doi: 10.17221/104/2015-CJGPB

Transgenic Flax/Linseed (*Linum usitatissimum* L.) – Expectations and Reality

Michaela LUDVÍKOVÁ and Miroslav GRIGA

Plant Biotechnology Department, Agritec Plant Research, Ltd., Šumperk, Czech Republic

Abstract

Ludvíková M., Griga M. (2015): Transgenic flax/linseed (*Linum usitatissimum* L.) – expectations and reality. Czech J. Genet. Plant Breed., 51: 123–141.

This review summarizes the history, important milestones, current status and perspectives of biotech flax/linseed (*Linum usitatissimum* L.), supplemented with some of our original research, breeding and data on environmentalsafety. We show how recent biotechnology methods and genetic engineering contributed to the flax/linseed breeding in order to speed up the breeding process (doubled haploids technology; *in vitro* selection with the use of pathogenic toxins or heavy metals; genetic transformation) and for the creation of new flax/linseed cultivars. The focus is laid on genetic engineering which represents an excellent technology to enrich the flax/linseed genepool with genes of interest, which are not naturally present in the flax/linseed genome. Different methods of flax transformation are mentioned, as well as various genes of interest that have been used for flax transformation to date aimed at improving transgenic flax properties, affecting both qualitative and quantitative traits. The fatty acid content and composition, the lignan (especially secoisolariciresinol diglucoside – SDG) content, flax fibre quality, tolerance to herbicides and resistance to diseases belong, among others, to flax traits that have already been modified by genetic engineering. Selection genes, reporter genes and also promoters that have been used for the vector construction are also summarized. This paper describes different fields of utilization of genetically modified (GM) flax with different improved properties. The history of the only so far officially registered transgenic linseed cultivar Triffid is described in detail. Finally, potential risks and benefits of flax modification are evaluated and also the prime expectations of GM flax and real current state of this technology compared. Unfortunately, the products created by this technology are under strict (albeit not scientifically-based) legislative/political control in the European Union (EU), which prevents the access of products, created by breeders using this top technology, to the EU market.

Keywords: biotechnology in plant breeding; fibre crops; heavy metal tolerance/accumulation; lignan content; oil composition; transformation/genetic modification

Over many centuries, flax has been selected for fibre production (fibre flax) or for the oil content of seeds (oilseed flax or linseed). Flax is an annual, dicotyledonous, highly self-pollinated plant species grown on almost all continents. *Linum usitatissimum* L. has three technological types: fibre flax, oil-fibre flax (dual-purpose type) and oil flax (oilseed, linseed). The botanical name of flax expresses its multipurpose uses (practically complete aboveground biomass may be processed for industrial, pharmaceutical and food products). Linseed oil was traditionally produced by seed extraction with an organic solvent. Flax oil is used for manufacturing varnishes, paints and dyes, printing ink, linoleum, bio-petroleum, oilcloths or plastics.

Flax fibre is used in the textile industry for linen cloth and also in the paper and pulp industry to make paper products including cigarette paper; it can also be useful for bio-product applications such as geotextiles and insulation (Kymäläinen & Sjöberg 2008). In addition, it offers new possibilities for non-traditional use, e.g. building and furniture industry or automotive and airplane industry. Flax is also considered to be an excellent candidate crop for *phytoremediation* and it has a raising importance as an energy crop.

doi: 10.17221/104/2015-CJGPB

It is also well known for its good quality oil and compounds favourable for human health. Flax oil is the richest plant source of linoleic and linolenic polyunsaturated fatty acids (PUFA), which are essential for humans, because they cannot be synthesized in the animal/human organism.

Flax belongs to the group of functional food, claimed to have health-promoting or disease-prevention properties in addition to basic nutritional properties. There have been many health claims for flax seed and flax oil. Published studies have shown that the consumption of flax seed has beneficial effects on coronary heart disease, hormonal and neurological disorders, some kinds of cancer or it can reduce the risk of diabetes (Huang & Ziboh 2001; Simopoulos 2002; Thompson *et al*. 2005). Lignans contained in flax seed (and especially secoisolariciresinol diglucoside) have been highlighted in recent studies due to their pharmacological activities (Zanwar *et al*. 2011; Patel *et al*. 2012).

Flax biotechnology: non-GM approaches

The utilization of tissue cultures has played a crucial role in the application of genetic engineering approaches. The techniques for regeneration from protoplasts, hypocotyl-, cotyledon- and leaf-derived callus (Barakat & Cocking 1983, 1985) made flax an attractive experimental system for somatic genetic manipulation.

Before the expansion of transgenic techniques or simultaneously with the application of methods of genetic engineering, non-transgenic methods including somaclonal variation (O'Conner *et al*. 1991) and mutagenesis induced both by γ-rays (Green & Marshall 1984) and chemicals (Green 1986; Nichterlein *et al*. 1988; Rowland & Bhatty 1990; Rowland 1991) were performed. The most interesting materials were obtained due to the use of ethyl methanesulfonate (EMS), mutant lines derived from cv. Glenelg (Green 1986) and cv. McGregor (Ntiamoah & Rowland 1997) with very low content of linolenic acid in seeds. The same method was applied for the creation of low-linolenic linseed Allan and medium linolenic linseed Raciol (Tejklová & Bjelková 2011; Tejklová *et al.* 2011).

Cunha and Ferreira (1996) assessed the organogenic and callus formation capacity for different types of source explants. Under the experimental conditions tested, 2,4-dichlorophenoxyacetic acid and zeatin were the most efficient combination of plant growth regulators for callus induction and biomass production. It was reported that the induction of somatic embryogenesis and shoot organogenesis was associated with increase in total sterols in the competent calli and increased ratio of stigmasterol to β-sitosterol in derived embryos and shoots (Cunha & Ferreira 1997). Further, Cunha and Ferreira (1999) found 4% glucose or fructose with MS media to be effective to give highly embryogenic cultures.

The technique for the transfer of mobile Ac/Ds elements known from maize (*Zea mays*), which could be useful in mutagenesis, genome mapping and gene isolation, was also successfully performed in flax (Finnegan *et al.* 1993).

Preťová and obert (2003) summarized the possibilities of flax embryogenesis. Agritec's contribution to flax/linseed anther culture was an extensive research of Tejklová (1996) testing *ca* twenty flax/linseed genotypes and their hybrids for morphogenic callus induction. Since that time, flax/linseed doubled-haploid technology has been routinely used in Agritec's breeding programme and has contributed to the release of several varieties (e.g. Raciol, AGT 297/12, AGT 318/08). Rutkowska-Krause *et al.* (2003) compared the regeneration of flax plants from anther culture and somatic tissues and established a flax regeneration system providing a basis for the creation of transgenic flax. Obert *et al.* (2004) also described the creation of flax haploid plants through anther culture, while McHughen (2000) obtained flax haploid plants through microspore-derived culture. McHughen (2000) and Millam *et al.* (2005) discussed these results and their possible applications in flax research and breeding.

Siegien *et al*. (2013) investigated the relationships between organogenesis of oil flax *in vitro*, cyanogenic potential of these tissues and light conditions. It was suggested that free HCN, released from cyanoglucosides potentially at a higher level under light conditions, may be involved in some organogenic processes which improve regeneration efficiency.

Non-genetically modified (non-GM) approaches have been very helpful for flax breeding and development of new varieties. However, genetic engineering is indispensable when a new trait is to be conferred to plants (when genes are not naturally present in the cultured flax/linseed genome) or when genes of interest are absent in accessible (compatible) gene pool. This approach enables to increase the genetic diversity and to produce new variant alleles that can be implanted from other plant species (and even from bacteria, animals etc.). The experiences to date with transgenic flax/linseed production are discussed in detail below.

doi: 10.17221/104/2015-CJGPB

The early history of flax transformation

Flax was among the first commodity crop species to be genetically engineered by recombinant DNA technologies. The first transgenic flax cells were created in 1983 (Hepburn *et al*. 1983), the transformation was performed via *Agrobacterium tumefaciens*. The first attempts aimed at transfer of whole unmodified T-DNA of *A. tumefaciens* and *A. rhizogenes* were later changed for integration of model prokaryotic genes (Basiran *et al*. 1987; Dong & McHughen 1993; Mlynárová *et al*. 1994; Bretagne-Sagnard & Chupeau 1996) and of specific genes coding economically important traits (Jordan & McHughen 1988; McHughen 1989; McHughen & Holm 1995) (and others as described in Table 1) into flax genome. Flax/linseed transformation has already been reviewed in a number of journal papers or book chapters (Rakouský & Tejklová 1999; McHughen 2000; Preťová *et al*. 2007; Pavelek *et al*. 2012; Badere 2014), nevertheless, our review deals with GM flax in more detail and to a greater extent, trying to provide a thorough summary of this topic.

Gene delivery methods in flax/linseed transformation

Different methods to deliver genes into flax genome have been developed over the years. The most frequently used procedure has been *Agrobacterium*-mediated hypocotyl transformation – flax, like most dicotyledonous crop species, being amenable to gene transfer via *Agrobacterium* (Hepburn *et al*. 1983).

Flax cells can be relatively easily transformed with *A. tumefaciens* and easily grown when the suitable inoculation/selection/regeneration procedure is applied (Jordan & McHughen 1988). Many attempts were made to enhance the efficiency of flax transformation procedures including a prolonged callus induction phase, removal of epidermis and prolonged co-cultivation. To enhance transformation efficiency, an improved procedure for flax was developed by increasing the cell transformation intensity in inoculated hypocotyls, a deliberate choice of selection agent and an optimization of the selection scheme (Dong & McHughen 1993). Flax transformation can also be performed with *Agrobacterium rhizogenes*. The first report of the regeneration of flax transformed by *A. rhizogenes* was described by Zhan *et al*. (1988). In their work the regeneration of flax plants after transformation by either *A. tumefaciens* carrying a disarmed Ti-plasmid vector, or *A. rhizogenes* carrying an unmodified Ri plasmid, was examined. Transformed plantlets with curled leaves and short internodes were obtained from hairy roots induced by *A. rhizogenes*. Some plantlets had a more developed root system characterized by plagiotropic behaviour. These results show that the transformation by *A. rhizogenes* can be an alternative to the transformation by *A. tumefaciens*.

Bleho *et al*. (2011) tested the stability of flax transformation by *A. tumefaciens* versus *A. rhizogenes* using the reporter *gfp* gene. Transformation with *A. rhizogenes* led to stable transformants for over two years, whereas transformation by *A. tumefaciens* resulted in non-regenerable transgenic calli and lasted only 6–8 weeks.

Transgenic flax plants were successfully obtained by Ling and Binding (1997) using protoplast transformation. However, results showed that direct gene transfer into isolated protoplasts is a more suitable procedure for wild species such as *Linum suffruticosum*, which is easily regenerated, while cultured flax *L. usitatissimum* seemed to be more recalcitrant to protoplast technology.

Another possibility is offered by the particle gun (biolistic) method, adapted to flax in the Crop Development Centre (University of Saskatchewan, Saskatoon, Canada). Wijayanto and McHughen (1999) documented a successful biolistic process to regenerate transgenic linseed from bombarded hypocotyls cultured on a standard selection medium. The results showed that particle bombardment could be an alternative method to flax/linseed *Agrobacterium* transformation.

Bastaki & Cullis (2014) described for the first time the *Agrobacterium* transformation of flax via floral dip. Two varieties of flax (fibre and oil) were used. Results showed that a floral-dip method could replace the previously used techniques for flax transformation, because of its simplicity, low cost and high transformation rate.

Selective and reporter genes used for flax transformation

Procedures based on the use of selectable genes coding for either herbicide tolerance or resistance to antibiotics were developed and successfully applied. Selection of transformed flax plants is often based on resistance to the antibiotic kanamycin (*nptII* gene), although the disadvantage of kanamycin as selective

doi: 10.17221/104/2015-CJGPB

Table 1. Milestones in flax transgenesis, agronomic and technological/quality traits – the summary

Year	Result	Reference
1983	first report on *Agrobacterium*-mediated transformation of flax cells	Hebpurn *et al.* (1983)
1987	chlorsulfuron (sulfonylurea) resistant transgenic cell lines	Jordan and McHughen (1987)
	Agrobacterium tumefaciens (*A.t.*)-mediated transformed shoots from callus	Basiran *et al.* (1987)
1988	first flax transgenic plants with glyphosate (Roundup®) tolerance	Jordan and McHughen (1988)
1989	*A.t.*-mediated transfer of sulfonylurea resistance to commercial flax cultivars	McHughen (1989)
1991	first field tests with sulfonylurea-resistant transgenic flax	McHughen and Holm (1991)
1991–1996	optimization of methodology of *A.t.*-mediated transformation protocols	mostly McHughen lab
1995	development and field tests of glufosinate-ammonium tolerant GM flax	McHughen and Holm (1995)
1996	registration of CDC Triffid	McHughen *et al.* (1997)
1997	transformation of flax protoplasts	Ling and Binding (1997)
1999	genetic transformation of flax by particle bombardment	Wijayanto and McHughen (1999)
	development of organ specific promoter from linseed for linseed transformation	Jain *et al.* (1999)
	first release of GM flax into environment in EU (flax lines after T-DNA insertional mutagenesis; randomly induced genetic/phenotype changes)	Rakouský *et al.* (1999, 2001)
since 2000	transformation of flax with various genes of interest (GOI) and environmental risk assessment studies	
2001	CDC Triffid transgenic flax was deregistrated	www.cabn.ca
2004	polyhydroxybutyrate (PHB) synthesis – production of biodegradable polymers	Wróbel *et al.* (2004)
	very-long polyunsaturated fatty acids biosysnthesis in linseed	Abbadi *et al.* (2004)
2005	Iincreased antioxidant capacity: expression of genes encoding chalcone synthase, chalcone isomerase and dihydroflavonol reductase	Lorenc-Kukuła *et al.* (2005)
2007	polyhydroxybutyrate (PHB) synthesis, improved elastic properties of flax fibers	Wróbel-Kwiatkowska *et al.* (2007a)
	lignin deficiency, improved mechanical properties	Wróbel-Kwiatkowska *et al.* (2007b)
	increased flavonoid content connected with *Fusarium* resistance	Lorenc-Kukula *et al.* (2007)
2008	introduction of *crtB* gene into flax, enrichment of carotenoids in flax seed	Fujisawa *et al.* (2008)
	reduction of pectin content, higher retting efficiency of transgenic fibres.	Musialak *et al.* (2008)
2009	glutathion synthesis, tolerance to oxidative stress and *Fusarium* tolerance	Czuj *et al.* (2009)
	omega-3-fatty acid (stearidonic acid) biosynthesis in flax seed, Δ6-desaturase gene from *Primula vialii* used	Rui-López *et al.* (2009)
	SsGT1 gene, higher resistance to *Fusarium* infection and significant increase of the flavonoid glycoside content	Lorenc-Kukula *et al.* (2009)
	risk assessment analysis (cross-polination, interspecific hybridization, escape of transgenes)	Tejklová *et al.* (2009)
2010	mitigation of adventitious presence of volunteer flax in wheat	Dexter *et al.* (2010)
2011	risk assessment analysis: field experiments with GM and organic flax	Jhala *et al.* 2011
2012	enhanced accumulation of heavy metals, introduction of *αMT1* human metallothionein gene	Vrbová *et al.* (2013)
2013	the expression of chimeric *gfp-TUA6* used for visualisation of microtubules	Shysha *et al.* (2013)
2014	RNAi silencing of pinoresinol lariciresinol reductase gene (*LuPLR1*), failed accumulation of SDG	Renouard *et al.* (2014)
2015	high oleic flax through RNAi-mediated multiple *FAD2* gene silencing	Chen *et al.* (2015)

doi: 10.17221/104/2015-CJGPB

agent in flax transformation is the recovery of a great number of escapes. Non-transformed shoots often originate from unmodified cells protected against selection agents by surrounding transformed cells (Jordan & McHughen 1988; McHughen 1989). Another obstacle of transformant selection may arise from a possible interaction of selective agent with antibiotics used for further agrobacterium elimination.

For instance, Koronfel (1998) tested the application of kanamycin for the selection of transformed flax plants together with cefotaxime and carbenicillin as bacteriostatic agents and their effect on differentiation of flax hypocotyls. The presence of cefotaxime and carbenicillin in the medium had a negligible negative impact on regeneration of plants and was found to be effective and safe for further use.

An alternative antibiotic selection method involving the use of hygromycin B has been aimed at an alternative protocol for the selection of transgenic flax applicable for the routine evaluation of broad sample numbers (Rakouský *et al*. 1999). Also the antibiotic spectinomycin was successfully applied in the selection of transformed flax plants (Bretagne-Sagnard & Chupeau 1996). In order to meet the requirement for using non-antibiotic resistance genes for the production of transgenic plants, the phosphomannose isomerase gene was used as an alternative selectable marker for flax transformation (Lamblin *et al*. 2007). They described that the mean transformation efficiency was comparable to that obtained routinely using the *nptII* selectable gene and these results indicate that the mannose selection system can be successfully used for the recovery of transgenic flax plants.

Reporter genes coding the enzymes β-glucuronidase (GUS), luciferase (LUC) or β-galactosidase (LazC) have been used for the monitoring of introduced gene expression in transgenic tissues. The histochemical GUS testing is the most useful assay in flax transformation (Dong & McHughen 1993), although it is a destructive method. Hraška *et al*. (2006) described in more detail the contribution of green fluorescent protein (GFP) as a visual marker for the establishment, evaluation and improvement of transformation procedure for flax plants which can continue in growth and development without damage to transgenic tissues.

Caillot *et al.* (2009) dealt with the influence of light intensity and selection scheme on the regeneration time of transgenic flax plants and established a protocol to increase the number of regenerated shoots and limit the recovery of escapes during regeneration.

Promoters used for flax transformation

The utilization of appropriate and tissue specific promoters is a key matter affecting the outcome of genetic modifications.

The constitutive *CaMV35S* promoter (covering the transgene expression in the whole plant tissues) is used in almost all GM crops and it has also been successfully used in many crucial studies dealing with flax transformation (Wrobel *et al*. 2004; Wrobel-Kwiatowska *et al*. 2007a; Lorenc-Kukula *et al*. 2007; Vrbová *et al*. 2013). On the other hand, a substantial experience with the use of tissue specific promoters in flax transformation has been published so far, as discussed below.

Jain *et al.* (1999) isolated two organ specific promoters from *sad1* and *sad2* members of a gene family encoding the enzyme stearoyl-acyl carrier protein desaturase (SAD). The *sad2* promoter was stronger than the *sad1* promoter in transgenic linseed, so it seemed to be a better candidate for use in linseed transformation. The sequences of promoters were subsequently patented.

Drexler *et al.* (2003) tested four different putative seed-specific promoters from linseed with the use of GUS reporter gene. The promoters included the regulatory regions of the gene coding β-ketoacyl-CoA synthase (KCS) and the napin protein gene from oilseed rape (*Brassica napus* L.), the promoter regions of the 'unknown seed protein' (USP), legumin protein gene (*leb4*) from faba bean (*Vicia faba* L.) and the CaMV 35S promoter (positive control). All the promoters showed some activity, but only CaMV 35S, LeB4 and USP promoters exhibited an expression level sufficient to be useful in linseed.

Truksa *et al.* (2003) identified cDNA and genomic clones of two conlinin genes (*cnl1* and *cnl2*) in flax. The analysis of transgenic flax and *Arabidopsis thaliana* containing the GUS reporter gene under the control of the *cnl1* promoter confirmed the seed specific pattern of expression. The promoter sequence was subsequently patented.

The use of stem-specific 14-3-3 promoter obtained by digestion of pBI101-14-3-3 plasmid was described by Wrobel *et al*. (2004).

Hano *et al*. (2006) studied the transcription activity of the flax pinoresinol-lariciresinol reductase gene (*luplr*) promoter with reporter GUS gene and the *luplr* gene expression during flax seed development. The reporter gene coding GUS protein under *luplr* promoter did not show any expression in vegetative

doi: 10.17221/104/2015-CJGPB

organs, while the control under CaMV35S promoter did. The promoter was found to drive the transcription of a *gus*-int reporter gene in the seed coats during flax seed development. USP (seed-specific promoter) from *Vicia faba*, originally described by Bäumlein *et al*. (1991), was used for flax transformation by Rui-López *et al*. (2009). This promoter is known to be active from early stages of seed development. Renouard *et al*. (2012) tested the expression of *gus*-int reporter gene under four promoters of different length based on sequences described earlier by Hano *et al*. (2006).

Genetically modified traits of flax

During the development of genetically modified flax technology the greatest attention was paid to agronomic traits, many of which were improved using new information about gene identification and molecular expression, including herbicide tolerance for weed control, fungal resistance, disease resistance, insect resistance and stress tolerance to adapt to climate and local factors.

Quality traits of flax were influenced as well; composition of flax ingredients was modified, such as composition and content of flavonoids (antioxidants) and omega-3 fatty acids with a preventive effect on hypertension and specific degenerative diseases. Renewable resources are also connected with modified flax properties, e.g. modified elasticity and thermoplastic characteristics of the flaxseed fibre for the synthesis of biologically degradable synthetic material (Wróbel *et al*. 2004). Another area of application of modified flax is the production of pharmaceutical agents (molecular pharming usage of GM flaxseed as a system to produce pharmaceuticals; to date only at the experimental level) and land reclamation and phytoremediation of heavy metal polluted soil (Broadley *et al*. 2001; Bjelková *et al*. 2011; Czemplik *et al*. 2012; Malik *et al*. 2014).

The milestones in flax transgenesis, including its agronomic and technological/quality traits are summarized in Table 1.

Agronomic traits

Flax was among the first crops to benefit from herbicide resistant constructs, as genes for glyphosate (Roundup) resistance, sulfonylurea and glufosinate resistance were inserted and modified plants were tested in the field. The first agronomic trait in flax influenced by genetic engineering was the tolerance to glyphosate obtained by delivering the modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene to flax (Jordan & McHughen 1988). McHughen (1989) developed flax with chlorsulfuron resistance via *Agrobacterium*-mediated transformation. No significant difference was observed in the overall agronomic performance of transgenic lines when grown in sulfonylurea-treated versus untreated soils in the field conditions (McHughen & Holm 1991; McShefrey *et al*. 1992). Another attempt to induce herbicide tolerance in GM flax was focused on the phosphinothricin acetyltransferase (PAT) gene conferring tolerance to the non-selective herbicide glufosinate (McHughen & Holm 1995).

The resistance of transgenic flax lines to *Fusarium oxysporum* and *F. culmorum* was improved by the expression of cDNA encoding potato β-1,3-glucanase in flax (Wróbel *et al*. 2004). Identically, Lorenc-Kukula *et al*. (2007) transformed flax with the aim of improving resistance to *Fusarium*. Wojtasik *et al*. (2013) analysed the biochemical composition of GM flax fibres overexpressing the β-1,3-glucanase gene, they accentuated their improved mechanical properties and increased antioxidant potential supporting their biomedical applications. Chen *et al*. (2008) focused on pyramiding of alleles with different rust resistance specificity in transgenic flax and creation of flax lines with multiple resistance characteristics.

Lorenc-Kukula *et al*. (2009) introduced the *Solanum sogarandinum* glycosyltransferase (SsGT1) gene into the flax genome. Flax overproducing SsGT1 showed higher resistance to *Fusarium* infection than wild-type plants, which correlated with a significant increase of the flavonoid glycoside content in transgenic plants. Overproduction of glycosyltransferase in transgenic flax also resulted in proanthocyanin, lignan, phenolic acid, and unsaturated fatty acid accumulation in seeds. Czemplik *et al*. (2012) dealt with the above described transgenic flax and its biomedical potential and realised that this GM flax is a good candidate for application in the repair and regeneration of human skin and might also be an alternative to antibiotic therapy for infected wounds.

Boba *et al*. (2011) reported the influence of carotenoid biosynthesis modification on the *Fusarium culmorum* and *Fusarium oxysporum* resistance in flax. The flax plants were transformed with a bacterial gene – *crtB*. The introduction of the *crtB* gene into flax, resulting in the enrichment of carotenoids in

doi: 10.17221/104/2015-CJGPB

flaxseed, was described even earlier by Fujisawa *et al.* (2008).

Flax as an industrial crop can be utilized for phytoremediation purposes as well. Vrbová *et al.* (2009, 2013) dealt with heavy metal binding proteins transformed to flax. However, to date, no transgenic flax/linseed has been permitted to be grown for commercial utilization.

Modification of fatty acid content and composition in flax

Flaxseed contains 35–45% of oil. Fatty acids in a typical linseed oil are of the following types: triply unsaturated α-linolenic acid (52–60%), doubly unsaturated linoleic acid (13–18%) and monounsaturated oleic acid (16–20%), saturated acids palmitic acid (about 6%) and stearic acid (about 3%).

Flax oil is also a well-known source of α-linolenic acid, a precursor of the very long chain polyunsaturated fatty acids: eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). The omega-3 fatty acids are often mentioned in connection with health benefits especially reducing the risk of cardiovascular diseases and cancer. Modern diet in the western world is high in total and saturated fats, ω-6 fatty acids and low in ω-3 fatty acids. This causes a nutritional imbalance, so the nutrition experts in general recommend the increased ω-3 fatty acid intake.

A high proportion of linolenic acid makes linseed oil optimal for industrial use, but inappropriate for using as cooking oil. Essential fatty acids in flax are highly susceptible to oxidation and therefore its oil has a very short shelf life. Only certain cultivars with very low linolenic acid content and appropriate lipid composition are suitable for the commercial preparation of edible oil.

One of the applied strategies aimed at the decrease of α-linolenic content was to replace α-linolenic acid in flax with palmitic acid (Rowland *et al.* 1995). Materials with very low content of linolenic acid in seeds were obtained even due to the use of EMS treatment: cv. Glenelg (Green 1986) and cv. McGregor (Ntiamoah & Rowland 1997). Flaxseed with zero percent of α-linolenic could not be obtained by traditional plant breeding methods, but may be achieved through methods of genetic engineering by reducing the activity of delta-15 desaturase (Jain *et al.* 1999). The new flax varieties with low content of α-linolenic acid (3%), higher content of oleic acid and about 70% linoleic acid were created in Agritec using EMS induced mutagenesis (Tejklová & Bjelová 2011; Tejklová *et al.* 2011).

The deficiency of ω-3 fatty acids is caused by their insufficient consumption on the one hand and as a result of the imbalanced ratio of ω-6 to ω-3 fatty acid intake on the other hand. This unfavourable state can be changed by consumption of oil with higher oleic acid content.

The approaches based on RNA interference of *fad2* gene encoding the enzyme fatty acid desaturase 2 have been successfully applied for a change in fatty acid content and composition (especially the increase of oleic acid content) in different plant species, e.g. *Arabidopsis thaliana* (Stoutjesdijk *et al.* 2002), tobacco (Yang *et al.* 2006), cotton plant (Liu *et al.* 2002), soybean (Chen *et al.* 2011; Wagner *et al.* 2011), rape (Jung *et al.* 2011; Tian *et al.* 2011) and rice (Zaplin *et al.* 2013). Very recently, this approach was also reported in flax (Chen *et al.* 2015). Flax transformations focused on RNA interference of *fad2* gene were performed also in Agritec and the experiments with the aim to change the fatty acid content and composition are still carried out (Ludvíková *et al.* 2014).

Changes in flax fatty acid composition have been reported in a number of papers dealing with flax genetic engineering. The synthesis of ω-3 fatty acids in flaxseed was increased by the introduction of Δ6-desaturase gene from *Primula vialii* (Rui-López *et al.* 2009). The production of stearidonic acid, commonly synthetized only by few plant families, instead of γ-linolenic acid was observed in transgenic plants.

Modification of oleic acid content and composition was mentioned as a by-product in many publications dealing with plant transgenesis usually aimed at different flax qualities. An increase of fatty acid accumulation in transgenic flaxseed oil as a result of generating flax plants with increased antioxidant capacity was described (Lorenc-Kukuła *et al.* 2005; Zuk *et al.* 2011a, b). The increased content of oleic and stearic acids was also noticed. Lorenc-Kukula *et al.* (2009) observed, apart from other changes, the accumulation of unsaturated fatty acids in seeds as a consequence of introduction of the *Solanum sogarandinum* glycosyltransferase (SsGT1) gene into the flax genome.

A few attempts aimed at increasing the synthesis of very long chain fatty acids in flax have also been already published. The accumulation of very long chain PUFAs (polyunsaturated fatty acids) in transgenic flax seed could represent a breakthrough in the

doi: 10.17221/104/2015-CJGPB

search for an alternative source of fish oil. Previous findings about the production of very long chain fatty acids (EPA, DHA) were reviewed by Vrinten *et al.* (2007). During expression of ω-6-desaturase (FAD2 desaturase) an evident increase in the accumulation of stearidonic acid (SDA) and γ-linolenic acid (GLA) in flax has been found.

Flax is considered to be a good candidate for SDA synthesis due to its abundance in endogenous α-linolenic acid (ALA). SDA and GLA were accumulated ten times more in transgenic flax containing the borage desaturase gene under the napin promoter control (Qiu *et al.* 2002). The total amount of GLA and SDA, however, represented only 0.1–2% of fatty acids in seed, probably because of the weak effect of the *Brassica* napin promoter in flax. The most successful experiment focused on increased synthesis of very long chain fatty acids included the usage of C18-Δ9 PUFA-specific elongase cDNA from *Isochrysis galbana* (Qi *et al.* 2002, 2004), Δ8-desaturase from *Euglena gracilis* and Δ5-desaturase from *Mortierella alpina* (Qi *et al.* 2004).

Abbadi *et al.* (2004) reported a high accumulation of 20C PUFAs including ARA and EPA in transgenic flaxseed after transformation with genes encoding acyl-desaturases and acyl-elongases from *Physcomitrella patens*, *Borago officinalis* and *Phaeodactylum.* Zuk *et al.* (2012) observed significantly increased PUFA levels in plants transformed with a chalcone synthase gene from *Petunia hybrida*.

Modification of fibre flax

Polyakov *et al.* (1998) transformed fibre flax cultivars and analysed the expression of introduced genes coding NPT II and GUS.

The fibre quality, strongly dependent on mechanical properties of fibres, is an important flax characteristic affecting its market value. The relationship between flax genes and fibre quality is revealed due to recent developments in plant genomics, the availability of microarray technology and development of metabolomics technology. Wróbel-Kwiatkowska *et al.* (2004, 2007b, 2009) reported the successful incorporation of bacterial genes involved in poly-beta-hydroxybutyrate (PHB) synthesis to flax plants and performed biochemical, mechanical, and structural analyses of transgenic flax stems and fibres. A reduction in the pectin and hemicellulose content and a significant increase in the lignin precursor content, which may lead to better extractability of fibres, were obtained in transgenic flax lines (Wrobel-Kwiatkowska *et al.* 2007a). The improved properties of fibres from genetically modified flax containing genes coding enzymes of PHB synthesis were studied also by Wróbel-Kwiatkowska *et al.* (2012a, b). This study showed the way for the environmentally safe production of biodegradable composites in the future. Dymińska *et al.* (2012) provided a study dealing with micronization improving the functional properties of fibre components of GM flax containing genes coding enzymes of PHB synthesis.

Musialak *et al.* (2008) aimed at generating transgenic flax plants that could be retted more efficiently. The constitutive expression of *Aspergillus aculeatus* genes resulted in a significant reduction in the pectin content in tissue-cultured and field-grown plants. This pectin content reduction was accompanied by a significantly higher retting efficiency of the transgenic plant fibres.

Modification of flax fibre composition was mentioned as a by-product in some publications dealing with plant transgenesis targeted at different flax qualities. Zuk *et al.* (2011a, b) tested the plants of the third generation overexpressing key genes of the flavonoid pathway (CHS, CHI, DFR), and regarding the flax fibre composition they found out an increased level of catechin and acetylvanillone and a decrease in phenolic acids upon flax modification.

Transgenic lines for functional studies of the role of microtubules were generated by genetic transformation of flax with chimeric *gfp-tua6* gene (green fluorescent protein and chimeric tubulin) (Shysha *et al.* 2013). The expression of chimeric *gfp-tua6* gene can be used for visualisation of microtubules with confocal laser scanning microscopy. It was found that GFP-labelled tubulin successfully copolymerizes with endogenous tubulin and participates in the formation of a cortical network of microtubules in cells of transgenic flax. The lines for in-depth studies regarding the role of microtubules in the formation of fibres and mechanical resistance to wind in flax were produced.

Genetic modification for higher lignan content in flax

Flaxseed is the richest source of the lignan secoisolariciresinol diglucoside (SDG). After ingestion, SDG is converted to secoisolariciresinol, which is further metabolised to the mammalian lignans enterodiol and enterolactone. Human and animal studies

doi: 10.17221/104/2015-CJGPB

identify the benefits of SDG consumption (Adolphe *et al*. 2010). The SDG content therefore belongs to the important flax traits already modified by the methods of genetic engineering. A key enzyme for SDG formation is called pinoresinol lariciresinol reductase (PLR). The *plr* gene is expressed in the seed coat of flax seeds and the synthesis of the PLR enzyme occurs where the flax main lignan is found stored in mature seeds, confirming its involvement in SDG synthesis (Hano *et al*. 2006).

Hano *et al*. (2006) studied the flax *plr* promoter activity with reporter *gus* gene and the *plr* gene expression during flax seed development. Renouard *et al*. (2014) successfully used the RNAi phenomenon for the silencing of flax pinoresinol lariciresinol reductase gene. The silencing led to the failed accumulation of SDG in flax seeds, while the synthesis of 8-5‘ linked neolignans dehydrodiconiferyl alcohol glucoside (DCG) and dihydro-dehydrodiconiferyl alcohol glucoside (DDCG) was observed in flax seeds for the first time. Further experiments using the generated transgenic lines devoid of SDG will be performed in order to compare their behaviour in the domain of insect resistance.

Lorenc-Kukuła *et al*. (2005) aimed at generating flax plants with increased antioxidant capacity via the expression of genes encoding chalcone synthase (CHS), chalcone isomerase (CHI) and dihydroflavonol reductase (DFR), an increased content of SDG was observed, although not in all transgenic lines.

As a result of introducing the *Solanum sogarandinum* glycosyltransferase (SsGT1) gene into the flax genome, not only a higher resistance to *Fusarium* infection in transgenic plants was observed, but also an increase in the flavonoid glycoside content, and an accumulation of proanthocyanin, lignan, phenolic acid, and unsaturated fatty acid in the seeds (Lorenc-Kukula *et al*. 2009).

Czemplik *et al*. (2012) and Zuk *et al*. (2011a) proved a further testing of transgenic plants of both aforementioned studies (transgenic flax with *chi*, *chs* and *dfr* genes originating from *Petunia hybrida* and with *ssgt1* gene) and consistently reported a significant increase in SDG content in transformants. The utilization of new flax dressing products from genetically engineered flax plants with the influenced phenylpropanoid pathway to treat long-standing venous ulcers was described by Skórkowska-Telichowska *et al*. (2010). The antioxidative and antibacterial activity of GM flax seedcake extract from transgenic flax plants overproducing compounds from the phenylpropanoid pathway was evaluated by Zuk *et al*. (2014).

Transgenic flax as a suitable candidate for phytoremediation

Most cultivars of flax and linseed are suitable candidates for phytoextraction of the pollutants from contaminated soils. Flax has therefore been gaining increasing attention for potential use in phytoremediation of soils polluted with cadmium (Cd), a highly toxic and abundant environmental contaminant, due to its cadmium-accumulating capability and cadmium-tolerance (Broadley *et al*. 2001; Kos *et al*. 2003; Angelova *et al*. 2004; Shi & Cai 2009; Hradilová *et al*. 2010; Smýkalová *et al*. 2010; Soudek *et al*. 2010; Bjelková *et al*. 2011; Najmanová *et al*. 2012).

GM flax expressing the fusion of the alpha-domain of mammalian metallothionein 1a (*alpha MT1a*) and beta-glucuronidase gene for GUS under the control of CaMV 35S promoter was created in Agritec (Griga *et al*. 2009; Vrbová 2013; Vrbová *et al*. 2009, 2013). The modified plants were genetically stable and had an improved ability to grow in contaminated soil (for field testing see below), extract heavy metals (particularly Cd) and accumulate them within the plant biomass, so they can be successfully used for reclamation and phytoremediation of heavy metal strained soil. While the first laboratory and field tests of alphaMT flax (2009) were connected with Cd, recent research is extended to other metals/metalloids (Pb, As, Se – Cvečková *et al.* 2014).

CDC Triffid story

The first and up to now the only transgenic cultivar of linseed introduced into agricultural practice was CDC Triffid with enhanced herbicide tolerance (namely tolerance to residues of sulfonylurea herbicides in soil) (McHughen *et al*. 1997). The gene of interest conferring resistance was a modified acetolactate synthase gene from *Arabidopsis*, originally cloned and described by Haughn *et al*. (1988). The gene was coupled to the bacterial-origin marker genes nopaline synthase and neomycin phosphotransferase II (*npt*II) in a plasmid and introduced into a disarmed *Agrobacterium tumefaciens* (McHughen 1989). Although this GM cultivar is commonly known as CDC 16 Triffid, formally it is known as FP967. “Much of the discussion surrounding this cultivar probably conjured up recollections of the science fiction novel, “The Day of the Triffids” by John Wyndham (1951), where man-eating plants wreak havoc and attempt to take over the world!” (Jhala *et al*. 2009).

doi: 10.17221/104/2015-CJGPB

Field trials of Triffid flax began at the end of the 1980s, and the regulatory approval process commenced in 1994 (Viju *et al*. 2014), which means that the legislation was distinctly overtaken by scientists. The Triffid cultivar was considered for commercial release in Canada in 1998. It was thought that Triffid would provide a broadleaf cropping option to summer fallowing or continuous cropping to flax growers (McHughen *et al*. 1997). In 1998, Triffid received Canadian and American feed and food regulatory approvals and entered a seed multiplication program. The following year, Europe threatened to stop importing Canadian flax if GM flax should enter into commercial production (Ryan & Smyth 2012).

CDC Triffid was deregistered in 2001 on the request of the Flax Council of Canada and the Saskatchewan Flax Development Commission, as a reaction to the EU's concern with importing GM flaxseeds and all remaining seed was supposedly destroyed. It has been probably the first and the only case of crop variety deregistration in the history. Thus, Triffid flax was not grown commercially and was thought to have been removed from the ecosystem. No tests existed that could detect the presence of Triffid and the exports of Canadian flax to the EU continued as normal until 2009 (Viju *et al*. 2014).

However, in late 2009, Triffid flax was unexpectedly detected in EU food products and in subsequent flax imports from Canada to Europe. In response to this, the EU immediately halted Canadian flax import (Ryan & Smyth 2012). At a meeting of the Standing Committee on the Food Chain and Animal Health of the European Commission, held in Brussels on 16th November 2009, Member States agreed that illegal flaxseed should not be allowed to enter the EU market (Viju *et al*. 2014). This event naturally led to an increased detection effort in the EU and the measures guaranteeing the absence of GMO in imported flaxseeds were requested. The Canadian Grain Commission (CGC), which is the agency in Canada responsible for ensuring quality, initiated its own testing which confirmed the presence of trace amounts of Triffid material in some Canadian flaxseed shipments (Viju *et al*. 2014).

Following the need of dependable Triffid identification a number of publications have been issued. A method to identify the CDC Triffid line using novel construct-specific real-time polymerase chain reaction (PCR) was developed and published by Nakamura *et al*. (2010). Both qualitative and quantitative PCR detection assays were developed to detect genetically modified Triffid flax (Vanella *et al*. 2014). The qualitative PCR revealed a limit of detection of 0.01% of GM flax in 100 ng of genomic DNA, while the quantitative PCR assay showed a limit of detection of about 9 haploid genome copies. The analysis of the prevalence of CDC Triffid transgenic flax in Canadian grain stocks was described by Booker *et al*. (2014). The study evaluated GM presence in Canadian grain stocks for the updated data set of 2009–2013.

How to finish/conclude the "Triffid story"? The authors of the variety elegantly used the most progressed scientific techniques of the time to quickly jump from fundamental research to real practical results, here – the creation of a novel organism/variety with high added value. Unfortunately, the society was not prepared for such progress and namely trade and political reasons resulted in the "deregistration" of the variety (an absolutely quite new phenomenon in the long-term history of plant breeding). Fortunately, another GM crop created in the New World (herbicide-tolerant soybean) had a more successful fate, and recently it has absolutely dominated the world soybean cultivation. Nevertheless, from the scientific and breeding point of view, the GM flax will forever represent the pioneering GM crop in history.

Alternative approaches to flax/linseed genome modification

Few things in science are as contentious or politically charged as genetically modified crops. In order to bring better public perception and as a consequence of the progress of molecular techniques, new approaches are applied and we can predict their expansion in future.

Intragenesis and cisgenesis were described as alternatives to common GM crop creation (Schouten *et al*. 2006; Schouten & Jacobsen 2008). Both concepts stress that plants must be transformed only with genetic material derived from the species itself or from closely related species capable of sexual hybridization. This attitude can be more acceptable to the public.

In the near future we can predict an expansion of new progressive techniques of targeted genome engineering, also known as genome editing (TALENs, ZFNs), as an alternative to classical plant breeding and transgenic methods to improve crop plants. These methods could be utilized in modifying flax qualities

doi: 10.17221/104/2015-CJGPB

including the flax oil composition and content as it was comparably proved in soybean by Haun *et al.* (2014). TALENs (Transcription activator-like effector nucleases*)* mediated targeted genome modification is rapidly becoming a powerful tool for targeted genome editing. In the last four years there has been an explosion in the number and diversity of applications of this technology in general (Joung & Sander 2012; Marx 2012; Pennisi 2012) and also in plant genomics (Chen & Gao 2013; Christian *et al.* 2013; Haun *et al.* 2014). Both zinc finger nucleases (ZFNs) and TALENs can be used to mutagenize genomes at specific loci, but these systems require two different DNA binding proteins flanking a sequence of interest, each with a C-terminal FokI nuclease module.

The above described methods represent effective tools for introducing site-specific double strand DNA breaks and targeted cleavage of genomic DNA. The indubitable advance of these methods is that the progeny of the transformed plant can result in transgene free lines with segregated selection genes and mutations created in the gene of interest. These techniques make possible introducing plant genome modifications, which are indistinguishable from those introduced by conventional breeding and chemical or physical mutagenesis (Belhaj *et al*. 2013). In response to this extension of methods of precise genome editing there is a need of the reform of regulations governing genetically modified crops in Europe. Nevertheless, these methods up to now have seemed to be less controversial than classical methods of genetic engineering and there may be a chance that the above technologies could be classified as non-GM. This would have a positive impact on the plant biotechnology and breeding sectors, especially in Europe (Belhaj *et al*. 2013).

Apart from TALENs and ZNFs, another method – CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) has often been mentioned due to its possible application in plant targeted genome editing (Belhaj *et al.* 2013; Xu *et al.* 2014). This new method is based on the bacterial adaptive immune system, there is an analogy between CRISPR and eukaryotic RNA interference. Nevertheless, recently published results (Gantz & Bier 2015) have caused serious concerns in the community of scientists dealing with genetic modifications of animals (Lunshof 2015). This technique could be considered to be undesirable (even to be used in plant genomics – especially in EU) because of possible risks connected with mutagenic chain reaction (MCR).

Potential risks of genetically modified flax

Genetic engineering, as a very new and not fully explored technology, naturally causes concerns, especially in connection with the commercialization of genetically engineered crops. The sceptics point out to potential movement of transgenes by pollen and seeds, subsequent introgression with weedy and wild relatives, impact on non-target organisms, changes in biodiversity and primarily possible impact on human health.

A prerequisite to the cultivation of transgenic flax must certainly be an environmental risk assessment analysis (ERA). GM flax may also need to be tested for its potential to become a plant pest and for the impact on non-target organisms and on biodiversity.

Cross-pollination between GM crops and wild weedy species was discussed in a number of publications (Beckie *et al*. 2003; Hall *et al*. 2003; Warwick & Stewart 2005; Jhala *et al*. 2008, 2009, 2011; Griga *et al*. 2008). Transgenes incorporated in the genomes of wild or weedy relatives of genetically modified cultural crops may cause changes in those populations. Unless a transgenic trait confers a significant fitness advantage, flax is unlikely to be invasive, but for example the herbicide resistance would grant it a selection advantage. Jhala *et al*. (2008) reported that flax has the ability to hybridize with at least nine species of *Linum* occurring in Asia and Europe with the same chromosome number as cultivated flax (n = 15). There are also eight *Linum* species identified in Canada, nevertheless only *L. rigidum* Pursh var. *rigidum* and *L. sulcatum* Riddell have the same chromosome number, indicating a potential for transgenic introgression (Jhala *et al*. 2009). However, experienced botanists know that the success of such spontaneous crossings is only hypothetical and has been raised just for actual discussion on GM flax environmental risks (which are minimal or tending to zero). Indeed, breeders would be very happy to cross wild *Linum* species with cultural flax, but this is extremely difficult (viz. the text below).

Nevertheless, pollen is only one possible source of the adventitious presence of transgenes coming from GM flax. Other sources of possible contamination that must be mitigated include seed-mediated gene flow through certified flax seed; volunteer flax and inadvertent mixing of products within the transportation system (Jhala *et al*. 2011). The study demonstrating effective mitigation strategies enabling the reduction

doi: 10.17221/104/2015-CJGPB

of seed-mediated gene flow from GM volunteer flax was published by Dexter *et al*. (2010). The prospects for increased future mingling between GM products and conventional products appear to be high. Testing will play a crucial role in whether trade barriers will arise. The experience with Triffid flax suggests that mingling can lead to the imposition of trade barriers through mechanisms that are not transparent and that can impose considerable disruptions to trade and ongoing costs (Viju *et al*. 2014). Nevertheless, all problems mentioned above do not represent a scientific, but just trade/political issue.

Rakouský *et al*. (2004), Griga *et al*. (2008), Tejklová *et al*. (2009) and Tejklová *et al*. (unpublished results) described model situations of uncontrolled cross-pollination between transformed and non-transformed linseed. The frequency of natural cross-pollination and maximum distance of pollen transmission were determined using a genetic line with recessive yellowish shoots obtained via T-DNA insertional mutagenesis and blue petals and a line with standard dominant green shoots and white petals. This work also dealt with uncontrolled interspecific hybridization between *L. usitatissimum* and *L. flavum*, the only wild *Linum* species in the Czech Republic (and Central Europe) potentially able to be hybridized with *L. usitatissimum* as well as possible escape of transgene seeds released during the matured plant processing. The results showed that the probability of unintended natural crossing between GM flax and L. *flavum* may be considered as extremely low and uncontrolled spreading of flax seeds without human help is not practically possible (Tejklová *et al*. – unpublished results).

In connection with possible concerns related with GM flax several studies dealing with impact of GM flax on the components of surrounding environment were published. Wróbel-Kwiatkowska *et al*. (2012b) studied the impact of genetic manipulation in flax on arbuscular mycorrhiza and plant performance. Five types of transgenic flax that were generated to improve fibre quality and resistance to pathogens, through increased levels of either phenylpropanoids, glycosyltransferase or β-1,3-glucanase or through producing polyhydroxybutyrate, were used. No significant influence of GM plants on interaction with arbuscular mycorrhizal fungi was found. The effect of transgenic flax seeds on rabbit caecal fermentation was reported by Miśta *et al*. (2011). This study suggested that tested seeds of GM flax with the modified flavonoid pathway did not have any unfavourable effect on the rabbit caecal microflora activity.

What were the expectations?

International trade with genetically modified organisms (GMOs) and agricultural products has been a contentious issue since the technology was first commercialised in the latter half of the 1990s (Isaac & Kerr 2003; Wugar & Cottier 2008).

However, the expectations about GM crops were in the past generally more optimistic in comparison with the present. GM crops have been framed by expectations that they would be an intrinsically "pro-poor" innovation that would contribute powerfully to international agricultural development. However, expectations typically have to be scaled back in the light of experience. Published reviews of the socio-economic impacts of GM crops among poor, small-scale farmers in the developing world indicate that these effects have been very mixed and contingent on the agronomic, socio-economic and institutional settings where the technology has been applied (Glover *et al*. 2010).

Dixon (1995) vindicated GM flax plants that could be grown in soil with high residual levels of sulfonylurea and that could be used with no yield penalty in the presence or absence of herbicides. The lesser requirement of chemicals leading to more sustainable agronomic practices during breeding of these transgenic plants was pointed out. It was argued that this experiment rebuffed criticisms of herbicide-tolerant plants that had been made by environmentalists and by plant breeders. McHughen *et al*. (1997) reported that there are no outstanding weaknesses of the Triffid cultivar, and the only major advantage is its ability to withstand sulfonylurea herbicide residues, so it will be of special interest to those farmers who use sulfonylurea herbicides. It was expected that CDC Triffid would provide a broadleaf cropping option to summer fallowing or continuous cropping to flax growers due to the transgene having conferred resistance to sulfonylurea herbicide residues in soil.

On the other hand, many scientists expressed grave concerns about ecological risks associated with GM crops during their introduction into the environment or release to the market (Rissler & Mellon 1996; Snow & Palma 1997; Ellstrand *et al*. 1999).

Potential risks to the environment are thoroughly assessed in ERA – an indispensable part of the Eu-

doi: 10.17221/104/2015-CJGPB

ropean Union (EU), Directive 2001/18/EC, and legislation of other countries on GMO release to the environment and to the market.

What is the reality (recent state and predictions for GM flax crop)?

Imports of GM product(s) have been embargoed or restricted by a number of countries, especially in the EU – the main protagonist in the international debate over genetically modified products. There predominates a circumspect and nearly sceptic attitude in the EU to GM crops (Directive EU 2015/412). This has been confirmed by the decreasing number of announced GM field trials, considerably lower number of GM crops approved for circulation and disproportionately lower number of GM crops approved for growing (only one at the moment compared to 25 in the world). In spite of reduced GM areas in the EU, Europe is a great importer of GM crops (mostly soybean and maize), and thus European growers are disadvantaged. It can be concluded that EU GM legislation prevents the progress of GM plant breeding (Directive EU 2015/412).

Field trials with GM flaxseed have been undertaken in the EU in three applications in three countries: Sweden, Poland and Czech Republic (http://www.gmo-compass.org; http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx). Traits studied in these experiments were oil composition, flavonoid content, elasticity (bioplastics), herbicide tolerance, insect resistance, insect and fungal resistance and heavy metal absorption. The area of GM flax field trials in the Czech Republic was 0.007 ha in 2014 (Ministry of Agriculture of the Czech Republic).

Despite approval in Canada, GM flaxseed has not been cultivated commercially to date (http://www.gmo-compass.org). The only herbicide-resistant GM cultivar Triffid was introduced in 2001 and it was soon taken off the market because European importers refused to buy it. The situation with up to now the only transgenic cultivar Triffid seems to move in a vicious circle: the only way that Canadian flax exports can escape the requirements of the EU policy of zero tolerance would be for Triffid to become an authorised GM product in the EU. This would require someone to pay for Triffid to undergo the very expensive and time-consuming EU registration process. Given that Triffid is no longer registered in Canada and not grown, no one will shoulder this burden (Viju *et al*. 2014).

At first Canada was considered to be a leader of flax transgenesis due to research activities related namely with Alan McHughen and his co-workers. In recent years Poland can be designated as the centre of flax transgenesis research. This situation seems like a paradox, because the Polish government does not want to allow GM crop cultivation. The probability that GM flax will be grown there is infinitesimal, although this country is traditionally connected with flax breeding, growing, processing and export.

It is clear that without the consent of society at large, GM crops in the EU will fail in the market place. It is thought that non-food application of GM crops (e.g. for phytoremediation) can be in general better accepted by the public than classical genetic modifications (resulting in food/feed products) as well as above-mentioned progressive technologies of cisgenesis or targeted genome editing.

Acknowledgements. This work was financially supported by Ministry of Education, Youth and Sports of the Czech Republic, Project No. LH12226. The authors are greatly acknowledged to Dr. S. Ochatt, Dr. S. Rakouský and Prof. J. Petr for reading the manuscript, critical comments and helpful discussion.

References

Abbadi A., Domergue F., Bauer J., Napier J.A., Welti R., Zähringer U., Cirpus P., Heinz E. (2004): Biosynthesis of very-long-chain polyunsaturated fatty acids in transgenic oilseeds: constraints on their accumulation. Plant Cell, 16: 2734–2748.

Adolphe J.L., Whiting S.J., Juurlink B.H., Thorpe L.U., Alcorn J. (2010): Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. British Journal of Nutrition, 103: 929–38.

Angelova V., Ivanova R., Delibaltova V., Ivanov K. (2004): Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). Industrial Crops and Products, 19: 197–205.

Badere R.S. (2014): Linseed improvement: a glance at the major developments in recent years. In: Makde K.H., Pawar S.E. (eds): Prof. A.D. Choudhary Commemoration – A Glimpse of Current Vistas in Plant Science Research. 1st Ed. Nagpur, Hislop College Publication Cell: 75–92.

Barakat M.N., Cocking E.C. (1983): Plant regeneration from protoplast derived tissues of *Linum usitatissimum* L. (flax). Plant Cell Reports, 2: 314–317.

Barakat M.N., Cocking E.C. (1985): An assessment of the culture capabilities of protoplasts of some wild species of *Linum*. Plant Cell Reports, 4: 164–167.

doi: 10.17221/104/2015-CJGPB

Basiran N., Armitage P., Scott R.J., Draper J. (1987): Genetic transformation of flax (*Linum usitatissimum*) by *Agrobacterium tumefaciens*: Regeneration of transformed shoots via a callus phase. Plant Cell Reports, 6: 396–399.

Bastaki N.K., Cullis C.A. (2014): Floral-dip transformation of flax (*Linum usitatissimum*) to generate transgenic progenies with a high transformation rate. Journal of Visualised Experiments, 94: e52189.

Bäumlein H., Boerjan W., Nagy I., Bassüner R., Van Montagu M., Inzé D., Wobus U. (1991): A novel seed protein gene from *Vicia faba* is developmentally regulated in transgenic tobacco and *Arabidopsis* plants. Molecular & General Genetics, 225: 459–67.

Beckie H.J., Warwick S.I., Nair H., Seguin-Swartz G. (2003): Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecological Applications, 13: 1276–1294.

Belhaj K., Chaparro-Garcia A., Kamoun S., Nekrasov V. (2013): Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods, 9: 39.

Bjelková M., Genčurová V., Griga M. (2011): Accumulation of cadmium by flax and linseed cultivars in field-simulated conditions: a potential for phytoremediation of Cd-contaminated soils. Industrial Crops and Products, 33: 761–774.

Bleho J., Obert B., Takáč T., Petrovská B., Heym C., Menzel D., Šamaj J. (2011): ER disruption and GFP degradation during non-regenerable transformation of flax with *Agrobacterium tumefaciens*. Protoplasma, 249: 53–63.

Boba A., Kulma A., Kostyn K., Starzycki M., Starzycki E., Szopa J. (2011): The influence of carotenoid biosynthesis modification on the *Fusarium culmorum* and *Fusarium oxysporum* resistance in flax. Physiological and Molecular Plant Pathology, 76: 39–47.

Booker H.M., Mischkolz J.M., St. Louis M., Lamb E.G. (2014): Analysis of the prevalence of CDC Triffid transgenic flax in Canadian grain stocks. AgBioForum, 17: 75–83.

Bretagne-Sagnard B., Chupeau Y. (1996): Selection of transgenic flax plants is facilitated by spectinomycin. Transgenic Research, 5: 131–137.

Broadley M.R., Willey N.J., Wilkins J.C., Baker A.J.M., Mead A., White P.J. (2001): Phylogenetic variation in heavy metal accumulation in angiosperms. New Phytologist, 152: 9–27.

Caillot S., Rosiau E., Laplace C., Thomasset B. (2009): Influence of light intensity and selection scheme on regeneration time of transgenic flax plants. Plant Cell Reports, 28: 359–371.

Chen K., Gao C. (2013): TALENs: Customizable molecular DNA scissors for genome engineering of plants. Journal of Genetics and Genomics, 40: 271–279.

Chen W., Song K., Cai Y., Li W., Liu B., Liu L. (2011): Genetic modification of soybean with a novel grafting technique: downregulating the *FAD2-1* gene increases oleic acid content. Plant Molecular Biology Report, 29: 866–874.

Chen Y., Singh S., Rashid K.Y., Dribnenki P., Green A. (2008): Pyramiding of alleles with different rust resistance specificities in *Linum usitatissimum* L. Molecular Breeding, 21: 419–430.

Chen Y., Zhou X.R., Zhang Z.J., Dribnenki P., Singh S., Green A. (2015): Development of high oleic oil crop platform in flax through RNAi-mediated multiple *FAD2* gene silencing. Plant Cell Reports, 34: 643–653.

Christian M., Qi Y., Zhang Y., Voytas D.F. (2013): Targeted mutagenesis of *Arabidopsis thaliana* using engineered TAL effector nucleases. Genes/Genomes/Genetics, 3: 1697–1705.

Cunha A.C., Ferreira M.F. (1996): Somatic embryogenesis, organogenesis and callus growth kinetics of flax. Plant Cell Tissue and Organ Culture, 47: 1–8.

Cunha A.C., Ferreira M.F. (1997): Differences in free sterols content and composition associated with somatic embyogenesis, shoot organogenesis and calli growth of flax. Plant Science, 124: 97–105.

Cunha A.C., Ferreira F.M. (1999): Influence of medium parameters on somatic embryogenesis from hypocotyl explants of flax (*Linum usitatissimum* L.) – effect of carbon source, total inorganic nitrogen and balance between ionic forms and interaction between calcium and zeatin. Journal of Plant Physiology, 155: 591–597.

Cvečková M., Vrbová M., Pavelková M., Větrovcová M., Smýkalová I., Griga M. (2014): Transgenic linseed (*Linum usitatissimum* L.) with alphaHMT1a: the effect on the accumulation of several metal elements. In: 11th Int. Phytotechnologies Conference, Sept 30–Oct 3, 2014, Heraklion: 155.

Czemplik M., Kulma A., Bazela K., Szopa J. (2012): The biomedical potential of genetically modified flax seeds overexpressing the glucosyltransferase gene. BMC Complementary and Alternative Medicine, 12: 251.

Czuj T., Zuk M., Starzycki M., Amir R., Szopa J. (2009): Engineering increases in sulfur amino acid contents in flax by overexpressing the yeast Met25 gene. Plant Science, 177: 584–592.

Dexter J.E., Jhala A.J., Hills M.J., Yang R., Topinka, K.C., Weselake R.J., Hall, L.M. (2010): Quantification and mitigation of adventitious presence of volunteer flax (*Linum usitatissimum*) in wheat. Weed Science, 58: 80–88.

Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC – Commission Declaration.

Directive (EU) 2015/412 of the European Parliament and of the Council (2015): Official Journal of the European Union, L68/1–8.

doi: 10.17221/104/2015-CJGPB

Dixon B. (1995): A vindication of plant transgenics. Nature Biotechnology, 13: 308.

Dong J.Z., McHughen A. (1993): An improved procedure for production of trans-genic flax plants using *Agrobacterium tumefaciens*. Plant Science, 88: 61–71.

Drexler H.S., Scheffler J.A., Heinz E. (2003): Evaluation of putative seed-specific promoters for *Linum usitatissimum*. Molecular Breeding, 11: 149–158.

Dymińska L., Szatkowski M., Wróbel-Kwiatkowska M., Zuk M., Kurzawa A., Syska W., Gągor A., Zawadzki M., Ptak M., Mączka M., Hanuza J., Szopa J. (2012): Improved properties of micronized genetically modified flax fibers. Journal of Biotechnology, 164: 292–299.

Ellstrand N.C., Prentice H.C., Hancock J.F. (1999): Gene flow and introgression from domesticated plants into their wild relatives. Annual Review of Ecology and Systematics, 30: 539–563.

Finnegan E.J., Lawrence G.J., Dennis E.S., Ellis J.G. (1993): Behaviour of modified Ac elements in flax callus and regenerated plants. Plant Molecular Biology, 22: 625–633.

Fujisawa M., Watanabe M., Choi S.K., Teramoto M., Ohyama K., Misawa N. (2008): Enrichment of carotenoids in flaxseed (*Linum usitatissimum* L.) by metabolic engineering with introduction of bacterial phytoene synthase gene *crtB*. Journal of Bioscience and Bioengineering, 105: 636–641.

Gantz V.M., Bier E. (2015): The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations. Science, 34: 442–444.

Glover D. (2010): Exploring the resilience of Bt cotton's "propoor success story". Development and Change, 41: 955–981.

Green A.G. (1986): A mutant genotype of flax (*Linum usitatissimum* L.) containing very low levels of linolenic acid in its seed oil. Canadian Journal of Plant Science, 66: 499–503.

Green A.G., Marshall D.R. (1984): Isolation of induced mutants in linseed (*Linum usitatissimum* L.) having reduced linolenic acid content. Euphytica, 33: 321–328.

Griga M., Tejklová E., Seidenglanz M. (2008): Assessment of possible risks associated with release of genetically modified flax and linseed (*Linum usitatissimum* L.) into environment of the Czech Republic. In: 10th Int. Symp. Biosafety of Genetically Modified Organisms, Symposium Handbook: Biosafety Research of GMOs: Past Achievements and Future Challenges, Nov 16–21, 2008, Wellington: 105.

Griga M., Vrbova M., Horacek J., Smykal P., Tejklova E. (2009): Agrobacterium-mediated transformation of flax (*Linum usitatissimum* L.) with heavy metal binding proteins genes. In: Joint Annual Meeting Amer Soc Plant Biol and Phycol Soc Amer, Honolulu: 242–243.

Hall L.M., Good A.G., Beckie H.J, Warwick S.I. (2003): Gene flow in herbicide resistant canola (*Brassica napus*): The Canadian experience. In: Lelley T., Balázs E., Tepfer M. (eds): Ecological Impact of GMO Dissemination in Agroecosystems. Vienna, Facultas Verlagsund Buchhandels AG: 57–66.

Hano C., Martin I., Fliniaux O., Legrand B., Gutierrez L., Arroo R.R., Mesnard F., Lamblin F., Lainé E. (2006): Pinoresinol-lariciresinol reductase gene expression and secoisolariciresinol diglucoside accumulation in developing flax (*Linum usitatissimum*) seeds. Planta, 224: 1291–301.

Haughn G., Smith J., Mazur B., Somerville C. (1988): Transformation with a mutant Arabidopsis acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Molecular and General Genetics, 211: 266–271.

Haun W., Coffman A., Clasen B.M., Demorest Z.L., Lowy A., Ray E., Retterath A., Stoddard T., Juillerat A., Cedrone F., Mathis L., Voytas F.D., Zhang F. (2014): Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnology Journal, 12: 934–940.

Hepburn A.G., Clarke L.E., Blundy K.S., White J. (1983): Nopaline Ti-plasmid, pTiT37, T-DNA insertions into a flax genome. Journal of Molecular and Applied Genetics, 2: 211–24.

Hradilová J., Řehulka P., Řehulková H., Vrbová M., Griga M., Brzobohatý B. (2010): Comparative analysis of proteomic changes in contrasting flax cultivars upon cadmium exposure. Electrophoresis, 31: 421–431.

Hraška M., Rakouský S., Čurn V. (2006): Green fluorescent protein as a vital marker for non-destructive detection of transformation events in transgenic plants. Plant Cell, Tissue and Organ Culture, 86: 303–318.

Huang S., Ziboh A. (eds) (2001): Gamma-linolenic Acid: Recent Advances in Biotechnology and Clinical Applications. Champaign, AOCS Publishing.

Isaac G.E., Kerr W.A. (2003): Genetically modified organisms and trade rules: Identifying important challenges for the WTO. The World Economy, 26: 29–42.

Jain R.K., Thompson R.G., Taylor D.C, Mackenzie S.L., McHughen A., Rowland G.G., Tenaschuk D., Coffey M. (1999): Isolation and characterization of two promotores from linseed for genetic engineering. Crop Science, 39: 1696–1701.

Jhala A.J., Hall L.M., Hall J.C. (2008): Potential hybridization of flax with weedy and wild relatives: an avenue for movement of engineered genes? Crop Science, 48: 825–840.

Jhala A.J., Weselake R.J., Hall L.M. (2009): Genetically engineered flax: potential benefits, risks, regulations, and mitigation of transgene movement. Crop Science, 49: 1943–1954.

Jhala A.J., Bhatt H., Topinka K., Hall L.M. (2011): Pollen-mediated gene flow in flax (*Linum usitatissimum* L.): can genetically engineered and organic flax coexist? Heredity, 106: 557–566.

doi: 10.17221/104/2015-CJGPB

Jordan M.C., McHughen A. (1987): Selection for chlorsulfuron resistance in flax (*Linum usitatissimum*) cell cultures. Journal of Plant Physiology, 131: 333–338.

Jordan M.C., McHughen A. (1988): Glyphosate tolerant flax plants from *Agrobacterium* mediated gene transfer. Plant Cell Reports, 7: 281–284.

Joung J.K., Sander J.D. (2012): TALENs: a widely applicable technology for targeted genome editing. Nature Reviews Molecular Cell Biology, 14: 49–55.

Jung J.H., Kim H., Go Y.S., Lee S.B., Hur C.G., Kim H.U., Suh M.C. (2011): Identification of functional BrFAD2-1 gene encoding microsomal delta-12 fatty acid desaturase from *Brassica rapa* and development of *Brassica napus* containing high oleic acid contents. Plant Cell Reports, 30: 1881–1892.

Koronfel M. (1998): Effects of the antibiotics kanamycin, cefotaxime and carbenicillin on the differentiation of flax hypocotyls. Arabian Journal of Biotechnology, 1: 93–98.

Kos B., Grčman H., Leštan D. (2003): Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant, Soil and Environment, 49: 548–553.

Kymäläinen H., Sjöberg A. (2008): Flax and hemp fibres as raw materials for thermal insulations. Building and Environment, 43: 1261–1269.

Lamblin F., Aimé A., Hano C., Roussy I., Domon J.M., Droogen-Broeck B. Van, Lainé E. (2007): The use of the phosphomannose isomerase gene as alternative selectable marker for *Agrobacterium*-mediated transformation of flax (*Linum usitatissimum*). Plant Cell Reports, 26: 765–772.

Ling H.Q., Binding H. (1997): Transformation in protoplast cultures of *Linum usitatissimum* and *L. suffruticosum* mediated with PEG and with *Agrobacterium tumefaciens*. Journal of Plant Physiology, 151: 479–488.

Liu Q., Singh S., Green A. (2002): High-oleic and high-stearic cottonseed oils: nutritionally improved cooking oils developed using gene silencing. The Journal of American College of Nutrition, 21: 205–211.

Lorenc-Kukuła K., Amarowicz R., Oszmiański J., Doermann P., Starzycki M., Skała J., Zuk M., Kulma A., Szopa J. (2005): Pleiotropic effect of phenolic compounds content increases in transgenic flax plant. Journal of Agricultural and Food Chemistry, 53: 3685–92.

Lorenc-Kukula K., Wróbel-Kwiatkowska M., Starzycki M., Szopa J. (2007): Engineering flax with increased flavonoid content and thus Fusarium resistance. Physiological and Plant Molecular Pathology, 70: 38–48.

Lorenc-Kukuła K., Zuk M., Kulma A., Czemplik M., Kostyn K., Skala J., Starzycki M., Szopa J. (2009): Engineering flax with the GT family 1 solanum sogarandinum glycosyltransferase SsGT1 confers increased resistance to Fusarium infection. Journal of Agricultural and Food Chemistry, 57: 6698–6705.

Ludvíková M., Hanáček P., Griga M. (2014): Modification of linseed oil composition using construct to induce gene silencing. In: 6. Metodické dny, sborník abstrakt, bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti. 1st Ed. Praha, Česká společnost experimentální biologie rostlin.

Lunshof J. (2015): Regulate gene editing in wild animals. Nature, 521: 127.

Malik S., Biba O., Gruz J., Arroo R.R.J., Strnad M. (2014): Biotechnological approaches for producing aryltetralin lignans from *Linum* species. Phytochemistry Reviews, 13: 893–913.

Marx V. (2012): Genome-editing tools storm ahead. Nature Methods, 9: 1055–1059.

McHughen A. (1989): Agrobacterium mediated transfer of chlorsulfuron resistance to commercial flax cultivars. Plant Cell Reports, 8: 445–449.

McHughen A. (2000): Transgenic linseed flax (*Linum usitatissimum*). In: Bajaj Y.P.S (ed.): Transgenic Crops I. Biotechnology in Agriculture and Forestry, 46: 338–351.

McHughen A., Holm F. (1991): Herbicide resistant transgenic flax field test: Agronomic performance in normal and sulfonylurea-containing soils. Euphytica, 55: 49–56.

McHughen A., Holm F. (1995): Development and preliminary field testing of a glufosinate-ammonium tolerant transgenic flax. Canadian Journal of Plant Science, 75: 117–120.

McHughen A., Rowland G.G., Holm F.A., Bhatty R.S., Kenaschuk E.O. (1997): CDC Triffid transgenic flax. Canadian Journal of Plant Science 77: 641–643.

McSheffrey S.A., McHughen A., Devine M.D. (1992): Characterization of transgenic sulfonylurea-resistant flax (*Linum usitatissimum*). Theoretical and Applied Genetics, 84: 480–486.

Millam S., Obert B., Preťová A. (2005): Plant cell and biotechnology studies in *Linum usitatissimum* – a review. Plant Cell, Tissue and Organ Culture, 82: 93–103.

Miśta D., Króliczewska B., Zawadzki W., Pecka E., Steininger M., Hull, S., Zuk M., Szopa J. (2011): The effect of Linola and W92/72 transgenic flax seeds on the rabbit caecal fermentation – *in vitro* study. Polish Journal of Veterinary Sciences, 14: 557–564.

Mlynárová L., Bauer M., Nap J.P., Preťová A. (1994): High efficiency *Agrobacterium*-mediated gene transfer to flax. Plant Cell Reports, 13: 282–285.

Musialak M., Wróbel-Kwiatkowska M., Kulma A., Starzycka E., Szopa J. (2008): Improving retting of fibre through genetic modification of flax to express pectinases. Transgenic Research, 17: 133–147.

Najmanova J., Neumannova E., Leonhardt T., Zitka O., Kizek R., Macek T., Mackova M., Kotrba P. (2012): Cadmium-induced production of phytochelatins and speciation of intracellular cadmium in organs of *Linum usitatissimum* seedlings. Industrial Crops and Products, 36: 536–542.

doi: 10.17221/104/2015-CJGPB

Nakamura K., Akiyama H., Yamada C., Satoh R., Makiyama D., Sakata K., Kawakami H., Mano J., Kitta K., Teshima R. (2010): Novel method to detect a construct-specific sequence of the acetolactate synthase gene in genetically-modified flax CDC Triffid (FP967). Biological and Pharmaceutical Bulletin, 33: 532–534.

Nichterlein K., Marquard R., Friedt W. (1988): Breeding for modified fatty acid composition by induced mutations in linseed (*Linum usitatissimum* L.). Plant Breeding, 101: 190–199.

Ntiamoah C., Rowland G.G. (1997): Inheritance and characterization of two low linolenic acid EMS-induced McGregor mutant flax (*Linum usitatissimum*). Canadian Journal of Plant Science, 77: 353–358.

Obert B., Dedicova B., Hricova A., Samaj J., Pretova A. (2004): Plant regeneration from flax anther culture: genotype, pretreatment and media effect. Plant Cell, Tissue and Organ Culture. 79: 233–238.

O'Conner B.J., Robertson A.J., Gusta L.V. (1991): Differential stress tolerance and cross adaptation in a somaclonal variant of flax. Journal of Plant Physiology, 139: 32–36.

Patel D., Vaghasiya J., Pancholi S.S., Paul A. (2012): Therapeutic potential of secoisolariciresinol diglucoside: a plant lignan. International Journal of Pharmaceutical Sciences and Drug Research, 4: 15–18.

Pavelek M., Tejklová E., Ondřej M., Vrbová M. (2012): Developments in fibrous flax breeding and cultivation. In: Kozlowski R. (ed.): Handbook of Natural Fibres, Vol. 1: Properties and Factors Affecting Breeding and Cultivation. Oxford, Woodhead Publishing Ltd.: 393–468.

Pennisi E. (2012): The tale of the TALEs. Science, 338: 1408–1411.

Polyakov A.V., Chikrizova O.F., Kalyaeva M.A., Zakharchenko N.S., Balokhina N.V., Buryanov Y.I. (1998): The transformation of fiber flax plants. Russian Journal of Plant Physiology, 45: 764–769.

Preťová A., Obert B. (2003): Flax (*Linum usitatisimum* L.) – a plant system for study of embryogenesis. Acta Biologica Cracoviensia, 45:15–18.

Preťová A., Obert B., Bartošová Z. (2007): Flax. In: Pua E.Ch., Davey M.R. (eds): Transgenic Crops VI. Series: Biotechnology in Agriculture and Forestry. Heidelberg, Springer: 129–148.

Qi B., Beaudoin F., Fraser T., Stobart A.K., Napier J.A., Lazarus C.M. (2002): Identification of a cDNA encoding a novel C18-Delta(9) polyunsaturated fatty acid-specific elongating activity from the docosahexaenoic acid (DHA)-producing microalga, Isochrysis galbana. FEBS Letters, 510: 159–65.

Qi B., Fraser T., Mugford S., Dobson G., Sayanova O., Butler J., Napier J.A., Stobart A.K., Lazarus C.M. (2004): Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nature Biotechnology 22: 739–745.

Qiu X., Hong H.P., Datla N., MacKenzie S.L., Tayler C.D., Thomas L.T. (2002): Expression of borage Δ-6 desaturase in *Saccharomyces cerevisiae* and oilseed crops. Canadian Journal of Botany, 80: 42–49.

Rakouský S., Tejklová E. (1999): Methods and prospects of flax transgenesis. Czech Journal of Genetics and Plant Breeding, 35: 125–129.

Rakouský S., Tejklová E., Wiesner I., Wiesnerová T., Kocábek T., Ondřej M. (1999): Hygromycin B – An alternative in flax transformant selection. Biologia Plantarum, 42: 361–369.

Rakouský S., Tejklová E., Wiesner I. (2001): Recent advances in flax biotechnology in the Czech Republic. In: Kozlowski R., Atanasov A. (eds): Natural Fibres: Bast Plants in the New Millennium, June 3–6, 2001, Borovets: 151–155.

Rakouský S., Ondřej M., Sehnal F., Habuštová O. Hussein H.M., Ovesná J., Kučera L., Kocourek F., Říha K., Dostálová R., Seidenglanz M., Tejklová E., Griga M. (2004): Transgenic plant products and their introduction into the environment and crop protection systems, a risk assessment. In: Nap J.-P., Atanassov A., Stiekema W.J. (eds): Genomics for Biosafety in Plant Biotechnology. Amsterdam, IOS Press: 173–184.

Renouard S., Corbin C., Lopez T., Montguillon J., Gutierrez L., Lamblin F., Lainé E., Hano Ch. (2012): Abscisic acid regulates pinoresinol–lariciresinol reductase gene expression and secoisolariciresinol accumulation in developing flax (*Linum usitatissimum* L.) seeds. Planta, 235: 85–98.

Renouard S., Tribalatc M.A., Lamblin F., Mongelard G., Fliniaux O., Corbin C., Marosevic D., Pilard S., Demailly H., Gutierrez L., Hano C., Mesnard F., Lainé E. (2014): RNAi-mediated pinoresinol lariciresinol reductase gene silencing in flax (*Linum usitatissimum* L.) seed coat: consequences on lignans and neolignans accumulation. Journal of Plant Physiology, 171: 1372–1377.

Rissler J., Mellon M. (1996): The Ecological Risks of Engineered Crops. Cambridge, MIT Press.

Rowland G.G. (1991): An EMS-induced low-linolenic-acid mutant in McGregor flax (*Linum usitatissimum* L.). Canadian Journal of Plant Science, 71: 393–396.

Rowland G.G., Bhaty R.S. (1990): Ethyl methane-sulfonate induced fatty acid mutations in flax. Journal of the American Oil Chemists Society, 67: 213–214.

Rowland G.G., Mchughen A., Bhatty R.S., Mackenzie S.L., Taylor D.C. (1995): The application of chemical mutagenesis and biotechnology to the modification of linseed (*Linum usitatissimum* L.). Euphytica, 85: 317–321.

Rui-López N., Haslam R.P., Venegas-Calerón M., Larson T.R., Graham I.A., Napier J.A., Sayanova O. (2009): The synthesis and accumulation of stearidonic acid in transgenic plants: a novel source of heart-healthy omega-3 fatty acids. Plant Biotechnology Journal, 7: 704–716.

doi: 10.17221/104/2015-CJGPB

Rutkowska-Krause I., Mankowska G., Lukaszewicz M., Szopa J. (2003): Regeneration of flax (*Linum usitatissimum* L.) plants from anther culture and somatic tissue with increased resistance to Fusarium oxysporum. Plant Cell Reports, 22: 110–116.

Ryan C., Smyth S. (2012): Economic implications of low level presence in a zero tolerance European import market: The case of Canadian Triffid flax. AgBioForum, 15: 21–30.

Schouten H.J., Jacobsen E. (2008): Cisgenesis and intragenesis, sisters in innovative plant breeding. Trends in Plant Science, 13: 260–261.

Schouten H.J., Krens F.A., Jacobsen E. (2006): Cisgenic plants are similar to traditionally bred plants: International regulations for genetically modified organisms should be altered to exempt cisgenesis. EMBO Reports, 7: 750–753.

Shi G., Cai Q. (2009): Cadmium tolerance and accumulation in eight potential energy crops. Biotechnology Advances, 27: 555–561.

Shysha E.N., Korkhovyu V.I., Bayer G.I.A., Guzenko E.V., Lemesh V.A., Kartel N.A., Yemets A.I., Blume Ya.B. (2013): Genetic transformation of flax (*Linum usitatissimum* L.) with chimeric GFP-TUA6 gene for visualisation of microtubules. Cytology and Genetics, 47: 3–11.

Siegień I., Adamczuk A., Wróblewska K. (2013): Light affects *in vitro* organogenesis of *Linum usitatissimum* L. and its cyanogenic potential. Acta Physiologiae Plantarum, 35: 781–789.

Simopoulos A.P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine and Pharmacotheraphy, 56: 365–379.

Skórkowska-Telichowska K., Żuk M., Kulma A., Bugajska-Prusak A, Ratajczak K., Gasiorowski K., Kostyn K., Szopa J. (2010): New dressing materials derived from transgenic flax products to treat long-standing venous ulcers – a pilot study. Wound Repair and Regeneration, 18: 168–179.

Smýkalová I., Vrbová M., Tejklová E., Větrovcová M., Griga M. (2010): Large scale screening of heavy metal tolerance in flax/linseed (*Linum usitatissimum* L.) tested in vitro. Industrial Crops and Products, 32: 527–533.

Snow A.A., Palma P.M. (1997): Commercialization of transgenic plants: Potential ecological risks. Bioscience, 47: 86–96.

Soudek P., Katrušáková A., Sedláček L., Petrová Š., Kočí V., Maršík P., Griga M., Vaněk T. (2010): Effect of heavy metals on seed germination in twenty three cultivars of flax (*Linum usitatissimum* L.). Archives of Environmental Contamination and Toxicology, 59: 194–203.

Stoutjesdijk P.A., Singh S.P., Liu Q., Hurlstone C.J., Waterhouse P.A., Green A.G. (2002): hpRNA-mediated targeting of the Arabidopsis FAD2 gene gives highly efficient and stable silencing. Plant Physiology, 129: 1723–1731.

Tejklová E. (1996): Some factors affecting anther culture in *Linum usitatissimum* L. Rostlinná výroba, 42: 249–260. (in Czech)

Tejklová E., Bjelková M. (2011): New Varieties: Low-linolenic linseed (*Linum usitatissimum* L.) Allan. Czech Journal of Genetics and Plant Breeding, 47: 41–42.

Tejklová E., Seidenglanz M., Griga M. (2009): Evaluation of risk assessment associated with release of genetically modified flax (*Linum usitatissimum* L.) into the environment in the Czech Republic. In: Sehnal F., Drobník J. (eds.): White Book of Genetically Modified Crops. EU Regulations and Research Experience from the Czech Republic. České Budějovice, Biology Centre AS CR: 74.

Tejklová E., Bjelková M., Pavelek M. (2011): New Varieties: medium-linolenic linseed (*Linum usitatissimum* L.) Raciol. Czech Journal of Genetics and Plant Breeding, 47: 128–130.

Thompson L.U., Chen J.M., Li T., Straaser W.K., Goss P.E. (2005): Dietary flaxseed alters tumor biological markers in post menopausal breast cancer. Clinical Cancer Research, 11: 3828–3835.

Tian B.M., Sun D.D., Lian Y.L., Shu H.Y., Ling H., Zang X., Wang B.N., Pei Z.Q. (2011): Analysis of the RNAi targeting FAD2 gene on oleic acid composition in transgenic plants of Brassica napus. African Journal of Microbiology Research, 5: 817–822.

Truksa M., Mackenzie S.L., Qiu X. (2003): Molecular analysis of flax 2S storage protein conlinin and seed specific activity of its promoter. Plant Physiology and Biochemistry, 41: 141–147.

Vanella R., Weston A., Brodmann P., Kubler E. (2014): Development of an event-specific assay for the qualitative and quantitative detection of the genetically modified flax CDC Triffid (FP967). Food Control, 41: 128–133.

Viju C., Yeung Mt, Kerr W.A. (2014): Zero tolerance for GM flax and the rules of trade. The World Economy, 37: 137–150.

Vrbová M. (2013): Molecular contingency of flax resistance to heavy metals and possibility of its influencing via transgenesis. [Ph.D. Thesis.] Brno, Mendel University.

Vrbová M., Horáček J., Smýkal P., Griga M. (2009): Flax (*Linum usitatissimum* L.) transformation with heavy metal binding protein genes. In: Sehnal F., Drobník J. (eds): White Book of Genetically Modified Crops. EU Regulations and Research Experience from the Czech Republic. České Budějovice, Biology Centre AS CR: 57.

Vrbová M., Kotrba P., Horáček J., Smýkal P., Švábová L., Větrovcová M., Smýkalová I., Griga M. (2013): Enhanced accumulation of cadmium in *Linum usitatissimum* L. plants due to overproduction of metallothionein α-domain as a fusion to β-glucuronidase protein. Plant Cell, Tissue and Organ Culture, 112: 321–330.

Vrinten P., Wu G., Truksa M., Qiu X. (2007): Production of polyunsaturated fatty acids in transgenic plants. Biotechnology and Genetic Engineering Reviews, 24: 263–280.

Wagner N., Mroczka A., Roberts P. D., Schreckengost W., Voelker T. (2011): RNAi trigger fragment truncation attenuates soybean FAD2-1 transcript suppression and

doi: 10.17221/104/2015-CJGPB

yields intermediate oil phenotypes. Plant Biotechnology Journal, 9: 723–728.

Warwick S.I., Stewart C.N. Jr. (2005): Crops come from wild plants – How domestication, transgenes, and linkage together shape ferality. In: Gressel J.B. (ed.): Crop Ferality and Volunteerism. Boca Raton, Taylor & Francis Group: 9–30.

Wijayanto T., McHughen A. (1999): Genetic transformation of Linum by particle bombardment. In Vitro Cellular and Development Biology, 6: 456–465.

Wojtasik W., Kulma A., Dyminska L., Hanuza J., Żebrowski J., Szopa J. (2013): Fibres from flax overproducing beta-1,3-glucanase show increased accumulation of pectin and phenolics and thus higher antioxidant capacity. BMC Biotechnology, 13: 10.

Wróbel M., Zebrowski J., Szopa J. (2004): Polyhydroxybutyrate synthesis in transgenic flax. Journal of Biotechnology, 107: 41–54.

Wróbel-Kwiatkowska M., Zebrowski J., Starzycki M., Oszmianski J., Szopa J. (2007a): Engineering of PHB synthesis causes improved elastic properties of flax fibers. Biotechnology Progress, 23: 269–277.

Wróbel-Kwiatkowska M., Starzycki M., Zebrowski J., Oszmiański J., Szopa J. (2007b): Lignin deficiency in transgenic flax resulted in plants with improved mechanical properties. Journal of Biotechnology, 128: 919–934.

Wróbel-Kwiatkowska M., Skórkowska-Telichowska K., Dymińska L., Maczka M., Hanuza J., Szopa J. (2009): Biochemical, mechanical, and spectroscopic analyses of genetically engineered flax fibers producing bioplastic (poly-beta-hydroxybutyrate). Biotechnology Progress, 25: 1489–1498.

Wróbel-Kwiatkowska M., Czemplik M., Kulma A., Żuk M., Kaczmar J., Dyminska L., Hanuza J., Ptak M., Szopa J. (2012a): New biocomposites based on bioplastic flax fibers and biodegradable polymers. Biotechnology Progress, 28: 1336–1346.

Wróbel-Kwiatkowska M., Turnau K., Góralska K., Anielska T., Szopa J. (2012b): Effects of genetic modifications to flax (*Linum usitatissimum*) on arbuscular mycorrhiza and plant performance. Mycorrhiza, 22: 493–499.

Wugar D., Cottier T. (2008): Genetic Engineering and the World Trade System. Cambridge, Cambridge University Press.

Wyndham J. (1951): The Day of the Triffids. New York, Doubleday & Company.

Xu R., Li H., Qin R., Wang L., Li L., Wei P., Yang J. (2014): Gene targeting using the *Agrobacterium tumefaciens*-mediated CRISPR-Cas system in rice. Rice, 7: 5.

Yang M., Zheng G., Zhang F., Xu Y. (2006): FAD2-silencing has pleiotropic effect on polar lipids of leaves and varied effect in different organs of transgenic tobacco. Plant Science, 170: 170–177.

Zanwar A.A., Hegde M.V., Bodhankar S.L. (2011): Cardioprotective activity of flax lignan concentrate extracted from seeds of *Linum usitatissimum* in isoprenalin induced myocardial necrosis in rats. Interdisciplinary Toxicology, 4: 90–97.

Zaplin E.S., Liu Q., Li Z.Y., Butardo V.M., Blanchard C.L., Rahman S. (2013): Production of high oleic rice grains by suppressing the expression of the OsFAD2-1 gene. Functional Plant Biology, 40: 996–1004.

Zhan X.C., Jones D.A., Kerr A. (1988): Regeneration of flax plants transformed by Agrobacterium rhizogenes. Plant Molecular Biology, 11: 551–559.

Zuk M., Kulma A., Dyminska L., Szołtysek K., Prescha A., Hanuza J., Szopa J. (2011a): Flavonoid engineering of flax potentiate its biotechnological application. BMC Biotechnology, 11: 10.

Zuk M., Dymińska L., Kulma A., Boba A., Prescha A., Szopa J., Mączka M., Zając A., Szołtysek K., Hanuza J. (2011b): IR and Raman studies of oil and seedcake extracts from natural and genetically modified flax seeds. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy, 78: 1080–1089.

Zuk M., Prescha A., Stryczewska M., Szopa. J. (2012): Engineering flax plants to increase their antioxidant capacity and improve oil composition and stability. Journal of Agricultural and Food Chemistry, 60: 5003–5012.

Zuk M., Dorotkiewicz-Jach A., Drulis-Kawa Z., Arendt M., Kulma A., Szopa J. (2014): Bactericidal activities of GM flax seedcake extract on pathogenic bacteria clinical strains. BMC Biotechnology, 14: 70.

Received for publication July 30, 2015
Accepted after corrections October 26, 2015

Corresponding author:

Ing. Michaela Ludvíková, AGRITEC, s.r.o., Oddělení biotechnologií, Zemědělská 2520/16, 787 01 Šumperk, Česká republika; e-mail: ludvikova@agritec.cz