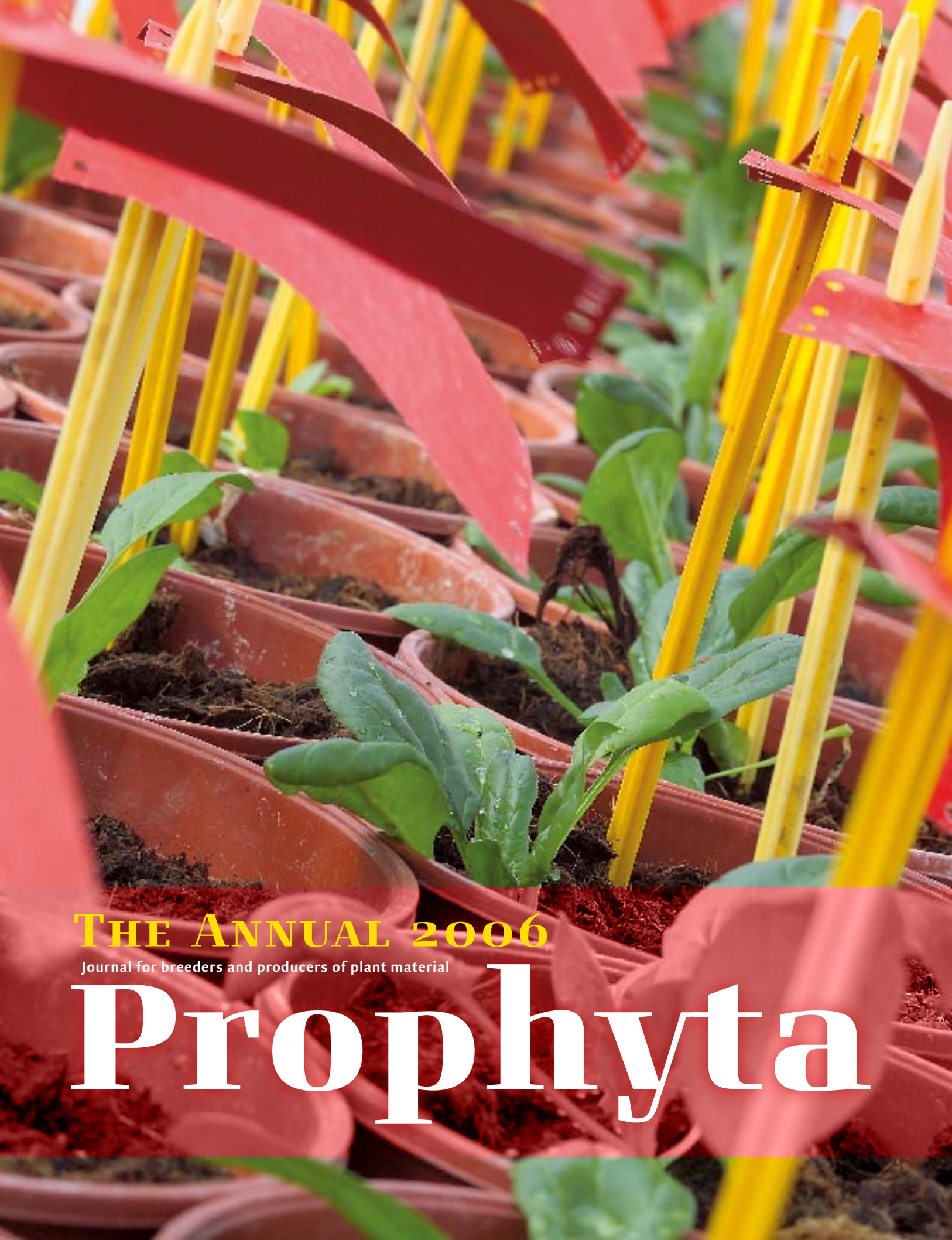

The Annual 2006

Journal for breeders and producers of plant material

Prophyta

Contents

Prophyta – The Annual
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cover photograph Rijk Zwaan

World Cup Football on Dutch grass

All German football stadiums where the World Cup matches will be played have had new grass. The organisation has chosen the mixture of Barenbrug because of its performance in heavy duty circumstances.

The mixture consists of English ryegrass and Kentucky bluegrass varieties that are tolerant to shade and form a thick layer of roots. Grass rolls of 2.4 metre width and a 4 cm thick were laid out in the stadiums, two to four weeks before the start of the World Cup finals on June 9th. These so-called big rolls make it possible to use the football pitches almost immediately. The German organisers decided that to create optimal circumstances for the football teams, the grass should be identical in all twelve stadiums in Berlin, Dortmund, Frankfurt, Gelsenkirchen, Hamburg, Hannover, Kaiserslautern, Cologne, Leipzig, Munich, Nuremberg and Stuttgart.

Search for Phytophthora-resistant potato

The Dutch Ministry of Agriculture has granted a 9.9 million euro subsidy to develop a genetically modified potato that is resistant to Phytophthora infestans. It is hoped that such a potato variety will halve the use of fungicides in potato. In the Netherlands 80% of all fungicides are used for potato growing. As Phytophthora also brings about more labour, a resistant variety would lower the costs in the primary sector by 150 million euro.
The research will be executed by Wageningen University and Research Centre and will take about ten years. Genes of resistant wild solanum varieties will be introduced in existing varieties. The research is focused on starch potatoes only.

Raspberries increase health

Raspberries have ten times as many anti-oxidants than tomatoes according to researchers of Plant Research International, Wageningen, the Netherlands. Moreover, besides vitamin C and anthocyanines the fruit also contains ellagitannines. Only strawberries have this substance too, but in much lower amounts. The researchers believe that ellagitannine is the effective ingredient in Chinese herbal medicines.
In test tubes ellagitannines have interesting characteristics. They are complex molecules that are probably fragmented during digestion. These small fragments are active in neutralising aggressive molecules in the body and prevent cells from being damaged.

Salt tolerant Arabidopsis created

In an article in Plant Science researchers show that Arabidopsis lines expressing YCF1 are also salt tolerant. Earlier it was proven that the so-called yeast cadmium factor 1, when introduced to Arabidopsis, allows the plant to grow in the presence of heavy metals.
According to the authors, this enhanced salt tolerance is due to the movement of salt ions from the cytosol to vacuoles, allowing the cell to maintain osmotic balance.
High soil salinity can severely limit agricultural productivity and lower crop quality. As saline soils are a growing problem in many countries, developing plants that can tolerate salinity is a key priority.

WTO reprimands Europe

The World Trade Organisation (WTO) has pronounced that EU member states should not close their borders to genetically modified plant varieties. According to the WTO the EU countries have not properly implemented the EU rules on biotech crops, nor is the EU approving products in a timely manner. The case was launched in 2003 by the USA, Canada and Argentina against the European Union. The complainants have been supported by a number of other countries including Australia, Brazil, Chile, China, Taiwan, Colombia, El Salvador, Honduras, Mexico, New Zealand, Norway, Paraguay, Peru, Thailand and Uruguay.
According to these countries the dispute over biotech crops is not about safety, as the crops are being grown around the world and have passed stringent food, feed and environmental safety standards. The UN Food and Agriculture Organization, the World Health Organization and the European Commission have all found that the approved crop biotechnology products on the market today do not show any risks to human health or the environment.
Farmers around the world have been choosing to plant biotech crops at unprecedented rates. Last year alone, more than 90 million hectares were sown with biotech crops by over 8.5 million farmers in 21 countries including European countries, such as the Czech Republic, France, Germany, Portugal, Romania and Spain.

New image should attract students

In the Netherlands a new approach has been developed to tackle the problem of the decreasing number of students that choose agricultural and 'green' studies. The marketing and communication company Pineapple Yellow discovered that these schools have a terrible image amongst youngsters. That is why future students don't visit open days, or if they do chose not tell their friends for fear of being ridiculed. According to them it are farmer schools, where the girls smell of cows and where everyone has dirty hands.
The communication company started to cluster the different trainings into seven perceptions with exiting names such as 'surprising nature', 'good food' and 'dynamic design'. The names are in English as using that language is trendy in the Netherlands. On the websites of the schools (www.aocterra.nl) new photography and new stories emphasise the exiting parts of the education, such as travelling to foreign countries, working with large machinery or using state-of-the-art technologies. The schools started to renew decorations to fit in with their new image.
The approach is now being translated into advertisements. The new approach seems to appeal to the teenagers. A first analysis by Wageningen University and Research Centre revealed that the number of students might be doubled or even tripled in this way.

Candy carrots

What does a candy bar have over a carrot? Certainly not its sweetness or crunchiness. Why then should children (and even adults for that matter) prefer a candy bar? It is not the taste of the snack, but the influence of a flashy packing, television commercials, shop displays and the multitude of other ways with which candy bar producers tempt consumers to eat more of their unhealthy snacks. According to the World Health Organization (WHO) there are more than 1 billion overweight adults, with at least 300 million of them obese. Among the youth the problem is rapidly increasing. On average 14% of boys and 17% of girls in Europe are overweight. This poses a major risk for chronic diseases, including cardiovascular disease, hypertension and stroke, certain forms of cancer and type 2 diabetes. The latter was confined to older adults for most of the 20th century, but today it affects obese children even before puberty.
The problem is not confined to North America and Europe, but is spreading throughout the world, often faster in developing countries than in the developed world. The cause of being overweight is well known: eating too much energy-dense food such as candy bars. According to the WHO, overweight will become the number one cause of death in the near future, overtaking cancer and cardiovascular diseases.
The seed industry has already found a way to help the world beating the latter two diseases. By identifying the protecting compounds in vegetables and creating varieties that contain more of these, they enable people to help themselves. Seed companies have the means to also relieve overweight problems. They already have created sweet carrots, colourful peppers, small-sized tomatoes and other vegetables that are suitable to become snacks, but more are needed.
With professional marketing and communication techniques to give these veggies a trendy, tempting and snack-like image a severe, worldwide problem might be solved. It is literally of life-saving importance that the promotion of healthy products such as carrots is taken up more firmly. If we start sowing that understanding now, it will certainly be a good beginning.

Monique Krinkels

BASF announces new starch potato

Next year the German company BASF will introduce a new, genetically modified starch potato on the European market. The new potato will produce the amylopectin (branched starch) and little or no amylase, which makes it more useful to starch production for industrial purposes. Normally, natural starches are mixtures of 10-20% amylose and 80-90% amylopectin. The latter is an insoluble substance. The potato was derived from the variety 'Prevalent', which was genetically modified. While not designed for human consumption BASF has executed a full health evaluation. The analysis of the DNA insert and flanking regions gave no cause for concern and it was concluded that sufficient evidence was provided for the stability of the insert structure.
BASF will increase the search for more transgenic varieties. The company has appropriated 825 million euro for biotechnological research for the next three years. One third of the money will be spent on transgenic crops. "Plant Biotechnology will play a crucial role in ensuring that the rapidly growing population continues to have a sufficient supply of high quality food. As such it is a key technology for the 21st century", said Dr. Hans Kast, Managing Director of BASF Plant Science. "We will compete with the Monsanto's and DuPont's of this world."

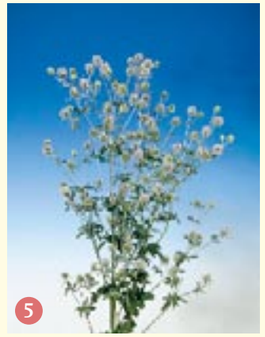
NAK introduces IRIS

The Dutch inspection service NAK has introduced a new internet service. The registration and information system IRIS enables seed companies and seed producers make their declarations for inspection online. It is also possible to follow the progress and results of the tests. IRIS became available for grain and fodder crops in March. Early next year seed potato growers will also be able to make use of the system. IRIS offers the advantage of a quick information exchange with the parties concerned. Furthermore it provides a 24-hours/seven days a week service. The efficiency is greatly improved as the data entry is fully automated and can be linked to management programmes.

Healthier and tastier tomatoes

A German-Israeli research team has discovered DNA fragments in wild tomatoes which could allow the development of healthier and tastier tomatoes. Researchers from the Max Planck Institute of Molecular Plant Physiology in Golm, Germany, in co-operation with Israeli scientists from Hebrew University, Jerusalem, Israel have created strains of tomatoes from crossing cultured and wild types. Their goal was to identify the biochemical composition of fruits and determine which factors control their development. The team used a combination of mass spectrometry and gas chromatography to analyse the composition of biological samples.
Dr. Alisdair Fernie, head of the Institute's central metabolism research group, discovered that there were 880 variations in the content composition in the descendants. "On one hand, we measured higher amounts of essential amino acids and vitamins, on the other hand the fruits showed an altered combination of various sugars and organic acids", Dr. Fernie says in Nature Biotechnology, March 12, 2006. "These contents have a great influence on the taste of tomatoes."
The scientists used molecular biological methods to identify parts of the tomato genomes responsible for biochemical changes. The researchers' findings could make it possible in the future to cross-breed wild tomatoes with cultured tomatoes in a targeted way to make them more nutritious.

Fleuroselect Gold Medal winners



This year Fleuroselect has awarded nine new flower varieties with the Gold Medal for innovative breeding and exceptional beauty.

'Sydney Blue Picotee' (Kieft Seeds Holland) adds a unique colour combination to the Sydney series: white with an attractive sky-blue edge. In addition, like the other Sydney's, it offers vertical branching, earliness and uniformity. (1)

'Presto' (Clause Tézier) is a so called semi-double, but produces more double flowers than any other and continues to flower over a significantly longer period. (2)

When the flowers of 'Noverna Clown' (Kieft Seeds Holland) open, they are white, but growing on they change to pink, salmon or violet. Flowering starts in the spring and continues well into the autumn. (3)

'Prairie Splendor' (Syngenta Seeds GmbH) is the first perennial Echinacea that flowers one hundred percent within the first year. Moreover, this variety is significantly earlier than any other Echinacea. (4)

The world's first 'FastraX' Eryngium 'Blue Glitter' (Ernst Benary Samenzucht GmbH) has high yields for fresh and dried cut flower production and adds interesting texture and colour as solitary planting in the garden and for the cut flower trade. (5)

Laurentia 'Avant-garde Blue' and 'Avant-garde Pink' (Thompson & Morgan UK Ltd) impressed the Fleuroselect judges by their earliness and their vigorous flowering. (6)

'Infiniti Scarlet' (Floranova Ltd) is exceptionally early flowering, with large blooms and an excellent garden performance. Until now, this combination of qualities has rarely been seen in seed-raised Pelargonium. (7)

The Fleuroselect judges' were charmed in their appraisal of Zinnia haageana 'Aztec Sunset' (Thompson & Morgan UK Ltd) by the remarkable bicolour mix and the amount of double flowers in comparison to existing cultivars. (8)

Austria bans transgenic rapeseed

The Austrian Minister of Health, Maria Rauch-Kallat has announced regulations to prevent the import of rapeseed variety GT73, bred by Monsanto. This variety has been allowed in the European Union since August last year. GT73 has been made resistant against glyfosates. The Austrian Ministry claimed that it was concerned about the allergic and toxic side effects the rapeseed might cause. Furthermore it said that the rapeseed might spread to non-GMO rapeseed. The ban extends through to the end of 2008, when the Minister will re-evaluate the scientific data. Earlier Austria banned three GMO maize varieties: BT-176 from Syngenta, MON-810 from Monsanto and T-25 from Bayer.

Support our initiative

The Prophyta Foundation is an independent non-profit organisation, aiming at informing interested parties worldwide about developments in e.g. plant breeder's rights, breeding techniques, genetics, biodiversity, technology, regulations, phytosanitary matters and more. Our communication methods include at present our Prophyta Annual and our website www.prophyta.nl
The Foundation primarily works with volunteers, but in order to recover costs for these activities we need advertisers for our annual magazine and/or direct financial support to the Prophyta Foundation.

We greatly acknowledge the companies mentioned underneath for supporting Prophyta, by either advertising or donating.

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Moerheim Roses and Trading	Leimuiden, the Netherlands	www.moerheim.com	Roses, bedding plants
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Naktuinbouw	Roelofarendsveen, the Netherlands	www.naktuinbouw.nl	Inspection, varieties, testing
Nunhems	Haelen, the Netherlands	www.nunhems.com	vegetable seeds
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Royalty Administration International	's-Gravenzande, the Netherlands	Info@royalty-adm-int.nl	Breeder's rights
Rijk Zwaan	De Lier, the Netherlands	www.rijkzwaan.nl	Vegetable seeds
SAHIN	Alphen aan den Rijn, the Netherlands	www.sahin.nl	Flower seeds
SBW	Roelofarendsveen, the Netherlands	www.stbw.nl	Tissue culture
Sande BV	't-Zand, the Netherlands	www.sandegroup.nl	Zantedeschia
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Please feel free to contact our secretariat (P.O. Box 40, 2370 AA Roelofarendsveen, the Netherlands, e-mail: info@prophyta.nl) to join membership of our distinguished group of agricultural and horticultural companies, both for further information or for donations.

In May Denmark shows at its best

Monique Krinkels

Jens Davidsen is looking forward to the ISF World Seed Congress 2006. Mid April he is almost certain that the targets the organising committee of the Danish Seed Trade Organisation has set will be met. "We hoped for 1,200 participants and it is looking very promising", the chairman says. He is pleased he will be able to show his home country Denmark to his colleagues from all over the world for the first time in over twenty years.

Jens Davidsen is understandably proud of his country. "Our farmers are about the best", he says. "They have laid down the basis on the success of our breeding and propagating industries." And of course he hopes that all delegates will take the opportunity to see more of his country. The last time that a world seed congress took place in Denmark was in 1984, when FIS held its yearly event in Copenhagen. For breeders it was even longer ago, as Assinsel convened in 1964 in the capital city. "We believe we have an excellent programme and social events for the delegates", he states.

Four organisations

These are hectic days for Jens Davidsen. As managing director and one of the founders owner of Vikima Seeds, one of the three largest vegetable seed producers, he has a busy job, besides organising the world seed congress. Vikima Seeds produces a wide variety of vegetable and flower seeds, although spinach is definitely the most important crop. Founded in 1983, production takes place in Denmark and also in France, where the company has had a subsidiary since 2001.
This annual congress is hosted by the Danish Seed Trade Organisation. It is the largest of the four seed organisations in Denmark. "In it the breeders and producers of field crops are united", Jens Davidsen explains. "Besides this we have a horticultural association, an association of cereal breeders and one for roses and other ornamentals."
When asked whether splintering breeders and propagators among four different organisations is not weakening their position, he firmly denies this. "We are all united in a trade organisation in which also farmers and growers are represented. We speak with one voice to the government", he states. However, he agrees that most regulations whether they are phytosanitary or else stem from Brussels. "Only few things are left to the individual countries", he remarks. But nonetheless the organisations have started talking about structural changes. "More cooperation might in the end be more efficient, especially as two of the organisations are rather small."
Denmark is a serious player when in comes to propagating material. It is the largest producer of grasses and clovers worldwide since DLF-TRIFOLIUM took over Cebeco Seeds three years ago. In the horticultural area the achievements of this Scandinavian country are also impressive with a leading position producing spinach seeds and a firm foothold in ornamental propagation material. Considering the size of the country (43,000 km2, of which 26,390 km2 is arable land) and the number of its inhabitants (5.5 million) these are remarkable facts.

Hygge

Culture in Denmark is permeated by the concept of 'hygge' which means cosy and snug. Eating together, especially during the dark winter evenings is therefore important. As food in Denmark is the most expensive in Europe consumers have high demands on quality. "However, the Danish public view GMOs as well as organics in a reasonably balanced way. Some are pro, some are against but with the Danish legislation in place each consumer has the free choice of buying what he or she likes," explains Ole Bech Bondesen, secretary general of the Danish Seed Trade Association.
Denmark stands number one in Europe when it comes to organic production. In 2004 6.2% of all agricultural produce was organic. More than 25% of the consumer's milk was organically produced. The 3,000 organic farmers had 160,000 hectare at their disposal. The total export of organic products was 267 million Danish crowns. Today, after years of continuous growth, the market for organics is stagnating. "At the moment we have a surplus of organic products in some areas, such as milk products. Therefore, we now see less interest among farmers to convert to organic production. For grass and clover seed the Danish area of organic has been 3,000 hectares in the two last years or 3.5% of the total Danish area with forage and turf. The quantities produced from the Danish crop of organic grass seed is probably enough to serve the total EU market. Lately we have seen a decrease of consumption of organic grass seed in the Danish market, probably a reaction of the slow-down in consumption of organic milk products", says Ole Bech Bondesen. He explains the situation with regards to vegetable seeds is much more complex as these are not as easily produced as grass seeds. "However, the Danish vegetable seed companies produce organic seed, but in rather small quantities."

As food in Denmark is the most expensive in Europe consumers have high demands on quality

Danish acreage with grass and clover seed in 2005

	Hectare	Tonnes
Red clover	327	121
White clover	4,757	2,293
Black medic	96	109
Clover total	5,180	2,523
Perennial ryegrass	36,393	43,397
Italian ryegrass	2,612	3,534
Hybrid ryegrass	1,438	1,712
Timothy	740	326
Small timothy	20	10
Cocksfoot	2,662	2,852
Meadow fescue	983	1,054
Red fescue	26,105	38,374
Sheep fescue	1,023	1,150
Tall fescue	2,747	3,309
Festulolium	262	265
Bentgrass	197	80
Rough bluegrass	243	258
Kentucky bluegrass	10,711	10,508
Grasses total	85,711	106,829

Co-existence

At present genetically modified plants are not an issue among Danish consumers. "The EU legislation on traceability and labelling is now in place giving consumers a free choice between conventional, GMO and organic food." Back in June 2004 the Danish Parliament adopted a law on co-existence which was afterwards approved by the European Commission. "Denmark was the first country in the European Union with legislation on co-existence. It means that on the level of production a set of rules is in place as to keep the production of GM plants, conventional and organic separated. The whole process of clearing the way for GM production has been promoted and supported by the farmers' organisations. In practice, no production has yet appeared on Danish fields except for some trials. The Danish climatic conditions are such that the GM varieties approved for cultivation, mainly maize varieties, have no particular advantages."

More to come

According to Jens Davidsen more is to be expected from his country in future. "We are discussing whether we can form an international agricultural university. He is not afraid that Denmark is too small for such an undertaking. Ole Bech Bondesen supports the initiative. "The idea is to bring all disciplines within agricultural education and research under one hat". The seed sector observed the need to strengthen disciplines such as biotechnology, plant breeding, seed technology and seed production science.
"A Nordic Agricultural University" would attract students from all over Scandinavia", Jens Davidsen believes. "Even from Germany or the Netherlands people might want to study in Denmark."

New species and new varieties of vital importance

Florentine Jagers op Akkerhuis

The pot plant sector in Denmark is small but sophisticated. Their growers are always on the lookout for exiting new varieties. Many nurseries are therefore actively breeding and selecting themselves. Besides new varieties of pot roses, Campanula's and Osteospermum, completely new pot plant species have been developed. With breeding and selection programmes the Danish breeders are securing their own future.

Danish pot plant breeders are enthusiastic, creative and assertive. For years they have been able to maintain a small but stable position on the world market with an exceptional assortment of flowering pot plants. Especially to neighbour Germany the export of pot plants is impressive. The biggest competitor confronting the Danish pot plant growers in Germany (and the rest of the world) are the Dutch. In the Netherlands the production of pot plants is many times higher, but they are nevertheless impressed by the Danish achievements. The Dutch and the Danes closely follow each others improvements in automation and especially the range of varieties. Every year the Danish achievements with new varieties are shown at the international trade fair IPM in Essen, Germany. Besides new colours and shapes of well known species such as Kalanchoë and Campanula, the Danes have also introduced new species, that are suitable for use as pot plants. Time and again the pot plant growers from Denmark have proved to be trendsetters in Europe. This year the trend is for small pot plants in large decorative pots.

Stimulated by competition

The many years of competition with the Dutch pot plant growers has been an important stimulus for the Danes to develop new varieties themselves, according to Jørgen Selchau, director of GPL International. GPL is an international organisation focused on registration and protection of new varieties. The company is specialised in ornamental crops and is based in Odense, in the middle of the Danish greenhouse area on the island Fynn. It was established by the two largest marketing cooperatives of growers of ornamentals, Gasa Arhus and Gasa Odense. Jørgen Selchau explains that the Danish pot plant growers have a long tradition in breeding and selecting novelties. "The Danes got tired of growing the old Dutch varieties and having to pay licences to them." In the seventies the Danes therefore started to breed plants themselves and that resulted in a large number of new varieties and species. Every year about hundred new varieties are added, according to the GPL director. Today, the Danes are famous in Europe for their novelties including pot roses, Kalanchoë, Schlumbergera (Christmas cactus), Aster, Osteospermum and Campanula. The breeding of these plants takes place in large, modern nurseries, which have in some cases their own breeding department. Roses Forever is one of these. The company started in 1996 as the daughter of two pot rose producers Rosa and Rosalina in Sabro on the peninsula of Jutland, north west of Arhus. Rosa Eskelund is joint owner and breeder. "We started as a small – 3,000 m² – pot rose producer at the end of the 1980s. We were not satisfied about the varieties that were available. The colours were disappointing and we had to pay expensive licences", she says. "That is why we started to breed and select ourselves." Rosa Eskelund manages the two nurseries together with her partner Harley Eskelund. The business is going well and their varieties are marketed successfully. "The demand for our varieties increased and nowadays our pot roses are grown on nurseries in the Netherlands, Norway, Poland, Japan and Korea." The Danish company takes up 15,000 m2 and has 60 employees now. It has developed a total concept, from variety to marketing including a brand and logo. "Our strong point is that we do the production as well as the breeding", Rosa Eskelund says. "Therefore we know what the market demands and we are able to break into new markets quickly. Our pot roses are doing well because we produce the colours and qualities the market demands."

'In the seventies the Danes got tired of growing the old Dutch varieties and having to pay licences to them. They therefore started to breed their own plants', explains Jørgen Selchau

Danish horticulture in numbers Horticulture in Denmark is a healthy sector, says the growers association Danskgartneri in their annual report of 2004. The production value was 4.2 billion Danish crowns (609 million euros). In the greenhouses the Danes produce mainly pot plants but also cut flowers and vegetables. In the open air it is especially arboriculture (Christmas trees) and vegetables that are important.
In 2002 there were 633 greenhouse companies of which 472 were growing pot plants. The number of nurseries is decreasing but the total area of about 500 hectares remains the same. So the world wide trend of scaling-up has also taken place in Denmark. More than halve of the nurseries can be found on the Southern island of Fynn where most of the expansion in size has taken place. Near Arhus on Jutland is a second concentration of greenhouses. Horticulture on the Eastern islands Sjaelland, Lolland and Langeland) has decreased.
The majority of the pot plants in Demark is grown for export. The total export value of the pot plants is 2.6 billion Crowns (378 million euros), while the home market is only 725 million crowns (105 million euros). Germany is the most important market with 33% of the export value. Vegetables and cut flowers are mainly grown for the home market.
The financial results of the nurseries has been under pressure in the last few years. The increased fuel prices had serious consequences for this northern European country. Furthermore, labour costs are high because the social legislation in Denmark has high standards for social services. Danish growers invest a great deal in expensive automation and Denmark is the number one in developing robots for logistics and sorting plant material.

Foreigners are impressed by the achievements of the Danes

Small nurseries

Not every Danish pot plant grower has the opportunity to set about their business on such a scale as Roses Forever. Many of the special novelties come from nurseries that are considerably smaller and that is the unique characteristic of Danish horticulture. "Preferably every grower has his own variety", explains Dorte Rhode Nissen. She is a biologist and consultant, who assists 20 smaller nurseries in their product development and breeding. "Many of these are too small or lack the knowledge to do this job themselves, that is why they leave part of the job to others. Sometimes it is only the administrative part, such as registration and application for plant breeder's rights", says Dorte Rhode Nissen. "In other cases I look after the complete breeding process, from finding the suitable material for crossing to the assessment of selections."
These smaller nurseries are especially good in finding species that are new to the pot plant business. Dorte Rhode Nissen says: "We are working on exiting novelties. Absolutely new is an Aloe species that is suitable for pot plant production. Another promising pot plant is a new variety of Muehlenbeckia. With these products Danish growers can stand out and that is an important marketing aspect."

Denmark is the number one in developing robots

photograph Roses Forever

Best of the world Pot roses and Kalanchoë from Denmark are household words. Poulsen Roser and for the last few years Roses Forever have also made a name with their large range of pot roses for houses and gardens. The Kalanchoë's of Knud Jepsen with the brand name Queen have conquered Europe. But it is not only the renewal of the assortment of well known pot plants that stems from Denmark. Osteospermum enjoys a growing popularity, thanks to breeder Sunny from Odense on the island of Fynn. With a large range in several colours for inside and out this company is doing well. PKM is renowned for its Campanula's. This company from Odense uses the famous fairytale writer Hans Christiaan Andersen in its marketing and the Fairytales Flowers can be recognised easily at every point of sale. Besides Campanula's, PKM also has a wide offer of lavender and cactuses (Schlumbergera and Rhipsalidopsis). In addition, many of the novelties in Euphorbia, Pelargonium, Solanum and Exacum come from Danish nurseries. The websites heralding 'Danish growers best in the world' can be found on www.sunnyaps.dk, www.rosborg-as.dk, www.group-unique.com/queen, www.pkm.dk, www.rosa.dk, www.tingdal.dk and www.ex-plant.com.

The Danish pot plant growers pay much attention to marketing and presentation. Several growers have registered their own brand. Some do it on their own, such as Queen Kalanchoë of Knud Jepsen and Fairytale Flowers of PKM. Others introduce a brand together, like the grower association Living Colors, which has several flowering pot plants, or Addenda, a cooperation of trade company Young Plants International and nursery Kjaergardsminde. Also the promotion organisation FloraDania is very active with some collective initiatives. A catalogue with the complete Danish pot plant assortment has been published, especially for the IPM. De Danes present all their products and use the country of origin Denmark as a quality characteristic. The largest competitor the Netherlands recognises that quality is one of the strong points of the pot plant assortment from Denmark.

World wide

The Danish breeding and selection of ornamentals is a combination of traditional crossing and an open eye for special plants. They do not rely on expensive technology so the costs are relatively low. It is important however to take care of breeder's rights as the trade in ornamentals is an international business. Danish propagation material is available throughout the whole world. These last few years the demand from Asia has increased, especially from Japan. Two Danish organisations, GPL International and PLA, take care of international breeder's right for the growers. An increasing number turn to the Dutch company Royalty Administration International of Nellie Hoek. Dorte Rhode Nissen: "It is important to also take care of the international breeder's rights. That is expensive, but vital to ensure income and therefore the future of the Danish nurseries." The margins in the Danish pot plant industry are small and it demands much more entrepreneurship. First of all the climate conditions in this Scandinavian country are more difficult. There is much less sunshine than in countries such as the Netherlands. Besides, labour is extremely expensive and the rising prices for fuels of the last few years are a source of concern. The marketing system of the Danish pot plants changed drastically in the early 1990s and that has not made things easier. Part of the trade has been transferred to the Netherlands and the transport to the most important destinations, the Dutch flower auction and the German wholesalers has become expensive. Thanks to their reputation and their exceptional assortment the Danish pot plant growers are able to hold their own. The development of new varieties is vital in their line of business. But luckily the Viking blood still streams strongly in the veins of many Danes and the pot plant branch has the fighting spirit it needs to survive.

Denmark offers ideal environment to multiply vegetable seeds

Monique Krinkels

For vegetable seed companies the word Denmark is synonymous with spinach. Not that the Danes are overly enthusiastic spinach consumers, but in spinach seed production they are the absolute number one in the world. This fact gave reason for Henning van Veldhuizen to emigrate from the Netherlands to Denmark in the eighties and join the vegetable seed company Jensen Seeds A/S. In 1992 he became main shareholder.

The production of vegetable seeds is an important part of the Danish seed industry. Spinach is by far the most important species the country multiplies. Of the nearly 5.5 thousand hectares that is used to produce vegetable seeds, 4.3 thousand are planted with spinach plants. This figure is increasing every year. "About seventy percent of all the spinach seeds in the world are grown in Danish soil. There are several reasons that Denmark has become the number one in the world", says Henning van Veldhuizen. "Climatic conditions, the latitude, the soil as well as the attitude of the Danish farmers and the way agriculture is structured in Denmark all play a vital role in making this country unique."

Ideal environment

To start with the latitude, Denmark is situated on the 55th Northern latitude, making the days in summer exceptionally long. In June and July plants receive 17 to 18 hours of daylight. "This is especially relevant to spinach, as breeders have created late varieties. These have the advantage tending not to set seed too quickly, which is a clear improvement for the growers. These plant varieties need more light, i.e. longer days to induce bolting, to produce seeds." The soil is very fertile. "We have a moraine soil, originating from the last ice age 10.000 years ago. It has a high pH value, which is excellent for vegetable growing", Henning van Veldhuizen explains. The climate is also favourable with its moderate temperatures and sufficient rainfall. There are therefore no environmental obstacles to produce spinach seeds. But there is more to it than that. Spinach is highly susceptible to pollination by unwanted pollen, as it is a wind pollinator. "Luckily spinach is not a very popular vegetable in Denmark. The scale on which the end product is grown is therefore limited. Besides, the Danes do not often grow spinach in their private gardens, minimizing the risk for inadvertent pollination. We have an agreement that the distance between spinach fields must at least be two kilometres and zones are created for the different varieties in order to reduce cross pollination." The farmer's attitude is highly professional. Their farms are well equipped with their own sowing machines, combines and drying/storage facilities. "We have contracts with 200 farmers, who in total have an available area of 2,500 hectares. Danes are true agriculturalists, so we do not have to compete with open field vegetables. Spinach is ideal replacement in crop rotation with for instance wheat", he says. Farmers in Denmark have large fields making it possible to have one farmer producing seeds on contract counting 5 to 40 hectares. "We do not need to bring in the seeds after harvesting. The farmers store the seeds until further notice. Only if our client asks for the seeds, it is delivered at our facilities in Birkum near Odense on the island of Funen."

Henning van Veldhuizen has serious ambitions for his company Jensen Seeds: 'The company will be so well known that if seedsmen think 'spinach seeds' they automatically think of Jensen Seeds'

Factory

Jensen Seeds is one of three major vegetable seed producers in Denmark. "We have expanded following the increasing production in Denmark. The area of spinach seeds has almost tripled in the past decade." The product has gained popularity worldwide as can be seen from the table. All the way from China to Guatemala and from Cyprus to the USA the production of spinach has increased. It is an easy product, since it can be sold as a deep frozen vegetable, fresh pre-packed spinach, as well as in the so-called baby leaf salads. "We multiply more than eighty varieties

History

According to Wikipedia, the internet encyclopaedia, the history of spinach is a long one. It was first cultivated in Southwest Asia. Iran is often named as its birthplace, because in a Chinese manuscript of 647 AD the vegetable is named 'the herb from Persia'. The word 'spinach' is derived from the French word 'espinache', which originates from the Persian word 'esfenaj'.
At the end of the first millennium, spinach travelled through Syria and Arabia to North Africa. In 1100 the Moors introduced spinach in Spain. Over the next century, prickly seeded spinach was spread throughout Europe being grown primarily in monastery gardens. A cookbook dating from 1390 belonging to King Richard II contains spinach recipes. Smooth seeded spinach seems to have spread through Europe shortly later. Its use in England was first documented in 1551.

Jensen Seeds has a full automatic processing line especially built for spinach

Most of the varieties produced nowadays are hybrid spinach late type (monoecious)

Main exporting countries of fresh spinach

COUNTRY	QUANTITY (million tonnes)	VALUE (x 1000 euro)
USA	28,614	22,406
China	12,135	1,532
Malaysia	9,501	1,836
Italy	7,548	9,298
The Netherlands	6,807	1,900
Mexico	6,374	2,323
Spain	5,742	9,506
Belgium	3,837	899
Canada	2,399	3,714
Portugal	1,391	201
France	1,262	2,103
Cyprus	1,018	939
Germany	654	366
Guatemala	274	18
Turkey	132	51

Source FAO 2003

Main importing countries of fresh spinach

COUNTRY	QUANTITY (million tonnes)	VALUE (x 1000 euro)
Canada	29,580	22,381
The Netherlands	12,922	3,135
Belgium	10,504	2,519
USA	9,210	7,429
Germany	5,379	4,324
United Kingdom	3,506	8,479
Singapore	3,122	2,164
Italy	1,804	1,370
Mexico	1,773	1,247
Switzerland	1,187	2,030
Sweden	1,078	112
France	1,074	1125
Portugal	552	844
Greece	379	363
Denmark	339	629

Source FAO 2003

Due to the excellent conditions almost all spinach bred in the Netherlands is multiplied in Denmark

photograph Rijk Zwaan

of which most are monoecious. Today eighty percent of the varieties have pistils as well as stamen, but twenty years ago the dioecious types, were common." When the seeds arrive in Birkum there are further preparations in the 'factory' of Jensen Seeds A/S. The company has four completely fully automatic processing lines to process the seeds. The capacity is 4,000 tons a year. "We had to automatise this, as labour is very costly in Denmark. Our staff is mainly focused on checking the process on the screens of their computers and maintaining the machinery. In 2003 Henning van Veldhuizen acquired a full automatic processing line especially built for spinach. "All processes are in line from pre-cleaning, debearding, brushing, air screen cleaning, indented cylinder, gravity separator, sizing/grading to colour sorting", he says. In 2004 an automatic PLC controlled processing line for all products that are sensitive to mechanical damage was added. Hybrid spinach seed is not the only product the company is involved with. "We also produce seeds of scorzonera. Denmark has 80% of the world production of that vegetable. Additionally, we have Chinese cabbage, hybrid pak choi, mizuna, radish, chrysanthemum garland, peas and the herbs chervil, chives, parsley and thyme", Henning van Veldhuizen informs.

Future

In Henning van Veldhuizen's view the worldwide market for spinach will steadily increase, because over the years spinach has become more accepted as a complementary vegetable in green salads. In general, people all over the world are more focused on healthy eating, which makes spinach an interesting supplement to mixed salads and in frozen meals. "But the market is very fragile. At the moment the demand for spinach is fulfilled, but should a new trend be to exchange the salad in hamburgers to spinach, the worldwide production could not keep up! On the other hand, if the demand for spinach in Asia suddenly decreased, this would also have a significant effect on production."
He is concerned about the lack of interest students have for breeding and seed production. "It is an unexpected and serious problem that seems to have grown worldwide too during the past few years. It is nowadays very difficult to find young people with seed expertise. That is a serious, indirect threat for the seed industry", Henning van Veldhuizen concludes.

Health

Even today consumers are convinced that spinach is the best source for iron. With 3.2 mg of iron in 100 grams of boiled spinach it has a relatively high level of iron, but the iron in vegetables is less easy to absorb in a human body than for instance from meat. The myth probably originates from a publication of Dr. E. von Wolf in 1870. He misplaced a decimal point, which led to an iron-content figure that was ten times too high. It took until 1937 before this mistake was officially corrected. Notwithstanding that, spinach is very healthy with high levels of vitamin A, vitamin C, vitamin E and several vital antioxidants.

Danish area with vegetable seed

YEAR	TOTAL VEGETABLE SEED (ha)	SPINACH SEED ha	%
1992	1,466	830	57
1993	2,057	1,327	65
1994	1,949	1,241	64
1995	2,626	1,576	60
1996	2,859	1,981	69
1997	3,456	2,067	60
1998	3,336	2,150	64
1999	4,210	3,168	75
2000	4,391	3,591	82
2001	4,430	3,672	83
2002	4,192	3,403	81
2003	4,776	3,958	83
2004	4,835	3,666	76
2005	5,475	4,316	79

CAP-reform has enormous consequences

Monique Krinkels

The EU sugar sector faces a radical reform. Over the next four years, European sugar beet growers will see the price level of beet dropping 40% while sugar prices are estimated to decrease by 36%. Sugar beet seed companies will have to rely on R&D investments to boost competitiveness of sugar beet versus sugar cane. A unique challenge lays ahead of them.

The geographical spread and split of crop acreage may shift over time. Breeders then need to adapt their programmes to a different environment by introducing new characteristics in the varieties. Marketing departments will have to open up and develop new markets. For sugar beet the story is very different as this product can be replaced by sugar cane.

Cane versus beet

A large majority of the sugar produced in the world comes from sugar cane, namely 110 million tons against 34 million tons of white sugar produced from sugar beet. The European Union is an important player in this field as 20 million tons of white sugar is produced in the 25 member states. Measured in area the differences are even more staggering with 26 million hectares of sugar cane against 5.8 million hectares of sugar beet. However, not all sugar cane is used for sugar production as it is also the raw material for bio ethanol, a major fuel produced in Brazil. For the last forty years the sugar regime in Europe has been based on two regulations. First the EU has established quotas per country which fixed the sugar production. To prevent fluctuations in production there also was a B-quota which had the possibility of being exported to the world market. Secondly, fixed prices for sugar are established, the so-called intervention price. If the sugar or the sugar containing products are exported to non-EU countries the exporter receives a restitution.

Reform

In November 2005 European Union agriculture ministers formally adopted a new sugar regime. The sugar price in the EU was three times higher than the world market level and some cane producing countries no longer accepted the subsidised exports from EU countries. The WTO panel ruled that these export subsidies were not in line with international trade rules. As a consequence, EU initiated a reform of its sugar regime and included sugar beet in the CAP reforms of 2003 and 2004. The key to the reform is a 36 percent cut in the guaranteed minimum sugar price and a restructuring fund to encourage uncompetitive sugar producers to leave the industry. It will provide a financial incentive to close down sugar factories, convert them to other uses and retrain workers. Also farmers will be compensated by direct payments, linked to the fulfilment of strict environmental and land management criteria. The way this is done varies by country, but it is expected to be at an average of 64% of loss of revenue from growing sugar beet. The EU will further stimulate farmers to diversify to other products. Countries which give up more than half of their production quota will be entitled to pay an additional coupled payment of 30 percent of the income loss for a temporary period of five years. Some developing countries, the so-called ACP and EBA countries, will continue to enjoy preferential access to the EU market at attractive prices. These are countries in Africa, the Caribbean and the Pacific, with whom there is a treaty focussed at integrating them in the world economy.

Countries producing sugar cane

COUNTRY	PRODUCTION IN MILLION TONS
Brazil	411,009,984
India	244,800,000
China	92,000,000
Thailand	63,707,272
Pakistan	52,040,000
Mexico	45,126,500
Colombia	37,100,000
Australia	36,892,000
Philippines	28,000,000
USA	27,501,310
Indonesia	24,600,000
Cuba	24,000,000
Argentina	19,500,000
South Africa	19,291,800
Guatemala	18,000,000
Vietnam	16,600,000
Egypt	16,335,000
Venezuela	8,800,000
Myanmar	7,500,000
Iran	6,500,000

SOURCE: FAO

Countries producing sugar beet

COUNTRY	PRODUCTION IN MILLION TONS
France	29,419,000
USA	27,002,000
Germany	25,486,732
Russian Fed	18,500,000
Turkey	13,965,000
Ukraine	13,660,000
Poland	11,471,800
Italy	10,000,000
United Kingdom	7,600,000
Spain	6,726,400
China	6,300,000
Netherlands	6,200,000
Iran	6,050,000
Belgium	5,914,200
Morocco	4,560,000
Japan	3,867,000
Czech Republic	3,367,380
Austria	3,045,000
Egypt	2,860,547
Denmark	2,857,295

SOURCE: FAO

'Throughout the world, the demand for sugar and energy from sugar beet is increasing rapidly. In a few years, new market opportunities for sugar and bio ethanol may also open up for our European farmers', says Philip von dem Bussche

(photograph copyright KWS Saat AG)

According to Philippe Rousseau sugar beet can complement or substitute cane. 'The crop has very attractive characteristics, which could be profitable to farmers in tropical areas'

(photograph copyright Syngenta Seeds AB)

One of the issues the treaty covers is, that the EU used to buy sugar from these countries at EU internal price. Those ACP countries which need help to deal with the new sugar regime will be eligible for an assistance plan worth 40 million euro for 2006. From 2009 the EU will open its market completely to imports from the world's 49 poorest countries.

Huge impact

According to the commissioner of agricultural issues, Mariann Fischer-Boel, the EU sugar production is expected to fall by between 6 and 7 million tonnes. "The new sugar reform will have a huge impact on the seed industry. It means a loss of business of over 25% in the EU market within the next four years", says Philippe Rousseau, crop manager sugar beet of Syngenta Seeds. "Of course we have anticipated and restructured our activity", he states. For seed companies he sees two solutions to cope with the loss. "You either chose cost savings or you try to strengthen your position. Cost savings would mean that breeding new improved varieties would come to a stand still. Syngenta has chosen a different path. We maintain our R&D investments in order to keep on developing innovative new varieties." The expected loss in the European Union might be offset by growing markets such as Eastern Europe, Turkey, Iran and NAFTA. "Besides we trial sugar beet growing in the subtropical and tropical countries. Although they have no tradition in sugar beet growing, sugar beet can complement or substitute cane. The crop has very attractive characteristics, which could be profitable to farmers in tropical areas. For instance beets need far less water than cane and has a good tolerance to saline soils. That makes it an interesting crop for countries where adequate potable water is the limiting factor. Ten years ago Syngenta set up breeding programmes to create varieties for tropical areas." Philippe Rousseau names other advantages. "Another benefit of sugar beet is that it only takes six months to grow. That makes it a good rotation crop." Breeding for tropical conditions means of course a whole new challenge. "We have to look for resistances and tolerances to diseases that do not occur in cooler climates. But of course our core markets remain the Europe and NAFTA."

Improving productivity

"It will be a particularly tough challenge for KWS to implement a forward-looking strategy in the totally new environment resulting from the sugar market reform", says Philip von dem Bussche, member of the executive board of KWS and responsible for sugar beet. "In four years time the current price of around 44 euros per tonne will decrease to 26 euros." In the medium term he foresees that cultivation will be concentrated in the most favourable locations in Central Europe. "This is the only region where competitive production will be possible at a price of 26 euros per tonne. As a result, cultivation in France, Germany, Benelux, the United Kingdom and Poland will probably remain stable. At the northern and southern extremities of the EU, however, cultivation is likely to decline rapidly, heading toward a complete standstill in just a few years." To further strengthen the market for sugar beet against competition from sugar cane, KWS will have to make more progress in improving the sugar beet productivity. "Until now, our yield has grown by around 2% per annum. This was attributable mainly to seed. Some farms and regions are already achieving and even exceeding a sugar yield of 15 tonnes per hectare. The KWS breeders aim to ensure that 15 tonnes of sugar beet becomes a realistic target yield for a large number of farms within just a few years", Philip von dem Bussche claims. "This breeding progress alone will enable farmers to compensate for a significant proportion of their lost profits over the coming years." In future, further variety developments will enable farmers to continue to control serious diseases affecting sugar beet production. "In many regions, such diseases would otherwise force farmers to

Sugar beet definitely has advantages over sugar cane, as well for the farmers as for the food industry

(photograph copyright Syngenta Seeds AB)

discontinue growing sugar beet. With the increasing density of cultivation in the vicinity of sugar refineries and the expansion of rapeseed cultivation, problems such as the sugar beet nematode present a serious threat to viable production. KWS has developed highly superior varieties in this segment."

Outside Europe

Philip von dem Bussche also sees opportunities outside Europe. "There are extensive markets for sugar beet seed, in Russia for example, Ukraine, USA and Turkey. Some of these markets are still dominated to a certain extent by local, uncompetitive varieties. There is certainly scope for us to increase our sales here." Besides KWS has genetically modified sugar beets that will be marketed in the USA in the near future. These crops will require significantly lower levels of herbicide treatment. "Until now, the use of multiple pesticides has created a significant cost burden for sugar beet cultivation. The replacement of pesticides with genetic technology will significantly increase sugar beet competitiveness, resulting in greater added value for the farmer and breeder", he explains. For SESVanderHave the new sugar regime is one more stimulant to invest in breeding new varieties. "Sugar beet is our only product", explains Rob van Tetering, commercial director of the company. "We invest 15% of our turnover in developing new varieties and that figure will remain the same in the years to come." The goal of the breeders at SESVanderHave is to increase the sugar production per hectare and to introduce varieties with disease resistances. Rob van Tetering sees opportunities in Eastern Europe, Russia and Ukraine. "In these countries the more productive varieties that are developed by Western European companies will rapidly gaining ground."

Strengths

The beet sugar industry developed a unique expertise which will be key to ensure the development of new uses for sugar beet, Philippe Rousseau claims. "Next to food industry needs, sugar is also used in pharmaceutical and chemical industries with strict requirements of traceability. In addition, the EU bio fuel policy is a clear possibility to develop the utilisation of beet for producing bio ethanol. With a continuously increasing worldwide sugar consumption and decreasing arable land, we remain convinced that the beet sugar industry keeps a positive future. The recent development of sugar prices on the world market is a clear sign." Rob van Tetering is convinced the high oil price will increase the interest in bio ethanol. "We believe that countries in Europe, Asia and Africa will in time switch over to this new fuel." Philip von dem Bussche agrees: "Throughout the world, the demand for sugar and energy from sugar beet is increasing rapidly. Because Brazil alone is unable to meet this demand, sugar prices have already risen sharply on the world market. In a few years, new market opportunities for sugar and bio ethanol may also open up for our European farmers. But these opportunities depend to a great extent on our breeding progress."

Dutch institute bridges gap between science and the field

Florentine Jagers op Akkerhuis

TTI Green Genetics

In research institutes much scientific knowledge is available. However, this knowledge does not always link up with the questions that the plant breeding and propagating sector has to cope with. This paradox is the reason for the foundation of a new top institute in the Netherlands, that will bridge this gap. Expectations run high.

The queen of hearts in the famous book Alice in Wonderland by Lewis Carroll had an interesting message for the girl travelling with the white rabbit. In the country where she had gone to in her dreams, her running was just sufficient to remain on the same spot. To go further Alice had to run at least twice as fast as the others. Nowadays the same goes for the world of scientific research in plant breeding and propagating. Going at the same speed is not enough anymore. For years Dutch companies as well as institutes have had a prominent role on the international scene. However, to maintain that position more effort is needed. Plantum NL, the Dutch association for breeding, tissue culture, production and trade of seeds and young plants, developed an ambitious plan, to help the 'green' industry to keep its leading position. Their goal is to found the Technological Top Institute Green Genetics that will bridge the gap between science and the field. The Dutch companies and institutes together stand surety for the 20 million euros that is needed for financing the plan. This spring, the Dutch government granted the initiative another 20 million as further financial support. The Dutch government views the plan as a structural impetus for the agro-food branch, which is an important pillar of the economy.

Research projects – The increasing efficiency of cultivation methods places higher demands on the quality of seeds. Fast and uniform germination is a basic requirement. Not only the genetic characteristics of a variety determine the germination quality of the seeds, the circumstances during the growth and the health of the seeds are also important. Research of TTI Green Genetics will provide new knowledge of the relationship between plant physiology, environment and genetics. These insights can be subsequently used to further improve seeds quality of lettuce for instance by using certain coatings. Another example of research that could take place within the top institute is increasing the disease resistance of the lily, an economically important crop in the Netherlands. Insight in the molecular background of crossing barriers will contribute to crossing for new resistances in lilies. A third example of research is to obtain more insight in the chemistry and plant physiology of volatiles that plants can produce. Aroma of flowers and and taste of vegetables are becoming more and more important characteristics in the eyes of the consumer. It is therefore essential to keep this in mind when breeding. Knowledge is required on the regulation of the emission of volatiles by the plant. Breeders can use these insights to improve the quality of the end product.

Knowledge paradox

Over the past few years policy makers in Europe and the Netherlands have been worried about the phenomena that while universities and institutes develop high-quality knowledge, the business community does not profit from the increasing know how. At the same time, modern economy has become more and more dependent on new science and technology. This so-called knowledge paradox is seen as a serious threat of the international competitiveness. By founding Technological Top Institutes (TTI's) the Dutch government wants to change this. In these top institutes public-private cooperation in research and development is possible. There are by now four institutes covering the fields of polymers, metals, telematics and food sciences. TTI Green Genetics, that is in the process of being established, follows the concept of these successful initiatives. Besides the government, partners in the new top institute are several breeders, seed companies, producers of propagation material, biotechnology companies and the Dutch universities, such as the Plant Science Group of Wageningen University and Research Centre. The various projects will be carried out at the participating organisations and companies. The aspiration is to start with the plans as soon as possible to have TTI Green Genetics operative for at least four years.

Market leader – The Netherlands is of old an important player in the field of plant propagating material. In plant breeding the Dutch research institutes still play a leading role. Eight out of ten of the largest vegetable seed companies have either their head office or an important office in the Netherlands. For propagating material of ornamentals as well as in seed potatoes the Netherlands is market leader. The export value of the Dutch propagating industry was 1.6 billion euros in 2004, twice as much as in 1988. It means that in 2004 24% of all exports of planting material world wide came from the Netherlands. For the Dutch economy the sector is of vital importance. The turnover is in total some 2.5 billion euros. It provides work for about 10,000 people, of which many have higher education qualifications. Furthermore, the quality of the propagation material is an important contribution to the thriving agricultural production chain.

Ambitions

The work of TTI Green Genetics will concentrate on the central theme 'innovative plants for sustainable flowers and food'. The research will be focussed on three important disciplines: genetics, phytopathology and plant physiology. In the plans two fields of activity are identified, namely the so-called core research and the cluster projects. The top institute will formulate and evaluate research proposals and will contract these out to research institutes. The goal of the core research is to enlarge the fundamental knowledge of growth and development of plants and their interaction with the environment. This new knowledge will then form part of the curriculum of the participating universities. The cluster projects have a common aim that they should generate knowledge and expertise that will strengthen the competitiveness of the business community. These projects are subdivided into three themes, namely growth and development, plant health, and quality for the end user. The programme has ambitious objectives when it comes to results. The expectation is that the participating companies will become more efficient in their breeding programmes. They will develop more new varieties for which they can apply for plant breeder's rights. The goal is to increase the revenues of the participating companies by three percent per year. They will also strive to obtain new patents and according to the initiators there should be at least four new patents a year. Furthermore, the top institute will offer 15 PhD students the possibility to write on their thesis. Participating researchers will produce a continuous stream of scientific papers in renowned magazines to report their findings and at symposia they will spread their knowledge.

Confiscations come as a shock

Annet Lamers

The battle against propagation without permission of the breeder is flaring up again. Breeders have taken the initiative to confiscate products or start legal procedures to collect the royalties. These actions have caused a shock wave in several countries. But it is not a battle for money only. It is meant to educate people and to point out the legal restrictions. The goal is to give growers who abide by the rules the opportunity to be more competitive.

It has become increasingly difficult in the last few years to uphold breeder's rights in some countries. The European breeder's rights itself offer a good protection of varieties. The security of newly developed technologies with patents is also well established. The problem is upholding the rights. That is partly due to the fact that varieties are grown in an increasing number of countries where repeated cropping as a spin off occurs more and more. Controlling repeated cropping is often difficult as the authorities in the countries concerned do not always want to cooperate.

Alarming developments

There are a number of developments for seed companies as well as breeders of vegetatively propagated ornamentals. Anton van Doornmalen, president of the vegetable section of the International Seed Federation ISF is worried about three developments. First he names the increased repeated cropping of true to type varieties, such as lettuce. It is relatively simple to grow lettuce from illegally obtained seed, even if the seeds are less uniform and have a lower quality. In the last five years the incidents with hybrid seeds, which are not easily reproduced, are increasing due to a new technology. But it happens also that material of one or even both parental lines are stolen and then reproduced, Tomatoes can be reproduced vegatatively by a simple cutting of a hybrid plant. The plants will grow less uniformly and have a greater risk of getting a disease, but some growers do it because of the low costs.
Rijk Zwaan, of which Anton van Doornmalen is the managing director, has encountered this problem with an aubergine variety in China. "If we sell one seed in that country, they will have six plants on the market. It happened two years ago and also last year. By the end of this season we will know how it went this year."
A third development that worries Anton van Doornmalen are the increased costs for breeding varieties and for introducing them onto the market. "The seed industry cannot survive without a clear and effective system for the protection and upholding of plant breeder's rights and patents. That is also in the interest of our customers."

Anti infringement bureau

According to Anton van Doornmalen the larger seed companies are considering forming an anti infringement bureau. "We are monitoring what is going on in the various countries, how things are organised and which authorities are responsible. The goal is to assemble all the information, in order to work out the way we can uphold the regulations better. A global, independent anti infringement bureau would be perfect but is not easily organised. It will be difficult enough to do it in Europe and it is still years away. That is why we will first improve the cooperation with the national authorities in the countries concerned and find lawyers who are specialised in breeder's rights."
At the moment this is done by the national seed organisations and the European Seed Association (ESA). Anton van Doornmalen is on the board of the vegetable section of ESA. As upholding is as yet a national affair, he emphasises the importance of the national sector organisations. "If you want to uphold your rights, you need the opportunities the national laws and regulations are giving. But the national seed associations are then of overriding importance.

Improving communications

Another important activity the vegetable seed companies in ESA are working on is the improvement of communications on intellectual property. "We want to improve communications within our sector, amongst others towards the seed companies in Eastern Europe and Turkey, but also towards third parties such as our customers and the supermarkets. For many it is unclear what plant breeder's rights contain and what the consequences of infringements are", says Anton van Doornmalen. "After all, under certain circumstances European breeder's rights can be enforced even at the retailer end. Many supermarket chains were unaware of that. We know that there is illegally produced lettuce in European supermarkets. If we can prove the lettuce is produced using illegally obtained seeds, we can enforce the supermarkets to empty their shelves. For growers it is also important to know what the consequences are of using illegal seeds. We will therefore inform the EurepGAP-organisation to make this clear, so that they can issue directions for the growers and their organisations."

Within the ornamental sector the battle against illegal propagation has been going on for some time, especially with the vegetatively propagated varieties. Maarten Leune, director of Royalty Administration International (RAI) says that Ciopora has been active to improve the facilities at customs in the European Commission. The outcome is that breeders can effectively confiscate illegal imports at the borders. This has resulted in a series of concrete cases often being done in cooperation with other companies. Peter van der Weijden, licence manager of the breeding companies Hilverda and Florist, has become the key figure in these cases. He has coordinated several actions which have led to lawsuits and confiscations of illegally imported flowers. He was successful with an action on the border between Romania and Hungary, in cooperation with the customs and an Hungarian representative of Kooij, who also acted as a translator. He has benefited from the information he obtained from the French rose breeding company NIRP, which has experience with these type of actions. At Moerheim New Plants they also know the ropes. This company has even employed their own man to check growers in Europe on illegal repeated cropping. According to Theo Ruys, director of Moerheim, this man easily recovers the cost of his salary. In the 'old' EU there is hardly any illegal repeated cropping. "It are mainly the countries on the fringes of the present EU, Southern Italy, the edges of Portugal and Spain, Eastern Europe, especially Poland and the Czech Republic. It happens there partly because the growers are not used to these regulations. Especially with summer flowers we found second and third propagations. But the situation is not so bad as five years ago. It has improved because we have educated the growers. We have been very active there. They now know that repeated cropping and using cuttings is illegal. These last few years we have tried a more positive approach, to improve the relationship with the growers."

Change of mentality

René le Clerq, president of the Dutch Seed Organisation Plantum NL and managing director of Fides, emphasises the importance of good relationships with propagators and growers. "When our customers are aware that a good relationship with the breeder is also in their interest and that using the newest varieties has a positive influence on the financial results, we will create a win-win situation. In newly producing countries it is different, as they often do not have the regulations that forbid propagation of someone else's plants. And when regulations have been instituted, as is been done step by step for a number of species in China, there should be authorities who check and have the power to punish infringements. In the ornamental sector we have specialised companies that check worldwide, establish infringement and collect license fees for the breeders. But even these companies have to rely on local authorities. Without local support there is not much one can do."
He welcomes the confiscations and lawsuits. "These actions cause a shock wave through these countries. It results in people respecting the law and pay royalties to continue legally. The ultimate goal is to collect royalties and stop infringement but that does not work out without a change of culture."

Serious consequences

Turkish carnations

Action: Confiscation of carnations grown in Turkey at the Romanian-Hungarian border.
When: March 2006, in the week before the English Mothers Day.
Initiative of: Carnation breeders Hilverda, Selecta Klemm and P. Kooij & Zonen.
Reason: In the Antalya region in Turkey a group of one hundred growers grow carnations. These flowers are not protected by the Turkish breeder's rights. However, when carnations are exported to Europe the EU breeder's rights apply. Therefore royalties should have been paid to the breeders. Visiting the 25 largest nurseries and the most important British importers to explain this did not have the desired effect.
Proceedings: Three trucks were stopped with carnations of the two largest growers and one exporter. The flowers were destined for the British market. The consignments were partly destroyed. The Turkish growers and exporters were shocked. At the moment discussions are taking place with the Turkish organisation Antalya Exporters Union and with the largest nurseries.

Spanish carnations

Action: Lawsuits against Spanish nurseries that produce carnation cuttings.
When: Spring 2006.
Initiative of: Carnation breeders Barbaret & Blanc, Selecta Klemm, Hilverda and P. Kooij & Zonen, in cooperation with Geslive, a Spanish company specialising in breeder's rights and control.
Reason: The companies mentioned above have been giving information on illegal cutting and breeders rights to growers and cooperatives for years. Contracts have been made up with some nurseries to prevent production of illegal cuttings. The nurseries that did not sign the contract are being dealt with.
Proceedings: With the help of the Spanish police seven nurseries were raided. In a lawsuit it will be demanded that illegally produced flowers will be destroyed. More raids will follow in another twenty nurseries.

Chinese carnations

Action: Control of illegally imported Chinese carnations in Japan.
When: Early April 2006, repeated in May/June 2006.
Initiative: Carnation breeders Hilverda and P. Kooij & Zonen.
Reason: Japan imports a great many carnations from China. In 2005 it were 37 million flowers. For part of these consignments no royalties have been paid.
Proceedings: Checks have been undertaken in a Japanese flower auction as a warning. This action was announced beforehand, so no illegal carnations were found. The next inspection will not be announced. If illegally imported flowers are found, breeders and importers will try to come to an agreement. According to Japanese regulations DNA analyses should establish the identity of the flowers.

Israeli gerbera growers

Action: Collecting royalties from Israeli gerbera growers, if needed by lawsuits.
When: Spring and Summer of 2006
Initiative: Gerbera breeder Florist
Reason: In Israel gerbera growers are used to producing their own cuttings. Verbal agreements on payment of royalties do not work anymore. For years growers have been fiddling. The man undertaking the counting of the plants is opposed by a number of growers and is sometimes not allowed to enter the greenhouse.
Proceedings: The verbal agreements have been terminated by Florist. The aim is to come to written contracts and collection of royalties that are due. It has partly succeeded. Lawsuits for illegal propagation will follow against growers that did not concur. In Europe the illegally produced gerbera's will be confiscated.

Roses from Ethiopia

Action: Containing of large consignments of illegally produced roses from Ethiopia at the Amsterdam airport Schiphol, the Netherlands.
When: Spring 2006.
Initiative: Meilland.
Reason: In a number of African countries rose varieties are propagated without royalties being paid. Because these countries do not have breeder's rights, the royalties can only be claimed when the flowers enter European territory.
Proceedings: By stopping the import of roses it is hoped the growers will abide by the law.

German chrysanthemum

Action: Lawsuit after inspection on a German chrysanthemum nursery.
When: Inspection in July 2005.
Initiative: Royalty Administration International (RAI) on behalf of 15 breeders.
Reason: Observation of repeated cropping of chrysanthemum multiflora in a German nursery.
Proceedings: RAI has observed during an inspection that the grower was producing chrysanthemums illegally. The grower refused to fulfil his obligations. The grower received a fine that equalled the royalties and was furthermore sentenced to pay all costs to the lawyers and the lawsuit. In total the grower has therefore paid many times more than the original license fee. Since the end of August 2005 the grower has been allowed to bring the flowers onto the market again.

Smart measuring device reveals transport conditions

Monique Krinkels

Seed companies do everything in their power to make sure seeds have the quality growers can count on. But there comes a moment that the seeds leave the premises on their way to the farmer, grower or plant raiser. During that trip things can go horribly wrong. Orbi Solutions has developed a solution to measure the conditions seeds on transit have to endure.

When a plant raiser discovers only half of the seeds have germinated and the seed company is certain the seeds it has sent had the standard quality, the problems must have originated during the transportation between them. It should be remembered that transporters often have no knowledge at all about seeds, so they might unintentionally do things a seeds man commonly would never consider doing. Taking a coffee break for instance and leaving a package of seeds in a stiflingly hot car. Or making a detour of several days in order to travel more efficiently. They are oblivious that the consequences of such deeds could be extremely costly.

Invention

The idea to develop a tracking system came up after an insurance man wondered aloud why no measures were taken to ensure seeds have a safe journey. He was staggered to discover that after all the measures a seed company takes to make sure that its products have a superior quality, seeds are just picked up by a common delivery service. The remarks triggered seed technologist Michèl Kockelkoren to search for a solution. “In my experience seeds lose quality in about ten percent of the dispatches. Transport companies often do things that are in our view completely irresponsible. Furthermore they are not informed of the effects of high temperatures on seeds. Especially with primed seeds, the consequences of overheating are disastrous.”

He remembered a data logger a friend had once shown him. “This solid device contained a microchip with temperature and humidity sensors. They are small sized and relatively cheap to buy and can be reused several times. Ideal therefore to include in a package of seeds.” With the data logger as a starting point he developed software with which the information could be efficiently applied to seeds. In November last year he proudly presented his invention, named ‘Verdict’ at the Hortifair, the international horticultural trade exhibition held in Amsterdam, the Netherlands. The tracing system consists of a data logger, a reader and software to interpret the results. Because of its minute size – no thicker than 5 mm and a diameter of only 16 mm – the data logger is secured on plastic card to avoid it getting lost between the seeds. It measures temperature and humidity every 15 minutes and records these data and the time. On arrival the device is placed on the reader, which is connected to a computer. The computer is linked through the internet with the computer of the seed company which sends all data automatically to their logistics department. When temperatures or moisture levels go outside a preset range at any time during the transport, the computer of the seed company gives a warning. It is able to give the exact temperatures together with the time it has measured these.

With only a diameter of 16 mm a data logger is very small

Michèl Kockelkoren has developed a tracing system that keeps track of the climate conditions during transit of seeds

The consignee is asked to return the logger by mail. Using Verdict a seed company has a choice whether or not to take action when temperature during transit has exceeded the specifications. “With this system it is like you are present at a customers warehouse to check the quality of the seeds while unpacking the package. If there is a problem you are able to take action before the seeds are sown, preventing loss of business for your customer”, says Michèl Kockelkoren.

Success

The system has proved to be exactly what the seed industry was waiting for. During the last few months many seed companies in the Netherlands have acquired either the research version or the integrated supply chain version. “It is unbelievable”, Michèl Kockelkoren admits. “Of course I had hoped this would happen, but I did not expect it to happen so quickly.” One of the reasons for its success is that working with the Verdict system is relatively simple. “During the last year the teething troubles of the software have been ironed out. The system is flexible and can be adjusted to the temperature range a species is sensitive to. A short training course of the staff is sufficient to start working with Verdict”, he says. Michèl Kockelkoren’s ultimate goal is to set the standard for the whole industry. Not only for seed companies, as other industries have already shown a keen interest. Pharmaceutical companies for instance face the problem that they have to concur to new regulations considering transporting medicines.

The customer is required to upload the data of the product in transit to the Verdict web server, which is located at a independent, professional web host. Companies automatically get a copy of the original data enabling them to analyse a mission or a whole supply chain through the year. However, it is important to stress that the original data cannot be changed and is stored for up to twelve months, where no customer has access to it. Whenever seeds arrive in bad shape, caused by high temperatures during transport, sowing the seeds can be prevented and new seeds can be sent to replace the damaged products. In this way a problem can be solved at the price of the production costs of the seeds. Without the Verdict system the damage for the grower is sometimes from 100 up to 1.000 times higher than the production value.

Legal base

In case of a dispute the original data is always available for independent investigation. An authorised insurance company will have the opportunity to check the conditions during transit and compare the results of the retained seed samples of the seed company with the low performing seeds in the field. Too high temperatures during transit can be proven. “Using the system does not mean that seeds will no longer suffer from overheating, but it prevents a plant raiser discovering a seeds failure after planting, ruining his schedule. And of course the costly judicial procedures that may follow such events”, concludes Michèl Kockelkoren.

New approach reduces costs enormously

Monique Krinkels

To reduce to the burden of farmers and growers, the Dutch Ministry of Agriculture has decided that in future only one inspector should examine the crops meant for import and export. Until now inspectors of the National Plant Protection Organisation as well as of the inspection services visit farm fields and nurseries. From January 2007 onwards, the tasks of the plant health authorities will be transferred to the inspection services.

The Dutch government has developed a new policy, named 'Another government'. According to the plans, the responsibilities for many affairs should foremost lie with the companies involved and not with government. The Ministry of Agriculture will implement this policy in its phytosanitary procedures. Mid April the Minister came to an agreement with the plant business community and the four inspection services the Netherlands has. The NAK will become responsible for the phytosanitary inspection of agricultural crops, the Naktuinbouw for vegetable plants, flowers, shrubs and trees, the BKD for flower bulbs and the KCB for the vegetable and fruit end products.

Cost reduction

Besides the advantage of a single inspector visiting nurseries and farm fields the Minister also gives the business community the opportunity to draw up the way the controls take place. Moreover, it will mean a hefty cost reduction, which could, according to a study, be as high as 30%, or 3 million euro per year. This is possible because the inspection services are able to combine the health inspections with the quality inspections they already carry out. The inspectors have done this already for exports within the European Union since the mid nineties.
The National Plant Protection Organisation will restrain its activities to monitoring the outlines of the system of the inspection services and to inspections that are not routine. One of the conditions is that the inspection services are accredited to ensure that their work complies with the regulations. The Minister will still have the final responsibility for plant health. He therefore demands that the inspection services give information on the diseases and pests the inspectors encounter. If general measures are needed to prevent spreading of pests or diseases, that will be the task of government and not of the inspection services.

Nothing lost

The inspectors of the inspection services already have experience with phytosanitary control of products exported within the EU. Besides becoming responsible for exports to the rest of the world, they also have to familiarise themselves with inspecting imports. But as many of the people employed by the plant health authority will go to the inspection services their experience will not be lost.
It is still not certain how the countries outside Europe will react to the new system. An analysis has been made whether Japan, USA and Canada will accept that the export inspections are done by a non-governmental institute. It is however expected that the new phytosanitary procedure will not cause much problems.

Epigenetic mechanisms cause aberrant plants

Geert-Jan de Klerk and René Smulders

Among plants produced in tissue culture, often aberrant phenotypes are found. It is becoming more and more evident that epigenetic mechanisms play a significant role.

Adventitious regeneration of complete plants from somatic tissues, cell suspensions, protoplasts and gametic cells was achieved in the 1950s and 1960s. Shoot formation by forced outgrowth of existing axillary buds was achieved in the same period. Initially, researchers expected that the plants produced in these ways would be exact copies of the original plants. However, frequently phenotypic variations were observed. The various types of variation are summarized in table 1. This paper deals with epigenetic variation.

Epigenetic versus genetic variation

Phenotypic variants are frequent in plants produced from protoplasts or callus, and sporadic after direct regeneration (i.e. regeneration without an intermediate callus phase) and in plants produced by outgrowth of axillary buds. The aberrant phenotypes may be detected already in tissue culture. However, some off-types can be distinguished only several years after planting, e.g., at the flowering stage. Variation in tissue-cultured plants may be genetic or epigenetic. Genetic variation is caused by changes in the information encoded in the genome; epigenetic variation is brought about by (long-lived) changes in the expression of the genomic information. Thus, the former concerns changes in the DNA sequence, the latter in (de)blocking of DNA transcription. Changes in the DNA-sequence occur randomly (although 'hot spots' may occur where the frequency is increased). Hence, phenotypic variation caused by genetic changes will be different at each event. For epigenetic variation, it has often been observed that the same variation is brought about in a predictable manner, suggesting that certain regions in the genome are more susceptible to epigenetic changes. Hence, repeated occurrence of similar off-types in various batches planted over several years and the occurrence of similar off-types in different cultivars suggest epigenetic variation. The term somaclonal variation is often used for variation in plants produced in vitro; some researchers use this term in the strict sense, only referring to genetic changes, while others use somaclonal variation for both genetic and epigenetic changes.

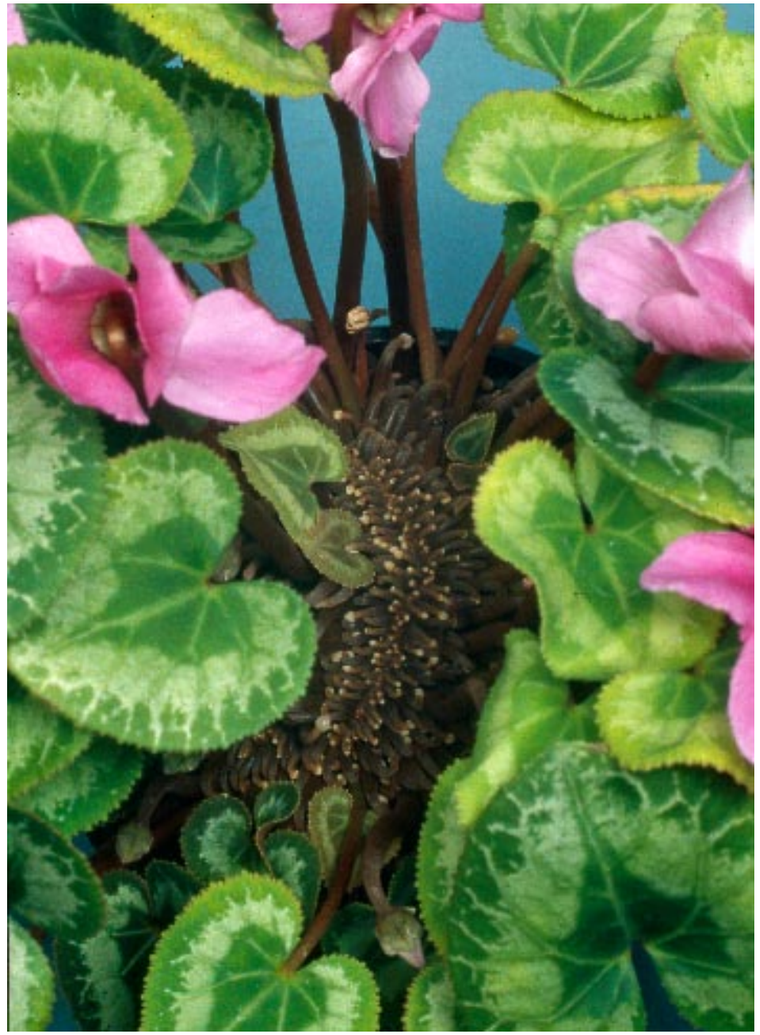
Molecular mechanisms

Even though each plant cell contains in principle the complete DNA sequence of the genome with over 20,000 genes, not all genes are expressed concurrently: expression depends on the type of tissue and the developmental state. Many of the non-expressed genes are marked by modification of the DNA (methylation of the cytosines) and/or methylation of lysines in histone tails. Conversely, active genes are often associated with acetylated histones. Such epigenetic modifications can be inherited from cell to cell during cell divisions and may therefore be long-lived. Epigenetic abnormalities as a result of tissue culture are thought to be the result of incomplete or erroneous resetting of DNA methylation and/or histone methylation / acetylation. In contrast, genetic changes consist of, e.g., point mutations and deletions of large chromosome fragments.

Table 2 Effect of NMU (chemical mutagens) and 2,4-D on the variation of the leaf shape.

	Regenerated plants (= 1st generation)	Plants regenerated from the 1st generation (=2nd generation)
Direct (control)	11.5	10.5
Direct, 0.2 mM NMU	14.2	13.4
Direct, 2 mM NMU	19.4**	18.1**
Direct, 10 mM NMU	22.1***	21.8***
Via callus-interphase (5 µM 2,4-D)	21.2***	13.9*

From plants of the 1st generation produced via direct regeneration in the presence of increasing concentrations of NMU or indirectly via an intermediate callus phase, a second generation was produced via direct regeneration. Note that the variation decreases strongly from the 1st to the 2nd generation in plants produced via callus and not in plants produced with NMU. The values are the coefficient of variation of the mean in the leaf shape obtained with populations of ca. 20 plants.



Cyclamen produced in tissue culture via direct regeneration. Note the rows of primordia in the middle (like a Mohawk haircut), indicating extreme bushiness. (courtesy of Dr. H.J. van Telgen, PPO-Aalsmeer, the Netherlands)

Vernalization

The molecular mechanisms of vernalization provide an excellent example of long-lived epigenetic changes under natural conditions. In many plants, flowering is promoted by a preceding period of cold. This process is known as vernalization. The ecological adaptation is obvious: vernalization ensures flowering after the winter season. Thus, plants someway 'remember' the exposure to low temperature. Initially, it was believed that during the cold period DNA demethylation occurred and that the demethylated state persisted for many months. More recent evidence suggests long-lived modifications of certain histones.

Examples

In woody plants, juvenile shoots undergo after some years a 'phase change' and become adult. Adult shoots can produce flowers, and often also exhibit a different growth habit and morphology, such as the development of spines. Buds taken from the adult portion of woody plants and used for tissue culture, become juvenile after repeated subculturing. Rejuvenation is very predictable, and can be observed early (in tissue culture) and also late (after several growth seasons in the field). For propagators, rejuvenation is highly relevant as rejuvenated shoots are rootable whereas the original shoots are recalcitrant. The phase change from juvenile to adult has been related to the methylation status of DNA. In Pinus radiata, in meristematic areas of juvenile material cytosine methylation was 30-35%, while in the same areas of adult material it was 60-65%. When the adult material was rejuvenated, methylation decreased. Well-known examples of epigenetic changes are flower aberrations in oil palm and date palm. These variations are observed after many years of growth in the field. In oil palm, the effect of tissue culture conditions has been well documented. Major factors were short culture cycles (2-4 weeks in stead of 8 weeks) and use of relatively high concentrations of kinetin. Furthermore, some genotypes were more sensitive than others. DNA

Table 1. The various classes of off-types in tissue-cultured plants

Underlying cause	Backgrounds	Example
Loss of pathogenic micro-organisms	Some micro-organisms influence morphology. During adventitious regeneration, they may be excluded from the newly formed meristematic cells together with other microorganisms.	E.g., Poinsettia in which a phytoplasm is lost during somatic embryogenesis from cell suspensions; this results in a restricted-branching pattern as compared to the normal bushy free-branching pattern (Lee et al., 1997). Thus, the phytoplasm has a cytokinin-like effect.
Loss of chimeric structure	*During adventitious regeneration* Plants are composed of three distinct cell layers that also exist in the meristems. As a rule, adventitious regeneration does not occur from neighbouring cells in the various layers but only from one layer. *During axillary branching* In axillary branching, the chimeric structure might be lost by irregularities in cell divisions in the meristems.	Loss of chimeric structure in Kohleria during adventitious regeneration; surprisingly, the chimeric structure may remain intact in adventitious regeneration in chrysanthemum. Loss of chimeric structure in hostas that are propagated via axillary branching
Epigenetic changes	Long-lived changes in gene-expression brought about by tissue culture	See main text
Genetic changes	Changes in the genetic information itself (e.g. point mutation, deletion of large fragment of the chromosome).	Many examples see various review papers

methylation is thought to be involved. It should be remembered that in both oil palm and date palm, an extended intermediate period of callus occurs. Propagation by axillary branching is based on forced outgrowth of axillary buds. After tissue culture, apical dominance should be restored. Frequently, though, the release of axillary buds persists resulting in bushy phenotypes. This has been studied to some extent in gerbera and in Zantedeschia. In both, high doses of applied cytokinins during propagation and high sensitivity of certain genotypes to cytokinin play a major role. Interestingly, in gerbera, bushiness is reduced by the fungicide imazalyl. This fungicide supposedly inhibits the synthesis of brassinosteroids, a recently discovered class of plant hormones.
In Zantedeschia, bushy and nonbushy plants of the cultivar 'Florex Gold' display many differences in the methylation pattern of DNA. In rhododendron, both bushiness and another type of variation, 'tissue proliferation', have been observed. Tissue proliferation is characterized primarily by the formation of galls or tumors at the crown not caused by bacteria. Both are correlated with increased sensitivity to cytokinin.
A final example is leaf shape in begonia. Leaf shape is determined as the ratio of the length of two perpendicular nerves. In a population of plants produced via an intermediate callus phase with high 2,4-D, the spread of the leaf shape was much larger than in a population of plants produced by direct regeneration. When during direct regeneration a chemical mutagens was added, the spread also increased sharply. As an increased spread indicates genetic mutation, it was initially believed that at high 2,4-D the rate of mutations (= genetic variation) had increased. However, the spread was sharply reduced by a second step of direct regeneration in the 2,4-D plants. This drop did not occur in plants produced with chemical mutagenesis. These data suggest that the increased spread at high 2,4-D was for the larger part easily reversible, so most likely epigenetic. This indicates that epigenetic variation may also be random (table 2).

Concluding remarks

Variation in tissue-cultured plants has been observed early. For plant propagators and genetic engineers, it is most important how variation may be reduced. For both genetic and epigenetic variation, a callus phase and protoplasts should be avoided. In axillary branching, epigenetic off-types may arise by application of high levels of cytokinins and by short subculture cycles.

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Phytosanitary Regulations

More species need a plant passport

Monique Krinkels

Seeds of lucerne, tomato, bean and sunflower should be accompanied by a plant passport within the European Union (Photograph: Naktuinbouw)

Due to new phytosanitary regulations, seeds of lucerne, tomato, bean and sunflower that are transported within the EU must have a plant passport. The change is minimal as the directive 2000/29/EC already required that introduction and spreading harmful organisms with these seeds should be prevented. But it means a change in the paperwork.

Until last year plant passports were not needed for seeds of lucerne, tomato, beans and sunflower. According to directive 2000/29/EG it was sufficient that the seeds were fulfilling the phytosanitary requirements for trade. Since May last year the obligation to have a plant passport has already been introduced for certified seeds, but now all seeds of these species are included in the new regulation. From May 1, 2006 it should be made clear by a plant passport that the seeds are free from the quarantine organisms Ditylenchus dipsaci en Clavibacter michiganensis ssp. insidiosus (lucerne), Clavibacter, PepMV and Xanthomonas (tomato), Xanthomonas (bean) and Plasmopara halstedii (sunflower) and fulfil the general phytosanitary requirements for trade.

Adjustments needed

Since 1993 numerous plants, bulbs tubers and seeds need a plant passport to be transported within the European Union. It replaced the phytosanitary certificate needed till then for trade between Member States of the EU. During the last 13 years the requirements have been revised regularly. The present revision had already been announced in 2004 and would have come into effect on January 1 last year, but was postponed several times.

The European phytosanitary regulations are focused on preventing the import and spread of harmful organisms throughout the EU. The quarantine list contains about 300 organisms for which there is a zero tolerance. But the regulations havd to be adapted to the actual circumstances. Notwithstanding all the efforts new organisms appear in the EU and have to be added to the list. Other organisms have spread throughout the continent or are less harmful then initially claimed, making plant passport demands superfluous.
As Switzerland is surrounded by EU-countries, this state has adjusted its phytosanitary regulations. Since April last year the requirements are (almost) the same as in the European Union. The EU plant passport and the Swiss phyto certificate are acknowledged back and forth. Nearly all species can be imported to and exported from Switzerland as if the country forms part of the EU. The import of citrus fruits from Switzerland into the EU is excluded from this regulation, as are the import and export of endangered plant species. For the latter trade is regulated by CITES.

Bulbs and tubers for which a plant passport is required

SPECIES	AREA
Allium ascalonicum	EU
Allium cepa	EU
Allium schoenoprasum	EU
Galtonia candicans	EU
Camassia	EU
Chionodoxa	EU
Crocus flavus 'Golden Yellow'	EU
Galanthus	EU
Gladiolus	EU
Hyacinthus	EU
Iris	EU
Ornithogalum	EU
Puschkinia	EU
Scilla	EU
Solanum spp.	EU*
Solanum tuberosum	Protected zones only
Tigridia	EU
Tulipa	EU

* All stolon or tuber forming varieties and hybrids

Seeds for which a plant passport is required

SPECIES	AREA
Abies	Protected zones only*
Allium ascalonicum	EU
Allium cepa	EU
Allium schoenoprasum	EU
Beta vulgaris	Protected zones only*
Dolichos	Protected zones only*
Gossypium	Protected zones only*
Helianthus annuus	EU
Lycopersicon lycopersicon	EU
Medicago sativa	EU
Phaeseolus	EU
Phaeseolus vulgaris	Protected zones only*

* Passport is required until the end user (ZP-code)

Growing interest in healthy nutrients

Fortified crops keep the doctor away

Gert van den Berg

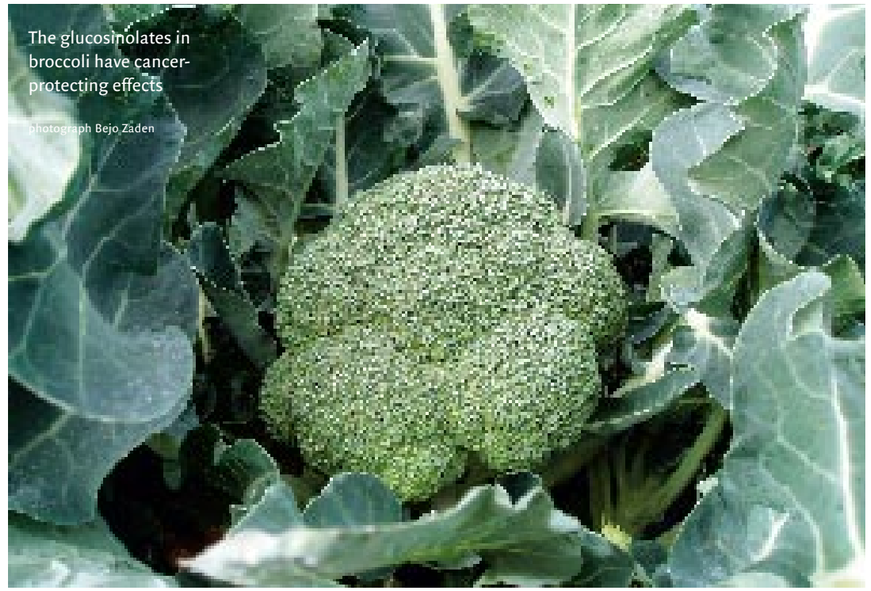
The lycopene-rich tomato has grown into a real market sector. Breeders have also other functional fruits and vegetables in the pipeline. These fortified fruits and veggies fit the increased interest in health-promoting foods, but breeding companies and marketeers have to toil before varieties with beneficial compounds are successful.

An apple a day keeps the doctor away. If fruit scientists from the Catholic University of Leuven have their way, this old adage will become an everyday reality. In collaboration with the university's spin off company 'Better 3 Fruit' NV, these Belgian researchers are aiming to develop nutritionally-enhanced apples using vitamin C rich cultivars as parents in their breeding programmes. According to Mark Davey from the Division of Crop Bio-engineering, apple varieties with a late harvest date generally contain more vitamin C than the early ones. This suggests that harvest date might provide an easy tool for selection of new varieties. According to their fruit specialist, apple cultivars also differ substantially in their ability to maintain vitamin C levels during storage, which Davey explains is primarily due to vitamin loss by the 'low-vitamin-C' cultivars. Apples with an enhanced vitamin C content may be beneficial from a human nutrition point of view, he says. In addition to these health-promoting effects, the vitamin also extends fruit shelf life and delays browning of apple slices. For the future selection of apples with high vitamin C content, the research group is currently in the process of developing molecular markers by QTL and genetic analysis.

Acceleration

Since research nutritionists regularly add new compounds to the already known beneficial substances, the development of fruits, veggies and other crops with an enhanced level of such substances is accelerated. A classic example of a health-promoting product is soymilk. In 1979 this product came onto the American market. Three decades after its introduction, the potential benefits of soyfoods are relatively well-known. In the wake of soyfoods other so-called functional foods come onto the market. A lycopene-rich tomato for instance, but also carrots with an enhanced carotene level. Vegetable breeders are developing new cultivars of broccoli and cabbages with a high content of glucosinolates that are thought to have health-promoting effects (see box Crucifers and glucosinolates). The last few years the search for such functional foods offer new leads for breeders. At the moment a range of more or less promising crops and substances are described: barley with an enhanced level of beta-glucan, cranberries with elevated content of anthocyanins, hempseed as a source of essential fatty acids, complex onion-type fructans in genetically modified sugar beet, folates and not forgetting the sulphur-containing substances in Allium species. Even toxicants like glycoalkaloids in potato tubers are mentioned to have disease-preventing properties. As a reaction to the large amount of studies, research nutritionists critically review the promises of functional foods.

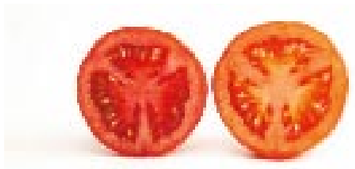
Crucifers and glucosinolates The year was 1992 when scientists from John Hopkins Medicine published the cancer-protecting effects of sulphoraphane. The sulphur-containing compound is one of the many glucosinolates in cruciferous plants and is found in large amounts in broccoli. Other cruciferous vegetables like Brussels sprouts, cauliflower, cress and mustard also contain this health-promoting substance. In plants the balance between the levels of the different glucosinolates is unsteady. Therefore, breeding for an enhanced level of the right compounds is a complicated matter, says research co-ordinator Henk Pennings from the Wageningen branch of Seminis. The company has an exclusive license for the use of patented glucosinolate-rich broccoli breeding material from the John Innes Institute in Norwich (UK). "Rather within five years than ten years", will the first varieties with elevated sulphoraphane levels come onto the market, is the most Pennings wishes to say. About the search for molecular markers regarding health-promoting substances he is also reticent. In years with much sunshine, broccoli makes more glucosinolates than in a year with much dull weather. This makes brassica breeder Jan de Geus reserved about the prospects of cultivars with an elevated level of these health-promoting substances. Although he already has some years of experience with breeding glucosinolate-rich broccoli and headed cabbage, he still stands aloof about this work. "If so, you can claim disease resistance, but about the glucosinolate content you can only say that cultivar A contains more than cultivar B". The Dutch breeder doesn't divulge details about the progress of this research programme of Bejo Zaden. His comment: "You need to look around and get experience." According to him, it becomes only an advantageous business if the industry makes a pill from your product.



The glucosinolates in broccoli have cancer-protecting effects

Photograph Bejo Zaden

Deep red tomato Lycopene fights cancer and cardiovascular diseases. It is found in the red pigment in ripe tomatoes and other fruits and vegetables and therefore receives much attention. It required however quite an effort from the Dutch breeding company De Ruiter Seeds before the lycopene-rich tomatoes were established on the marketplace. Three years ago the production of these fortified tomatoes started on a modest scale under the label of 'Harvest of health', a Dutch sales organisation specialising in fresh functional foods. "At the moment lycopene-rich tomatoes have grown into a real market sector", says product manager Len de Kok, De Ruiter Seeds. After the launch of some cocktail tomato varieties, De Ruiter Seeds has now started to introduce a more robust truss tomato under the name of 'Internal Red'. For the deep red colour a new slogan will be introduced: 'See the taste'. The lycopene-rich 'Red Star Safari' tiger cocktail tomato from De Ruiter Seeds is exclusively marketed by the Dutch market garden company Red Star Trading. The export of the lycopene-rich products to the USA goes successfully, says Len de Kok. He attributes this success to the great interest of Americans in health-promoting effects of foods. "Health is a trend", he says. According to him the lycopene content will become a popular aspect of tomatoes in North America and North-Western Europe. Functional foods fit well the increased care for your body, he explains and therefore he expects a great deal of such products. De Ruiter Seeds believes it has a lead on their competitors, as the seed company believes to have maintained the breeding programme more intensively than the competition. "At the beginning the communication about lycopene-rich tomatoes was difficult", Len de Kok remembers, "but still as one of the few we have continued." At USDA Agricultural Research Service and breeding companies several programmes regarding the development of lycopene-rich water melon and autumn olive varieties are ongoing. Are those fruits a threat to the tomatoes of De Ruiter Seeds? "No", says Len de Kok, "because choices and diversity are important for the consumer." Besides, he thinks the market is large enough for several players. The breeding company has also selected an orange-coloured tomato with an enhanced beta-carotene content. Since consumers associate tomatoes with red, the orange tomato is not easily accepted by the public. Only in the USA this product is somewhat popular. "A consumer doesn't buy only a functional food", is the conviction of Arjan Bimmel from the Dutch branch of Seminis. According to the breeder, a lycopene-rich cultivar must therefore win on more points in order to be able to gain an advantage over other tomato varieties with an enhanced lycopene level. At the moment, Seminis has no commercial tomatoes for this market sector; because the lycopene-rich varieties are still under examination.



Lycopene-rich tomatoes have grown into a real market sector in the USA.
Photograph De Ruiter Seeds

Influence of crop management

Climate, soil and crop management influence the level of health-promoting substances of fruits and vegetables. A study of the Texas Agricultural Experiment Station and the USDA Agricultural Research Service for instance shows different concentrations of ascorbic acid and folic acid in green-flesh muskmelon fruit, whether grown on a clay loam or a sandy loam soil. Similar differences have been found in experiments with other crops. So, scientists from the German Geisenheim Research Center have found that reduced irrigation and nitrogen

Rick Pedersen grew orange cauliflowers on the Pedersen Farm in Seneca Castle, USA, last autumn. Consumer interest was good, he said. People like the colour and the flavour

fertilisation enhances the sulphoraphane content in broccoli. At the Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt (Germany), researchers have therefore investigated suitable crop management strategies in order to increase the quantity of health-promoting substances. According to Monika Schreiner, specific crop management strategies increased contents of individual phytochemicals tenfold in broccoli and cauliflower, and twofold in radish. A strong influence of growth conditions on the content of phytochemicals is also shown in experiments with apples and tomatoes. So, HortResearch scientists measured significant differences in polyphenolic concentrations in apple fruits from different regions in New Zealand. Remarkably enough this finding didn't hold true for all the analysed cultivars.

Rainbow carrots

Molecular markers are handy tools for an intelligent selection of carrots rich in carotenoids. Philip Simon therefore investigates traits that correspond with enhanced levels of these antioxidant pigments in this vegetable. For more than a quarter century he has developed carrot breeding material at The Vegetable Crops Research Unit of the USDA Agricultural Research Service, which makes the germplasm available for commercial breeding companies. Seminis is one of these companies that use the breeding material. On the website, Seminis explains about their Rainbow Crunch Carrots that it is 'originally created by nature, but rediscovered by Seminis'. According to Seminis the company developed the carrots with Colorful Harvest, a growing, shipping and marketing company in the west of the USA. The carrots are now available in North America and Australia. Kirsten Brandt eats one or two carrots a day because of the presence of falcarinol. With Danish colleagues, the plant biochemist from the British University of Newcastle upon Tyne published recently a rat study in which it is shown that this antifungal substance can reduce the risk of cancer. Kirsten Brandt now wonders if breeding for an enhanced level of beta-carotene is the right way to fortify carrots. She has put up the question whether falcarinol and not carotenes cause the beneficial effects of carrot on health. Philipp Simon has his doubts about this. "In all the studies that I know, beta-carotene from fruits and vegetables does promote health."

Furthermore researchers from Texas A&M University (US) demonstrated that greenhouse-grown cluster and round cultivars contain more lycopene than the same varieties which were grown in the field. The opposite however is also found: under field circumstances the tested cherry tomato types produced more lycopene in the fruits than the cherry tomatoes which were grown in the greenhouse.

Brands

Functional foods should be distinguishable in the market. So, breeders and marketeers develop brands, labels and marketing strategies in order to tout for consumers. An example of such a label is Colorful Harvest, under which this US-based growing, shipping and marketing company sells Rainbow Crunch Carrots and an orange cauliflower. Both the colourful carrots (see box Rainbow carrots) and the funny cauliflower contain enhanced levels of beta-carotene. The lycopene-rich tomatoes from De Ruiter Seeds are also put on the market under special brands and slogans like 'Harvest for health', 'Internal red' and 'See the taste' (see Box Deep red tomato). For brassica breeders, brands are also envisioned. The success of branding however will heavily depend upon the type of health claim that can be put on the label, says research co-ordinator Henk Pennings from the Dutch branch of Seminis.

Embryo accurately predicts plant quality

Monique Krinkels

For over a decade researchers have tried to solve the problem of not uniformly grown tomato plants. The final solution has come from medical sciences. The X-ray machines, commonly used by doctors to view the inside of a human body, are also applicable viewing the internal features of seeds. For many years this has been used for quality inspection of sugar beet seeds to determine multi-germ and empty seeds. This summer the first tomato seeds, whose embryos are individually and thoroughly checked on quality by Incotec, will be selected and marketed.

The outward appearance of a seed might be perfect, its genes just what its parentage promised. Nevertheless the plant that it produces might not be meeting the quality criteria. Especially with tomatoes, the number of seeds that germinate is much higher than the number of plants that find their way to the grower. It is the invisible physiological processes inside the seeds that are responsible for these stragglers. At first sight that might appear only bothersome to the plant raiser, but even at nurseries inadequate plants have to be removed. Besides, plant raisers just have raised the prices for tomato plantlets. In short, some seeds are a waste of time, effort and money.

Buzzing activity The X-ray machine at Incotec is softly buzzing. But for the fine rustling of seeds it would be easy to imagine you are in a hospital, instead of in a seed research laboratory. Inside a glass cubicle on the one side, tomato seeds drop in the tiny holes of one of the trays on the conveyor belt. When filled with one hundred seeds it moves automatically onwards on the steel covered belt to arrive underneath the X-ray machine. The low intensity rays quickly scan the embryos.
A few steps away the computer shows their digitalised snail shell shaped images. With a speed of 25 seeds per second, the computer decides which seeds meet the quality standard. Passing further on the conveyor belt, the seeds enter the sorting machine where the seeds with the best shaped embryos are selected. These seeds will grow out to plants with maximum uniformity.
"The automatic image analysis was the hardest part of the system to develop", says Ed Nobel, international product research director of Incotec. "We have succeeded in developing our own software, but it took a long time to do so. Not only are the forms of embryos complicated for a computer to analyse, there are remarkable differences between varieties."
Incotec's involvement in the development of the system dates back to 1992/1993, when researchers at Incotec parallel with researchers at Wageningen University came to the same conclusion that – theoretically anyway – selection based on the X-ray images of the embryos would solve the problems of the plant raisers. The latter had to cope with tomato seeds of which only 75% would ultimately produce saleable plantlets. It takes much work to select the plantlets and of course the space the unmarketable plants occupy is wasted. "And according to growers they have to remove up to another 10% of the plants in order to have a truly uniform crop. It is a time-consuming activity and leaves them too with unproductive spaces."

Improving quality From the start Incotec showed interest in researching this phenomena, as it offered one more way to improve seed quality. In-company research took place in the early days to determine the parameters which are of importance for the final quality of the plant. The company is the largest worldwide involved in vegetable seed treatment, using seed coating, pelleting, priming and upgrading technologies. Currently the company provides products for a large number of vegetable, flower, tobacco and agricultural seed species.
Since 1963, the year Incotec started developing its coating technology, research has always formed an important part of the company's activities. Today most of the product oriented research is done locally, to ensure close contact with customers and to help find the best solutions in the market.
It has production facilities in the Netherlands, Japan, Brazil, India, Australia and the USA, which also have local integrated product research departments. That is why Incotec can rapidly develop new products to meet the changing needs of seed companies, plant raisers and growers.
However, fundamental and highly expensive research such as the X-ray image analyser is concentrated in its head office at Enkhuizen, the Netherlands, where the International Technology Research group has its offices and laboratories. From the onset it was clear that manually selecting X-ray images would be possible. Henry Bruggink, responsible for the X-ray project, says: "The only requirement is that the seeds have to be primed first. There are two reasons for this. First, priming minimises physiological differences between seeds, making the germination result largely dependent on physical properties. Secondly, in unprimed seeds the differences between the embryos are not detailed enough for selection purposes. To analyse the image the embryo has to be loosened from its endosperm."

Selecting seeds based on the form of the tomato embryo ensures the grower will have uniform plants

Selection criteria Of course there also needs to be an experienced judge to evaluate the image of the embryo. Observing differences between embryos is not enough. The researchers at Incotec first had to discover which embryo form would lead to the best plants. In order to do so seeds were analysed and subsequently sown in a greenhouse.
"By evaluating the young tomato plants it was possible to establish the ideal form of the embryos", explains researcher Henry Bruggink.
A complicating factor was that there are significant differences between varieties. "We had to determine the appearances of the perfect embryo on a variety by variety basis. So far, we have a limited number of varieties which are fully tested, but as we now know what to look for, this number will increase quickly", Bruggink predicts.
The second obstacle was the automated image analysis. It proved to be a much more difficult hurdle to take. "We started to seriously invest in this technology in 1997. Many attempts by various researchers to translate the selection criteria into a format a computer can understand failed.
After six years of research, the computer still could not distinguish the embryos without flaws.
The breakthrough came when Incotec was put in touch with a computer programmer. "He proved to be a true 'wiz kid', solving the riddles the others had struggled with in a relatively short period of time because of his creative approach", praises Ed Nobel his programmer. "We now have reached a situation that with respect to imaging we are on almost the highest level of what is possible to achieve technology-wise."

Ready to go Today, after nine months of struggling with all kinds of technical issues, the machine is ready for operation working at a lower capacity. Minor problems that always occur when a new apparatus is put to the test, will have to be dealt with, but for introduction purposes the apparatus is working properly. Digitalised images of seeds, the computer could not decide on at first, have been evaluated manually, thereby enhancing the computers selection criteria. This summer the first commercial tomato seeds are expected to be X-rayed and selected, which will be for sale from October 2006 onwards.
So far the system has only been used for upgrading tomato seeds. "For tomatoes the problem of selection at the plant raisers was the most costly. But also low quality seed lots can be upgraded in an efficient way to create a saleable and usable quality. In the next phase, seeds of tomato rootstock varieties are expected to be X-rayed and upgraded. But of course our research goes on. We are fairly sure we can have stunning results with triploid water melons and peppers too. The embryos of triploid water melon and peppers look completely different, but we know how to proceed and our software programmer is able to adapt the software."
However, this technique will not be applicable to all vegetable seeds. As X-raying, the imaging and selection of the individual seeds is an expensive operation, it is only cost effective for high-priced seeds. Besides the seeds should be flat shaped to be able to always position them in the same way.
The seeds will become available to growers all over North Western Europe. "At the start the X-ray service will only be offered at our headquarters in the Netherlands. But of course our facilities elsewhere will follow in due course. Ultimately seed companies might want to licence the system. We will keep that into consideration", concludes Ed Nobel.

New bill improves breeder's position

Judith Blokland

On the first of February 2006 the new seed and plant law came into force in the Netherlands. In comparison to the old law dating from 1967 nothing substantial has changed regarding the content of breeders' rights, as these requirements all originate from the UPOV 1991 Convention. Still there are some remarkable changes on subjects where UPOV leaves the member states some freedom to act.

In the Netherlands breeders have gained a better position to protect their new varieties. The duration for which plant breeder's protection is given has been prolonged, the regulation for farm saved seeds is improved and plant breeders have a new weapon in their struggle against infringers. Unfortunately, the text of new law is only available in Dutch at the moment. It can be found on the website of Plantum NL (www.plantum.nl).

Extended protection

One of the most important improvements is that the duration of the breeder's right has been extended. In the Netherlands this protection period is in general 25 years, but it can be extended to 30 for some specific crops. In the old law this extension was already granted to acacia, apple, ash, cherry, elm, pear, poplar, potato and willow. In the new law this extension is also granted to strawberry, freesia, lily, tulip, maple, cornel, currant, rowan, linden and magnolia. The improvement is due to the strong lobby of Plantum NL in order to acknowledge the longer time needed to breed flower bulbs, strawberries and trees. The main argument is that a breeder needs a certain period in which he effectively can regain his investments. If a breeder already protects his variety in the period of testing and multiplication, because of the possibility that the variety is stolen from the field, he loses costly years. The 30 year protection period applies to all varieties that still fall under PBR protection on the first of February 2006 or will fall under it in the future.

Obligation to inform

The new law has a completely new approach to the farm saved seed (FSS) principle. A farmer who makes use of FSS is obliged to report the amount of farm saved seed he has planted to the breeder before the 15th of May of the year he will harvest his crops. This means the breeder no longer needs to enquire for information. But if a farmer has used farm saved seed and has not reported this in time to the breeder, he is without any doubt infringing the plant breeder's right.

In the Netherlands the use of farm saved seeds is limited to potatoes and cereals. In these crops around 30 percent of the seed used is farm saved seed and 70 percent is certified. In comparison to other countries this is relatively low, but still this makes it hard for the breeder to be able to regain his investments on the intellectual property of his varieties. It is therefore important that he receives the remuneration that the farmer pays for the use of farm saved seed.
To make it easier for farmers to consent to this regulation, the members of Plantum NL have agreed that the report can be sent to the organisation, instead of to the individual breeders. Plantum NL will then inform the breeders.
Another change in the new Plant Breeder's Right Law is that it offers breeders new methods to enforce their rights in case of an infringement. A holder of a plant breeders' right can undertake a range of legal actions on the basis of the new law where in the past this had to be done on the basis of Civil Law. This possibility already existed in other Dutch intellectual property laws such as the Patent Law.

Better structured

Most changes in the new seed and plant law are improvements in the structure of the law, as the old one had been revised several times in the past. Other changes relate to illogical or outdated regulations. For instance the procedures of applying for PBR and/or the national list. Both procedures have become fully separated, whilst in the past it was obligatory to apply for plant breeder's right in order to put a variety on the National List of Varieties. In case a breeder wants to apply for both, the results of one DUS-test can be used for either of them.
The government has also introduced a new Board that takes care of all applications, the 'Raad voor Plantenrassen' (board for plant varieties). It replaces the old 'Raad voor het Kwekersrecht' (board for breeder's rights).
All in all the new law gives breeders some new means in order to improve the possibilities to exercise their rights.

Temporary immersion beats traditional techniques on all fronts

Maritza Escalona

In comparison with conventional micropropagation on semi-solid medium, the temporary immersion technique gives more output for less input. Indeed, the proliferation rate is higher, the quality of plants is better and labour efficiency is improved.

For many food, ornamental and forestry crops, micropropagation (propagation in tissue culture) has become the most important means for propagation of selected elite varieties. However, the present techniques are not always reliable and scaling-up is often difficult. In addition, because much labour is involved, production costs are high. For these reasons, micropropagation is carried out commercially only with a limited number of crops.
To overcome shortcomings of the conventional micropropagation techniques, new methods have been developed using bioreactors and liquid medium. Bioreactors constitute one of the most effective ways to reduce the costs of micropropagation. The use of liquid culture media for plant micropropagation has many advantages and is a key factor for automation. Major problems in liquid medium are asphyxiation and – in agitated liquid medium – shear damage. Hyperhydricity of shoots is another main problem. Temporary immersion reduces these problems: Because of the short periods of immersion, asphyxiation does not occur, and because of limited convection of the medium, shear damage is minimized. With most crops, growth is sharply enhanced and high quality propagules are produced.

Semi-automated system

Based on the concept of temporary immersion, researchers at the Bioplant Center of Ciego de Avila, Cuba, developed a new type of bioreactor, the Temporary Immersion Bioreactor (TIB(r)). In TIB, explants are temporarily immersed by pneumatic driven transfer of liquid medium without medium replenishment. The system is simple and easy to use. It enables contact of the liquid medium with all parts of the explants without the risk of asphyxiation. Pneumatically-driven temporary immersion is achieved by connecting two glass or plastic flasks (250 ml to 20 l) using silicone tubes, and applying overpressure to force the medium from one flask to the other (see figure). In addition, during each immersion period, the gas phase is renewed by the forced ventilation so that detrimental gases like ethylene do not accumulate. Injection of CO_2 may improve photosynthesis. TIB has been used for a wide range of crops, among others Ananas, Saccharum sp, Musa sp, Colocasia sp, Araceaes, Eucaliptys sp, Rosaceae, Bromelias, Paeony. It may also be used for microtuberization, among others in potatoes and yam. The efficiency is often sharply increased (see table).

Unique characteristics

The main reason for the efficacy of TIB is that it allows full contact between explants and medium as in liquid medium without asphyxiation. In order to establish a micropropagation procedure and to increase the efficacy of TIB-technology, various parameters should be optimized. Among them, immersion time, immersion frequency, the volume of nutrient medium, the volume of culture container (headspace volume of the container), the duration of the proliferation phase, the type of explant, the use of plant growth retardants and the number of cycles in TIB. Depending on the crop, these parameters have had a major influences. The clonal fidelity of the plants produced using TIB have been assayed using molecular probes and evaluations in the field. For example a field experiment was carried out at the National Corporation of Bananas in Costa Rica (CORBANA) with plants produced in TIB. Up to now, data on survival, growth, flowering, harvesting time, fruit production and somaclonal variation indicate the efficiency of TIB for plantain production. TIB has a major positive impact on production costs. For the proliferation of Saccharum sp shoots, it was calculated that costs were reduced by 46% compared with the standard procedure on semi-solid medium. In pineapple propagation, TIB resulted in an increased multiplication rate and better shoot quality. This protocol reduced production costs per propagule by 20% as compared to conventional liquid medium.

Optimizing conditions

A research on the effect of temporary immersion on physiology is essential to optimize culture conditions in this simplified bioreactor. Plantain and pineapple have been used as model plants to study physiological behaviour in this new technique. The composition of the headspace during proliferation and prior to acclimatization of plantain shoots in TIB and semi-solid culture showed large differences in gases concentrations. There was a higher CO_2 and a lower O_2 peak during the elongation phase compared to the multiplication phase. Renewal of the headspace results in a supply of O_2 and the prevention of CO_2 and ethylene accumulation, which is important to shoot quality. TIB increased respiratory activity of shoots, but this fact did not seem to affect the intrinsic quality of plantlets. For this reason, a good understanding of temporary immersion culture and its physiology at the moment of transfer to ex vitro conditions can provide guidance in how to optimize the acclimatization procedure in order to reduce plant losses. Studies along this idea continue in progress.
The system did not improve photosynthetic capacity of pineapple shoots before transplanting. Shoot growth did not totally depend on the photosynthesis process. The shoots appeared to use more nutrients from the culture medium than from photosynthesis. TIB-derived pineapple shoots showed a remarkable nutrient uptake indicating higher photomixotrophic metabolism. However, TIB-derived plantain-shoots improved photosynthesis. Starch was the most important sugar accumulated by the stems. Plantain plantlets start to uptake the starch during the first days of the acclimatization. This accumulation, as a nutrient reserve, is important to overcome the stress period during subsequent growth under ex vitro conditions. The growth parameters of plantain shoots before acclimatization indicated that the system improves the plant quality in comparison with semi-solid culture. In this culture method, there is a better relationship with photosynthesis/transpiration, which permitted major nutrients assimilation for the plantlets and further shoot quality before acclimatization.

Useful tool

For the last 20 years, plant mass propagation systems have played an important role in Cuban agriculture and in the international market. Cuban specialists have introduced a new concept for low costs at commercial laboratories which are called Bio-factories. They have incorporated an affordable design for culture rooms using natural light, the use of simple culture media adding sugar and macro- and microelements of a commercial grade, chemical sterilization in the laminar flow using calcium or sodium hypochlorite, liquid culture media on the majority of those species responding on, the chemical sterilization adding to the culture media substances like Vitro-Fural (G-1), and finally implementing Temporary Immersion Bioreactors. Quantity and plant quality in a short time have been proven by the rigorous quality control and good practices for in vitro mass propagation of plants like ornamental, fruits, plantain, forest and other kind of crops. The Cuban Bio-factory conception has been transferred and is also available to several countries, especially to those placed in tropical and sub-tropical regions.

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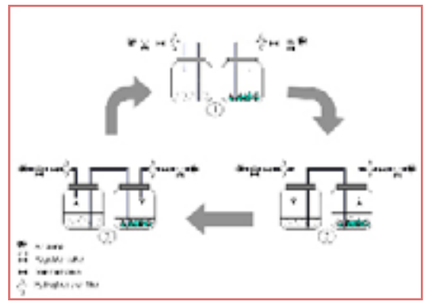
Summary of effect of TIB® on yield compared with conventional methods (semi-solid and liquid). The parameters which more influenced on the efficacy of the success of the protocol are also indicated.

Family-Species (common name)	Morphogenetic pathway	Conventional method	TIB®	Culture parameter affecting the efficacy
Ananas comosus (pineapple)	Shoot proliferation	3.8	20.2	Shooting duration and volume of culture medium
Saccharum spp (sugarcane)	Shoot proliferation	4.0	39	Plant growth retardant and volume of culture medium.
Musa sp (bananas)	Shoot proliferation	3.6	11.4	Plant growth retardant
Musa sp (plantain)	Shoot proliferation	2.8	16.0	Number of cycle in TIB® and type of explant.
Araceae				
Syngonium	Shoot proliferation	7.3	28.0	Plant growth retardant
Phylodendron	Shoot proliferation	3.0	103	Plant growth retardant and shooting duration.
Anthurium	Shoot proliferation	3.5	79.8	Culture container volume and shooting duration
Spathyphylum	Shoot proliferation	3.7	61.5	Culture container volume and shooting duration.
Eucalyptus Grandis	Shoot proliferation	2.5	16.5	Forced ventilation and immersion frequency
Eucalyptus Urograndis	Shoot proliferation	2.7	12.3	Forced ventilation and immersion frequency
Paeonias sp SeSu	Shoot proliferation	2.1	6.8	Number of subculture in TIB®
Bromeliad				
Vriesea	Shoot proliferation	2.0	10.6	Volume of culture medium and type of explant
Aechmea	Shoot proliferation	2.2	24.6	Plant growth retardant, culture container volume.
Neoregelia	Shoot proliferation	2.1	32.4	Plant growth retardant, culture container volume
Cryptanthus	Shoot proliferation	1.8	22.9	Plant growth retardant, culture container volume
Solanum tuberosum (potatoe)	Microtuberization	15.0*	62.0*	Not evaluated
Dioscorea (yam)	Microtuberization	1.7 **	4.7 **	Not evaluated

* Percentage of tubers higher 6 mm. ** number of microtuber/plant.



Operating cycle of TIB®. (1) Non-immersed stage, shoots are free-standing on the bottom of the culture vessel. (2) Beginning of the immersed stage; an overpressure is applied and the medium is pushed up into the plant container, immersing the shoots for a few min. (3) End of the immersed stage, a second solenoid valve is opened and the culture medium is removed into the reservoir. These steps are performed, e.g., every 3 h. The air pump and electric valves were under control of a timer.

DUS-testing transferred to Naktuinbouw

Monique Krinkels

Until this year it depended on the type of crop where breeders had to send their material for DUS-testing in the Netherlands. For vegetables it was the inspection service Naktuinbouw, for agricultural and ornamental crops the Centre for Genetic Resources. To improve efficiency, the Raad voor de Plantenrassen (board for plant varieties) has decided to put all DUS-testing in one establishment. At the Naktuinbouw, the Netherlands over 2,300 applications are being tested for Distinctness, Uniformity and Stability (DUS). Since January 2006 the responsibility for these tests has been transferred to the Varieties & Trials business unit of the Dutch inspection service for ornamentals and vegetables. "We now are the largest DUS-testing organisation worldwide", claims Kees van Ettekoven, head of the business unit.

Current DUS tests at the Naktuinbouw

Crop	Approval	National PBR	EU PBR	Total
Ornamentals				
Alstroemeria	8	22	30
Carnation	9	19	28
Freesia	8		8
Gerbera	27	39	66
Lily	41	96	137
Rose	23	89	112
Tulip	109	7	116
Other field plants	64	168	232
Pot plants	43	140	186
Cut flowers	15	71	86
Agricultural crops			
Potato	11	3	114
Sugar beet	51		51
Grains	16		16
Flax	4		4
Chicory	3		3
Grasses	144	1	145
Vegetables			
Tomato	139	24	23	186
Lettuce	109	1	68	177
Pepper	94	20	2	116
Cauliflower	42	4	13	59
Cucumber	29		6	35
Bean	28		19	47
Melon	26	9		35
Onion	25		14	39
White cabbage	25	1	8	34
Spinach	15		22	37
Leek	14		20	34
Endive	14		9	23
Courgette	14			14
Gherkin	11			11
Aubergine	11		2	13
Water melon	10			10
Pea	10	1	19	30
Others	89	3	29	121
Total	684	743	927	2,354

Until this year it depended on the type of crop where breeders had to send their material for DUS-testing in the Netherlands. For vegetables it was the inspection service Naktuinbouw, for agricultural and ornamental crops the Centre for Genetic Resources. To improve efficiency, the Raad voor de Plantenrassen (board for plant varieties) has decided to put all DUS-testing in one establishment. At the Naktuinbouw, the Netherlands over 2,300 applications are being tested for Distinctness, Uniformity and Stability (DUS). Since January 2006 the responsibility for these tests has been transferred to the Varieties & Trials business unit of the Dutch inspection service for ornamentals and vegetables. "We now are the largest DUS-testing organisation worldwide", claims Kees van Ettekoven, head of the business unit.

Experienced

For several years Naktuinbouw has been the appointed authority for testing new vegetable varieties on distinctness, uniformity and stability for breeder's rights applicants. It was a logical choice, as the organisation already undertook these tests for the approval and registration of vegetable varieties since 1974. As most breeder's of hybrid vegetables deem it sufficient to protect their varieties by registration only, the organisation has considerable expertise on these crops. All other crops were being tested in Wageningen by the Centre for Genetic Resources (CGN). "But as we can include them in our whole range of activities we can do it more efficiently", says Kees van Ettekoven. "Because the government does no longer finance DUS-testing, all costs have to be paid by the breeders themselves. It makes it very important to work cost-effectively and that is easier when all testing is carried out in one organisation."
"The knowledge of the people in Wageningen is however not lost. The complete staff of CGN involved in DUS-testing, now works for Naktuinbouw. So the experts on agricultural and ornamental crops will remain the same." Furthermore the cooperation with the agricultural inspection service NAK and the inspection service for flower bulbs BKD will be strengthened, increasing the expertise even more. Based on the findings of Naktuinbouw, the Dutch board for plant varieties can decide to add a variety to the variety list or grant plant breeder's rights. The same work is carried out for the Community Plant Variety Office (CPVO), which grants EU plant breeder's rights. The Naktuinbouw has an ISO 9001 certificate and recently acquired an ISO 17020 accreditation for the most important crops, making it clear that the quality of the tests meets the highest standards.

Assembling knowledge

The people involved in DUS-testing can use their expertise more widely at the Naktuinbouw. One of the other activities of the Varieties & Trials unit is identity testing and investigations into breaches of plant breeder's rights. It requires more or less the same knowledge used in DUS-testing. These activities form part of the 'Variety Tracer' service. Especially for mutants it is important to establish morphological differences as DNA tests are not conclusive. The Varieties & Trials unit does not only work for the Dutch market. Many foreign breeders call on the services of this unit to carry out the DUS inspection for them. These numbers are expected to increase due to globalisation and the expanding EU. That makes it even more important to unite DUS-testing in one expertise centre, where all necessary knowledge has been assembled.

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