Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.01

DEVELOPMENT OF TRANSGENIC WHEAT PLANTS FREE OF HERBICIDE MARKER GENES AND PLASMID BACKBONE SEQUENCES THROUGH "CLEAN GENE" TECHNOLOGY AND POSITIVE SELECTION

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Pmi, bar, phosphomannose isomerase, marker gene, clean gene

The development of novel biotechnologies for direct gene transfer has accelerated plant improvement programs, especially for monocotyledonus species such as wheat that have been traditionally recalcitrant to transformation. Identification of transgenic plants in important crop species, which have relatively low transformation efficiencies, requires the use of selectable marker genes to minimize regeneration of non-transformed plants.

The most frequently used selectable markers are genes conferring resistance to herbicides or antibiotics. Moreover the routine generation of transgenic plants involves transformation with foreign DNA carried on plasmids, and causes the integration of vector backbone sequences into the genome along with any transgenes. Integrated vector DNA has been detected in transgenic plants generated by *Agrobacterium*-mediated transformation and direct delivery procedures such as particle bombardment. However, in both plant and animal systems, vector backbone sequences may exert undesirable negative effects on transgene or endogenous genes expression and can promote transgene rearrangements. The use of minimal expression vectors comprising linear DNA fragments containing only promoter, transgene coding region and terminator/polyadenylation sites shows significant advantages in reducing these events. In the present work durum wheat transformation of cv. Svevo was carried out in parallel experiments (simultaneously) by using either whole plasmids containing suitable gene constructs, or the minimal gene cassettes, which were linear DNA fragments lacking vector sequences excised from the plasmid. Trasformation experiments were carried out using as target genes two wheat sequences encoding the Dx5 and Dy10 HMW glutenin subunits and the *phosphomannose isomerase (pmi)* gene as the selectable gene and mannose as the selective agent. The integration and expression of genes in T_1 generation was confirmed by PCR analysis with specific primers and chlorophenol red assay.

The average biolistic transformation frequencies obtained using the plasmids and linear DNA were approximately the same: 1.14% in bombardment with entire plasmids and 1.50% with linear DNA.

An efficient selection method was established in durum wheat transformation, without the use of herbicide or antibiotic resistance genes.

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ISBN **88-900622-6-6**

Poster Abstract - H.02

EU REGULATION ON TRACEABILITY AND LABELING OF GENETICALLY MODIFIED FOOD AND FEED: PERCEPTIONS OF STAKEHOLDERS AND CITIZENS

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decision-making process, Focus groups method, GMOs, labels, risk perception

With the aim of perceiving reactions, opinions, attitudes and feelings of the various actors of the agro-food chain involved in the GMO debate, we carried out a qualitative research in the Trento Autonomous Province. UE regulation on traceability and labeling of GM food and feed (1829/2003/CE a 1830/2003CE) was the topic of the analysis. The final goal was providing local institutions with guidelines for managing the policy on food and feed traceability on the basis of the Informed Choice Principle (Reg. 178/2002/EU, art. 8 and 10). Besides, we wanted to assay the suitability of a deliberative democracy approach in the view of an improved public perception analysis. In 2004, two focus groups with selected stakeholders, and two public meetings with citizens were organized. Firstly, we analyzed the position of the stakeholders, i.e. people who work, study or directly deal with agrobiotechnologies. We selected delegates of public institutions, of private and public analysis laboratories, of farmers, breeders and industry associations and trade-unions, and representatives of consumer and environmental associations. Then, we evaluated citizens' perceptions and points of view on GM food and feed consume, and their trust in the institutions. The discussions were organized on the basis of a public debate and a dialogue with experts of the question. A DVD with a short documentary of the happenings was produced. This was presented during a final meeting, and given to the participants as a gift for their cooperation.

Our research confirmed a general non-acceptance of the GMO products. Worth stressing, stakeholders admitted that their choice was based on market motivations, while citizens expressed a concern based on a complex combination of emotional, political, cultural and ethical aspects. Stakeholders showed familiarity with the matter. Citizens, at the opposite, resulted dazed and worried on their own competence in label understanding. Both groups judged the EU regulation on traceability and labeling a promising tool toward an enhanced defense of the consumer's right. Both groups, however, revealed a notable diffidence and doubts on the Authorities' effectiveness in managing the control activity. Stakeholders faced the debate whit a *problem solving* approach and expressly focused on the technical questions concerning the proper application of the UE regulation. Conversely, the lay-public tended continuously to diverge the discussion from the principal theme of the debate (UE regulation on food labeling) and to direct it to wide issues concerning the whole humanity (i.e. word famine, biodiversity defense, multinational resources exploitation, long term consequences of GMO spread, etc). Moreover, they shoved a strong feeling of *outrage* against the GM technology applications, and a marked need of participation in the decision-making process (Martinelli, 2004, NDA, 3:69-76). Finally, stakeholders and citizens expressed a notable feeling of incommunicability with the scientific community. We believe that a more aware attitude of the scientists on the risk management and communication strategies would empower their role in the public decision-making process on biotechnologies.

This research was supported by the Autonomous Province of Trento, Project *OSSERVA3*. Authors thank Elena Collavin for her contribution in the research, and stakeholders of the Trento Province, and citizens of Rovereto and Levico Terme for their participation.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
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ISBN **88-900622-6-6**

Poster Abstract - H.03

DEFINITION OF AN ANALYTICAL METHOD TO QUANTIFY GM SOYBEAN CONTAMINATION IN FEED

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Real-time PCR, plasmid, endogenous gene, feed, soybean contamination

Italian livestock system can not provide a convenient soybean supply to satisfy internal animal feed need. Thus, over the 95% of the soybean employed in the formulation of complementary and concentrated feed in Italy is imported from countries where GMO cultivation is allowed. As a consequence, soybean is often present as accidental contamination in feed, and often, such component results genetically modified. At the present, however, no analytical solution for quantifying soybean contamination entity is published. In the aim of giving our contribution to solve this problem, we developed an analytical method based on Real Time PCR. Firstly, in the view of settling up the method, we exploited a single component feed. Corn meal was chosen, being the major energy source in feed. As calibrator for the standard curves, we constructed four pGEM-t plasmids (Promega), each one containing a single copy of soybean (*lectin*) and corn (alcohol dehydrogenase *Adh1*, high mobility group protein *hmga*, invertase 1 *inv*1 and *zein*, respectively) endogenous genes. The Real-time quantification experiments were carried out on samples prepared by mixing together defined amounts of corn meal with soybean RR powder certified material (Fluka). In this strategy, the percentage of soybean contamination can be calculated from the ratio between the copy numbers of the soybean and corn endogenous genes, on the basis of the relative haploid genome sizes. Besides, the quantification of the transgenic component percentage on the total sample can be estimated. For each plasmid, method accuracy and precision were evaluated. Further assays are in progress where feed samples at more complex composition are analysed.

Present research is supported by the Autonomous Province of Trento (Project OSSERVA3).

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.04

VALIDATION OF REAL-TIME PCR METHODS FOR GRAPEVINE DETECTION AND IDENTIFICATION: A PRACTICAL APPROACH

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Real-time PCR, endogenous gene, GMO detection, Vitis vinifera, validation

Genetic engineering of commercial crops has progressed very rapidly in the last decades and recently genetic modification of grape has been taken into consideration as a powerful tool to improve quality of vine varieties and their derived products. In view of the scientific and technical results already achieved and expected in the near future, European Union considered the necessity to introduce specific provisions for risk assessment, labelling and monitoring of genetically modified (GM) vine in the legislation and the marketing of material for the vegetative propagation of the vine (Council Directive 2002/11/EC) and implemented a comprehensive GM food and feed legislation (Regulation EC 1829/2003). The enforcement of this legislative framework entails the development, validation and application of analytical methods for the specific detection and quantification of transformation events of grape varieties. Real-time PCR is currently the most powerful technique for the quantification of specific nuclei acid sequence. As for grapevine, however, this technique is not a routine yet and needs to be improved. Being embarked in projects on grape molecular breeding and GMO detection, we developed a protocol for standardizing and optimizing grape Real-time PCR identifying a valuable endogenous gene to be applied as genotype referee and gene copy number standard. Finally, we developed a practical approach for the validation of our technique, and provided a detailed protocol for the evaluation of detection and quantification limits, linear dynamic range, precision and trueness, specificity and robustness.

Research supported by Italian Ministry of Health (Project IZS UM 18/2002, National Coordinator C. Rondini) and by the Autonomous Province of Trento (Project OSSERVA3).

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.05

GENOMIC INSTABILITY IN *NICOTIANA* TRANSGENIC PLANTS DETECTED BY AFLP ANALYSIS

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Nicotiana langsdorffii, Gr receptor, AFLP analysis

A relevant part of the discussion on genetically modified plants (GMP) focuses on the possible consequences due to the insertion and expression of one or more foreign genes in the plant genome. Moreover, controlled transgene integration into a specific and pre-determined site remains to be achieved, in spite of several gene targeting approaches already developed (Kumar and Fladung, 2001).

The transformation of *Nicotiana langsdorffii* plants with a binary *Agrobacterium* vector containing the *gr* gene, coding for the rat glucocorticoid receptor under the constitutive viral promoter CaMV35S (Irdani et al. 1998), produced different primary transformants showing alterations in the morphology and development with respect to the wild type. A detailed analysis of four different transgenic *gr* plants, maintained in *in vitro* culture over a period of 8-10 years, allowed us to detect differences in the copy number of transgenes but sequence homology of the transgene insertion sites. Moreover, in order to investigate the possible perturbations due to the transformation process, AFLP (Vos et al. 1995) and MSAP (Reyna-Lopez et al. 1997) analyses were carried out both in transgenic and untransformed control plants. Between and within groups genetic distances were established to discriminate differences derived from somaclonal variation effects. Finally, analyses of MSAP profiles suggested that qualitative differences in DNA methylation patterns could be attributed to the insertion of *gr* in the genome of *Nicotiana*.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
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ISBN **88-900622-6-6**

Poster Abstract - H.06

DEVELOPMENT OF A NEW ANALYTICAL TOOL FOR TRACEABILITY OF TRANSGENES IN GENETICALLY MODIFIED PLANTS

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GMO, traceability, DNA-based sensors

Analytical techniques to track transgenes in GMO are essential both for the production and trade of modified crops in the view of the adoption of different new regulations by Government Institutions. A great effort has been devoted to the development of new devices for the detection of transgene sequences in the environment and in the food chain. In particular, PCR and, more recently, real time PCR are widely used to amplify target DNA fragments corresponding to marker genes, promoter and terminator sequences or portions of genes of interest and transgene-host integration junctions (Hernandez et al. 2003; 2005; Taverniers et al. 2004). However PCR-based techniques being prone to false positive/negative results are still time consuming, and require the sequencing of the amplified products. Recently, we have demonstrated the ability of different DNA-based sensors to detect PCR-amplified sequences complementary to part of CaMV35S viral promoter and present in transgenic *Nicotiana* plants (Minunni et al. 2001; Giakoumaki et al. 2003).

Here we present a method based on piezoelectric sensor for direct detection of specific sequences in the whole genomic transgenic plant DNA, suggesting that biosensors may represent an interesting and alternative candidate analytical tool for GMO traceability (Minunni et al., in press).

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.07

AN ALTERNATIVE TO ANTIBIOTICS FOR ALFALFA GENETIC ENGINEERING

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chloroplast, gabaculine, GSA-AT, Medicago sativa, transgenic plants

Concern over the presence of antibiotic resistance genes in genetically engineered plants has been expressed because of the risk that these genes are horizontally transmitted to pathogenic bacteria that become resistant to antibiotics. Although this risks has been judged to be very low, the scientific community has been stimulated to develop alternative selection systems, and several are available.
Gabaculine (3-amino-2,3-dihydrobenzoic acid) is toxic to a range of plants through a potent inhibition of chlorophyll synthesis via binding to the enzyme glutamate 1-semialdehyde aminotransferase (*GSA-AT*). In plants, this enzyme catalyses the conversion of glutamate-1-semialdehyde into aminolaevulinic acid, a step in the synthesis of tetrapyrrole compounds, including chlorophyll. Gabaculine has been used as a selecting substance for tobacco transformation (Gough et al. 2001). The gene conferring gabaculine resistance to tobacco encodes a mutant, gabaculine-insensitive *GSA-AT* form from *Synechococcus*. The coding sequence of the *hem*L gene with a chloroplast transit peptide (kindly provided by K. C. Gough) was introduced into the T-DNA of the pPZP201BK binary vector, along with the conventional selection marker gene for alfalfa, *Npt*II, conferring kanamycin resistance, in order to directly compare the efficiency of the two selection systems. Both genes were placed under the control of the dual CaMV*35S* promoter and the *Nos* terminator. This vector was introduced in the *Agrobacterium tumefaciens* strain LBA4404 for a transformation experiment using alfalfa (genotype Regen-SY1) leaf explants. Half of the *Agrobacterium*- treated leaf explants were placed on gabaculine selection (25 and 50 μM, previously shown to completely inhibit regeneration) and half on kanamycin (25 mg/l) selection. Sixty six % of the leaf explants produced at least one green somatic embryo on gabaculine selection *vs* 54% on kanamycin. In a functional test of marker gene expression, 78 % (95 in 121) of the embryos obtained with gabaculine selection regenerated again in a second regeneration cycle on gabaculine, whereas only 33% (32 in 94) of the embryos obtained with kanamycin regenerated again on kanamycin. PCR with primers specific for the bacterial *GSA-AT* demonstrated that the regenerated plants contained the gene. The higher efficiency of gabaculine vs kanamycin selection in this first experiment is probably due to the mechanism of action of the enzymes encoded for by the two genes: *Npt*II detoxifies the selective substance (kanamycin), whereas the mutant *GSA-AT* substitutes for the gabaculine-sensitive plant *GSA-AT*; therefore, gabaculine in the culture medium is not depleted and this would reduce the chance of non-transgenic plant cells to give rise to somatic embryos. Safety of *GSA-AT* for animal or human consumption has not yet been directly tested, but this enzyme is found in many organisms, including plants, and *Arabidopsis thaliana GSA-AT* is 69 % identical to the bacterial enzyme used in this experiment. Gabaculine-resistant *GSA-AT* could be an useful tool to develop more acceptable genetically engineered alfalfa varieties and may be applicable to a variety of crop species.

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Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.08

ANALYSIS OF GENE FLOW FROM CROP TO WILD FORMS IN LETTUCE AND CHICORY

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molecular marker, introgression, gene flow, Chicorium intybus, Lactuca serriola

Transgenic plants have a high potential for improving both agriculture and the environment. Their introduction, however, has led to serious public concern about the accompanying risks, amongst others, the unpredictable consequences for the natural environment. Chicory and lettuce are two important horticultural species, belonging to the Compositae family. The biotechnological approach could be solution of choice to overcome some problems related to their cultivation at present not affordable with conventional techniques. The introduction in chicory of male sterility genes to obtain uniform F1 hybrids and in lettuce of resistance genes (es chitinase) could represent the proper solution at some of that problems. Despite of the agricultural practices are rather similar for both species the reproductive biology is quite different since lettuce is mainly self-crossing whereas chicory is out-crossing. We have studied the degree of out-crossing under field circumstances in *C. intybus* and *L. serriola* by using molecular marker (SSAP and SSR). This work was performed for accessing the safety of introduction of genetically modified chicory and lettuce by providing the important elements of establishing the risks of escape and its consequences for the natural environment. We report the final remarks of the research carried out on three years in the frame of EU project Angel "Analysis of gene flow from crop to wild forms in lettuce and chicory and its population-ecological consequences in the context of GM-crop biosafety".

The populations of lettuce sampled across North Italy and Switzerland were genetically different, and some SSR alleles were specific and diagnostic for some populations. The populations were highly homozygous at most loci, with the significant exception of one population growing at 200 m of altitude. The results indicate that the gene flow between populations by pollen is very limited or absent and also inside the populations the paternity analysis confirmed the self pollination of the species. The gene flow was restricted to seed dispersal and, combining the molecular profile of plants with their position in the field, it was possible to analyze genotype distribution. The limit of seed dispersal was estimated at 20 m, since genotypes were randomly distributed over this distance.

The molecular profile of wild chicory populations was analyzed to determine genetic parameters. Fis and Fst values were calculated and the results pointed out that the populations were in Hardy–Weinberg equilibrium and the allelic distribution was different across populations. No simple correlation was found between genetic and geographic distance of populations. The gene flow inside populations was estimated by paternity analysis taking in account distance between plants. It was not possible to draw a general conclusion because results were strictly related with the peculiar conditions of each location. In general pollination was in inverse relation with distance, possibly for mechanisms preventing crossing between relatives. This conclusion is supported by seed dispersal that is very limited in this species. In our conditions the maximum distance of out-crossing was estimated around 60 m.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
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ISBN **88-900622-6-6**

Poster Abstract - H.09

FACTORS AND TIME THAT CARACTERIZE THE APPROVATION OF TRANSGENIC PLANTS IN USA AND EUROPE

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GMO, field testing, commercial release, access to market, US regulations

Genetically modified organisms (GMO), specifically crops, are constantly increasing their international market share, both in terms of seeds, fields grown and food and feed produced; attention to them has escalated in sight of the resumption of approvals in Europe. While a moratorium has been in place for several years in Europe and more stringently in Italy, research and development of new genetically modified plants have never entirely ceased in Europe and in Italy as well. If research is ever to turn into commercial deployment, it becomes important to understand which GM-specific factors drive, accelerate or slow approvals of GMOs; such factors may be measured as the time that elapses between an application for approval (or even for field testing) and the final outcome. Also, to identify the context in which Italian GMOs may compete, it may be as important to ascertain the trends in GM plants released for field trials, considering for example which species, which modifications have been tested in the recent years. Europe has resumed only recently approvals of GMOs; therefore it is difficult to use European data for this purpose. In the US, on the other hand, all data concerning approved, withdrawn and pending applications for field testing and commercial release of GM plants are publicly available since the 1985. Therefore, we have decided to conduct our analysis on US data first, and then compare the results with available European data.

Specifically, by using univariate and multivariate statistics, we have identified key factors, taking time into account, for rapid versus slow approval of GM plants (from first field testing to market release) in the database maintained by the Biotechnologies Regulation Service (BRS), an office within the US Deparment of Agriculture's APHIS (Animal and Plant Health and Inspection Service) which is responsible for apmonitoring of field trials and commercial releases.

Also, we have identified different, well defined time periods, characterized by different GM events brought to field testing, Variables scrutinized included the species modified, the institution that has requested the approval, the genotypic and phenotypic characteristics and the dates of petition (notification) and granting of approval. We have then compared these results with existing European data in order to bring to light the differences between the factors that may lead to approval of the events in the two analyzed systems.

Although US data cannot be extrapolated to the European Union without caution, our analysis provides insights into which crops and types of modification may be marketed more readily. Also, GM plant developers may understand the current scenario in terms of plants brought to testing (and eventually to market) in the US by different institutions, within a historical perspective.

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Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.10

ETHICAL AND BIOETHICAL ASPECTS OF BIOTECHNOLOGIES

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biotechnology, ethical and bioethical aspects, case study

During some working sessions of the project *Ecogenetic.com* ("*Eco-friendly" genes: from the scientific research to the risk management, ethical issues and communication*), we have followed a case-studies based approach to analyze and discuss ethical and bioethical questions related to some biotechnological applications. The new discipline of bioethics tends to identify, analyze and discuss some ethical aspects of the life sciences, biology, biotechnology and biomedicine.

The analysis of a case can show the different ethical and social perceptions about biotechnological innovations, from a general and a specific point of view.

In this paper we will adopt the same methodology to examine a specific case study for identifying the main questions and the most relevant scientific, ethical, legal and social aspects that guide the discussion. In this way, we can better discuss and manage political and legal dimensions involved into these practices.

The present research is supported by the Autonomous Province of Trento – Project "Ecogenetic.com".

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.11

DETECTION OF TRANSGENE IN WHEAT FLOUR AND DERIVED BREAD AFTER FOOD PROCESSING

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GMO detection, durum wheat, multiplex PCR

According to the European Commission Regulation (E. C. R.) 1830/2003 of 22/09/2003, the limit of Genetically Modified Organism (GMO) derived material accidentally present in a foodstuff, without the necessity of labelling it, is 0.9% w/w for each ingredient. Therefore it is necessary to have an analytical and reliable methods to detect GMO in foodstuffs.

This paper describes the use of Polimerase Chain Reaction (PCR) techniques to evaluate the possibility of amplifying the GM DNA at two stages of food production, using as experimental model a GM durum wheat carrying the GUS (β–glucuronidase) reporter gene. In particular two matrixes prepared in the lab were used: the raw material (wheat flour) containing only traces of GM wheat flour and the derived foodstuff (bread) produced in laboratory using the same wheat flour. The aim of this work is to develop a protocol able to detect the GM DNA via PCR amplification capable of measuring below the E. C. R. limit of 0.9% w/w after food processing.

In qualitative PCR analysis, the detection sensitivities found were 0.05% w/w and 0.5% w/w for flour and bread respectively.

Additionally a multiplex PCR protocol was optimised to simultaneously detect three genes, two internal controls in addition to the transgene. The internal controls are: I) a chloroplast gene to identify a vegetal matrix, II) a gene specific to wheat to evaluate the DNA quality; III) the transgene specific to the GM wheat line.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.12

DEVELOPMENT OF A REAL-TIME PCR BASED METHOD FOR IDENTIFICATION OF GENETICALLY MODIFIED WHEAT WITH LUX PRIMERS

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Real-Time, LUX primer, GMO, wheat

Current E.U. legislation limits for the accidental presence of Genetically Modified Organism (GMO) derived products in foodstuff is 0.9 % w/w. To quantitatively determine the level of GMO derived products found in foodstuff, the most sensitive method currently available is Real-Time Polymerase Chain Reaction (PCR). All quantitative PCR (qPCR) strategies involve using interchelating dyes or hybridizing probes.

Common qPCR probes used are TaqMan probes, molecular beacons, FRET probes and Scorpion probes. A new innovation of the hybridizing probe method are the Light Upon extension (LUX) primers from Invitrogen (www.invitrogen.com). This technique is very sensitive and requires a labelled and unlabelled primer without the addition of an internal probe of the amplified product. This use of only two primers without the necessity of a probe allows a greater choice in selecting the amplicon for the sequence of interest.

One critical point in the development of quantitative PCR methods is DNA extraction method, different sources may require different extraction protocols. A modified CTAB protocol for the extraction of DNA from wheat has proven effective for good DNA yields and quality.

Accurate quantification of source DNA for amplification is also critical. We have found that quantification of DNA is best performed with appropriate standards on agarose electrophoresis gels, confirmed by spectrophotometry and analytical PCR to be the most reliable.

As no standards for the detection of GMO wheat flour are currently available on the market, it is necessary preparing standards containing mixtures of control and GM wheat flour.

The aim of this work is to develop a quantitative method for detecting the presence of GMO derived products in durum wheat with Real-Time PCR in combination with LUX primers. Critical points of the method were identified and modified in order to maximize the sensitively of the protocol.

Results from the Real-Time PCR experiments shows the high degree of sensitivity for the derived protocol while accurate reference standards or alternative systems are required.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.13

VIABILITY OF DNA TEMPLATE FROM PROCESSED TOMATO FOR THE IDENTIFICATION OF GENETICALLY MODIFIED DNA TRAITS AND SUBSEQUENT DEVELOPMENT OF A MULTIPLEX PCR SYSTEM

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Genetically Modified (GM), tomato, multiplex PCR

According to current European legislation, processed food products containing greater than 0.9% w/w genetically modified derived ingredients must be labelled as GMO. It is therefore important that a system be developed to identify and quantify GMO products as well as understand the limits of such assays.

In the present work the limits in detecting DNA from genetically modified organisms was investigated using genetically modified tomato, transformed with the SGP-CP gene derived from cucumber mosaic virus (patent number: US5959181, inventor: Cellini F. and Grieco P.D.). Commercial tomato crops undergo various industrial treatments, both thermal and chemical, which increase the stability of the final foodstuff product but make it very difficult to obtain good quality template DNA for amplification. In fact available techniques used to detect transgenes via PCR are not reliable or repeatable when DNA is prepared the final processed product.

The aim of this work was to establish the limits to detecting the transgene after tomato processing. To determine this limit the food matrix was subjected to thermal treatment at different temperatures (60, 110, and 120°C) for different periods of time (3, 10, 20, 30, 60, and 120 minutes). DNA from the resulting food products was then extracted and subjected to PCR.

The results show that only DNA extracted from material treated to temperatures or 120°C for 30 minutes contained viable target template for amplification. This demonstrates that the temperature as well as time of incubation greatly influences the recovery of good quality template DNA.

In addition a multiplex PCR was developed for processed tomato sauce prepared from GM fruit which allows the simultaneous identification of the transgene and internal controls. The three target genes for amplification were: 1) a chloroplast specific DNA trait; 2) an endogenous tomato specific gene (PDOLL); 3) the 35S CaMV promoter specific for transgenes.

Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.14

PRODUCTION OF MARKER-FREE WHEAT (*TRITICUM AESTIVUM*) PLANTS TRANSFORMED BY *AGROBACTERIUM*

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wheat transformation, marker-free, Cre/loxP

Wheat (*Triticum aestivum*) is an important food crop, because of both the nutritional value of its seeds and the unique technological properties of flours prepared from those seeds.

Plant genetic transformation has become an important tool for functional genomics and as an adjunct to conventional breeding programmes.

Removal of a selectable marker gene from genetically modified crop alleviates the risk of its release into the environment and hastens the public acceptance of genetically modified crops, it is therefore desiderable to generate marker-free transgenic wheat plants.

Recently, chemical-inducible Cre/*loxP* DNA recombination system (CLX) (1) have emerged and seem to provide a highly reliable method to generate marker-free transgenic *Arabidopsis* plants after a single transformation.

Here we report the production of marker-free transgenic wheat using CLX. We have used *Agrobacterium* strain AGL1 harbouring pX6-GFP , which contains the CLX system, *nptII* and *GFP*. Untill now we have obtained transgenic lines (cv. Bobwhite) with a transformation frequency of 2.3% demonstrated by marker-gene expression and molecular analysis.

Seeds from transgenic lines have been used to propagate the material. We are analysing T_1 plants for the presence of the transgene by PCR and Paromomicyn leaves spray. Furthermore we are performing chemical-induction tests on leaf protoplasts from these lines in order to verify the functionality of the system.

The aim of this project is to obtain complete marker free plants expressing GFP.

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Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - H.15

GENE-ESCAPE STUDIES ON TOMATO GROWN IN OPEN FIELD AND GREENHOUSE

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gene flow, GM crop, wild species, weeds, markers

Agriculture has always drawn great usefulness from genetic improvement. Most of the species improved as a result of such activity are crossable not only each other, but also with their wild ancestors or genetically close weeds. Therefore it can be asserted that the "gene flow" has been a phenomenon started together with species evolution.

The hybridization and the introgression of characters between wild species or weeds and cultivated ones are therefore always happened, with different extent according to the species considered. With the exponential widening of the GM crops grown worldwide, the problem of the "gene flow" has assumed greater interest. The chance that a transgene flow toward wild or similar species could occur has always been one of the major concern about the presumed "ecological risk" of GM crops.

The entity of the gene flow, the possible environmental consequences, and the strategies taken into account to limit such phenomenon, are deeply described in the scientific literature of last the 10 years. Single species are considered, case for case, particularly grains, canola and sugar beet. Less literature is available on tomato, and no indications are available regarding the influence of the different cultivation methods (open field or greenhouse).

The experiences carried out has concurred to verify the natural gene transfer between the various cultivar and Italian ecotypes of tomato grown, both in field and greenhouse. The analysis of the offspring obtained from the plants tested, and the search of spontaneous crossings, has been carried out by means of phenotypic and molecular markers.