CRA-W NEWS

PERIODICAL PUBLISHED BY THE WALLOON AGRICULTURAL RESEARCH CENTRE N°12 AUTUMN 06

Diversity in aneuploid apple trees from *in vitro* culture

The range of pome fruits on the market is currently based on a small number of varieties which are genetically very close. The heterozygosity and long period of juvenility preceding sexual reproduction in fruit trees have in fact obliged conventional breeders to use the few genotypes that preserve most of the commercial properties originally present. This explains the drive to develop methods for creating diversity independently of genetic engineering.

Although in vitro culture is now widely recognised as an efficient methodology to clean and propagate true-to-type cultivars of fruit species on a large scale, its usefulness in creating genetic diversity without genetic transformation is less known.

Generally, fruiting depends on successful fertilisation, which determines the fruit's remaining on the tree until maturity and also its shape and size. However, seeds interfere sometimes with the fruit consumption. In the case of the grape, breeders thus create aneuploid genotypes after crossing diploid and triploid parents. Since, essentially, aneuploidism characterises the state of a genome where the chromosome set does not contain the basic number of chromosomes, due to the gain or loss of chromosomes, the berries from such hybrids are seedless. Aneuploidism therefore causes sterility.

In apple trees, aneuploids are not viable on their own roots; the plants die shortly after germination. After in vitro sowing, the genotypes are saved and it is possible to make true-to-type copies through axillary budding. Grafting of the microshoots on a rootstock followed by growing under glass then leads to the development of complete trees which grow and flower naturally in the orchard. Contrary to expectation, however, such trees bear fruit. The apples actually contain pips as a result of conventional pollination and these in turn can be sown and saved in vitro.

A study in progress involving Jonagold (3n) and the McIntosh «Wijcik» columnar apple tree (2n) has shown the diversity of the fruits and growth habits (columnar, spurs and weeping types) in the first aneuploid generation. Second generation aneuploids are also able to bear fruit naturally.

The genetic instability of certain aneuploid genotypes has been revealed in vitro through adventitious buds induced on leaves. The extent of the changes varies according to the regeneration event, in most cases involving very small quantities of DNA that affect plant behaviour and can also lead to polyploidisation.

In vitro germination techniques and micrografting thus enable apple tree genotypes that are not naturally viable to be developed, while off-type regeneration processes, such as in vitro adventitious budding, also create diversity. Not only can such diversity be integrated directly into conventional breeding programmes, but it could also facilitate screening for new agronomic traits using molecular analysis techniques.

Contact: Philippe Druart
druart@cra.wallonie.be

Walloon Agricultural Research Centre

WALLOON AGRICULTURAL RESEARCH CENTRE (CRA-W)

Rue de Liroux, 9 - B – 5030 Gembloux

Tél : +32(0) 81/62.65.55 -Fax :+32(0) 81/62.65.59

cra@cra.wallonie.be - http://www.cra.wallonie.be

In vitro tubers, minitubers and plantlets are having babies ...

Potato *in vitro* micropropagation techniques to produce initial material for the start of the potato plant multiplication chain are an integral part of the multiplication scheme today. Such techniques offer the following advantages:

- Assurance of health at the start of the chain,
- Propagative power,
- Smaller number of field propagation cycles and hence improved plant health,
- Flexibility by permitting swifter adaptation to the demands of the market in terms of varieties, for example.

The Walloon Agricultural Research Centre has been developing these techniques for over fifteen years now and produces a significant quantity of starter material for the Walloon regional potato plant multiplication sector in the form of in vitro tubers, minitubers or acclimatised vitroplants.

A sizeable collection – unique in the Walloon region – of potato varieties has been created *in vitro*, from which starter material is produced to order.

Minitubers are potato tubers originally obtained from vitroplants or in vitro tubers in an environment protected from viral infections (greenhouses). Ranging in size from 10 to 45 mm, these tubers are produced in a hydroponic system, designed by CRA-W, which has proved capable of economically producing tubers of very high health value. After harvesting and cold storage, the minitubers are then propagated in the field to produce the first generation of field tubers.

In vitro tubers or microtubers are small tubers between 5 and 10 mm in size which are produced in the laboratory The production process developed is fairly straightforward and does not require any special equipment. Furthermore, as production takes place in the laboratory, it offers the best possible guarantee of health.

The acclimatised and rooted plantlets are produced by transferring the vitroplants into compressed balls of peat. The plants are grown under glass for four to five weeks, during which time they root and reach a height of approximately 20 cm. They are then planted out in the field manually or with the aid of a horticultural transplanter.

The adoption of these working techniques by our growers is guided by a constant drive to improve plant production quality. One of the major health problems today in plants produced in Western Europe and generally exported to Eastern Europe and the Mediterranean is infection, latent or otherwise, with the bacterias that cause soft rot (Erwinia sp.). To overcome this, the development of the use of plant material micropropagated in vitro in shorter multiplication chains can significantly improve overall product quality levels.

Contact : Jean-Louis Rolot
rolot@cra.wallonie.be

Community Reference laboratory for detection of animal proteins

Since 1998, CRA-W has coordinated various national and international studies aimed at developing analytical methods for the detection, identification and quantification of animal proteins in feedingstuffs. As a result of these programmes, two methods based on infrared spectroscopy and one method based on molecular biology techniques have been developed. CRA-W has also acquired expertise in carrying out interlaboratory studies and in organising training in the detection of animal proteins by conventional microscopy (Council Directive 126/2003/EC).

In recognition of its expertise the CRA-W's Quality of Agricultural Products Department was recently appointed **Community Reference Laboratory (CRL) for the detection of animal proteins in animal feed** for the 2006-2011 period (by Commission Regulation no. 776/2006 of 23rd May 2006). As a CRL, CRA-W is expected to:

(i) Provide advice and scientific support to the European Commission;

(ii) Coordinate the network of national reference laboratories (NRL) and assist the member laboratories;

(iii) Organise ring tests and comparative studies;

(iv) Participate in the development and assessment of new analytical methods;

(v) Organise training and workshops.

Contact : Vincent Baeten
baeten@cra.wallonie.be
Gilbert Berben
berben@cra.wallonie.be

CRL Animal Proteins

Wood pellets, a versatile fuel

Used for the first time for heating in the United States in the seventies after the first oil crisis, wood pellets are now regarded as an excellent home heating fuel.

A wood pellet is a small cylinder of highly compressed sawdust. Its high calorific value (it takes around 2 kg of pellets to obtain the energy equivalent of one litre of heating oil) and its fluidity make it a suitable fuel for fully automated heating systems today. There are currently various pellet-fired boiler and stove models on the Belgian market. While the cost of some systems is still relatively high, the fuel price has become very competitive in comparison to heating oil. In current market conditions (with pellets at €185 per tonne and heating oil at €0.60 per litre), the use of 5 t of pellets to replace 2,500 litres of oil means an annual saving of €575. Taking into account the subsidies offered by the Regional Government and the possibility of tax breaks, the return on investment time is under ten years (and in fact very much less with some boiler models). From an environmental point of view, using 5 t of pellets avoids the emission of 6.75 t of fossil CO_2.

Exclusively imported a few years ago, pellets are now produced in the Walloon Region. Since the beginning of this year two pellet production plants have started up, with total output of just under 70,000 t per year. Further units should come on stream shortly.

According to a survey conducted by the ValBiom organisation, there are thought to be around 2,000 pellet-fired heating systems currently in operation in the Walloon Region, including nearly 300 boilers (as at end 2005) compared to only just over 50 boilers in operation one year previously. Besides being used for domestic heating, wood pellets are also used in the tertiary sector and even as a fuel for power stations!

To underpin the development of the pellet sector in the Walloon Region, it is important to ensure that quality fuel and efficient heating systems are available on the market. To this end the CRA-W's Biomass Laboratory carries out pellet lot analyses for quality assessment purposes, while the ValBiom organisation is involved in putting the sector on a professional basis by distributing advice and information about wood heating and assisting with setting up "wood energy" projects, etc. ValBiom is also a partner on the EUBIONET II project, one of the aims of which is to validate the Technical Specification CEN/TS 14961 related to solid biofuels.

Contact : Didier Marchal, marchal@cra.wallonie.be

Animal welfare… for pregnant sows

The Royal Decree of 15th May 2003 on the protection of pigs at pig farms takes account of sow welfare by establishing an obligation for them to be housed in groups for a period extending from four weeks after insemination until one week before the scheduled date of giving birth. Various types of housing for groups of pregnant sows have therefore been designed, one of which uses Automatic Concentrate Feeders (ACFs), also known as electronic feeders, and is based on managing the sows in large groups. Sow management is then organised into either dynamic or stable groups, depending whether new sows are periodically introduced. The ACF is designed for about 45 sows and allows each individual to feed in turn without being disturbed by the others. However, the restricted "eating space" available and the need for the sows to take turns to feed are potential sources of stress, especially for subordinate individuals or new entrants to the group. Likewise, the regrouping phases of unfamiliar sows are followed by changes in the pecking order, causing acute stress among the sows.

A joint research project by the ULg Faculty of Veterinary Medicine and CRA-W, financed by the Federal Public Service (Public Health, Food Chain Safety and Environment) has therefore been undertaken to investigate how the integration of new sows into housing can be facilitated and the social pressure within a group lessened. Three assumptions are being tested, namely:

- Increasing the available area per sow,
- Delaying sow introduction in relation to the feeding cycle,
- Providing an outdoor run.

Initial results with respect to the available area per sow show that sow welfare is enhanced by increasing the available area from 2.25 m² (standard according to the Royal Decree) to 3.00 m². These results will be presented in a paper to the 40th International Congress of the International Society for Applied Ethology in Bristol. It has been found that in a larger area, there are fewer agonistic interactions and sows have a lower injury rate and a lower salivary cortisol level (stress indicator) two hours after regrouping.

Contact : José Wavreille, wavreille@cra.wallonie.be

Plants know their own nitrogen needs best!

Like any living being, plants need a balanced diet. In agriculture, correct feeding of growing crops results in a financially profitable yield together with a harvest that meets the quality expectations of users (food processing industry, supermarkets and consumers) while avoiding the negative side-effects on the environment of excessive exogenous nutrient application.

Nitrogen is one of the major components of plant nutrition and making it more efficient at field level has been the subject of on-going research at CRA-W for over forty years. Practical recommendations for crop nitrogen fertilisation have for a long time been based mainly on striking a balance between the supply of mineral nitrogen (from the soil and synthetic nitrogen fertilizers), on the one hand, and crops' standard nitrogen requirements, defined *a priori*, on the other. To avoid using these standard estimates, which are subject to often significant errors compared to actual field conditions, CRA-W along with other European research institutes is looking at techniques for refining and estimating crop nitrogen requirements in real time, at regional level. The plant is then considered to be its own nitrogen demand indicator!

Various tools already on the market or under development can be used to "question" the crop during the season and find out whether an additional application is needed in order to meet requirements. In the last few years CRA-W has studied the potential for measuring nitrate levels in stalks or leaf petioles in cereals and potatoes and has also investigated the use of a chlorophyll meter to measure light absorption by leaf chlorophyll. The chlorophyll meter has led to the development of a decision support system for potato nitrogen fertilisation management in the Walloon Region (CRA-W Info No. 2, Spring 2004). For the last three years, the light reflected by potato leaves has also been measured with the aid of a radiometer. The chlorophyll meter and the radiometer both give quick measurements at farm field level, without having to uproot plant samples. They are both based on the use of sensors and specific wavelengths in the electromagnetic spectrum which are closely linked to plant nitrogen levels.

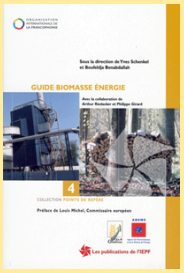
With the rapid development of modern data acquisition techniques in space and time, a change in the use of these tools is to be expected in the next ten years. The most efficient of them will have to meet several criteria if they are to be usable at farm field level. These criteria are sufficient sensitivity and specificity of measurement in relation to plant nitrogen levels, repeatability and precision of measurements and, lastly, ease and rapidity of use of data acquisition, while keeping the cost at an economically acceptable level. CRA-W is continuing its investigations aimed at applications meeting these criteria in the interests of the players in the Walloon Region's plant sectors.

Contacts : Jean-Pierre Goffart, goffart@cra.walllonie.be

Biomass Energy Guide

The second edition of the new Biomass Energy Guide is now available from CRA-W.
Following the first edition, the guide puts the subject of biomass into the context of emerging global issues, notably sustainable development and climate change.
The guide describes biomass-to-energy techniques in the light of acquired knowledge and recently developed technologies.

Contact: Yves Schenkel, schenkel@cra.wallonie.be



Sixth in the series of our traditional Pig and Poultry Products seminars.
"Which future for the pig and the poultry in Wallonia"

Wednesday 8th November 2006
will look at the topic of "**Developing the pig and poultry sectors in the face of economic, social and political pressures"**
Espace Senghor, Gembloux

Contact: Geneviève Minne, minne@cra.wallonie.be