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The Use of Herbicides in Biotech Oilseed Rape Cultivation and in Generation of Transgenic Homozygous Plants of Winter Oilseed Rape (*Brassica napus* L.)

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1. Introduction

Oilseed rape (rapeseed, *Brassica napus* L.) is one of the most important crop plants, taking the third place after palm oil and soybean among oil plants and the fifth among economically important crops, following rice, wheat, maize and cotton (Snowdon et al., 2007). Oilseed rape is cultivated primarily in temperate regions, where climatic conditions prevent growth of most other oilseed crops. Spring cultivars of oilseed rape dominate in the USA, Canada, Australia and China, while winter varieties are cultivated mainly in northern European countries (Great Britain, France, Germany, Poland, Scandinavia, etc.).

Over the past few decades, a number of crucial results in oilseed rape breeding research have been found. Improvement of oilseed rape by reducing erucic acid content in oil and glucosinolates in meal, were the two most prominent milestones, which contributed to the obtaining of '00' cultivars (also known as a canola), and subsequent world-wide expansion in the production of oilseed rape (Vollmann & Rajcan, 2009). Therefore, rapeseed oil has become one of the basic cooking oils and provides ca. 10 - 15% of total world production of vegetable oils. Moreover, rapeseed oil, free of anti-nutritional substances, has a higher dietary value than many other oils due to the lowest amount of saturated fats among vegetable oils, high oleic acid content and a favourable ratio of unsaturated fatty acids (linoleic and linolenic acids), as well as sterols and fat-soluble vitamins. Rapeseed oil is also used for varnish production and is increasingly being exploited as a bio-fuel. Meal remained after oil extraction as well as specially grinded seeds are valuable high-protein fodder.

Therefore, oilseed rape is an object of extensive efforts of breeding programmes to generate high yielding cultivars that are resistant to several biotic and abiotic stress conditions. Although breeding programmes based on natural genetic variation in *Brassica napus* are crucial, biotechnological approaches are essential as a complement to traditional procedures. Several methods, based on tissue cultures, e.g. interspecific and intergeneric hybridization

combined with embryo rescue techniques, protoplast fusion or production of doubled haploids (DH) have been successfully exploited in oilseed rape breeding programmes for over 30 years. Since the last two decades, genetic transformation has proven to be a significant method employed in commercial oilseed rape improvement.

2. Gene transfer in improvement of oilseed rape

Due to its economical importance, *Brassica napus* L. was among the first genetically transformed crops in the history of genetically transformed plants (Maheshwari et al., 2011). Various physical techniques of gene transfer to oilseed rape have been tested, including microprojectile bombardment (Chen & Beversdorf, 1994; Fukuoka et al., 1998), electroporation (Chapel & Glimelius, 1990), microinjection (Jones-Velleneunes et al., 1995) and PEG-mediated DNA uptake (Golz et al., 1990). However, *Agrobacterium tumefaciens*-mediated method is the preferred choice since it is an easy and effective way to transfer foreign genes to plant cell. (Cardoza and Stewart, 2003; Friedt & Snowdon, 2009; Jonoubi et al., 2005; Moghaieb et al., 2006; Maheshwari et al., 2011; Sharma et al., 2005; Takahata et al., 2005). Many factors have been investigated to elaborate transformation protocols, including oilseed rape varieties, *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* strains, binary vectors, type of explant (leaf pieces, cotyledons, hypocotyls, stem segments etc.) and the selection system such as antibiotics, herbicides etc. (De Block et al., 1989; Pechan, 1989; Poulsen, 1996; Wang J. et al., 2005; Wang W.C. et al., 2003; Wang Y.P. et al., 2005). Beside efficient transformation methods, crucial parameters for the use of transgenic crops are stable inheritance of the transgene and its expression and harmonization of input traits with the host genome and physiology. Transgenic cultivars or production lines with proper characteristics have to be selected from among hundreds and thousands primary transformants.

Nowadays, with the development of transformation techniques, targeting precisely defined genes directly into the biological characteristics of oilseed rape, biotechnology has become very important for the improvement of oilseed rape breeding. Although, so far only spring oilseed rape was successfully transformed, up till now 40 genes (transgenes) have been introduced into the oilseed rape genome (ISAAA, 2011). Transformation has been used for basic research purposes, for example deciphering the functioning of the myrosinase system (Troczyńska et al., 2003). Yet, most of reports on transgenic, i.e. genetically modified (GM) oilseed rape, were referred to new utilitarian and agricultural traits such as resistance to herbicides, viruses, fungi, insects and environmental factors such as drought, salinity, as well as male sterility and changes in the composition of fatty acids and proteins in the seeds (Dill, 2005; Kahrizi et al., 2007; McGloughlin, 2010; Poulsen, 1996; Senior & Bavage, 2003; Sharma et al., 2005; Wang J. et al., 2005; Zhong, 2001). However, the predominant genetic modification of commercially cultivated transgenic oilseed rape, also called GM canola, is herbicide resistance (Table 1).

3. Herbicides in GM canola agriculture

The first commercial launch of GM plants tolerant to herbicide, which was glyphosate-resistant soybean in 1996, signalled the beginning of a new era in weed management in row crops. Since that time several crops, mainly soybean, maize, cotton and canola, have been transformed that have allowed crop applications of many classes of herbicide chemistries. Such crops tolerate herbicides which completely eliminate weeds. As a result, one-two

sprays (e.g. after crop emergences) per vegetative season reduce the number of agronomical interventions, labour cost, fuel, machine consumption etc., and consequently generate lower economical costs. However, there is still ongoing discussion on the risk of uncontrolled release of herbicide-resistant crops into the environment and a potential danger of spreading of genes determining resistance to herbicides which could lead to the rise of herbicide-tolerant weeds. Nevertheless protests of some organisations such as Greenpeace etc., generally low public acceptance and governmental stands in some countries (mostly European ones), it appears that GM crops will continue to grow in species and cultivar number and in hectares planted, ca. 5-10% per year. In 2010, GM crops were cultivated on an area of 148 mln ha, in 29 countries. Dominant producers of GMO (Genetically Modified Organism) are the USA, Argentina Brazil, Canada and China, but the number of countries admitting GMO is growing, mainly in Asia, Africa and Latin America, and even in European Union, GM plants are cultivated in 9 countries on over 100,000 ha. So far, field trials of GMO have been conducted for almost 140 species in 11,400 experiments and approved commercial events have reached 80 cultivars among 16 species. The largest part of entire GMO area is occupied by soybean, maize, cotton and canola. Since the beginning of GMO agriculture, herbicide resistance is the predominant genetic modification. Nowadays, more than 70% of cultivated GM plants or 61% of GM occupied area over the world are herbicide resistant (ISAAA, 2011).

The herbicides utilized in the cultivation of GM canola and other crops are characterized by non-selective and systemic mode of action and foliar penetration. There are three main groups of these herbicides, distinguished by different active constituents: glyphosate (N-(phosphonomethyl)glycine) (Fig. 1a), oxynils (Fig. 1b) and phosphinothricin (glufosinate, glufosinate ammonium) (Fig. 1c).

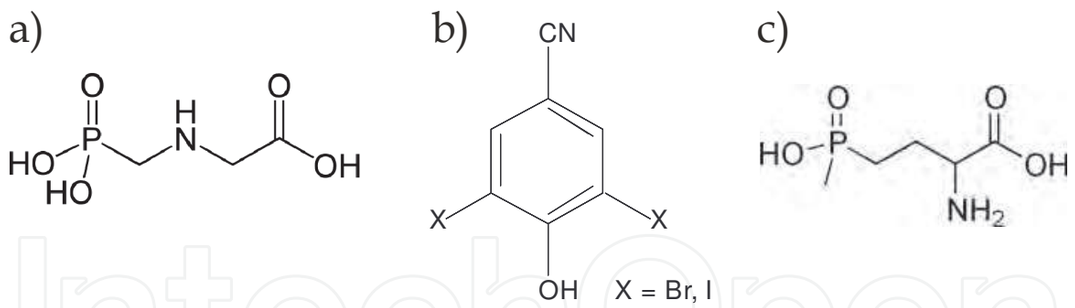


Fig. 1. Chemical structure of active constituents of herbicides used in GM canola agriculture. a) glyphosate - N-(phosphonomethyl)glycine; IUPAC name - 2-[(phosphonomethyl)amino]acetic acid; b) oxynil; IUPAC name - 4-hydroxy-3,5-dihalogenobenzonitrile, halogene – bromine or iodine; c) phosphinothricin – glufosinate, glufosinate ammonium; IUPAC name - 2-amino-4- (hydroxy(methyl)phosphonoyl)butanoic acid.

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of 5-enolpyruvyl-shikimate-3-phosphate (ESP) formation. ESP is a precursor for the aromatic amino acids: phenylalanine, tyrosine and tryptophan, used as protein building blocks and in synthesis of such essential metabolites as folic acid and quinones. Glyphosate-based herbicides such as Roundup®, are produced by Monsanto. Roundup Ready™ crops, i.e. transgenic plants resistant to glyphosate, contain genes

originated from *Agrobacterium tumefaciens* strain C4 and encoding the C4 EPSPS form insensitive to glyphosate, and/or the *gox* gene encoding glyphosate oxidase derived from *Ochrobactrum anthropi*.

Oxynils are lethal to plants, as they inhibit photosynthesis by blocking electron transport at photosystem II. Herbicides containing oxynils - Actril®, Totril®, are provided by Bayer Crop Science Company. Resistant crops contain *bxn* gene from *Klebsiella ozaenae*, which encodes a nitrilase enzyme that hydrolyzes oxynils to non-toxic compounds.

Phosphinothricin or glufosinate (glufosinate ammonium) is an active ingredient in several herbicides -- Basta®, Rely®, Finale®, Ignite®, Challenge® and Liberty® and other proprietary pesticides, produced by Bayer Crop Science Company. Glufosinate is a glutamine synthetase inhibitor that binds to the glutamate site of the enzyme. Consequently, ammonia builds up to toxic concentration, next pH increases to alkaline level as well as a cessation of photosynthesis resulting in the lack of glutamine and plant death. Resistance of transgenic plants to glufosinate is due to the activity of phosphinothricin N-acetyltransferase (PAT) encoded by the gene *bar* from *Streptomyces hygroscopicus* or *pat* from *Streptomyces viridochromogenes.* This is the most common resistance system used in GM canola production.

Herbicide tolerance is the predominant trait introduced into commercially cultivated transgenic oilseed rape (Table 1). Among 21 events approved, 19 are herbicide-resistant. Beside the above-mentioned economical reasons, herbicide selection system is also exploited due to GMO safety regulations and health requirements, (e.g. Regulation EC No. 1829/2003), which forbid the use of genes determining antibiotic resistance in GM plants utilized as food and feed sources. Herbicide resistance in GM canola was introduced either as a single trait or combined with other agronomical features (Table 1). Nowadays the cultivation area of GM canola constitutes ca. 7.5 mln ha which is 5% of the total GMO global area. The area occupied by GM canola reached 18-20% of the entire oilseed rape area and is distributed mainly in the USA, Canada and China (ISAAA, 2011). However, it should be stressed that so far GM canola events released to agriculture have been obtained only for spring varieties of oilseed rape. Therefore, a transformation method of winter oilseed rape is necessary due to its particular importance in countries of moderate and cool climate.

4. Transgenic homozygous herbicide-resistant winter oilseed rape

Production of transgenic winter oilseed rape, especially herbicide-resistant one, has been delayed in comparison to spring varieties, due to the recalcitrance of winter varieties of *Brassica napus* to transformation via *Agrobacterium* spp. or other techniques. So far transformation attempts have shown very limited success, despite using selection system based on *npt II* gene, conferring resistance to the mildly acting antibiotic kanamycin (Grison et al., 1996; Poulsen, 1996; Takahata et al., 2005; Wang Y.P. et al., 2005). A major constraint for the advance of transgenic breeding has been limitations of initial regeneration systems as well as problems in combining regeneration and transformation within the same cells. The choice of cells and organs as recipients of introduced gene and an efficient system for selection of cells containing an integrated foreign gene in genome, are essential elements of successful plant transgenesis. For the obtained successful gene transfer, cells or tissues have to represent a high regenerative capacity to develop complete and normal transgenic organism. Therefore, it is assumed that under specific conditions, obtaining of transgenic winter oilseed rape is possible.

Event	Description
GT200 (RT200) (MON-89249-2) Roundup Ready™ Canola Developer: Monsanto	Canola tolerant to the herbicide glyphosate produced by inserting genes encoding the enzymes EPSPS and GOX.
RT73 (GT73) (MON-ØØØ73-7) Developer: Monsanto	Glyphosate herbicide tolerant canola (cv. Westar) produced by inserting the *epsps* gene encoding the enzyme EPSPS and GOX.
HCN10 Liberty-Link™ Independence Developer: Aventis Crop Science	HCN10 (Independence) is an open pollinated canola line, which is tolerant to the glufosinate-ammonium (phosphinotricin), the active constituent of the non-selective broad-spectrum herbicides Basta®, Finale®, Buster®, Harvest® and Liberty®.
PGS2 (MS1 x RF2) (B91-4 x B94-2) (ACS-BNØØ4-7 x ACS-BNØØ2-5) Developer: Aventis Crop Science	The canola lines MS1 and RF2 were developed using genetic engineering techniques to provide a pollination control system for the production of hybrid oilseed rape (MS1xRF2) expressing male sterility and tolerance to glufosinate ammonium. The novel hybridization system involves the use of two parental lines, a male sterile line MS1 and a fertility restorer line RF2. The transgenic MS1 plants do not produce viable pollen grains and cannot self-pollinate. In order to completely restore fertility in the hybrid progeny, line MS1 must be pollinated by a modified plant containing a fertility restorer gene, such as line RF2. The resultant F1 hybrid seed, derived from the cross between MS1 x RF2, generates hybrid plants that produce pollen and are completely fertile.
OXY-235 (ACS-BNØ11-5) Navigator™ , Compass™ Canola Developer: Aventis Crop Science	Canola (variety Westar) tolerant to the oxynil herbicides created through insertion of the *bxn* gene encoding a nitrilase enzyme that hydrolyzes oxynils to non-toxic compounds.
MS8 (ACS-BNØØ5-8) InVigor™ Canola Developer: Bayer Crop Science	Canola with male-sterility system displaying glufosinate herbicide tolerance. Contains the barnase gene from *Bacillus amyloliquefaciens* and the *bar* gene encoding PAT. Also contains the *npt II* gene conferring resistance to the antibiotic kanamycin.
PHY14, **PHY35**, **PHY36** Developer: Bayer Crop Science	These lines are high yielding fertile hybrids and tolerant to the herbicide glufosinate-ammonium, used for the selection of the transformants.
RF3 (ACS-BNØØ3-6) InVigor™ Canola Developer: Bayer Crop Science	Canola fertility restoration system displaying glufosinate herbicide tolerance. Contains the *barstar* gene from *Bacillus amyloliquefaciens*, and the *bar* gene encoding PAT to confer tolerance to the

	herbicide glufosinate (phosphinothricin).
T45 (HCN28) (ACS-BNØØ8-2) InVigor™ Canola Developer: Bayer Crop Science	Glufosinate tolerant canola with insertion of the *pat* gene, conferring tolerance to glufosinate ammonium herbicide.
MS8 x RF3 (ACS-BNØØ5-8 x ACS-BNØØ3-6) InVigor™ Canola Developer: Bayer Crop Science, Aventis Crop Science (AgrEvo)	Canola with male-sterility, fertility restoration, pollination control system displaying glufosinate herbicide tolerance.
PGS1 (MS1(B91-4) x RF1(B93-101)) (ACS-BNØØ4-7 x ACS-BNØØ1-4) MS1 x RF1 Developer: Bayer Crop Science, Aventis Crop Science (AgrEvo)	Canola with male-sterility, fertility restoration, pollination control system, and glufosinate herbicide tolerance. MS1 line contained the barnase gene from *Bacillus amyloliquefaciens* (with pTa 29 pollen specific promoter from *Nicotiana tabacum*). RF1 line contained the *barstar* gene from the same bacteria with anther-specific promoter, and both lines contained the *bar* gene encoding PAT (with PSsuAra promoter from *Arabidopsis thaliana*) to confer tolerance to the herbicide glufosinate. Also includes *npt II* gene conferring resistance to the antibiotic kanamycin.
Topas 19/2, HCN92 (ACS-BNØØ7-1, HCN92) Liberty-Link™ Innovator Developer: Bayer Crop Science, Aventis Crop Science (AgrEvo)	Glyphosate herbicide tolerant canola produced by inserting the pat gene conferring tolerance to glufosinate ammonium herbicide and *npt II* conferring resistance to the antibiotic kanamycin.
MPS961, MPS962, MPS963, MPS964, MPS965 **Phytaseed**™ Canola Developer: BASF AG	Canola which expresses a phytase gene derived from *Aspergillus niger*. Altered quality product -- canola seeds contain higher content of a usable form of inorganic phosphorus released from phytic acid.
23-18-17, 23-198 (**CGN-89111-8, CGN-89465-2)** **Laurical™ Canola** Developer: Calgene Inc.	Lines 23-18-17 and 23-198 are high laurate- and myristate-containing canola produced by inserting a thioesterase (te) encoding gene from the California bay laurel (*Umbellularia californica*). The *npt II* gene confers resistance to the antibiotic kanamycin.

Abbreviations:
EPSPS -- 5-enolypyruvylshikimate-3-phosphate synthase non-sensitive to glyphosate, encoded by *epsps* gene from *Agrobacterium tumefaciens* strain C4
GOX – glyphosate oxidase, encoded by the *gox* gene from *Ochrobactrum anthropi*
PAT - phosphinothricin N-acetyltransferase, encoded by the *bar* gene from *Streptomyces hygroscopicus* or the *pat* gene from *Streptomyces viridochromogenes*
npt II – gene encoding neomycin phosphotransferase II

Table 1. GM canola events released to agriculture (on the basis of ISAAA, 2011a; Genescan, 2011).

One of the first successful attempts to obtain transgenic herbicide-resistant winter oilseed rape was the transformation of haploid microspore-derived embryos (MDEs) using hypervirulent *Agrobacterium tumefaciens* EHA105 strain (Cegielska et al., 2008). In this method, two directions of oilseed rape improvement and new cultivar production have been combined – doubled haploids and genetic modification.

Doubled haploids, obtained by tissue cultures of microspores and microspore-derived embryos (MDEs), show uniformity and complete homozygosity, thus they are particularly useful for breeding programmes and have been extensively used to produce homozygous breeding lines and cultivars of oilseed rape (Cegielska et al., 2002; Friedt and Zarhloul, 2005; Takahata et al., 2005). DH plants can also be efficiently used in basic studies, for example in genetic analysis and genetic transformation (Cegielska-Taras et al., 2008; Fukuoka et al., 1998; Huang, 1992; Jardinaud et al., 1993; Kazan et al., 1997; Nehlin et al., 2000; Oelck et al., 1991; Swanson & Erickson, 1989; Takahata et al., 2005; Troczyńska et al., 2003). It is postulated that in the process of genetic transformation, the gene introduced into the haploid genome, in this case MDE and subsequent chromosome doubling (diploidization), it will give rise to homozygous transgenic oilseed rape (Dorman et al., 1998; Huang, 1992).

Herbicide resistance as a marker trait of transgenic winter oilseed rape was chosen due to three reasons. First of all, glufosinate ammonium (phosphinothricin) was able to effectively select transformants of winter oilseed rape in contrast to kanamycin or other antibiotics. Moreover herbicide-tolerant winter oilseed rape conforms GMO regulations and has potential utilitarian value. Appropriate binary vector pKGIB was constructed (Fig. 2), which contained two chimeric genes (transgenes) inside a DNA fragment transferred into plant cells (T-DNA): the *bar* marker gene coding for phosphinothricin N-acetyltransferase under the control of constitutive NOS promoter (nopaline synthase originated from *Agrobacterium tumefaciens* C58 strain) and g7 terminator and the *uid* gene with an intron encoding β-glucuronidase (GUS), under the control of constitutive 35S promoter (35S RNA of Cauliflower Mosaic Virus) and NOS terminator, which was used as a reporter gene.



Fig. 2. Organisation of T-DNA of binary plasmid pKGIB. P35S = CaMV 35S promoter; PNOS = nopaline synthase promoter; GUS-INT = gene of β-glucuronidase with an intron; *bar* = gene of phosphinotricin N-acetyltransferase; NOSt = nopaline synthase terminator; g7t = g7 terminator; LB and RB = left and right T-DNA border sequences. (Cegielska et al., 2008)

Hypervirulent *Agrobacterium tumefaciens* EHA105 carrying the vector, after treatment in minimal medium with acetosyringone as an inducer of transformation process, was able to transfer T-DNA into slightly injured and pre-chilled MDEs. Although both oilseed rape microspores and MDEs characterise high regeneration potential (Fig. 3) (Cegielska et al., 2002, Friedt and Zarhloul 2005; Takahata et al., 2005), only MDEs were able to regenerate post *Agrobacterium* inoculation (Fig. 4). Plant regeneration under selection conditions, i.e. in tissue cultures on media supplemented with 10 mg/L of glufosinate ammonium, occurred via somatic embryogenesis, similarly to the usual regeneration method in MDEs during regular DH production (Fig. 5a). Nine putative haploid transformants were regenerated

from 100 primary explants. The plants were completely or partially resistant to glufosinate-based herbicide Basta® as observed in leaf-painting test and expressed the reporter gene at satisfactory level (Figs. 5b, 5c).

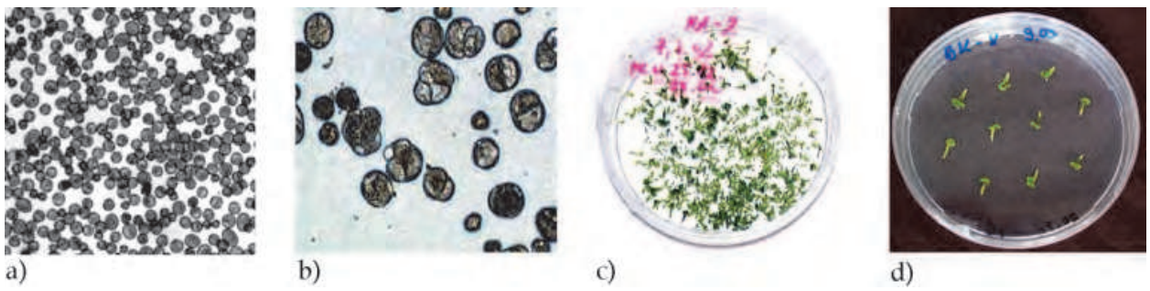


Fig. 3. Development of haploid microspore-derived embryos (MDEs) of winter oilseed rape. a) microspores (pollen grains); b)cellular divisions in regenerating microspores: c) culture of microspore-derived embryos; d) pre-chilled 21day-old microspore-derived embryos at the stage suitable for *Agrobacterium*-mediated transformation.

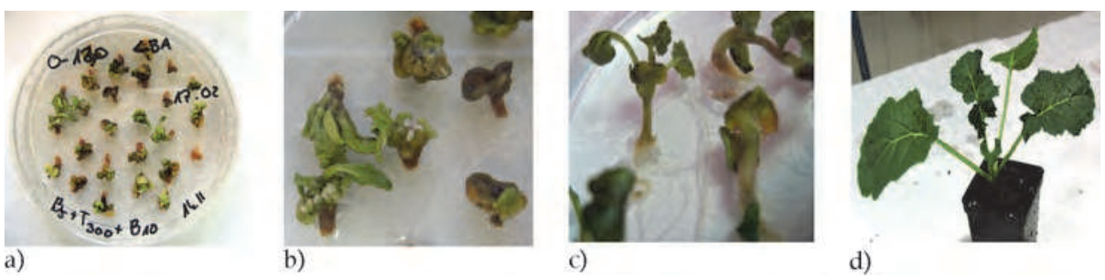


Fig. 4. Regeneration of transgenic haploid winter oilseed rape. a) transformed MDEs on a selection medium supplemented with 10 mg/L of glufosinate ammonium; b) plantlet development from transformed MDEs; c) regenerated and rooted transformants before transferring into soil; d) a transformant adapted to *ex vitro* conditions before colchicine treatment and vernalization.

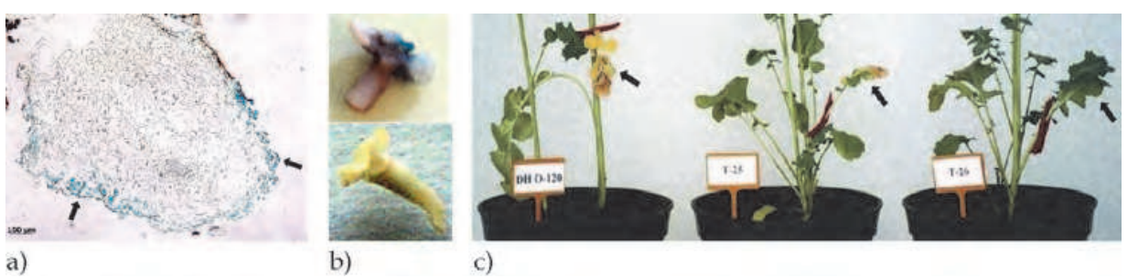


Fig. 5. Expression of transgenes in transformed MDEs and regenerated plants of winter oilseed rape. a) Localization of expression of the GUS reporter gene in MDE apical bud 10 days after transformation using *A. tumefaciens.* Putative sites of secondary bud formation are indicated by arrows. b) GUS expression (blue staining) in the meristematic region of transformed MDEs (top) and control embryos (bottom). c) Herbicide test – painted leaves (clamped, indicated by arrows) 5 days after herbicide application: control rape DH O-120 (left) and transgenic plants, i.e. partially resistant plant T-25 (centre) and completely resistant T-26 (right). (based on Cegielska et al., 2008)

However, after colchicine treatment and diploidization (Fig. 6a), only one primary transformant (T_0 generation) set vital seeds. In contrast to others characterised by one integration site, this fertile transformant contained two loci of T-DNA integrated in the genome (Fig. 6b). Nevertheless, all its progeny plants (T_1 generation) revealed the presence of *bar* gene present in the genome (Fig. 6c), were diploid and tolerant to herbicide spray.

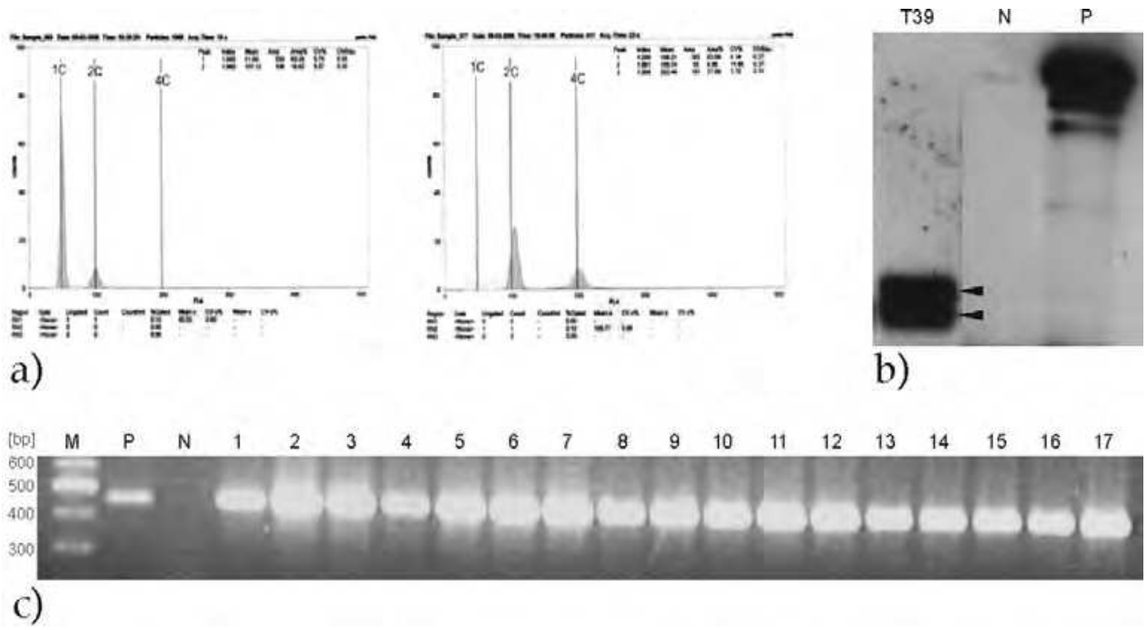


Fig. 6. Molecular analysis of transgenic winter oilseed rape. a) Determination of ploidy level of regenerated transgenic oilseed rape plants by flow cytometry analysis of DNA content (1C, 2C and 4C): haploid T39 transformant (left) and control diploid plant (right).
b) Southern-blot analysis of transgenic plant of DH winter oilseed rape, 25 μg of DNA cut with *Eco*R I. Lanes: T-39 = DH transgenic plant; N = nontransgenic plant, negative control; P = plasmid pKGIB, positive control. c) PCR analysis of the *bar* gene in T_1 plants of DH T-39 transformant of oilseed rape DH line O-120, Lanes: M = DNA size marker; P = plasmid pKGIB, positive control; N = untransformed O-120 plant, negative control; 1-17 = T_1 plants of DH T-39 transformant. (based on Cegielska et al., 2008)

While only one reliable transgenic plant was obtained in the first trial, following experiments verified the *Agrobacterium*-mediated MDE transformation as useful method of gene transfer. For every transformation round, 3-4 fertile transformants were obtained and progeny plants were resistant to Basta® as well as expressed introduced genes (unpublished). Although verification is still required for transgene functionality (including potential gene silencing) in the next generations, so far results strongly indicate that the repeatable method of winter oilseed rape transformation is worked out.

It may be assumed that contrary to routinely produced hemizygotic transformants, transgenic homozygotes will stably express integrated genes due to reduction or even elimination of at least one reason of gene silencing. A two-allelic transgene will probably resemble natural plant genes in more extent than an one-allelic transgene in a hemizygote. This aspect of both theoretical and practical significance is planned to be investigated in the future. However, *Agrobacterium*-mediated MDE transformation makes such research possible.

5. Conclusion

The presented method of winter oilseed rape MDE transformation using *Agrobacterium* appears promising. It allows for obtaining truly genetically modified winter oilseed rape of potential practical application. Main advantage of this method is that, as a result of chromosome duplication in transformed haploid, the introduced trait can be evaluated in a single step in a transgenic homozygote. Production of transgenic homozygous oilseed rape provides unique material for further studies of inheritance and functionality of introduced genes trough subsequent generation, for both basic research, breeding programmes and utilitarian purposes.

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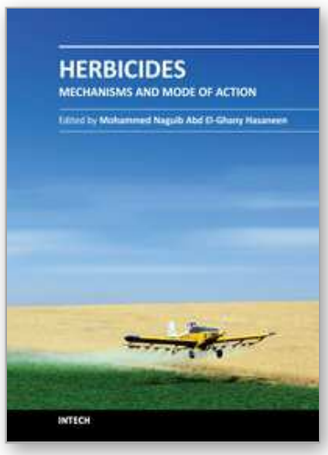
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Herbicides - Mechanisms and Mode of Action

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This volume contains two sections: Mechanisms of herbicidal action (chapters 1-4) and Mode of action of selected herbicides on controlling diseased, weed growth and productivity and/or growth and development of field crops (chapters 5-10). Topics by chapters are: molecular mechanism of action, immunosensors , laboratory studies, molecular modeling, weed resistance, community response, use of herbicides in biotech culture, gene flow, herbicides and risk, herbicides persistence. These recurring themes reinforce my view, held over a very long time, that experience with one crop or problem can sometimes be relevant, often to an unexpected extent, to an apparently dissimilar situation in a different crop. I hope that readers interested in herbicides and pesticides will be satisfied with all the chapters in the book as its content might be of interest and value to them in the future.

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