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BioOK – a Comprehensive System for Analysis and Risk Assessment of Genetically Modified Plants

BioOK – ein umfassendes System zur Analyse und Risikobewertung von genetisch veränderten Pflanzen

Abstract

Genetically modified (GM) plants have to be analyzed for their potential impacts on the environment and on human or animal health before authorisation by the EU.

The approval process currently refers to a conglomeration of diverse analytical methods and is intensive in time and costs. The intention of BioOK as a multidisciplinary scientific network is the development of tailor-made approaches for GM plants based on a cause-effect hypothesis to obtain an effective and qualified risk assessment system. The research activity of BioOK aims to renew the current approval process. It is based on a modular system covering all aspects of risk assessment: molecular characterisation, compound analysis, agronomic traits, target and non-target organisms, soil and micro organisms, toxicology, allergenicity and post-market monitoring, each module containing several test methods.

The renewal of the risk assessment procedure intended by BioOK consists of two phases: first the optimization of test methods and second the establishment of a decision support system (DSS) based on baselines, indicators and thresholds developed for each of the methods.

Optimized test methods have been developed mainly during the first phase: For compound analysis methods have been developed to ease the analysis of substantial equivalence of the events by GC-MS, LC-MS and HPLC/RI. A newly introduced testing scheme for the detection of potential effects of GM plants on soil combines an *in-vitro* system to collect rhizodeposits from plants grown under controlled environmental conditions and the corresponding bulk soil, and their characterisation by untargeted and highly sensitive molecular-chemical screening and fingerprinting technique. A novel *in vitro* system simulating the transport of substances from the gut into the blood that detects the risk of incorporation in human or animal at an early time point was developed. In order to increase the effectiveness and reproducibility of the sampling procedure we developed a valid defined sampling scheme. Finally, complementing the actual General Surveillance methodology, an approach for a Europe-wide case specific monitoring referring to cause-effect scenarios was developed.

The second phase concentrates on the development of a Decision Support System (DSS). A computer-based system will implement and merge all standardized methods

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in a decision tree system following decision rules defined by baseline and thresholds for indicators.

Key words: Environmental risk assessment, human and animal health, genetically modified (GM) plants, transgenic plants, decision support system, in vitro systems, field release

Zusammenfassung

Gentechnisch veränderte (GV) Pflanzen müssen im Rahmen des Zulassungsverfahrens in der EU auf ihre potentiellen Auswirkungen auf die Umwelt und die menschliche oder tierische Gesundheit analysiert werden.

Der gegenwärtige Zulassungsprozess ist ein Konglomerat verschiedenster Analysemethoden und extrem zeit- und kostenaufwendig. Das Anliegen von BioOK als ein multidisziplinäres wissenschaftliches Netzwerk ist die Entwicklung von maßgeschneiderten Ansätzen zur Risikoanalyse von GV Pflanzen auf der Grundlage von Ursache-Wirkungshypothesen mit dem Ziel des Aufbaus eines effektiven und qualifizierten Risikobewertungssystems. Die Forschungsaktivitäten von BioOK zielen auf einen Paradigmenwechsel im aktuellen Zulassungsprozess. Sie basieren auf einem modularen System, das alle Aspekte des Risikomanagements umfasst: molekulare Charakterisierung, Inhaltsstoffanalyse, agronomische Eigenschaften, Ziel- und Nichtzielorganismen, Boden und Mikroorganismen, Toxikologie, Allergenität und Überwachung nach Markteinführung, wobei jeder Modul unterschiedliche Analysemethoden beinhaltet.

Die durch BioOK angestrebte Reform des Risikobewertungsprozesses von GV Pflanzen umfasst zwei Phasen: zunächst die Optimierung der Analysemethoden selbst und dann die Etablierung eines Entscheidungsunterstützungssystems (Test Decision System – DSS), basierend auf biologischen Schwankungsbreiten (baselines), Zeigermerkmalen (indicators) und Grenzwerten (thresholds) für jede Analysemethode.

BioOK hat in einer ersten Entwicklungsphase bereits optimierte Testmethoden entwickelt: Für die Inhaltsstoffanalyse wurde die Untersuchung auf substantielle Äquivalenz durch GC-MS, LC-MS und HPLC/RI Methoden vereinfacht. Ein neu eingeführtes Analyseschema zur Ermittlung potentieller Effekte von GV Pflanzen auf den Boden kombiniert ein in vitro System zur Beprobung von Rhizodepositaten von Pflanzen, die unter kontrollierten Umweltbedingen gewachsen sind, sowie die entsprechenden Bodentypen und deren Charakterisierung mit offenen und hochsensitiven molekular-chemischen Screening und Fingerprinting-Methoden. Ein neues in vitro System zur Simulation des Transports von Substanzen aus dem Darm ins Blut, das das Risiko der Aufnahme durch Mensch oder Tier zu einem frühen Zeitpunkt misst, wurde entwickelt. Um die Effektivität und Reproduzierbarkeit von Probenahmen an der Pflanze zu erhöhen, wird ein genau definiertes Probenahmeschema entwickelt. Schließlich, in Ergänzung der aktuellen Methodik zur Allgemeinen Überwachung (General Surveillance) von GV Pflanzen im Anbau, wurde eine Herangehensweise zur Abschätzung der Notwendigkeit für ein europaweites fallspezifisches (Case Specific) Monitoring beruhend auf Ursache-Wirkungsszenarien, erarbeitet.

Die zweite Phase der BioOK F&E-Arbeiten konzentriert sich auf die Entwicklung eines Entscheidungsunterstützungssystems (Decision Support System, DSS). Dazu wird ein computergestütztes System implementiert, in dem alle standardisierten und validierten Methoden zu einem Entscheidungsbaum mit Knotenpunkten, definiert über biologische Schwankungsbreiten und potentielle Risiken definierenden Grenzwerten für Zeigermerkmale, zusammengeführt sind.

Stichwörter: Risikobewertung, Umwelt, menschliche und tierische Gesundheit, gentechnisch veränderte Pflanzen, transgene Pflanzen, Entscheidungsunterstützungssystem, Freisetzung, in vitro System

1 Introduction

The authorisation of the environmental release of transgenic plants especially within the EU is regulated on a risk assessment basis. The potential adverse effect of transgenic plants on the environment and the consumer or user has to be considered before they can be approved for commercialisation by the EU (EFSA, 2006). Nevertheless, since today's authorisation procedure refers to a laborious conglomeration of diverse analytical methods, it is too expensive to allow small and medium sized breeding companies to bring transgenic events on the market.

Risk assessment of GM plants today covers all aspects of environmental or food/feed issues these plants might come into contact with. The different aspects of the risk assessment can be summarized in eight modules that are described in Fig. 1. The procedures used to date to assess these points have on the one hand been extremely extensive, time-consuming and expensive, and are on the other hand not concerted or dedicated to GMP specific needs, thus allowing only the approval of plants with very high economic potential.

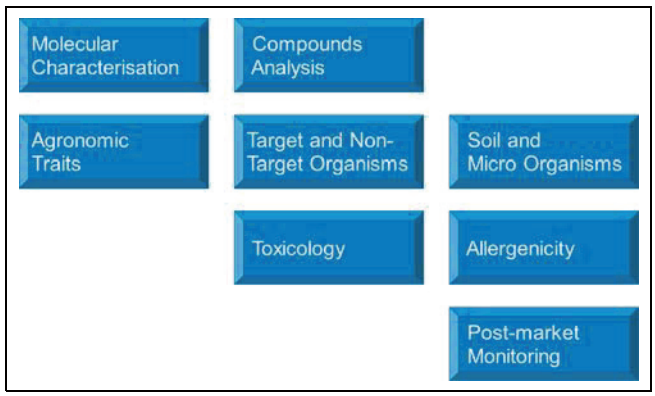

Fig. 1. Modular system for the biosafety research of GM plants.

In particular the risk assessment is based on several investigations:

The risk assessment strategy as proposed by the European Food Safety Authority (EFSA, 2006) is based on a comparative analysis of GM and conventional plants and the concepts of familiarity and substantial equivalence. Application of the substantial equivalence concept and therefore a thoroughly and comprehensive compound analysis is a starting point for the safety assessment. Compound changes, their possible effects and detection are of high importance (KUIPER et al., 2003). The analysis of unexpected effects is extremely difficult, considering the number of possible metabolites (between 10.000 and 25.000) in plants. Generally as many compounds as possible are extracted, detected and identified from the corresponding plant matrices. Analytical methods with GC/MS have been proven to be particularly effective (ROESSNER et al., 2000; FIEHN et al., 2000). Nevertheless, due to the huge number of compounds it is desirable to identify key compounds as indicators since it is not possible to determine all metabolites in GM plants and to compare them with conventional ones.

An important parameter of GM plants is the expression value of the inserted transgene and therfore the amount of recombinant protein produced. Until now scientific work mainly resticts to the variability of the transgene expression between different loci (QIN et al., 2003; NAGAYA et al., 2005). Several approaches aim to perform the selective integration of transgenes and thereby reduce variability (SRIVASTAVA et al., 2004). However, safety assessment should refer to the „worst case“ (usually the highest amount of the new compound) and therefore it should be secured that this case will be covered during risk assessment – and for all thinkable European environmental conditions as well.

There are many reports in the literature about possible impacts of GM plants on the soil ecosystem (e.g. ANGLE, 1994; JEPSON et al., 1994; MORRA, 1994; ICOZ and STOTZKY, 2008; LIU, 2010). Often these studies looked at only one or a few individual groups of soil organisms or soil properties. State-of-the-art in this research and in a more comprehensive approach to assess possible environmental risks of GM plants on soil microbial communities and biochemical processes was reviewed by BRUINSMA et al. (2003). They emphasized that major gaps in knowledge concern the poor understanding of structural and functional responses of microbial community to “normal” variation in soil systems and versatile laboratory procedures that are easy to perform and interpret with practical relevance. Plants form a rhizosphere during growth by the release of organic compounds (rhizodeposits), and this compartment is a hot spot of microbial activity in soil. Little is known about general transgene effects on rhizodeposition and SOM composition although a few relevant studies were published (MELNITCHOUCK et al., 2005; FILLION, 2008). However, a comprehensive, versatile and sensitive method for the evaluation of trangene induced changes in this soil compartment is still missing.

Assessing the risk of GM plants as well as its indicators also requires the analysis of possible effects of intended or accidental incorporation into human or animal organisms. A method for in vitro-digestion has shown that particularly resorption processes have a key position in the assessment (BRUCH et al., 2010). Until now resorption studies have been performed in Ussing chambers. Hints from literature indicate that prior estimations on transport rates and metabolisms also can be achieved with cell culture monolayers (GRUNWALD et al., 2007).

The post-market monitoring of GM plants under cultivation in the EU is amended by a mandatory General Surveillance for which tools and methods have been established (SCHMIDT et al., 2008) may include a case specific monitoring to reduce substantial uncertainties in relevant risk scenarios identified in the environmental risk assessment and should be based on the outcomes of the risk assessment. Nevertheless, clear methodological rules to deduce the scientific need for a case specific monitoring from the risk assessment studies have not been developed until now.

Due to the lack of a scientific based determination of the required scope of examination, the contemporary assessment of food/feed safety of GM plants is based on a huge conglomeration of different toxicological and allergological methods. The risk assessment procedure for GM plants needs further improvement – mainly in the whole structural approach. The enumeration of the status of single test methods underlines the potentials for further optimization also in the nodal points of the whole system.

2 Materials, Methods

2.1 Establishment of a Modular Risk Assessment System

Based on the EFSA guidelines (EFSA, 2006) the main areas of risk assessment issues have been identified (see Fig. 1). The areas were structured in modules. Each module addresses a subject area where different specialised know how, an analytical methods and equipment are needed. Within each module a collection of test methods was pooled to cover the several questions of this subject area. Since GM plant analysis is based on experiences from other fields (e.g. plant protection, food quality control, and pharmaceutical studies) we brought together specialists from these fields to incorporate their knowledge. As a result we got a modular system combining all the possibly needed risk analysis test methods under the umbrella BioOK. The modular system has several advantages: it might address all risk analyses demanded, but it might also deliver single components of a risk assessment desired by the customer. The main result of the development of the risk assessment modular system was a comprehensive compilation of test methods from different subject areas. We therefore inevitably came to an interdisciplinary understanding of the whole process and realised the scientific challenge for developing a compre-

hensive system to optimize the process and systematically reduce it to a stepwise, decision based approach.

2.2 Development of a Decision Support System (DSS)

A decision support system (DSS) is a computer-based information system including knowledge-based systems that support decision-making activities. DSSs serve the management, operations, and planning levels of an organization and help to make decisions, which may be rapidly changing and not easily specified in advance.

The BioOK DSS is developed to implement an integrated test system for the risk assessment of GM plants where the amount and order of analyses to be performed will be optimized plant-, transgene- and event-specific, and selected sequentially depend on the outcome of previous examinations. The DSS links the modules and analytical methods of the so-far used modular system to decision trees and rules in a logical sequence (Fig. 2). Based on this system, an extensively IT supported dynamic planning of the procedure of the assessment of a GM plant can be established. The outcome of the comparison supports the final decision making of a scientific expert in a multiplicative way (HÖFLICH et al., 2010).

To achieve the functionality within the DSS several components are developed:

a) **Decision trees and rules**. Depending on the plant, transgene, event and under consideration of previous results, decision trees and rules are developed that partially automatic control the way of a concrete question through the decision system. Prerequisite is the expert modelling of the paths within the risk assessment.
b) **Indicator**. The analytical methods focus on indicators defined as selected traits with significance to assess a potential risk. Changes in indicator values exceeding a threshold indicate a potential risk. Indicators can be substances, organisms, functional measures like transport rates etc.
c) **Baselines and thresholds**. Decision criteria for the branching points of the test system have to be established. They base on indicators, baseline data and thresholds defining a potential risk. Baseline is defined as the "normal" range of an indicator in conventional plant species which is classified as safe for consumer and environment. Studies were conducted to determine baseline data for each detection system and indicator. Baseline data are also determined by literature search. Threshold is defined as marginal value given by baseline data resulting from own studies or rules of the regulatory authorities. If the determined values of the GM plant are outside the "normal" range or exceed the threshold, the potential risks associated with this result will be analyzed and interpreted.

Indicators, baseline data and thresholds as basis for the decision tree and decision rules are technically implemented into the DSS which is developed on the basis of standard software. For this purpose the technological developments will be realized up to the prototype of the DSS. The DSS will have an interface to the partners and the consumers (Fig. 3). With this focused analytical system and the resulting standardized decision rules the integrated approval system can identify potential risks of the investigated GM plant in comparison to the non-GM plant.

The DSS focuses the procedure for risk estimation on standardized methods to reveal an effective science-based and objective approval system. The identification of indicators and the definition of baseline values and thresholds are the basis for the standardization.

2.3 Validation and verification of the DSS for the risk assessment of genetically modified plants

In order to establish the system also for novel traits we decided to use plants of the third transgenic generation producing biopolymers or pharmaceuticals. For all projects addressing the development of new analytical tools, potato (*Solanum tuberosum* L.) was chosen as model plant, since its tubers are suitable for biomaterial production and, due to their vegetative propagation and low invasive potential, they considered to be an environmen-

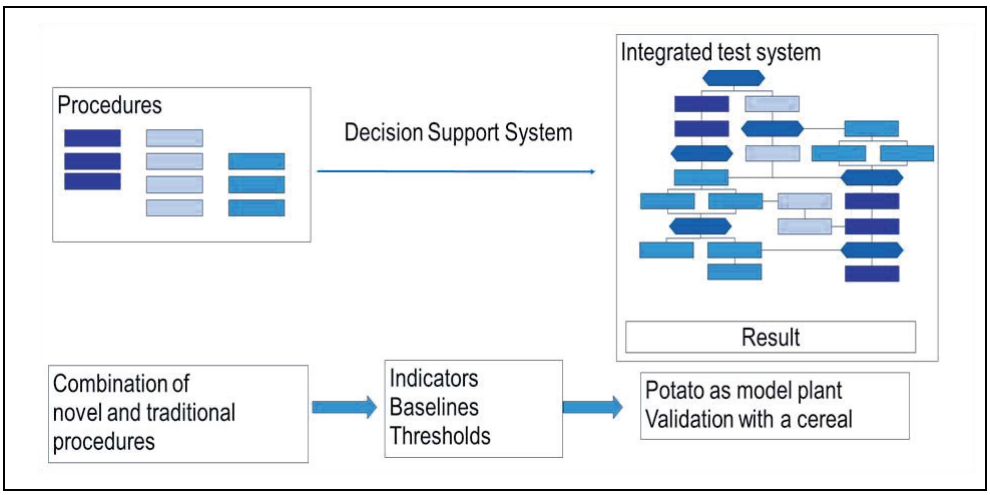

Fig. 2. **Integrated decision support system (DSS).**

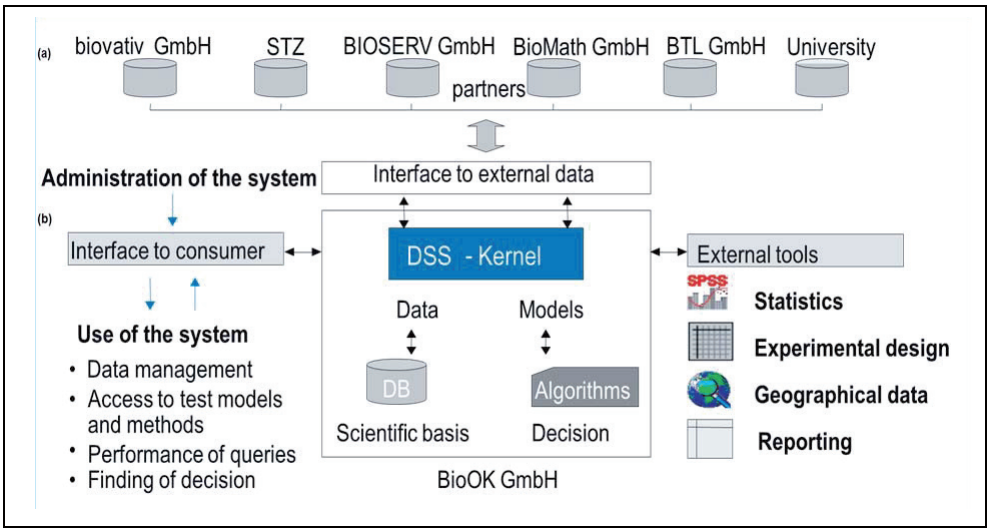

Fig. 3. Protoype of DSS.

tally safe production system. The transgenic variants produced either the biopolymer cyanophycin (cultivar Albatros), or the vaccine VP60, all plants carried the marker gene nptII (cultivars Albatros, Desirée, Fasan). Comparators were the near-isogenic variants (NIV) and, additionally, the cultivar Saturna.

The biopolymer, the cyanobacterial polypeptide cyanophycin is composed of a polyaspartat backbone and arginine residues (SIMON, 1976; SIMON and WEATHERS, 1976). It is produced as a by-product to starch in the potato cultivar Albatros via non-ribosomal protein synthesis by the transgene encoded enzyme cynaophycin-synthetase (HÜHNS et al., 2009). The pharmaceutical produced in the transgenic potato cultivars Albatros, Desirée and Saturna is the viral antigen VP60 that can be used to immunize rabbits against the haemorrhagic disease RHD (MIKSCHOFSKY et al., 2009). The neomycinphosphotransferase II (NPTII), which is used as marker in most of the GM plants today, was present in all transgenic events analyzed. The basis for the comparison are conventional plants which have a history of safe use as traditionally cultivated crops for many years. Thus, comparators for the GM plant are the near isogenic variants (NIV) Albatros, Desirée and Saturna and, in addition, the cultivar Fasan.

Field releases until now were performed over five years to assess the environmental effects, the agronomic traits and to produce plant material (Tab. 1) for compositional and food/feed analyses. The dried potato tubers were distributed to all partners of BioOK (see annex) in order to allow comparable results.

3 Results

With the intention to develop a comprehensive, effective and qualified risk assessment system, experts from different scientific institutions and SMEs were joined in the interdisciplinary network BioOK (Annex). The scientific projects of BioOK are funded by the Federal Ministry of Education and Research (German BMBF). Since 2005 BioOK has been establishing a structured and sequential approach to analyze cultivar, transgene and event specific risks (case-by-case). At first, we theoretically identified all possible risks caused by our model transgenic crops that are based on a scientifically founded hypothesis of

Tab. 1. GM and non-GM cultivars of potato (*Solanum tubersoum* L.) grown in the field 2009

GM potato cultivar	Vector	Event	Transgenic product
Albatros	PsbY-cphA	9, 10, 12, 23, 24, 31, 35	Cyanophycin
Albatros	35Svp60SEK	204	VP60
Desirée	35Svp60SEK	6	VP60
Fasan	35Svp60SEK	201	VP60
Albatros	35S (NPTII)	205	NPTII
Desirée	35S (NPTII)	6	NPTII
Fasan	35S (NPTII)	203	NPTII

Non-GM potato cultivars
Albatros (NIV), Desiree (NIV), Fasan (NIV), Saturna

cause and effects. Second, standardized methods specifically developed for the analysis of transgenic plants were used to generate a data base for the analysis of the potential risks. These methods fulfil the following criteria: 1. They target a specified problem, 2. they identify and simulate the exposure, 3. they focus on the analysis of the potential cause of hazard, 4. they simulate a worst case scenario, 5. they are linked to thresholds for risk identification and baselines for conventional variety data to deliver solid bases for the decision whether a difference between a GM plant and its near isogenic variant (NIV) causes a hazard or not, 6. they focus on indicators being representative for risk phenomena.

To optimize the risk assessment of GM plants, BioOK on the one hand adapted existing methods so far used for non-GM plants and on the other hand developed new and effective methods, if no adequate methods were available. For example, in vitro systems and cell culture methods for laboratory or greenhouse use were established in order to substitute extensive feeding studies or field trials whenever possible. Nevertheless, event specific field experiments have to be carried out to investigate environmental effects e.g. on non-target organisms or the variability of transgene expression.

As a first outcome, the compilation of the novel and improved methods resulted in a comprehensive modular system for an accelerated and low cost risk assessment for GM plants and derived food and feed. Starting with the compound analysis and molecular characterisation, it creates the fundamental data to localize the necessary research for a case specific risk assessment on the environment and on human and animal health and also considers the post-market environmental monitoring.

Within the module "**Compound Analysis**" as the basis for all risk assessment, methods have been developed to ease the analysis of substantial equivalence of the events by GC-MS, LC-MS and HPLC/RI. Beside that the content analysis of key nutrients was performed with conventional methods known for food and feed examinations according to Food and Animal Feed Statute Book.

A newly introduced testing scheme for the detection of potential effects of GM plants on soil (module: "**Effects on soil and micro-organisms**") combines two innovative approaches. First, an *in-vitro* system, is used to collect rhizodeposits from plants grown under controlled environmental conditions and the corresponding bulk soil. This substitutes more expensive field trials. Second, the rhizodeposits and soil samples are characterised by an untargeted and highly sensitive molecular-chemical screening and fingerprinting technique that records all volatilized and ionized molecules and fragments. This versatile mass spectrometric screening is capable to detect a priori unexpected changes in rhizodeposits and soil organic matter. Therefore, we expect that this technique will potentially replace target analyses like phospholipid fatty acid (PLFA) profiling for the characterisation of soil microorganism population.

The prediction of potential adverse effects on human or animal health arising from genetic modification of a plant is essential in the context of risk analyses of GMP compared to near isogenic variants (NIV). The toxic or allergenic potentials can not only be assessed in vivo but also in vitro. Therefore within the module "**Toxicology**" a novel highly sensitive in vitro system simulating the transport of substances from the gut into the blood that detects the risk of incorporation in humans or animals at an early time point was developed. The extent of resorption is an important information for further toxicological and allergological investigations. Dependent on the data achieved selected in vivo systems will be applied. Suitable animal models and in vitro tests were established to assess the allergic and toxic potential of GMP as well as the stability of novel proteins against digestion. Due to the integration of the established methods into a decision tree based on indicator parameters, baseline values obtained from NIV and thresholds it is possible to assess allergic and toxic health risks in an effective, rapid and low cost manner.

One of the critical steps of risk analysis and a precondition for a valid statistical analysis is the field sampling. In order to increase the effectiveness and reproducibility of the sampling procedure within the module "**Molecular Characterisation**" we developed a valid defined sampling scheme. It describes sample size, time point and sampled plant organ based on the variability of the protein content influenced by transgene, location, and plant organ and integration site.

Finally, complementing the actual General Surveillance methodology, within the module "**Post Market Monitoring**" an approach for a Europe-wide case specific monitoring referring to cause-effect scenarios, requested in Europe after the approval of a transgenic event, was developed.

The actual research and development activities of BioOK are based on this modular system and aim to merge all analytical methods within a computer-based decision support system (DSS). The aim is to establish a structured and sequential approach, where on a scientific basis only the methods for analyzing theoretically identified risks are performed.

In the following we introduce methods of the individual BioOK partners representing the main, but not the entire BioOK research work.

3.1 Compound analysis: baselines and threshold for the analysis of plant metabolites

The first question in risk assessment is whether the GM plant differs from its conventional counterpart or whether plants are substantially equivalent – with the exception of the intended product of the genetic modification –. The compound analysis shows changes in the amount of a plant metabolite or the occurrence of new metabolites.

The comparator plants give the **baseline** data for the metabolite concentrations, the measured values of the GM plant have to be compared with. The GM plant is substantially equivalent to the comparator if the indicator values are within the baseline range. If thresholds are exceeded, a further detailed risk assessment has to follow and the biological relevance has to be proven.

The use of LD50-values is a first approach to characterize **thresholds**. LD50-values are well-established for a huge amount of different chemical substances and describe the amount of a material, given all at once, which causes the death of 50% of a group of test animals.

We analyzed the metabolites of the four different potato cultivars Albatros, Desiree, Saturna and Fasan in comparison to the GM potato cultivar Albatros PsbY-*cph*A_{Te} clone 12 (Tab. 1). For the detection of the metabolites we established a GC-MS method with one extraction step related to the method of ROESSNER et al., 2000. For semi-quantitative calculation of metabolite concentrations we used the deconvolution software AMDIS (Automated Mass Spectral Deconvolution and Identification System). In a second step we calculated baselines out of the results from the four non GM potato cultivars. Subsequently, we compared the metabolites of the GM potato cultivar with the baselines to prove substantial equivalence or to identify any changes in the metabolite composition of the GM potato (NEUBAUER et al., 2010).

Approximately 60 compounds from various substance classes, e.g. sugars, sugar alcohols, amino acids, organic acids, fatty acids and sterols, were analyzed. In Fig. 4 an exemplary GC-MS chromatogram is shown. Fig. 5 illustrates three possible results of the statistical evaluation in the comparison of GM plant with non-GM plant: Box plot A demonstrates for L-aminobutyric acid the outcome that the metabolite concentration in a GM plant is below the baseline of the four different conventional cultivars. In box plot B the values for L-phenylalanine of the GM plant are within the baseline range. Box plot C shows in case of D-fructose-6-phosphate the possibility that the concentration in a GM plant is above the baseline range.

In case of box plot A, no risk will normally occur for customers. Only if the lowered value is a metabolite, which is known as an essential nutrient of food or feed, the biological relevance has to be proved. For the possibility B, substantial equivalence can be confirmed. In the case of the possibility C, however, the biological relevance has to be proven and a detailed risk assessment has to follow.

Indicators for plant metabolites have to be identified to ease the compound analysis and to reduce the expenditure of time. Here, an indicator is defined as a plant metabolite or protein that shows a reaction in concentration or activity reflecting a change in plant metabolism. A definition of a substance as an indicator for changed metabolic processes is hardly to find in the literature. Consequently, own experiments are conducted to identify indicators. We presently investigate light-induced changes in metabolites of potatoes.

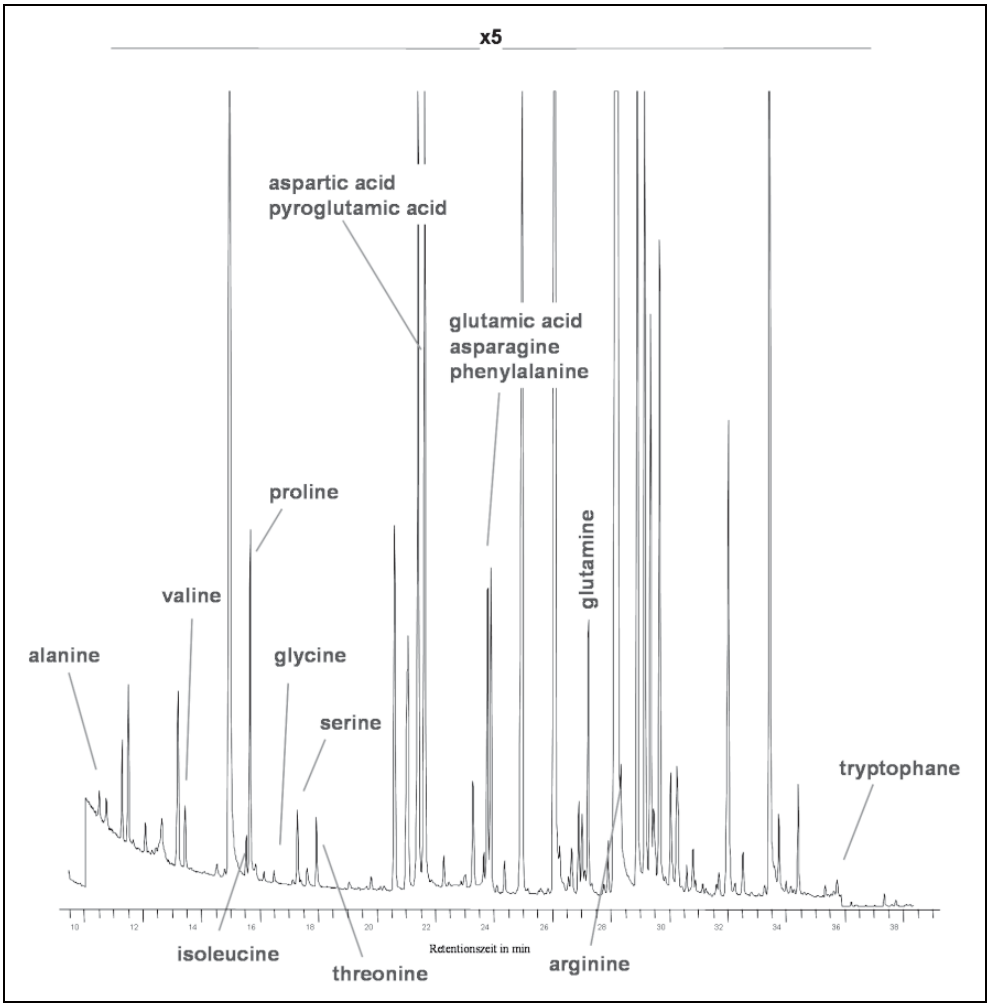

Fig. 4. GC-MS chromatogram with exemplary illustrated compounds.

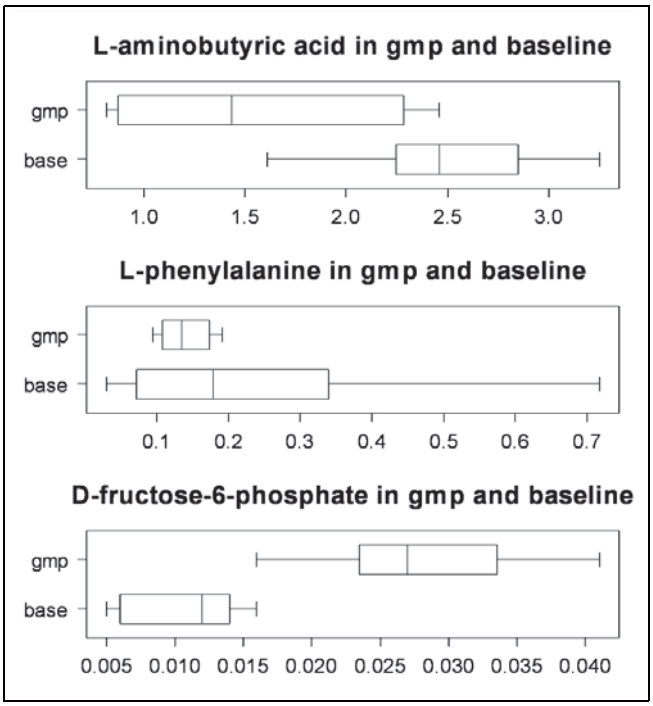

Fig. 5. Comparison of metabolite analysis of a GM plant and referring baseline data from non-GM plants (GM plant = Albatros PsbY-cphA$_{Te}$ clone 12; concentration of substances in µmol/mg freeze-dried potato sample).

The outcome of the compound analysis is linked to the environmental risk assessments such as investigations on non-target organisms and soil as well as the risk assessment on human and animal health: Potential effects of a changed substantial composition have to be investigated.

3.2 Influence on soil

3.2.1 Fast and efficient in vivo system for detection of transgene-specific effects on soil microorganisms. The influence of transgenic plants on soil microorganisms is, to date mainly analyzed in several years of field releases which are expensive, time- and labour consuming. Moreover, lots of these studies showed no transgene-specific effects (BECKER et al., 2008; MIETHLING-GRAFF et al., 2010; O'CALLAGHAN et al., 2005) compared to other factors such as the plant developmental stage (GYAMFI et al., 2002; SESSITSCH et al., 2005; VAN OVERBEEK and VAN ELSAS, 2008), growing season (DUNFIELD and GERMIDA, 2003), field heterogeneities (BAUMGARTE and TEBBE, 2005; WEINERT et al., 2009) or the variation in non-transgenic cultivars (MILLING et al., 2005). Since these trials have only been carried out for a small number of different transformation events, the results are event specific but not transgene specific (LOTTMANN et al., 1999). Nevertheless, transgene specific tests are preferable to evaluate possible effects in early phases of the development of transgenic lines and to reduce the effort necessary for subsequent field trials. These tests have to be controlled, but nature-orientated worst case experiments that allow the selective addition of specific environmental factors.

We therefore developed an efficient *in vivo* test system based on composite plants composed of transgenic roots emerging from a conventional shoot of the model legume *Vicia hirsuta* (QUANDT et al., 1993) or other plant species (COLLIER et al., 2005).

The legume model is of importance since the symbiotic interaction between leguminous plants and Rhizobia as well as mycorrhizal fungi represents sensitive **indicators** (ABD-ALLA et al., 2000; CASTILLO et al., 2006; DE VRIES et al., 1999; IKEDA et al., 2010) for impacts on soil bacteria and fungi and their activities.

Composite *Vicia hirsuta* plants expressing the transgene-encoded proteins NPTII, T4-lysozyme, CTB, VP60 or cyanophycin synthetase in roots were generated within three months for further analysis. Since three plants carrying each two to four transgenic roots that represent an integration event can be cultivated on one petridish, hundreds of events can be analyzed simultaneously to lead to statistically significant result. The roots developed were analyzed concerning parameters like biomass, content of transgene encoded protein and influence of the transgene encoded protein on symbiotic interaction. Since the nodulation of the roots is an important indicator for the effectivity of bacteria plant interaction (WANG et al., 2011) the number and phenotype of nodules was documented. Furthermore, *Rhizobium leguminosarum* bv. *vicieae* strains which were isolated from the nodules and characterized by genetic fingerprinting. Changes in the diversity of rhizobial populations in the nodules, the effectivity of N fixation or nodulation indicate effects of the transgene encoded protein on the close interaction between the bacteria and the plants and represent a sensitive system to identify potential adverse effects prior to the production of transgenic plant. The effect of the transgene encoded protein on soil can be determined by the combination of composite plants and the soil analysis described below.

3.2.2 Baselines and thresholds for the detection of transgene effects on rhizodeposition . Soil is among the first environmental components that could be affected by GM plants. In particular the rhizosphere is the susceptible interface between plant, soil and soil microorganisms as is releases rhizodeposits that feed soil organism communities. Furthermore, the rhizodeposition controls fundamental portions of the carbon (C) and nitrogen (N) cycling in ecosystems, that may be altered by GM plants. Previously, the characterization of the molecular-chemical composition of rhizodeposits often was based on *a priori* known, pre-selected compounds such as low molecular weight organic acids, carbohydrates or amino acids. *A priori* not known, possibly minor or trace compounds remain undetected in such an approach. Therefore, a promising alternative approach is the screening of the bulk molecular-chemical fingerprint of rhizodeposits by Pyrolysis-Field Ionisation Mass Spectrometry (Py-FIMS). This technique previously was successfully applied to detect day/night-cycles in maize rhizodeposition (MELNITCHOUCK et al., 2005) and diurnal as well as growth-stage effects on

potato rhizodeposition (SCHLICHTING and LEINWEBER, 2009). Thus, an untargeted molecular-chemical screening of the bulk rhizodeposits by Py-FIMS was proved to be sensitive in the detection of diverse impacts to soil induced by GM-plants.

In order to evaluate potential risks from GM-plants which are significantly above the natural variation according to plant species or varieties, growth stage, soil type etc., we developed a sensitive testing system. This system enables either an event- or transgene-specific risk assessment and comprises four main steps:

(1) acquisition of the molecular-chemical "fingerprint" of rhizodeposition and soil organic matter and the evaluation of the whole mass signal pattern by multivariate statistics,

(2) aggregation of marker signals to main compound classes of soil organic matter and as first order **indicators**,

(3) application of biomarkers representing the bacterial and fungal community as well as inhibitors and promoters of enzymatic and microbial activity as second order **indicators,** and

(4) a scheme for the detection, isolation, structural identification/verification and adjustment of unknown substances.

The steps (1) and (4) are primary aimed to denote *a priori* unexpected effects. The other steps are baseline-orientated, i.e. they are embedded in the DSS of BioOK. **Baselines** mean thresholds reflecting natural abundances of substances and compound classes characterized by marker and/or indicator signals.

A major advantage of the proposed testing system is the collection of rhizodeposition from plants which were grown under controlled nature orientated environmental conditions in climate chambers, eliminating the uncertainties of masking effects by alternating environmental parameters. The second two steps were presented recently (SCHLICHTING et al., 2010).

Previous experiments included various plant species (*Zea mays*, *Solanum tuberosum*, *Vicia hirsuta*, *Salix* and *Populus* spp.), which were grown in leaching vessels under controlled environmental conditions in different soils (topsoil from Gleysols and Cambisols). The numerous experiments covered different cultivars of maize (Sandrina, Pioneer, Gavott), transgenes, with composite vetch (*Vicia hirsuta*) and potato (cultivar Albatros) expressing the transgene-encoded proteins NPTII, CTB, VP60, cyanophycin synthetase (HÜHNS et al., 2008; MELNITCHOUCK et al., 2006; MIKSCHOFSKY et al., 2009; QUANDT et al., 1993; SCHLICHTING and LEINWEBER, 2009), inoculation with mycorrhizal fungi (poplar (*Populus nigra* × *maximowiczii*)) and stages of plant development (*Solanum tuberosum*). All rhizodeposits were leached at defined growth stages, immediately freeze-dried and subsequently analysed by Py-FIMS. The mass spectra were evaluated by multivariate statistics.

A huge relevant data set compiled so far comprises the Py-FI mass spectra of 134 rhizodeposits. For a more concise visualisation, mass spectra and thermograms were condensed according to the plant species (Fig. 6), whereas the mass spectra for potato rhizodeposits were published elsewhere (SCHLICHTING and LEINWEBER, 2009; LEINWEBER et al., 2009). The comparison of the Py-FIMS data clearly indicates plant-specific differences in *m/z*-signal patterns and total ion intensity (TII in 10^6 counts mg^{-1} with its thermal release curve in the upper right position). The statistical data evaluation by discriminant analysis disclosed significant differences in the mass spectral patterns among which 281 *m/z*-signals significantly ($P < 0.001$, multiple F-test for univariate Wilks's Lambda) contributed to the discrimination. The plot of the principle components 1 and 2 (explaining in sum 67.6% of the total variance) in Fig. 7 exhibits more or less pronounced clusters of the discrete plant species. The variability within the subsets followed the order potato > willow > poplar > maize > vetch. A more detailed data evaluation (not shown) revealed, that the variance among potatoes is mainly affected by the plant growth stage, whereas for willow and poplar most significant effects can be attributed to soil properties and mycorrhiza formation, respectively. In consequence, the effects of the transgenes, investigated for potato and vetch, were subordinate to the above more pronounced factors. The small cluster dimension for the maize rhizodeposit-subset, the coincidence of the maize and the vetch clusters and the partial overlap of both with the potato cluster are strong indicators for minor impacts of cultivars (maize) but a major impact of soil properties, because potato, vetch and maize were grown in the same soil (topsoil from a Gleysol).

Based on our mass spectrometric in-house library and/or the literature about 90% of *m/z*-signals with highest discriminant power can be assigned to known substances and related compound classes. Among those, carbohydrates, monolignols, lipids, non-peptidic N-compounds and free fatty acids were relevant for the significant differences between the plant taxa (Tab. 2, different letters indicate significant differences, $P < 0.05$). Generally, these results confirm CATCHPOLE et al. (2005), who also showed that the mass-spectrometric „fingerprint" of the bulk metabolome is suitable to detect substantial equivalence or differences among non-GM and GM-plants. From our dataset we can derive the following order of factors influencing the composition of rhizodeposits: soil properties > plant taxa > growth stage = mycorrhiza formation > cultivar = transgene.

Ongoing work is focused on the acquisition and/or validation of cultivar- and soil-specific baselines. That means the data base will be expanded to cover the "normal" variation in the above influencing factors. Then the accomplished test procedure is valuable to detect effects on soil by GM plants and compatible to the DSS. It provides a time and cost efficient, standardised risk assessment.

3.3 Influence on consumer: baselines and threshold for the establishment of an early detection system for the risk of incorporation

The effects of an intended or coincidental incorporation of transgene encoded proteins in the human or animal

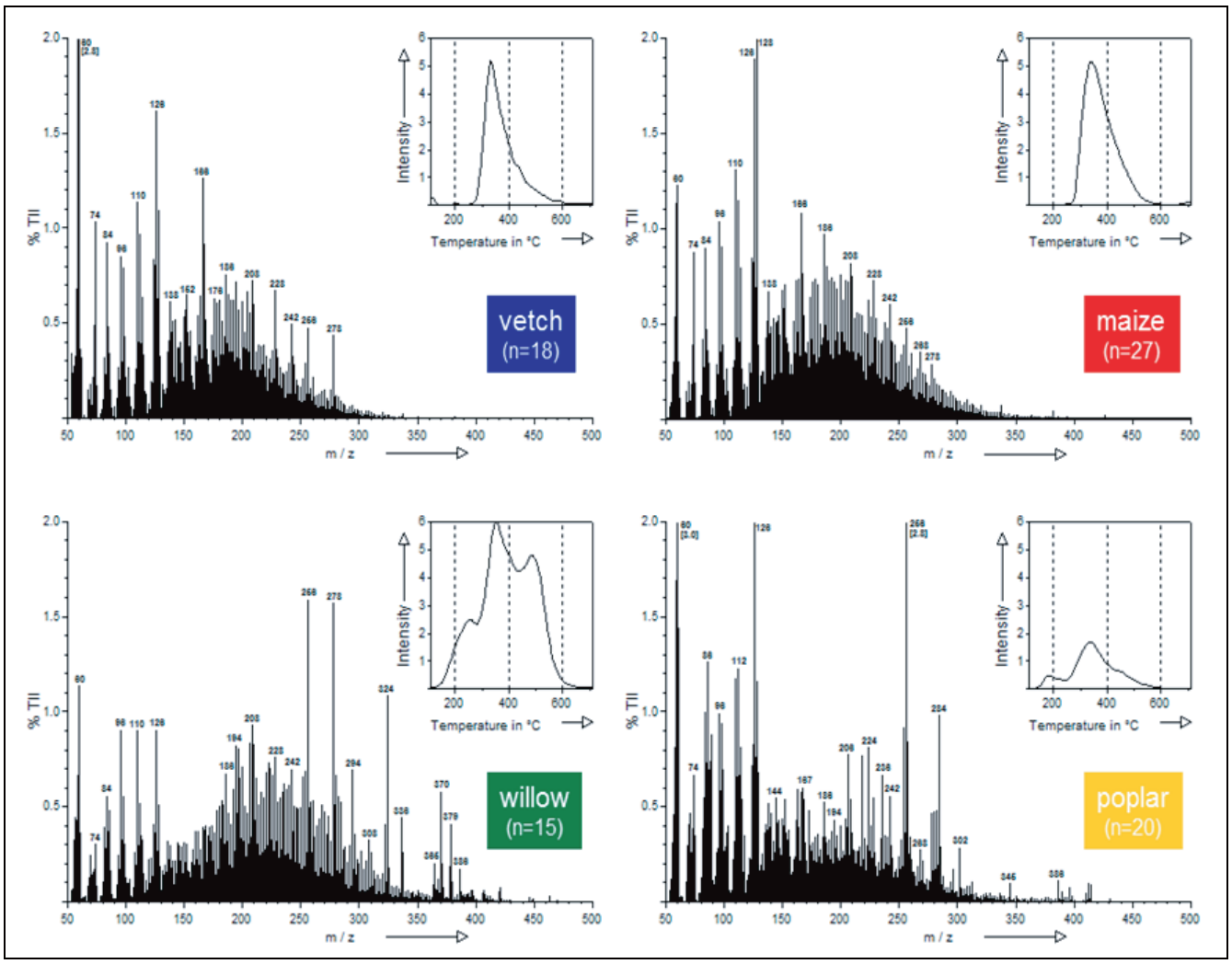

Fig. 6. Thermograms of total ion intensity (TII in 10^6 counts mg^{-1} lyophilisate) and summed and averaged pyrolysis field ionisation mass spectra of leachates from different plant taxa.

organism play a dominant role in the risk assessment of GM plants on consumer. The grade of resorption is an important information for the potential allergenicity or toxicology of a protein and give a hint for further toxicological and allergological studies.

In previous works the transport of proteins, e.g. green fluorescent protein (GFP), through native porcine epithelia in the Ussing-chamber simulated the transport from the gut into the blood in the animal (LODEMANN et al., 2006). The biological unevenness of the tissue involved transport rates of GFP with high inter- and intra-individual variability. The establishment of a cell culture (IPEG-J2) model minimized these effects (BRUCH et al., 2010).

In this experimental design **indicators** are defined as the following functional measures of the monolayer and the tissue probe in the Ussing-chamber: Transport rate for the amino acid lysine/measurement of the mannitol flux for the determination of the extracellular density of the epithelial layer/Change of the electric resistance of the epithelial layer/Electrical reaction of the tissue on the addition of glucose. The results in all measures give **baselines** and **thresholds** defined as mean + 2 s (standard deviation). The effect of an investigated substance e.g. a recombinant protein on these functional measures can be examined by adding this substance. The risk of a possible negative influence of the investigated substance on the normal functionality can be estimated even without a resorption of the substance. Irrespective of the effect of a substance on the functionality, the transport and resorption of the substance through the monolayer or the tissue can be simultaneously investigated. The risk of incorporation can be estimated.

First results showed a transport of intact GFP (Fig. 8). Glucose and sodium influence was detected in Ussing-chambers by measuring electrical current. GFP transport was detected in the same system but measured by ELISA. All described transports could also be detected on native porcine epithelium.

Experiments with GM and non-GM potatoes (Tab. 1) are performed, the results are incorporated into the DSS. The *in vitro* test system will be validated with a transgenic cereal in comparison to *in vivo* methods.

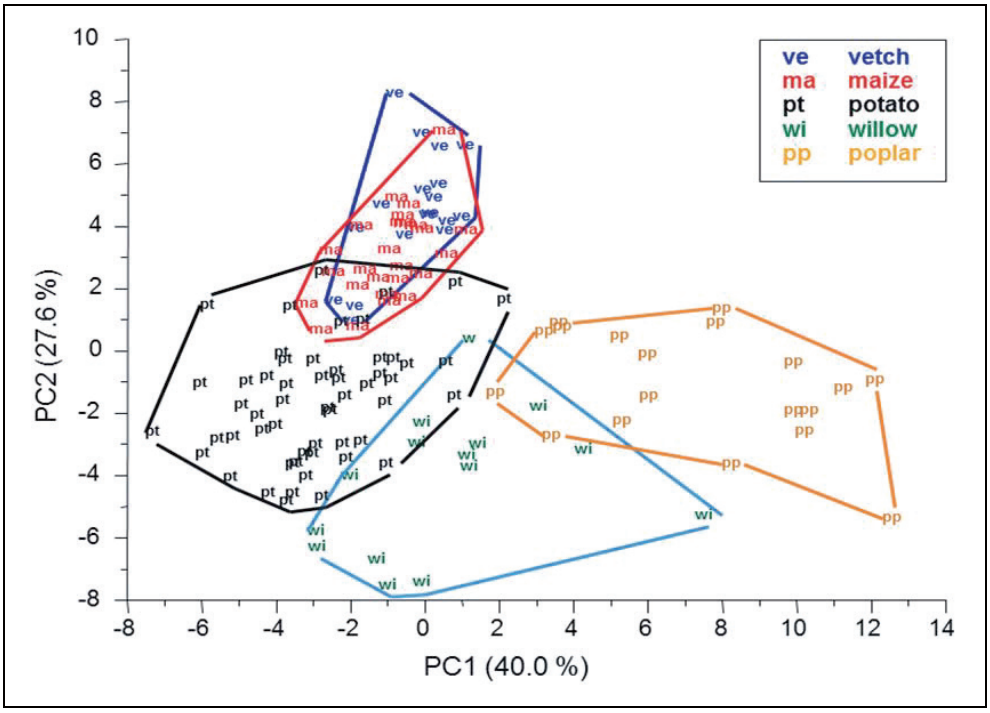

Fig. 7. Plot of the principle components 1 and 2 calculated from Py-FI mass spectra of rhizodeposits; discriminant analysis, grouped by the plant taxa (referring to the legend).

Tab. 2. Relative abundance (TII in %, mean ± standard error) of main compound classes: carbohydrates (CHYDR), monolignols (PHLM), lignin dimers (LDIM), lipids (LIPID), alkylaromatics (ALKYL), non-peptidic N-compounds (NCOMP), sterols (STEROL), peptides (PEPTI), suberin (SUBER), free fatty acids (FATTY)

Plant taxa	n	CHYDR	PHLM	LDIM	LIPID	ALKYL	NCOMP	STEROL	PEPTI	SUBER	FATTY
Poplar	20	21.8 ± 1.4 a	11.3 ± 0.7 a	2.7 ± 0.4 ab	1.9 ± 0.3 a	9.8 ± 1.1 a	10.6 ± 0.3 a	0.5 ± 0.1 ab	12.8 ± 1.0 a	0.02 ± 0.01 ab	4.2 ± 0.7 ab
Willow	15	10.6 ± 1.1 b	12.5 ± 0.9 ac	5.5 ± 0.7 b	4.6 ± 0.5 bc	14.1 ± 1.3 ab	7.2 ± 0.4 b	2.1 ± 1.2 ab	5.5 ± 0.6 b	0.09 ± 0.05 a	3.4 ± 0.5 a
Maize	27	16.2 ± 0.5 c	17.9 ± 0.3 b	3.2 ± 0.2 b	4.0 ± 0.2 b	15.5 ± 0.2 b	9.2 ± 0.2 c	0.3 ± 0.0 a	7.3 ± 0.2 b	0.02 ± 0.00 b	1.4 ± 0.1 bd
Vetch	18	16.8 ± 0.5 ac	17.4 ± 0.4 b	1.8 ± 0.2 a	2.7 ± 0.2 ac	13.0 ± 0.3 a	10.2 ± 0.3 ac	0.1 ± 0.0 b	7.5 ± 0.2 b	0.01 ± 0.00 cd	0.9 ± 0.1 c
Potato	54	12.9 ± 0.4 b	15.3 ± 0.3 c	3.0 ± 0.2 b	3.5 ± 0.2 b	14.1 ± 0.4 ab	8.1 ± 0.2 b	0.1 ± 0.0 b	6.5 ± 0.2 b	0.00 ± 0.00 bd	1.3 ± 0.1 cd

3.4 Influence on consumer: baselines and threshold for the assessment of allergological and toxicological risks of GM plants

The EU Authorities demand information on any potential toxic, allergenic or other harmful effects on human or animal health arising from the GM plant. Determination of the potential allergenicity or toxicity of GM plants compared to near isogenic variants (NIV) and other cultivars is a key issue in this context.

BIOSERV could introduce a model system for the assessment of a potential toxic or allergenic potential of GM plants (STEINMANN et al., 2010). Beside animal models a suitable test battery of methods for analysis of toxicity and allergenicity was established. Brown Norway rats (KNIPPELS et al., 1999; KNIPPELS and PENNINKS, 2002) and Balb/c mice (DEARMAN et al., 2003) were treated with model plant material (Tab. 1) orally and systemically for a period of 42 days to assess allergy type I. Guinea pigs and rabbits were treated with extracts of the plants to determine potential allergy type IV and toxicity and mice were fed with plant material to assess the acute, sub-acute and chronic toxicity. Using positive controls it could be shown that these animal models are capable to determine allergenic and toxic effects. Different *ex vivo in*

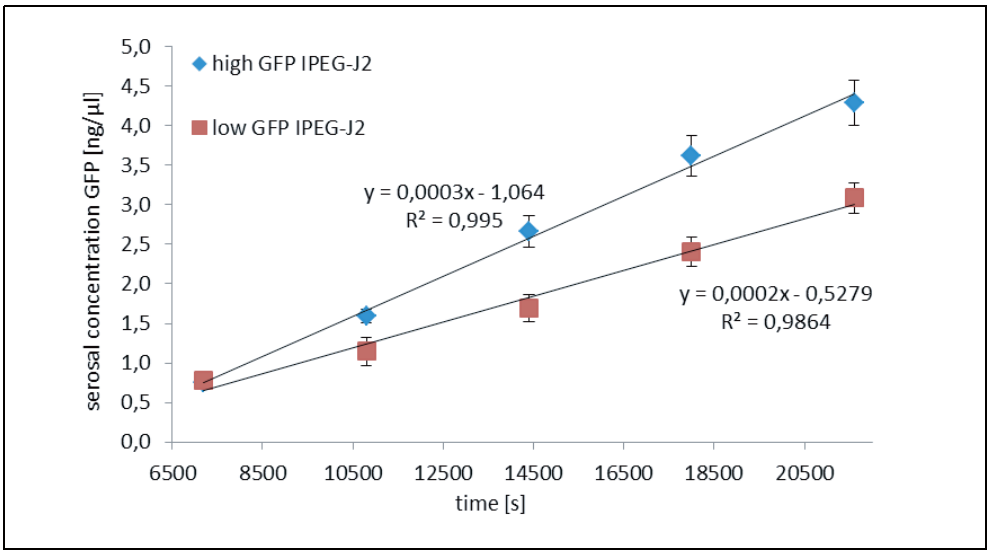

Fig. 8. Transport of GFP (different concentrations) by IPEG-J2 cell monolayer.

vitro test systems as ELISA-Systems or β-hexosaminidase-assay (Fig. 9) for determination of allergy specific cell products were established. Furthermore an *in vitro* test system to compare the potential toxic effects of the GM plants and the non- GM plant on a cell culture was introduced. *In vitro* and *in vivo* tests were done to assess the stability of the novel proteins against digestion.

For every single established test method **indicators** are defined. The indicators show the reactions of the systems, e.g. immunoglobulin E (IgE) content in sera of test animals, increase in weight of animals from feeding studies, etc. Various conventional potato lines will be analyzed with all test methods to define **baselines** of the variation of the reactions for every single test method. With these data **thresholds** have to be appointed and the results of an analyzed GM plant will be interpreted on the basis of these thresholds.

It can be summarized that the present results demonstrate that the tested transgenic potatoes did not cause toxic or allergic alterations.

Furthermore we will refine the method of detection of allergic reactions in test animals by establishing a miniaturized multiplex assay. With this test it is possible to examine a huge panel of potential allergens in one step with highly reduced consumption of test material.

Validating the test model system a transgenic cereal will be analyzed with all test methods.

The development of adequate and standardized analytical methods embedded in the DSS will assess health risks in a rapid and low cost manner.

3.5 Molecular characterization: development of a sampling regime based on the variability of transgene expression in the plant

The expression of transgene encoded proteins in GM plants depends on many factors. Thus, protein concentrations can differ in the various plant organs. Even isogenic plants show differences in transgene expression caused by physiological variability. The identification and definition of an optimal sampling regime is the basis for specific procedures within the environmental risk assessment and the post-market environmental monitoring.

The development of a valid test scheme defines sample size and sampling organ to enable a statistically valid and efficient analysis of transgene expression and its variability (STRUZYNA-SCHULZE et al., 2010). The scheme will be based on data obtained for the variability in expression

- of different recombinant proteins in one cultivar;
- of different integration sites of one transgene in one cultivar;
- of the same recombinant protein between different cultivars and between isogenic clones in a cultivar.

The variation of transgene expression in the field was analyzed using the recombinant proteins VP60, NPTII and the biopolymer-forming enzyme cyanophycin-synthetase. Data of transgene expression, generated in the first funding phase of BioOK, were evaluated. Variability was determined and sample size was defined.

In the field trial 2009 13 transgenic events expressing three different recombinant proteins and four non GM potato plants (Tab. 1) were randomly arranged in six times repeated blocks.

Statistical interpretation of transgene encoded protein contents show differences between locations, plant organs (leaf and tuber) and differences between similar integration sites (Fig. 10). Hence, an individual optimal sample size for the detection of the complete expression range is required for each event. Therefore, a universally valid sample size means the maximum of all deduced sample sizes.

The optimization of experimental procedures shall enable the development of valid, cost and time efficient sampling systems that will be implemented in the DSS.

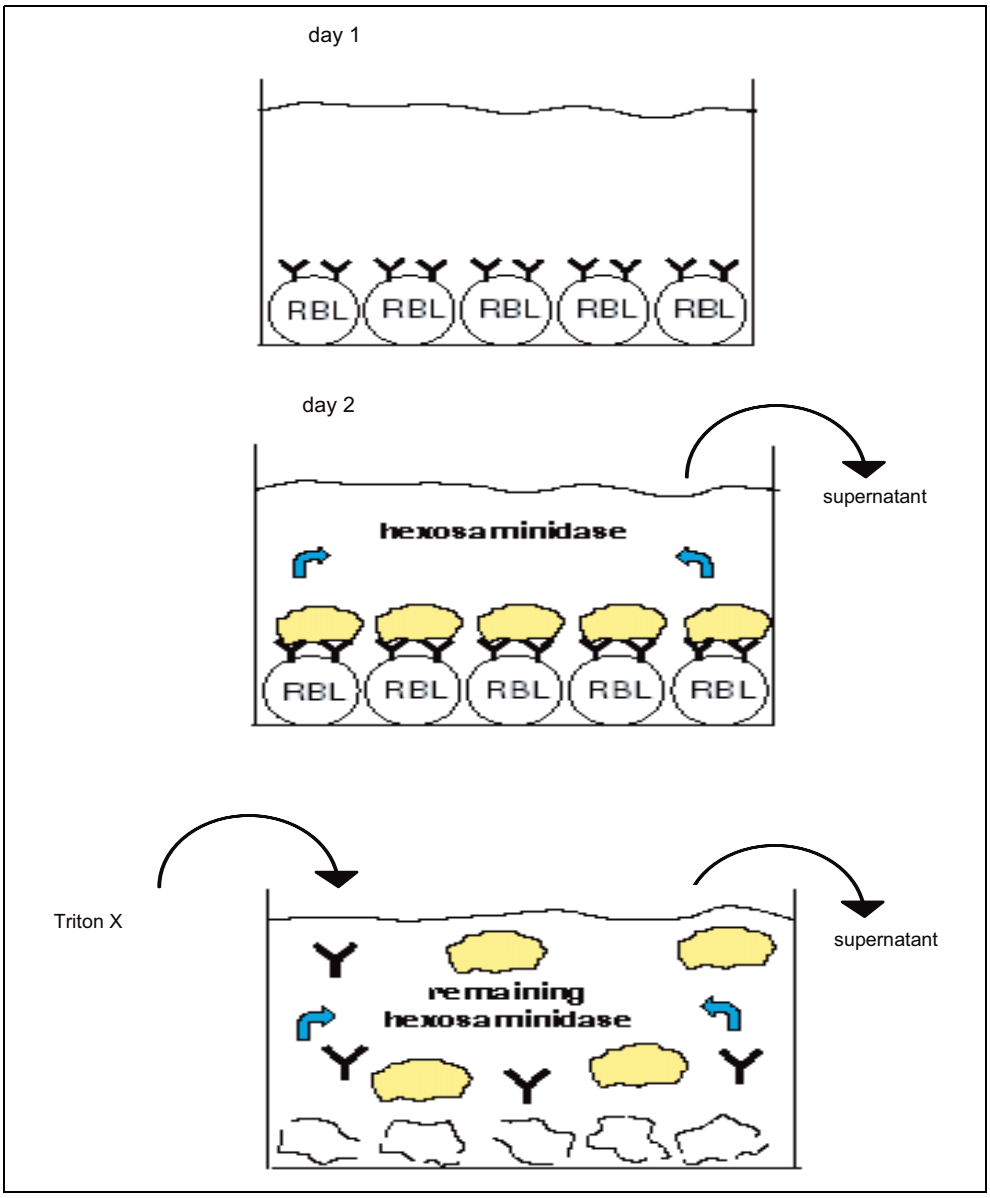

Fig. 9. Reaction scheme of β-hexosaminidase-assay.

3.6 Post-market environmental monitoring: development of a Europe-wide case specific monitoring

Directive 2001/18 EC demands the cultivation of genetically modified organisms (GMO) to be accompanied by post-market environmental monitoring (PMEM). It is composed of a general surveillance for unanticipated adverse effects and case specific monitoring (CSM), which is set up to reduce substantial uncertainties in relevant risk scenarios identified in the environmental risk assessment (ERA). PMEM should also allow early identification of potential long-term effects of cultivation of GM plants. It should facilitate decisions in risk management. PMEM forms not a component of the (pre-market) risk assessment, but a monitoring plan is a mandatory part of the application files.

In this project, an approach for a Europe-wide CSM was to be developed (ZIEGLER et al., 2010). Starting with critical questions from ERA, exposure scenarios – first for non-target organisms, later for all other parts of the ERA – were to be designed and potential monitoring characters ought to be identified. This was meant to facilitate the design of a CSM approach. Consideration was focussed on potatoes and wheat. Relevant pathways of plant-organism interactions through nutrients, toxins or mechanisms of attraction and defence were reviewed. The employment of CSM was reconsidered, taking into account the variability among existing potato and wheat cultivars, respectively, and environmental conditions.

Secondary metabolites are involved in communication and defence against pests and pathogens and in the attraction of natural enemies (relevant to biocontrol, integrated pest management). The secondary metabolites show a high degree of variability and are influenced by a multitude of biotic and abiotic factors.

Prominent groups of metabolites are the glycoalkaloids in potatoes and the hydroxamic acids in wheat, which have functions for defence and allelopathy. Only for glycoalkaloids effect thresholds for defence and toxicity have been discussed to some extent. Such threshold levels

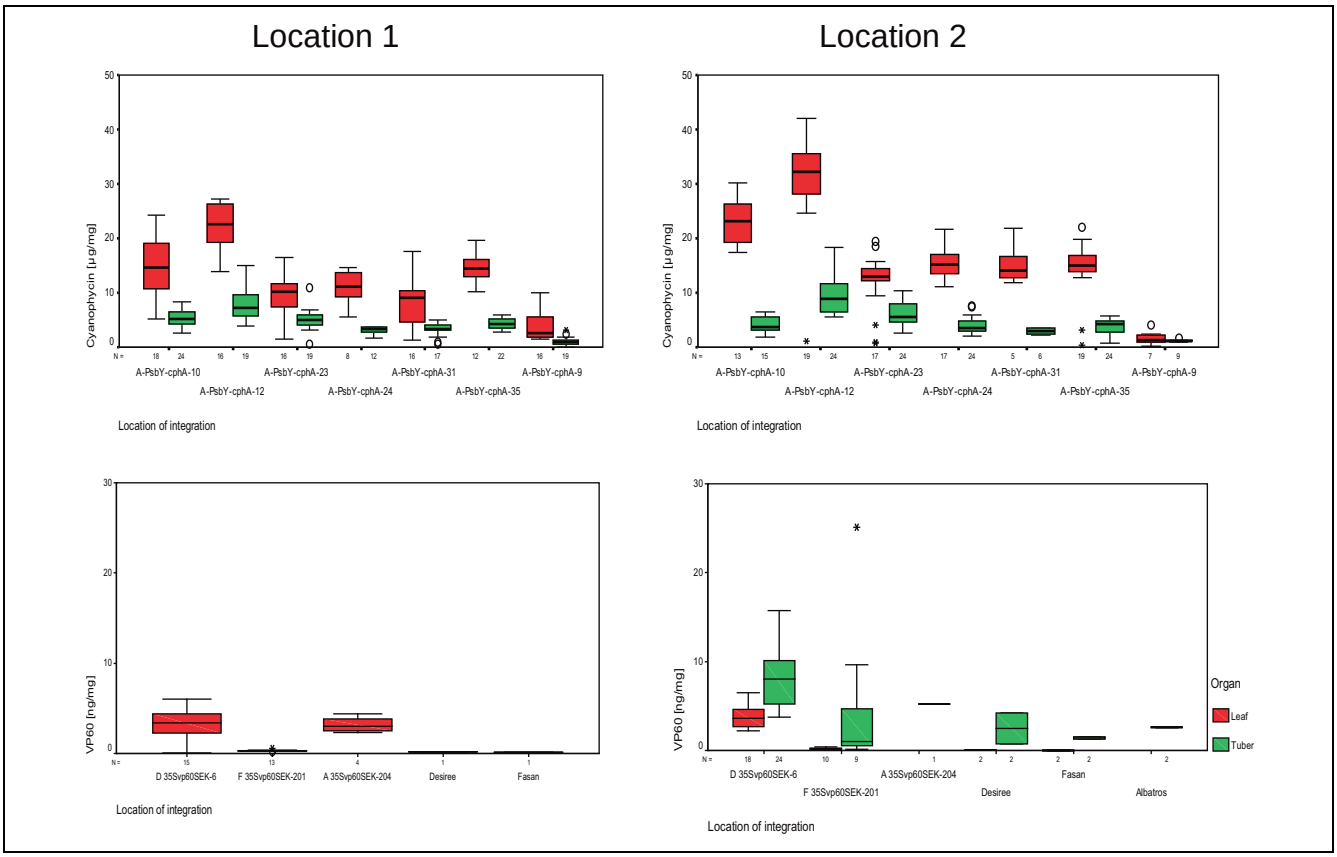

Fig. 10. Exemplary results of determination of cyanophycin and the VP60 content. Variation between clones is shown depending on the integrationsite, the organ analyzed (leaf, tuber) and the location.

lie within the natural variability of glycoalkaloids in tubers of commercial cultivars (Tab. 3).

In addition, volatile allelochemicals are discussed performing a remarkable range of functions. Insects interact with plants by detecting volatiles. Plant volatiles attract pollinators, act as indirect defences by attracting parasites and predators that prey upon herbivores and they are involved in plant-plant or within-plant communication. A genetic modification that interferes with metabolism of volatiles might thus affect, e.g. the attraction of predators

Tab. 3. Ecologically active compounds and their pathways of interactions

Compound	Function/ effect	Target organism	Natural variability	Scientifically accepted threshold	Pre-market testing	Case specific monitoring
glycoalkaloids (potato)	defence toxicity in humans	insects herbivores	0.08–5 g/kg in leaves 0.01–0.75 g/kg in tubers of commercializes potato varieties	3 g/kg (EC 50 CPB) 0.2 g/kg (ADI food)	compounds: HPLC, ELISA pest susceptibility: field trials	not recommended
hydroxamic acids (Hx) (wheat)	defence allelopathy	insects herbivores fungi weeds	0.6–3 mmol/kg in modern cultivars (higher in wild species)		LC/MS-MS	not recommended
volatile allelochemicals	defence (direct and indirect) allelopathy communication	insect herbivores plants	changing composition		E-nose (volatile signature)	not recommended

and parasitoids. However, conventional cultivars already show a remarkable variability in their volatile patterns.

While already different cultivars exhibit considerable differences, additional environmental factors drive variations and fluctuations in metabolites as well as in populations of potentially affected organisms. This raises the question whether possible adverse effects can be reasonably assessed prior to an environmental release, i.e. within the risk assessment process, or whether and how they are efficiently detected by monitoring after market release of the GM plant. In addition, breeding goals for the considered metabolites are missing. This counteracts the concept and usability of CSM approaches referring to such compounds to derived comparative cause-effect scenarios. Dose-response analyses under field conditions as well as reasonable thresholds for decision making are currently not available.

4 Discussion

The introduction of the decision support system (DSS) by the BioOK network aims to be an extensive improvement of the existing risk assessment in the framework of the approval and monitoring system for GM plants. In the current situation where neither a comprehensive standardized assessment scheme nor standards rules for baseline or threshold data exist, this system might contribute to render the whole risk assessment procedure more transparent, effective and science-based. Such improvement is necessary to assist both the applicants and the Competent Authorities to improve the management of the authorisation procedure. It might even support the public acceptance.

The new DSS contains only procedures that are science-based and safety-relevant and therefore essential but sufficient. A scientifically founded cause-effect hypothesis will form the platform of the evaluation scheme. The consequent application the DSS therefore reduces the total amount of analyses necessary on a highly science-based basis. This substantiates our belief in an early acceptance of the system in the scientific community and consequently in administrative processes.

Since the molecular composition of plants varies between different cultivars and even between individual plants, baselines and thresholds taking the respective "natural variability" into consideration have to be defined in order to compare the potential risk of a transgenic plant with those already present in the cultivation or consumption of other (conventional) varieties of the same cultivar. The baseline-threshold approach will not only help to quantify a "risk" but also to better rank GM plants within the actual range of wild and cultivated plants and varieties, and so – independent from the controversial discussion of GM plant cultivation especially in Europe – contribute to basic knowledge and understanding of biological and chemical processes in nature.

The reduction of risk analyses on indicator substances or species is a substantial step to focus on scientifically based risk scenarios and to identify early warning systems that are not only of relevance for the assessment of GM plants but also for other technologies or environmentally significant changes.

The methods assembled in the DSS are sufficient to characterise and analyse the GMO and their potential risks as required by the authorities. The implied methods take the plant, the new compound(s) and the planned use of the plant into account. Depending on to the intended use of the GMO (as for example food/feed or non food/feed) the system is flexible enough to focus on the toxicological or the environmental risk assessment – due to the hazard potential and expected exposition.

The modular system fulfils all biosafety requirements and contains all test methods requested by the authorities for analysing GMO plants.

Other comparable systems with a similarly practical approach are not known to us. Although other companies or networks work on developing support systems to pass through the authorization process (e.g. Flanders-UNIDO Risk Assessment Research Network FURARN), these developments do not include scientific biologic, agronomic or methodological work.

The implementation of the decision-making procedure in an IT-supported program allows a high grade of automatisation. The system itself is designed to be a "learning" system, i.e. by feeding it with data from analysing several GM plants the database for setting baselines and thresholds broadens with each risk assessment. This again might help to reduce the total afford for single risk assessments in the future. The intended optimizations, mainly the reduction of the whole risk assessment procedure only to scientifically founded analyses will extremely increase the efficiency of the total approval process. We aim to reduce the costs of this process drastically so that also small and medium sized plant breeders may apply the biotech technologies.

Annex: Partners in BioOK

Having experience in the production of GM plants and the associated risk assessment for more than 20 years, **the chair of Agrobiotechnology and risk assessment for bio- and gene technology** (Faculty of Agricultural and Environmental Sciences, University of Rostock) accompanied as scientific project coordinator the network in all scientific questions concerning GM plants. In addition, they constructed the model plants used in the project. A fast and effective in vivo system for the analysis of soil effects was developed in cooperation with the Steinbeis-Transferzentrum Soil Biotechnology and the molecular characterization of plants in the field was optimized together with the biovativ GmbH.

The chair of Technical Chemistry (Institute for Chemistry, Faculty of Mathematics and Natural Sciences, University of Rostock) has the expertise for the compound analysis of plants, especially for the structural analysis with GC/MS and LC/MS. New methods for the analysis and identification of indicators – as selected traits of an organism with significance to assess a potential risk – are

developed and can simplify the determination of substantial equivalence.

The chair of Technical Chemistry (Institute for Chemistry, Faculty of Mathematics and Natural Sciences, University of Rostock) has the expertise for the compound analysis of plants, especially for the structural analysis with GC/MS and LC/MS. New methods for the analysis and identification of indicators are developed and optimized together with biovativ GmbH.

The chair of Soil Science (Faculty of Agricultural and Environmental Sciences, University of Rostock) has developed expertise in the assessment of agronomic measures, including cropping of GM plants, on almost all important biological and chemical soil properties. In soil biology this involves, e.g., rhizosphere microorganisms such as mycorrhizal fungi and enzyme activities.

The Steinbeis-Transfercentre (STC) Soil Biotechnology has specific expertise in the molecular-chemical characterization of complex biomaterials by various mass- spectrometric techniques (Py-FIMS, Py GC-MS, HPLC-MS/ MS). The STC is closely associated with the chair of Soil Science; they together provide the complete range of soil- related investigations in risk assessment.

The chairs for Nutrition Physiology and Animal Nutrition, chair for Animal Health (Institute for Farm Animal Sciences and Technology, Faculty of Agricultural and Environmental Sciences, University of Rostock) are well-appointed with analytical facilities and *in vitro* techniques like nutrient analysis, GC, HPLC, simulation of digestion *in vitro*, cell culture, histological examination, immunological tests (ELISA, Western Blot). The institute assesses allergenic and toxic risks of GM plants in closely cooperation with bioserv.

BioMath GmbH is a service company for applied statistics and informatics in life sciences. BioMath implements the mathematical modelling and the technical development of the computerized DSS. Additionally, BioMath makes the experimental design for all within the BioOK network arranged field trials and statistically analyses the outcomes of the trials. BioMath has comprehensive experience in designing and performing the post-market monitoring, especially the General Surveillance (GS). For GS BioMath was involved in the development of farm questionnaires and also developed criteria and selection procedures for using existing networks for post market environmental monitoring.

Biovativ GmbH is a service company for agriculture and plant breeding. It has modern equipment for the cultivation, release and analysis in conventional and particular GM plants: agricultural area for field trials, greenhouse area of security level S1 experiments, S1 laboratories and special equipped rooms like cold storage rooms, acclimatized rooms, plant breeding rooms, rooms with special laboratory equipment. With its extensive experience in breeding and biosafety relevant issues in the areas of laboratory, greenhouse and field trials biovativ performs experiments and analyses in the fields of molecular characterization, compound analysis and agronomic traits. Additionally, biovativ produces the plant material for all other investigations of the partners.

BIOSERV Analytics and Medical Devices Ltd. is an independent provider of comprehensive testing services in the fields of food, feed and water, medical devices, cosmetics and hygiene monitoring. As an innovative enterprise BIOSERV operates modern research laboratories for biocompatibility testing, protein purification and production of antibodies as well as ELISA test kits. In cooperation with the chairs for Nutritional Physiology, Animal Nutrition and Animal Health BIOSERV handles the assessment of allergological and toxicological risks of GM plants.

Bio-Testlabor (BTL) is a private enterprise with expertise in the analysis of anthropogenic impacts on the ecosystem in general and with interactions between crops and their pathogens and herbivorous arthropods in particular. BTL investigates the influence of GM plants on target and non-target organisms.

Part of the work was performed in research co-operation with the **Julius Kühn-Institut** – Federal Research Centre of Cultivated Plants, at the Institute for Biosafety of Genetically Modified Plants. These results are published without restrictions according to intellectual property rights.

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