

EPSO-Conference 2002

Session

System Approaches to Plant Biology Poster 2.1 – 2.13

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Session	System Approaches to Plant Biology
Title	Identification of MADS box transcription factor functions based on protein-protein interactions
Author(s)	Gerco Angenent and Richard Immink Plant Research International, Bornsesteeg 65 6708 PD Wageningen The Netherlands Author Email: g.c.angenent@plant.wag-ur.nl
<p>Abstract:</p> <p>The MADS box transcription factor family is one of the most important families of regulatory proteins in plants. In Arabidopsis this large family of transcription factors consist of at least 100 members. The challenge of this moment is to identify the functions for all unknown MADS box proteins. A complicating factor for this functional analysis is the existence of many close homologues, which are functionally redundant. Therefore, common functional genomic tools like gene knockouts often do not give clues. Yeast two-hybrid experiments performed for a few plant MADS box proteins showed that MADS box transcription factors are able to form specific homo- and heterodimers and even ternary complexes. Because these interactions determine the function of a transcription factor, mapping of protein-protein interactions can be a next step to elucidate MADS box protein functions. We identified the protein-protein interactions among 23 petunia MADS box transcription factors and found that a few known functional redundant proteins have similar interaction patterns [1,2]. Based on this criterion some more putative functional redundant proteins could be identified and to some proteins a function could be addressed based on interactions with functionally well-characterised proteins. To proof that even proteins from different species are indistinguishable in their ability to form heterodimer transcription factors, a new heterologous two-hybrid screen has been developed. The results obtained from a pilot experiment revealed that this is a fast and reliable way to identify functional homologues from a specific protein into another species [3]. In addition, heterologous three hybrid experiments were performed and showed that also ternary complex formation is conserved and occurred specifically between MADS box proteins from different species. The above mentioned results clearly demonstrate the power of protein-protein interaction analysis as an additional tool for comparative genomics. At this moment we are developing a new high-throughput technology to identify protein-protein interactions based on flow sorting. It can be envisioned that the potential of above mentioned proteomics strategies will expand in the near future due to these developments and the emerging protein-array technology.</p>	

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Session	System Approaches to Plant Biology
Title	Comparative mapping between Arabidopsis and rice and between rice and maize
Author(s)	Michel Delseny, Jerome Salse , Benoit Piegu , Richard Cooke Genome et Développement des Plantes UMR 5096 CNRS-UP-IRD Université de Perpignan 66860 Perpignan cedex France Author email: delseny@univ-perp.fr
<p>Abstract :</p> <p>Complete genomic sequence is available for Arabidopsis as well as large portions of high quality sequences for rice. We have carried out direct sequence comparisons between the available sequenced regions and identified more than 60 regions involving from 4 to 22 rice genes which are orthologues of Arabidopsis genes occurring in the same order. Most of these regions are less than 3cM on the genetic map of rice. 35 rice loci match at least 2 Arabidopsis loci as expected from the duplicated nature of the Arabidopsis.</p> <p>The available rice sequence was also aligned with all the publically available mapped maize EST so that a detailed correspondence between the two genomes could be established involving 270 orthologous pairs. This analysis revealed a much more complex situation than the one usually described by concentric circles and many colinearity breakages were observed. This analysis also provided further evidence for large scale duplications between rice chromosomes.</p>	

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Session	System Approaches to Plant Biology
Title	Proteomics of <i>Medicago truncatula</i> seed filling.
Author(s)	Karine Gallardo, Christine Le Signor, Françoise Moussy, Gérard Duc, Richard Thompson and Judith Burstin INRA-URGAP Legume Unit BP 86510 F-21065 Dijon France Author email: karine.gallardo@epoisses.inra.fr
<p>Abstract:</p> <p>The objective of the Plant Breeding and Genetics Research Unit at the INRA of Dijon is to improve seed quality in legumes, especially in peas, by plant breeding and integrated agronomic practice. To provide new information regarding genes involved in legume seed quality, we undertook a proteomic study of the model species <i>Medicago truncatula</i> during seed development. The analyses of two-dimensional (2-D) protein patterns of seeds collected during development clearly show a progressive accumulation of the most abundant proteins between 12 and 20 days after pollination (DAP). Matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF) analyses of 31 abundant proteins revealed the major storage protein families, the vicilins, legumins and convicilins. To focus on key events occurring in legume seeds during synthesis and deposition of storage proteins we quantitatively compared 2-D protein patterns of <i>Medicago truncatula</i> seeds collected 12 DAP (before the accumulation of storage proteins) and after 14, 16, 18 and 20 days. Statistical analyses of the 273 detected protein spots revealed a significant variation in the quantity of 133 polypeptides of low abundance during these five stages. Some of them were associated with stages preceding and/or following storage protein accumulation, whereas others show various patterns during storage protein accumulation. Thirty four spots showing significant variations were analyzed by MALDI-TOF or Electrospray ionization tandem mass spectrometry and classified according to their function (e.g. cell division, photosynthesis, metabolism, translation, folding, stresses). These data will be used to provide reference maps of <i>Medicago truncatula</i> seed proteins at various stages of development to enable us to focus on the effects of genetic and environmental factors.</p>	

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Session	System Approaches to Plant Biology
Title	Large complexes of nucleus-encoded factors control post-transcriptional steps of chloroplast gene expression.
Author(s)	<p>M. Goldschmidt-Clermont, E. Boudreau, S. Depp, V. Fiechter, S. Lemaire, K. Perron, C. Rivier and J.-D. Rochaix</p> <p>Departments of Plant Biology and of Molecular Biology University of Geneva 30 quai E. Ansermet CH - 1211 Geneva 4 Switzerland</p> <p>Author email: michel.goldschmidt-clermont@molbio.unige.ch</p>
Abstract	<p>Numerous nuclear mutations in <i>Chlamydomonas reinhardtii</i> affect chloroplast gene expression and reveal trans-acting factors that are required in post-transcriptional steps such as RNA splicing, mRNA stability or translation. The factors are surprisingly specific since they are usually required for the expression of a single chloroplast gene or gene cluster. We have cloned and identified several of these factors, which are part of large multi-protein complexes.</p> <p>Three novel nuclear genes encode factors required for trans-splicing of the <i>psaA</i> introns: Raa3 for intron 1, Raa1 and Raa2 for intron 2. The Raa2 protein shares similarity with conserved domains of pseudouridine synthases, but this catalytic activity is not required for <i>psaA</i> trans-splicing. Raa2 may thus have been recruited during evolution to facilitate splicing of the second intron. Raa1 and Raa2 are associated with a membrane fraction of the chloroplast. They are part of a large multiprotein complex which is altered in some of the other trans-splicing mutants. A different complex, containing Raa3, is required for splicing of the first intron. Such large ribonucleoprotein particles may function in groupII-intron splicing as counterparts of the spliceosome.</p> <p>Stable accumulation of the mRNAs from the <i>psbB</i> / <i>psbT</i> / <i>psbH</i> cluster depends in trans on the nucleus-encoded Mbb1 protein and in cis on the <i>psbB</i> 5' untranslated region (5'UTR). Site-directed mutagenesis of the cis-acting elements in the <i>psbB</i> 5'UTR suggests that RNA processing and translation may be coupled. The Mbb1 protein, a member of the family of TPR (tetratricopeptide repeat) proteins, is imported in the chloroplast and is part of two large complexes in the stroma.</p>

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Session	System Approaches to Plant Biology
Title	Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection
Author(s)	Asano, T., T. Masumura, H. Kusano, S. Kikuchi, A. Kurita, H. Shimada and K. Kadowaki National Institute of Agrobiological Sciences Kannondai 2-1-2 Tsukuba, Ibaraki 305-8602 Japan
<p>Abstract:</p> <p>Laser capture microdissection (LCM) is an effective system which allows the isolation of selectively targeted cells from a tissue section for the analysis of gene-expression profiles of individual cells. The LCM has never been reported to be applied to the gene expression profile of plant cells. We successfully applied it to target and isolate the phloem cells of rice leaf tissue whose morphology was different from the surrounding cells. Total RNA was extracted from microdissected (~150) phloem cells and the isolated RNA was used for construction of a cDNA library following the T7 RNA polymerase amplification. Sequence analysis of 413 randomly chosen clones from the library revealed that they could be classified into a total of 124 different groups. The results also showed that 37% of these clones were novel and had no significant homology with any known sequences in the databases. Rest of 63% clones were either homologues to the known genes reported to be localized in phloem tissues of different plant species, or were homologues to other known genes. In situ hybridization revealed that putative amino acid permease, one of the cDNAs, was specifically expressed in the phloem tissues. The results proved the effectiveness of construction of a specialized cDNA library for isolation and characterization of genes expressed in the specific plant cells.</p>	

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Session	System Approaches to Plant Biology
Title	SVISS - A novel transient gene silencing system for gene function discovery and validation in tobacco plants
Author(s)	Michael Metzloff, Veronoqué Gosselé, Ina Faché, Frank Meulewaeter and Marc Cornelissen Bayer BioScience N.V. Jozef Plateaustraat 22 B-9000 Gent Belgium Author email: Michael.Metzloff@bayercropscience.com
<p>Abstract:</p> <p>We developed a novel, two-component transient gene silencing system, in which the satellite tobacco mosaic virus (STMV) is used as vector for the delivery of inhibitory RNA into tobacco plants and the tobacco mosaic virus strain U2 (TMV-U2) is used as helper virus for supplying replication and movement proteins in trans. The main advantage of the system is that by uncoupling virus replication components from silencing induction components the intensity of silencing becomes more pronounced. We call this system satellite-virus-induced silencing system (SVISS) and will demonstrate its robustness, speed and effectiveness. We were able to obtain pronounced and severe knockout phenotypes for a range of targeted endogenous genes belonging to various biochemical pathways and expressed in different plant tissues, such as genes involved in leaf and flower pigmentation, genes for cell wall synthesis in leaf, stem and root tissues or an ubiquitous RNA polymerase gene. By tandem insertion of more than one target gene sequence into the vector we were able to induce simultaneous knockouts of an endogenous gene and a transgene. SVISS is the first transient gene silencing system for <i>Nicotiana tabacum</i>, which is a genetically well-characterized bridging species for the Solanaceae plant family.</p>	

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Session	System Approaches to Plant Biology
Title	Diverting tropane alkaloids in <i>Hyoscyamus muticus</i>
Author(s)	Leonardo Nora John Innes Centre Norwich Research Park Norfolk, NR4 7UH UK Author email: leo.nora@bbsrc.ac.uk
<p>Abstract:</p> <p><i>Hyoscyamus muticus</i> (Egyptian Henbane) is an important medicinal plant because it is a source of tropane alkaloids (TPAs), mainly hyoscyamine and scopolamine, widely used as pharmaceutical products to treat a variety of diseases including Parkinson, motion sickness and poisoning. The use of <i>H. muticus</i> TPAs extracts can be traced back to the last century BC. In the contemporary world TPAs remain as important pharmaceuticals and studies have been carried out for decades to elucidate the formation of these compounds in the plants in order to understand and overcome low productivity problems. More recently the importance of TPAs to the plant that produces them became of interest and some evidences connecting TPAs (e.g. calystegines) to the plant self-defence mechanism are under investigation. As a result of decades of studies on TPAs, key enzymes have been well characterised and their corresponding genes have been cloned however the mechanisms that control the TPAs metabolic flux remain unknown. The well-characterised TPAs pathway in <i>H. muticus</i> provides an excellent model to test the feasibility of transgenic strategies to modify metabolic flux through secondary metabolic pathways and to gain valuable insight into regulatory mechanisms that control pathway activity. Recently, for the first time, <i>H. muticus</i> plants have been transformed with either antisense constructs or virus based constructs and regenerated to adult plants. The constructs used were designed to suppress the expression of two endogenous genes, trI and trII, acting at a branch-point in TPA metabolism, encoding tropinone reductase I (TR I) and tropinone reductase II (TR II), respectively. By suppressing the gene controlling the competing branch we are expecting to divert the secondary metabolic flux exclusively through either the TR I branch leading to the production of hyoscyamine and/or scopolamine or through the TR II branch, leading to the production of calystegines.</p>	

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Session	System Approaches to Plant Biology
Title	Current understanding of cereal seed development, genes involved and their regulation
Author(s)	Hilde-Gunn Opsahl-Ferstad, Lene T Olsen and Heidi Rudi Dept. of Chemistry & Biotechnology, Agricultural University of Norway P.O.Box 5040 1432 Ås, Norway Author email: hilde-gunn.opsahl-ferstad@ikb.nlh.no
<p>Abstract:</p> <p>Having increasingly numbers of genomes sequenced, global transcription profiles and promoter studies from transgenics, our understanding of the eukaryotic 'promoter' and other regulatory sequences involved in gene regulation are changing. Our lab is focusing on genes setting up cell identity in cereal seed development and their corresponding regulation (Olsen et al. 1999; Becraft et al. 2001). The cereal endosperm represents a major source for food, feed and industrial raw material in the world. When fully developed, the endosperm is a simple plant system consisting of four major cell types, the starchy endosperm, the aleurone layer, the transfer cells, and cells of the embryo surrounding region. The Crinkly4 and Dek1 genes from maize are necessary to develop and keep aleurone cell identity (Lid et al. 2002), and we are about to characterize the corresponding barley genes. Even though several members of receptor kinases have been shown to play key regulatory roles in plant development, it was not until recently CLAVATA1 was shown to physically interacted with the small peptide and presumed ligand CLAVATA3 (CL3). Using mutant studies, functional genomics, genomics and a more directed tBlastn surge a novel gene family the CLE (CLV3/ESR-related) has been identified (Cock and McCormick 2001). The ESR genes are specifically expressed in a restricted region of the endosperm surrounding the basal part of the embryo (Opsahl-Ferstad et al. 1997; Bonello et al. 2002). We are studying promoters and regulatory elements preferentially directing gene expression in developing endosperm for potential uses in metabolic engineering, using Agrobacterium transformation (Philippe Vain unpublished).</p> <p>Becraft PW, RC Brown, BE Lemmon, O-A Olsen, and H-G Opsahl-Ferstad. 2001. Developmental biology of endosperm development. (ed. S.S. Bhojwani) 353-374 Bonello et al. 2002. J Exp Bot 53: 1559-1568. Cock JM & S McCormick. 2001. Plant Phys. 126: 939-942. Lid et al. 2002. Proc Natl Acad Sci U S A 99: 5460-5. Olsen et al. 1999. Trends in Plant Science 5: 253-257. Opsahl-Ferstad H-G, E Le Deunff, C Dumas, and P Rogowsky. 1997. The Plant Journal 12: 235-246.</p>	

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Session	System Approaches to Plant Biology
Title	Array-based identification of genes involved in flower development in gerbera hybrida
Author(s)	<p>Teeri Teemu H1., Laitinen Roosa1, Rintala Miia1, Koskela Satu1, Hämäläinen Jaana1, Kotilainen Mika1, Raukko Elina1, Ruokolainen Satu1, Pöllänen Eija1, Paulin Lars2, Auvinen Petri2 and Elomaa Paula1</p> <p>1Department of Applied Biology, P.O. Box 27, 00014 University of Helsinki, Finland. 2Institute of Biotechnology, P.O. Box 56, 00014 University of Helsinki, Finland.</p> <p>Author email: teemu.teeri@helsinki.fi</p>
Abstract:	<p>We are using <i>Gerbera hybrida</i> as a model plant to study inflorescence development in Asteraceae. The inflorescence of gerbera consists of three morphologically different types of flowers. During the early stages of development, floral primordia of each flower type are identical, but later in development differences are found. For example, in the female ray and trans flowers stamens abort, while the central disc flowers bear both carpels and functional stamens. Also petal symmetry varies flower type specifically and, in different cultivars, anthocyanin pigmentation. We are going to use cDNA microarrays to identify genes involved in differentiation of the various flower types. For the construction of the chip, altogether 8 different cDNA libraries have been established and approximately 15000 ESTs have been sequenced (size of the unigene set is 8700). The chip will be used for several applications, but our first goal is to compare gene expression profiles during differentiation of ray and disc flowers in gerbera.</p>

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Session	System Approaches to Plant Biology
Title	Histone acetylation as regulatory switches of plant development processes
Author(s)	Bertrand C, Bergounioux C, Delarue M, Zhou DX IBP, B630 Université Paris-sud 91405 ORSAY, FRANCE Author email: zhou@ibp.u-psud.fr
<p>Abstract:</p> <p>Histone acetyltransferases (HAT) and deacetylases (HDAC), which are able to acetylate or deacetylate histone and non-histone proteins, play an essential role in gene regulation. A few subclasses of HAT and HDAC have been defined in animal and yeast systems. Implication of HAT in gene transcription turns out to be complex. HAT activity has been found not only in cofactors and general transcription factors, but also in DNA-binding transcription factors and transcription elongation factors. It is suggested that HAT are involved in the expression of subsets of genes. Loss of function mutation of HAT genes induces changes of expression of about 5% genes in yeast genome. However, similar mutations cause embryo lethal phenotypes in animals. More than twenty putative HAT encoding genes were found in the Arabidopsis genome. A number of T-DNA insertion mutants have been identified in some of the HAT genes. Reduction of histone overall acetylation levels was observed in some of the mutants. Analysis of a few mutants showed specific phenotypes, such as homeotic transformation of flower organs, alteration of leaf shape, defaults in greening, photosynthetic gene expression and responses to environmental cues, etc.. Gene expression analysis showed that the expression of key developmental regulatory genes was altered in some of the mutants. A combination of functional genomics and biochemical analysis of plant HAT will be needed to investigate the regulatory networks involving HAT in specific gene activation and repression required for the regulation of plant developmental processes.</p>	

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Session	System Approaches to Plant Biology
Title	H.A.P.P.Y. Mapping: a cost-effective solution for large and very-large scale genome mapping projects
Author(s)	<p>Madan Thangavelu, Alan T. Bankier, Helen F. Spriggs, Bernard A. Konfortov, Justin A. Pachebat and Paul H. Dear</p> <p>Medical Research Council Laboratory of Molecular Biology, Hills Road Cambridge CB2 2QH U.K.</p> <p>Author email: madan@mrc-lmb.cam.ac.uk</p>
Abstract	<p>Various genome mapping approaches and technologies have been developed and are being used for constructing and comparing DNA-marker linkage maps of genomes. Almost all these procedures have deficiencies and drawbacks – for instance, many are not applicable for certain genomes, some are many orders of magnitude too slow, some too expensive and most too cumbersome - and are unlikely to offer realistic means for future large-scale genome mapping and comparative genomics projects. The ability to construct DNA-marker maps rapidly and in an efficient and cost-effective manner is, therefore, of considerable interest to researchers worldwide.</p> <p>HAPPY mapping is a simple, rapid, inexpensive, cloning-free, PCR-based procedure for defining the order and spacing of DNA markers directly on native genomic DNA. The technique is based on analysing the co-segregation of markers amplified from limiting dilutions of randomly sheared high-molecular weight genomic DNA. HAPPY mapping has been applied for high-resolution, error-free mapping in phylogenetically diverse genomes ranging from humans to fish and from plants to parasites.</p> <p>This presentation will provide a brief overview of the problems and challenges in genome mapping, the principles of HAPPY mapping, ongoing and completed projects, results of plant genome mapping studies and finally the implications and prospects for large and very-large scale plant and agrigenome mapping projects. Details of the technology and results will also be presented in accompanying poster(s).</p>

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Session	System Approaches
Title	Functional genomics of rice grain development : Establishment of a database of mutant phenotypes and enhancer trap gus expression in mature seeds.
Author(s)	Barnola I., Perez P. Biogemma, Gene Function and Maize Traits Group, 24, Avenue des Landais, 63 170 Aubière, France Gay C., Larmande P., This D., Guiderdoni E. UMR 1096 GACA, CIRAD Amis, TA 40/03, 34398 Montpellier, France Guillemint J., Devic M. Modat S. Laboratoire Génome et Développement des Plantes, CNRS-UMR 5096, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan-cedex, France.
<p>Abstract:</p> <p>In the framework of the French national initiative Génoplante, a T-DNA/Tos17 insertion lines collection in rice is being developed in Montpellier. This mutants library gives the very interesting opportunity to collect knowledge on grain development for rice and cereals in general.</p> <p>We are screening the insertion lines for seed mutant phenotypes and for expression of a reporter gene in mature seeds. In most cases, the collection allows to directly observe some seed phenotypes on the primary transformants' progenies without additional sowing. Indeed, we can easily observe a mendelian segregation where a quarter of the seeds presents a mutant phenotype in a case of a recessive mutation. We have already evaluated several thousand lines mainly for seed and endosperm morphology. We will present the different types of mutations found and their frequencies. Using <i>the gusA</i> gene enhancer trap located in the T-DNA, we have also evaluated the <i>gus</i> expression pattern in dry seeds. Examples and frequencies of <i>gus</i> expression in seeds will be presented. Our first results in rice and the results obtained in well-characterised <i>Arabidopsis</i> mutants collections will be compared. Moreover, FST sequences available for a large proportion of the library will give us access to putative key genes involved in seed development and filling. Cytological and molecular aspects of key developmental stages of rice seed are also investigated in order to further characterise more precisely interesting mutants.</p> <p>Finally we will discuss how the development of such rice kernel phenotype database will allow <i>in silico</i> reverse genetics for any rice and cereals genes and will serve most of the Genoplante Seed and Grain projects.</p>	

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Session	System Approaches
Title	A genome-wide library of insertion lines in rice: distribution of T-DNA inserts over the rice genome
Author(s)	<p>Christophe Sallaud (1), Céline Gay (2), Pierre Larmande (1), Emmanuelle Bourgeois (1), Benoit Piégu (3), Farid Regad (1), Martine Bès (1), Alexander Johnson (5), Pietro Piffanelli (1), Christophe Périn (1), Alain Ghesquière (4), Mark Tester (5), Jullian Hibberd (5), Michel Delseny (3) and Emmanuel Guiderdoni (1)</p> <p>1) Cirad-Amis and 2) Inra-Ensam, Biotrop program, Avenue Agropolis, F-34398 Montpellier Cedex 5, France 3) Laboratoire Génome et Développement des Plantes, UMR5096, CNRS/UP, 52, avenue de Villeneuve, F-66860, Perpignan Cedex, France 4) Genetrop, Ird, BP5045, F-34032 Montpellier Cedex 01, France 5) Department of Plant Sciences, University of Cambridge, Downing St, Cambridge CB2 3EA, UK</p>
<p>Abstract:</p> <p>In the framework of the genomics initiative Génoplante, we have embarked on a project of creation of a genome-wide library of insertion lines of rice (<i>Oryza sativa</i> L. japonica cv. Nipponbare). The generation of the library relies first on a highly efficient <i>Agrobacterium</i>-mediated transformation procedure for delivery of T-DNA inserts (Sallaud <i>et al.</i> Theor Appl Genet, <i>in press</i>). This method allowed the production of 30,000 primary transformants (T0) with an average efficiency of 5 independent transformation events per co-cultured callus in a 18 month-time span. The equipment of the pC-4978 and pC-4956:ET15 T-DNAs with a <i>gusA</i> and a <i>gal4:UAS:gfp</i> enhancer trap respectively allows gene detection through visualisation of GUS activity and fluorescence in specific cell types and organs. Histochemical assays conducted on more than 2,000 T0 plants allowed detection of GUS-specific activity in leaves, roots and floral organs with respective frequencies of 26.9%, 6.9% and 3%. Observations of mature leaf and floral organs of 2,000 pC-4956:ET15 primary transformants allowed fluorescence detection in more than 10% of the plants.</p> <p>The T0 plants allowed to set seeds in the greenhouse have been first selected at the <i>in vitro</i> stage for amplification of a unique product of the genomic region flanking the T-DNA left border. 15,316 plants have been so far selected with an overall frequency of 60%, which is remarkably stable over transformation experiments. To date, 9,700 (90%) of the 10,862 PCR2 products sequenced with a T-DNA specific primer produced a readable sequence. BlastN search allowed identification of the T-DNA footprint in most (94.5%) sequences. Survey of the 5,603 (57.8%) genomic sequences larger than 30bp (average length 250 bp) against the rice BAC/PAC sequences (total : 333.5 Mb as of June 2002) detected 4,261 hits and allowed to assign 3170 (56%) T-DNA insertion sites to at least one position on the rice genome. This is consistent with the percentage of the rice genome sequenced at that time (57%). The T-DNA insertion sites density for each chromosome averages 13.1 insertions per Mb sequenced and merely varies by a factor of 1.5 in ranging from 10 to 15 insertions per Mb across the 12 chromosomes. A more detailed examination of the distribution of T-DNA inserts along the Chromosome 1 showed that 578 (92,5%) over 626 T-DNA insertions are assigned to a unique location. The results clearly demonstrate that the centromeric region exhibits a lower insertion density whereas higher insertion density is observed in the subtelomeric regions. Aside from the T-DNA insert(s), 75% of T0 plants harbour a mean of 1.5 new copies of the rice endogenous <i>Tos17</i> retrotransposon which have been specifically amplified and reinserted during the transformation/ regeneration procedure. Walk-PCR based amplification of flanking regions of nearly all new inserts proved to be possible and 60% of them produced a PCR product that can be directly sequenced using an LTR-specific primer.</p>	