



Najaarsymposium Autumn Symposium

Biotechnology and Horticulture

Nederlandse Vereniging voor Plantenbiotechnologie en -Weefselkweek
Netherlands society for Plant Biotechnology and tissue Culture

in collaboration with

Benelux Society for Horticultural Science

19 December 2002

Gorlaeus Laboratorium

Einsteinweg 55, Leiden, Netherlands

- 9.30 Registration and coffee
- 10.00 Arie Altman (The Hebrew University, Rehovot) Tolerance to drought and salinity stress: Biotechnological and molecular aspects of plant
- 10.40 Piet van der Linde (Stichting Bedrijfslaboratorium voor Weefselkweek: SBW) Aging in production processes
- 11.05 Bart Panis (Catholic University Leuven) Cryopreservation of plant germplasm: Techniques, limitations and applications
- 11.30 Frank Van Breusegem (Ghent University) The dual face of oxidative stress: from acclimation to cell death
- 11.55 Marc de Loose (Agricultural Research Centre Gent) GMO detection and quantification: the legislation versus the technical capacity and the theoretical limitations.
- 12.20 Lunch
- 13.45 Maarten Koornneef (Wageningen University) Arabidopsis genomics en horticulture
- 14.25 Tom Gerats (Catholic University Nijmegen) Plant Biotechnology as a tool and as a promise.
- 14.50 Ludmilla Khurstaleva (Plant Research International) Physical mapping of protein encoding genes on plant chromosomes.
- 15.15 Kerstborrel/Christmas Drinks

Biotechnological and molecular aspects of plant tolerance to drought and salinity stress

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Many countries face periodical serious drought periods and a continuous deficit of good-quality fresh water resources. Most countries will face it within 20-25 years. Therefore, agriculture must use alternative water resources such as saline water and recycled sewage effluents. It is clear that salt accumulation in irrigated soils can increase salinity to levels that will reduce growth and yield of even the more tolerant crops. In non-irrigated areas, salinization is even more devastating, causing famine and death. Therefore, breeding for drought and salinity tolerance in crop plants (for food supply) and in forest trees (being a central component of the global ecosystem) should be given the highest research priority in all future plant biotechnology programs.

The molecular basis of drought and salinity tolerance is rather limited. Discovery of new genes for abiotic stress tolerance, combined with controlled molecular breeding will have an important role in shaping agricultural plants in the post-genomic era. Examples of several molecular control mechanisms of drought and salinity tolerance will be presented, including our recent findings on the molecular characterization of a novel class of plant chaperone-like stress-associated proteins (SP1 and related proteins), which are able to protect the structure and activity of other proteins and to sustain growth.

Biotechnological-oriented breeding for drought and salinity tolerance includes: (1) Functional genomics analysis, (2) Use of molecular probes and markers for selection among natural and bred populations, (3) Transformation with specific genes and generation of transgenic plants. Biotechnology cannot solve all problems, however it is a most powerful technique that can and will be integrated in all classical plant breeding programs. At the end of the day, the only criterion to evaluate the efficiency of genetic engineering will be its cost effectiveness and benefits to society.

The dual face of oxidative stress: from acclimation to cell death

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Adequate responses to environmental changes are crucial for plant growth and survival. However, the molecular and biochemical mechanisms involved are poorly understood and the signaling networks remain elusive. The accumulation of active oxygen species (AOS) is a central theme during plant responses to both biotic and abiotic stresses. In both situations, AOS can play two divergent roles: either exacerbating damage or activating multiple defense responses hereby acting as

signal molecules. Such a dual function was first described in pathogenesis but has also recently been demonstrated during several abiotic stress responses.

In transgenic tobacco plants with reduced catalase activity we can modulate the levels of hydrogen peroxide (H₂O₂). We show that in these transgenics H₂O₂ production can trigger both the induction of an active cell death program and an acclimatory response.

To identify the genes involved in this dual response we followed changes in gene expression using cDNA-AFLP transcript profiling during both processes. In this way we have built an inventory of genes modulated by an H₂O₂ signal. Sequence analysis revealed, in addition to genes with unknown function, several homologues of genes known to be involved in cell death signal transduction, genes involved in the defence response to biotic and abiotic stress.

CRYOPRESERVATION OF PLANT GERMPLASM : Techniques, limitations and applications

Bart Panis

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Cryopreservation refers to the storage of biological material at ultra-low temperatures i.e. at -196°C, the temperature of liquid nitrogen. At this temperature, all chemical and metabolic and most physical processes are arrested. For this reason, cryopreservation is the method of choice for the long term conservation of plant germplasm since both the problem of genetic instability and the risk of losing accessions due to contamination or human error during subculturing are overcome. Most cryopreservation endeavours deal with recalcitrant seeds, *in vitro* tissues from vegetatively propagated crops, species with a particular gene combination (elite genotypes) and dedifferentiated plant cell cultures. Other applications of cryopreservation in plant science are also discussed, i.e. cryotherapy and its potential for storing the causal agents of pest and diseases.

Sofar, cryopreservation procedures are developed for *in vitro* tissues and recalcitrant seeds of about 100 and 40 species, respectively. There is still a limited number of cases where cryopreservation is used routinely for plant germplasm conservation, mainly because the techniques need to be adapted for each species in function of its natural freezing resistance, explant size and type, and its water content. Care must be taken to avoid ice crystallisation during the freezing process, which otherwise would cause physical damage to the tissues. The existing cryogenic strategies rely on air-drying, freeze dehydration, osmotic dehydration, addition of penetrating cryoprotective substances and adaptive metabolism (hardening) or combinations of these processes.

GMO detection and quantification: the legislation versus the technical capacity and the theoretical limitations.

Marc De Loose

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The use of transgenic plants in Europe is strictly restricted and regulated by directives and legislation's. Seeds, food and feed containing GMOs have to be labelled. Only in the case of an accidental contamination below a fixed threshold,

expressed per ingredient, no labelling is required. For seeds on the one hand and food/feed on the other hand other thresholds are used, respectively 0.3%/0.5%/0.7% for seeds and 1% for food. The reason for lower thresholds in seeds is to be able to guarantee farmers that wish to produce GMO, that they can do so with certified GMO free seeds. Recently the European legislation concerning the thresholds has been changed. By this occasion also a threshold for non-authorized but positively evaluated GMO has been introduced.

Of course legislations and thresholds will only be implemented in real life when adequate testing systems are available. Demonstrating the presence of a GMO is possible at phenotypic, protein and DNA level. Quantification is most practical at protein and DNA level. Protein and DNA based methods are not necessarily resulting in the same experimental result. In Europe there is a preference for DNA based methods.

During the presentation different aspects relating to GMO analysis will be discussed: sampling, which unit to use for expression of the percentage of contamination, analysis methods, strategies for quantification, standardisation of methods, how to get access to the necessary information, standards, etc.

Arabidopsis genomics and horticulture

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Arabidopsis is considered as the major model plant for molecular and genetic studies. Especially the ease to do genetics and transformation experiments together with the resources available (including the complete genome sequence) make that research in Arabidopsis leads to faster results than similar research using crop plants. The cloning of genes controlling complex quantitative traits has been illustrated already on flowering time genes.

An important question is, if plants are so similar that what we can learn from Arabidopsis can be applied to crop plants. The comparisons of the DNA sequence of genomes (especially the genes that are expressed = EST's) and the study of individual genes controlling specific traits show strong similarity between plants. This implies that Arabidopsis knowledge often can be used to identify functional similar genes in crop plants. However differences between plants also exist, probably especially in the regulating factors. Examples that show similarity and differences between Arabidopsis and crop plants will be demonstrated for flowering time, plant length, pigmentation and glucosinolate biosynthesis.

Plant Biotechnology as a tool and as a promise

Tom Gerats

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The human population of the world is still growing and at a total of around 6 billion there are 800 million people that have a chronic shortage of food. Yearly around 10-15 million people die from malnutrition, the majority of which are children. The latest FAO report on the state of the world food situation indicates that, as in the two years before, there are no improvements in this situation for the year 2001.

World food production is sufficient to feed all people. Much is lost in transport, storage and distribution. There are many political issues that hamper an efficient

food trade and distribution. The loss of fertile land due to mismanagement and especially in coastal regions due to salination is increasingly endangering food production. Predictions are that even in the most optimistic scenarios, the human population will need more food than can be produced with the presently available set up around the year 2040. 'It took 10.000 years to expand food production to the current level; within 25-30 years we will have to double that' (Norman Borlaug).

So, among other things we have to aim at developing agriculture further by improving methods, equipment, storage/transport/distribution, but also by selecting improved varieties by classical breeding methods and indeed, where appropriate, by biotechnological means. A recent example of a very promising development in this area is the creation of rice varieties that produce trehalose in their plant body. Trehalose provides protection against salt stress, against cold stress and against drought. As such it will enable cultivation in precisely the increasingly endangered agricultural areas. Trehalose rice is offered to the world community and the trait can be used as a parent in breeding schemes. I will present an overview of the "Breeding by Design" (Keygene trademark) approach that combines classical breeding with biotechnological tools. For further reading on these issues I can strongly recommend the following book: ***Plants, Genes, and Crop Biotechnology, Second Edition*** by Maarten J. Chrispeels and David E. Sadava. (see: <http://www.plantbiotech.jp.org>)

Physical mapping of protein-encoding genes on plant chromosomes

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The strategy for physical mapping of genes on plant chromosomes was based on developing of deletion or translocation lines. An other approach for gene physical mapping was applied specifically for Arabidopsis and rice genomes, which were chosen as model organisms for sequencing in plants. Recently developed BAC-based mapping using *in situ* hybridization allows visualising genes on plant chromosome. However, this method is not always realistic for plants with large genomes. Moreover, BAC clones might contain next to unique sequences also repetitive DNA sequences, which are dispersed over genome and can not be even blocked with total genomic DNA or Cot-100 DNA fraction. Physical localization of small DNA sequences like ESTs (expressed sequence tags) is difficult on high-condense plant chromosomes. Therefore we recently adapted a new signal amplification method termed Tyramide-FISH for plant cytogenetics. This technique allows for the detection of small (700bp) DNA sequences on *Allium* metaphase chromosomes (Khrustaleva & Kik 2001, Plant J 25:699-707). Using the Tyramide-FISH technique we analyzed the chromosomal organization of onion bulb tissue expressed genes (ESTs). We found that 600 EST clones were not uniformly distributed on the *Allium* genome and that a high proportion of actively transcribed genes were present in clusters. The distribution of ESTs in *Alliums* was not even along entire chromosomes and also not among chromosomes. Some chromosome regions, such as the long arm of the chromosome 2 contained few ESTs whereas others, such as the short arm of the chromosome 1,7 and 8, had a high density of ESTs. Genes are thought to be relatively rare in the centromeres of higher

eukaryotes. In contrast, we found that some of the EST clones hybridized to the centromeric region of the chromosomes 1, 3 and 5.

Mapping of single EST clones showed that these ESTs are syntenic in *A. cepa* and *A. fistulosum*. For example, an EST clone coding for a putative pectinesterase gene was localized on the long arm of the chromosome 6 in both species. However, comparison of the pectinesterase gene location in *A. fistulosum* and *A. cepa* showed difference in its position towards the centromere. The difference in location might be explained by a regional increase in copy number of repetitive sequences through tandem, transpositional, or retrotranspositional duplication, or by loss, of specific chromosomal regions. Also the difference in chromatin condensation of this region might play a role.

The advantage of direct mapping ESTs on chromosomes is that the obtained transcriptional map provides a description of the organization of expressed genes and, what is very important, it can fill uncovered regions with a high suppression of recombination like centromeres. Combination of EST chromosome mapping and other marker techniques will accelerate gene mapping in plant with large genome. Visualisation of specific chromosomal regions with ESTs will also assist microdissection of chromosome fragments and subsequent development of molecular markers based on intron polymorphism.

see also: www.plant.wag-ur.nl/expertise/alliumresearch