ANNEX 2

LITERATURE REVIEW

A literature review was conducted in January 2014 based on searching relevant STN databases. Executing the search profile given below 38 hits were found in the following traditional bibliographic databases:

- Chemical Abstracts

  The Chemical Abstracts database covers all areas of biochemistry, chemistry and chemical engineering, and related sciences. CA contains records for documents reported in printed Chemical Abstracts (CA) from 19th century to the present

- Biosis

  The largest and most comprehensive life science database in the world, BIOSIS Previews® covers original research reports, reviews, and selected U.S. patents in biological and biomedical areas, with subject coverage ranging from aerospace biology to zoology.

- Caba

  The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine.

- Medline

  MEDLINE contains information on every area of medicine. The MEDLINE database corresponds to Index Medicus, Index to Dental Literature, and International Nursing Index; OLDMEDLINE, with data from NLM's from the Cumulated Index Medicus (1960-1965) and Current List of Medical Literature (1958-1959); and, since August 2001, IN-PROCESS records, the latest documents before they have been completely indexed for inclusion on MEDLINE. Sources

The search profile was set as follows:

- Amflora

  or

- EH92-527-1 or "EH92 527 1" or eh925271

  or

- gbss or gbssi or "granule bound starch synthase"

  or

- (nptII or kanamycin resistance or neomycin phosphotransferase) and (potato or solanum)

  or

- (potato or solanum) and (amylopectin* or "amylo pectin*" or waxy starch or basf) and (gmo or transgen* or "genet* engine*)

Search performance:

- The focus was on scientific literature or news, patents were not included.
- All searches were done for the publication year 2013.
- All results were intellectually checked for relevance.

In the following all results from this literature review are listed including the abstracts.

Paper 1

Paradoxical EU agricultural policies on genetically engineered crops

Author(s)

Masip, G., Sabalza,M., Pe´ rez-Massot, E., Banakar, R., Cebrian, D., Twyman, R.M., Capell, T., Albajes, R., and Christou,P.

Source

Trends in Plant Science (2013) Volume 18, Number 6, pp. 312-324. 77 refs. Published by Elsevier Ltd.

Abstract
European Union (EU) agricultural policy has been developed in the pursuit of laudable goals such as a competitive economy and regulatory harmony across the union. However, what has emerged is a fragmented, contradictory, and unworkable legislative framework that threatens economic disaster. In this review, we present case studies highlighting differences in the regulations applied to foods grown in EU countries and identical imported products, which show that the EU is undermining its own competitiveness in the agricultural sector, damaging both the EU and its humanitarian activities in the developing world. We recommend the adoption of rational, science-based principles for the harmonization of agricultural policies to prevent economic decline and lower standards of living across the continent.

Paper 2

WTO Law and genetically modified products

Author(s)
Papic Brankov, T. and Lovre, K.

Source
135 EAAE Seminar Challenges for the Global Agricultural Trade Regime after Doha. (2013), 40 refs

Abstract
The paper discusses the mechanisms by which World Trade Organization (WTO) influence the diffusion of genetically modified (GM) products. We have analyzed the connection between the international trade of GM products and the three WTO Agreements: the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), the Sanitary and Phytosanitary (SPS) Agreement and the General Agreement on Trade in Services (GATS). It can be concluded that the mechanisms of the WTO organization are often used as instruments of threat to nations seeking to ban imports of GM food. In failing to acknowledge and support the precautionary principle, the WTO may have further weakened its authority to make decisions affecting the human health and environment and, in so doing, lessened its legitimacy in the world arena.

Paper 3

The GMOseek matrix: a decision support tool for optimizing the detection of genetically modified plants

Author(s)
Block, A., Debode, F.,Grohmann, L.,Hulin, J., Taverniers,I., Kluga, L., Barbau-Piednoir, E., Broeders,S., Huber,I., Van den Bulcke,M., Heinze, P., Berben, G., Busch,U., Roosens, N.,Janssen, E., Žel, J., Gruden, K., and Morisset,D.

Source
The GMOseek matrix: a decision support tool for optimizing the detection of genetically modified plants. BMC Bioinformatics (2013) Volume 14, Number 256, pp.14, 65 refs.
DOI:10.1186/1471-2105-14-256 http://www.biomedcentral.com/1471-2105/14/256

Abstract
Background: Since their first commercialization, the diversity of taxa and the genetic composition of transgene sequences in genetically modified plants (GMOs) are constantly increasing. To date, the detection of GMOs and derived products is commonly performed by PCR-based methods targeting specific DNA sequences introduced into the host genome. Information available regarding the GMOs' molecular characterization is dispersed and not appropriately organized. For this reason, GMO testing is very challenging and requires more complex screening strategies and decision making schemes, demanding in return the use of efficient bioinformatics tools relying on reliable information.

Paper 4

Tools for a scientifically rigorous and efficient monitoring of genetically modified organisms (GMOs) – VDI Guidelines to ensure high quality of GMO-monitoring data

Author(s)
Züghart, W., Beismann, H., and Schröder, W.

Source
BioRisk (2013) Volume 8, pp.3-13, 33 refs. DOI: 10.3897/biorisk.8.4036,www.pensoftonline.net/biorisk

Abstract

The deliberate release of genetically modified organisms (GMOs) implies the potential occurrence of environmental impacts which are either unexpected or only partially predictable and, thus, necessitates development of appropriate monitoring methodology. Therefore, new challenges have to be met when implementing the post market environmental monitoring (PMEM) of genetically modified organisms (GMOs), which is mandatory according to the European legal framework. According to Directive 2001/18/EC PMEM has to follow standard methodologies, wherever available and appropriate. To provide all involved parties with appropriate standard monitoring methods, the so called VDI Guidelines are developed by working groups established by the Association of German Engineers (VDI). These working groups are composed by external experts participating on a voluntary basis. The VDI is an independent technical standardization body. All Guidelines are published in German and English and can therefore be used throughout Europe. VDI Guidelines are available in the field of exposure of the environment to GM plants (e.g. standardized sampling of pollen, standardised observation of hybrids or ferals), biomolecular analyses (e.g. standardised extraction and detection of transgenes or their products in different environmental compartments), and the standardised monitoring of effects on non-target organisms (e.g. butterflies, wild bees, amphibians or soil organisms). The aim beyond this work is to facilitate generation of reliable and comparable monitoring data and enable an effective and efficient PMEM with high acceptability to the scientific community as well as the general public.

Paper 5

GMO environmental impact monitoring

Author(s)

Züghart, W. and Settele, J.

Source

BioRisk (2013) Volume 8, pp. 1–2, 4 refs. DOI: 10.3897/biorisk.8.5949,. www.pensoftonline.net/biorisk

Abstract

When new technologies are introduced and uncertainties for risks exist, appropriate environmental monitoring of potential hazards is needed (EEA 2001). This also applies to genetically modified organisms (GMO). European regulations foresee an environmental monitoring for every GMO imported, processed, used for food or feed, or cultivated (EC 2001). The aim of post market environmental monitoring (PMEM) is to serve as an early warning system to facilitate early and appropriate mitigation measures (EC 2002). To reach this objective, specific monitoring strategies are needed. Incompleteness of knowledge and uncertainties about environmental impacts of GMOs as well as the wide range of potential ecological effects pose big challenges for the development of PMEM. At present no routine environmental impact observation procedure for GMOs exists. Current PMEM plans and reports (e.g. MON810, Amflora) reveal fundamental shortcomings concerning objectives, design and methodology (BfN, EEA, BAFU 2011). With the present focal section of BioRisk we want to contribute to the improvement of PMEM. Bearing in mind that science-based methodology is a premise for appropriate and reliable monitoring data and sound monitoring results, the contributions here aim at filling gaps for suitable and harmonized monitoring protocols for PMEM. The papers present the work of experts, who developed standardized methods for different aspects of the monitoring of genetically modified organisms, in cooperation with the Association of German Engineers (VDI) and the Federal Agency for Nature Conservation (BfN). The final products are technical standards (VDI guidelines) for PMEM. They are published in German and English, and therefore accessible to all European stakeholders (www.beuth.de).

Paper 6

Development and validation of a multiplex real-time PCR method to simultaneously detect 47 targets for the identification of genetically modified organisms

Author(s)

Cottenet, G., Blancpain, C., Sonnard, V., and Chuah, P. F.

Source

Anal Bioanal Chem (2013), Volume 405, pp. 6831‑6844, 39 refs.
DOI 10.1007/s00216-013-7125-5, Published by Springer-Verlag Berlin Heidelberg.

Abstract

Considering the increase of the total cultivated land area dedicated to genetically modified organisms (GMO), the consumer’s perception toward GMO and the need to comply with various local GMO

legislations, efficient and accurate analytical methods are needed for their detection and identification. Considered as the gold standard for GMO analysis, the real-time polymerase chain reaction (RTi-PCR) technology was optimised to produce a high-throughput GMO screening method. Based on simultaneous 24 multiplex RTi- PCR running on a ready-to-use 384-well plate, this new procedure allows the detection and identification of 47 targets on seven samples in duplicate. To comply with GMO analytical quality requirements, a negative and a positive control were analysed in parallel. In addition, an internal positive control was also included in each reaction well for the detection of potential PCR inhibition. Tested on non-GM materials, on different GM events and on proficiency test samples, the method offered high specificity and sensitivity with an absolute limit of detection between 1 and 16 copies depending on the target. Easy to use, fast and cost efficient, this multiplex approach fits the purpose of GMO testing laboratories.

Paper 7

New SYBR_Green methods targeting promoter sequences used for screening of several GM events pending for authorization in Europe

Author(s)

Broeders, S., Barbau-Piednoir,E., Vandermassen, E.,Debode, F., Mazzara, M., Roosens, N.

Source

Eur Food Res Technol (2013) Volume 236, pp. 537–547, 37 refs.
DOI 10.1007/s00217-013-1910-4, Published by Springer-Verlag Berlin Heidelberg.

Abstract

Seen the growing number of genetically modified (GM) crops being developed, the need for cost- and time-effective detection methods is increasing to enable continuing the necessary effective control on food and feed products. This need can be achieved by performing an intensive screening combined with decision support tools like the CoSYPS matrix which permits reducing the number of events to be identified. To allow an extra covering power of the CoSYPS and to be able to include new EU-authorised GM events, two new SYBR_Green real time PCR (qPCR) methods targeting two promoter sequences (pNOS and pFMV) were developed. These methods were validated using acceptance parameters such as the specificity, sensitivity and repeatability. In addition, the methods were transferred to a second laboratory, namely the Institute for Health and Consumer Protection, to test the reproducibility. Furthermore, the applicability and practicability of the methods were tested by using proficiency test samples. The two methods allow a specific and sensitive detection of the targets in food and feed samples and can be used efficiently in different laboratories.

Paper 8

Development of a CTAB buffer-based automated gDNA extraction method for the surveillance of GMO in seed

Author(s)

Guertler, P., Harwardt, A., Eichelinger, A., Muschler, P., Goerlich, O.,and Busch, U.

Source

Eur Food Res Technol (2013) Volume 236, pp.599–606, 20 refs.
DOI 10.1007/s00217-013-1916-y, Published by Springer-Verlag Berlin Heidelberg.

Abstract

Seed imported into the EU from countries growing genetically modified (gm) plants may contain traces of these gm crops. As a result of the zero tolerance policy of the EU, these products must be removed from the market. Along with the amount of biotech crops produced worldwide, the work load for seed surveillance authorities increases. Since the commonly used CTAB buffer-based extraction methods are manual and laborious, a large part of the work load is caused by DNA extraction. In order to reduce labour input and accelerate the DNA analysis workflow, we developed an automated CTAB buffer-based DNA isolation method for seed. Several isolation and chemistry parameters were altered to combine a thorough cell lysis, removal of inhibitors and a highly efficient binding of gDNA to paramagnetic beads. This optimized procedure was compared with manual CTAB buffer-based and Wizard-based DNA extraction methods for maize, soya bean and rapeseed. Automated DNA extraction was faster, less laborious and resulted, on average, in higher DNA yield and purity. The applicability of our method was successfully proven with in-house routine samples.

Paper 9

Towards a more open debate about values in decision making on agricultural biotechnology

Author(s)

Devos, Y., Sanvido, O.,Tait, J.,and Raybould, A.

Source

Transgenic Res 90 refs. DOI 10.1007/s11248-013-9754-z Published by Springer

Abstract

Regulatory decision-making over the use of products of new technology aims to be based on science-based risk assessment. In some jurisdictions, decision-making about the cultivation of genetically modified (GM) plants is blocked supposedly because of scientific uncertainty about risks to the environment. However, disagreement about the acceptability of risks is primarily a dispute over normative values, which is not resolvable through natural sciences. Natural sciences may improve the quality and relevance of the scientific information used to support environmental risk assessments and make scientific uncertainties explicit, but offer little to resolve differences about values. Decisions about cultivating GM plants will thus not necessarily be eased by performing more research to reduce scientific uncertainty in environmental risk assessments, but by clarifying the debate over values. We suggest several approaches to reveal values in decision-making: (1) clarifying policy objectives; (2) determining what constitutes environmental harm; (3) making explicit the factual and normative premises on which risk assessments are based; (4) better demarcating environmental risk assessment studies from ecological research; (5) weighing the potential for environmental benefits (i.e., opportunities) as well as the potential for environmental harms (i.e., risks); and (6) expanding participation in the risk governance of GM plants.

Paper 10

EFSA's scientific activities and achievements on the risk assessment of genetically modified organisms (GMOs) during its first decade of existence: looking back and ahead

Author(s)

Devos; Y., Aguilera, J., Diveki, Z., Gomes, A., Liu, Y., Paoletti, C., Jardin, P., Herman, L., Perry, J.N., and Waigmann, E.

Source

Transgenic Res. 155 refs. DOI 10.1007/s11248-013-9741-4

Abstract

Genetically modified organisms (GMOs) and derived food and feed products are subject to a risk analysis and regulatory approval before they can enter the market in the European Union (EU). In this risk analysis process, the role of the European Food Safety Authority (EFSA), which was created in 2002 in response to multiple food crises, is to independently assess and provide scientific advice to risk managers on any possible risks that the use of GMOs may pose to human and animal health and the environment. EFSA's scientific advice is elaborated by its GMO Panel with the scientific support of several working groups and EFSA's GMO Unit. This review presents EFSA's scientific activities and highlights its achievements on the risk assessment of GMOs for the first 10 years of its existence. Since 2002, EFSA has issued 69 scientific opinions on genetically modified (GM) plant market registration applications, of which 62 for import and processing for food and feed uses, six for cultivation and one for the use of pollen (as or in food), and 19 scientific opinions on applications for marketing products made with GM microorganisms. Several guidelines for the risk assessment of GM plants, GM microorganisms and GM animals, as well as on specific issues such as post-market environmental monitoring (PMEM) were elaborated. EFSA also provided scientific advice upon request of the European Commission on safeguard clause and emergency measures invoked by EU Member States, annual PMEM reports, the potential risks of new biotechnology-based plant breeding techniques, evaluations of previously assessed GMOs in the light of new scientific publications, and the use of antibiotic resistance marker genes in GM plants. Future challenges relevant to the risk assessment of GMOs are discussed. EFSA's risk assessments of GMO applications ensure that data are analysed and presented in a way that facilitates scientifically sound decisions that protect human and animal health and the environment.

Paper 11

Risk communication discourse among ecological risk assessment professionals and its implications for communication with non-experts

Author(s)

Hunka, A.D., Palmqvist, A., Thorbek, P., and Forbes, V. E.

Source

Integrated Environmental Assessment and Management − Volume 9, Number 4, pp. 616‐622, 34 refs. © 2013 SETAC DOI: 10.1002/ieam.1426. Published online in Wiley Online Library (wileyonlinelibrary.com).

Abstract

Risk communication, especially to the general public and end users of plant protection products, is an important challenge. Currently, much of the risk communication the general public receives is via the popular press, and risk managers face the challenge of presenting their decisions and their scientific basis to the general public in an understandable way. Therefore, we decided to explore the obstacles in risk communication, as done by expert risk assessors and managers. Using the discourse analysis framework and readability tests, we studied perspectives of 3 stakeholder groups−regulators, industry representatives, and academics across Europe. We conducted 30 confidential interviews (10 participants in each group), with part of the interview guide focused on communication of pesticide risk to the general public and the ideas experts in the field of risk assessment and management hold of the public perception of pesticides.We used the key informant approach in recruiting our participants. They were first identified as key stakeholders in ecological risk assessment of pesticides and then sampled by means of a snowball sampling technique. In the analysis, first we identified main motifs (themes) in each group, and then we moved to studying length of the sentences and grammar and to uncovering discourses present in the text data. We also used the Flesch Reading Ease test to determine the comprehension difficulty of transcribed interviews. The test is commonly used as a standard for estimating the readability of technical documents. Our results highlight 3 main obstacles standing in the way of effective communication with wider audiences. First of all, ecological risk assessment as a highly technical procedure uses the specific language of ecological risk assessment, which is also highly specialized and might be difficult to comprehend by non-experts. Second, the idea of existing “expert‐lay discrepancy,” a phenomenon described in risk perception studies is visibly present in the experts' opinions. Finally, the communication flow among stakeholders was perceived as flawed, e.g., our participants did not consider themselves fully included in the communication process, despite taking part in many networks. Interestingly, both studies on the role of trust in risk perception, and research on links between daily choices and perceived risk, show that the public is more likely to rely on experts they can trust, than the experts in our study were inclined to think

Paper 12

An overview of the last 10 years of genetically engineered crop safety research

Authors

Nicolia,A., Manzo, A., Veronesi, F., and Rosellini, D.

Source

Crit Rev Biotechnol, Early Online: 1–12, 115 refs. @2013 Informa Healthcare USA, Inc. DOI: 10.3109/07388551.2013.823595 http://informahealthcare.com/bty. ISSN: 0738-8551 (print), 1549-7801 (electronic)

Abstract

The technology to produce genetically engineered (GE) plants is celebrating its 30th anniversary and one of the major achievements has been the development of GE crops. The safety of GE crops is crucial for their adoption and has been the object of intense research work often ignored in the public debate. We have reviewed the scientific literature on GE crop safety during the last 10 years, built a classified and manageable list of scientific papers, and analyzed the distribution and composition of the published literature. We selected original research papers, reviews, relevant opinions and reports addressing all the major issues that emerged in the debate on GE crops, trying to catch the scientific consensus that has matured since GE plants became widely cultivated worldwide. The scientific research conducted so far has not detected any significant hazards directly connected with the use of GE crops; however, the debate is still intense. An improvement in the efficacy of scientific communication could have a significant impact on the future of agricultural GE. Our collection of scientific records is available to researchers, communicators and teachers at all levels to help create an informed, balanced public perception on the important issue of GE use in agriculture.

Paper 13

Risk assessment and regulation of molecular farming - A comparison between Europe and US

Authors
Sparrow, P., Broer, I., Hood, E. E., Eversole,K., Hartung, F., and Schiemann, J.

Source
Current Pharmaceutical Design(2013) Volume 19, Number 00, 91refs. © 2013 Bentham Science Publishers

Abstract
In this article, the general principles of genetically modified (GM) plant risk assessment and the regulatory framework for contained use and open field production of plant-made pharmaceuticals/plant-made industrials (PMP/PMI) are described. While significant progress has been made for the containment grown (plant cell culture) production of PMPs, with the first regulatory approval made by the FDA in 2012, the commercialization of medicinal or industrial products produced in the field has yet to emerge in either Europe or the US. In the current paper, we describe the regulatory environment in Europe and the US surrounding GM crops, and provide case studies for experimental field releases of PMP and PMI producing plants in both regions. Suggestions for reducing the regulatory burden for GM plants will be discussed, also in light of the emerging new technologies to modify the genetics of plants. Since regulations surrounding the commercialization of GM crops are very costly and not appropriate for most of the PMP/PMI applications in Europe, we propose that amendments to the EU Directive 2001/18/EC are necessary to allow for the commercialization of products from GM plants without the need of an 'authorization'. To fully acknowledge the overall outcome of adopting plants to produce PMP/PMI, the conclusion is that broader and more balanced legislative oversight is needed in Europe; while specific legislation is not needed in the US.

Paper 14

Developing a policy for Low-Level Presence (LLP): A Canadian case study

Author(s)
Tranberg.J.

Source
AgBioForum, Volume 16, Number 1, pp. 37-45, 14 refs. @2013 AgBioForum

Abstract
Agricultural biotechnology research and adoption is increasing. It is estimated that by 2015 there will be a three- to four-fold increase in the number of commercialized biotech products. Also increasing are the complications with international trade given the wide range of acceptance and regulatory capabilities currently in practice globally, specifically, the increasing low level presence (LLP) of biotech products that have received full regulatory approval in one or more countries but not in the country of import. Canada, recognizing the impact of LLP on international trade, is taking a leadership role. Using a government-Industry collaborative model, the Canadian government is developing a domestic regulatory policy to manage LLP from imports and building international collaborations to raise awareness of the impacts of LLP on trade globally. This article details the collaborative government- industry process and the current status of the draft domestic LLP policy and international engagement.

Paper 15

The European union regulatory framework on genetically modified organisms and derived foods and feeds

Author(s)
Schauzu, M.

Sources
The European Union's Regulatory Framework on Genetically Modified Organisms and Derived Foods and Feeds. (2013) Adv Genet Eng Volume 2,Number 109, 35 refs. DOI: 10.4172/2169-0111.1000109

Abstract
Many countries have established policies and laws with regard to the introduction of genetically modified organisms into the environment already in the early 1990s. Although these jurisdictions differ, approaches to risk assessment are similar since they are following general principles and guidelines elaborated by international organizations. The European Union's regulatory framework and the approach to risk assessment of genetically modified organisms and derived food and feed are reviewed in this paper.

Paper 16

Benefits and risks associated with genetically modified food products

Author(s)
Kramkowska, M., Grzelak,T., Czyżewska,K.

Source
Annals of Agricultural and Environmental Medicine (2013), Volume 20, Number 3, pp 413–419. 63 refs.

Abstract
Scientists employing methods of genetic engineering have developed a new group of living organisms, termed 'modified organisms', which found application in, among others, medicine, the pharmaceutical industry and food distribution. The introduction of transgenic products to the food market resulted in them becoming a controversial topic, with their proponents and contestants. The presented study aims to systematize objective data on the potential benefits and risks resulting from the consumption of transgenic food. Genetic modifications of plants and animals are justified by the potential for improvement of the food situation worldwide, an increase in yield crops, an increase in the nutritional value of food, and the development of pharmaceutical preparations of proven clinical significance. In the opinions of critics, however, transgenic food may unfavourably affect the health of consumers. Therefore, particular attention was devoted to the short-and long-lasting undesirable effects, such as alimentary allergies, synthesis of toxic agents or resistance to antibiotics. Examples arguing for the justified character of genetic modifications and cases proving that their use can be dangerous are innumerable. In view of the presented facts, however, complex studies are indispensable which, in a reliable way, evaluate effects linked to the consumption of food produced with the application of genetic engineering techniques. Whether one backs up or negates transgenic products, the choice between traditional and non-conventional food remains to be decided exclusively by the consumers.

Paper 17

Bringing a transgenic crop to market: Where compositional analysis fits

Author(s)
Privalle,L.S., Gillikin, N., and Wandelt, C.

Source
J. Agric. Food Chem (2013), Volume 61,pp 8260−8266, 28 refs. dx.doi.org/10.1021/jf400185q

Abstract
In the process of developing a biotechnology product, thousands of genes and transformation events are evaluated to select the event that will be commercialized. The ideal event is identified on the basis of multiple characteristics including trait efficacy, the molecular characteristics of the insert, and agronomic performance. Once selected, the commercial event is subjected to a rigorous safety evaluation taking a multipronged approach including examination of the safety of the gene and gene product, the protein, plant performance, impact of cultivating the crop on the environment, agronomic performance, and equivalence of the crop/food to conventional crops/food − by compositional analysis. The compositional analysis is composed of a comparison of the nutrient and antinutrient composition of the crop containing the event, its parental line (variety), and other conventional lines (varieties). Different geographies have different requirements for the compositional analysis studies. Parameters that vary include the number of years (seasons) and locations (environments) to be evaluated, the appropriate comparator(s), analytes to be evaluated and statistical analysis. Specific examples of compositional analysis results will be presented.

Paper 18

***GSL2* over-expression confers resistance to *Pectobacterium atrosepticum* in potato**

Author
Mohan,S., Meiyalaghan,S., Latimer,J.M., Gatehouse,M.L., Monaghan, K.S., Vanga, B.R., Pitman, A.R., Jones,E.E., Conner,A.J., and Jacobs, J.M.E.

Source
Theor Appl Genet (2013), 39 refs. DOI 10.1007/s00122-013-2250-2 Published by Springer

Abstract
The *Gibberellin Stimulated-Like 2* (*GSL2*) gene (also known as *Snakin 2*) encodes a cysteine-rich, low molecular weight antimicrobial peptide produced in potato plants. This protein is thought to play important roles in the innate defence against invading microbes. Overexpression of the *GSL2* gene in potato (cultivar

Iwa) was achieved using *Agrobacterium*-mediated gene transfer of a plant expression vector with the potato *GSL2* gene under the regulatory control elements of the potato light-inducible *Lhca3* gene. The resulting plants were confirmed as being transgenic by PCR, and subsequently analysed for transcriptional expression of the *Lhca3- SL2-Lhca3* chimeric potato gene. Quantitative RT-PCR analysis demonstrated that the majority of the transgenic potato lines over-expressed the *GSL2* gene at the mRNA level. Based on qRT-PCR results and evaluation of phenotypic appearance, eight lines were selected for further characterization and evaluated in bioassays for resistance to *Pectobacterium atrosepticum* (formerly *Erwinia carotovora* subsp*. atroseptica*), the causal agent of blackleg in potato. Three independent pathogenicity bioassays showed that transgenic lines with significantly increased transcriptional expression of the *GSL2* gene exhibit resistance to blackleg disease. This establishes a functional role for GSL2 in plant defence against pathogens in potato.

Paper 19

RNA interference: concept to reality in crop improvement

Author (s)

Saurabh,S. Vidyarthi, A. S. and Prasad,D.

Source

Planta (2013), 229 refs. DOI 10.1007/s00425-013-2019-5 Published by Springer

Abstract

The phenomenon of RNA interference (RNAi) is involved in sequence-specific gene regulation driven by the introduction of dsRNA resulting in inhibition of translation or transcriptional repression. Since the discovery of RNAi and its regulatory potentials, it has become evident that RNAi has immense potential in opening a new vista for crop improvement. RNAi technology is precise, efficient, stable and better than antisense technology. It has been employed successfully to alter the gene expression in plants for better quality traits. The impact of RNAi to improve the crop plants has proved to be a novel approach in combating the biotic and abiotic stresses and the nutritional improvement in terms of bio-fortification and bio-elimination. It has been employed successfully to bring about modifications of several desired traits in different plants. These modifications include nutritional improvements, reduced content of food allergens and toxic compounds, enhanced defence against biotic and abiotic stresses, alteration in morphology, crafting male sterility, enhanced secondary metabolite synthesis and seedless plant varieties. However, crop plants developed by RNAi strategy may create biosafety risks. So, there is a need for risk assessment of GM crops in order to make RNAi a better tool to develop crops with biosafety measures. This article is an attempt to review the RNAi, its biochemistry, and the achievements attributed to the application of RNAi in crop improvement.

Paper 20

Vector integration in triple *R* gene transformants and the clustered inheritance of resistance against potato late blight

Author(s)

Zhu,S. , Duwal,A., Su, Q., Vossen, J.H., Visser, R.G.F., and Jacobsen, E.

Source

Transgenic Res (2013) Volume 22, pp. 315–325, 46 refs. DOI 10.1007/s11248-012-9644-9. Published by Springer. CODEN: TRSEES; ISSN: 0962-8819.

Abstract

Genetic transformation with resistance (*R*) genes is expected to enhance resistance durability against pathogens, especially for potato, a vegetatively propagated crop with tetrasomic inheritance and a long-term breeding program. In this study, 128 potato transformants were analysed for the presence of vector T-DNA genes, borders and backbone sequences. They were harvested after transformation using a construct containing *neomycin phosphotransferase* II (*npt*II) and three *R* genes against potato late blight (*Phytophthora infestans*). Our analysis revealed that 45 % of the *R* gene-containing transformants possessed a low T-DNA copy number, without the integration of vector backbone and borders. The integration of vector backbone sequences was characterized using eight genes, and backbone gene *tetA* was selected for the early prediction of plants with backbone sequence integration. Three transformants, two plants harbouring one T-DNA copy and one plant harbouring three T-DNA copies, were crossed with susceptible cv. Katahdin. Based on our results, we conclude that all four T-DNA genes were inherited as one cluster and segregated in a Mendelian fashion. The three T-DNA inserts from the transformant harbouring three T-DNA copies were statistically proven to be un-linked and inherited into the offspring plants independently. All of the *R* genes were functionally expressed in the offspring plants as in their parental transformants. This functional gene stacking has important implications towards achieving more

durable resistance against potato late blight.

Paper 21

Genetically modified foods: safety, risks and public concerns—a review

Author(s)
Bawa, A.S. and Anilakumar, K. R.

Source
J Food Sci Technol (2013) Volume 50, Number 6, pp.1035–1046, 70 refs. DOI 10.1007/s13197-012-0899-1

Abstract
Genetic modification is a special set of gene technology that alters the genetic machinery of such living organisms as animals, plants or microorganisms. Combining genes from different organisms is known as recombinant DNA technology and the resulting organism is said to be 'Genetically modified (GM)', 'Genetically engineered' or 'Transgenic'. The principal transgenic crops grown commercially in field are herbicide and insecticide resistant soybeans, corn, cotton and canola. Other crops grown commercially and/or field-tested are sweet potato resistant to a virus that could destroy most of the African harvest, rice with increased iron and vitamins that may alleviate chronic malnutrition in Asian countries and a variety of plants that are able to survive weather extremes. There are bananas that produce human vaccines against infectious diseases such as hepatitis B, fish that mature more quickly, fruit and nut trees that yield years earlier and plants that produce new plastics with unique properties. Technologies for genetically modifying foods offer dramatic promise for meeting some areas of greatest challenge for the 21st century. Like all new technologies, they also pose some risks, both known and unknown. Controversies and public concern surrounding GM foods and crops commonly focus on human and environmental safety, labelling and consumer choice, intellectual property rights, ethics, food security, poverty reduction and environmental conservation. With this new technology on gene manipulation what are the risks of "tampering with Mother Nature"?, what effects will this have on the environment?, what are the health concerns that consumers should be aware of? and is recombinant technology really beneficial? This review will also address some major concerns about the safety, environmental and ecological risks and health hazards involved with GM foods and recombinant technology.

Paper 22

Starches—from current models to genetic engineering

Author(s)
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Source
Plant Biotechnology Journal (2013) Volume 11, pp. 223–232, 96 refs. DOI: 10.1111/pbi.12029

Abstract
As the world's second most abundant biopolymer, starch serves as food, feed and renewable resource for bioenergy production and other industrial applications. Unlike storage lipids, starch is stored in the form of semi- crystalline granules, which are tissue- and species-specific in number, shape and size. Over the last decades, most biosynthetic and degradative enzymes of starch metabolism have been identified in the model species *Arabidopsis thaliana*. Based on this, biotechnological applications have arisen that led to a number of transgenic crop plants with elevated starch content or improved starch quality. Irrespective of this great success, there are still numerous open questions including the regulation of starch metabolism, the initiation of granule formation, the regulation of granule shape and size and many more, which will be tackled over the next decades. Here, we briefly summarize current knowledge concerning starch metabolism and its regulation and biotechnological use.

Paper 23

Loop-mediated isothermal amplification: Rapid visual and real-time methods for detection of genetically modified crops

Author(s)
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Source
Journal of Agricultural and Food Chemistry (2013), Volume 61, Number 47, pp. 11338-11346, 35 refs. ISSN: 0021-8561.

Abstract

A rapid, reliable, and sensitive loop-mediated isothermal amplification (LAMP) system was developed for screening of genetically modified organisms (GMOs). The optimized LAMP assays using designed primers target commonly employed promoters, i.e., Cauliflower Mosaic Virus 35S (P-35S) and Figwort Mosaic Virus promoter (P-FMV), and marker genes, i.e., *aminoglycoside 3'-adenyltransferase (aadA*), *neomycin phosphotransferase* II (*npt*II), *and beta-glucuronidase* (*uidA*). The specificity and performance of the end-point and real-time LAMP assays were confirmed using eight genetically modified (GM) cotton events on four detection systems, employing two chemistries. LAMP assays on the isothermal real-time system were found to be most sensitive, detecting up to four target copies, within 35 min. The LAMP assays herein presented using alternate detection systems can be effectively utilized for rapid and cost-effective screening of the GM status of a sample, irrespective of the crop species or GM trait. These assays coupled with a fast and simple DNA extraction method may further facilitate on-site GMO screening.

Paper 24

***In vitro* regeneration and *Agrobacterium*-mediated transformation of potato (*Solanum tuberosum* L.) cultivars grown in Mexico**

Author(s)

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Source

Plant Tissue Culture and Biotechnology (2013) Volume 22, Number 2, pp. 93-105, 27 refs.
ISSN 1817-3721, DOI: 10.3329/ptcb.v22i2.14193

Abstract

Prior to *Agrobacterium*-mediated genetic transformation *in vitro* regeneration protocol was established for three potato cultivars (Alfa, Cambray Rosa Morelos and Atlantic) grown in Mexico using leaf, node and internodal explants. Regeneration protocol was developed with or without the intervention of callus. Two potato cultivars, namely, Cambray Rosa Morelos and Alpha were transformed using *Agrobacterium tumefaciens* strain LBA4404 harboring binary plasmid *pBI121* containing the *GUS* and *nptII* genes. GUS histochemical assay and PCR analysis were conducted on rooted shoots grown in media without hormones but supplemented with antibiotics. Transformed shoots tested positive through *GUS* histochemical assay and integration of *nptII* gene was confirmed by PCR analysis.

Paper 25

Production of high-amylopectin potato plants by using ihpRNAi technology

Author(s)

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Source

Zhongguo Nongye Kexue (Beijing, China) (2013), Volumen 46, Number 18, pp. 3750-3757.
CODEN: CKNYAR; ISSN: 0578-1752

Abstract

Objective: The objective of this study was to develop *transgenic potato* (*Solanum tuberosum* L.) plants with high-amylopectin starch in its tubers. Method: RNA interference expression vector *pBI121g-PgABI* driven by Patatin was transformed into elite potato cultivar 'Gannongshu 2' by *Agrobacterium*-mediated transformation. The transgenic plants were identified by PCR, Southern-blotting, semi-quant. RT-PCR and real-time quant. PCR, and the starch content of transgenic potatoes was detd. Result: Ten transgenic potato lines were confirmed by PCR and Southern blot anal. that the target gene integrated into the plant genomes. Result of semi-quant. RT-PCR indicated that the accumulation of mRNAs derived from *granule bound starch synthase* I (*GBSS*I) was inhibited significantly in all transgenic lines, which were not detectable in 6 transgenic lines. Result of real-time quant. PCR showed that the inhibition ratio was 66.27%-93.53%. There were significant changes of starch content in ten transgenic microtubers, of which the amylopectin content was up to 90.16%-98.84%, 10.31%-20.92% higher than the non-transgenic microtuber. A significant correlation was found between inhibition ratio of mRNA and amylopectin content of transgenic potato plants ($r=0.937$, $P<0.01$). Conclusion: The ihpRNAi technol. could be used effectively in the prodn. of high-amylopectin potato or pure-amylopectin potato by silencing endogenesis gene *GBSS*I.

Paper 26

Practical experiences with an extended screening strategy for genetically modified organisms (GMOs) in real-life samples

Author(s)

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Source

Journal of Agricultural and Food Chemistry (2013), Volume 61, Number 38, pp. 9097-9109
CODEN: JAFCAU; ISSN: 0021-8561

Abstract

Nowadays most animal feed products imported into Europe have a GMO (genetically modified organism) label. This means that they contain European Union (EU)-authorized GMOs. For enforcement of these labeling requirements, it is necessary, with the rising no. of EU-authorized GMOs, to perform an increasing no. of analyses. In addn. to this, it is necessary to test products for the potential presence of EU-unauthorized GMOs. Anal. for EU-authorized and -unauthorized GMOs in animal feed has thus become laborious and expensive. Initial screening steps may reduce the no. of GMO identification methods that need to be applied, but with the increasing diversity also screening with GMO elements has become more complex. For the present study, the application of an informative detailed 24-element screening and subsequent identification strategy was applied in 50 animal feed samples. Almost all feed samples were labeled as contg. GMO-derived materials. The main goal of the study was therefore to investigate if a detailed screening strategy would reduce the no. of subsequent identification analyses. An addnl. goal was to test the samples in this way for the potential presence of EU-unauthorized GMOs. Finally, to test the robustness of the approach, eight of the samples were tested in a concise interlab. study. No significant differences were found between the results of the two labs.

Paper 27

Identification of an abundant 56 kDa protein implicated in food allergy as *granule-bound starch synthase*.

Corporate Source

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Source

Journal of Agricultural and Food Chemistry (2013), Volume 61, Number 22, pp. 5404-5409 ISSN: 0021-8561 DOI: 10.1021/jf4014372 Published by: American Chemical Society, Washington URL (Availability): http://pubs.acs.org/doi/abs/10.1021/jf4014372

Abstract

Rice, the staple food of south and east Asian counties, is considered to be hypoallergenic. However, several clinical studies have documented rice-induced allergy in sensitive patients. Rice proteins with molecular weights of 14-16, 26, 33, and 56 kDa have been identified as allergens. Recently, it was documented that the 56 kDa rice allergen was responsible for rice-induced anaphylaxis. The 14-16 kDa allergens have been identified as .alpha.-amylase inhibitors; the 26 kDa protein has been identified as .alpha.-globulin; and the 33 kDa protein has been identified as glyoxalase I. However, the identity of the 56 kDa rice allergen has not yet been determined. In this study, we demonstrate that serum from patients allergic to maize shows IgE binding to a 56 kDa protein that was present in both maize and rice but not in the oil seeds soybean and peanut. The 56 kDa IgE-binding protein was abundant in the rice endosperm. We have purified this protein from rice endosperm and demonstrated its reactivity to IgE antibodies from the serum of maize-allergic patients. The purified protein was subjected to matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry analysis, resulting in identification of this rice allergen as granule-bound starch synthase, a product of the Waxy gene. Immunoblot analysis using protein extracts from a waxy mutant of rice revealed the absence of the 56 kDa IgE-binding protein. Our results demonstrate that the 56 kDa rice allergen is granule-bound starch synthase and raise the possibility of using waxy mutants of rice as a potential source of the hypoallergenic diet for patients sensitized to the 56 kDa rice allergen.

Paper 28

Molecular characterization of *SSI* gene in *Triticum L*. and *Aegilops tauschii.*

Corporate Source

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Author(s)

Li, W., Liu, A.J., Sheng, Y.Z., Pu, Z.E., Liu, Y.X., and Cheng, G.Y.

Source

Journal of Biological Sciences (2013), Volume 13, Number 6, pp. 528-533, 31 refs. ISSN: 1727-3048 DOI: 10.3923/jbs.2013.528.533 Published by: Asian Network for Scientific Information, Faisalabad URL (Availability): http://scialert.net/fulltext/?doi=jbs.2013.528.533

Abstract

In order to knowing the variation of starch synthase I (SSI) in *Triticum L*. and *Aegilops* and exploiting new gene resources for the improving of starch quality in common wheat, using PCR cloning, eight sequences of *SSI* genes were obtained from AS60 (DD, *Aegilops tauschii*), AS2255 (AABB, *Triticum turgidum*), SHW-L1 (AABBDD, *synthetic hexaploid wheat*) and *Chuanmai* 32 (AABBDD, *Triticum aestivum*), respectively. These sequences of *SSI* gene showed the higher conservative characterization between different materials. A total of 10 variable nucleotide bases and two substitutions of amino acid residues (*Ser/Asn* and *Ala/Val*) were observed in the cloning sequences. Alignment of all sequences, including *SSI* gene from *T. aestivum, Hordeum vulgare, Brachypodium distachyon, Zea mays* and *Oryza sativa* suggested that the sequences from *Ae, tauschii, T. turgidum* and synthetic hexaploid wheat, were more similar to *SSI* gene from common wheat and barley and far distance with sequences from *Brachypodium distachyon, Zea mays* and *Oryza sativa*. Using neighbor-joining method, phylogenetic tree including a total of 28 sequences could be clustered into four groups. Most of sequences of *SSI* from different species were clustered together and *SSI* gene showed the clear difference with other starch synthases, including *Granule-bound Starch Synthase* (*GBSS*), *SSII, SSIII* and *SSIV*. These results suggested that there was variation from *SSI* gene in *Triticum L.* and *Aegilops.* It necessary to developing molecular markers for exploit genetic resources and the improving of wheat starch quality.

Paper 29

SNPs and an insertion sequence in five Wx-A1 alleles as factors for variant Wx-A1 protein in wheat.

Corporate Source

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Source

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Abstract

The waxy (Wx) gene encodes a *granule-bound starch synthase* (also called Wx protein) that is involved in synthesizing amylose in the starch grains of cereals, including common wheat (*Triticum aestivum* L.). Because amylose content affects the quality of food products made from wheat flour, Wx alleles affecting amylose content are of interest. Five wheat Wx alleles (Wx-A1c, -A1d, -A1e, -A1i and -A1j) that produce polymorphic Wx proteins on electrophoretic gels were investigated in terms of amylose content in starch and DNA sequences. Measurement and electrophoresis of gelled starch showed that apparent amylose contents of the genotypes were as follows: Wx-A1e, 2.9% (=waxy phenotype) <-A1i, 8.0% <-A1c, 16.8% <-A1j, 22.6%=level of wild type allele -A1a. DNA sequencing of the five alleles identified single nucleotide polymorphisms (SNPs) and insertion/deletion variations compared to Wx-A1a. A particular SNP causing amino acid changes in Wx-A1e and -A1c was identified as the factor responsible for decreased amylose. A SNP in Wx-A1d should cause an amino acid change and be responsible for an altered Wx-A1d protein. A transposable-like element of 376 bp present in the 3' *untranslated region* (*UTR*) of Wx-A1i most likely lowered the levels of Wx protein and amylose through aberrant mRNA. The fifth allele, Wx-A1j, possessed four SNPs, two of which altered amino acids in the Wx-A1j protein and should cause polymorphism in the Wx protein. Based on the DNA sequences, functional markers for Wx-A1c, -A1d, -A1e and -A1i were developed.

Paper 30

A novel waxy allele in sorghum landraces in East Asia.

Corporate Source

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Source
Plant Breeding (2013), Volume 132, Number 3, pp. 305-310, 30 refs.
ISSN: 0179-9541 DOI: 10.1111/pbr.12054 Published by: Wiley-Blackwell, Berlin URL (Availability): http://onlinelibrary.wiley.com/doi/10.1111/pbr.12054 /full

Abstract
A loss of *granule-bound starch synthase* I (*GBSS* I) activity results in starch granules that contain mostly amylopectin and little or no amylose, a phenotype described as waxy. Previously, two phenotypic classes of waxy alleles*, wxa*, associated with no detectable *GBSS* I, and *wxb*, associated with apparently inactive *GBSS* I in the endosperm, were reported in sorghum (*Sorghum bicolor* (L.) Moench). In this study, the waxy alleles in a sorghum core collection were investigated using DNA markers. Of the 337 sorghum accessions examined, 17 accessions that were confirmed to be waxy by a negative iodine staining result and 16 were found to be *wxa*. A novel waxy allele, *wxc*, was found in a Taiwanese landrace. This allele consists of a +1G to C mutation in the 5' splice site at the intron 10-exon 11 boundary, a mutation that most likely resulted in the suppression of *GBSS* I gene expression. A DNA marker specific for *wxc* was produced to distinguish the *wxc* allele from other alleles, allowing the identification of heterozygous non-waxy plants.

Paper 31

Identification of two novel waxy alleles and development of their molecular markers in sorghum.

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Source
Research Press, Ottawa URL (Availability): http://www.nrcresearchpress.com/doi/full/10.1139/gen-2013-0047

Abstract
High amylopectin grains of waxy sorghum have a high economic value in the food and bioenergy industries because of their increased starch digestibility and higher ethanol conversion rate compared with wild-type sorghum grains. Mutation in the *granule-bound starch synthase* (*GBSS*) gene contributes to the waxy phenotype. Two classes of waxy alleles, *wxa* and *wxb*, have been characterized previously. In the present work, we identified two novel types of waxy mutations in the sorghum *GBSS* gene, designated as *wxc* and *wxd*. The *wxc* allele has a G deletion at the 5' splicing site of the ninth intron, causing a shift of the 5' cleavage site; in turn, a reading frame shift occurred and resulted in an early translation termination. The *wxd* allele contained a mutation at the 3' splicing site of the 10th intron, which led to a splicing site shift and resulted in the deletion of five amino acids (GTGKK) in the predicted translation product. Furthermore, cleaved amplified polymorphic sequence (CAPS) markers were developed to detect the *wxc* and *wxd* alleles. With these markers, classification of waxy alleles was performed in nearly 100 sorghum accessions from our breeding program. Most waxy sorghum cultivars in China were either *wxa* or *wxc*, implying that these two mutations are preferentially maintained during domestic selection in glutinous sorghum production.

Paper 32

Characterization and expression analysis of waxy alleles in barley accessions.

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Source
Genetica (2013), Volume 141, Number 4/6, pp. 227-238, 48 refs.
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http://rd.springer.com/journal/10709

Abstract
Granule Bound Starch Synthase I (*GBSS* I) encoded by the waxy gene plays an important role in accumulating amylose during the development of starch granules in barley. In this study, we isolated and characterized waxy alleles of three waxy (GSHO 908, GSHO 1828 and NA 40) and two non-waxy barley accessions (PI 483237 and CIho 15773), estimated the expression patterns of waxy genes via Real-time quantitative PCR (RT-qPCR), investigated promoter activity by analyzing promoter-GUS expression, and examined possible effects of waxy alleles on starch granule morphology in barley accessions by scanning electron microscopy (SEM). A 193-bp insertion in intron 1, a 15-bp insertion in the coding region, and some single nucleotide polymorphic sites were detected in the waxy barley accessions. In addition, a 397-bp deletion containing the TATA box, transcription starting point, exon 1 and partial intron 1 were also identified in the waxy barley accessions. RT-qPCR analysis showed that waxy accessions had lower waxy expression levels than those of non-waxy accessions. Transient expression assays showed that GUS activity driven by the 1,029-bp promoter of the non-waxy accessions was stronger than that driven by the 822-bp promoter of the waxy accessions. SEM revealed no apparent differences of starch granule morphology between waxy and non-waxy accessions. Our results showed that the 397-bp deletion identified in the waxy barley accessions is likely responsible for the reduction of waxy transcript, leading to lower concentrations of *GBSS* I protein thus lower amylose content.

Paper 33

Waxy phenotype evolution in the allotetraploid cereal broomcorn millet: mutations at the *GBSSI* locus in their functional and phylogenetic context.

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Source
Molecular Biology and Evolution (2013), Volume 30, Number 1, pp. 109-122, 45 refs.
ISSN: 0737-4038 DOI: 10.1093/molbev/mss209 Published by: Oxford University Press, Oxford URL (Availability): http://mbe.oxfordjournals.org/

Abstract
Waxy mutants, in which endosperm starch contains 100% amylopectin rather than the wild-type composition of 70% amylopectin and 30% amylose, occur in many domesticated cereals. The cultivation of waxy varieties is concentrated in East Asia, where there is a culinary preference for glutinous-textured foods that may have developed from ancient food processing traditions. The waxy phenotype results from mutations in the *GBSSI* gene, which catalyzes amylose synthesis. Broomcorn or proso millet (*Panicum miliaceum* L.) is one of the world's oldest cultivated cereals, which spread across Eurasia early in prehistory. Recent phylogeographic analysis has shown strong genetic structuring that likely reflects ancient expansion patterns. Broomcorn millet is highly unusual in being an allotetraploid cereal with fully waxy varieties. Previous work characterized two homeologous *GBSSI* loci, with multiple alleles at each, but could not determine whether both loci contributed to *GBSSI* function. We first tested the relative contribution of the two *GBSSI* loci to amylose synthesis and second tested the association between *GBSSI* alleles and phylogeographic structure inferred from simple sequence repeats (SSRs). We evaluated the phenotype of all known *GBSSI* genotypes in broomcorn millet by assaying starch composition and protein function. The results showed that the *GBSSI*-S locus is the major locus controlling endosperm amylose content, and the *GBSSI*-L locus has strongly reduced synthesis capacity. We genotyped 178 individuals from landraces from across Eurasia for the 2 *GBSSI* and 16 SSR loci and analyzed phylogeographic structuring and the geographic and phylogenetic distribution of *GBSSI* alleles. We found that *GBSSI* alleles have distinct spatial distributions and strong associations with particular genetic clusters defined by SSRs. The combination of alleles that results in a partially waxy phenotype does not exist in landrace populations.

Our data suggest that broomcorn millet is a system in the process of becoming diploidized for the *GBSSI* locus responsible for grain amylose. Mutant alleles show some exchange between genetic groups, which was favored by selection for the waxy phenotype in particular regions. Partially waxy phenotypes were probably selected against - this unexpected finding shows that better understanding is needed of the human biology of this phenomenon that distinguishes cereal use in eastern and western cultures.

Paper 34

Effect of genetic modification of potato starch on decomposition of leaves and tubers and on fungal decomposer communities.

Corporate Source

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Source

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Abstract

As part of a risk evaluation of growing genetically modified crops, we investigated the effects of a genetic modification of starch quality (increased level of *amylopectin*) in potato tubers (*Solanum tuberosum* L.) on the decomposition of tissues (tubers and leaves) as well as on the associated fungal functional and phylogenetic diversity. The weight loss of both leaves and tubers in litterbags was analysed after 1, 3 and 6 months of incubation in soils and combined with measurements of fungal extracellular enzyme activities (laccases, Mn-peroxidases and cellulases) as well as molecular analyses of the fungal community (*ITS* regions and *cellobiohydrolase* I (*cbhI*) genes). The study revealed that initial (after one month) decomposition of both tubers and leaves of the parental isoline was significantly faster than that of the genetically modified (GM)-variety. This coincided with differences in fungal community composition. After this initial difference, no significant differences in any of the parameters measured could be detected after 3 and 6 months of decomposition illustrating the transient nature of the initial difference between the cultivars. Thus, it can be concluded that the starch modified tubers are not harmful to the fungal decomposer community and that despite initial differences in decomposition, the final decomposition rate of tissues from the GM-variety was similar to that of tissues from the parental variety. Furthermore, interesting dynamics of fungal phyla and species during decomposition were observed; the basidiomycetal yeasts and *ascomycetes* were primary colonizers of the *potato* tissue while *basidiomycetes* were dominant in the more decomposed and lignin-rich litter.

Paper 35

Genetic diversity of null alleles of waxy gene in *Triticum L.*

Corporate Source

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Source

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Abstract

In order to exploit new genetic resources for the improving of starch quality of common wheat, the genetic diversity of null alleles of *Granule-bound starch synthase* I (waxy gene) was investigated by special PCR molecular markers in *Triticum* L. The results indicated that there was relative abundant genetic diversity of waxy alleles in all accessions. Accession AS2347, AS2356, AS2317 and AS2308 with null allele at Waxy-B1 locus and AS2310 and AS2335 with null alleles at Waxy-A1 and Waxy-B1, were observed in 81

landraces of *Triticum turdigum* L. from China. In 53 landraces of *Triticum aestivum* L. from Sichuan, China, eight accessions at Waxy-A1, Waxy-B1 and Waxy-D1 loci and accession AS1668 at Waxy-D1, were observed null alleles. In 29 *Triticum macha*, Accession PI361862 and PI572911 at three Waxy loci, PI572913 at Waxy-B1 and Waxy-D1, PI572910 at Waxy-A1 and Waxy-D1, PI 290507 at Waxy-B1 and PI572906 at Waxy-D1, respectively, were observed null alleles. Seven accessions with null alleles at Waxy-B1 locus was observed in 28 *Triticum sphaerococcum*. Specially, the accessions of two regions, Anyue in Sichuan, China and Georgia, had the high frequency of the mutations with null alleles of waxy gene. Landraces of *Triticum aestivum* L. with the high frequency of waxy wheat, could be considered as a unique genetic resource for improving of waxy wheat. These result suggested that the special molecular marker could be used reliably in evaluation of genetic resources and these mutations also could be directly used in the improving of common wheat.

Paper 36

Scientific opinion on the annual post-market environmental monitoring (PMEM) report from BASF plant science company GmbH on genetically modified potato EH92-527-1 in 2012.

Corporate Source

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Source

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Abstract

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) assessed the monitoring report, provided by BASF, on the genetically modified (GM) potato EH92-527-1 (variety Amflora) for the 2012 growing season. Because of the discontinuation of the GM potato cultivation in the European Union in 2012, the 2012 monitoring report contained a limited information package, mainly the results of the 2012 monitoring study for volunteers within and around the fields cropped with the GM potato in 2010. The EFSA GMO Panel concludes that GM potato volunteers can be controlled by the applied weed control practices but cannot conclude on the absence of enhanced fitness of the GM potato due to data limitations and flaws in the study design. Hence, the EFSA GMO Panel makes appropriate recommendations. Accounting for the biology of the crop, the GM trait and the common management practices in potato cropping, the EFSA GMO Panel considers it is unlikely that a potential change in fitness or persistence would significantly alter the ability of GM volunteers to establish. Moreover, the EFSA GMO Panel does not consider the occurrence of potato volunteers as an environmental concern but rather as a crop management issue. Therefore, the EFSA GMO Panel concludes that the information provided in the 2012 monitoring report does not indicate any adverse effects of potato EH92-527-1 on the environment or human and animal health. Therefore, the outcomes of the 2012 monitoring report do not invalidate the conclusions of the EFSA GMO Panel's previous opinions on potato EH92-527-1.

Paper 37

Genome-Specific *Granule-Bound Starch Synthase* I (*GBSSI*) influences starch biochemical and functional characteristics in near-isogenic wheat (*Triticum aestivum* L.) lines.

Corporate Source

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Source

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Abstract

Near-isogenic wheat (*Triticum aestivum* L.) lines differing at the Waxy locus were studied for the influence of genome-specific *granule-bound starch synthase* I (*GBSSI*/Waxy; Wx-A, Wx-B, Wx-D) on starch composition, structure, and *in vitro* starch enzymatic hydrolysis. Grain composition, amylose concentration, amylopectin unit-chain length distribution, and starch granule size distribution varied with the loss of functional *GBSSI*. Amylose concentration was more severely affected in genotypes with *GBSSI* missing from two genomes (double nulls) than from one genome (single nulls). Unit glucan chains (DP 6-8) of amylopectin were reduced with the complete loss of *GBSSI* as compared to wheat starch with a full complement of *GBSSI*. Wx-A and Wx-B had an additive effect toward short-chain phenotype of waxy amylopectin. Loss of Wx-D isoprotein alone significantly ($p < 0.05$) reduced the C-type starch granules. However, the absence of Wx-D in combination with Wx-A or Wx-B increased the B-type and C-type starch granules but decreased the volume of A-type starch granules. The rate of *in vitro* starch enzymatic hydrolysis was highest in completely waxy grain meal and purified starch. However, the presence of Wx-D reduced wheat starch hydrolysis as it increased the large A-type starch granule content (volume %) and reduced short chains (DP 6-8) in amylopectin. Factors such as small C-type starch granules, amylose concentration, and long chains of amylopectin (DP 23-45) also influenced wheat starch hydrolysis.

Paper 38

Association mapping of starch physicochemical properties with starch biosynthesizing genes in waxy rice (*Oryza sativa* L.).

Corporate Source

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Abstract

Waxy (glutinous) rice is widely used in traditional foods, and understanding the genetic bases of its diverse physicochem. properties will contribute to breeding of new waxy rice with unique qualities. The objective of this study was to investigate the genetic relationship between the starch biosynthesis related genes and the physicochem. properties of waxy rice using assocn. mapping. A total of 36 mol. markers representing 18 genes were used to genotype 50 waxy rice accessions for which starch properties were previously available. Most of the starch properties differed between high and low gelatinization temp. (GT) groups, whereas most traits were similar between the low-GT indica rice and low-GT japonica rice, suggesting GT was the main determinant of the starch quality of waxy rice. Assocn. mapping indicated that the starch properties of waxy rice were mainly controlled by *starch synthase IIa* (*SSIIa* or *SSII*-3, a major gene responsible for the gelatinization temp.) and *SSI*. It was found that gene-gene interactions were also important for the genetic control of starch properties of waxy rice. This study suggests that application of the functional *SNPs* of *SSIIa* in mol. breeding may facilitate quality improvement of waxy rice.