ANNEX 14

LITERATURE REVIEW

A literature review was conducted in January 2012 based on searching 18 databases. Of main importance were the following databases:

- BIOSIS
- Web of SCIENCE
- Medline
- Chemical Abstracts
- CABA
- PIRA

The search parameters were set as follows:

- Amflora or EH92-527-1 or potato in general in combination with a word profile for genetic engineering and in combination with the enzymes granule bound starch synthase or neomycin phosphotransferase.
- In addition Amflora or EH92-527-1or potato in general in combination with a word profile for genetic engineering and in combination with amylopectin or waxy starch or antibiotic resistance or kanamycin resistance or BASF.
- The focus was on scientific literature or news, patents were not included.
- All results were checked for intellectual relevance.

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

Paper 1

Structural characterization of novel cassava starches with low and high-amylose contents in comparison with other commercial sources

Author(s)

Rolland-Sabate, Agnes; Sanchez, Teresa; Buleon, Alain; Colonna, Paul; Jaillais, Benoit; Ceballos, Hernan; Dufour, Dominique

Source

Food Hydrocolloids (2012), 27(1), 161-174 CODEN: FOHYES; ISSN: 0268-005X

Abstract

Two new mutant cassava starches with extreme amylose contents (0 and 30-31%) have been recently reported. These mutants are drastically different from normal cassava starch whose amylose content typically ranges between 15 and 25%. The new mutants were compared with five normal cassava starches (ranging from 16.8 to 21.5% amylose) and com. versions of amylose-free or normal potato and maize starch. Macromol. features, crystallinity, granule sizes, and thermal properties of these starches were compared. The structure of cassava amylopectin was not modified by the waxy mutation and waxy cassava starch exhibited properties similar to the ones of waxy maize starch. Waxy cassava and maize show similar Mw and RG of amylopectin (between 408 .times. 106 g mol-1 and 520 .times. 106 g mol-1; 277-285 nm, resp.), whereas waxy potato amylopectin has lower Mw and RG. On the contrary, the higher-amylose mutations induced by gamma rays radiation in cassava, modified deeply the branching pattern of amylopectin as well as the starch characteristics and properties: Mw and RG decreased, while branching degree increased. These modifications resulted in changes in starch granule ultrastructure (e.g. decreased starch crystallinity), a weak organized structure, and increased susceptibility to mild acid hydrolysis. The distinctive properties of the new cassava starches demonstrated in this article suggest new opportunities and com. applications for tropical sources of starch.

Paper 2

Cisgenic inhibition of the potato cold induced phosphorylase L gene expression and decrease in sugar contents

Author(s)
Kamrani, Morteza; Kohnehrouz, Bahram Baghban; Gholizadeh, Ashraf

Source
African Journal of Biotechnology (2011), 10(50), 10076-10082 CODEN: AJBFAH; ISSN: 1684-5315 URL: http://www.academicjournals.org/AJB/PDF/pdf2011/5Sep/Kamrani%20et%20a_ l.pdf

Abstract
To decrease the accumulation of reducing and non-reducing sugar in potato tubers stored at low temp., a single gene silencing vector pARTPhL-IR, harboring a part of starch phosphorylase L gene as inverted repeats with pdk intron within was constructed and transformed into potato (Solanum tuberosum L.) cultivars Agria and Marfona. Polymerase chain reaction (PCR) of nptII gene and pdk intron indicated that the RNA interference construct was transformed successfully into the genome. Real time RT-PCR anal. of starch phosphorylase L gene in stored microtubers for 90 days at 4.degree.C showed that the expression level of this gene in transgenics ranged from 1.63 to 7.54 % of that in the non-transgenic plants. Anal. of sugar content in these plants showed that the total sugar content in transgenic microtubers was significantly reduced compared to the control, up to 34% in line M4. The accumulation of reducing sugars in transgenic lines at 4.degree.C was reduced from 9.13 (in Agria) to 5.57 mg/g fresh wt. (transgenic line A5) and from 9.56 (in Marfona) to 6.52 mg/g fresh wt. (transgenic line M4), implying that silencing of starch phosphorylase L gene reduced starch breakdown during cold storage conditions.

Paper 3

Preparation and characterization of new and improved soluble-starches, -amylose, and -amylopectin by reaction with benzaldehyde/zinc chloride

Author(s)
Johnston, David A.; Mukerjea, Rupendra; Robyt, John F.

Source
Carbohydrate Research (2011), 346(17), 2777-2784 CODEN: CRBRAT; ISSN: 0008-6215

Abstract
Seven different starches from potato, rice, maize, waxymaize, amylomaize-VII, shoti, and tapioca, and potato amylose and potato amylopectin have been reacted with benzaldehyde, catalyzed by ZnCl2, to give new water-sol. starches and water sol.-amylose and sol.-amylopectin. In contrast to the native starches, aq. solns. of the modified starches could not be pptd. with 2-, 3-, or 4-vols. of ethanol. .beta.-Amylase gave no reaction with the modified starches, in contrast to the native starches, indicating that the modification occurred exclusively at the nonreducing-ends, giving 4,6-benzylidene-D-glucopyranose at the nonreducing-ends. Reactions of .alpha.-amylase with native and modified potato and rice starches gave a decrease in the triiodide blue color and an increase in the reducing-value that were similar for the native- and modified-starches, indicating the modified starches had not been significantly altered by the modification. The benzaldehyde-modified starches and benzaldehyde-modified potato amylose and potato amylopectin components, therefore, have a starch structure very much like their native counterparts, in contrast to the Lintner, Small, and the alc./acid-hydrolyzed sol.-starches that have undergone acid hydrolysis. The benzaldehyde-modified starches and starch components have significantly higher water soly. than their native counterparts even though the structures of the modified starches had only been slightly altered from the structures of their native counterparts. They all gave crystal-clear solns. that did not retrograde.

Paper 4

Impact of structural changes due to heat-moisture treatment at different temperatures on the susceptibility of normal and waxy potato starches towards hydrolysis by porcine pancreatic alpha amylase

Author(s)

Varatharajan, V.; Hoover, R.; Li, Jihong; Vasanthan, T.; Nantanga, K. K. M.; Seetharaman, K.; Liu, Q.; Donner, E.; Jaiswal, S.; Chibbar, R. N.

Source

Food Research International (2011), 44(9), 2594-2606 CODEN: FORIEU; ISSN: 0963-9969

Abstract

The objectives of this study were to det. the impact of structural changes within the amorphous and cryst. domains of normal potato (NP) and waxy potato (WP) starches subjected to heat-moisture treatment (HMT) at 80, 100, 120 and 130 .degree.C for 16 h at a moisture content of 27% and to det. the impact of structural changes at each of the above temps. on the susceptibility on hydrolysis by porcine pancreatic .alpha.-amylase (PPA). The results showed that structural changes due to HMT were influenced by differences in starch chain mobility at the different temps. of HMT. Starch chain mobility in turn was influenced by the interplay between the extent to which B-type crystallites were transformed into A + B-type crystallites, kinetic energy imparted to starch chains and amylose content. The main type of structural changes influencing physicochem. properties at the different temps. of HMT was starch chain interactions (at 80 and 100 .degree.C), disruption of hydrogen bonds between amylose (AM)-amylopectin (AMP) and AMP-AMP chains (at 120 and 130 .degree.C), disorganization of AMP chains near the vicinity of the hilum (at 100, 120 and 130 .degree.C) and formation of interrupted helixes (at 130 .degree.C). The susceptibility of NP and WP starches towards .alpha.-amylase decreased at 80 .degree.C, but increased in the range of 100 to 130 .degree.C. This suggested that .alpha.-amylase hydrolysis of HMT starches was influenced by the interplay of: 1) amt. of A-type crystallites, 2) starch chain interactions and 3) changes to double helical conformation. Differences in granule morphol. in PPA hydrolyzed NP and WP starches were largely influenced by the higher granular swelling in the latter. NP and WP starches exhibited heterogeneity in degrdn. (NP > WP) in both their native and HMT states.

Paper 5

Anthocyanin production as a potential visual selection marker during plant transformation

Author(s)

Kortstee, A. J.; Khan, S. A.; Helderman, C.; Trindade, L. M.; Wu, Y.; Visser, R. G. F.; Brendolise, C.; Allan, A.; Schouten, H. J.; Jacobsen, E.

Source

Transgenic Research (2011), 20(6), 1253-1264 CODEN: TRSEES; ISSN: 0962-8819

Abstract

A mutant allele of the transcription factor gene MYB10 from apple induces anthocyanin prodn. throughout the plant. This gene, including its upstream promoter, gene coding region and terminator sequence, was introduced into apple, strawberry and potato plants to det. whether it could be used as a visible selectable marker for plant transformation as an alternative to chem. selectable markers, such as kanamycin resistance. After transformation, red colored calli, red shoots and red well-growing plants were scored. Red and green shoots were harvested from apple explants and examd. for the presence of the MYB10 gene by PCR anal. Red shoots of apple explants always contained the MYB10 gene but not all MYB10 contg. shoots were red. Strawberry plants transformed with the MYB10 gene showed anthocyanin accumulation in leaves and roots. No visible accumulation of anthocyanin could be obsd. in potato plants grown in vitro, even the ones carrying the MYB10 gene. However, acid methanol exts. of potato shoots or roots carrying the MYB10 gene contained up to four times higher anthocyanin content than control plants. Therefore anthocyanin prodn. as result of the apple MYB10 gene can be used as a selectable marker for apple, strawberry and potato transformation, replacing kanamycin resistance.

Paper 6

Cryo-milling of starch granules leads to differential effects on molecular size and conformation

Author(s)
Dhital, Sushil; Shrestha, Ashok K.; Flanagan, Bernadine M.; Hasjim, Jovin; Gidley, Michael J.

Source
Carbohydrate Polymers (2011), 84(3), 1133-1140 CODEN: CAPOD8; ISSN: 0144-8617

Abstract
Milling of starch granules is important for many food applications and involves a combination of mech. and thermal energy. In order to understand the effects of mech. force alone, four com. starches including maize starch (MS), potato starch (PS), and two high amylose maize starches (HAMS) (Gelose 50 and Gelose 80) were cryo-milled for 20 min under the same conditions. The structural and conformational changes of the starches after cryo-milling were evaluated using X-ray diffraction, NMR spectroscopy, IR and Raman spectroscopy, and size exclusion chromatog. (SEC). The cryo-milled starches had less crystallinity (15-35%) and 35-50% less ordered structure (double and single helixes) than the native starch counterparts. The gelatinization temps. of the starches were not significantly altered by cryo-milling, but the gelatinization enthalpies were significantly reduced in line with the redns. in the amt. of double helixes. Although, all four starches showed similar extent of degrdn. of cryst./ordered structure, SEC results showed a greater degrdn. of amylopectin mol. in MS and PS than in HAMS. Increased amylose content in starch seemed to reduce the mol. degrdn. during milling, which is consistent with a role for amylose as a mech. plasticizer in starch granules. It is concluded that (i) cryo-milling has differential effects on mol. size and conformation depending on starch granule type, and (ii) deterioration of starch cryst. and mol. order by mech. treatment is not necessarily linked with the redn. in mol. size. The implication from the results is that the mech. forces acting during cryo-milling are capable of disrupting helical and cryst. structures without breaking covalent bonds of starch mols.

Paper 7

Identification of plant-derived genetically modified organisms in food and feed using a hydrogel oligonucleotide microchip

Author(s)
Gryadunov, D. A.; Getman, I. A.; Chizhova, S. I.; Mikhailovich, V. M.; Zasedatelev, A. S.; Romanov, G. A.

Source
Molecular Biology (Moscow, Russian Federation, English Edition) (2011), 45(6), 894-903 CODEN: MOLBBJ; ISSN: 0026-8933

Abstract
A method of multiplex polymerase chain reaction (PCR) followed by hybridization on a hydrogel oligonucleotide biochip was developed for simultaneous identification of ten different transgenic elements of plant DNA in food and feed products. The biochip contained 22 immobilized oligonucleotide probes that were intended for (1) detection of plant DNA, (2) detn. of plant species (soybean, maize, potato, and rice), and (3) identification of transgenic elements, including sequences of 35S CaMV, 35S FMV, rice actin gene promoters, nos, 35S CaMV, ocs, pea rbcS1 gene terminators, and bar, gus, and nptII marker genes. The limit of detection was 0.5% for genetically modified (GM) soybean and maize in the analyzed samples. The tests on food and feed products using the developed approach and real-time PCR showed full agreement in detn. of transgenic DNA in the samples. The proposed assay can be used for selection of GM samples by screening food and feed products for subsequent quant. detn. of GM component based on the identified transgene.

Paper 8

Production of marker-free disease-resistant potato using isopentenyl transferase gene as a positive selection marker

Author(s)
Khan, Raham Sher; Ntui, Valentine Otang; Chin, Dong Poh; Nakamura, Ikuo; Mii, Masahiro

Source
Plant Cell Reports (2011), 30(4), 587-597 CODEN: PCRPD8; ISSN: 0721-7714

Abstract
The use of antibiotic or herbicide resistant genes as selection markers for prodn. of transgenic plants and their continuous presence in the final transgenics has been a serious problem for their public acceptance and commercialization. MAT (multi-auto-transformation) vector system has been one of the different strategies to excise the selection marker gene and produce marker-free transgenic plants. In the present study, ipt (isopentenyl transferase) gene was used as a selection marker gene. A chitinase gene, ChiC (isolated from Streptomyces griseus strain HUT 6037) was used as a gene of interest. ChiC gene was cloned from the binary vector, pEKH1 to an ipt-type MAT vector, pMAT21 by gateway cloning and transferred to Agrobacterium tumefaciens strain EHA105. The infected tuber disks of potato were cultured on hormone- and antibiotic-free MS medium. Seven of the 35 explants infected with the pMAT21/ChiC produced shoots. The same antibiotic- and hormones-free MS medium was used in sub-cultures of the shoots (ipt like and normal shoots). Mol. analyses of genomic DNA from transgenic plants confirmed the integration of gene of interest and excision of the selection marker in 3 of the 7 clones. Expression of ChiC gene was confirmed by Northern blot and Western blot analyses. Disease-resistant assay of the marker-free transgenic, in vitro and greenhouse-grown plants exhibited enhanced resistance against Alternaria solani (early blight), Botrytis cinerea (gray mold) and Fusarium oxysporum (Fusarium wilt). From these results it could be concluded that ipt gene can be used as a selection marker to produce marker-free disease-resistant transgenic potato plants on PGR- and antibiotic-free MS medium.

Paper 9

Effects of genetically modified starch metabolism in potato plants on photosynthate fluxes into the rhizosphere and on microbial degraders of root exudates

Author(s)
Gschwendtner, Silvia; Esperschuetz, Juergen; Buegger, Franz; Reichmann, Michael; Mueller, Martin; Munch, Jean Charles; Schloter, Michael

Source
FEMS Microbiology Ecology (2011), 76(3), 564-575 CODEN: FMECEZ; ISSN: 0168-6496

Abstract
A high percentage of photosynthetically assimilated carbon is released into soil via root exudates, which are acknowledged as the most important factor for the development of microbial rhizosphere communities. As quality and quantity of root exudates are dependent on plant genotype, the genetic engineering of plants might also influence carbon partitioning within the plant and thus microbial rhizosphere community structure. In this study, the carbon allocation patterns within the plant-rhizosphere system of a genetically modified amylopectin-accumulating potato line (Solanum tuberosum L.) were linked to microbial degraders of root exudates under greenhouse conditions, using 13C-CO2 pulse-chase labeling in combination with phospholipid fatty acid (PLFA) anal. In addn., GM plants were compared with the parental cultivar as well as a second potato cultivar obtained by classical breeding. Rhizosphere samples were obtained during young leaf developmental and flowering stages. 13C allocation in aboveground plant biomass, water-extractable org. carbon, microbial biomass carbon and PLFA as well as the microbial community structure in the rhizosphere varied significantly between the natural potato cultivars. However, no differences between the GM line and its parental cultivar were obsd. Besides the considerable impact of plant cultivar, the plant developmental stage affected carbon partitioning via the plant into the rhizosphere and, subsequently, microbial communities involved in the transformation of root exudates.

Paper 10

Genetic transformation of potato with soluble starch synthase SSIII gene

Author(s)
Du, Honghui; Wen, Yikai; Zhang, Ning; Si, Huaijun; Wang, Di

Source
Jiyinzuxue Yu Yingyong Shengwuxue (2011), 30(3), 303-307 CODEN: JYYSAZ; ISSN: 1674-568X

Abstract
Potato is one of the most important crops in the prodn. of starch. Sol. starch synthase SSIII is the main active compn. of sol. starch synthase. Therefore, it is possible to alter starch quality and quantity and investigate function of SSIII gene in potato starch synthesis via genetic engineering methods. Sol. starch synthase SSIII gene of RNA interference expression vectors driven by the constitutive expression promoter CaMV 35S was introduced into two potato cultivars Kexin1 and Kexin4 by Agrobacterium-mediated transformation method. Then 65 kanamycin-resistant plants with kanamycin resistance were obtained. PCR detection showed that the interference fragment of SSIII gene was integrated into potato genome. RT-PCR anal. showed that the expression of SSIII gene was repressed apparently on the transcription level. These results pave the way for improvement of potato starch quality.

Paper 11

Greenhouse and field cultivations of antigen-expressing potatoes focusing on the variability in plant constituents and antigen expression

Author(s)
Mikschofsky, Heike; Heilmann, Elena; Schmidtke, Joerg; Schmidt, Kerstin; Meyer, Udo; Leinweber, Peter; Broer, Inge

Source
Plant Molecular Biology (2011), 76(1-2), 131-144 CODEN: PMBIDB; ISSN: 0167-4412

Abstract
The prodn. of plant-derived pharmaceuticals essentially requires stable concns. of plant constituents, esp. recombinant proteins; nonetheless, soil and seasonal variations might drastically interfere with this stability. In addn., variability might depend on the plant organ used for prodn. Therefore, we investigated the variability in plant constituents and antigen expression in potato plants under greenhouse and field growth conditions and in leaves compared to tubers. Using potatoes expressing VP60, the only structural capsid protein of the rabbit hemorrhagic disease virus (RHDV), CTB, the non-toxic B subunit (CTB) of the cholera toxin (CTA-CTB5) and the marker protein NPTII (neomycinphosphotransferase) as a model, we compare greenhouse and field prodn. of potato-derived antigens. The influence of the prodn. organ turned out to be transgene specific. In general, yield, plant quality and transgene expression levels in the field were higher than or similar to those obsd. in the greenhouse. The variation (CV) of major plant constituents and the amt. of transgene-encoded protein was not influenced by the higher variation of soil properties obsd. in the field. Amazingly, for specific events, the variability in the model protein concns. was often lower under field than under greenhouse conditions. The changes in gene expression under environmental stress conditions in the field obsd. in another event do not reduce the pos. influence on variability since events like these should excluded from prodn. Hence, it can be concluded that for specific applications, field prodn. of transgenic plants producing pharmaceuticals is superior to greenhouse prodn., even concerning the stability of transgene expression over different years. The authors expect equal or even higher expression levels with lower variability of recombinant pharmaceuticals in the field compared to greenhouse prodn. combined with approx. 10 times higher tuber yield in the field.

Paper 12

Regeneration of multiple shoots from transgenic potato events facilitates the recovery of phenotypically normal lines: assessing a cry9Aa2 gene conferring insect resistance

Author(s)
Meiyalaghan, Sathiyamoorthy; Barrell, Philippa J.; Jacobs, Jeanne M. E.; Conner, Anthony J.

Source
BMC Biotechnology (2011), 11, 93 CODEN: BBMIE6; ISSN: 1472-6750

Abstract
Background: The recovery of high performing transgenic lines in clonal crops is limited by the occurrence of somaclonal variation during the tissue culture phase of transformation. This is usually circumvented by developing large populations of transgenic lines, each derived from the first shoot to regenerate from each transformation event. This study investigates a new strategy of assessing multiple shoots independently regenerated from different transformed cell colonies of potato (Solanum tuberosum L.). Results: A modified cry9Aa2 gene, under the transcriptional control of the CaMV 35S promoter, was transformed into four potato cultivars using Agrobacterium-mediated gene transfer using a nptII gene conferring kanamycin resistance as a selectable marker gene. Following gene transfer, 291 transgenic lines were grown in greenhouse expts. to assess somaclonal variation and resistance to potato tuber moth (PTM), Phthorimaea operculella (Zeller). Independently regenerated lines were recovered from many transformed cell colonies and Southern anal. confirmed whether they were derived from the same transformed cell. Multiple lines regenerated from the same transformed cell exhibited a similar response to PTM, but frequently exhibited a markedly different spectrum of somaclonal variation. Conclusions: A new strategy for the genetic improvement of clonal crops involves the regeneration and evaluation of multiple shoots from each transformation event to facilitate the recovery of phenotypically normal transgenic lines. Most importantly, regenerated lines exhibiting the phenotypic appearance most similar to the parental cultivar are not necessarily derived from the first shoot regenerated from a transformed cell colony, but can frequently be a later regeneration event.

Paper 13

A novel light-dependent selection marker system in plants

Author(s)
Koh, Serry; Kim, Hongsup; Kim, Jinwoo; Goo, Eunhye; Kim, Yun-Jung; Choi, Okhee; Jwa, Nam-Soo; Ma, Jun; Nagamatsu, Tomohisa; Moon, Jae Sun; Hwang, Ingyu

Source
Plant Biotechnology Journal (2011), 9(3), 348-358 CODEN: PBJLAE; ISSN: 1467-7644

Abstract
Photosensitizers are common in nature and play diverse roles as defense compds. and pathogenicity determinants and as important mols. in many biol. processes. Toxoflavin, a photosensitizer produced by Burkholderia glumae, has been implicated as an essential virulence factor causing bacterial rice grain rot. Toxoflavin produces superoxide and H2O2 during redox cycles under oxygen and light, and these reactive oxygen species cause phytotoxic effects. To utilize toxoflavin as a selection agent in plant transformation, we identified a gene, tflA, which encodes a toxoflavin-degrading enzyme in the Paenibacillus polymyxa JH2 strain. TflA was estd. as 24.56 kDa in size based on the amino acid sequence and is similar to a ring-cleavage extradiol dioxygenase in the Exiguobacterium sp. 255-15; however, unlike other extradiol dioxygenases, Mn2+ and dithiothreitol were required for toxoflavin degrdn. by TflA. Here, our results suggested toxoflavin is a photosensitizer and its degrdn. by TflA serves as a light-dependent selection marker system in diverse plant species. We examd. the efficiencies of two different plant selection systems, toxoflavin/tflA and hygromycin/hygromycin phosphotransferase (hpt) in both rice and Arabidopsis. The toxoflavin/tflA selection was more remarkable than hygromycin/hpt selection in the high-d. screening of transgenic Arabidopsis seeds. Based on these results, we propose the toxoflavin/tflA selection system, which is based on the degrdn. of the photosensitizer, provides a new robust non-antibiotic selection marker system for diverse plants.

Paper 14

Transformation methods for obtaining marker-free genetically modified plants

Author(s)
Schaart, Jan G.; Krens, Frans A.; Wolters, Anne-Marie A.; Visser, Richard G. F.

Source
Plant Transformation Technologies (2011), 229-242. Editor(s): Stewart, C. Neal, Jr. Publisher: Wiley-Blackwell, Chichester, UK. CODEN: 69NZGD; ISBN: 978-0-8138-2195-5

Abstract
A review on two different methods for obtaining marker-free genetically modified (GM) plants without the need for genetic segregation. The first method concerns transformation without the use of any selectable marker gene, whereas the second method employs site-specific recombination-mediated excision of the gene used for selection of transgenic plants or tissues. Both methods are particularly suitable for prodn. of marker-free GM plants in vegetatively propagated crops, such as potato and many fruit crops, or in crops with a long-generation time, such as woody species.

Paper 15

Positive-selection and ligation-independent cloning vectors for large scale in Planta expression for plant functional genomics

Author(s)
Oh, Sang-Keun; Kim, Saet-Byul; Yeom, Seon-In; Lee, Hyun-Ah; Choi, Doil

Source
Molecules and Cells (2010), 30(6), 557-562 CODEN: MOCEEK; ISSN: 1016-8478

Abstract
Transient expression is an easy, rapid and powerful technique for producing proteins of interest in plants. Recombinational cloning is highly efficient but has disadvantages, including complicated, time consuming cloning procedures and expensive enzymes for large-scale gene cloning. To overcome these limitations, we developed new ligation-independent cloning (LIC) vectors derived from binary vectors including tobacco mosaic virus (pJL-TRBO), potato virus X (pGR106) and the pBI121 vector-based pMBP1. LIC vectors were modified to enable directional cloning of PCR products without restriction enzyme digestion or ligation reactions. In addn., the ccdB gene, which encodes a potent cell-killing protein, was introduced between the two LIC adapter sites in the pJL-LIC, pGR-LIC, and pMBP-LIC vectors for the efficient selection of recombinant clones. This new vector does not require restriction enzymes, alk. phosphatase, or DNA ligase for cloning. To clone, the three LIC vectors are digested with SnaBI and treated with T4 DNA polymerase, which includes 3' to 5' exonuclease activity in the presence of only one dNTP (dGTP for the inserts and dCTP for the vector). To make recombinants, the vector plasmid and the insert PCR fragment were annealed at room temp. for 20 min prior to transformation into the host. Bacterial transformation was accomplished with 100% efficiency. To validate the new LIC vector systems, they were used to coexpress the Phytophthora AVR and potato resistance (R) genes in N. benthamiana by infiltration of Agrobacterium. Coexpressed AVR and R genes in N. benthamiana induced the typical hypersensitive cell death resulting from in vivo interaction of the two proteins. These LIC vectors could be efficiently used for high-throughput cloning and lab.-scale in planta expression. These vectors could provide a powerful tool for high-throughput transient expression assays for functional genomic studies in plants.

Paper 16

Agrobacterium-mediated Genetic Transformation for Local Cultivars of Potato (Solanum tuberosum L.) Using Marker Genes.

Author(s)
Borna, Rita Sarah; Hoque, M. I.; Sarker, R. H. [Reprint Author]

Source
Plant Tissue Culture + Biotechnology, (DEC 2010) Vol. 20, No. 2, pp. 145-155. ISSN: 1817-3721.

Abstract
Genetic transformation using nodal and internodal segments from three economically important potato (Solanum tuberosum L.) varieties namely, Diamant, Cardinal and Granola was conducted using an Agrobacterium tumefaciens strain LBA4404 harbouring binary plasmid pBI12 containing the GUS and nptII genes. Node and internodal segments were used for direct regeneration as well as regeneration with the intervention of callus. Best responses were obtained for direct regeneration of shoots when the explants were cultured on MS supplemented with 4.0 mg/l BAP +1.0 mg/l IAA, 1.5 mg/l BAP + 0.5 mg/l IAA and 5.0 mg/l BAP +1.0 mg/l IAA in Diamant, Cardinal and Granola, respectively. In Diamant spontaneous in vitro microtuberization was obtained from these proliferated shoots. Further culturing of these in vitro grown green microtubers regenerated a large number of shoots on MS containing 4.0 mg/l BAP +1.0 mg/l IAA. By combining the best treatments, this protocol yielded an average transformation rate of 87% of treared explants. Stable expression of GUS gene was visualized in the various parts of transformed shoots through histochemical assay. Genomic DNA was isolated from transformed shoots and stable integration of the GUS and nptII genes was confirmed by PCR analysis.

Paper 17

Development of a multiplex PCR method for simultaneous detection of diagnostic DNA markers of five disease and pest resistance genes in potato.

Author(s)
Mori, Kazuyuki; Sakamoto, Yu; Mukojima, Nobuhiro; Tamiya, Seiji; Nakao, Takashi; Ishii, Takashige; Hosaka, Kazuyoshi [Reprint Author]

Source
Euphytica, (AUG 2011) Vol. 180, No. 3, pp. 347-355. CODEN: EUPHAA. ISSN: 0014-2336. E-ISSN: 1573-5060.

Abstract
Multiplex PCR is practically a reasonable choice for molecular marker-assisted selection in potato breeding. We had developed and were using a multiplex PCR method for selection of resistance genes to cyst nematode (H1), Potato virus X (Rx1) and late blight (R1 and R2). Since then, more reliable and tightly linked markers for H1 and R2, and a new marker for resistance to Potato virus Y (Ry (chc)) were developed. In this article, all these superior markers, including a positive marker to eliminate PCR-failed samples, were incorporated into one multiplex PCR assay. Using the newly developed multiplex PCR technique, five plants potentially harboring all five resistance genes were selected from 96 hybrid plants approximately 5 h after DNA extraction, which is a third of the operation time compared with separate PCR reactions for each marker.

Paper 18

Cultivation of GMO in Germany: support of monitoring and coexistence issues by WebGIS technology.

Author(s)
Kleppin, Lukas [Reprint Author]; Schmidt, Gunther; Schroeder, Winfried

Source
Environmental Sciences Europe, (2011) Vol. 23, No. 1, pp. Article No.: 4. ISSN: 2190-4715. E-ISSN: 2190-4715.

Abstract
Background: In Germany, apart from the Amflora potato licensed for cultivation since March 2010, Bt-maize MON810 is the only genetically modified organisms (GMO) licensed for commercial cultivation (about 3,000 ha in 2008). Concerns have been raised about potential adverse environmental impacts of the GMO and about potential implications on the coexistence between conventional and genetically modified production. These issues should be considered on a regional base. The objective of this article is to describe how GMO monitoring that is required after risk assessment and GMO release can be complemented by a Web-based geoinformation system (WebGIS). Secondly, it is also described how WebGIS techniques might support coexistence issues with regard to Bt-maize cultivation and conservation areas. Accordingly, on the one hand, the WebGIS should enable access to relevant geodata describing the receiving environment, including information on cultivation patterns and conservation areas containing protected species and habitats. On the other hand, metadata on already established environmental monitoring networks should be provided as well as measurement data of the intended GMO monitoring. Based on this information and based on the functionality provided by the WebGIS, the application helps in detecting possible environmental GMO impacts and in avoiding or identifying coexistence problems.Results: The WebGIS applies Web mapping techniques to generate maps via internet requests and offers additional functionality for analysis, processing and publication of selected geodata. It is based on open source software solely. The developments rely on a combination of the University of Minnesota (UMN) MapServer with the Apache HTTP server, the open source database management systems MySQL and PostgreSQL and the graphical user interface provided by Mapbender. Important information on the number and the location of Bt-maize fields were derived from the GMO location register of BVL. The 'WebGIS GMO Monitoring' provides different tools allowing for the application of basic GIS techniques as, for instance, automatic or interactive zooming, distance measurements or querying attribute information from selected GIS layers. More sophisticated GIS tools were implemented additionally, e. g. a buffer function which enables generating buffers around selected geoobjects like Bt-maize fields. Finally, a function for intersection of different maps was developed. The WebGIS comprises information on the location of all Bt-maize fields in Germany according to the official GMO location register of the Federal Office of Consumer Protection and Food Safety between 2005 and 2008. It facilitates, amongst others, access to geodata of GMO fields and their surroundings and can relate them with additional environmental data on climate, soil, and agricultural patterns. Furthermore, spatial data on the location of flora-fauna-habitats and environmental monitoring sites in the federal state of Brandenburg were integrated. The WebGIS GMO monitoring was implemented according to the concept for an 'Information System for Monitoring GMO' (ISMO) which was designed on behalf of the German Federal Agency for Nature Conservation. ISMO includes hypotheses-based ecological effects of GMO cultivation and suggests checkpoints for GMO monitoring to test whether impacts may be observed in the receiving environment.In contrast to the public GMO register, the WebGIS GMO monitoring enables mapping of GMO fields and provides relevant geodata describing environmental and agricultural conditions in their neighbourhood of the cultivation sites as well as information derived from monitoring sites. On this basis, spatial analyses should be enabled and supported, respectively. Further, the WebGIS GMO monitoring supplements PortalU which, in Germany, is the technical realisation of the Infrastructure for Spatial Information in Europe directive (Directive 2007/2/EC) released by the EU in 2007.Conclusions: The article should have shown how to support and complement GMO monitoring with the help of the WebGIS application. It facilitates co-operation and data access across spatial scales for different users since it is based on internet technologies. The WebGIS improves storage, analysis, management and presentation of spatial data. Apart from the improved flow of information, it supports future long-term GMO monitoring and modelling of the dispersal of transgenic pollen, for instance. Additional information (e. g. data on wind conditions or soil observation sites) provided by the WebGIS will be helpful to determine representative monitoring sites for detecting potential GMO impacts by means of monitoring or modelling. Thus, the WebGIS can also serve as part of an early warning system. In the near future, the integration of locations of all Bt-maize fields in Germany into the WebGIS as a continuous task should be automatised. Additionally, a methodology should be developed to detect maize fields by means of remote sensing data to manage coexistence problems on the basis of actual field patterns.

Paper 19

Development of multiplex and construct specific PCR assay for detection of cry2Ab transgene in genetically modified crops and product.

Author(s)
Kamle Suchitra; Kumar Arvind; Bhatnagar Raj K

Source
GM crops, (2011 Jan-Mar) Vol. 2, No. 1, pp. 74-81. Journal code: 101556751. E-ISSN: 1938-2006. L-ISSN: 1938-1999.

Abstract
An efficient detection system for trait validation and screening of GMOs is a much sought after procedure, which could also help in regulatory compliance. Currently, in India, a number of cry2Ab transgene carrying GM crops are undergoing field trial i.e., MON15985 cotton, Bt rice, Bt okra, Bt corn, Bt brinjal, Bt potato and Bt tomato. In this study, we report a qualitative assay for detection for cry2Ab (326 bp). Further, the amplification compatibility with promoter, p35S (195 bp), terminator, t-nos (180 bp) and marker gene, npt II (215 bp) was also confirmed using Bt cotton event MON15985 as reference material. The detection sensitivity was 0.1% that is far below the requirement of the stringent European Union (EU) regulations of 0.9%. The target DNA when spiked with either MECH-12 (cry1Ac), RR-soya (epsps) or MON-810 (cry1Ab) showed no inhibitory effect on cry2Ab detection. Moreover, the cry2Ab specific transgene construct (1.9 kb) was amplified and its identity confirmed by a nested PCR. Hence, a comprehensive multiplex PCR method for detection of cry2Ab gene in a GM crop/products was established. This is possibly a first report showing concurrent amplification of cry2Ab transgene, promoter, terminator and marker gene.

Paper 20

Post-moratorium EU regulation of generically modified products: trade concerns.

Author(s)
Viju, C.; Yeung, M. T.; Kerr, W. A.

Source
Trade Policy Brief - Canadian Agricultural Trade Policy Research Network (CATPRN) (2011), Number 2011-07, 3 p., 1 refs. Published by: Canadian Policy Research Network (CPRN), Ontario

Abstract
This article discusses the initial reluctance and later approval of the EU for genetically modified products. The new EU regulatory regime of 2003 is now in place and accepting applications for the approval of GM-products. The first product to successfully work its way through the revised EU-level approval process - BASFs Amylopectin (Amflora) potato - received its approval on March 15, 2010 based on an application made in February 2005. Thus, only now can the EU's GM regulatory regime be assessed. Canada and other countries have a clear interest in whether the new EU GM regulatory regime is compliant with the SPS and with the Panel ruling of 2006. Based on the procedures outlined in EU Commission Directives, the new decision criteria appear not to comply with the EU's WTO commitments and are sufficiently cumbersome that they may not be the least trade restricting means of achieving the official policy objectives. The European Food Safety Authority (EFSA) has jurisdiction over the scientific assessment of GMO authorization applications. Its GMO Panel reviews each GMO authorization application on a case by case basis as no GMO is presumed to be safe. The GMO Panel consists of 21 independent experts supported by a number of specialized Working Groups drawing on a pool of more than 40 external experts in fields such as allergenicity, ecology, microbiology, toxicology, plant physiology and molecular genetics. The EFSA can refuse to approve an application to allow a new GM product on a scientific basis. The problem arises, however, in the instance where the EFSA recommends approval on the basis of its scientific assessment. At this point, the approval moves into the political arena and a scientifically acceptable GM product can be denied approval for non-scientific reasons. This runs directly counter to the interpretation of the SPS rules that is taken by Canada and the US and suggests that the EU may also not be in compliance with the Panel ruling in 2006. Thus, the new EU regulatory regime would seem open to a WTO disputes challenge. Of course, the political consequences of such a challenge would have to be carefully weighed.

Paper 21

Optimization of genetic transformation system for potato variety 'Desiree' and obtainment of transgenic lines.

Author(s)
Xin CuiHua; Guo JiangBo; Huang SanWen; Qu DongYu; Xin, C. H.; Guo, J. B.; Huang, S. W.; Qu, D. Y.

Source
China Vegetables (2011), Number 6, pp. 15-21, 16 refs. ISSN: 1000-6346 Published by: Institute of Vegetables and Flowers, Beijing

Abstract
The aim of this work was to optimize the transformation system for potato (Solanum tuberosum L.) variety 'Desiree' and to obtain transgenic potato. Explants derived from potato leaves and stems were used as receptor materials to compare transformation efficiency with different time of pre-culture, Agrobacterium tumefaciens content, duration of infection and the proportion of hormones. The results showed that stem was most suitable for the induction of callus and shoots. Stem explants were pre-cultured for 2 days before infection by A. tumefaciens strain Agl-0 (OD600=0.5, 10 min) and selected on MS20 containing 1.0 mgL-1 zeatin-trans (ZT-t), the highest transformation efficiency was obtained with 80% of callus induction and 70% of differentiation. R1, the gene resistant to potato late blight, was introduced into potato through above improved transformation system. PCR analysis to the kanamycin-resistant transformants revealed that R1 gene was integrated into potato genome. Transfer of R1 gene into potatoes had not any impact on agronomic traits compared to the non-transgenic line (CK) and the most transgenic lines had strong resistance to potato late blight verified by inoculation with isolate 89148-9 (0).

Paper 22

Biotechnology in postharvest research of fruits: prospects and achievements.

Author(s)
Owino, W. O.; Ezura, H.

Source
Stewart Postharvest Review (2011) Volume 7, Number 1, article 4 p., 38 refs. ISSN: 1745-9656 DOI: 10.2212/spr.2011.1.4 Published by: Stewart Postharvest Solutions Ltd, London

Abstract
Purpose of review: This article highlights inroads made by biotechnological approaches in improving the quality and postharvest shelf-life of fruits among various reports in the recent five years. Biotechnological research has fostered new approaches that aid in understanding ripening mechanisms and can be applied in addressing challenges of postharvest losses of fresh fruits. Recent findings: Recent studies have demonstrated that ripening comprises regulatory networks, rather than a pathway. These studies have provided evidence that positive transcriptional regulation for ACS and ACO genes and the potential genetic network involving ethylene and homeotic proteins, including CNR, HB-1 and MADS-box proteins (such as RIN, TAG1 and ALQ/TAGL1), may regulate the fruit ripening process. The RIN and CNR may be good candidates for controlling ripening and prolonging shelf-life in both climacteric and non climacteric fruits than is currently possible via ethylene control alone. Characterisation of a tomato APETALA2 gene, (SlAP2a) transcription factor, demonstrated its negative role in influencing the ripening process by modulating ethylene and carotenoid pigmentation, essential for tomato fruit colour and nutrient quality. The identification of SlAP2a as a negative transcriptional regulator of fruit ripening presents an additional biotechnological tool for modifying the quality and nutritional value of fruit crops. Screening of mature mutagenised tomato plants, combined with extensive fruit metabolic QTL mapping, holds the promise of numerous additional functionally defined loci that will become increasingly accessible as part of expanding genomics resources. The generation and integration of 'omic' data (transcriptome, proteome, metabolome, and phenome), in combination with functional analysis has been useful in pinpointing candidate regulatory genes linked to compositional changes and fruit development. Targeting Induced Local Lesions IN Genomes (TILLING) has been applied in identifying a melon mutant line which exhibits longer fruit maturation, enhanced fruit firmness and delayed fruit yellowing, which are economically important traits for melon fruit breeders. A new and simple vector- and selectable marker-free melon transformation system which bypasses in vitro culture and plant regeneration, has been demonstrated to be a cost efficient system for obtaining positive transgenic plants. Limitations: New approaches are needed that involve more comprehensive models of the biochemical and physiological elements that contribute to fruit 'firmness'. A major constraint in making further

advances in biotechnological control of fruit ripening is the lack of large mutant populations required for gene identification in tomato and other important fruit species. Directions of future research: The discovery of additional transcriptional factors that control the members of ACS and ACS genes demonstrate the interactions between the identified regulatory networks and other genes. Application of reverse genetics approaches such as TILLING, screening of mature mutagenised fruits, combined with extensive fruit metabolic QTL mapping and generation of 'omics' data to narrow the expressional candidate genes to specific subsets of genes that can be further used for biotechnological applications aimed at increasing the sensorial, nutritional values and postharvest shelf-life of fruits. Further, there is an urgent need to develop high-throughput transformation and regeneration protocols for various other fruits which are economically important and where postharvest spoilage is high.

Paper 23

Modification of potato starch granule structure and morphology in planta by expression of starch binding domain fusion proteins.

Author(s)

Huang XingFeng; Huang, X. F.

Source

Modification of potato starch granule structure and morphology in planta by expression of starch binding domain fusion proteins (2010), vi + 140 p. ISBN: 978-90-8585-811-9 Published by: Wageningen Universiteit (Wageningen University), Wageningen

Abstract

This thesis aims to (1) modulate starch in planta by expressing different bacterial proteins fused to a starch binding domain (SBD) from carbohydrate-binding module (CBM) family 20 in potato, (2) explore the possibility of using SBDs from other CBM families for applications in starch bioengineering, and (3) better understand how the expression of heterologous proteins interferes with starch biosynthesis. In this thesis, 2 different microbial enzymes, Escherichia coli glycogen branching enzyme (GlgB) [1,4-alpha-glucan branching enzyme] and Neisseria polysaccharea amylosucrase [sucrose--glucan glucosyltransferase], were chosen to modify starch composition or structure by fusing with an SBD. Furthermore, the GlgB/SBD and amylosucrase/SBD transformants were analysed at the transcriptional level using the POCI array to investigate whether and how the expression of these heterologous genes affect the expression levels of genes involved in the starch biosynthesis pathway. Previous study showed that the expression of an E. coli maltose acetyltransferase (MAT) fused with an SBD resulted in amalgamated starch granules. In Chapter 2, the starch granule morphology at different stages during potato tuber development was analysed to elucidate when and how the deposition of MAT/SBD fusion protein interferes with starch biosynthesis, and the transcriptomic analysis of tubers from the controls and transformants with altered morphology of the granules was performed. Branching degree of amylopectin plays an important role in the physicochemical behaviour of starch. In Chapter 3, a glycogen branching enzyme (glgB) from E. coli fused to an SBD was expressed in both amylose-containing potato background (Kardal) and an amylose-free potato mutant (amf) to increase the amylopectin branching degree of potato starch. The POCI array was used to investigate how the expression of glgB interfered with the transcription level of other genes in the starch biosynthesis pathway. In Chapter 4, an amylosucrase from N. polysaccharea fused to an SBD was introduced in 2 potato genetic backgrounds (wild type and amylose-free mutant) to modify amylose/amylopectin ratio, and thereby, broaden starch applications. Moreover, transcription profiling of genes in an amylosucrase/SBD transformant was analysed using the POCI array. Besides the CBM20, there are 8 CBM families with affinity for starch granules. In Chapter 5, a tandem CBM25 domain of -amylase from Microbacterium aurum was introduced in potato to assess whether it is suitable for applications in starch bioengineering. In Chapter 6, results from all above chapters are discussed. Furthermore, future perspectives on starch modification in planta are discussed.

Paper 24

Amflora: great expectation for GM crops in Europe.

Author(s)
Abdallah, N. A.

Source
GM Crops (2010) Volume 1, Number 3, pp. 109-112 ISSN: 1938-1999 DOI: 10.4161/gmcr.1.3.12398 Published by: Landes Bioscience, Austin

Abstract
Amflora provides a plausible reason for commercially cultivation of GM Crops in Europe. For improving the industrial application of potato, the amylose-less Amflora was genetically engineered to produce only amylopectin component in its starch. Amflora was developed by silencing the expression of the starch synthase enzyme using antisense strategy to eliminate the expression of amylose. Amylopectin is known to be the required starch component for industrial purpose because of its thickening properties, in the contrary amylose component has a gelling property which interfere with the industrial processes and it makes the dissolved potato starch unstable. Therefore, it is requested to separate the two components. Separating amylopectin and amylose in potato starch would require energy and water consumptions and therefore be uneconomical.

Paper 25

Accumulation of multiple-repeat starch-binding domains (SBD2-SBD5) does not reduce amylose content of potato starch granules.

Author(s)
Firouzabadi, F. N.; Vincken, J. P.; Ji Qin; Suurs, L. C. J. M.; Buleon, A.; Visser, R. G. F.; Ji, Q.

Source
Planta (2007) Volume 225, Number 4, pp. 919-933, 30 refs. ISSN: 0032-0935 Published by: Springer Berlin, Heidelberg

Abstract
This study investigates whether it is possible to produce an amylose-free potato starch by displacing the amylose enzyme, granule-bound starch synthase I (GBSSI), from the starch granule by engineered, high-affinity, multiple-repeat family 20 starch-binding domains (SBD2, SBD3, SBD4, and SBD5). The constructs were introduced in the amylose-containing potato cultivar (cv. Kardal), and the starches of the resulting transformants were compared with those of SBD2-expressing amylose-free (amf) potato clones. It is shown that a correctly sized protein accumulated in the starch granules of the various transformants. The amount of SBD accumulated in starch increased progressively from SBD to SBD3; however, it seemed as if less SBD4 and SBD5 was accumulated. A reduction in amylose content was not achieved in any of the transformants. However, it is shown that SBDn expression can affect physical processes underlying granule assembly, in both genetic potato backgrounds, without altering the primary structure of the constituent starch polymers and the granule melting temperature. Granule size distribution of the starches obtained from transgenic Kardal plants were similar to those from untransformed controls, irrespective of the amount of SBDn accumulated. In the amf background, granule size is severely affected. In both the Kardal and amf background, apparently normal oval-shaped starch granules were composed of multiple smaller ones, as evidenced from the many 'Maltese crosses' within these granules. The results are discussed in terms of different binding modes of SBD.

Paper 26

Potato, a valuable tuber vegetable
La pomme de terre: legume et tubercule de valeur Pommes de terre

Author(s)
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Source
Cahiers de nutrition et de dietetique, (2010), 45(HS1), S60-S67, 14 refs. ISSN: 0007-9960 CODEN: CNDQA8

Abstract
Bien que largement consommee depuis longtemps en France, en Europe et dans le monde, la pomme de terre est parfois encore mal consideree parce que mal connue du monde de la nutrition. Pourtant elle s'inscrit parfaitement dans les recommandations nationales et internationales visant a maintenir ou accroitre les apports en glucides, et notamment en glucides complexes. A ce titre, mais pas seulement, elle peut repondre aux besoins de l'ensemble de la population mais aussi a des populations specifiques avec des situations physiologiques particulieres: enfants, femmes enceintes, personnes agees, sportifs. Sa specificite est en effet, non seulement d'etre une source de glucides complexes, mais aussi d'avoir les caracteristiques nutritionnelles de la categorie des legumes. Elle a donc cette double capacite dans la construction d'un repas d'etre a la fois ' garniture ' de legumes et source de glucides: elle complete parfaitement les aliments sources de proteines animales pour contribuer a l'equilibre alimentaire du repas. Ainsi s'est developpee une culture culinaire particulierement abondante autour de la pomme de terre. Il convient cependant d'ameliorer certains usages culinaires et certaines caracteristiques issues de la transformation. Les industries agroalimentaires doivent s'y employer, c'est leur role.

Paper 27

Influence of cooking processes on nutritional composition and potato digestibility; Influence des procedes de cuisson sur la composition nutritionnelle et la digestibilite de la pomme de terre

Author(s)
MOREIRA Tracy S.; WOLEVER Thomas M. S.; DAVIGNON Jean; YADA Rickey FRUCHART Jean-Charlesa (limin.)

Source
Cahiers de nutrition et de dietetique, (2010), 45(HS1), S37-S43, 25 refs. ISSN: 0007-9960 CODEN: CNDQA8

Abstract
Les pommes de terre constituent un choix alimentaire sain, car elles sont riches en nutriments et pauvres en calories ; elles renferment de grandes quantites de nutriments utiles, dont le potassium et l'acide ascorbique. Neanmoins, la consommation de pommes de terre continue a decroitre dans plusieurs pays, peut-etre en raison de leur index glycemique (IG) pretendument eleve. L'IG est un systeme permettant de classer les aliments en fonction de leur potentiel a elever la glycemie. Les regimes a IG eleve ont ete associes a un risque accru de developper du diabete de type 2 et des maladies cardiovasculaires. Un facteur important dans la determination de l'IG des aliments est la vitesse de digestion de l'amidon. L'amidon des pommes de terre crues est tres resistant a la digestion. Cependant, lorsqu'on le cuit, l'amidon se gelatinise et peut rapidement etre degrade par les enzymes digestifs. Inversement, lorsqu'il refroidit, l'amidon des pommes de terre subit le processus de retrogradation, qui le rend a nouveau resistant a la digestion. La mesure de l'IG de diverses varietes de pommes de terre revele d'importantes variations: de seulement 23 jusqu'a 111. Plusieurs facteurs affectent l'IG des pommes de terre, dont la variete, la methode de cuisson, la maturite et le procede de transformation. Des pommes de terre venant d'etre cuites ont un IG plus eleve que des pommes de terre cuites puis refroidies avant d'etre consommees, mais l'amplitude de ces effets varie en fonction des differentes varietes de pommes de terre. Le degre de ramification de l'amylopectine est moindre chez les pommes de terre nouvelles, qui ont un IG plus faible que les pommes de terre recoltees a maturite. Les pommes de terre peuvent etre transformees en differents produits alimentaires, comme les flocons de pommes de terre, les frites, les chips et les gnocchis, ce qui influe aussi sur leur IG. Il est necessaire de poursuivre les recherches pour etudier les effets des divers procedes de cuisson sur l'IG des pommes de terre et produits a base de pommes de terre. .

Paper 28

Cloning, recombinant expression and characterization of a new glucoamylase gene from aureobasidium pullulans NRRL 12974 and its potential application in raw potato starch degradation.

Author(s)
Li, Haifeng; Sun, Wei; Gao, Yunyun; Wu, Yudan; Huang, Lifeng; Huang, Eric Zhijian; Wang, Anming; Yin, Xiaopu; Wang, Qiuyan; Xie, Tian (correspondence); Zeng, Zhaowu

Source
African Journal of Biotechnology, (17 August 2011) Vol. 10, No. 45, pp. 9122-9131. Refs: 27 ISSN: 1684-5315

Abstract
A new amylase gene APGA1 was cloned from Aureobasidium pullulans NRRL 12974 and expressed in Pichia pastoris. This is the first report on cloning and expression of amylolytic gene from the industrially important microorganism A. pullulans. The purified recombinant protein with MW of 66 kDa and specific activity of 298.02 Umg-1 protein was verified as a glucoamylase by its hydrolytic mode. This recombinant glucoamylase with optimal pH of 4.5, and temperature of 60.degree.C, showed good hydrolytic activity against raw potato starch. At 60.degree.C, 83.1% of raw potato starch slurry (150 gl-1) was hydrolysed into glucose by 0.1 U mg-1 starch purified recombinant glucoamylase in less than 2.5 h. This is the highest raw starch hydrolysis efficiency report about recombinant fungal glucoamylase. This useful property indicated that this glucoamylase may find important applications in the starch saccharification industry and in bioethanol production.

Paper 29

Phosphate esters in amylopectin clusters of potato tuber starch.

Author(s)
Wikman, Jeanette (correspondence)

Source
International Journal of Biological Macromolecules, (01 May 2011) Vol. 48, No. 4, pp. 639-649. Refs: 53 ISSN: 0141-8130 CODEN: IJBMDR

Abstract
Starch phosphate is important in starch metabolism and in order to deduce its location and structural effects in clusters and building blocks of amylopectin, these were isolated from a normal potato (WT) and two starches with antisense suppressed glucan water dikinase (asGWD) activity and starch branching enzyme (asSBE) activity possessing suppressed and increased phosphate contents, respectively. Neutral N-chains and phosphorylated P-chains of the amylopectin macromolecules were similar in WT and asGWD, whereas asSBE possessed considerably longer P-chains. Cluster .beta.-limit dextrins were isolated by .alpha.-amylase treatment and successive .beta.-amylolysis. Cluster sizes were generally smaller in asSBE. The building block composition of neutral N-clusters were very similar in WT and asGWD, while asSBE was different, containing less blocks with degree of polymerization (DP) > 14. Phosphate content of the P-clusters of WT and asGWD was rather similar, while asSBE contained highly phosphorylated P-clusters with proportionally more P-chains and a low degree of branching. The average chain lengths of the P-clusters were, however, similar in all samples. Our data demonstrate only minor effect on the cluster structure in relation to phosphate deposition suggesting conserved reaction patterns of starch phosphorylation. Models are suggested to account for the principle structural and functional effects of starch phosphate esters.