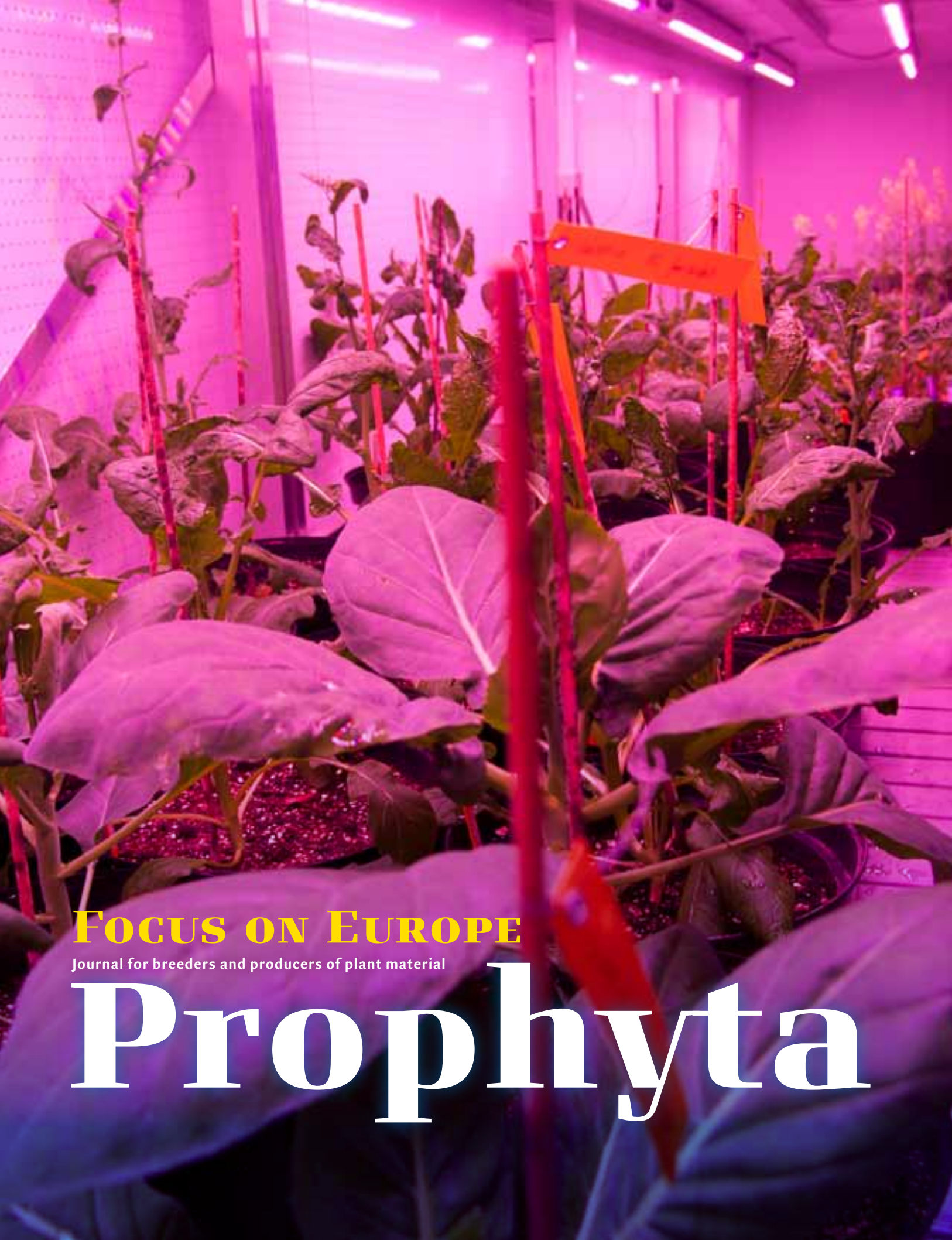
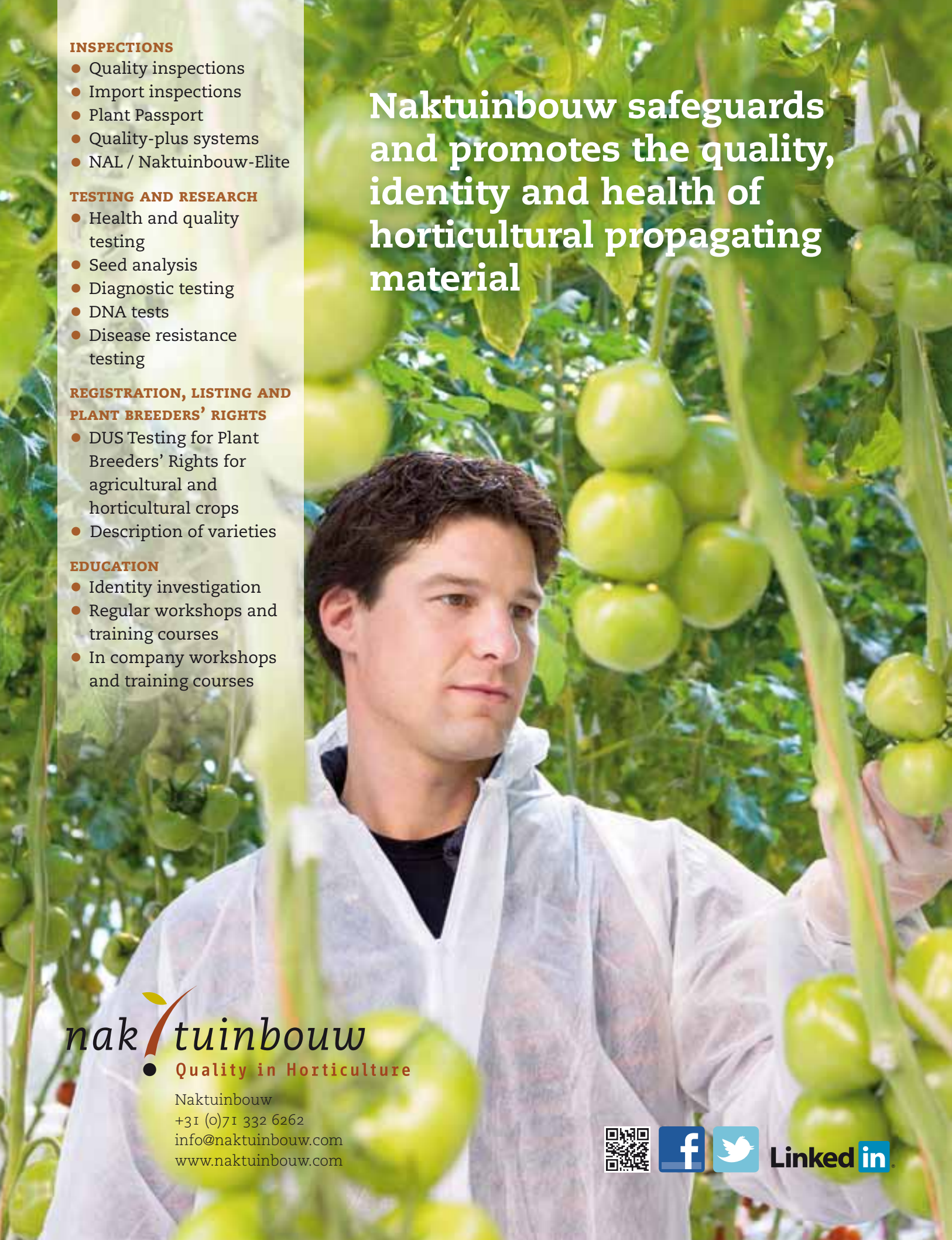


Focus on Europe

Journal for breeders and producers of plant material

Prophyta



INSPECTIONS

- Quality inspections
- Import inspections
- Plant Passport
- Quality-plus systems
- NAL / Naktuinbouw-Elite

TESTING AND RESEARCH

- Health and quality testing
- Seed analysis
- Diagnostic testing
- DNA tests
- Disease resistance testing

REGISTRATION, LISTING AND PLANT BREEDERS' RIGHTS

- DUS Testing for Plant Breeders' Rights for agricultural and horticultural crops
- Description of varieties

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- Regular workshops and training courses
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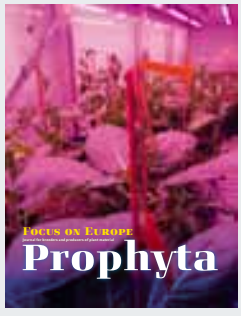
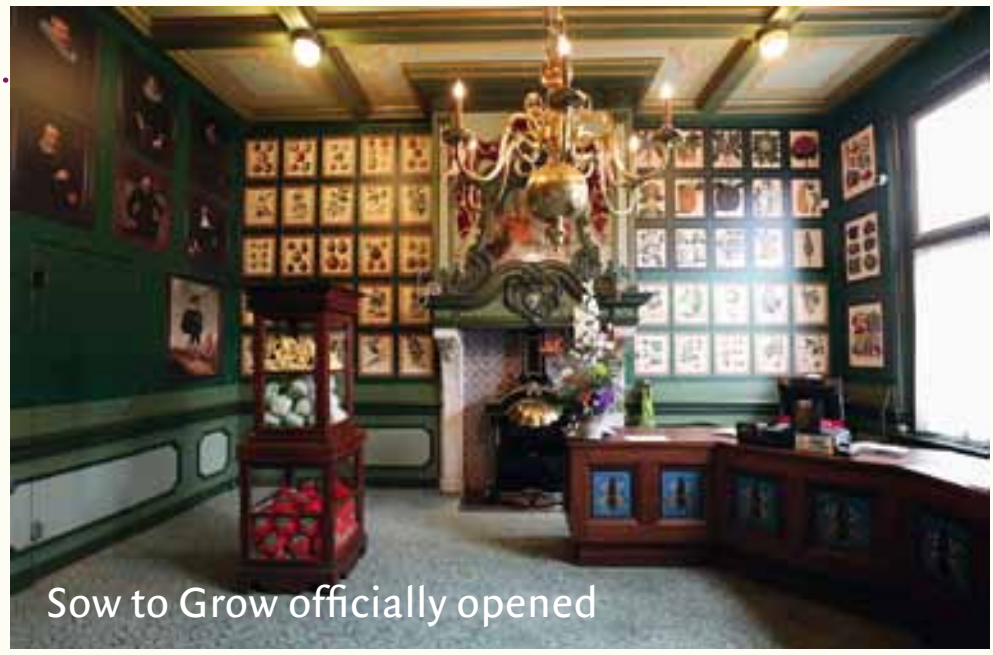


On the cover: In the climate cells of KeyGene cabbage varieties are tested for their ability to cope with urban farming conditions

FOCUS ON EUROPE
Journal for breeders and producers of plant material
Prophyta

Organics will suffer due to Brexit

UK EXPORTERS OF ORGANIC food could face a 9-month block to sales if there is no Brexit deal. That is because UK organic certification bodies will have to be approved by the EU, but they cannot apply until the UK leaves. It can then take 9 months for approval to be granted, though the government says: 'we are exploring alternative approaches that should speed up this process'. Farm subsidies, currently paid by the EU, will be paid to farmers by the UK government if there is no deal. The government has pledged the same total cash 'until the end of this parliament', expected in 2022.



Sow to Grow officially opened

PROFESSOR LOUISE FRESCO, President of the Executive Board at Wageningen University & Research, was impressed by the interactive plant science experience Sow to Grow. At the official opening on Friday 14 September, she praised the initiative of the former Saet & Cruyt Museum on the modern way of introducing laypeople to the exciting world of plant breeding and the production of seeds. In interactive ways, they became acquainted with the role of plants in feeding and making the world more pleasant. Sow to Grow can be visited from Tuesday till Sunday in the former City Orphanage, Westerstraat 111 in Enkhuizen.

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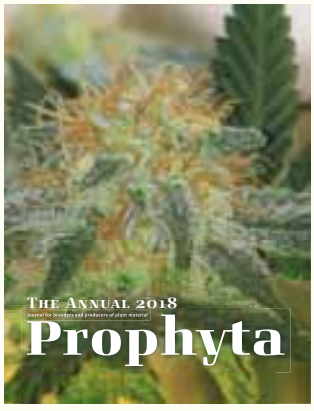

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Satellite images identify infection

An international team of scientists has discovered a new way to map out an infection of *Xylella Fastidiosa* at such an early stage, that preventative action could be taken. This bacterium is an EU quarantine organism harmful to olive trees, almond trees and grape vines. On satellite images, the infected trees are recognizable by a specific 'fingerprint' months before the trees show signs of the disease. For their research, scientists analyzed satellite images of 200,000 olive trees in southern Italy, for 2 years. They compared the reflection of sunlight on their images with field and laboratory tests to identify diseased trees. In the reflected light spectrum, they discovered a 'fingerprint' that corresponds specifically to *Xylella*. The fingerprint was already observable 4-12 months before the trees were visibly affected.

Saving biodiversity

Anthura, the world leading specialist in orchids and Anthurium, celebrated its 80th anniversary by reintroducing a rare orchid in Switzerland. *Cypripedium calceolus*, the Lady's Slipper Orchid, is one of the most spectacular wild European orchids. Unfortunately, this has a downside: this also makes the plant popular in illegal trade. In Switzerland, the plants grow in open forests as well as on mountain pastures, as they tolerate extreme cold. Together with The Swiss Orchid Foundation, Anthura started a pilot experiment with mature plants that Anthura had cultivated. Cypripediums are very vulnerable in their first stage of life and specialist knowledge is required, otherwise the plants usually do not survive. After 5 years, all the plants are still alive and have flowered with 150 flowers. The large number of scented flowers attracted many pollinating insects. The ultimate culmination of this test was the number of seedlings that had come up.
This year, Anthura delivered 3,000 mature plants, which have been planted in the wild at 44 different locations in Switzerland, among others in the Chasseral Regional Park and Gantrisch Nature Park. With the replanting of the *Cypripedium*, *Anthura* hopes to contribute to the conservation of biodiversity.

Farmageddon

What will happen during the night of 29 and 30 March 2019, midnight in Brussels and 11 p.m. in Britain? Will it be a smooth transition, a cliff edge departure or a jump into the abyss? Given the lack of consensus of what the future relationship between the UK and the EU should be and the preparations in the UK on a no-deal outcome, the latter seems more and more likely. The British government has recently published a series of technical notices setting out what will happen in the event of the UK leaving the EU without reaching a deal. From trade barriers, border inspections, GMO-regulations and Plant Breeders' Rights, to the use of seed enhancement chemicals and organic seeds (not to speak about the financial consequences), from 'day one' after Brexit everything that has an EU-Britain link will have new rules.
So far, the British farmers and growers are preparing for the worst. "If we can't get our relationship right with Europe, we have nowhere else to sell or buy at the right price. After all, 90% of British food and drink exports go to Europe and 29% of food comes from the EU. Food will be left to rot in the fields due to labour shortages. At present, a third of the workforce in food manufacturing comes from the EU. At peak season, farmers hire 75,000 temporary labourers, almost all from the EU," are some of the laments. Even the green groups warn about the negative effects it may have on the environment. In their view, everything from the ability to tackle climate change to the UK's bird populations could be under threat. The sudden lack of regulation could make the UK a 'dumping ground' for harmful chemicals banned in EU countries, threatening both human and environmental health, they believe. In short, the British farmers are afraid of a 'farmageddon'.
The agricultural sector in the other European countries is not much more positive. Being prepared is crucial is the advice governments give to the companies in their countries. Extra staff at customs and in the inspection services is one of the focal points. Research shows that the costs for all imports and exports will increase by 387 to 627 million euros annually. This does not include customs duties, VAT expenditures and unknown sector-specific market entry requirements, such as phytosanitary certificates.
And what to do with the tasty Armagh Bramley apples, the Pembrokeshire Earlies and the Yorkshire forced rhubarb? These EU protected traditional quality products rely on the recognition of their authenticity by EU's Food Name Scheme. Hopefully, the negotiators in Brussels will take their taste buds into account and settle for a friendly divorce.

Monique Krinkels

European Court does not do **CRISPR-Cas** justice

Monique Krinkels

The European Court ruled on 25 July that some modern breeding techniques, such as CRISPR-Cas, fall under the strict European Directive on genetically modified organisms. Contrary to expectations, the Court ruled that modern mutagenesis techniques are indeed covered by that directive. This puts European plant breeders at a disadvantage compared to their competitors elsewhere in the world.

Is a variety obtained by modern mutagenesis techniques, such as CRISPR-Cas, a GMO or a 'normal' mutant? That was the question the EU Court of Justice had to decide upon this summer. The case was first addressed by the Confederation Paysanne, an organization of small (hobby) farmers in France, who wanted to know whether mutant varieties would be allowed in the EU. Their goal was to prevent admission of herbicide-resistant varieties on the European market. In 2016, the French Conseil d'État, the Council of State, passed it on to the European Court of Justice in Luxembourg. Contrary to the positive advice of the Advocate General at the Court of Justice, Michal Bobek, on 18 January 2018, the Grand Chamber of the Court of Justice decided that the European Union's GMO legislation applies.

Classic or not

The focal point is whether mutants that are a result of radioactive irradiation or chemicals – the so-called 'classic' mutants should be treated differently from the modern mutants obtained by gene editing. The first are exempt from the EU rules for GMOs and, therefore, do not have to comply with the strict rules on precautionary measures, risk assessment and traceability.

Michal Bobek, Advocate General at the Court of Justice of the EU: "I am of the view that it is necessary to interpret Directive 2002/53, taking into account the scope of the GMO Directive, and to conclude that the exemption laid down in the GMO Directive also applies to Directive 2002/53. Thus, organisms created by mutagenesis are subject to the general obligations laid down in Directive 2002/53 that apply to all kind of varieties of agricultural plant species to be put in the common catalogue. They are not, however, subject to the specific obligations laid down for genetically modified varieties."

In practice, the Court usually follows the Advocate's opinion. It was, therefore, the cautious expectation that these new mutagenesis techniques would be exempted from GMO legislation. And it came as a

'In many cases, we cannot distinguish whether a product was made with a classical mutagenesis technique or with a new technique, or via a conventional cross. I think it is a pity that the European Court explains the law by focusing on technology, instead of on the product.' Esther Kok, Head of Department of Novel Foods and Agrichains, Wageningen University & Research.

'It is good news for organic breeders, farmers and processors, but also for all European producers and consumers, as it brings clarity and will ensure the freedom to avoid such GM products and the protection of the environment from the potential risks of these new technologies.' Jan Plagge, EU President of IFOAM Organics International.

'Innovations made possible by genome editing hold enormous promise to keep Europe at the forefront of socioeconomic development, continuing to generate jobs and growth in the EU. Unfortunately, this court ruling, which is inconsistent with the Advocate General's Opinion published in January, does not provide the necessary regulatory clarity needed by EU researchers, academics and innovators.' John Brennan, Secretary General EuropaBio.

'It is now likely that much of the potential of these innovative methods will be lost for Europe with significant negative economic and environmental consequences. That strikes a serious blow to European agriculture and plant science.' Garlich von Essen, ESA Secretary General.

'This statement makes it more difficult for companies to innovate and also to keep up with the rest of the world. Because in Asia and the United States, for example, the rules are smoother and developments are moving forward.' Kai Purnhagen, expert in European and international law at Wageningen University & Research.

'One third of the food produced for human consumption is wasted. Innovative gene editing methods, like CRISPR, could help reduce food waste.' Michael Keller, Secretary General ISF.

'We believe that with targeted mutations less changes

The European Court of Justice (ECJ) is the supreme court of the European Union in matters of European Union law. The Court, based in Luxembourg, is composed of one judge per member state, led by president Koen Lenaerts

complete surprise that the Court of Justice took the opposite point of view. While the European GMO Directive includes an exception for mutagenesis, the Court states that this can only be used for traditional mutation techniques as these have a history of safe use. It gave the sector the long-awaited clarity, but not the clarity breeders had hoped for.

Future

The Court's ruling puts Europe well behind the rest of the world. A fully-fledged risk assessment will cost between 10 and 100 million euros per edited characteristic due to the multitude of tests on human and environmental impacts. This might be an option for the largest arable crops, such as maize, wheat or rapeseed, but it is a NO GO for vegetables, for instance. This will have a chilling effect on research, in the same way that GMO legislation has had a chilling effect for 15 years now. So, all in all, it is a severe blow to innovation in EU agriculture.

in the DNA are induced than with traditional mutation techniques and that the risks are comparable or even smaller. If we have to wait for a history of safe use, then perhaps we might have to wait half a century.' Niels Louwaars, director Plantum.

'The Netherlands (..) will be committed to the application and admission of new breeding techniques, such as CRISPR-Cas9, in Europe, provided that no species boundaries are exceeded.' The Dutch government in the coalition agreement.

'It follows from the Court's case-law that under the precautionary principle, "risk uncertainty" does not mean mere general doubts. Concrete risks for human health or the environment must be identified, supported by a minimum amount of serious and independent scientific research. A fear of a risk, or risk of a risk, is not enough.' Michal Bobek, Advocate General at the Court of Justice of the EU.

'The Court makes it crystal clear that plants and animals derived from gene editing are subject to the same safety and labelling requirements as other GM organisms. These requirements exist to prevent harm and inform consumers about the food they eat.' Franziska Achterberg, Greenpeace EU food policy director.

'The important point is that this ruling ignores assessment of the safety of the trait developed, and rules only on the technology used. So, introduction of higher yielding crops, engineered by mutagenesis (traditional or by new breeding technologies), could be blocked by NGOs in the absence of an approved environmental impact evaluation. This is going to impact on plant breeding in Europe hugely and negatively.' Cathie Martin, Professor at the John Innes Centre.

'We need a future-proof risk-based regulatory framework based on the traits being introduced, not the way in which they were introduced. It should include all breeding techniques, from conventional to whatever the latest approaches might be. The idea that things that could occur 'naturally' are distinct and somehow automatically safe for people and for the environment is untenable. The distinction between GMO and not GMO is contrived.' Ottoline Leyser, Professor at the University of Cambridge.

'I have developed a 'CRISPR cabbage' that I have consumed, and which was growing in my home garden. I took a photo yesterday (ed. 24 July), and I took another after the ruling. It's still the same plant. Yesterday it wasn't a GMO, and now it's a GMO. I'm a bit curious what I have to do. Do I have to remove it?' Stefan Jansson, plant physiologist at Umeå University, Sweden.

Fostering European Research – **but for what?**

Garlich von Essen

The European Union's third biggest budget line is its framework programme for research and innovation. Over the past decades, it has consistently grown both in scope and financial magnitude; and today again, Commission, Member States and European Parliament are discussing yet another significant budget increase for the next edition to cover the years post 2020.

Everybody in Europe seems to just love research. And spending lots of money on it. But growing ambition is not only marked by numbers. While earlier programmes will go down in history merely as numbers (former framework programmes were called just 'FP1', 'FP2', 'FP3' and so forth), the current one was, or better is, named 'Horizon 2020', and the title for the latest proposal now is a catchy 'Horizon Europe'. What's in a name? Well, more than just a marketing pitch if one takes a little closer look.

Up, up, up

More and more, the EU's research policy and funding are supposed to support the general European policy agenda. That may sound not only logical but even desirable. Who would oppose overarching objectives such as supporting economic growth, strengthening Europe's competitiveness, creating jobs or establishing a knowledge-based (bio-)economy that sustainably preserves resources? Correct – probably nobody. But this widespread principal support from all sides of the political spectrum, across sectors and stakeholder groups comes at a cost. Not as far as budget is concerned. Here, the trend has always been up, up, up. But it seems that with every increase of budget (and ambition), the actual political debate on scientific priority areas and preconditions for EU support has – successfully – been pushed to the sidelines. Gone are the days when the discussion of 'the FP' was THE highlight of the work of the respective European Parliament Committee, when even full plenary debates were spent on exactly WHAT Europe's role in this policy area should be and on what the money should actually be spent. Partly, this was due to the fact that specifically the EP understood the FP as a political instrument where it could exercise both its budgetary but most of all its policy shaping powers.

The widespread support from all sides of the political spectrum comes at a cost

What kind of energy policy, what kind of biotechnology policy did Europe want? A good part of the answer can be found in the final agreement of e.g. framework programmes 3 to 5.

Implementation phase

These debates were long and difficult; and one might argue that a research agenda should first and foremost try to foster scientific excellence as a base for future political considerations and decisions, not the other way around. But one might also underline the importance of having this debate at all; and even triggering the interest of almost the entire European Parliament as well as industry groups and other stakeholders.

Over time, this discussion on detailed objectives has more and more disappeared. True, efficiency and effectiveness of decision making probably improved with that. But at the cost of having a more in-depth debate on principal policy choices as well as more clearly described objectives for EU level funding of specific research and development projects between MEPs and national ministers. This discussion has now been pushed to the actual implementation phase of the programme. Once the overarching agreement is signed off, it is largely left to Commission and programming committees to hammer out concrete calls for projects and decide on the allocation of funding. Consequently, the influence specifically of the Commission on the specific focus (and objectives) of projects has grown considerably. In practice, this often means that projects are designed to deliver expected results that can then be used to argue for or justify and back-up certain regulatory initiatives for which one (the Commission) expects to get the necessary political support for adoption.

Credibility

Again, all of this is not entirely new. Bits and pieces have always been present in policy making and are for sure not specific for research policy. But unlike other policy areas, R&I funding also carries a specific 'seal of approval', one of impartiality, of a greater cause and common good behind it; and behind its results. But if calls are developed in a manner that is supposed to already in advance assure a desired outcome to then justify a political initiative, there is a risk that

Garlich von Essen is Secretary General of the European Seed Association, Brussels, Belgium, vonessen@euroseeds.eu.

Sunday speeches on plant breeding innovation and research funding become absurd if EU regulations subject them to multi-million-Euro authorisation requirements

science actually loses rather than wins credibility, irrespective of formal independence.

Another element largely absent in the current discussion is the necessary consistency of policies that is precondition to in the end turn research into innovation, or – to put the focus on our specific of plant science – to, in the end move from company labs to farmers' fields and citizens' kitchens. Two examples: Europe's upcoming protein plan is supposed to address the well-known huge import requirements of the EU of animal feed, mainly from GM soy from North and South America. Any plant scientist knows that successfully and radically improving the relative competitiveness of a species, e.g. of oilseed rape, vis-à-vis another (here soy) on the one hand can only be based on a sustainable business model and in any case requires decades of plant breeding work and a predictable policy environment. Cutting back biofuels, banning neonicotinoids – all of this is actually harming the competitiveness of European oilseed rape and with that the availability of competitive EU derived protein supply. Some financial support via some R&I projects for a few years will prove to be incapable of compensating for this.

Similarly, Sunday speeches on plant breeding innovation and some specific research project funding on the use and important potential impact of the latest mutagenesis techniques in specific crops become absurd if EU regulations at the same time subject them to multi-million-Euro authorisation requirements. The maths is easy: get a few million from the EU for your CRISPR research, subtract some 30 to 50 million EUR for the authorisation of a single new variety, add the costs for post-market monitoring, and take account of the fact that your product may not be planted in 19 out of 27 EU Member States in the future due to national restrictions... Easy, isn't it?

Consistency

The EU needs a much more fundamental and much broader policy discussion when it comes to its objectives and available tools. Research and innovation support and funding for sure is an important element, even one that may help to drive further societal and political debate with excellent scientific results, conclusions and recommendations. As to how far the next EU framework programme can help to deliver on this also largely depends on not overburdening it with a pre-defined agenda and acknowledging that consistency in policy-making and stamina in its implementation are probably more important than increasing funding levels.

Stop stealing protected varieties

Monique Krinkels

10 **About half of the area sown with potatoes in Europe is planted with farm-saved seeds or illegally obtained propagation material. "It is completely unacceptable that the market is manipulated by cheaters," says Geert Staring, director of Breeders Trust in Brussels, Belgium. His organisation strives for fair play in the potato and grass sector. "The hunt for thieves is on."**

Breeders Trust is regularly in the news with the most spectacular part of the work, namely conducting legal proceedings or organising a police raid and seizure of goods or paperwork. "That is always with permission of the judge, so a lot of homework precedes it," says Geert Staring. Since 2011, he has been director of Breeders Trust, an organisation founded in 2008 by ten international operating seed potato breeding and trading companies. As the development of a new potato breed takes at least ten years and costs up to 3 million euros, they were fed up with violators of plant breeders' rights. The ten companies together account for over 80% of the newly developed potato varieties within the EU. In 2012, the six largest grass seed companies in Western Europe joined the organisation.

What is the goal of the shareholders?
"The companies were frustrated that, even though their varieties were protected by plant breeders' rights, they were stolen just like that. International, illegal trade and propagation took place without anyone raising a finger. They concluded that doing nothing was no longer an option. Our aim for fair play and a level playing field for everyone working through the whole potato and grass seed chain is non-competitive. What binds our shareholders and members is that they all subscribe to our mission. It is in the interests of the entire chain (including those who are not affiliated with Breeders Trust) that growers receive what they are entitled to and can continue to search unperturbed for new, better varieties in a market that is not troubled by malicious producers and traders."

How does it work?
"We spend a lot of time on providing information, tracking tips and conducting our own research and making contacts with the authorities, such as inspection services, fraud investigators, customs, food safety authorities, etc. We also assist in setting up an infrastructure and the enforcement of plant breeders' rights.
"In every country where we operate, we have our own lawyer, because every country and EU member state has its own laws and regulations. In close consultation with our lawyers, we build a case and outline the strategy. Incidentally, that does not always lead to a lawsuit in each case. Depending on the nature and size of the violation, this can lead to a warning or a mutual arrangement. Sometimes a friendly settlement with an abstention declaration is sufficient, but always under our conditions. If we do not have the conviction that a lesson has been learned, we will prosecute the fraud."

How many people work at Breeders Trust?
"It is not necessary to put an enforcer behind every tree. That would also be unaffordable. But if you know that there is a fair chance that your fraud will be discovered, it is often enough to keep the majority in check. Compare it to traffic cameras to stop speeding cars. We receive tips from our own people and shareholders who are in the field daily. They know who they are dealing with in practice and know when the market is troubled. We also conduct mystery shopping and test buys and use modern detection and analysis techniques that accurately indicate where seed lots are and which varieties are at stake."

Can you give some concrete examples in the potato sector?
"Farm Saved Seed (FSS) plays an important role in potatoes. A few years ago, ESA made an inventory and it showed that up to 50% of the total area of potatoes in Europe is planted with FSS or illegal propagation material. The re-use of last year's harvest is permitted, but in the case of the use of varieties protected by plant breeders' rights, a fair payment must be paid to the breeder. The latter is sometimes 'forgotten'. This involves only a small amount of often less than €100 per hectare, but in total we are talking about far over 10 million euros. In many countries, we only receive a fraction of what we are entitled to. So, there is still a lot of work to be done for us, but we are on the right track.
"Another recent example is that by the time plant breeders' rights nearly expire, which in the case of potatoes lasts for a period of 30 years, there are quite a few companies that start building up a stock of seed potatoes, either by propagating or by importing from a country where the variety is not protected. If that is the case, we come down very hard and prosecute the offender. This is sufficiently frightening to prevent others from enriching themselves in such a way.

Fraud can have catastrophic consequences for a farmer. An unsuspecting buyer believed he bought a reliable batch of Italian ryegrass with an official certificate. Instead, infringers put a cheap Westerwold ryegrass of unknown origin in the bag

"Finally, we are and always remain vigilant that no consumption potato is traded as seed potato and that the material is only propagated with the breeder's consent. Only last year, we detected several cases of this type of fraud. It meant that the farmers planted uncertified seed potatoes, as they had not been inspected. That is illegal and it can create huge phytosanitary problems. Therefore, we could either inform the food authorities or go to court. In both cases, the growers ran the risk of having to harvest the seed potatoes again only one week after planting."

And in the grass seed sector?

"In the past five years, we have bought certified bags of English and Italian ryegrass in various shops in France, Italy and Belgium. We have had the contents of these bags examined at a recognised laboratory. They found that in no less than 35% of the sampled batches, there was something else in the bag than stated on the label.

Fraud can have catastrophic consequences for a farmer. An unsuspecting buyer thinks he is buying a reliable batch of Italian Rye Grass, in this case with an official certificate, but when sowing, it appears that a cheap Westerwold ryegrass of unknown origin has been put in the bag. For Breeders Trust that is reason enough to inform certifying authorities in the EU countries and take action.

"Another example was the discovery of counterfeit labels. Bags of grass seed were sold with 'home-made' Polish labels. The swindlers, however, had not noticed that the Polish authorities had modified the layout of the labels in some places. Our report to the Polish authorities was sufficient to stimulate action throughout Europe. In this case, the bags in Poland, France and Portugal were removed from the shelves by order of the authorities within 24 hours of our notification.

"One final example with grasses. Last year, we started to investigate the presence of pesticides in organic seeds that had been sold on the French market from Germany and Italy. The results were nothing short of shocking. An independent laboratory recognized that pesticide residues were detected in 40% of the sampled batches. We targeted batches of certain origin/exporters. All these origins, however, were officially certified. Reason enough for us to start a follow-up study this autumn and to continue to exert pressure on not only the certifying authorities, but also on the Fraud Investigation Services and Food Products Authorities to enforce the regulations adequately."

These are harsh measures!

"We realise very well that conducting legal proceedings is a tough remedy and has a big impact on a company. But we cannot and will not accept that the effort of breeders remains unrewarded because an infringer runs off with a protected variety. Going to court is a final resort to show the parties involved and the chain that it is unacceptable for our members that the market is being manipulated by cheaters. There are lawsuits that last three years or longer, especially if it leads to a higher appeal, but Breeders Trust has the time. We are not only building up international case law, but our actions also have a deterrent effect. All in all, it is in the general interest."

BASF Agricultural Solutions

When **chemistry** meets seeds

Monique Krinkels

12 **When Bayer acquired Monsanto, the antitrust authorities forced the company to sell, among others, their vegetable seed business. On 16 August 2018, BASF completed the acquisition of Bayer's vegetable seeds business – mainly consisting of the Dutch seed company formerly known as Nunhems. Today, it is part of BASF's Agricultural Solutions division.**

This year, BASF extended its portfolio with several businesses that formerly belonged to Bayer. Among others: the global glufosinate-ammonium business; seeds businesses including traits, research and breeding capabilities, and trademarks for key row crops; the global vegetable seeds business; the R&D platform for hybrid wheat; a range of seed treatment products; certain glyphosate-based herbicides in Europe, used predominantly for industrial applications; the complete digital farming platform xarvio™; as well as certain non-selective herbicide and nematicide research projects. In total, the all-cash purchase price amounts to € 7.6 billion and about 4,500 employees joined BASF through the acquisition. Vincent Gros, Senior Vice President Global Integration Management and Joachim Schneider, Head of the vegetable seeds business, about the first steps together in BASF's Agricultural Solutions division.

BASF has over 9,000 chemical raw materials and semi-finished products and has an oil & gas division. How will seeds fit into this? And how can seed contribute to the financial success of the business?

Vincent Gros: "BASF's 'We create chemistry' strategy targets seven customer industries – transport, construction, consumer goods, health and nutrition, electronics, energy & resources and agriculture – where BASF can contribute to solving global challenges and thus continue to grow profitably. The recent acquisition – including the key field crop and vegetable seeds businesses and assets – strengthens our solutions portfolio for tackling the challenge of feeding a growing world population in an economic, social and environmental balance.
With our new seeds activities in key field crops (canola, cotton, soy, wheat) in important agricultural markets, along with a global vegetable seeds business, we will give farmers worldwide a real choice in a consolidating industry. Our strategy is to become a global seed player. This acquisition is a key milestone on this journey and establishes BASF as a seed player in important crops in key countries across the globe."

In the past, did BASF consider adding seeds to its portfolio?

Vincent Gros: "We evaluated opportunities for seed assets in the market in the past. We looked at trends and opportunities in the agricultural industry and what they are influenced by. We also looked at our portfolio and what farmers need to be successful, today and in the future (e.g. game-changing thinking, relentless innovation, ambitious investment in new technologies, a correspondingly responsible treatment of our environment). Our goal is to even better combine innovative technologies, solutions and know-how to support farmers to grow more high-quality food more efficiently and safely.
We used a unique opportunity in our consolidating industry to add very attractive seed assets to enhance and expand our solutions portfolio in a targeted way. This acquisition was the best next step for BASF."

Vincent Gros, Senior Vice President Global Integration Management: 'In terms of people and culture, this acquisition is a fantastic opportunity to build one stronger BASF team in agriculture. We share similar values, a focus on innovation and a passion for agriculture'

Was BASF immediately interested in the acquisition of the vegetable seed business when Bayer decided to opt for Monsanto and sell Bayer Crop Science?

Vincent Gros: "The acquisition was a targeted, strategic complement to our well-established, successful crop protection business and biotechnology activities. It marks our entry into the seeds, non-selective herbicide and nematicide seed treatment businesses. It increases and broadens our R&D and digital capabilities considerably.
All the businesses and assets we acquired play a key role in successfully shaping our future in agriculture. The vegetable seeds business is highly complementary to our portfolio and allows us to increase our technology base and footprint globally. It creates new opportunities for sustainable growth and innovation, strengthening our overall market position and competitiveness in the long term.

Passport BASF

Original name: Badische Anilin & Sodafabrik (Baden Aniline and Soda Factory)
Foundation: 6 April 1865 in Mannheim, Baden, Germany
Founder: Friedrich Engelhorn
Portfolio: Chemicals, Performance Products, Functional Materials & Solutions, Agricultural Solutions and Oil & Gas
Subsidiaries and joint ventures: more than 80 countries
Employees: 115,000
Sales: € 64.5 billion (2018)

Passport BASF's vegetable seeds business

Main brand name: Nunhems
Original name: NV Zaaizaadvereeniging Nunhem
Foundation: 16 October 1916 in Nunhem, the Netherlands
Founder: Herman Meddens
Portfolio: 24 vegetable crops and over 2,600 varieties
Subsidiaries: more than 40 countries
Employees: 2,000

Joachim Schneider, Head of BASF's vegetable seeds business: 'It is our goal to continue to be a reliable, long-term partner in a fast-changing, consolidating industry'

Through the vegetable seeds business, mainly marketed under the brand Nunhems®, we offer hybrid varieties with traits and characteristics that are suited to different climates, growing conditions and cultural preferences."

Nunhems has a history with different chemical companies. From Hoechst, Aventis and Bayer, and now BASF. How did that influence the goals and choices made by the company in the past?
Joachim Schneider: "Whether we are part of a chemical company or not, for us it is important that we are part of a company that shares our passion for agriculture – with R&D, an understanding for the needs of farmers, and a vision for the future of agriculture. BASF is a well-known brand in the agricultural market. This also creates opportunities for the Nunhems® brand. We are confident that our combined, complementary R&D portfolio brings even better innovation capabilities and scale."

With its 2,000 employees, Nunhems will only be a small part of BASF. Will Nunhems be a completely separate operation or will the people working at Nunhems become foremost BASF-employees. And, if so, how will you integrate these people into the BASF-family?
Vincent Gros: "We are very excited to welcome our new colleagues in commercial, regulatory, production, R&D and other functions to BASF and look forward to growing our business with them. As a growing team, we become stronger and more diverse. This is a great opportunity to learn from each other and try new ways of working and finding solutions. Having just completed the acquisition of the vegetable seeds business on 16 August, it is too early to give any specifics. Clear is that the vegetable seeds business is part of our Agricultural Solutions division and the newly established Global Business Unit Seeds & Traits. We will spend the next weeks and months carefully evaluating options for building one strong future organization that best supports the innovation and market potential and decide accordingly."

What will the employees notice? And the customers?
Joachim Schneider: "For our customers, very little will change, apart from different email addresses and branding. We will gradually change our visual identity on all relevant materials, such as packaging, shipping documents and invoices. Documents with BASF branding have been implemented already, whereas for seed packaging, we plan a gradual implementation in 2019.
We will continue to deliver the same portfolio and varieties under the brand Nunhems®, and customers will see the same key contact. Our customer focus will also stay the same – while vegetable seeds are now a key part of BASF's expanded portfolio in agriculture, we will continue to be responsible for all vegetable seeds activities worldwide, including breeding, research, operations, logistics and sales. It is our goal to continue to be a reliable, long-term partner in a fast-changing, consolidating industry.
Most of our vegetable seeds employees will notice mainly a visual change, as the business unit will remain intact and most functions are dedicated to vegetable seeds activities."

Will BASF invest in growth of Agricultural Solutions division, expanding it with new crops or will it focus on stabilizing the present business?
Vincent Gros: "We are building on our combined strength in chemistry and broadening our position as a global seeds and traits player. Our considerably expanded global R&D capabilities and targeted funding will ensure our profitable growth as an agricultural solution provider, for decades to come."

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New EU Phytosanitary Legislation Coming Up

Crucial decisions are awaiting

John van Ruiten

At the end of 2016, the EU published its new Plant Health Regulation 2016/2031/EU. Before it will come into force on 14 December 2019, a number of implementation aspects have to be sorted out and decisions have to be taken. Some of them are very crucial for the seed and plant sector in the Union.

Basically, the new legislation is making control of diseases and marketing of seeds and plants within the Union itself easier. The number of quarantine organisms (Q) is brought down to a really low level. Only those pathogens and plagues for which eradication measures will be taken by the national plant protection organisations of the EU Member States will have the quarantine status in the future.

New category

Many current quarantine pathogens will be transferred to a new category: RNQP (regulated non-quarantine pests). Among the pathogens on this long list of RNQP's are important ones such as Clavibacter, Pospiviroids, Xanthomonas, fireblight and leafminers. There will be no more official phytosanitary measures in the production of commercial crops if they are infected with these pathogens. It will be up to growers to manage these diseases. However, the spread of these diseases also needs to be prevented in the future. Therefore, the requirement for marketing seeds and plants will be that the marketed lot/shipment must be free of these pathogens.
Over the last year, within the organisation EPPO, groups of EU experts have been evaluating over 1,500 quarantine and quality pests with the purpose of deciding if diseases qualified for the Q or RNQP status, and which inspection norms and standards would be the best to apply. EPPO has finished its task and has sent a huge list of proposals to the EU Commission. It is expected that a decision about the final list will be taken in Brussels at the end of the year.

Voorbeeld plantenpaspoort Rosa

With the start of the new regulation an EU harmonized/uniform plant passport (with EU logo) has to be used

Objectives of the EU Plant Health Regime

The new rules aim to modernise the plant health regime, enhancing more effective measures for the protection of the Union's territory and its plants. They also aim to ensure safe trade, as well as to mitigate the impacts of climate change on the health of our crops and forests. Different stakeholders will benefit from this new approach:
Citizens: better protection of landscapes and forests, public and private green spaces, reduced need for pesticide use;
Growers and farmers: simpler and more transparent documentation (plant passport), better protection of their production, more financial support for fighting pests;
Other business operators: common operators' register, harmonised traceability;
Public authorities: EU financial support for the implementation of surveillance and eradication/containment measures.

Severe control

A crucial point in the new EU legislation is that imports of seeds, plants and plant products will come into a more intensive and more severely controlled system. Imports will become more difficult, especially for so-called high-risk seeds and plants. For other products, there will be a need to be imported with an accompanying phytosanitary certificate (in fact, this will be obligatory for all seeds and planting materials). More stringent inspection procedures will apply for specific 'very important' quarantine pathogens. Imported seeds and plants also (of course) have to fulfil the RNQP requirements, which will be part of the import inspection procedures.

High-risk plants

A point that is currently causing a lot of practical concern is the new EU approach for so-called high-risk plants. It has been assessed by EFSA that plants of about forty different species are known to potentially

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The import of for instance maples (Acer) is only possible if an assessment makes clear that the species/country combination does not pose phytosanitary risks

host pathogens that are dangerous to the EU. Therefore, the import of seeds and plants of these species, originating in third countries, will be forbidden, unless a pest risk assessment has been carried out. It should be clear from that assessment that the species/country combination does not pose phytosanitary risks (to be decided by the EU Commission).
There are economically very important species in the draft list that was published by the EU Commission in July 2018, especially from woody ornamental, fruit and/or forestry plants such as Acer, Ficus, Malus, Prunus and Syringa. There are different reasons for putting certain species on that list, such as that they are frequently "collected from nature, grown in non-protected circumstances, known organisms (sometimes symptomless) in the species that can have big impact, or they are older plants." Examples of Xylella fastidiosa, red palm weevil, Asian long horn beetles and nematodes, such as pine wood nematode, have created very noticeable EU concerns about new pests coming in.
The final decision on the composition (species) on the list will be taken in December 2018. From September 2018 onwards, third countries can already send PRA documents to be studied by the EU, which will be done by EFSA. That can possibly lead to exemptions from the list. For the agricultural and horticultural sector itself, a stakeholder consultation (during August 2018) was organised. The results (objections, concerns, requests for more specified measures) will be evaluated by the EU Commission and might result in changes in the approach.

Nematodes

Another item of relevance is that the EU requirements for plants in growing medium/soil will be sharper and more intensified. Basically, because of concerns about nematodes (strains or species sometimes yet unknown to the EU). In fact, the standard will be that only clean/new growing medium will be allowed, and that plants in that medium must have been watered with clean water. In other cases, soil attached to roots of plants will not be accepted.
Furthermore, the new EU directives require official national registration systems of all operators, active in the production and/or marketing of plants and plant products. The objective is to have a full traceability system in place, that allows forward and backward tracing in case phytosanitary calamities suddenly occur. This registration goes further than the systems up until now, in which the registration was necessary for companies working with plant passport products and companies active in import/exports, but not for so-called 'end product producers'. The national plant protection organisations of the EU Member States have to make these registers and keep them updated.
One element of the new legislation was finalized last year: the layout/form of the new plant passport. Since the plant passport system was introduced in 1993, very many different forms/types of plant passports were developed and used. The EU has now decided that with the start of the new regulation, an EU harmonized/uniform plant passport (with EU logo) has to be used, containing all the necessary information. These requirements are published in implementing regulation 2018/2313/EU. The passport has to be fixed/attached to the smallest undividable unit of seed/plants. Another new element is that plant passports have to be used for all plants for planting, bedding and including pot plants.

Ancient newspapers reveal true **motivation of Mendel**

Monique Krinkels

Most scientists regard Gregor Mendel as the founder of genetics. Why he was interested in the heredity of characteristics had thus far remained unknown. Until the recent discovery by Dutch plant breeder, Peter van Dijk, who found two newspapers published in 1861 in Brünn (Brno), the capital of Moravia. It makes clear that Mendel's intention was improvement of crops.

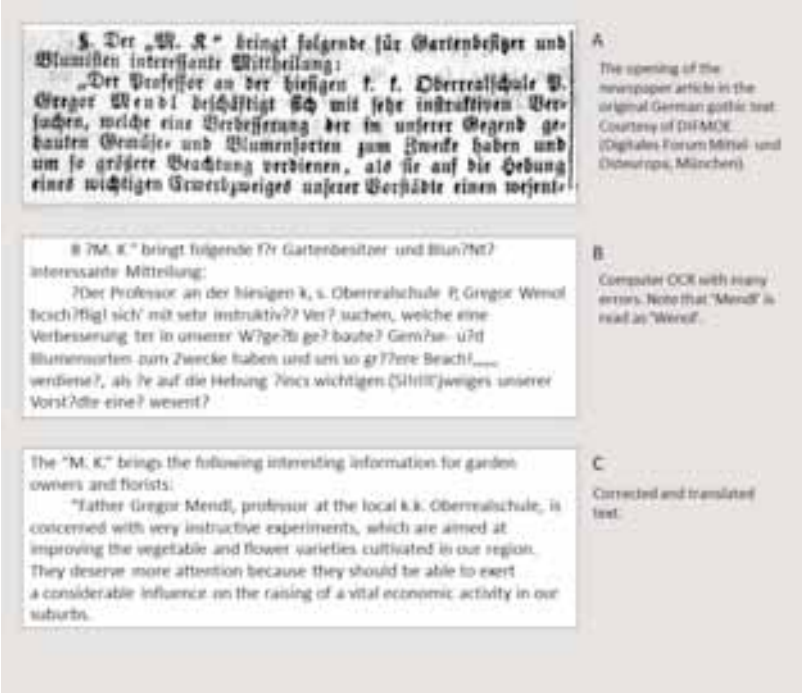
It is hard to find information which provides us with an insight into his thinking. Few documents have survived more than 150 years since Gregor Mendel made his famous discoveries. There is, of course, his well-known scientific pea paper, 'Versuche über Pflanzenhybriden' (Experiments on plant hybrids) published in 1866. In addition, he wrote two short papers about pests in crops in 1853 and 1854 and a brief paper on artificial hybridisation in Hieracium (hawkweeds) in 1870. But these writings do not disclose the origin and motivation for his work.
The written notes he made during his experiments have unfortunately been lost. It is speculated that the succeeding abbot burned all papers in Mendel's collection, to end disputes with the government over taxation. Only two pages of his notes are still to be found at the Mendel Museum, an institution of Masaryk University in Brno. Another source could have been letters to contemporary scientists, but of these only a few survived the decades. And in the 18 remaining letters he wrote to family and friends, he did not mention his experimental work. A meagre record compared to Darwin, who wrote many thousands of letters of which over 15,000 can be found in the archives.

Filling the gap

It triggered Peter van Dijk to track down the personal considerations of Mendel. "There is a gap in the crucial period between Mendel's graduation in 1853 and his famous pea publication in 1866. I became fascinated by that 12-year gap and started looking for information from that period."
Van Dijk is a geneticist specialised in plant reproduction traits, such as apomixis and cytoplasmic male sterility. He investigated these traits at the Netherlands Institute of Ecology (KNAW-NIOO) from an evolutionary / ecological point of view. Today, he is senior scientist in reproduction traits at KeyGene. "I am fascinated by the history of science and specifically genetics. Together with Noel Ellis, I published two papers about the work of Gregor Mendel. In the first paper, it is argued that Mendel studied hawkweeds as an example of constant hybrids, which contrasted variable hybrids, such as pea. The commonly held idea that Mendel was frustrated because he could not repeat his pea results is therefore incorrect. He

Gregor Mendel (20 July 1822-6 January 1884) was a farmer's son who became a friar and later an abbot at the Augustinian monastery of St. Thomas Abbey in Brünn (Brno) in what is currently the Czech Republic (Photo: Mendel Museum of Masaryk University, Brno, Czech Republic)

was - just as I am - interested in what today is called apomixis," says Van Dijk. The second paper discusses a newly discovered newspaper article, dating from 1861, which reports on Mendel's breeding and horticultural work while he was making his famous pea crosses.
In the last decade, many old newspapers have become available online. Using Optical Character Recognition (OCR) software, it is possible to search these digitalised papers for keywords. The Brünn newspapers were however printed in German gothic fonts, which



A

The opening of the newspaper article in the original German gothic text. Courtesy of DIFMOE (Digitales Forum Mittel- und Osteuropa, München)

B

Computer OCR with many errors. Note that 'Mendl' is read as 'Wenol'.

C

Corrected and translated text:

The "M. K." brings the following interesting information for garden owners and florists:

"Father Gregor Mendl, professor at the local k.k. Oberrealschule, is concerned with very instructive experiments, which are aimed at improving the vegetable and flower varieties cultivated in our region. They deserve more attention because they should be able to exert a considerable influence on the raising of a vital economic activity in our suburbs.

A search for 'Mendel' or 'Mendl' did not find the article, but a search for 'Befruchtung' (fertilisation) did

are often not well converted by OCR software. A search for 'Mendel' or 'Mendl' did not find the article, but a search for 'Befruchtung' (fertilisation) did. The first article appeared on 26 July 1861 in 'Neuigkeiten' (News), a daily newspaper in Brünn. It is a copy of an article in Mährischer Korrespondent (M.K.) (Moravian Correspondent), another newspaper in Brünn and its surroundings. It reads:

"The 'M.K.' brings the following interesting information for garden owners and florists: Father Gregor Mendl, professor at the local k.k. Oberrealschule, is concerned with very instructive experiments, which are aimed at improving the vegetable and flower varieties cultivated in our region. They deserve more attention because they should be able to exert a considerable influence on the raising of a vital economic activity in our suburbs. Through artificial fertilization, truly surprising results could be achieved. The vegetables grown by the professor, such as peas, fisols, cucumbers and beans, are high towering bushes that are distinguished by a massive production of fruit which, in size and taste, leave nothing to be desired. For the cultivation of these plants, mainly seeds from abroad were used. Of the foreign vegetables so far, the New Zealand spinach, which thrives in our soil, was acclimatized. The very fleshy leaves not only contain more nutritious substances than the currently cultivated varieties, but the plant is characterized by luxuriant growth, so that some specimens cover their rather large experimental plot almost entirely with their leaves. Until now, the experiments carried out with potatoes were less successful. The plants showed a very vigorous development, but the fruits started to rot and, so far, no remedy has been found. Professor Mendl has temporarily extended his experiments [also] to several species of flowers, which up to now have had to be imported at great expense from abroad. The carnations and fuchsias, of which the Professor grew several 100 pots, stand out by their abundance and colourful splendour in an astonishing way. Considering the efforts and diligence which these experiments require to obtain a successful result, one must give all recognition to the professor's endeavour. The substantial amounts of money that are currently spent on buying seed abroad can better be preserved for domestic production."
(Anonymous 1861a)

Mendel was not interested in understanding the inheritance of plant traits, but rather in agricultural innovation

Innovation

Van Dijk: "In other words, in his early years, Mendel was not interested in understanding the inheritance of plant traits, but rather in agricultural innovation." These two articles date from the period in which Mendel was in the middle of his Pisum experiments and are the only significant sources so far about his work between the finishing of his university studies at Vienna in July 1853 and the two pea lectures in February and March 1865. The articles in local newspapers reflect the interest from the general public for the type of work that Mendel was doing. These newspapers were widely read and together had a daily circulation of over 6,000 copies.
"We can conclude that Mendel's horticultural activities were quite well known in Brünn at the time. Partly because of his intellectual environment and the possibilities Mendel had for doing research, he increasingly focused on clarifying the laws of inheritance. At the time, the common belief was that crossing of plants was like mixing two fluids, by which the traits from each parent are averaged, whereas he proved it to be passing on 'hereditary particles' to the progeny. That research ultimately led to the famous 1866 publication," says Van Dijk.

The scientific article 'How Mendel's interest in inheritance grew out of plant improvement' by Peter van Dijk of KeyGene and his co-authors Franjo Weissing of the University of Groningen and the Institute for Advanced Study in Amsterdam, the Netherlands, and Noel Ellis of the University of Auckland, New Zealand, is published in the series 'Perspectives' in the October edition of Genetics. The article has open access and can therefore be read at www.genetics.org.

Crop Innovation Center

Turning the **matter** around

Monique Krinkels

Where seed companies select among their varieties the ones best fitted to hydroponics, led lights and the ability to grow in climate chambers, KeyGene has chosen another route: to develop methods for optimal selection of varieties especially meant for urban farming. It is one of the activities the organisation is focussing on in their brand-new Crop Innovation Center.

"We want to surprise our shareholders with innovative research," says Rolf Mank, manager of KeyGene's Crop Innovation Center. "We need to concentrate on the possibilities these four seed companies Enza, Takii, Vilmorin and Rijk Zwaan are not even aware of. Being one step ahead in researching how to combat the global challenges, that is what we strive for." He proudly shows the three climate chambers in which the first plants have recently been put down on the shelves. Besides cauliflower and Chinese cabbage, a trial with dandelions has just been started. The led lighting can be changed in colour purple and there are several options for watering the plants.

Purple

Using far red, red and blue leds is quite common in urban farming. "But it is hard to determine the quality of a plant as the leaves look almost black under purple light. That is one of the reasons why we also use a little green light," explains Rolf Mank. "We started with a few cauliflower and Chinese cabbage varieties. But we will certainly try other crops as well."
He believes that urban farming will acquire an important market share. "Not in the Netherlands though, as our country is fairly small, so transport to the cities is not an issue. Besides, the climate is moderate, so the existing greenhouses satisfy our needs perfectly. In the Netherlands, vertical farming may be an attractive approach to supply certain niche markets, to grow plants having a higher content of certain substances or to get the farm to the people. But in other climates, urban farming can become a game changer." For instance, in the Scandinavian countries, vegetables are expensive as much has to be imported. With urban farming, growers can produce crops in any season and in any climate. And it is also a solution for consumers in large cities such as London, New York or Beijing, where transport from grower to retailer will become an increasing problem. That is why the development of fitting varieties has to broaden to other crops. "Take, for instance, tomatoes. If we could develop a bushy type instead of the lengthy plants that are grown in greenhouses, tomatoes could be produced in urban farming cells. The same goes for cucumber, sweet pepper and similar crops."
There is more to see in the Crop Innovation Center. Researcher Fernando Garcia is for instance trying to create a banana that is resistant to Fusarium oxysporum f. sp. cubense (Foc). The so-called Tropical Race 4 (TR4) is an aggressive form of this fungi and spreading rapidly in Southeast Asia. The most grown banana variety, Cavendish, is susceptible to infection and it cannot be controlled by fungicides. "We have a large collection of banana species that are resistant. Unfortunately, most of them are not edible as they produce a large number of seeds. But we hope that by crossing these species with Cavendish, we will obtain an edible resistant variety," he explains.
In another part of the centre, business developer, Christiaan Biemond, works on phenotyping rice plants. A conveyor belt moves the plants in transpar-

Happy living in the blue zone

At the official opening of the Crop Innovation Center on 20 September, KeyGene organised a symposium 'Living in the blue zone'. It underlined the benefits of a diet based on vegetables. Michel Poulain, professor emeritus demography at the university of Louvain, Belgium, was the first to discover the so-called blue zones. These are areas in which the population experiences exceptionally high longevity.

So far five blue zones have been identified:
Barbagia region, Sardinia, Italy, with the world's highest concentration of male centenarians.
Ikaria, Greece, with one of the world's lowest rates of middle age mortality and the lowest rates of dementia.
Nicoya Peninsula, Costa Rica, with the world's lowest rates of middle age mortality, second highest concentration of male centenarians.
Loma Linda, California, USA, where the Seventh Day Adventists live ten years longer than their North American counterparts.
Okinawa, Japan, where females over 70 are the longest-lived population in the world.
One of the characteristics that the blue zones share is that vegetables form the main part of the menu. Beans, including fava, black, soy and lentils, are the

The cabbage leaves look almost black under purple light

ent pots slowly through the system. Every day, they are weighed to determine the evaporation and, every day, the amount of evaporated water is supplemented. The aim is for instance to discover which varieties thrive with less water. To make comparison easy, the plants are photographed on all sides, even the roots. "We have developed an algorithm to calculate the total amount of roots with a picture of just the outside of the pot."

Dandelions

Eight years ago, KeyGene joined a European research project on dandelions as a source of natural rubber and also started its own breeding research on dandelions. Kazakh dandelions (Taraxacum koksaghyz) to be precise, as this plant contains more rubber than other Taraxacum species. Natural rubber is an important product, as the petrochemical industry never succeeded in matching the quality with their synthetic product. Even car tyres contain a certain percentage of natural rubber, up to 24%, as said by the Société Internationale de Plantations d'Hévéas. At the same time rubber tree plantations face problems with diseases, such as the South American Leaf Blight Disease. Moreover, the rubber tree plantations are a threat to the shrinking rain forests.

Marina Arias tries to increase the amount of rubber in the roots of the small Kazakh dandelions by crossbreeding them with some other Taraxacum species that have a more exuberant root system. The aim is to increase the amount of rubber in the root from 200 kg/ha to up to 750 kg/ha. And she experiments with growing the young plants in one of the climate chambers. "It would be a breakthrough if we can replace rubber tree plantations with an environmentally friendly dandelion rubber production unit. That would be just the thing the world is waiting for," concludes Rolf Mank.

cornerstone of most centenarian diets. Small servings of meat are eaten but, on average, only five times per month.

According to Michel Poulain, you have to have won the genetic lottery to make it to age 100. But most of us have the capacity to make it well into our early 90s and largely without chronic disease. As the Adventists demonstrate, the average person's life expectancy could increase by 10-12 years by adopting a Blue Zone lifestyle.

Paediatric Intensive Care specialist, Koen Joosten, of the Erasmus University Medical Centre, Nijmegen, the Netherlands, steps into the breach for the sprout, the pea, the carrot and chicory, so long as it is a vegetable. And if toddlers learn to eat it. Preferably, from the age of four to six months, because a taste once acquired, remains.

It worries him that the vast majority of children, 75%, eat less than half the amount of vegetables they should eat. In adults, this shortage only increases. The consequences of an unhealthy diet, including lack of vegetable intake, are known: obesity, cardiovascular diseases and type 2 diabetes. According to Koen Joosten, schools must offer vegetables to their pupils and in offices 'meeting vegetables' should replace the biscuits.

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PANAMERICAN SEED
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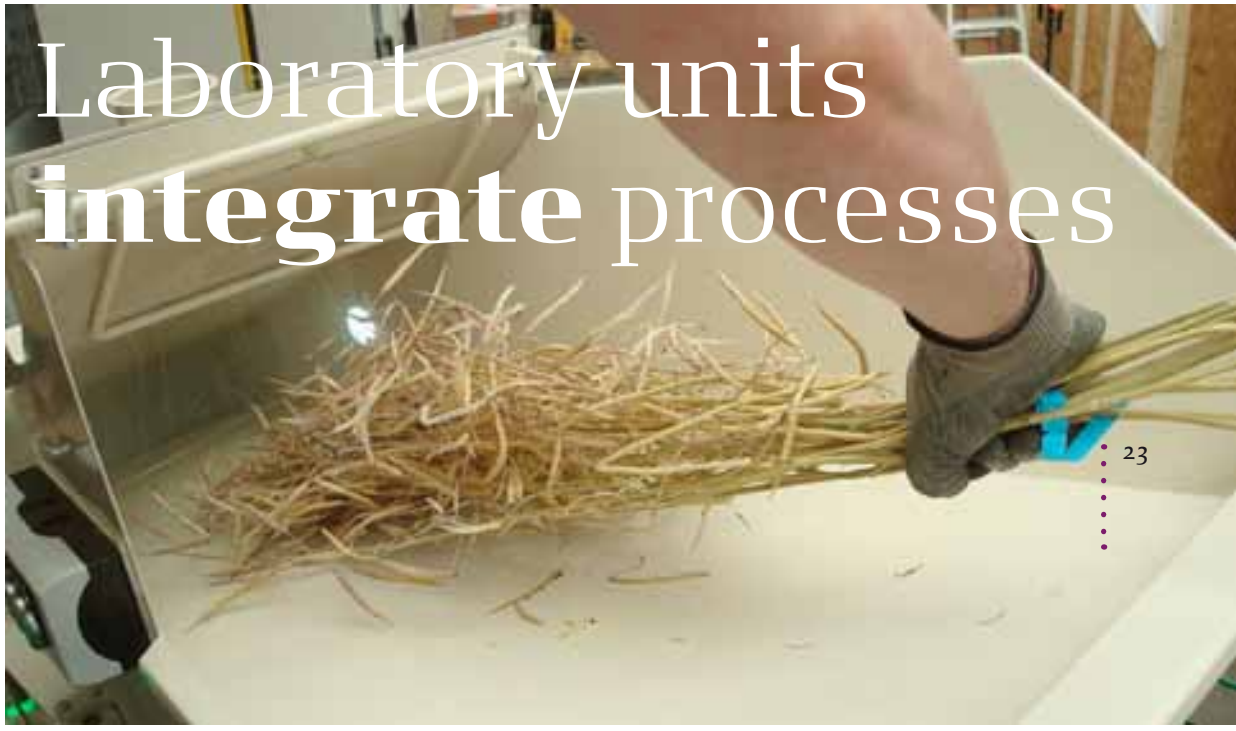
Laboratory units **integrate** processes

Monique Krinkels

Seed Processing Holland used to be focused on the vegetable and flower seed businesses. But that is changing, as agricultural seed companies also discover the potential of, for instance, the threshing equipment. Especially when small quantities need threshing in the breeding and selection process, the threshers have proven their worth.



The threshers are able to remove the chaff without any seed damage

Processing up to 600 small batches a day, with an automatic air cleaning programme (to clean the inside of the machine automatically) and dust suction connections to avoid endotoxins (the remains of bacterial death that may cause severe lung problems) spreading into the air. Seed Processing Holland has two machines that can be used by breeders of agricultural crops. "Both are integrated systems that combine threshing with cleaning and air separation," says Willem van Dok, Area Sales Manager Europe. "Whether it is oil rape or wheat, the threshers are able to remove the chaff without any seed damage. All suitable for selections (breeding samples)."

Basic model

The smallest one, STM-196, is a machine used for threshing seeds out of single plants, capsules, umbels and heads (ears). This machine is mainly used for rotary threshing of very small quantities of seeds. It is designed in such a way that it can be cleaned very quickly and easily. Therefore, it is very suitable for breeding stations and research stations. The use of rubber beaters and a perforated basket ensures that this machine has many applications in damage-free threshing of flower, herbs, grains and vegetable seeds.

"The dry plants are fed manually into the threshing chamber," Willem van Dok explains. "Inside this chamber, you can choose either a metal mesh or rubber perforated basket. In the middle, there is a variable speed rubber beating system that breaks the plant and threshes the seeds out of it. The seeds must go through the perforated basket and are collected in a small bin. Cleaning of the machine itself is manual.

More advanced model

The other threshing machine, STM-350, is somewhat more advanced. This machine does the same as the previous machine, but additional features a screen where chaffs are separated from the seeds. The next step is that the seeds enter an adjustable speed air separation unit. The clean seeds are collected in a bag, while the chaffs and empty seeds are collected in a waste bin. The machine has integrated air nozzles, so after threshing the machine will be automatically cleaned and dust is exhausted."

"It is possible to save instructions for the different crops to make automation possible. Furthermore, the automatic air cleaning reduces the cleaning time significantly," adds Willem van Dok. It can process small quantities of umbels, siliques or pods. It is possible to collect three separations: heavy seeds, less heavy seeds and chaffs with dust. The good seeds will be directly packed in small bags, while the dust that comes free is exhausted and the chaffs ends up in an anti-static bin.

LED lights

"A special characteristic is that visual inspection is possible during the process, as this threshing machine has polycarbonate windows and LED lights. All in all, I believe this threshing equipment is very suitable for breeding stations or processing stock seed production," concludes Willem van Dok.

Gipmans Plants

Growing plants requires **careful attention**

Tijs Kierkels

24 **For a company where there is a lot of manual work, the large percentage of highly educated employees is very noticeable. Plant nursery, Gipmans Plants, places a strong emphasis on knowledge and innovation.**

The outer edge of the largest vegetable nursery in the Netherlands is less than a hundred meters from the German border. A deliberately chosen location, close to the main importers and far away from other greenhouses with regard to the transfer of diseases and pests.

You really are entering a 'glass city' here. There are a total of 45 hectares of greenhouses on both sides of the road. A further four hectares can be added - and that is certainly going to happen - and then the limit of expansion will have been reached here. And after that? There are several options, as director Erik Gipmans indicates later in this interview. Contrary to the robustness of the greenhouse area is the vulnerability of the process that takes place in these greenhouses. That is why the same words always reappear in Gipmans' account: conscious choice, extreme diligence. "Growing plants is a profession in which it comes down to the smallest details. That only works if you do not waste any time. That is why we have always chosen to concentrate everything here in Venlo and not to work with multiple branches. We enter the chain just after the seed companies. If we do anything wrong, there is an immediate 'oil slick effect' on the entire sector. That is why hygiene and extreme diligence are very important issues," he says.

Every year, the company produces some 600 million plants: almost all greenhouse and field vegetables. They go to more than a thousand customers in Western and Central Europe. The buyers of the young vegetable plants are primarily the larger horticultural companies.

In addition to the plant nursery, the family business also has its own cultivation of herbs and vegetable plants for retail, under the name Especia. In total, there are 130 employees; supplemented with temporary employees at peak times.

Erik Gipmans: 'Size has never been our goal, but it is a result of the development among the customers'

Choice for seed

"Everything starts with seed here," Gipmans indi-

CRISPR-Cas

Developments in breeding have direct consequences for the plant grower. Gipmans is closely following the seed companies: "There is a development towards fewer and fewer growers in the vegetable sector. At a given moment, the number of companies is really too low and oligopoly characteristics appear. On the other hand, CRISPR-Cas (the technique of disabling or adding genes) is so easy to implement that many more small businesses can start up in the future. How do you then monitor the high-quality requirements? CRISPR-Cas makes the development of a new variety so much faster, that it can be at the expense of care in the process. The European Court of Justice has decided that the technique must meet the strict European requirements for genetic modification. But that really seems like an intermediate position to me. In the long run, you will invariably be involved."

In total, there are 130 employees; supplemented with temporary employees at peak times

cates. “We always opt for generative propagation if possible, because that gives the most certainty about the health and varietal purity of the initial product, provided you use good seed sources.” Only strawberries are still grown from cuttings. It is striking that there are no ornamental plants in the entire company. “That is a conscious choice: we concentrate on food crops. Decorative plants do not fit well with that. For example, you have to deal with other crop protection agents and because we recirculate 100% of the irrigation water, you could transport something from the ornamental cultivation to the vegetable cultivation. This applies not only to substances but also to diseases. Petunia, for example, is from the same plant family as tomato - you could end up with cross-contamination of viruses. If you focus on food production, you will always be on the safe side,” he says.
In the past, a plant grower supplied the plants at the beginning of the season and then looked out at large empty greenhouse space. Then the temptation is great to fill that space and you almost automatically end up with short-term floriculture. However, Gipmans keeps the greenhouse green in a different way. “The old seasonal pattern has already disappeared due to the advancement of the assimilation lighting cultivation. In addition, our sales throughout Europe provide diversification. After the start of the Dutch traditional tomato season, comes the tunnel cultivation in spring in the Pfalz, followed by the one in northern Germany,” he says.
Even the crop consultants are no longer bound to a strict season. “We believe distribution of knowledge about varieties, cultivation and market is an essential part of our service. Our advisors visit customers five or six times per year and talk about the state of the crop, adaptation to climate conditions and evaluation of variety choice. The customer really appreciates that. For some horticulturalists, we are the only advisers who come to visit,” says the director.

Growth and innovation

Vegetable growers are developing well all over Europe. In parallel to this, the plant grower is also developing. “Size has never been our goal, but it is a result of the development among the customers. They will double in surface area over the next 5-10 years and we have to go along with that. That poses choices for us if we can no longer grow here. Sustain-

We share Jose's eagerness to grow and develop

"After studying agronomics in Santiago, I returned to the village where I'd been raised and still felt at home. I started working at Rijk Zwaan, and just two years later I was asked to become Station Manager. It was a tough decision for me because I knew I had to move away from my village. But in the end I went for it, and I'm glad I did. I've been able to strongly develop personally and the combination of working with plants and people is perfect for me. When I look back on my time at Rijk Zwaan, I feel really thankful for the steps that I've been able to take – and I'm still only 31 years old and see so many possibilities ahead of me!"

Jose Marcelo Caro Tobar is Station Manager for Rijk Zwaan in Chile. He seizes the opportunities that come by and thus keeps on developing. Rijk Zwaan – a worldwide player in vegetable breeding – shares this approach. We are working together towards a healthy future. Learn more at **rijkzwaan.com**

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There is hardly any manual labour involved with lettuce, with tomato there is a lot

ability considerations also play a role here. Should you deliver from Venlo to the Greek border? Maybe we should move a bit with a branch office in Central Europe. But as said, this is only possible if we can keep 100% control over the process. Furthermore, our substantial investments in R&D, hygiene and ICT require volume scale. In the long run, collaboration with other plant breeders in Europe may also be an option," he says.

Automation

Focal points for innovation are further automation, crop protection in the case of a narrowing range of substances, water quality, cultivation using LED lighting (including plant factories) and cultivation on water.

There is hardly any manual labour involved with lettuce, with tomato there is a lot. This is because almost every customer requires a different type of tomato plant. This phenomenon is an obstacle for automation. "Yet something must be done in that area: employment is under severe pressure, even more so with the horticulturalists than with us. However, you can only achieve more automation or robotization if you standardize the plant. That therefore requires a contradictory development to what you have seen in recent years with tomatoes," Gipmans suggests.

Meanwhile, 8000 m2 of greenhouse is equipped with LED lighting. Quality improvement and optimization for the customer are the focal points here. The plant breeder also experiments with cultivation in cells without daylight - a plant factory. The results, initially in the cultivation of herbs, are very promising. "Because of the great steerability and planning ability, we also think that it is possible to improve the quality of tomato and pepper varieties: faster growth, stronger generative parts, better roots, no crop protection. In addition, we also have a week's profit on a 60-day cultivation," he says.

The good results give a taste for more, and there is already consultation with seed companies about upscaling. "In the long run, we may no longer build additional greenhouses, but plant factories," he thinks. Because this makes the company much more capital-intensive, it requires a much closer connection with both seed companies and customers.

you can only achieve more automation or robotization if you standardize the plant

Corporate Social Responsibility

In order to test corporate social responsibility in a verifiable manner, the Gipmans Group commits itself to the NEN-ISO 26000 standard. This includes publicly reporting on all progress. That is why the website contains the so-called 'Self-declaration ISO 26000' about the years 2017/2018.

This not only highlights the accomplished successes, but also the goals yet to be achieved. Key points are lighter work, safety, employee health, less use of plant protection products, less energy consumption, 100% recirculation of water and fertilizers and reduction of the CO_2 footprint.

Designing plant varieties with **information technology**

Richard Visser and Richard Finkers

A field which could potentially lead to a further boost in plant breeding is the information technology. By a clever use of computers, a wide variety of (partially) curated (linked) data, and smart software and bioinformatics techniques, we can start to much better predict, design and select potential future improved varieties.

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The use of plants for human purposes (as food, feed, fuel, housing and clothing materials, medicines etc.) has been around since the earliest civilizations were build. While already in the 16- and 17 hundreds people were actively trying to achieve what might be considered the first breeding efforts it took until the end of the 19th century when the laws of genetics became an integral part of the breeding process. In the 20th century breeding took off and many new and improved varieties were the result of this. 'Breeders eyes' and 'green fingers' became well known terminologies for those skills required to produce outstanding varieties.

Boosts

Roughly speaking seven major improvements gave plant breeding a boost in the last century. 1) The creation and use of mutations using irradiation and chemicals around the 1940ties; 2) The introduction and use of hybrids in the 1950/60ties; 3) The use of fertilizers and pesticides in the 1960/70ties; 4) The use of cell-biological techniques, like embryogenesis and protoplast fusion, in the 1970ties; 5) The introduction and use of genetic modification in the late 1980ties and 6) The use of molecular markers in the 1990ties and 7) genome sequence information around the turn of the century.

New developments, tools and techniques are manifold as plant breeding is a very innovative field. Whether these new developments will persist and lead to new boosts remains to be seen but one can rather safely speculate that for instance one of the New Plant Breeding Techniques known under its acronym CRISPR/Cas9 (a targeted mutagenesis technique) will certainly in countries where the technique is accepted and not falling under the strict GMO regulations as they exist e.g. in the European Union lead to a further boost. Using information technology might speed up the development of new varieties.

One of the global challenges of the future is how to feed an ever growing world population with an expectation that food demand will increase by 50% in 2030. One approach to address this challenge is to breed new crop varieties that will yield more than the current ones, even under unfavourable conditions (like drought, heat, massive attacks by pests and pathogens). Another possibility is to resort to new or as yet marginally deployed plant species, which will not be discussed in this paper. However, designing a breeding programme is a very laborious and time consuming effort. Depending on the reproduction system of the crop, the available genetic variation and the level of knowledge it can take anywhere from 3 to 15 years (and sometimes even longer) to come up with an improved variety ready to enter the market.

Often also the capacity is lacking to generate new improved cultivars quickly and in response to the required traits. Our advances in all fields of research including biotechnology and genomics data science have the potential to accelerate and precise breeding programs greatly. More and more large-scale genomic and phenomic data sets are becoming available for many (major and minor) crop species. These multiple independent data sets are generated by researchers, by the breeding companies themselves, by growers and are very diverse in size, type and structure.

Linking these data sets together is a major challenge but is improving as many groups who are active in generating these data have underwritten the agreement to have data stored and available according to the so called FAIR principles. This means that data should be Findable, Available, Interoperable and Re-usable. These principles make it possible for machines to find data which should be and in fact is related. Likewise, it is needed to agree on terminology for words and processes. PhD student Eliana Papoutsoglou collaborates within European and world-wide consortia on the definition of the Minimal Information About a Plant Phenotype Experiment (MIAPPE) v1.1 data standard and formalisation of the data standard in a RESTful specification to enable interoperability among plant breeding databases (Plant Breeding API, BrAPI).

The implementation of these standards and technologies build further on, for example, crop specific trait ontology terms and FAO standards as the multiple crop passport descriptor specification. Crop plant trait ontologies are hierarchically organized controlled vocabularies describing key phenotypes of a plant. Together, these efforts should enable her to address the key research question which focuses on proving that once formalized, datasets obtained from previous (plant breeding) field and glasshouse trials

In-silico genomic assistance helps researchers and breeders in the design of a precise and improved breeding programme

could be more easily analysed in a new context. This, while many researchers still consider that re-use of datasets by other scientist is impossible, mostly due to the lack of context (or proper meta data description), while not realizing that more-and-more PhD theses are actually written by providing new insights from existing datasets by using novel data scientists' skills. For example, we speculate that such a step is made four our datasets, combined with datasets from growers, and machine learning technologies, which should help to uncover novel leads for complex traits, such as yield.

Mining data

Standardisation of experimental data is a first step; however, we have a lot of information buried (implicit) in literature. Approaches to allow computers to 24/7 mine this data are not trivial and require a lot of input from different experts. For instance text mining, which is quite common and already advanced, will have to be done on the relevant plant science literature. Based on the quality of the text mining programs useful and relevant information will be retrieved by the computer. A recent improvement by PhD student Gurnoor Singh within Plant Breeding has enabled us not only to mine text, but also to mine tables. This is a big improvement over existing programmes because in many cases tables contain more, relevant, information and results.
Again, an important step in this process, and text mining in general, is in the use of controlled dictionaries to understand the text. For example, the crop trait ontologies are used in this approach to identify the columns containing information about traits. In the resulting datafile, explicit standardized links, for example of a marker flanking a QTL region to this marker on the genome reference sequence, is made, adding semantics. If this is done on a combination of datasets like for instance a set of varieties, with relational (or pedigree) information linked to the DNA sequence information and the mapping results thereof than this will enable identification of novel genomic regions, such as quantitative trait loci (QTLs), which positively or negatively affect the traits of interest. Furthermore, text mining approaches can allow identification of potential genes of interest in different crop species, which a breeder on its own would never have been able to spot due to the enormous amount of scientific papers which grow exponentially in numbers. By using semantic web technology it will be possible to integrate large data sets across heterogeneous data resources resulting in more insight and more meaningful results. For example, a paper about melons might not be of interest to tomato fruits, though we have seen many examples in which fruit biology is conserved across plant genera and knowledge discovered in other plant species might be the missing pin in understanding the trait in the crop under investigation.
The expectation is that this approach will allow

Knowledge discovered in other plant species might be the missing pin in understanding the trait in the crop under investigation

the prediction of an optimal genotype with a set of defined traits for production in a given environment. Hence, this in-silico genomic assistance, helps researchers and breeders in the design of a precise and improved breeding program. The identification of the most important genomic regions and the required optimal characteristics for particular traits will enable the selection of the most appropriate parental lines. Before these optimal parental lines can be chosen the most important candidate genes (or rather their most important allelic forms) underlying the traits must be identified. Traditionally, plant breeders are introgressing chromosomal regions containing genes or rather quantitative trait loci (QTL), positively affecting a trait of interest into their elite breeding lines. However, a QTL region can easily contain 1000s of genes, including genes negatively influencing the trait of interest or negatively influencing other traits. The introgression is normally done by crossing two generally well combing lines or (if one or more traits are very important) specific combining lines to create one or more populations of offspring plants. In these offspring plants subsequently the quest for a superior variety is performed and from anywhere between 10.000 to 100.000 seeds in a process of many years one or a few varieties will be selected.

This so called offspring based breeding is rather inefficient and tedious. Breeding using the actual causative gene(s) is a much more efficient and effective approach. So knowing which parents contain the best allelic forms for let us say the genes of the 15 to 40 traits which are really important for your crop, would mean that you could predict the chance of creating the desired genotype (with the expected phenotype) and would know how large the offspring population should be and by using molecular markers for these 15-40 traits would allow you to focus on selecting those plants that are also performing well for those traits for which not yet the underlying genes are known and markers are available.

To be able to achieve this all of course a lot of basic knowledge should be acquired and stored in a preferably well curated manner. Ideally these data would be proven by experimental set ups but that will not be possible. What will be possible is to combine and learn from other systems especially those which deal with generic aspects. Not all knowledge, available for one species, can be transferred to another (e.g. tomato does not form tubers, like its relative potato) or resources describing different subsets (e.g. between species) of similar data are not yet properly mapped.

Artificial intelligence

Finally, we would like to go one step further and also let the computer tell us, even before experiments have been done and proven that a certain gene is responsible for a given trait, what the most likely gene and its best allelic form is to obtain the superior genotype expressing the desired phenotype. To this end deep learning Artificial Intelligence (AI) supporting business critical decisions like Watson (IBM) can be used. At Plant breeding we have a project on potato with Watson and we have learnt the computer first what a potato is and what potato tubers are and what flesh colour of the potato tubers is. In essence it is like teaching a child to read and write and understand what the meaning of the different words is. In doing so we used the simple trait flesh colour as an example to see with what available literature Watson could predict which gene the most important gene in determining flesh colour was. This was done by feeding the programme first about 50 selected papers on potato on tubers and flesh colour followed by feeding it with thousands (between 3-5000) of abstracts from publications from the 1980 till now and see how much sooner it was able to predict a result which only years later was proven.

This is only the start of using all available information and letting a supercomputer come up with one or more answers, which can be tested for accuracy. The final goal of all this should be that for a specific new variety of a crop for a certain target area with an ensured yield it is possible to make the cross between two selected parents, sow out a couple of thousand seeds and identify or select (using molecular markers and other tools) the 20 best ones for further analyses. This will change breeding from offspring based to parental based breeding. The future for sure looks exciting and promising!

More information on MIAPPE and BRAPI: http://www.miappe.org; http://www.brapi.org

Contamination still causes headaches and sleepless nights

Geert-Jan de Klerk

The first attempts to grow plant tissues on an artificial medium were made in the early part of the 20th century. After that, it took some 50 years until plant tissue culture became a routine practice. During that time, various problems had to be solved. A short overview about contamination, a problem that continues to cause headaches and sleepless nights.

We live in a world in which we are surrounded by microbes. Tissue culture, though, requires that the cultures are free from microbial contamination. The nutrient media, the growth containers and the plant material should all be made free from microbes before the culture is initiated. Handling and processing are done in laminar flow cabinets that ensure an air flow with microbe-free air, and instruments are used that are sterilized, usually by heat. Still, sterility is far from certain. For example, mites in the culture rooms may be a source of contamination that completely ruins tissue culture.

The media and containers are sterilized by autoclaving or gamma-irradiation and occasionally by other techniques, such as filter-sterilizing. Sterilization of plant material is more complicated and the plant material is generally considered as the topmost source of contamination in plant tissue culture. For the sterilization of plant material, it is important to distinguish microbes that have colonized the epidermis and those that live within the plants. The former is dealt with by surface-sterilization.

For the endogenous microbes several methods have been developed that are often less successful. In the practice of tissue culture, the source of contamination is easily detected. When the microbes originate from the explants, both the ones that live on the epidermis and those that live inside the tissue, the colonies are in the direct vicinity of the explant. Otherwise, for example, in the case of inadequate autoclaving, the colonies are 'everywhere' in the medium.

Surface sterilization

The above-ground parts of plants are usually colonized by a variety of bacteria, yeasts, and fungi. Compared to field grown plants, the extent of microbial contamination is usually much less on the epidermis of stock plants reared in a greenhouse, laboratory or growth room. Plants should be potted in clean containers using clean and preferably pasteurized soil. Hydroponic culture is a helpful alternative. If aerial parts are used as source of explants, it is essential to avoid wetting the upper part of the stock plant (or splashing it with soil). Field-gathered shoots may be treated with fungicides (e.g. 0.05% Benlate + 0.05% Captan) before buds are forced open. Shoot apices need to be surface-sterilized before explants (meristems) are dissected from them and isolated meristems may be sterilized once more with a diluted NaOCl solution.

The epidermis of a plant is not a smooth surface, rather it is uneven with hairs, stomata and hyda-

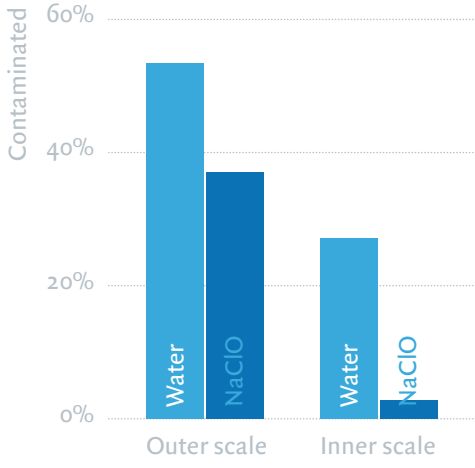

Fig. 1. Contamination of explants cut from inner and outer lily scales, rinsed after the surface-sterilization in 1% NaClO with water or with 0.03% NaClO

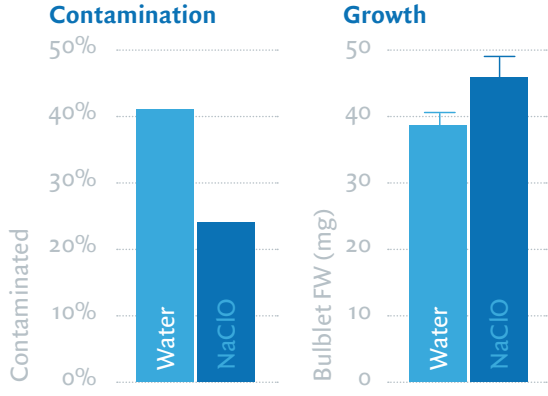

Fig. 2. Left: Contamination of explants cut from inner and outer scales after detaching the scales in streaming water or streaming 0.03% NaClO. Right: Growth of bulblets from scales detached in water or NaClO

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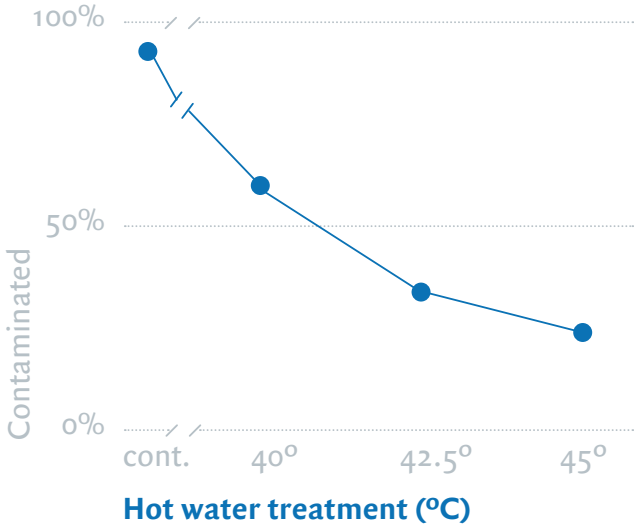


Fig.3. Effect of a 2-h HWT at various temperatures on contamination in Acer lobelii axillary buds

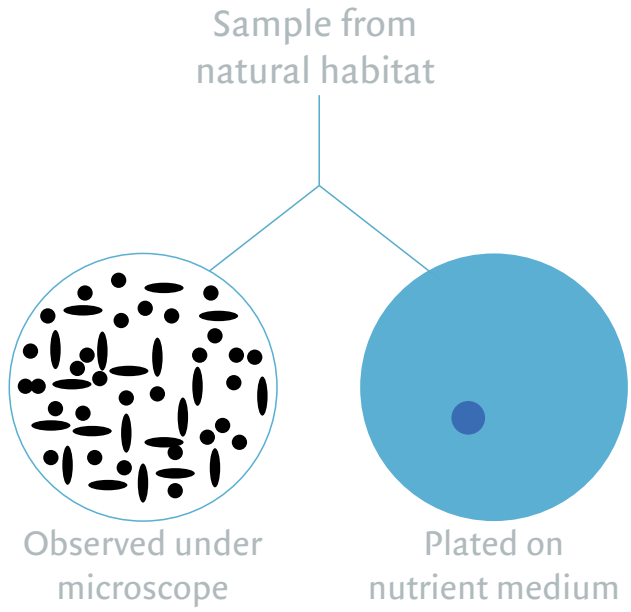


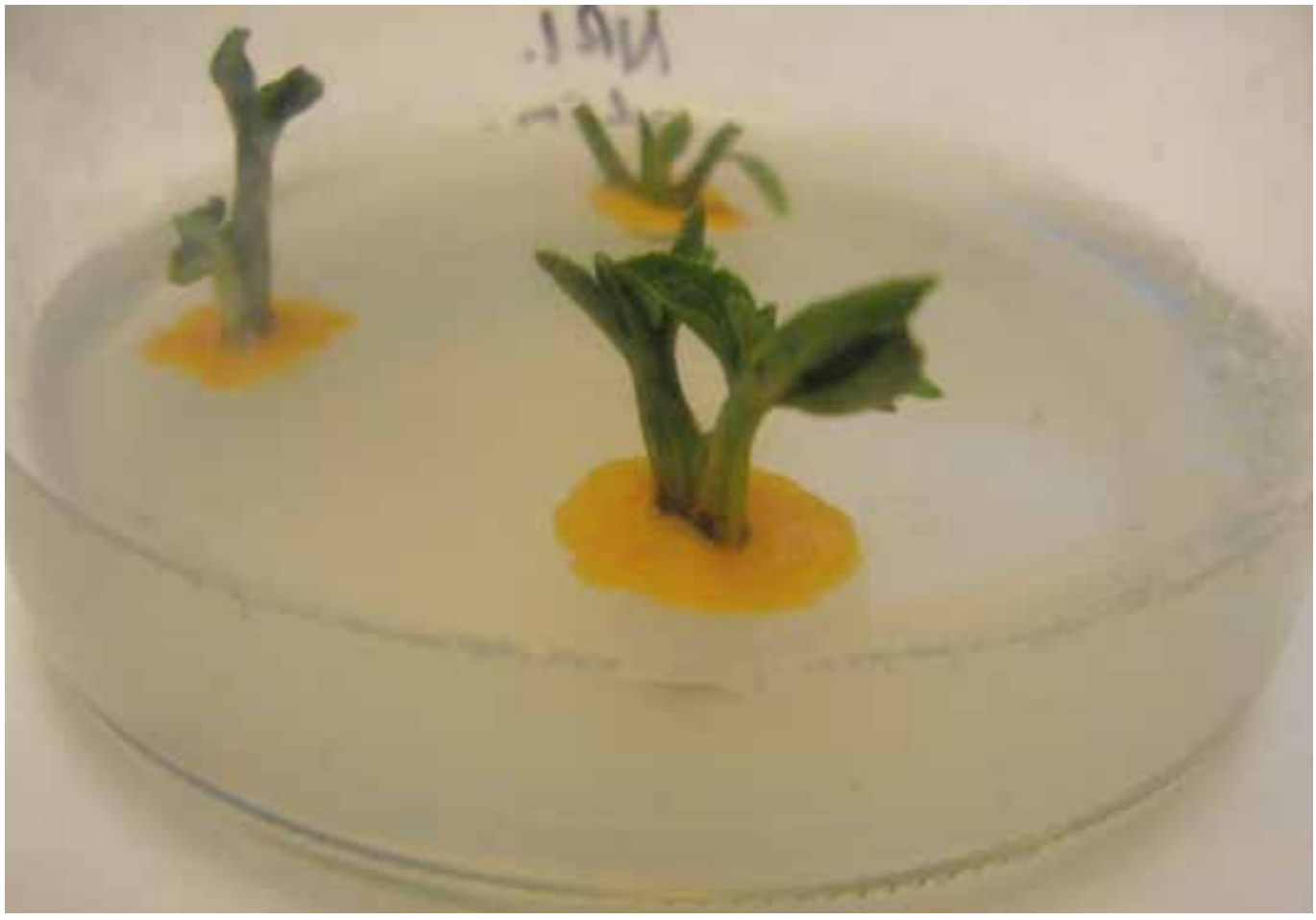
Fig. 4. The great plate count anomaly. Two ways of detecting microorganisms in a sample: by microscope or by plating on a bacterial nutrient medium. Note that the nutrient medium allows only the growth of very few microorganisms

Disinfectants cannot eradicate microbes without severely damaging plant tissues

thodes. Colonization often occurs close to the latter three. In lily scales, we observed that scales treated with ABA before surface-sterilization showed significantly more contamination. This indicates that microbes were living in the cavity below the stomata and were protected from the sterilizing agent by the closure of the stomata. Several sterilizing agents have been used: hypochlorite solutions (NaOCl, Ca(OCl)2) and other oxidizing compounds such as H2O2 and permanganate, heavy metals (HgCl2, AgNO3), alcohols (ethanol 70-96%) and PPM (Plant Preservative Mixture is comprised of two isothiazolones).
The most common disinfectant is NaOCl. Ethanol is less effective and heavy metals are very poisonous and the waste is very polluting for the environment. For optimal effect, to the NaOCl solution a detergent (a few drops of Tween-20) is added and the exposure to NaOCl may be preceded by a short rinse in 70% ethanol. The concentration of NaOCl varies from 0.25-2% (w/v) according to the plant material and the duration of exposure. This equates to 5-20% (v/v) of a domestic bleach solution.
The bactericidal action of hypochlorite solutions is due to both hypochlorous acid (HOCl) and the OCl- ion. The former is probably much more active than the latter, because the disinfecting efficiency of chlorine is best in slightly acid hypochlorite solutions and decreases with an increase in the pH, corresponding to conversion of hypochlorous acid to OCl-. For the most effective disinfection of plant material, hypochlorite solutions should be used at pH 6-7. After sterilization, the disinfectant is removed, usually by three rinses with sterile water. Routinely, the explants are not processed individually but in samples of 5 to 20. When one of the explants contains hidden endogenous contamination after the sterilization, other explants in the sample may be infected by this explant. We found that this problem can be easily solved by adding a low percentage NaOCl (0.03%) in the final rinse (see fig. 1). Here it should be mentioned that there are some papers in which a low level of NaOCl is added during the culture, without a negative effect on the plants. PPM may also be added to the nutrient media.

Endogenous microbes

Sterilizing compounds relatively easily deals with microbes inhabiting the epidermis. Those living within plants, though, are too difficult to reach for the disinfectants, not least because of the extreme slowness of diffusion. In particular, the microbes inside the cells are a problem. Disinfectants cannot eradicate these microbes without severely damaging the plant tissues. However, most endogenous microbes - in particular in the temperate climate zones - live in between the cells: they have colonized the vessels and the intercellular spaces. For these microbes, there are several possible means of eradication.



Bacterial contamination originating from explant

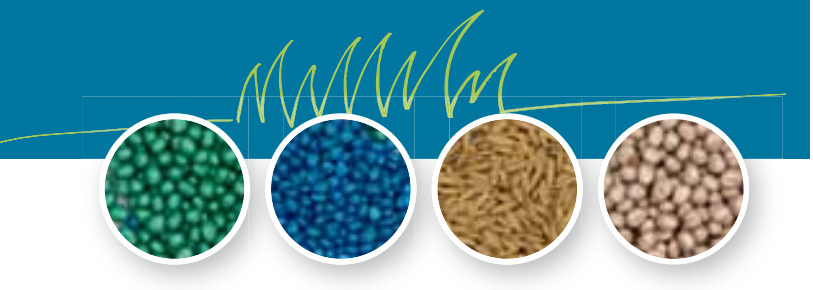
Firstly, it should be considered how the endogenous microbes entered the plant body. This may occur during the preparation of the explants. Thus, the operator initiating the culture is actually the guilty person and the plant material itself was initially clean! What happens is that, inside plants, a negative hydrostatic pressure occurs and when a cut is made when taking an explant, tissues suck up air and liquid from the vicinity of the cut edge. If microbes are present when the air or liquid is sucked up, they enter the vessels for some centimetres. In this way, the explant becomes endogenously contaminated. The explants release the microbes until wounding tissue has been formed and they become captured within the tissue. Release of microbes resumes when new cuts are being made during sub culturing. The solution to this problem is either to excise first long stems (30 cm), sterilize these and excise the apical buds after the sterilization. Because of the length of the vessels, the entrance of microbes is not sufficiently far. A second method is to excise the apical buds when the stem is submerged in diluted NaOCl (0.03%) (see fig. 2)

A method to remove pre-existing contamination before excision of the explant from shoots has been explored by some researchers. Shoots are first cultured for an extended period, with the lower cut ends in a solution of a weak sterilizing agent. The sterilizing liquid is sucked up and supposedly disinfects the shoots internally. Because the concentration of disinfectant is low, the plant tissue itself survives. Another method is a hot water treatment (buds are submerged for a few hours at 40-55 °C). The buds should be able to survive the high temperature. In Acer, we had excellent results with dormant buds (see fig. 3).

Mites

Usually, cultures are grown in containers to which small organisms may gain access. Mites seem to be most common, but thrips have also been identified. 'Footprints' of the mites, consisting of populations of microbes, can be detected on the surface of the agar. Although fungal contamination is the most obvious sign of mite infestation, bacterial contamination also occurs, as mites carry both fungal spores and bacteria on and in their bodies. A review in the UK revealed that one-third of the laboratories had major, and another fifth minor, problems with micro-arthropods. Other pathogens in tissue cultures, such as viruses, bacteria and fungi, are spreading passively by carry-over during manipulations, turbulence or by specific vectors. Micro-arthropods, however, spread actively in culture rooms by crawling from one container to the next. Because most of the life-cycle of the mites takes place within a closed tissue culture container, it seems unlikely that a population can be eradicated by simple pest control in the culture room. Nevertheless, we found that spraying with a miticide was very effective.

Seeds & Customised Services



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SEED & SERVICES

Micro-arthropods spread actively in culture rooms by crawling from one container to the next

A normal life cycle of mites in tissue culture takes 10-20 days. Each female generates an offspring of 100 to 200 mites, 98% of which are female. The first mite born is a male. It attaches itself to the swollen abdomen of the female parent and copulates with the successive female descendants during their birth. Once fertilized, the young mites will spread in the cultures. Next, they leave containers via the smallest cracks, actively looking for plant material. Parafilm does not prevent spreading. Nine days after their birth, the first mites of the next generation propagate. Hatching will continue for about 7 days. A tissue culture container infected with a single mite can theoretically contain 25,000 descendants after 30-40 days and more than 5 million after 60 days. Young mites will spread in the culture rooms and infect nearby containers.

Great plate count anomaly

At the end of this article, it is thought-provoking to mention the inability to cultivate the large majority of microorganisms taken from natural habitats using routine bacteriological nutrient media. This has been referred to as the 'great plate count anomaly': when observed under the microscope, a sample contains hundreds of microorganisms, but when the same sample is plated on a bacterial nutrient medium, there seem to be only very few. It has been estimated that 99% (!!!) of the microorganisms is unculturable.
As plant nutrient media are most probably not optimal for microorganisms, many more plant-derived microbes fail to grow on plant media. To illustrate this, it has been found that most bacterial species isolated from contaminated plant tissue cultures and multiplied on bacterial media, were incapable of growing on Murashige Skoog medium. However, they did grow when plant material was also cultured at the same medium. Apparently, the plant tissues supplied the bacteria with necessary compounds. Molecular techniques are a new way of establishing the presence of endogenous microbes. In this way, the ubiquitous presence of non-culturable endophytic bacteria in field shoot-tips of banana has been demonstrated. Finally, it should be mentioned that bacteria living in the plants may be beneficial for the plants, but there is not yet a coherent picture of this phenomenon.

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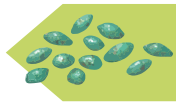

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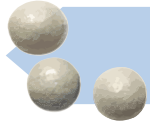

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