějovice

Faculty of Science

Traditional plant breeding, advanced biotechnological engineering with emphasis on GM crops and environmental risk assessment of GMs

Bachelor thesis

Klára Kopicová, Bc.

Supervised by RNDr. Zdeňka Svobodová, Ph.D.

České Budějovice 2020

Kopicová, K., 2020: Traditional plant breeding, advanced biotechnological engineering with emphasis on GM crops and environmental risk assessment of GMs. Bc. Thesis, [in English.] – 57p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation:

While searching for the best approach that would ensure an adequate supplement of the current still growing world population, the national governments support different strategies to multiply crop production and to stabilize profits. It is obvious that traditional plant breeding techniques are not capable to meet this challenge. Hence, scientists developed an advanced plant breeding techniques that cope with the adversity of the environment (e. g. pest and weed pressure, drought, salinity) as well as satisfy major needs of human society (e. g. sufficient yields, nutrient content). However, advanced bioengineering arouses distrust in consumers. This review compares both, the advanced plant breeding techniques with traditional breeding approaches; and the debate is further extended by critical assessment. Further focusing on genetic modifications (GMs) and genetically modified (GM) crops, this study describes a status of their growing on the example of two completely different strategies – strategy of the United States (U.S.) and the European Union (EU). Additionally, this thesis suggests a new design for testing of GM crops environmental risk assessment (ERA) – more precisely, a very little explored, potential transfer of GM gene constructs into invertebrates.

Prohlašuji, že svoji bakalářskou práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své bakalářské práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

Date, Location: **Signature:**

22-05-2020, České Budějovice

Keywords

Agricultural crops, advanced biotechnological engineering, environmental project, environmental risk assessment, genetic modification, genetic transfer, mutation, recombination, selection, traditional plant breeding.

Acknowledgment

I would like to express my gratitude to my supervisor, Zdeňka Svobodová, who guided me and helped me all the way long, and to Doc. Mgr. Eva Kaštovská, Ph.D. for her valuable comments at the time of writing.

List of content

1. Preface

It was a Californian company, Calgene, who in 1992 commercially released the first series of GM crops (Zhang et al., 2016). Since then, aiming at the current industrial and agricultural demands, this progressive biotechnological approach has noticed immediate expansion worldwide. As summarized by International Service for the Acquisition of Agri-biotech Applications (ISAAA, 2018a): five industrial and 21 developing countries currently give rise to biotech crops , whereas 44 additional countries (including 26 members of EU) only actively support their import; in contrast to 1.7 million hectares in 1996 (first year of commercial growing of GM crops), the global area with GM crops was 2.5 billion hectares in 2018. Five world largest GMs producers (ordered from the smallest): India, Canada, Argentina, Brazil, and the U.S. that dominate the GM crops production by growing them at 75 million hectares. These five GMs giants represent 91 % of the total global GMs area; which means that nearly 26 % of the current world population avails of GM agriculture (ISAAA, 2018a).

These statistics underline not only the long-termed continuum of GM crops, but also their "one-sided" popularity. Despite the current world trend, which is in general very supportive towards planting GM crops, the majority of European countries mainly refuse to grow them on their agricultural land. There are only few exceptions like the Spanish GM corn. Nonetheless, the size of Spanish fields cannot be seriously compared with any of large GMs world producers: Spain manages only 0.1 million hectares of GM fields; it has 17^{th} place between world producers (ISAAA, 2018).

Together with still developing genetic instruments, GM approaches further strengthen their position against the traditional (non-GM) management. GM products represent an innovative market article embracing new challenges: GMs producers face on economic issues connected with high input costs; and potential GMs costumers question the possible health and environmental risk assessments.

The majority of ecological concerns turn to how an ecosystem reacts to GM-management and what are its possible consequences. To fully accept the novelty of GM approach, its profits must overbalance not only economic difficulties, political debates, and bioethics, but also these environmental aspects.

In this thesis, by comparing them against each other, I am presenting a systematic overview of all: basic, more advanced, and new plant breeding techniques (NPBT) which stayed behind the success and later expansion of still progressing GM agricultural management.
I further describe and evaluate the currently most GM-friendly policy of the U.S. in comparison to a rather GM-sceptical European attitude. The second part of this thesis refers to a proposal that designs a new methodology for testing of gene transfer from a GM corn to invertebral species.

2. Introduction

Since humans domesticated first corn at the region of Fertile Crescent 8400 years ago (Bilgic et al., 2016), their lifestyle has transformed into full settlement characterized by a continuous population growth and locally-bound agriculture (Kılınç et al., 2016). First farmers then unintentionally laid down the basics of first traditional breeding techniques when selected the most advantageous crop forms. Without knowing deeper genetic principles, small holders preferred targeted crops based on the internally encoded potential of possibly highest profit.

2.1. Plant and its heritable material

Each self-reproducing organism carries deoxyribonucleic acid (DNA) as its genetic material (Alberts et al., 2014). The polymeric DNA consists of four basic monomers that differ in their structure and are known as bases (adenine, guanine, cytosine, and thymine) (Jobling et al., 2014). The majority of current modern plant breeding approaches aim their targets at this monomeric DNA level.

If plant breeding, it is very important to understand the basic proteosynthetic model that forms the final nature of plant: DNA bases provide a template that is transcribed into the second polymeric form, ribonucleic acid (RNA) (Soultanas, 2011). RNA complexes are further active in protein translation.

After finishing their conformation changes, the proteins works as structural or functional units in each living individual (Alberts et al., 2014). Phenotype is then a comprehensive result of allover protein assemblies and their complex functional manifestations (Geiler-Samerotte et al., 2013). Epigenetic is a next factor that can further significantly affect the phenotype (Pikaard & Scheid, 2014).

To fully characterise main plant specifications, it is also required to mention a totipotency of somatic cells. The plant totipotency is the capability of each plant somatic cell to initiate the complete organogenesis resulting in the complete mother plant clone (Fehér, 2019). This plant plasticity is very advantaging for the regeneration of cell and tissue cultures that has also become a stable part of culture-based-on approaches in food and cosmetic industry (Eibl et al., 2018). However, its actual contribution to current advanced breeding methods is rather minor, and therefore will not be discussed here.

2.2. Evolutionary selective approach

Evolution enables survival of those, who adapt quickly enough in reaction to changing circumstances, and who are able to pass this adaptability to their offspring. These adaptable phenotypic characteristics are passed by encoded in DNA (Alberts et al., 2014). Before any human-by-initiated breeding activities have started, an "evolutionary selective" approach was the only source of plant variability. The evolutionary selective approach may serve as comparative default stage for current modern breeding methods.

The only natural chance of how to preserve adaptability are sudden modifications (Alberts et al., 2014). To reach that, the evolutionary modulating relies on two important tools: spontaneous or induced mutations and the selection (Kreuzer & Massey, 2014).

2.2.1. Spontaneous and induced mutations

Mutation is any bases change that suddenly occurs in DNA/RNA, in contrast to its previous template. Several types of modifications are grouped into categories based on their character or structure (described in detail in Alberts et al. 2014).

Mutations can interpose the coding or non-coding parts of the genome. All coding parts are directly responsible for the protein synthesis. The non-coding parts rather fulfil the regulative demands. Mutations can be both, benefiting or lethal (Mahdieh & Rabbani, 2013).

They occur either spontaneously via enzymatic error, transposons and insertion sequence elements (Chatterjee & Walker, 2017), and as reaction to external physical signal (UV radiation) or chemical compounds (alkylating and intercalating agents).

The specific type of mutation is meiotic recombination. It is source of genetic variability. It is a process of a spontaneous reciprocal DNA exchange between parental homologous chromosomes during meiosis (Hunter, 2015). The meiotic recombination accumulates genotypic changes and determines the final intergenerational variability (Hunter, 2015).

Genetic changes can be successfully passed on to the next generation only when they affect regions in the germ line of gametes, escape from all presented miss-match DNA repairing mechanisms, are compatible with fertility and survive till the reproductive age (Jobling et al., 2014).

2.2.2. Natural selection

Natural selection is a process when individuals with the best adaptive phenotype (with higher chances to reproduce) better survive over individuals carrying the adaptability-worsening characteristics (Griffiths, 2002). As Grifftihs (2002) further explains, since Charles Darwin's time, three main principles have been accepted to explain the natural selection: the principle of variation (1), the principle of heredity (2), the principle of selection (3). The principle of variation considers the phenotypical similarity and differences that are observable between individuals of one single species generation. The second principle, the principle of heredity explains the obvious children-parents inter-similarity which cannot be traced between unrelated subjects. The third (most decisive) principle of selection comments advantaging individuals with highest adaptability and reproductive potential. Thanks to natural selection, the benefiting characteristics are passed more frequently to offspring, and later dominate in population.

2.3. Human selective approach

Natural crossings, the first way of an artificial crop selection, relies fully on the accidental natural changes when aiming mainly at the favourable phenotypic markers like the colour or sizes of fruits.

However, our current fast growing society faces on new, more alarming demands: rough estimations suggest that the global number of Earth's inhabitants will increase up to ca. 9 billion people by the middle of the century (Godfray et al., 2010). So concerning supplying, the current breeding values are different: a sufficient quantity and high nourish value of food around the world (Brookes, 2018). By using target DNA modifications, the current approaches of advanced biotechnological engineering are able to successfully produce those of additional features.

2.3.1. Natural crossing

Natural crossing is the most primitive reproductive incident that relies fully on principles of natural selection. Being naturally crossed, a plant offspring in the field results from a mixture of self- and cross-pollination (Stephens & Finkner, 1953).

The global diversity then arises thanks to attacks of the seasonal variability and still new diseases or environmental outbreaks across different regions. Local attempts of adaptation bring dissimilarities in between two (e.g. geographically separated) populations, previously belonging to the one common group.

The in vitro methods of natural plant crosses can be simply run as follows (Sahadevan & Namboodir, 1963): based on previous observations, the coloured-marked pollens (carrying the improving characteristics) are selected, and then cross-fertilized giving the parental generation. Later, during harvesting, hundreds of single parental plants are again selected and preserved for additional growing. The adult variations of their seeds finally carry the dominant target characteristics that may be further used in the next round of cross-fertilisations.

Those of procedures performed by Agricultural Research Stations mostly took up to six seasons (Sahadevan & Namboodir, 1963). These simulations only confirm the lengthiness of breeding process that relies only on sudden natural modifications.

The current calculations (Whitlock & Gomulkiewicz, 2005) considered the fixation of new mutations as very unique (only a 2% chance of eventual fixation. Despite this very low mutation's ration (occasionally also weakened by e.g. the lack of pollen vectors [Stephens & Finkner, 1953], or increased because of plant eldering [D'Amato & Hoffmann-Ostenhof, 1956]), even natural crosses may provide very useful products.

An outstanding example of that enabling the Green Revolution, the semi-dwarf varieties of rice, weas successfully derived from both, natural and artificial independently induced modifications within the gene for gibberellin 20-oxidase (Zakir, 2018).

As Breseghello and Coelho (2013) comment, before the laws of heredity were defined, natural crossing was the only possible way of breeding, applied over and over again, until the time of first proper breeding experiments that were carried out by Kölreuter in the 1760s, as mentioned by (Roberts, 1929).

2.3.1.1. Landraces

Landraces represent the initial, regionally specific plant products resulting from the long-termed cultivations. Being formed fully naturally, landraces represent the consequences of locally typical biotic and abiotic factors, in most cases cultivated to fulfil local consumption demands (Breseghello & Coelho, 2013).

As Breseghello and Coelho (2013) further claim, the landraces share three major characteristics: a variable genetic background among populations contrasting to the homogeneous genetic among individuals; their adjustment to the local ecological and environmental circumstances; and finally, being a favourite fraction of the regional cuisine. The most advantageous benefit of landraces is their very well balanced plant population stability. Such a locally reached equilibrium helps to maintain the primary ecological setting of the region. This naturally balanced selection is very challenging to be hold while growing the modern artificial hybrids (Breseghello & Coelho, 2013).

The examples of landraces-based-on management can be nowadays found all around the world. For example in Brazil, the local variant of common beans (*Phaseolus vulgaris L.*), referred as Serro Azul, took a part in the regional complex research, whose results aimed at a new potential of genetic crosses in the region (Konzen & Tsai, 2012).

In the Kashmir province, a descriptive survey revealed approximately four dozens of different landraces of Asian rice (*Oryza sativa L.)* that were without artificial interventions recorded in such high-altitude regions as Indian Himalayan (Sultan & Subba Rao, 2013).

Landraces nowadays represent the purest line among natural crops. Until proven otherwise, modern cultivars are questionable at their quality and possible harmful side effects. Therefore, landraces serve as negative control group (an original unaffected plant stage) which any breeding results are compared to (Kaplan & Fehrer, 2006). In majority, these tests are required to be so transparent, that it is possible to follow their final reports via different internet databases (e.g. available from: sasa.gov.uk).

2.3.2. Modern plant breeding

After all domestication processes, humans currently use only 15-20 plant species for food production from the complete world plant reservoirs o 200,000 plant species (Sikora et al., 2011). As further claimed by Sikora et al. (2011), such a "crop evolution" significantly reduced the genetic variability among crops. Hence, breeders have started to aim at its increasing: in 1920s, Stadler (1928) firstly noticed increased diversity among seedlings of barley after X-ray treatment. Since then, plant breeders have started to take advantage of another mutagenesis: physical (gamma and neutron radiation) and chemical (ethyl methane sulfonate, azide or methylnitrosourea) (Sikora et al., 2011).

2.3.2.1. Cultivars

Cultivars represent the plant products of modern breeding techniques cultivated on the basis of artificial induced mutations. More than 2,200 cultivars (IAUE-GEF-HH Committee & NRC, 2004) were produced by modern breeding, as possible to verify directly in the FAO/IAEA Mutant Variety Database (available from: mvd.iaea.org) (Ahloowalia & Maluszynski, 2001). Modern cultivars are utilized on a broad-spectrum level, although the agriculture sector still represents the biggest customer (Oliver, 2014).

Physical and chemical modifiers cause the changes in cultivars unpredictably and in the uncontrollable way (IAUE-GEF-HH Committee & NRC, 2004). Achieving "the maximum spatial homogeneity", modern cultivars respect the highest "breeding and agricultural paradigm", but lose their ability for a long-term adaptation (Breseghello & Coelho, 2013).

If facing on stress (especially diseases), modern cultivars (monomorphic for most of their genes) are unable to adjust adequately (Breseghello & Coelho, 2013). With that said, the modern breeding methods basically supress the sensitiveness in cultivars making them intact. Paradoxically, the importance of the local habitat has been revealed as the most influential source of variation (44,5%) compared to the rest of external modifiers like e.g. weather conditions (Ewing et al., 2019).

One outstanding example among modern cultivars represents a modified variety of barley, called Diamond, that overmastered the market in 1965 (Bouma, 1967). The author, a Czech breeder Josef Bouma, exposed the wild form of barley to very high amount of radiation. After selecting the morphologically most suitable modified grains, a new form of the barley was set up. It consisted of shorter internodes, which were not as much decumbent as the previous ones, and strongly remarkable taste (Bouma, 1967)

Thanks to this experiment, 150 of barley lineages based on Diamond could have been cultivated in Europe (Kuczyńska et al., 2013).

2.3.3. Advanced biotechnological engineering

Low adaptability of cultivars and unpredictable nature of modern plant breeding methods resulted in search of the next self-monitored approach based on the genetic engineering. Advanced bioengineering produces GM hybrids so, that it inserts a DNA-motif in at its specific position in the genome. These structural modifications happen basically in three possible ways: transgenic, cisgenic or intragenic (Kamle et al., 2017).

Whereas the "traditional" GM hybrids are formed by the method of transgenesis, the newly available NPBT rather display a higher success in gene transfer in comparison with transgenesis.

Genetic constructs usually consist of two parts: a gene of additional characteristic, e.g. gene variants of Cry proteins or Cyt toxins (Kamle et al., 2017) and the repair fractions that are responsible for a target implement of the insert (Cathomen, 2004). Considering the latter, several repairing pathways take the responsibility for the DNA re-unifying, and can be therefore used as inserting vector. Namely, there are four most useful variants: the mismatch repair (MMR), homologous direct recombination (HDR), non-homologous end joining (NHEJ), and base /nucleotide excision repair (B/NER) (Ceccaldi et al., 2016).

The first, pilot biotechnological strategies have been developed by using viral vectors together with the HDR, however, these methods generally had very low efficiencies to be further extended (Cathomen, 2004). Current alternatives introduce DNA double-strand breaks (DSBs) straight within the locus of interest, which is then repaired either by increased expression of NHEJ (Bibikova et al., 2002) sometimes also extended by HDR-mediated genes (Jasin, 1996).

Nowadays, four standardized DNA-binding proteins have been designed to introduce site-specific DSBs: mega-nucleases on the basis of microbial mobile genetic elements (Smith et al., 2006); zinc-finger nucleases (ZFNs) derived from the eukaryotic transcription factors (Urnov et al., 2005); transcription activator-like effectors (TALEs) from *Xanthomonas* bacteria (Christian et al., 2010); and most recently the RNA-guided DNA endonuclease Cas9 from the type II bacterial adaptive immune system CRISPR (Cong et al., 2013).

Although especially ZFNs, TALEs, and CRISPR/Cas opened the doors to a completely revolutionary way of plant breeding, there are also other methods available, like the marker assisted selection (MAS) or RNA interference (RNAi) that can be also classified to NPBT, but work on completely different principles.

2.3.3.1. Transgenesis

Transgenesis transformations can be mediated either via bacterial messengers (*Agrobacterium tumefaciens*), via direct gene transfer into protoplasts, or by the particle bombardment; and although these methods have a low mutagenesis-success rate, the most of commercially available GM crops were originated this way (Podevin et al., 2012).

2.3.3.2. Zinc-fingers

Zinc-fingers (ZFs) are a man-made tool of genetic engineering that consists of two DNA-binding domains. Each domain contains one target specific DNA sequence (mostly 3-6 individual finger motifs of three base pairs), and one (5-7-nucleotides) catalytic spacer of the nuclease domain of the restriction enzyme (Yee, 2016).

Whereas the target specific part recognizes its binding motif and based on the compatibility associates with the DNA template, the spacer causes the catalytic reaction which ends with site-specific DSBs. The inter-compatibility of ZFNs happens through protein-DNA cross-talks resulting in DSBs that are consequently repaired by either NHEJ or HDR (Yee, 2016). Currently, a number of families of ZFs are available (Miller et al., 2011) and customized to be applied in wide range of model organisms like *Drosophila melanogaster*, *Caenorhabditis elegans*, plants, or human cells (Maeder et al., 2008).

There are also several potential disadvantages of ZFN approach: not only that the ZFN domains bind randomly (only every 200bps), but the consequently produced DSBs also happen randomly, which consequently gives to arise to the off-targets residuals (Gupta & Musunuru, 2014). Despite that, ZFN tools are still being used for in various novels of modern crop arrangements (Davies et al., 2017). For example, in 2016 in Greece, a ZFN-based-on targeted gene mutagenesis in tomato seeds (*Solanum lycopersicum*) increased the heterochronic phenotypic diversity of both, plant and fruit organs (Hilioti et al., 2016).

2.3.3.3. TALEs

Transcription activator–like effector (TALE) proteins build the largest family of gene effectors (Boch & Bonas, 2010). Alike to ZFs, the TALE structure consists of a central repeat domain (repeated units of typically 34 amino acids) and catalytic domain that recognizes the DNA target with two parallel, left and right TALE sequences (Moscou & Bogdanove, 2009). Instead of grouping three nucleotides together (as ZFs clamp), the TALEs linkage procedure uses individually each of its structural nucleotides separately. The reliability and success of TALE nucleases is therefore defined by much higher specify.
Genome cleavage is then mediated by a catalytic restriction enzyme (Fok1) and a successful construct insertion is again induced by NHEJ or HRD (Miller et al., 2011).

Artificial designing of TALE nucleases (TALENs) is far less challenging than of ZFs; on the other hand, they catalyse DSBs so insufficiently that even their previously favourite size (18-bp sequences or even longer) loses its attractiveness (Gupta & Musunuru, 2014).

2.3.3.4. CRISPR-Cas

The CRISPR-Cas system combines the advantages of the clustered regularly interspaced short (30-40 base pairs) palindromic repeats (CRISPR) together with its associated proteins (Cas9) (Marraffini, 2015). Since 2013, the expression of a CRISPR-Cas has been easily targeted to any genomic location of choice - yeast, plants, mammals, even humans (Mali et al., 2013) with a help of a short RNA guide mediating the genome cleavage (Cong et al., 2013).

The system runs in two phases: firstly, an external sequence is incorporated into the bacterial CRISPR array, and consequently transcribed by RNA polymerase III into a short guide RNA, which includes one crucial element: the spacer sequence. The second phase works with the catalytic function of Cas9-endonuclease (being associated with short guide RNAs) that based on the compatibility cleavages all targets bound to the spacer (Marraffini, 2015). DSBs catalysed by Cas9 are later processed through either NHEJ or HDR (Cong et al., 2013).

2.3.3.5. Marker Assisted Selection

MAS is a method based on indirect selecting; a target gene of trait of interest is (based on its compatibility) associated with a marker (morphological, biochemical or DNA/RNA variation). When an adult variety later displays the marker like a resistance to diseases and pest, or a tolerance against stress), such an individual is then selected and preferably used in the next conventional breeding (Ben-Ari & Lavi, 2012).

2.3.3.6. RNA interference

RNAi, a silencing complex interferes with the target regions of mRNAs in order to eliminate them - based on the degradation cell-machinery that destroys any possible double-strand(ds) RNA to prevent possible expression of doubled structures (Fire et al., 1998).

2.3.3.7. Crop production based on advanced biological engineering

The "traditional" GM crops (designed to fight with pest and weed) resulting from transgenesis are being planted all around the world in many variants. In the majority of the world countries, GM crops have been grown and distributed without restrictions - somewhere under strict conditions of coexistence only. However, there is still a simultaneous debate (running in many countries) whether NPBTs should be implemented to the same governmental regulations (Whelan & Lema, 2015)

The more advanced NPBTs are gradually used as the only fully capable solution to prevent various numbers of pests and diseases. Furthermore, they support the crop productivity; two cases of the successfully increased crop production have been reported so far - in rice and tomatoes (CRISPR-Cas; Baltes et al., 2017). NPBTs are also able to successfully increase the resistance of plants to their pathogens - e.g. in rice against bacterial *Xanthomonas oryzae* attacks (TALENs) or in barley, tomato or wheat against *Blumeria graminis* fungal diseases (TALENs, Baltes et al., 2017). NPBT can be also used in fights against weed. Using of CRISPR/Cas this time together with ZFNs helped to introduce mutations into target regions of acetolatctate synthase genes, which afterwards appeared to be very efficient in enhancing of plant tolerance (in tobacco, soybean, maize, or rice) to herbicides (Baltes et al., 2017).

As a part of the industrial sector, NPBT are able to produce e.g. fruits with an increased anthocyanin pigment expression (functioning as antioxidants; using TALENs and CRISPR/Cas), oils with improved composition of fatty acid from soybean (using TALENs) and potatoes, in which the natural accumulation of carcinogenic acrylamide (mostly while storage) was reduced by a knockout of sugar-reducing invertase gene (using TALENs) (Baltes et al., 2017).

MAS was extensively researched and proposed for plant breeding, especially for purposes of fight against diseases in cereals (rust fungi), potato (late blight) and beet (Rhizomania disease); however, this trend has been recently reported as very weakening (Ricroch et al., 2016). In contrast to that, RNAi has been still applied, especially, when dealing with diseases in cereals (rust) or potato (late blight, viruses') (Ricroch et al., 2016).

2.4. Applying of genetic modifications in agriculture

In 1989, the Health and Safe Executive of United Kingdom (UK) published (and fifth-times has re-edited since then) a guideline on the genetically modified organisms (GMOs). In terms of that, a genetic modification is a material genetic alternation in that organism that is not possible to occur naturally via mating or natural recombination (Report of Health and Safety Executive, 2014).

The first successful endonuclease-based-on transformation was performed when newly constructed plasmids were inserted into *Escherichia coli* (Cohen et al., 1973). But the key moment for genetic modifications came seven years later, in 1980, when the U.S. Food and Drug Administration (U.S. FDA) Supreme Court officially allowed to patent genetically modified bacteria (Chakrabarty, 2010). Since then, an official permission over GM ownerships-rights (Roorda, 2017) has enabled that (inter-)national developers were allowed to continue with GM experiments. In 1987, researchers from Calgene institute in Davis successfully engineered the first GM tomato fruit, referred as Flavr Savr (Bruening & Lyons, 2000).

After that, researchers genetically suppressed the expression of an enzyme polygalacturonase (responsible for dissolving cell-wall pectin and consequent softness of fruits), and increased the fruit resistance against damage by transport (Bruening & Lyons, 2000).

In 1990's, beside commercially preferred features - like a nutrient-enriched Golden rice - Swiss-German collaboration (Potrykus, 2001), or a tear-supressing onion, New Zealand, (Eady et al., 2008) - two very alarming goals for GM technologies were defined: an increase of crop resistance against pests and a herbicide tolerance. Nowadays, the most commonly inserted transgenes to crop are responsible for insect resistance and herbicide tolerance.

Insect resistance is based on crystalline (Cry) delta proteins (toxins) from bacteria *Bacillus thuringiensis*. Cry toxins are endotoxic to certain invertebrates (Bravo et al., 2007). These "natural insecticides" popular in organic farming are very special in comparison to other conventional insecticides due to their limited toxicity affecting only minor fraction of related species. Specific pH levels, enzymes, and midgut receptors are required to specific activation of Cry toxins in invertebrate's midgut. Then they bind to specific locations on the cadherin-like proteins present on the epithelial cells of the midgut and form ion channels allowing outflow of potassium ions. The affected epithelial cells subsequently lyse and die creating holes in midgut; all of this is consequently lethal for the caterpillars (Palma et al., 2014).

The most common herbicide tolerance in GM crops is tolerance to glyphosate. It is based on transgen for protein CP4 EPSPS (5-enolpyruvylshikimate 3-phosphate) found in *Agrobacterium* sp. CP4. EPSPS is enzyme catalysing synthetisation of aromatic amino acids in plants and bacteria. Glyphosate is a competitive inhibitor for EPSPS. However EPSPS from *Agrobacterium* sp. CP4 is not sensitive to glyphosate (Funke et al., 2006). Tolerance to glufosinate is the second most common type of herbicide tolerance in GM crops. Glufosinate with phosphinothricin as active ingredient targets glutamine synthetase. Glufosinate-tolerant crops have been modified by insertion of phosphinothricin acetyltransferase that detoxifying glufosinate (Carbonari et al., 2016).

2.4.1. GM crops

At these days, the major GM production is represented by soybeans (50%; within 10 countries), corn (30.7%; within 14 countries), cotton (13%; within 15 countries), canola (5.3%; within 3 countries) and others: sugar beets, papaya, squash, eggplant, potatoes, and apples (1%) (ISAAA, 2018b).

The amount of till these days developed GM crop varieties is large; under the management of ISAAA, an online database publishes a list of those of GM crop varieties currently available on the world market (available from isaaa.org/gmaprovaldatabase/croplist). This study describes in detail three most popular of them: GM soybeans, GM corn, and Bt cotton.

GM Soybeans are economically very important since being a source of both, industrial (biofuel production) and food products (supplement for humans and animals) (Homrich et al., 2012). Because of that, GM soybeans have become a very desired target of genetic improvements, firstly applied in very late 1980s (Christou et al., 1988; Hinchee et al., 1988). The early produced lineages of commercial GM soybeans were predominantly aiming at such qualitative traits as being herbicide tolerant and insect resistant (Homrich et al., 2012). As Homrich et al. (2012) also discuss, in contrast to that, current GM soybean production is aimed mainly at the enhanced nutrition value, and the food functionality for medical and industrial purposes.

Approximately 85% of the world soybean areal was in 2011/2012 managed with a GM technology (Homrich et al., 2012).. Economical study from South America summarized the financial benefit of south-American farmers (based on their total profit from GM-soybeans): taken together, locals earned 4.37 billion dollars extra in 2016; and 54.6 billion dollars extra in total, since 1996 (Brookes & Barfoot, 2018b).

As Brookes and Barfoot (2018a) further explain within their later environmental analysis of previously described data, beside the financial profit itself, the GM-soybean-technology-based-on oil production has also significantly reduced the local fuel use (reducing of car use); which finally resulted in a significant decrease of the greenhouse gas emissions from the GM cropping area. As they both also add, in 2017/2018, this was equivalent to removing 3.3 million cars from the roads (Brookes & Barfoot, 2018a).

GM corn plants (so-called Bt corn due to expressing insecticidal Cry toxins from *Bacillus thuringiensis* Berliner) have been cultivated since 1996 (Ferry & Gatehouse, 2009). Bt corn MON810 (resistant to *Ostrinia nubilalis*) is one of two GM plants that have been cultivated even in the European Union, mainly in Spain (Gómez-Barbero et al., 2008). As observable in executive summary done by Brookes (2007), in certain regions across EU, Bt corn has truly caused important improvements in grain quality when significantly reduced levels of grain mycotoxins.

GM corn resistant to lepidopteran pest has caused important improvements in grain quality, when significantly reduced levels of grain mycotoxins (Brookes 2007). Even non-GM-corn growers have profits from growing GM corn resistant to lepidopteran pest in their neighbourhood; GM corn decreases level of damage in their non-GM corn field (Hutchison et al., 2010).

Bt cotton is an insect-resistant GM crop carrying genetic constructs from *B. thuringiensis* expressing proteins to vanquish effects of the bollworms. Those of actively designed anti-insecticidal crops formatted from *B. thuringiensis* have been processing to face on commercial biological pesticides, and further re-evaluating from the environmental point of view for over 50 years (Koch et al., 2015).

In 2012, an Indian study analysed the regional dynamic of Bt cotton benefit together with the economic influence on locals (Kathage & Qaim, 2012). Having assembled their measurements between 2002 and 2008, Kathage and Qaim (2012) were finally able to show final trends within those of six years. According to them, Bt technology reduced pest damage in cotton fields by 24% per acre and increased the cotton profit by 50% gain in contrast to previous years (Kathage & Qaim, 2012).

2.4.2. Critical statements referred to GM crops

The artificial nature of GM approach invokes a number of critical discussions. Moreover, there are many other issues that are connected with GM crops growing although they are often rather a result of human factor failure that occurs in other sectors as well.

Herbicide tolerant crops can cause a shift in local weed spectrum that could lead, instead of an immediate decrease of herbicide use, to the application of herbicides that can even overcome the level before growing herbicide tolerant crop (Benbrook, 2012).

Since GM crop became an economical topic in the first place, the illegal market has immediately started to react by extending their offers and selling unapproved GM seeds. For example in India, 80 % of growth GM cotton varieties appeared to be unlicensed after three years from the official approval of insect resistant GM cotton (Jayaraman, 2004).

Opponents of GM technology often argue that introduction of GM crop raised up the numbers of suicides enormously in India. However, the situation in the cotton-seed-sector has already evolved very unstably before introduction of GM crops due to the previous, very intensive monopolization (Thomas & De Tavernier, 2017).

There are also several publications that found serious negative effect of GM crops or connected with GM crops. These publications were largely repeated without confirmation of the original result or even revealed as a hoax. However original results are still used for argumentation in various negotiations, even at a high political level. One example is publication of Séralini et al. (2012).

They found serious negative effect of management connected with growing herbicide tolerant corn – application of glyphosate that was served in the diet to rats. Séralini's study has provoked a number of responses from the scientific community (e. g. Resnik, 2015), resulted in several review GM-crop-tests on animals (e. g. Snell et al. 2012), in statistical review of Jiang et al. (2019) and launched a call for further studies about effect of herbicide tolerance to animals from European Food Safety Authority (FSA). Moreover, since Séralini's refused to publish raw data, many journals have made a demand to provide raw data from every single publication that they accept. This everything happens despite the fact that their results were marked at least as unreliable (e. g. MONSANTO, 2005).

2.4.3. Comparison of GM-crop legislation between U.S. and EU

In the U.S., although being the world biggest GM producer, GM farmers must follow any federal legislation specified for GMOs only: according to the Federal Register (F.R.), GM regulations belong in general to the same category as other biological technologies (e.g. as aerosol technologies or nanotechnologies), managed by The Coordinated Framework for Regulation of Biotechnology published in 1986 (Federal Register of United States, 1986). The regulations of GM crops (considering the conventional products themselves rather than the process of their origin) are in the U.S. divided among the U.S. Department of Agriculture (USDA); the Food and Drug Administration (FDA); and the Environmental Protection Agency (EPA) (FDA, 2020).

If comparing the U.S. to the rest of the world, the GM regulations there are rather liberating. Being intensively supporting towards GM research and GM applications, U.S. contradicts the EU. The regulations of GM crops (and other GMOs) were established in EU after 2001, and have not become less strict since then. Especially, a free release of GM crops into environment is very strictly judged by European Commission (EC) (Brandt, 2003). Manipulations with GM crops within the EU territory are strictly regulated and controlled by multiple monitoring. For example, the European Directive 2001/18/EC, prescribes very precisely circumstances when GM crops are allowed to be exposed to the environment: firstly, for experimental purposes, and secondly, for the purpose of placing GM crops on the market.

The European Regulation No. 1829/2003 differentiates strictly food and feed products in terms of being considered as directly GM on their own, or secondly being produced with a help of GM source. The GM crops waiting for a cultivation permit are considered by the EC. If the GM crop is approved for growing, this permission is valid for all EU members (E.C., 2007). However, approval for European members to cultivate GM crops does mostly not come at all (Ujj, 2016).

In Europe, there are just two GM crops approved for cultivation: GM corn MON810 resistant to lepidopteran pest *Ostrinia nubilalis*, and GM potato Amflora producing amylopectin that is processed to waxy potato starch (EC, 2013). However, in 2012, German Baden Aniline and Soda Factory (BASF), the Amflora developer, itself ended the entire project. These restrictions resulted in very limited amount of GM crops producers among EU members: Spain and Portugal (Brookes, 2019).

Different attitudes of the U.S. and the EU politics may origin at that times when the first American colonists left Europe and its old traditions - practice of tillage, which appeared to be a very helpful tool while weed destroying (Ujj, 2016). Another dividing element might have been an experiencing of two world wars that could have left a sense of technology's dangers in European inhabitants; although the same aspect could have helped to raise up the U.S. national confidence and beliefs in scientific and technological progress (Ujj, 2016).

2.4.4. Application of GM plants in Czech Republic

Because of its membership in EU since 25th February 2004, Czech Republic (CZE) has also duty to follow Act 78/2004 Sb. and Act 209/2004 about the treatment with GMO and genetic product, agreed by the Ministry of the Environment (M.E.), the Ministry of Health and the Ministry of Agriculture (M.A.) (M.E., 2004a, 2004b).

As both acts in detail add, each GMO treatment must be conducted under the control of specialists, further well documented, and significant sub-results including side-effects must be regularly reported - if new information related to GMO was published, M.E. is allowed to change conditions, break the GMO application or cancel the GMO application completely.

Within EU, CZE used to belong to the most experienced GM crop producers having planted the varieties of GM corn MON810 since 2005 (Stratilová & Jedličková, 2016). Starting 2009, the Czech areal with GM management has been continuously reducing, till 2017, when CZE gained the GM-free status (Trnková et al., 2019).

If stepwise following the annual reports of M. A. (available from: eagri.cz/public/web/mze/rostlinna-vyroba/gmo-geneticky-modifikovane-organismy/-archiv), since the GM revolution started, CZE has focused on both of two GM crops approved to be grown in the EU: Bt corn MON810 and Amflora potatoes. According to those of annual reports of M.A., whereas at the beginning of 2005, 51 producers planted Bt corn at 150 hectares, in 2016, the last producer announced his Bt-corn-based-on management at 75 ha (Pardubice district). Since then, any other official producers have been registered (Trnková et al., 2019).

As M.A. within its reports further publishes, the second attempt of GM planting in CZE - Amflora potatoes for non-food industry - ran only in 2010 (at 150 hectares, Vysočina disctrict), and since then has never appeared in CZE again.

2.5. Environmental risk assessment of GM crop

The EU precautionary principles assume that planting of GM crops cannot be considered as environmentally completely safe, and therefore have initiated a number of studies focusing on the impact of herbicide tolerant crops to the environment.

Insect resistant GM crops are continuously prepared to kill specific target insect species but there are many other invertebrate species that are in direct contact with the Cry toxins by feeding on a GM plants; via (non-)target prey or from soil/water (Leslie et al., 2009).

Several insect species come into contact with Cry toxins with a higher probability than the others. For example in aphids, a limited, or rather zero contain of Cry toxins was monitored (Raps et al., 2001). In case of on-whole-plant-feeding herbivores matters if the concentration of Cry toxin reaches the higher levels, e. g. larvae of pest *Spodoptera littoralis* (Svobodová et al., 2017), as was also recorded in mites (Álvarez-Alfageme et al., 2008).

In modern GM crops, the expression of Cry toxins in pollen is low, which makes GM crops safe for pollinators. Remainig safe is an essential component of the ecosystem. The results of many studies confirmed the safety status of GM pollen for bees; meta-analysis done by (Duan et al., 2008).

Predators and parasitoids of various invertebrates are exposed to different amounts of Cry toxins. Cry toxins have been found in their bodies, e. g. coccinellid *Adalia bipunctata* (Álvarez-Alfageme et al., 2011). Several studies informed about negative effects of Cry toxin on predators and parasitoids. However these results can be explained by fact that predators were fed with target species representing nutritionally poor food source - indirect effect (Lawo et al., 2010). Similarly, parasitoid's larvae negatively affected by GM crops developed in nutritionally poor food source. Parasitoids have narrow specialization so they are the most endangered group. Indeed, the abundance of parasitoids specialized on target pest is often lower in GM crops. They are affected by host loss or their reduced fitness (Wolfenbarger et al., 2008). However parasitoids of non-target pests are not affected (Pons et al., 2011).

Soil decomposers and microbial communities may come in contact with Cry toxins from plant residues and from root exudates. Several studies did not show any negative effect of cultivation of GM crops on soil fauna, e. g. earthworms (Hönemann & Nentwig, 2009).

With some differences in different conditions, Cry toxins degrade in soil. However, their small amount binds to clay and humic particles and retain their insecticidal activity being resistant to biodegradation and also to any effort to extract it (Saxena & Stotzky, 2000).

Cry toxins can be probably flushed to water with soil, pollen or plant material. Further studies are needed in this area because they are rare and those which exists inform about negative effect on life table parameters of caddisflies and *Daphnia magna* (Bøhn et al., 2008; Chambers et al., 2010).

According to the results of meta-analyses, non-target invertebrate herbivores, predators, parasitoids, and decomposers are not endangered by the presence of Cry toxins in GM crops(Marvier et al., 2007; Wolfenbarger et al., 2008).

It was shown that Cry toxins are portable in the food chain without any negative effect on its components. The question arise, if there is any mechanisms that would allowed transfer of transgene, referred here to as gene transfer (GT), from GM crops to some of above mentioned invertebrate group. This concern originating in the fear of spreading the antibiotic resistance gene (selection of successful transformation) that, besides gene for desired trait, is transferred to GM crops as well (Pontiroli et al., 2007). The process of gene transfer occurs naturally especially at the bacterial level (Maheshwari et al., 2017) but there is a very low probability that both genes could be transferred to irrelative species in the field (Keese, 2008).

This thesis is further structured as funding proposal that covers an innovative environmental project (that is also specially designed on purposes of this thesis) to be run in GM and non-GM corn fields.

3. Detecting potential GT from GM-corn fields

A detailed, 2-years-long terrain study that would focus namely on the GT from GM crops has never been performed in Czech Republic, and rarely within Europe. The first reason is probably a sceptical attitude of regional farmers towards GM. The second reason is most likely a limited (rather zero) evidences of so far detected genetic transmission in-between unrelated organism. Nonetheless, when previously sampling in GM crop fields, free forms of Cry protein have been detected in arthropods (Priesnitz et al., 2013). Regarding these recent conclusions, a new extended survey should further inspect a potential GT into arthropods that would be this time collected outside the GM field.

If free GM-constructs (genes or proteins) released from a GM-corn-plant into its surrounding, if further transferred, they can hypothetically harm the metabolism of non-target living communities. Exposing an earlier lethality, these attacked communities decrease in numbers of individuals, which can later reverse the species-distribution and destabilize the entire ecosystem. Hence, this study investigates a presence of free GM-constructs within few critical arthropod families (as categorized in chapter 3.2.3). The arthropods were selected based on two criterions: for being eukaryotic (in contrast to bacterial sampling); and if affected, they display very soon and transparently a decrease in the abundance. Being territorially limited, arthropods can be also feasibly monitored (in contrast to large habitats of vertebral species).

This project also engages selected local Czech farmers (co-partners) together with their traditional agricultural management (in detail in chapter 3.2.1.1.). A 30-months-running experiment (comparing GM and non-GM approaches) is planned to be run by using the fields of co-partners; environmentally monitored by authors of this project. This GT-GM risk assessment builds an informative platform (politic-economically fully independent) for everyone (farmers, scientific communities, and laic public).

Although the entire concept of GT between plants and invertebrates is rather provocative, as long as the biotechnological approaches develop into being more invasive (affecting the evolutionary conservative parts of genome), similar questions are becoming still more topical and should not be underrated.

3.1. Aims of the study

Stated goals:

- To directly detect a change of individual numbers of non- and targeted local vertebral species (arthropods; as categorized in chapter 3.2.3.); in the field and in a far distance (0m – 25m – 50m – 100m) from the field (as described in chapter 3.2.3).
- To directly detect a possible transfer of *Cry* gene from GM corn into non- (e.g. *Anthocoridae* or *Lepidoptera*) and targeted (e.g. *Diabrotica* species or Chrysomelidae) arthropods (as categorized in chapter 3.2.3.); in the middle of the field and in a far distance (0m – 25m – 50m – 100m) from borders of GM- and control plots.
- To directly detect a possible transfer of free forms of Cry protein (firstly expressed in GM corn, and then released) into soil; in the middle of the field and in a far distance (0m – 25m – 50m – 100m) from borders of GM- and control plots.

Stated hypotheses:

- The presence of GM-corn causes a target-specific decrease of pests (e.g. *Diabrotica virgifera virgifera* species or *Ostrinia nubilalis*). However, the non-target species should remain intact. The intensity of decreasing trend weakens with an increasing distance from the source (Bt-corn plants).
- *Cry* gene transfer between GM crops and non-target arthropods, as noticed before in (Thomson, 2001) are expected to be observable, however, in very minor fractions and low frequencies without being considered as influencing. In another words, final numbers of non-target individuals must remain comparable with numbers from the control plot.
- Free forms of released Cry proteins degrade naturally very quickly. Therefore, any (or close to zero levels of) free forms of Cry proteins are expected to be detected in soil. If a minimal presence of Cry protein detected, its amount is still expectable to weaken with an increasing distance from the source (Bt-corn plants).

3.2. Planning of the environmental study

The overall proceeding is planned to be divided into four phases: designing (1), planting and cultivation (2), sampling (3), and final analyses including a compendious evaluating (4), described in detail below.

3.2.1. Design

The project is planned for two vegetative seasons; to be run "in vivo". In terms of that, being displayed to various external interferences (natural and man-made modifiers), the experimental results will provide more reliable conclusions than if gained under artificial conditions in labs. In each step during the monitoring, a set of environmental modifiers (that could possibly affect the actual numbers of arthropods) will be recorded: local temperature, cloudiness, current precipitations, wind situation (all available from portal.chmi.cz).

Furthermore, sudden changes in soil structure (caused e.g. by erosion or acid rain) and soil composition (caused e.g. by an application of chemical modulators newly available on the market) will be recorded from the field (by interviewing field managers).

If monitoring the water situation (e.g. a free presence of Cry protein in streams or water reservoirs; nearby to experimental fields), final detections could also offer an interesting conclusions, but in that case, the final numbers of observations would be too extended. Being estimated as poorly contributing anyway, the water aspect was excluded from this comparative study.

3.2.1.1. Contact with co-operators

Before the project will start, I will contact two former GM corn producers in Czech Republic to suggest potential collective field collaboration on this project. These are: Czech agricultural company Rostěnice a.s. (Vyškov district) managing the habitat in Southern Moravia because of the long tradition of Bt corn cultivation (1) and the Czech agricultural company ZD Mořina (Pardubice district), where the last GM cultivation (GM corn with resistance against corn insect pest) existed in 2017 (2). The motivating aspect for farmers will be a renting budget for two of experimental parcels (further discussed in chapter 3.3.).

3.2.1.2. Plot establishment

Two plots will be established on localities ideally neighbouring with different habitats (suitable for monitoring of possible co-incidences) like a corn field, oilseed rape field, and forest. GM and non-GM corn will be each grown on one 0.16 ha plot (40 x 40 m^2) in localities of either South Moravia or Central Bohemia. The distance of 1 km between GM and non-GM plots will be preserved, and a buffer zone of five meters width (covered with uncultivated soil) will be established around plots (Fig. 1).

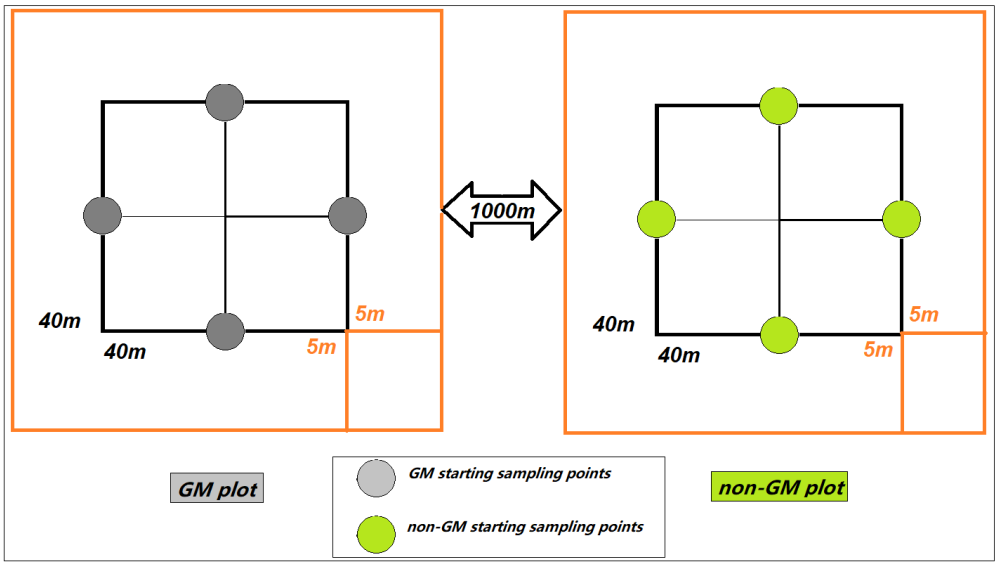

***Fig.1. The sampling design**. This comparative study designs two monitored plots: one GM and one non-GM treatment. Each plot will be staked as square (40 x 40m^2), surrounded by a 5m wide pathway, and distant from the second one by 1km.*

Before the project will start, all characteristics possibly slowing down the local GM growth (like the elevation, terrain slope, a local average amount of precipitation, or local soil characteristics – e.g. nutrient composition or alkalinity) will be evidenced based on official records done by co-partners in previous years (probably listed in the annual field reports). By interviewing field management services, the character and level of currently (and previously) applied agricultural technologies will be also documented (to exclude any side effects like e.g. unwanted chemicals in soil from previous years).

3.2.2. Planting and cultivation

For purposes of this study, GM corn SmartStax hybrid (Bayer) will be used. It expresses the sequences of Cry34Ab1, Cry35Ab1, and Cry3Bb1 (active against *Diabrotica* species (Coleoptera: Chrysomelidae), the sequences of Cry1F, Cry1A.105, and Cry2Ab2 (protect the plants against various lepidopteran pest), and two herbicide-tolerance genes (tolerance to the glufosinate-ammonium and glyphosate herbicides). This project will focus on Cry protein because protein for glyphosate and glufosinate-ammonium tolerance is commonly present in plants and soil, respectively.

In control plot, their non-GM variants, the closest conventional hybrid EXP258 will be planted. Non-GM plots will be treated with insecticide commonly used against *Ostrinia nubilalis* (Karate Zeon (lambda-cyhalothrin)) and *Diabrotica virgifera virgifera* (Karate Zeon, Force 1,5 G (tefluthrin)).

The complete farming process (a purchase of seeds, seed planting, and plant cultivation) will be fully under shield of cooperating partners (sponsored from this funding proposal). The later management (watering, controls) will follow usual procedures, and will be the same on GM and non-GM plots. The only two differences: the name, producer, character and amount of applied pesticides in non-GM plot will be monitored by cooperating partners.

3.2.3. Sampling

All calculations in this chapter are related to just one plot (with one treatment only).

Sampling will be performed in seven stages: before sowing (1), when corn germinating (2), in a stadium of 6 leaves of corn (3), while flowering (4), in a stadium of wax matureness (5), during harvesting (6), and three weeks after harvesting (7). Sampling before sowing (in the first year of experiment) will monitor default parameters during the initial state of the field and will confirm the pre-experimental absence of Cry protein.

Five fiducial points of sampling will be established: one in the absolute middle of each of the plots; plus one in the middle of each of sides of the square. From that point, five measurements in linear direction will be performed: one in the absolute middle of the plot and four more in a distance of 0m, 25m, 50m, and 100m (Fig.2). Each of sampling events will consist of three activities: a soil sampling; a Cry protein sampling, and insect sampling.

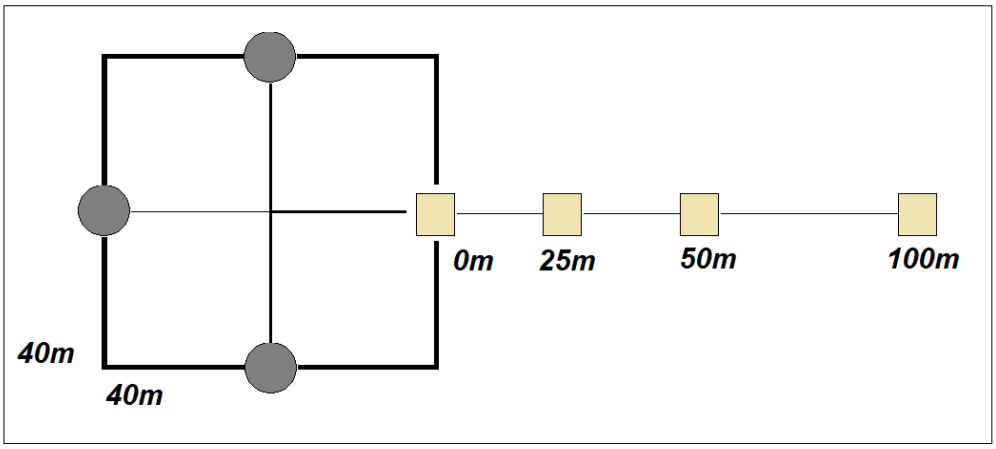

Fig.2. The sampling design of distances. *Each plot includes four starting sampling points in the middle of each side following the pattern of 0m – 25m – 50m – 100m from borders towards all four sides.*

3.2.3.1. Soil sampling

The soil sampling collects basic features about the soil material characterising its quality in each of five sampling points.

In agreement with prescriptions of standardized soil samplings for Czech Republic (ÚZKZÚZ, 2016), a simple procedure will be performed as follows: by using a garden vane, 200 grams of homogeneous surface soil will be gathered, sowed through a 2mm-scieve, and only those of passed soil grains will be stored (in paper bags) and transported for later analyses (Fernandez, n.d.).

For analysis of Cry protein presence, all soil samples must be kept in freezer during the transport. To monitor following three characteristics, a set of working procedures in lab will be used as published in (Fernandez, n.d.): the temperature regime of soil (a method of mulching cultures); the soil texture (a quantitative analyse according to Tames); and alkalinity (pH-indicator-based-on test).

In total, there will be a sevenfold collection including five samples by the end of the experiment (counted up 35 samples).

3.2.3.2. Cry protein sampling

The soil samples collected during the second activity serve as testing for presence of free forms of *Cry* protein.

The procedure of sample gathering is the same as described in chapter 3.2.3.1.

The next analysing will be procedured in lab as described in (Chen et al., 2009) by using protein-soil extracting SDS buffers, followed by phenol extraction. After completing of diluting steps, potential protein presence in samples will be visualized by SDS-PAGE electrophoresis and further detected using immunodetection.

In total, there will be a sevenfold collection including five samples by the end of the experiment (counted up 35 samples).

3.2.3.3. Arthropods sampling

The last of triad sampling activities, the collecting of insects will be performed as suggested in (Z. Svobodová et al., 2016), to trace the possible GT of *Cry* gene from GM crops into different insects.

At each of sampling points, three randomly selected plants will be covered with fabric sacs to inspect dwelling insect (e. g. *Anthocoridae, Aphididae, Thysanoptera, and Lepidoptera*).

One of those three plants will be sampled entirely with the root system to further detect a possible presence of root pests (*Diabrotica virgifera virgifera* in corn, *Elateridae*). The complete collection will be gathered in terrain, but finally analysed in the lab:

- At each sampling point, the yellow sticky traps (Bio Plantella, Unichem d.o.o., Slovenia) will be used for monitoring of flying parasitoids (*Braconidae*) and predators (*Coccinellidae, Syrphidae, Chrysopidae*).
- At each of sampling points, one sticky trap will be placed on wooden stick at a height of surrounding vegetation for one day at each corn growth stage.
- At each of sampling points, one pitfall trap covered with an aluminium cap will be used for the monitoring of ground dwelling invertebrates (*Carabidae, Araneae* and *Staphylinidae*). The trap consisting of doublets of an inner and outer plastic cup (volume 0.5 litre) will be placed in sampling points for one day. The pitfall trap will be filled with 300 ml of 10% NaCl supplemented with 2–3 drops of a detergent.

In total, there will be a seven times fivefold collection including three plant samples, two sticky-traps samplings, and one pitfall trap sampling by the end of the experiment (counted up 210 samplings). For analysis of *Cry* gene presence, samples of all invertebrates must be kept in freezer during the transport. To detect *Cry* gene presence, well-known primer pairs for PCR amplification as discussed e.g. in (Noguera & Ibarra, 2010).

During the seventh sampling when highest amount of larvae are presented in soil, a triple independent soil sampling to monitoring dipteran larvae will be collected. Samples will be processed in Kempson's extractors.

3.2.4. Sampling evaluating

Taken into account the entire gained dataset, firstly, there will be a list of basic features available characterizing the pre-experimental stage of parcels. Secondly, there will be a terrain dataset consisting of: a soil sampling (35), a secondary soil sampling focusing on Cry protein presence (35), and finally the total insect collection sampled from terrain traps (210+3). Having two plots (and therefore a double amount of samples), there is 70 Cry-protein-presence-orientated-on sampling collections and 426 *Cry*-gene-presence-orientated-on sampling collections, whose individuals will be determined (families and orders), counted, and analysed.

All in chapter 3.2.3 characterized insect families and orders are the most common groups in Czech corn fields, as reviewed in several previous Czech experiments (Z. Svobodová et al., 2015, 2016, 2020). They are expected to be found in the field samples; however, if new monitored, the predated list will be extended.

To analyse the change of individual numbers between detected non-target and target insects, the total numbers of collected species within each sampling event (counting together all collecting methods from the third activity) will be compared vice versa for GM and control (non-GM) treatments (using STATISTIKA or R software; t-test or ANOVA method). Having a structured dataset, basic statistical analyses can be further extended by considering possible numbers-disturbing effects of e.g. the distance between GM source and each of sampling pointes or the changing level of soil features (e.g. pH) towards limiting levels.

The across-insect-PCR bands will give proofs about an occurred *Cry* gene transfer from Bt-corn into detected non-target and target insects. If primers (designed to amplify specifically *Cry* gene sequence) used correctly and the PCR amplification multiplies the compatible DNA segment presented in the genome (although misinterpretations like e.g. the PCR non-target residuals have to be also taken into account).

To directly detect a possible transfer of free forms of Cry protein, the results from SDS-PAGE electrophoresis and from immunodetection will be controlled for presence of positive markers. However, the effect of consumed Cry protein (possible to be detected in insect digestive system) must be excluded, as discussed in chapter 4. In all cases, the parcel with GM treatment will be judged as experimental plot and the parcel with non-GM treatment as a negative control. Final results will be properly commented and published.

3.3. Suggested time schedule and costs of experimental procedures

The complete duration of the entire experiment is meant to be 30 months (Tab. I.).
The process starts with an initial administrative contacting that includes three subjects: the negotiations regarding an authorization for growing GM corn from the M. E. (1), M. A. (2), and finally the negotiation with seed supplier (Bayer) (3). The entire agreement with possible co-partners starts to be pre-aligning so, that all final approvals can be validated immediately after grant awarding. During next 28 months, the experimental plots will be established (in the third month after the official initiation of project), and since then in detail monitored.

Tab. I. Time schedule of experimental procedures, planned for 30 months since opening.

Activities	**Months**							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII - XXIV**	**XXV - XXX**
Administration								
Plots establishment								
Planting and cultivation								
Sampling								
Final analyses								
Informative reports								

The fourth month after plots established, the phase of planting and cultivating will start as described in chapter 3.2.2. Parallel to that, the first sampling will be run to monitor the initial (default) stages at the parcels.

The sampling procedures will continue within the next two years until finishing the final analyses (starting six months after beginning). During the entire period (of 30-months), sub-messages and reports will be published as informative reports giving a basic overview about sub-successes and the next perspectives.

The total costs of this environmental project are 159.020 EUR. The financial proposal (seen in Tab. II) is divided into two parts: according to the foundation of material investments, and according to wages.

Tab. II. Financial schedule of experimental procedure, planned for 30 months since opening.

1. Material investments

Investments:	**Required (EUR)**		
	1st year	**2nd year**	**3rd year (6 months)**
Long–termed material belongings (up to 1.400 EUR)	2.200	-	-
Long-termed non-material belongings (up to 2.200 EUR)	7.680	7.680	3.840
Material	2.750	2.750	1.370
Statutory enlistment (34%)	5.058	5.058	2.529
Overhead costs			
• Field management	6.130	6.130	3.060
• Laboratory management	7.840	7.840	3.920
Travelling	3.200	3.200	1.600
Services	11.200	11.200	5.600
Total	**46.058**	**43.858**	**21.929**

2. Wage investments

2.1. Wages

Investments:	**Annual Tariff**	**Contract**	**Number of hours/ year/ person**
Worker – Researcher	8.500	Full	1.920
Worker – Technician	6.375	75%	1.440
DPP	-	-	300

2.2. Personal wages investments (PWI)

PWI Specifications	Required (EUR)		
	1st year	2nd year	6 months
Researcher	8.500	8.500	4.250
Technician	6.380	6.380	3. 190
DPP Student 1	1.330	1.330	565
DPP Student 2	1.330	1.330	565
DPP Student 3	1.330	1.330	565
Total	**18.870**	**18.870**	**9.435**
Total costs of the project	**159.020**		

The material investments will be used as follows:

At the time of writing this proposal, three computers as long-termed material belongings are required. For purposes of this study, are various additional software tools necessary (like for the DNA primer designing or the statistical analyses).

Materials (price/1 package) include: traps for monitoring (~27 EUR), soil-sampling kits (~1.350 EUR), DNA-extraction kits (83–370 EUR), alkalinity-measuring kits (~18 EUR), chemicals, transportable freezing boxes (~100 EUR), and stationery (informative books [~270 EUR], papers and pens).

Concerning others investments, the field management requires to be covered due to parcels renting, whereas laboratory managment costs shield the rental financing of the cooperative analytical lab at Biology Centre CAS in České Budějovice (BC CAS). Overhead costs are settled on 20 % from other financial expenses (as observable in table Tab. II). Field management implies the field rent, sowing, pesticide application, harvest, other processes connected with corn growing.

Travelling covers all transports to the trial sites; including both, travel allowance, and travel insurance. Finances in terms of services will be used for DNA sequencing, further for English language spelling & mistakes corrections of manuscripts and papers, and finally for open access publication fees.

The wage investments will be used as follows:: one researcher: full time job (21.250 EUR); one technician: 75% part time job (15.938 EUR); 3 master students as DPPs. Whereas the researcher will respond for the entire communication between cooperating agriculture companies and the laboratory (including the communication between technician and students), the technician will be responsible for terrain sampling and the next lab analyses. To the responsibility of researcher further belong the compliance of financial budget and time schedule.

Both, a researcher and a technician participating in this study are well-experienced considering mentioned procedures and the final dataset evaluating. No special skills are required from students, who will be rather helpful with manual terrain activities.

In terms of that, the co-operating laboratory (BC CAS; CZE) is also well-equipped (necessary machines, kits, and analytical software instruments) and certificated to run biological analyses.

No foundations have so far covered this experiment, and none are so far contracted.

4. Discussion

Initially, I would like to discuss here several parameters concerning the way of how the project was designed:

Although suggesting a very complex sampling, the length of this experiment was agreed on 30 months. Terrain sampling itself consumes two years, which is a very minimum for a field study. On the other hand, since the main aim of the study is to detect a presence of free-GM constructs (without any tempts to deeply evaluate arthropod population dynamic), the suggested time schedule is considered to be sufficient.

By evidencing the initial parcel parameters, we will prove how much the local conditions are supportive towards growth of GM corn. Those of recorded terrain parameters (e.g. slope or a level of precipitations) may serve as standardized quotas in case of future comparative studies. The initial soil analyses will further show the pre-experimental chemical-level control. If increased, a chosen locality displays clear marks of being not recovered from previous chemical approaches. Therefore, possible effect of GT would be influenced by residual chemical bias in soil.

Regular monitoring of soil within each sampling event will help to detect any possible switches that would cause an unexpected changes in arthropods communities (increased/decreased pH, (too high/low temperature; too/not enough compact soil). Hence, number of arthropods in samples could decrease, however, not in linking to GM corn.

Concerning the Cry toxin/*Cry* gene presence analyses, the positive results have to be evaluated critically. When Cry toxin will be detected in sample, intestinal dissection of other species individuals will be performed to eliminate false positive (Cry toxins in food).
If PCR bands positively confirmed a presence of *Cry* gene, the effect of non-target amplification still has to be excluded by running negative controls. In other case, the amplified, non-related but still compatible insect genome sequences (resulting from accidental, not enough specific annealing of PCR primers) could be miss-interpreted as presence of *Cry* gene.

This comparative study will assess three stated goals that will be together with the possible results-modifying scenarios discussed here. I summarized them into three main categories concerning the arthropod families/orders distribution between plots (1), the challenges of technical background required for purposes of the project (2), and finally, impacts of possible GT detection (3).

The first aspect focuses on a problem of final (un-)equal distribution of numbers of insect families and individuals between plots (inside and in surrounding of). The reliable pattern of real arthropod distribution will be created by sampling in the middle of each plot as well as on its borders. Thanks to that, the edge effects (on the borders of plots) will be fairly monitored if any.

In general, the application of pesticides, if ran successfully, should decrease the numbers of non-target arthropods in the traditionally pesticide-treated non-GM plot in contrast to the GM plot, as was previously many times reported (Bhatti et al., 2006; Leslie et al., 2010; Svobodová et al., 2020).

Nonetheless, it can happen that the final measurements show the in-between insect comparison as equal as was found in many studies (Ahmad et al., 2005; Al-Deeb & Wilde, 2003; Svobodová et al., 2015, 2016). An equal distribution between the pesticide-managed-by and pesticide free (GM) plots would mean that the pesticide effect is not functioning so strongly in non-GM plot. Therefore, all present arthropod communities there can locally profit and further develop, without being chemically disturbed from outside. This situation very favours our arthropod collecting phase (in the field and on its borders) and raise our chances for potential GT detection in the plot.

In general, a long-termed pesticide-based-on treatment can permanently exclude non-target insect species from a certain locality. If planting without using pesticides, a return of such communities to their previous habitats is highly expected. This fact is far more important: being eliminated chemically does not mean being excluded from the GT risk. In other words, the returned insect groups (that were not detected before the start of the experiment) need to be additionally added to for a testing of possible GT.

Concerning the second (technical) aspects, if running a complex study and aiming at reliable results, it is highly required to keep the planting management including all sorts of used chemicals (like the pesticides or nutrients) in an unified mode. Difficulties as sudden outage of previously applied chemicals, an unexpected ending of crop/pesticide production, or unanticipated legal ban can easily harm the fluent happening of the experiment.

Agreement with seed suppliers will ensure an access to GM corn that is well-accommodated and also very popular GM crop in U. S. Simultaneously, the current available pesticides are accessible in many variants, therefore if one banned, another alternative is able to easily replace the previous efficacy.

Planting GM corn for two growing seasons (as suggested in this proposal) gives results that pretty much lack reliability if considering possible environmental fluctuations (like e.g. the prolonged, hot and dry spring seasons). However, in case of repetitive monitoring (triple-fold monitoring or more), the seasonality may also vary every year. Therefore, the gained results do not display much higher reliability.

Beside the GT-GM discussion, this study is also able to provide a side adding value while monitoring the effect of used pesticides. The stronger application will be performed, the lower presence of target insect communities should be observable, in contrast to GM plot. Furthermore, if the application appears to be too strong, the amount of all possible arthropod families will be in general endangered.

The power of the endangering will be measured when numbers of non-target arthropod families (and their approximate individual composition) will be compared against results from GM plot sampling. Therefore, this dataset (assembled actually for purposes of different study) may also serve as an argumentative material providing the terrain evaluation of pesticides at the internal market.

The third aspect discussed here regards the environmental risks if GT between GM crop and its surrounding will be detected. GT presence can have three possible impacts on individuals and populations: positive, neutral, or negative.

The genomic structure can be spontaneously re-assembled via e.g. the mechanism of mobile genetic elements (Sahebi et al., 2018). If such an element incorporates itself into genome (carrying a GM-construct) the previous gene order can be modified in the same way as if affected by spontaneous mutation (knock out of regulating or coding gene regions). If GT-affected, any of important sequences (within a genome of GM-construct-carrying individual) could have come through an accidental gene knock out (or over-expression) so, that a novel trait appeared. This trait may be all, beneficial, neutral, or disadvantaging impact. Beneficial traits would advantage the individual and later the entire insect population over competitors, whereas the disadvantaging characteristics usually cause a mortality. If any GT detected in arthropods, its strength and further impacts would then require next investigations.

The final aspect discussed here evaluates the current mood towards GM crops. European farmers hesitate both to produce GM crops as well as to test them. It is possible that thanks to GT detection (as revealed here), several environmental discussions will be refreshed, and therefore farmers gain more decisive, refusing opinion against GM crop.

In 2018, an interesting discussion from China was published, when after more than 25 years of GM research, the open public was asked about their altitude - for or against the additional governmental support concerning GM technology in food-production sector. In reaction to that, Cui & Shoemaker (2018) then surveyed consumers in China for their awareness and sympathy to GM food. The basic GM principles were understood by only a minority of respondents (11.7%), whereas the remaining number of interviewed assigned themselves as rather unfamiliar with the topic or neutral (Cui & Shoemaker, 2018). The survey also showed that the information on GM food was gathered via the Internet (69.3 % of respondents) and such a through media obtained impression was mostly negative (for 64.3 % of respondents) (Cui & Shoemaker, 2018).

Similarly to the Chinese example, in 2012, in reaction to the ore published publication of Séralini et al. (2012) criticism (as described in chapter 2.4.2.), an open platform showed that 79 % of French people were worried about the possible presence of GM organisms in their food, compared with 65 % respondents in 2011 (Houllier, 2012).

This example from China and France only confirm the generally known fact about how official reports influence a public opinion and how necessary is to provide such audience with reliable facts collected within independent studies.

There is for example, another interesting, rarely-discussed topic concerning how the strong selections for the rare mutant plants adapted to cultivation by early farmers dropped most of the variation present in the wild populations from which cultivated forms arose (Zamir, 2001). It is obvious now that solid and meaningful genetic reservoirs were left - especially genes associated with pest resistance.

However, this loss could be without bigger difficulties reversed back by artificial genetic engineering - as Breseghello and Coelho (2013) very correctly summarize, bringing back of those genes into modern cultivars is of the most relevant applications of modern advanced-technologies-based-on breeding programs.

This study describes both, traditional breeding methods compared to modern and advanced ways of breeding. Having presented those of their most important principles, the advanced engineering shows a faster and more precise approach than natural crosses and chemical-mutagens-based-on breeding. However, since the first application of GM crop, many discussions have considered their safety and negative impact on the human health, food quality and environment.

Their environmental potential risk plays for sure an important question that decides about popularity of GM crops among mainly consumers of GM products and is therefore needed to be studied carefully and deeply.

5. Conclusion

This study describes the evolution of traditional breeding methods, from the applied natural-selection-based-on approaches over natural crosses to modern plant breeding procedures. These traditional breeding methods are compared to the advanced way of biotechnological engineering. Furthermore, this study also considers the current applying of GMs while stressing out its both, positive and critical aspects. Next, the political situation linking to national GMs support is commented here. Since the origin of authors and the placement of the environmental project are referred to Czech Republic, the local situation is also mentioned here.

The second part of this study works with a new design of environmental project that focuses on the impact of GM Bt corn on the local arthropods. With a help of arranged co-partners, two established testing plots will monitor differences between the traditional and GM approach. Initially, the survey monitors the possible changes of individual insect numbers inside and outside of the chemically-non- and treated parcels. Then, the possible transfer of *Cry gene* and free forms of Cry proteins are detected.

Here suggested procedure is high probably the only one of its kind. Results of this study would be highly evaluated in context of European insect risk-assessment monitoring.

6. List of acronyms

BC CAS	Biology Centre CAS in České Budějovice
B/NER	Base/Nucleotide Excision Repair
Bt	*Bacillus thuringiensis*
CP4 EPSPS	5-enolpyruvylshikimate 3-phosphate protein
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cry	Crystalline delta protein
CUPs	Currently Used Pesticides
CZE	Czech Republic
DNA	Deoxyribonucleic Acid
DSB	Double Strand Breaks
EC	European Commission
EU	European Union
EPA	Environmental Protection Agency
ERA	Environmental Risk Assessment
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
F.R.	Federal Register
FSA	Food Safety Authority
GM	genetically modified
GMs	Genetic Modifications
GMO	Genetically Modified Organism
GT	Genetic Transfer
HDR	Homologous Direct Recombination
IAEA	International Atomic Energy Agency
ISAAA	International Service for the Acquisition of Agri-biotech Applications
MAS	Marker Assisted Selection
M.A.	Ministry of Health and the Ministry of Agriculture
M.E.	Ministry of the Environment
MMR	Mismatch Repair
NHEJ	Non-homologous End Joining
NPBT	New Plant Breeding Techniques
PCR	Polymerase Chain Reaction
POV	Personal Wages Investment
RNA	Ribonucleic acid
RNAi	RNA interference
TALE	Transcription Activator-like Effectors
UK	United Kingdom
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. FDA	U.S. Food and Drug Administration
ZF	Zinc-Finger
ZFN	Zinc-Finger Nucleases

7. References

Ahloowalia, B. S., & Maluszynski, M. (2001). Induced mutations - A new paradigm in plant breeding. *Euphytica*, *118*(2), 167–173. https://doi.org/10.1023/A:1004162323428

Ahmad, A., Wilde, G. E., & Zhu, K. Y. (2005). Detectability of Coleopteran-specific Cry3Bb1 Protein in Soil and Its Effect on Nontarget Surface and Below-Ground Arthropods. *Environmental Entomology*, *34*(2), 385–394. https://doi.org/10.1603/0046-225x-34.2.385

Al-Deeb, M. A., & Wilde, G. E. (2003). Effect of Bt Corn Expressing the Cry3Bb1 Toxin for Corn Rootworm Control on Aboveground Nontarget Arthropods. *Environmental Entomology*, *32*(5), 1164–1170. https://doi.org/10.1603/0046-225x-32.5.1164

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2014). *Essential Cell Biology* (Fourth). Garland Science, Taylor & Francis Group, LLC This.

Álvarez-Alfageme, F., Bigler, F., & Romeis, J. (2011). Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of Adalia bipunctata (Coleoptera: Coccinellidae): The importance of study design. *Transgenic Research*, *20*(3), 467–479. https://doi.org/10.1007/s11248-010-9430-5

Álvarez-Alfageme, F., Ferry, N., Castañera, P., Ortego, F., & Gatehouse, A. M. R. (2008). Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator Stethorus punctillum Weise (Coleoptera: Coccinellidae). *Transgenic Research*, *17*(5), 943–954. https://doi.org/10.1007/s11248-008-9177-4

Baltes, N. J., Gil-Humanes, J., & Voytas, D. F. (2017). Genome Engineering and Agriculture: Opportunities and Challenges. In *Progress in Molecular Biology and Translational Science* (1st ed., Vol. 149). Elsevier Inc. https://doi.org/10.1016/bs.pmbts.2017.03.011

Ben-Ari, G., & Lavi, U. (2012). Marker-assisted selection in plant breeding. In *Plant Biotechnology and Agriculture* (First Edit). Elsevier Inc. https://doi.org/10.1016/B978-0-12-381466-1.00011-0

Benbrook, C. M. (2012). Impacts of genetically engineered engineered. *Environmental Sciences Europe*, *24*(24), 1–13.

Bhatti, M. A., Duan, J., Head, G. P., Jiang, C., McKee, M. J., Nickson, T. E., Pilcher, C. L., & Pilcher, C. D. (2006). Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)–Protected <I>Bt</I> Corn on Foliage-Dwelling Arthropods. *Environmental Entomology*, *34*(5), 1336–1345. https://doi.org/10.1603/0046-225x(2005)034[1336:feotio]2.0.co;2

Bibikova, M., Golic, M., Golic, K. G., & Carroll, D. (2002). Targeted chromosomal cleavage and mutagenesis in Drosophila using zinc-finger nucleases. *Genetics*, *161*(3), 1169–1175.

Bilgic, H., Hakki, E. E., Pandey, A., Khan, M. K., & Akkaya, M. S. (2016). Ancient DNA from 8400 Year-Old Çatalhöyük Wheat: Implications for the origin of neolithic agriculture. *PLoS ONE*, *11*(3),

e.0151974. https://doi.org/10.1371/journal.pone.0151974

Boch, J., & Bonas, U. (2010). Xanthomonas AvrBs3 Family-Type III Effectors: Discovery and Function . *Annual Review of Phytopathology*, *48*(1), 419–436. https://doi.org/10.1146/annurev-phyto-080508-081936

Bøhn, T., Primicerio, R., Hessen, D. O., & Traavik, T. (2008). Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety. *Archives of Environmental Contamination and Toxicology*, *55*(4), 584–592. https://doi.org/10.1007/s00244-008-9150-5

Bouma, J. (1967). *New variety of spring barley, "Diamant" in Czechoslovakia* (pp. 177–182). Abh Dt AkadWiss Berl Akademie-Verlag.

Brandt, P. (2003). Overview of the current status of genetically modified plants in Europe as compared to the USA. *Journal of Plant Physiology*, *160*(7), 735–742. https://doi.org/10.1078/0176-1617-01031

Bravo, A., Gill, S. S., & Soberón, M. (2007). Mode of action fo Bacillus thuringiensis Cry and Cyt toxins and their potential for Insect control. *Toxicon*, *49*(4), 423–435. https://doi.org/10.1016/0168-9002(90)90614-C

Breseghello, F., & Coelho, A. S. G. (2013). Traditional and modern plant breeding methods with examples in rice (Oryza sativa L.). *Journal of Agricultural and Food Chemistry*, *61*(35), 8277–8286. https://doi.org/10.1021/jf305531j

Brookes, G. (2018). The farm level economic and environmental contribution of Intacta soybeans in South America: the first five years. *GM Crops and Food*, *9*(3), 140–151. https://doi.org/10.1080/21645698.2018.1479560

Brookes, G. (2019). Twenty-one years of using insect resistant (GM) maize in Spain and Portugal: farm-level economic and environmental contributions. *GM Crops and Food*, *10*(2), 90–101. https://doi.org/10.1080/21645698.2019.1614393

Brookes, G., & Barfoot, P. (2018a). Environmental impacts of genetically modified (GM) crop use 1996-2016: Impacts on pesticide use and carbon emissions. *GM Crops and Food*, *9*(3), 109–139. https://doi.org/10.1080/21645698.2018.1476792

Brookes, G., & Barfoot, P. (2018b). Farm income and production impacts of using GM crop technology 1996–2016. *GM Crops and Food*, *9*(2), 59–89. https://doi.org/10.1080/21645698.2018.1464866

Bruening, G., & Lyons, M. J. (2000). We believe that the. *Calif. Agric.*, *54*(4). https://doi.org/http://calag.ucanr.edu/Archive/?article=ca.v054n04p6#Calag-FullText

Carbonari, C. A., Latorre, D. O., Gomes, G. L. G. C., Velini, E. D., Owens, D. K., Pan, Z., & Dayan, F. E. (2016). Resistance to glufosinate is proportional to phosphinothricin acetyltransferase

expression and activity in LibertyLink® and WideStrike® cotton. *Planta*, *243*(4), 925–933. https://doi.org/10.1007/s00425-015-2457-3

Cathomen, T. (2004). AAV vectors for gene correction. *Current Opinion in Molecular Therapeutics*, *6*(4), 360–366.

Ceccaldi, R., Rondinelli, B., & D 'andrea, A. D. (2016). Repair Pathway Choices and Consequences at the Double- Strand Break Mechanisms of DNA DSB Repair. *Trends in Biochemical Sciences*, *26*(1), 52–64. https://doi.org/10.1016/j.tcb.2015.07.009

Chakrabarty, A. M. (2010). Bioengineered bugs, drugs and contentious issues in patenting. *Bioengineered Bugs*, *1*(1), 2–8. https://doi.org/10.4161/bbug.1.1.9850

Chambers, C. P., Whiles, M. R., Rosi-Marshall, E. J., Tank, J. L., Royer, T. V., Griffiths, N. A., Evans-White, M. A., & Stojak, A. R. (2010). Responses of stream macroinvertebrates to Bt maize leaf detritus. *Ecological Applications*, *20*(7), 1949–1960. https://doi.org/10.1890/09-0598.1

Chatterjee, N., & Walker, G. C. (2017). Mechanisms of DNA damage, repair, and mutagenesis. *Environmental and Molecular Mutagenesis*, *58*(5), 235–263. https://doi.org/10.1002/em.22087

Chen, S., Rillig, M. C., & Wang, W. (2009). Improving soil protein extraction for metaproteome analysis and glomalin-related soil protein detection. *Proteomics*, *9*(21), 4970–4973. https://doi.org/10.1002/pmic.200900251

Christian, M., Cermak, T., Doyle, E. L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A. J., & Voytas, D. F. (2010). Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*, *186*(2), 756–761. https://doi.org/10.1534/genetics.110.120717

Christou, P., McCabe, D. E., & Swain, W. F. (1988). Stable Transformation of Soybean Callus by DNA-Coated Gold Particles. *Plant Physiology*, *87*(3), 671–674. https://doi.org/10.1104/pp.87.3.671

Cohen, S. N., Chang, A. C. Y., Boyer, H. W., & Helling, R. B. (1973). Construction of biologically functional bacterial plasmids in vitro. *Proceedings of the National Academy of Sciences of the United States of America*, *70*(11), 3240–3244. https://doi.org/10.1073/pnas.70.11.3240

Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, & Counci, N. R. (2004). Safety of Genetically Engineered Foods. In *Board on Life Science, Food*. The National Academies Press.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). *Multiplex Genome Engineering Using CRISPR/Cas Systems*.

Cui, K., & Shoemaker, S. P. (2018). Public perception of genetically-modified (GM) food: A Nationwide Chinese Consumer Study. *Npj Science of Food*, *2*(1), 1–8. https://doi.org/10.1038/s41538-018-0018-4

D'Amato, F., & Hoffmann-Ostenhof, O. (1956). Metabolism and Spontaneous Mutations in Plants. *Advances in Genetics*, *8*(C), 1–28. https://doi.org/10.1016/S0065-2660(08)60497-6

Davies, J. P., Kumar, S., & Sastry-Dent, L. (2017). Use of Zinc-Finger Nucleases for Crop Improvement. In *Progress in Molecular Biology and Translational Science* (Vol. 149, pp. 47–63). Elsevier B.V. https://doi.org/10.1016/bs.pmbts.2017.03.006

Duan, J. J., Marvier, M., Huesing, J., Dively, G., & Huang, Z. Y. (2008). A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). *PLoS ONE*, *3*(1). https://doi.org/10.1371/journal.pone.0001415

E.C. (2007). *Questions and Answers on the Regulation of GMOs in the European Union*. http://ec.europa.eu/food/plant/gmo/legislation/future_rules_en.htm

E.C., E. C. (2013). *Questions and Answers on EU ' s policies on cultivation and imports of GMOs What is the current procedure for authorising the cultivation of Are any GMOs already cultivated in the EU ? Have Member States already prohibited GMO cultivation ? Why is the Com*. *November*, 2012–2014.

Eady, C. C., Kamoi, T., Kato, M., Porter, N. G., Davis, S., Shaw, M., Kamoi, A., & Imai, S. (2008). Silencing onion lachrymatory factor synthase causes a significant change in the sulfur secondary metabolite profile. *Plant Physiology*, *147*(4), 2096–2106. https://doi.org/10.1104/pp.108.123273

EC. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food and Chemical Toxicology*, *50*(11), 4221–4231. https://doi.org/10.1016/j.fct.2012.08.005

Eibl, R., Meier, P., Stutz, I., Schildberger, D., Hühn, T., & Eibl, D. (2018). Plant cell culture technology in the cosmetics and food industries: current state and future trends. *Applied Microbiology and Biotechnology*, *102*(20), 8661–8675. https://doi.org/10.1007/s00253-018-9279-8

Ewing, P. M., Runck, B. C., Kono, T. Y. J., & Kantar, M. B. (2019). The home field advantage of modern plant breeding. *PLOS ONE*, *14*(12), e0227079. https://doi.org/10.1371/journal.pone.0227079

FDA. (2020). *How GMOs Are Regulated for Food and Plant Safety in the United States | FDA*. https://www.fda.gov/food/agricultural-biotechnology/how-gmos-are-regulated-food-and-plant-safety-united-states

Federal Register of United States. (1986). *Coordinated Framework for Regulation of Biotechnology*.

Fehér, A. (2019). Callus, dedifferentiation, totipotency, somatic embryogenesis: What these terms mean in the era of molecular plant biology? *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00536

Fernandez, J. G. (n.d.). *Analýza půdy, soubor pracovních postupů, 419.0023*.

Ferry, N., & Gatehouse, A. M. R. (2009). *Environmental Impact of Genetically Modified Crops*. CAB International.

Fire, A., Xu, S., Montgomery, M. K., & Kostas, S. A. (1998). Orignal Papers. ON ETHER-VAPOUR, ITS MEDICAL AND SURGICAL USES. *Nature*, *391*(6669), 806–811. https://doi.org/10.1038/35888

Funke, T., Han, H., Healy-Fried, M. L., Fischer, M., & Schönbrunn, E. (2006). Molecular basis for the herbicide resistance of Roundup Ready crops. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(35), 13010–13015. https://doi.org/10.1073/pnas.0603638103

Geiler-Samerotte, K. A., Bauer, C. R., Li, S., Ziv, N., Gresham, D., & Siegal, N. (2013). Why and How to Study Phenotype Variability. *Curr Opin Biotechnol*, *24*(4), 752–759. https://doi.org/10.1016/j.copbio.2013.03.010.The

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, *327*(5967), 812–818. https://doi.org/10.1126/science.1185383

Gómez-Barbero, M., Berbel, J., & Rodríguez-Cerezo, E. (2008). Bt corn in Spain - The performance of the EU's first GM crop. *Nature Biotechnology*, *26*(4), 384–386. https://doi.org/10.1038/nbt0408-384

Gupta, R. M., & Musunuru, K. (2014). Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *Journal of Clinical Investigation*, *124*(10), 4154–4161. https://doi.org/10.1172/JCI72992

Hilioti, Z., Ganopoulos, I., Ajith, S., Bossis, I., & Tsaftaris, A. (2016). A novel arrangement of zinc finger nuclease system for in vivo targeted genome engineering: the tomato LEC1-LIKE4 gene case. *Plant Cell Reports*, *35*(11), 2241–2255. https://doi.org/10.1007/s00299-016-2031-x

Hinchee, M. A. W., Connor-Ward, D. V., Newell, C. A., McDonnell, R. E., Sato, S. J., Gasser, C. S., Fischhoff, D. A., Re, D. B., Fraley, R. T., & Horsch, R. B. (1988). Production of transgenic soybean plants using agrobacterium-mediated DNA transfer. *Bio/Technology*, *6*(8), 915–922. https://doi.org/10.1038/nbt0888-915

Homrich, M. S., Wiebke-Strohm, B., Weber, R. L. M., & Bodanese-Zanettini, M. H. (2012). Soybean genetic transformation: A valuable tool for the functional study of genes and the production of agronomically improved plants. *Genetics and Molecular Biology*, *35*(4 SUPPL.), 998–1010. https://doi.org/10.1590/S1415-47572012000600015

Hönemann, L., & Nentwig, W. (2009). Are survival and reproduction of Enchytraeus albidus (Annelida: Enchytraeidae) at risk by feeding on Bt-maize litter? *European Journal of Soil*

Biology, *45*(4), 351–355. https://doi.org/10.1016/j.ejsobi.2009.03.001

Houllier, F. (2012). Bring more rigour to GM research. *Nature*, *491*(7424), 327–327. https://doi.org/10.1038/491327a

Hunter, N. (2015). Meiotic recombination: The essence of heredity. *Cold Spring Harbor Perspectives in Biology*, *7*(12), 1–35. https://doi.org/10.1101/cshperspect.a016618

Hutchison, W. D., Burkness, E. C., Mitchell, P. D., Moon, R. D., Leslie, T. W., Fleischer, S. J., Abrahamson, M., Hamilton, K. L., Steffey, K. L., Gray, M. E., Hellmich, R. L., Kaster, L. V., Hunt, T. E., Wright, R. J., Pecinovsky, K., Rabaey, T. L., Flood, B. R., & Raun, E. S. (2010). Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science*, *330*(6001), 222–225. https://doi.org/10.1126/science.1190242

ISAAA, B. 54. (2018). Global Status of Commercialized Biotech/GM Crops in 2018. In *Brief 54, Executive Summary*. https://doi.org/10.13523/j.cb.20190801

ISAAA, P. 16. (2018). Biotech Crop Highlights in 2018. In *Pocket K No.16*. https://doi.org/10.1017/CBO9781107415324.004

Jasin, M. (1996). Genetic manipulation of genomes with rare-cutting endonucleases. In *Trends in Genetics* (Vol. 12, Issue 6, pp. 224–228). Elsevier Ltd. https://doi.org/10.1016/0168-9525(96)10019-6

Jayaraman, K. S. (2004). Illegal seeds overtake India's cotton fields. *Nature Biotechnology*, *22*(11), 1333–1334. https://doi.org/10.1038/nbt1104-1333

Jiang, C., Meng, C., & Schapaugh, A. (2019). Comparative analysis of genetically-modified crops: Part 1. Conditional difference testing with a given genetic background. *PLoS ONE*, *14*(1), 1–17. https://doi.org/10.1371/journal.pone.0210747

Jobling, M., Hollox, E., Hurles, M., Kivisild, T., & Tyler-Smith, C. (2014). *Human Evolutionary Genetics* (Second). Garland Science, Taylor & Francis Group, LLC This.

Kamle, M., Kumar, P., Patra, J. K., & Bajpai, V. K. (2017). Current perspectives on genetically modified crops and detection methods. *3 Biotech*, *7*(3), 1–15. https://doi.org/10.1007/s13205-017-0809-3

Kaplan, Z., & Fehrer, J. (2006). Comparison of natural and artificial hybridization in Potamogeton. *Preslia*, *78*(3), 303–316.

Kathage, J., & Qaim, M. (2012). Economic impacts and impact dynamics of Bt (Bacillus thuringiensis) cotton in India. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(29), 11652–11656. https://doi.org/10.1073/pnas.1203647109

Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research*, *7*(3), 123–149. https://doi.org/10.1051/ebr:2008014

Kılınç, G. M., Omrak, A., Özer, F., Günther, T., Büyükkarakaya, A. M., Bıçakçı, E., Baird, D., Dönertaş, H. M., Ghalichi, A., Yaka, R., Koptekin, D., Açan, S. C., Parvizi, P., Krzewińska, M., Daskalaki, E. A., Yüncü, E., Dağtaş, N. D., Fairbairn, A., Pearson, J., … Götherström, A. (2016). The Demographic Development of the First Farmers in Anatolia. *Current Biology*, *26*(19), 2659–2666. https://doi.org/10.1016/j.cub.2016.07.057

Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., & Hammond, B. G. (2015). The food and environmental safety of Bt crops. *Frontiers in Plant Science*, *6*(APR), 1–22. https://doi.org/10.3389/fpls.2015.00283

Konzen, E. R., & Tsai, S. M. (2012). Genetic Variation of Landraces of Common Bean Varying for Seed Coat Glossiness and Disease Resistance: Valuable Resources for Conservation and Breeding. *Intech*, *i*(tourism), 13. https://doi.org/10.1016/j.colsurfa.2011.12.014

Kreuzer, H., & Massey, A. (2014). Evolutionary Mechanisms. *Biology and Biotechnology*, 287–318. https://doi.org/10.1128/9781555816094.ch13

Kuczyńska, A., Surma, M., Adamski, T., Mikołajczak, K., Krystkowiak, K., & Ogrodowicz, P. (2013). Effects of the semi-dwarfing sdw1/denso gene in barley. In *Journal of Applied Genetics* (Vol. 54, Issue 4, pp. 381–390). Springer. https://doi.org/10.1007/s13353-013-0165-x

Lawo, N. C., Wäckers, F. L., & Romeis, J. (2010). Characterizing indirect prey-quality mediated effects of a Bt crop on predatory larvae of the green lacewing, Chrysoperla carnea. *Journal of Insect Physiology*, *56*(11), 1702–1710. https://doi.org/10.1016/j.jinsphys.2010.06.012

Leslie, T. W., Biddinger, D. J., Mullin, C. A., & Fleischer, S. J. (2009). Carabidae Population Dynamics and Temporal Partitioning: Response to Coupled Neonicotinoid-transgenic Technologies in Maize. *Environmental Entomology*, *38*(3), 935–943. https://doi.org/10.1603/022.038.0348

Leslie, T. W., Biddinger, D. J., Rohr, J. R., & Fleischer, S. J. (2010). Conventional and Seed-Based Insect Management Strategies Similarly Influence Nontarget Coleopteran Communities in Maize. *Environmental Entomology*, *39*(6), 2045–2055. https://doi.org/10.1603/en10049

M.E. (2004a). *Act No.209/2004 Coll.*

M.E. (2004b). *Act No.78/2004 Coll.*

Maeder, M. L., Thibodeau-Beganny, S., Osiak, A., Wright, D. A., Anthony, R. M., Eichtinger, M., Jiang, T., Foley, J. E., Winfrey, R. J., Townsend, J. A., Unger-Wallace, E., Sander, J. D., Müller-Lerch, F., Fu, F., Pearlberg, J., Göbel, C., Dassie, J. P. P., Pruett-Miller, S. M., Porteus, M. H., … Joung, J. K. (2008). Rapid "Open-Source" Engineering of Customized Zinc-Finger Nucleases for Highly Efficient Gene Modification. *Molecular Cell*, *31*(2), 294–301. https://doi.org/10.1016/j.molcel.2008.06.016

Mahdieh, N., & Rabbani, B. (2013). An overview of mutation detection methods in genetic

disorders. *Iranian Journal of Pediatrics*, *23*(4), 375–388.

Maheshwari, S., Ishii, T., Brown, C. T., Houben, A., & Comai, L. (2017). Centromere location in Arabidopsis is unaltered by extreme divergence in CENH3 protein sequence. *Genome Research*, *27*(3), 471–478. https://doi.org/10.1101/gr.214619.116

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science*, *339*(6121), 823–826. https://doi.org/10.1126/science.1232033

Marraffini, L. A. (2015). CRISPR-Cas immunity in prokaryotes. In *Nature* (Vol. 526, Issue 7571, pp. 55–61). Nature Publishing Group. https://doi.org/10.1038/nature15386

Marvier, M., McCreedy, C., Regetz, J., & Kareiva, P. (2007). A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science*, *316*(5830), 1475–1477. https://doi.org/10.1126/science.1139208

Miller, J. C., Tan, S., Qiao, G., Barlow, K. A., Wang, J., Xia, D. F., Meng, X., Paschon, D. E., Leung, E., Hinkley, S. J., Dulay, G. P., Hua, K. L., Ankoudinova, I., Cost, G. J., Urnov, F. D., Zhang, H. S., Holmes, M. C., Zhang, L., Gregory, P. D., & Rebar, E. J. (2011). A TALE nuclease architecture for efficient genome editing. *Nature Biotechnology*, *29*(2), 143–150. https://doi.org/10.1038/nbt.1755

MONSANTO. (2005). Glyphosate: Response to "'Differential effects of glyphosate and Roundup on human placental cells and aromatase.'" In *Environmental Health Perspectives*. https://doi.org/10.1289/ehp.7728

Moscou, M. J., & Bogdanove, A. J. (2009). Recognition by TAL Effectors. *Science (New York, N.Y.)*, *326*(December), 1501. https://doi.org/10.1126/science.1178817

Noguera, P. A., & Ibarra, J. E. (2010). Detection of new cry genes of Bacillus thuringiensis by Use of a Novel PCR primer system. *Applied and Environmental Microbiology*, *76*(18), 6150–6155. https://doi.org/10.1128/AEM.00797-10

Oliver, M. J. (2014). Why we need GMO crops in agriculture. In *Missouri medicine* (Vol. 111, Issue 6, pp. 492–507). Missouri State Medical Association.

Palma, L., Muñoz, D., Berry, C., Murillo, J., Caballero, P., & Caballero, P. (2014). Bacillus thuringiensis toxins: An overview of their biocidal activity. *Toxins*, *6*(12), 3296–3325. https://doi.org/10.3390/toxins6123296

Pikaard, C. S., & Scheid, O. M. (2014). Epigenetic regulation in plants. *Cold Spring Harbor Perspectives in Biology*, *6*(12), 1–31. https://doi.org/10.1101/cshperspect.a019315

Podevin, N., Devos, Y., Davies, H. V., & Nielsen, K. M. (2012). Transgenic or not? No simple answer! New biotechnology-based plant breeding techniques and the regulatory landscape. *EMBO*

Reports, *13*(12), 1057–1061. https://doi.org/10.1038/embor.2012.168

Pons, X., Lumbierres, B., Antoni, R., & Starý, P. (2011). Parasitoid complex of alfalfa aphids in an IPM intensive crop system in northern Catalonia. *Journal of Pest Science*, *84*(4), 437–445. https://doi.org/10.1007/s10340-011-0383-0

Pontiroli, A., Simonet, P., Frostegard, A., Vogel, T. M., & Monier, J. M. (2007). Fate of transgenic plant DNA in the environment. *Environ. Biosafety Res*, *6*, 15–35. https://doi.org/10.1051/ebr:2007031

Potrykus, I. (2001). Golden Rice and Beyond International Society for Plant Molecular Biology 2000 Kumho Science International Award. *Society*, *125*(March), 1157–1161.

Priesnitz, K. U., Benker, U., & Schaarschmidt, F. (2013). Assessment of the potential impact of a Bt maize hybrid expressing Cry3Bb1 on ground beetles (Carabidae). *Journal of Plant Diseases and Protection*, *120*(3), 131–140. https://doi.org/10.1007/BF03356464

Raps, A., Kehr, J., Gugerli, P., Moar, W. J., Bigler, F., & Hilbeck, A. (2001). Immunological analysis of phloem sap of Bacillus thuringiensis corn and of the nontarget herbivore Rhopalosiphum padi (Homoptera: Aphididae) for the presence of Cry1Ab. *Molecular Ecology*, *10*(2), 525–533. https://doi.org/10.1046/j.1365-294X.2001.01236.x

Report of Health and Safety Executive. (2014). The Genetically Modified Organisms (Contained Use) Regulations 2014. In *Health and Safety Executive* (Fifth). http://www.hse.gov.uk/pubns/books/l29.htm

Ricroch, A., Harwood, W., Svobodová, Z., Sági, L., Hundleby, P., Badea, E. M., Rosca, I., Cruz, G., Salema Fevereiro, M. P., Marfà Riera, V., Jansson, S., Morandini, P., Bojinov, B., Cetiner, S., Custers, R., Schrader, U., Jacobsen, H. J., Martin-Laffon, J., Boisron, A., & Kuntz, M. (2016). Challenges facing European agriculture and possible biotechnological solutions. *Critical Reviews in Biotechnology*, *36*(5), 875–883. https://doi.org/10.3109/07388551.2015.1055707

Roberts, H. F. (1929). *Plant hybridization before Mendel*.

Roorda, J. (2017). Patents, Hidden Novelty, and Food Safety. *Florida Law Review*, *68*(2), 657.

Sahadevan, B. Y. P. C., & Namboodir, K. M. N. (1963). *NATURAL*.

Sahebi, M., Hanafi, M. M., van Wijnen, A. J., Rice, D., Rafii, M. Y., Azizi, P., Osman, M., Taheri, S., Bakar, M. F. A., Isa, M. N. M., & Noor, Y. M. (2018). Contribution of transposable elements in the plant's genome. *Gene*, *665*(December 2017), 155–166. https://doi.org/10.1016/j.gene.2018.04.050

Saxena, D., & Stotzky, G. (2000). Insecticidal toxin from Bacillus thuringiensis is released from roots of transgenic Bt corn in vitro and in situ. *FEMS Microbiology Ecology*, *33*(1), 35–39. https://doi.org/10.1016/S0168-6496(00)00041-6

Sikora, P., Chawade, A., Larsson, M., Olsson, J., & Olsson, O. (2011). Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *International Journal of Plant Genomics*, *2011*. https://doi.org/10.1155/2011/314829

Smith, J., Grizot, S., Arnould, S., Duclert, A., Epinat, J.-C., Chames, P., Prieto, J., Redondo, P., Blanco, F. J., Bravo, J., Montoya, G., Pâques, F., & Duchateau, P. (2006). A combinatorial approach to create artificial homing endonucleases cleaving chosen sequences. *Nucleic Acids Research*, *34*(22), e149. https://doi.org/10.1093/nar/gkl720

Snell, C., Bernheim, A., Bergé, J. B., Kuntz, M., Pascal, G., Paris, A., & Ricroch, A. E. (2012). Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. *Food and Chemical Toxicology*, *50*(3–4), 1134–1148. https://doi.org/10.1016/j.fct.2011.11.048

Soultanas, P. (2011). The replication-transcription conflict. *Transcription*, *2*(3), 140–144. https://doi.org/10.4161/trns.2.3.15908

Stadler, L. J. (1928). Genetic Effects of X-Rays in Maize. *Proceedings of the National Academy of Sciences*, *14*(1), 69–75. https://doi.org/10.1073/pnas.14.1.69

Stephens, S. G., & Finkner, M. D. (1953). Natural crossing in cotton. *Economic Botany*, *7*(3), 257–269. https://doi.org/10.1007/BF02984952

Stratilová, Z., & Jedličková, M. (2016). *GMO bez obalu* (4th ed.).

Sultan, S. M., & Subba Rao, L. V. (2013). *GERMPLASM COLLECTION FROM LAST REMNANTS OF RICE LANDRACE GENETIC DIVERSITY IN HIGH ALTITUDE AREAS OF KASHMIR HIMALAYAS*. *4*(4), 467–476.

Svobodova, Z., Burkness, E. C., Skokova Habustova, O., & Hutchison, W. D. (2017). Predator Preference for Bt-Fed Spodoptera frugiperda (Lepidoptera: Noctuidae) Prey: Implications for Insect Resistance Management in Bt Maize Seed Blends. *Journal of Economic Entomology*, *110*(3), 1317–1325. https://doi.org/10.1093/jee/tox098

Svobodová, Z., Skoková Habuštová, O., Boháč, J., & Sehnal, F. (2016). Functional diversity of staphylinid beetles (Coleoptera: Staphylinidae) in maize fields: Testing the possible effect of genetically modified, insect resistant maize. *Bulletin of Entomological Research*, *106*(4), 432–445. https://doi.org/10.1017/S000748531500111X

Svobodová, Zděka, Habuštová, O. S., Hutchison, W. D., Hussein, H. M., & Sehnal, F. (2015). Risk assessment of genetically engineered maize resistant to Diabrotica spp.: Influence on above-ground arthropods in the Czech Republic. *PLoS ONE*, *10*(6), 1–16. https://doi.org/10.1371/journal.pone.0130656

Svobodová, Zdeňka, Skoková-Habuštová, O., Spitzer, L. S., & Sehnal, F. (2020). Importance of

functional classification in the use of carabids for the environmental risk assessment of the GE crops and other agricultural practices. *Insect Science*, *27*(2), 375–388. https://doi.org/10.1111/1744-7917.12643

Thomas, G., & De Tavernier, J. (2017). Farmer-suicide in India: debating the role of biotechnology. *Life Sciences, Society and Policy*, *13*(1). https://doi.org/10.1186/s40504-017-0052-z

Thomson, J. A. (2001). Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science*, *66*(2), 188–193. https://doi.org/10.1111/j.1365-2621.2001.tb11314.x

Trnková, J., Hanák, J., Křístková, M., & Dvořáková, M. (2019). *Organizace a kontrola pěstování GM plodin v ČR*.

Ujj, O. (2016). European and American Views on Genetically Modified Foods. *The New Atlantis, Journal of Technology and Society*, *49*, 77–92. http://www.thenewatlantis.com/docLib/20170101_TNA49Ujj.pdf

Urnov, F. D., Miller, J. C., Lee, Y. L., Beausejour, C. M., Rock, J. M., Augustus, S., Jamieson, A. C., Porteus, M. H., Gregory, P. D., & Holmes, M. C. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature*, *435*(7042), 646–651. https://doi.org/10.1038/nature03556

ÚZKZÚZ. (2016). *Jednotné pracovní postupy ÚZKZÚZ - Analýza půd - I*.

Whelan, A. I., & Lema, M. A. (2015). Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. *GM Crops & Food*, *6*(4), 253–265. https://doi.org/10.1080/21645698.2015.1114698

Whitlock, M. C., & Gomulkiewicz, R. (2005). Probability of fixation in a heterogeneous environment. *Genetics*, *171*(3), 1407–1417. https://doi.org/10.1534/genetics.104.040089

Wolfenbarger, L. L. R., Naranjo, S. E., Lundgren, J. G., Bitzer, R. J., & Watrud, L. S. (2008). Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. *PLoS ONE*, *3*(5). https://doi.org/10.1371/journal.pone.0002118

Yee, J. K. (2016). Off-target effects of engineered nucleases. *FEBS Journal*, 3239–3248. https://doi.org/10.1111/febs.13760

Zakir, M. (2018). Mutation Breeding and its Application in Crop Improvement under Current Environmental Situations for Biotic and Abiotic Stresses. *International Journal of Research Studies in Agricultural Sciences (IJRSAS)*, *4*(4), 1–10.

Zamir, D. (2001). Improving plant breeding with exotic genetic libraries. *Nature Reviews Genetics*, *2*(12), 983–989. https://doi.org/10.1038/35103590

Zhang, C., Wohlhueter, R., & Zhang, H. (2016). Genetically modified foods: A critical review of their

promise and problems. *Food Science and Human Wellness*, *5*(3), 116–123. https://doi.org/10.1016/j.fshw.2016.04.002