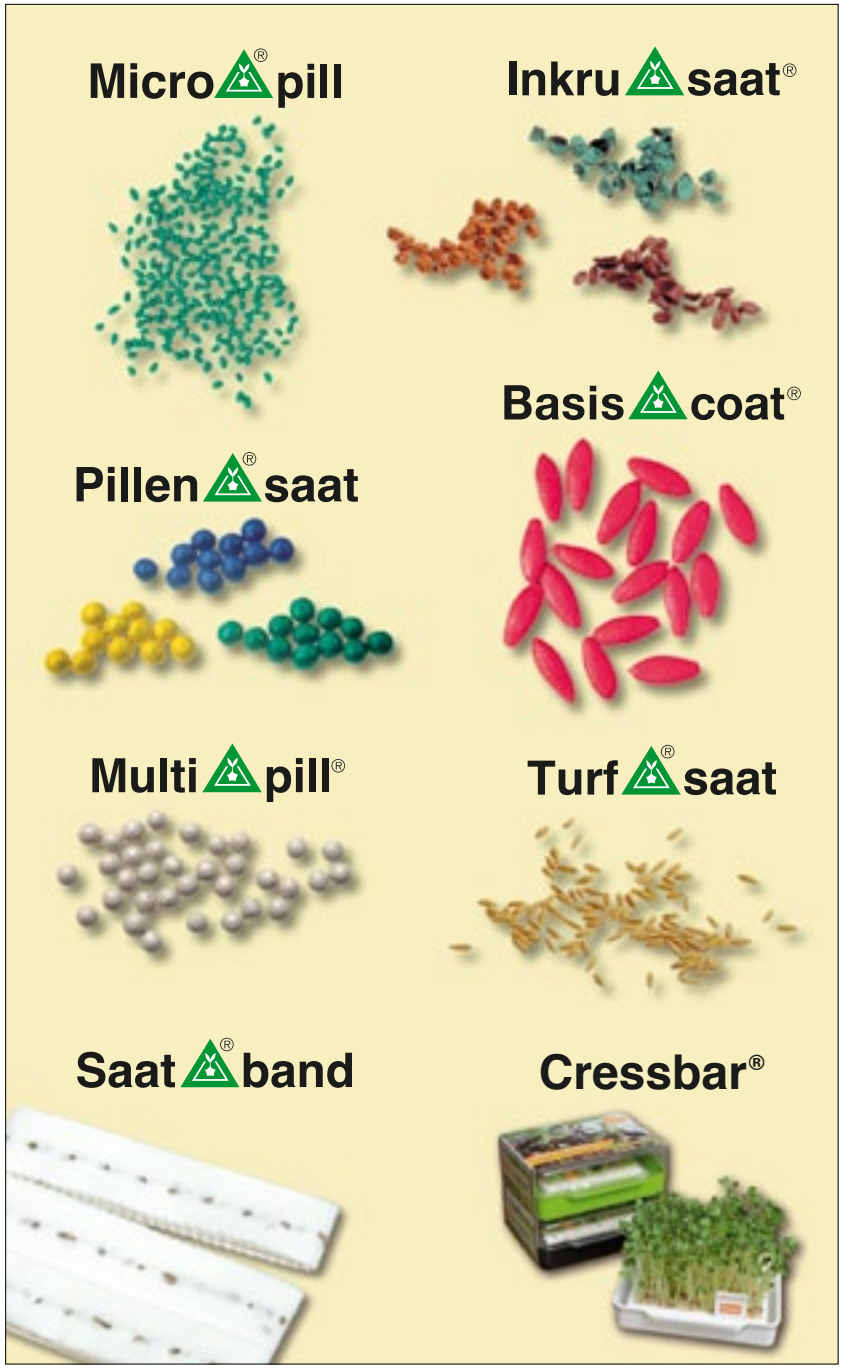
The Annual 2016

Journal for breeders and producers of plant material

Prophyta

Seed at its best



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THE ANNUAL 2016
Journal for breeders and producers of plant material
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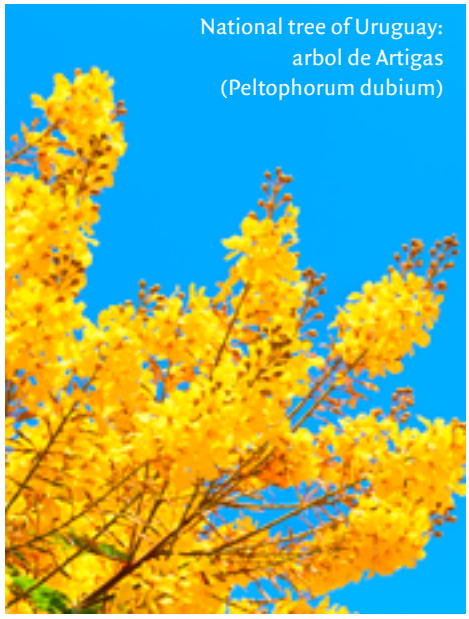
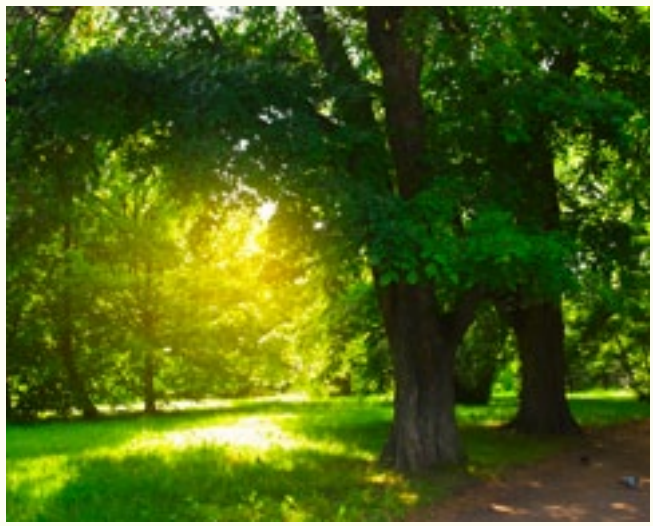
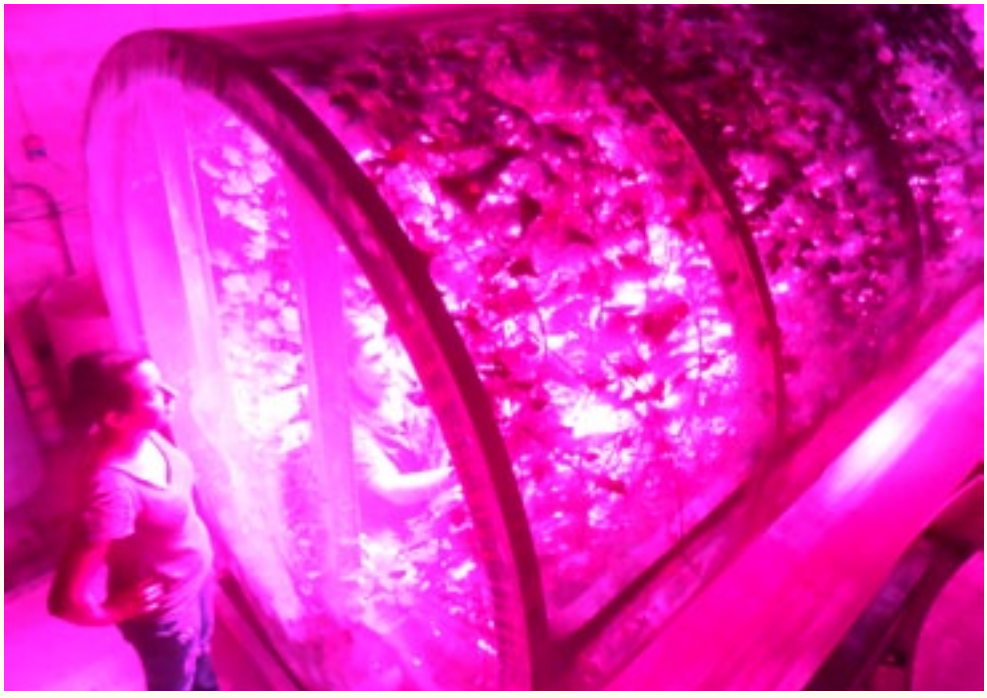


National tree of Uruguay: arbol de Artigas (Peltophorum dubium)

Healthier thanks to greenery

People who spend time in a green environment are healthier and happier than people who walk in a busy street, researchers have discovered. Even a picture of a tree in a park is able to lower the heart rate after a stressing experience. Apparently, viewing nature influences the parasympathetic nervous system.
"Short durations of viewing green pictures may help people to recover from stress," says Magdalena van den Berg, who led a study at the VU University Medical Center. "Finding an effect with regard to even boring visual stimuli - no spectacular green views, no sound, no smells, etc - is surprising. The effects would probably be magnified if someone could visit nature or even look out of a window and see actual greenery."





LED light speeds up lettuce growth

Philips Lighting, a Royal Philips company and global leader in lighting, has collaborated with The University of Arizona Controlled Environment Agriculture Center (CEAC) to test energy-efficient ways to grow food that will help feed astronauts on missions to the moon, Mars and beyond. A recent study, conducted over a 9-week period, found that replacing water-cooled high-pressure sodium (HPS) systems with energy efficient LED lighting from Philips in a prototype lunar greenhouse resulted in an increased amount of high-quality edible lettuce, while dramatically improving operational efficiency and use of resources. Lettuce grown under Philips LED modules achieved up to 54 grams/kWh of fresh weight, edible lettuce compared to lettuce grown under a high pressure sodium system which achieved only 24 grams/kWh of fresh weight, edible lettuce. This represents an energy savings of 56 %.
"The lunar greenhouses, equipped with Philips LED modules, provided the light needed to produce the same amount of indoor crops that the specialized water-cooled sodium systems provide, while significantly decreasing the amount of electrical energy used," said Gene Giacomelli, CEAC Director. "Findings from this study are critical in that not only can it be applied to growing food in space, but it can be applied to farming techniques in places where there is a shortage of water and good agricultural land right here on this planet."

Peruvian potatoes on Mars

In cooperation with the International Potato Centre (CIP) in Lima, Peru, NASA has started to research whether potatoes can be grown on Mars. The potatoes are planted in Peruvian desert land, as this is very similar to the soil on Mars. In the greenhouse, CO_2 is added to mimic the atmosphere on the red planet, although the thin air is not imitated. The scientists expect that the potato plants will produce two to four times as many potatoes as under earthly conditions. According to NASA, it is a major step towards building a controlled dome on Mars, capable of farming the invaluable crop in order to demonstrate that potatoes can be grown in the most inhospitable environments.
For years, CIP has been testing the robustness of potatoes in the most unlikely places. The global research and development organization is focused on the conservation of the genetic resources of potato and sweet potato. It maintains the largest collections of potato (more than 4,000 varieties) and sweet potato (more than 8,000 varieties) in the world. The genebank holds over 80% of the world's native potato and sweet potato cultivars and over 80% of the known species of wild potato.

In preparation for third edition ‘Seed meets Technology’

THE PREPARATIONS for the third edition of ‘Seed meets Technology’ have started. The event is taking place from 27-30 September (week 39) in the Seed Valley region (North West Holland). Besides coating systems, varieties, automated phenotyping, seed processing, specialized software, climate and growing systems, the organization is also focussing on the trial fields and growers this year. Different meetings and symposia will again take place about new developments in the seed breeding sector.
Last year’s edition was a resounding success, with approximately 1,500 people coming to visit Proeftuin Zwaagdijk, which was above all expectations. There was a clear increase in the amount of international visitors, about 25 % of the total number of visitors came from foreign countries. Consequently, we can say that the Dutch seed breeding sector is a leading player, and there is potential for ‘Seed meets Technology’ to grow internationally.
The bulk of the visitors have a background in seed companies, resellers of seeds and related products, suppliers and growers, technologists, operators and growing specialists in the Dutch seed breeding sector.
For more information: www.seedmeetstechnology.com or contact Ronald Hand, telephone +31 (0)228 563164, ronaldhand@proeftuinzwaagdijk.nl.

The power of green

Please do me a favour and look at the photograph of the tree on the left hand page. Only a few moments will suffice. And: has it lowered your heart rate and do you feel healthier? According to medical researchers you should. For the research, university students were assessed after a complex maths test and their heart rate lowered significantly by simply looking at a photograph of a tree on a computer screen. A photograph of buildings and cars had no effect at all.
But maybe ‘greeneries’ have less effect if you work in them all day. It might even have the opposite effect and cause stress if you think about the difficulties the sector encounters. Take the photograph of the bumble bee on the cover. For laypersons, it is a fine example of nature at its best (unless you are afraid of stinging creatures). But for people working in agriculture, it is also a reminder of the discussion on neonicotinoids and the decreasing numbers of pollinating insects. Other plant pictures might remind you of the barriers you encounter when applying for access to wild species. Does that raise your heart rate to an unhealthy speed?
It is striking that when governments want to solve problems that concern all of humanity, they tend to send the bill to easy targets instead of sharing the burden. In the case of biodiversity, plant breeders and pharmaceutical companies are supposed to pay. As if they are the only producers using nature. And when it comes to insects, some governments have suggested that farmers should be obliged to plant flowers along the sides of their fields, as a food source for the pollinators who starve due to monocultures. Of course, pollinators are important to all people involved in agriculture and horticulture and, of course, everyone should lend a helping hand. But why only farmers? Why not choose a more general approach? If governments should decide to plant flowers on the banks of roads, it would help insects so much more as they can travel alongside the tarmac throughout the country.
Since even a boring picture of a tree can promote health and happiness, how much more beneficial will real nature be, with its colourful scenery, rustle of leaves, buzzing of insects and fragrance of blooming plants. It is the responsibility of all mankind to ensure that everyone can enjoy a lowered heart rate.

Monique Krinkels

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Feed and fodder crops form the main part of the seed production

Monique Krinkels

Traditionally, Uruguay is a land of sheep, cattle and gauchos. Over 20 million livestock (of which 6.7 million are sheep) roam the vast pampas in het centre of the country. It is one of only five countries in the world which has more cattle than people - of which there are only 3.4 million. But the agriculture sector has more to offer than wool and meat: the country also exports rice, soybeans and barley.

The 'Oriental Republic of Uruguay', as is the official name of the country, is traditionally more prosperous than most countries in South America. It is well-known for its advanced education and social security systems and liberal social laws. It was the first nation in the region to establish a welfare state and developed a democratic tradition. Its gross national product of US$ 16,882 per head is by far the highest of the Mercosur partners. That is why Uruguay earned the nickname 'the Switzerland of South America'.
The main activity of the country is extensive cattle and sheep rearing; more than 13.5 million hectares are under permanent pasture, almost 83% of the agricultural area. Besides livestock, wheat, soybean, barley, beans, onions, vegetables, grapes and fruits are grown in the west and south of Uruguay. Around Lagoa Mirim, a lagoon in the northeast, rice production takes place.

Holiday resort

For the ISF World Seed Congress of 2016, the organisers have chosen a very special location. Instead of the capital, Montevideo, the organising committee proposed Punta del Este, a holiday resort with a scenic coastline, where the Rio de la Plata meets the Atlantic Ocean. "We expect between 1,100 and 1,200 delegates to attend the congress", says Pablo Civetta, Chair of the National Organising Committee. He sketches the Uruguayan agricultural landscape.

How many members do the Uruguayan Seed Chamber (CUS) and the Uruguayan Plant Breeders Association (URUPOV) have?
"At the moment, the Uruguayan Seed Chamber (CUS) has 35 members, and URUPOV has 50 members. These organisations focus on the national seed business, and in the region of mainly Argentina and Brazil, due to their similar environments. The main objectives of CUS are to promote the seed business, represent the common interest of its members and work closely with government and authorities to develop and update the legal framework, trading protocols and new guidelines to harmonize the seed industry. URUPOV's main objectives are to represent their members nationally and internationally on all issues related to Plant Breeders' Rights (PBR), to develop royalty collection systems and to enforce PBR. Uruguay meets all the international standards regarding production certification such as ISTA, OECD and the Cartagena Protocol."

The national flower is the ceibo-tree flower (Erythrina crista-galli), also known as the cockspur coral tree

Are seed companies in your country mainly seed trade, seed producing or plant breeding companies?
"Seed companies in Uruguay are seed traders, and many of them produce seed in Uruguay (soybeans, wheat, barley, rice and temperate forage seeds). Hybrids are mainly imported from Argentina, and in much less volume from the USA and Europe. Breeding is done in cooperation with foreign companies. The companies are mostly locals, some of them with representation and distribution of varieties of multinationals. Only one or two multinationals operate directly in the country.

One of the most peculiar trees in Uruguay is the ombú (Phytolacca dioica), with its soft and fluffy bark

Seeds	Quantity (t)	Value
Field crops seed exports	1,500	US$ 7,000,000
Field crops seed imports	N/A	US$ 15,000,000
Vegetable seed imports	30	US$ 3,000,000

Source: ISF

Agricultural products	Quantity (t)	Value
Rice	819,433	US$ 167,872,000,000
Barley malt	184,086	US$ 51,694,000,000
Sorghum	44,976	US$ 3,054,000,000
Wheat flour	22,478	US$ 4,592,000,000
Sunflower	22,281	US$ 3,841,000,000
Soybean	10,848	US$ 1,592,000,000
Barley (beer)	10,020	US$ 1,366,000,000
Oat	2,112	US$ 289,000,000
Sunflower oil	1,725	US$ 779,000,000
Rice oil	878	US$ 213,000,000
Wheat	65	US$ 20,000,000

Source: FAO

Feed and fodder crops form the main part of the seed producing activities in Uruguay, but we have some vegetables production, basically for national consumption. The two main areas are around the capital city of Montevideo, and in the north-west of the country where, due to climate conditions, growers can produce 1-1.5 months earlier in the year. Citrus and deciduous fruits are also important."

Together with Brazil, Argentina and Paraguay, Uruguay was one of the founding nations of Mercosur in 1991. Later Venezuela and Bolivia joined and Chile, Peru, Colombia, Ecuador, Surinam and Guyana are associate members, while Mexico and New Zealand are observers. Does the seed business in Uruguay profit from the international collaboration in Mercosur?

"It is especially advantageous regarding phytosanitary issues. Our country benefits from the standards set by the Mercosur Commission as our products can be traded in the region with the same permits as those of other countries. Uruguay has good trade relations with its Mercosur partners."

Does Uruguay grow GM-crops? How does the general public react to that?

"Yes, all soybeans (1.4 million hectares) and nearly all maize (0.15 million hectares) are GMOs. Farmers are satisfied with the results, as it improves the margins and reduces the use of herbicides and pesticides, thus lowering the environmental impact. The general public, however, remains ignorant of the truth about biotechnology and fearful of potential health risks, but in general we don't have many problems with the public opinion on GMOs."

In the invitation, you mention the unique soil conservation plan. Is Uruguay suffering from erosion?

"Like many other countries, Uruguay is suffering from erosion although, in the past 25 years, agriculture landscape has changed a lot and even more the way farmers carry out their farming. Direct drilling and all its technological package has reduced erosion significantly. To reduce and prevent further damage, the Ministry of Agriculture developed a conservation plan, based on always having a crop and avoiding leaving the land unused.
The plan is called 'Use and management of soil plan' and it aims to prevent and reduce the erosion and degradation. This regulation contributes to the goal set in Uruguay to have sustainable agricultural production systems. Based on more than 50 years of research on which variables affect erosion and loss of soil quality,

Uruguay is the fourth-largest producer of wine in South America, with a production of 67,000 tonnes and 8,023 hectares of vineyards in 2012. Wine making started in 1870 when Don Pascual Harriague, a Basque, introduced the Tannat, a blue grape that presumably originates from south-west France and which is also used in the Cahors wines

the prevention policies have been developed, applying mathematical models predicting erosion and soil loss. With the use of this predictive model, and the input of soil slope and crop rotation, we predict the soil loss per year, which has to be below a maximum set for each soil type. Therefore, the combination of crops (C4 grasses and soybeans, winter crops, rapes, etc.), pastures or cover-crops needs to be included in the proposed crop rotation in order to be approved by the authorities. Fiscalisation and fines complete the enforcement of the conservation plan."

In daily life, you are the CCO of Gentos Uruguay. Can you tell me more about the company?

"Gentos started in Argentina more than 30 years ago. It was the first company breeding temperate forage species in the region. It is the market leader in forage species in Argentina. In Uruguay, Gentos started its operation in 2005 and has grown to be one of the main companies in the forage business, trading, producing and breeding. We have several projects and agreements with the most important international institutions, working from the early stages of the breeding programme. Our main concept is breeding in the same region where the varieties will be used in the future.

Besides forage crops, our main products are grasses and legumes. Our focus today is based on adapting temperate forage species to harder environments, with less rainfall and higher temperatures. We work with the challenge of developing better pastures for the new environments where livestock activities are moving in our country. We also work with forage and grain sorghum seeds, being one of the main players in the market.

Gentos' forage varieties are tested in different parts of the world: Australia, New Zealand, USA, Europe, South America and China, amongst others. In some of those countries, we have already landed commercially. And now we see that Gentos' varieties, obtained in Argentina and Uruguay, are being chosen by overseas farmers. We are very proud of it!"

Is seed a **pest** risk?

The ISF Pest List Team

The seed industry today is a global business. It is common for a seed company to have breeding programmes and produce seed in numerous countries, sometimes both in the northern and southern hemispheres, and to distribute commercial seed to a large number of countries from a central location where seeds are cleaned, treated, tested and packed.

Phytosanitary regulations play an important role in the international movement of seed. Seeds may present a pest risk when they are introduced to environments where associated pests could establish and spread. Countries often exercise their sovereign right to utilize phytosanitary measures to regulate the entry of plants and plant products and other materials capable of introducing plant pests into their territory.

Challenge

The basis of phytosanitary regulations instituted by a country is a Pest Risk Analysis (or PRA). However, many countries do not have the resources to perform all the PRAs needed, neither in a reasonable period of time nor with the thoroughness they require.
There are numerous research papers on plant diseases in which the authors note that the pest in question was found on seed. But what is the relevance of such papers? The presence of a plant pathogen on seed does not necessarily mean that it transmits a disease. Furthermore, the conclusion drawn of a disease being seed borne or seed transmitted may be based on experimental evidence or limited observations. Many pests that are neither seed transmitted nor seed borne are, nevertheless, regulated.
Seed companies have to satisfy each country's requirements, technically justified or not, in order to move seed.

Responses (in %) to the question 'is seed a pathway' in ISF's Regulated Pest Lists

	Is seed a pathway?				
Crop Species	Regulated pests (no.)	Yes	Pathway not proven	No	Not a host
Carrot	92	4	8	46	42
Cucumber	90	4	9	56	31
Lettuce	64	5	16	56	23
Melon	69	12	20	45	23
Onion	94	7	13	44	36
Pepper	106	8	16	42	33
Spinach	38	21	8	34	34
Squash & pumpkin	54	9	17	50	24
Average (%)	9	14	47	31	

Source: http://www.worldseed.org/our-work/phytosanitary-matters/pest-lists/

Is seed a pathway?

Yes = Seed is a known pathway
Pathway not proven = Seed as a pathway is not certain because: 1) the evidence has not been verified or proven, or 2) the evidence is limited or doubtful, or 3) the evidence is conflicting
No = No references found indicating seed is a pathway nor is it known within the industry that seed is a pathway
Not a host = No references found nor information known that the crop is a host of this pest
Yes, but crop not a host = The pest may be found with the seed, however, the crop is not a host of the pest

ISF's Regulated Pest List Initiative

In 2007 the seed industry decided there was a need to assess the risk of seed as the means for the movement of regulated pathogens and pests based on sound scientific information, and the Regulated Pest List Initiative was born. The objective of the initiative is to develop a dynamic database with a science-based summary of pertinent information to serve as a resource for the industry as well as regulatory bodies. The starting point to building the database is a species specific compilation of all Phytosanitary Additional Declaration (Phyto AD) requests made by NPPOs to seed companies; or in other words, species specific lists of regulated pests. These lists are country non-specific, meaning that the Phyto AD could be required by one or multiple countries.
In the first endeavor, twelve vegetable species that represent a significant volume of seed traded internationally have been selected. The compilation of Phyto ADs shows that the number of regulated pests range from 38 in spinach to 150, and even more in tomato.

Pathway

To assess whether seed is a pest risk and to determine appropriate phytosanitary measures, a PRA requires a key question to be answered: is seed a pathway, i.e. is seed a means for the entry or spread of a pest? In nature, the designations seed borne and seed transmitted often represent a continuum which can be dependent upon the host, the environment and the pest itself. And so by using this concept the difficult task of distinguishing between seed borne and seed transmitted is avoided.
The answer to the question 'Is seed a pathway' is the

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There is little doubt about the pests for which seed is and is not a pathway

primary focus of the Regulated Pest List Initiative. The answer to this question for each pest in the species specific regulated pest list determines if the request for a Phyto AD is technically justified. The assessment is based on a review of the scientific literature as well as industry research, knowledge and experience.

In the ideal world one expects two answers to the question whether seed is a pathway: 'yes' and 'no'. However, early attempts at making this assessment quickly revealed there are 5 potential answers (see box).

There is little doubt about the pests for which seed is and is not a pathway. Watermelon and melon seed are known to be a pathway for bacterial fruit blotch just as tomato seed is a pathway for bacterial canker. Carrot and cucumber seeds are not a pathway for cucumber mosaic virus. Unfortunately, there are scores of pests for which the vegetable crops being studied are not even a host! Some examples of this are Alternaria brassicicola on carrots, Pantoea stewartii on cucumber, and bacterial spot of tomato and pepper on onion.

As all the assessments of whether seed is a pathway must be supported by scientific literature, cases where the scientific evidence couldn't be verified or proven, or was limited or doubtful, fall into the category 'pathway not proven'. This answer poses a problem when industry experience suggests seed is not a pathway. However, as the database aims to be science based, the existence of scientific literature, however doubtful, must be acknowledged.

The assessment of whether seed is a pathway in the database is supported by references and remarks. The remarks are vital for an accurate interpretation because they summarize the findings from the literature and also give the industry perspective.

Eight pest lists are already available on the online database (see http://www.worldseed.org/our-work/phytosanitary-matters/pest-lists/). All pertinent references cited are also readily available for database users as a summary PDF file.

Database

The breakdown of answers in percentages to the question 'is seed a pathway' for the eight pest lists in the table indicates that most of the Phyto AD requests received by companies are not warranted. Eighty four percent (84 %) of the total number of regulated pests associated with the eight species have a 'regulatory' statement in the remarks stating that the available information indicates there is no scientific basis for regulating the pest. Clearly, the industry is being asked to address problems that do not exist.

Future

ISF will continue adding regulated pest lists for other crop species with a science based assessment of whether seed is a pathway for the regulated pest lists. It is hoped that every seed company, large and small, will use the database to ensure their seed health programs are up to date. Some NPPOs have informed the author that they routinely refer to the database. In time, ISF hopes that regulatory bodies will use the database to assess whether their import restrictions are based on sound scientific information. This should help eliminate irrelevant Phyto ADs that act as non-tariff trade barriers.

The database is dynamic and has a feedback mechanism available. If you have any comments on the information provided, you are encouraged to let the ISF know.

Concern about Xylella in Europe

Olive trees under attack

Peter Lentjes

Additional measures to prevent the further spread of the bacterial disease Xylella were taken in the EU in December 2015. Planting material from a number of genera/species can only be marketed if it comes from an officially inspected source/ location and provided it is accompanied by an EU plant passport.

In 2013, researchers in Bari, Italy, rang the alarm bell. They identified the presence of Xylella fastidiosa in olive trees (Olea), and therewith identified the cause of a disease that had already been occurring for a number of years in Apulia, Lecce province, in Southern Italy. The disease was spreading and many old olive trees were already dying. The research institute in Bari identified X. fastidiosa strain pauca (also called CoDiRO, the abbreviation from the Italian name 'Complesso del Disseccamento Rapido dell'Olivo') as the main suspect for the dieback disease. Immediate action was taken by authorities to try to stop further spread of the disease. Many infected

A bacterial infection threatens to infect 11 million olive trees. As these trees have a life expectancy of more than a century it predicts dire consequences for farmers

trees were destroyed. Up till now, further spread of infection has been, as far as is known, limited to a region 50 kilometres north of the earlier boundaries. Xylella can be spread through (planting) material and through some insect vectors (mainly cycads).

Spreading

In 2015, a large number of infestations of Xylella were also identified in Corsica, France and an incidental infection in the PACA-region (Provence-Alpes-Côte d'Azur) in France, mainly in the species myrtle-leaf milkwort (Polygala myrtifolia). But the bacteria were also found in other species. The identified strain (X.f.subsp multiplex) is very different from the strain in the olive trees in Italy. There seems to be no relationship at all between the two sources. Furthermore, in 2013 and 2014, in various monitoring activities throughout the whole of the EU, infections with Xylella were also identified in coffee (Coffea) plants from Central America. Various Xylella strains have already been causing big problems for many years in the Americas in fruit tree cultivation (Prunus, Citrus) and grapevines (Pierce's Disease). If present in a plant, in trunks or branches, the bacteria can block water and nutrient transport in xylem, and thereby cause wilting and dieback.

Growing concern

Considering the very large number of species that (based on evidence elsewhere in the world) can be attacked by Xylella, there is now great concern over this disease, both within EU authorities and certainly also amongst growers and their organizations. Vectors of Xylella are present in the EU, mainly in southern European countries.

As the main method of spreading the disease is via plants, the EU has taken emergency measures to try and curb any further spread. A very wide range of possible host plants has been identified and included in EU measures (EU Implementing Decision 2015/789/EC, of 19 May 2015). A regular update of species will take place. Imports from third countries of these host plant species can only take place if the country of origin has officially declared to the EU authorities that the country or the production sites are free of Xylella fastidiosa.
If the authorities in the EU identify a new region with an outbreak of Xylella in the future, a protection zone of 10 kilometres surrounding the location of infestation will be established. During the next five years, no host plants from that location are to be moved to other locations and/or outside the zone. Such a measure would, of course, severely damage the nurseries and trade in these areas and countries.

Track and trace

After finding infestations on Corsica and in France, concern in the EU has increased further. That is why, since 17 December 2015 (EU Implementing Decision 2015/2417), the production locations of plants for planting of a number of genera/species in the EU must be inspected, and marketed plants from these locations must have plant passports in order to identify their background and to have traceability systems in operation if, despite this inspection, the disease should manifest itself. It concerns the genera/species that were infested in Italy by the pauca-strain of Xylella, and in France by the multiplex strain of Xylella.

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We share Johan's ambition

to surprise consumers time after time

Johan Solleveld comes from a tomato-growing family and has been involved in variety development at Rijk Zwaan for over 30 years. Thanks to his extensive experience, and to the fact that he really speaks the growers' language, he knows exactly what to look out for when selecting new tomatoes. Over the years, Johan has gained an ever-greater appreciation of the tomato's versatility and potential. He knows that nature can sometimes have surprises in store, and how important it is to remain open to the resulting opportunities. In close collaboration both with colleagues and customers, he strives to make a valuable contribution to creating tasty new products every day.

It is Johan's ambition to surprise consumers time after time. Rijk Zwaan - a global specialist in vegetable breeding - shares this ambition. We are working together towards a healthy future. Learn more at **rijkzwaan.com**.

Sharing a healthy future

RIJK ZWAAN

International Seed Testing Association

Boosting the knowledge of **seed sampling** and seed testing

Monique Krinkels

With 223 Member Laboratories, 39 Personal Members and 56 Associate Members, from 81 countries around the world the International Seed Testing Association is the acknowledged centre of excellence in the field of seed testing. ISTA might be nearly a century old, but there is nothing old-fashioned in its daily operations. On the contrary.

For Secretary General Dr. Beni Kaufman it is clear: ISTA should be a lean and keen organization which supports its members. The Association entrusts the development and evolution of the testing methods to its members, through its Technical Committees. ISTA's control over the implementation of these methods is done through its Accreditation Program. So far, 136 of the ISTA Member Laboratories have gained accreditation. Since July 2013 Beni (officially Benjamin) Kaufman has headed the secretariat in Bassersdorf, Switzerland. The molecular geneticist has over twenty years of experience in research and seed testing.

Auditing revised

The latest development is the revision of the auditing process. Auditing used to be done by a system auditor, who was employed by ISTA and also served as the lead auditor and a technical auditor. "We have three system auditors travelling nearly full-time around the globe. This system has reached saturation, we did not have any more room for growth while the demand for ISTA Accreditation is increasing. It would be far more efficient if this could be done by engaging a larger number of system/lead auditors. We therefore are creating a pool of System Auditors", says Beni Kaufman. These System Auditors should meet certain requirements. "They have to work according to the ISTA Standard and Rules and, of course, they should be completely independent", he adds.

More science

This year, the seed scientists and technologists will meet in Tallinn, Estonia, from 14-21 of June, for the triennial ISTA Seed Congress."The Congress allows for more time to be dedicated to science than in our regular Annual Meetings", Kaufman states. "The event will provide an opportunity to present scientific discoveries and technical innovations, as well as their practical applications. Besides the general meetings there will be poster presentations and several workshops. At the congress, the new ISTA website is expected to be launched, which will be more up-to-date, interactive, and with added functionalities. Since last year, ISTA has its own Marketing Head, a new function for the organisation. Pierrick Marcoux will make sure no one overlooks ISTA. "We want to be more visible at congresses and international meetings. Besides, we want to boost the knowledge of seed quality and seed testing in the world. Each year, we organise between 5-9 workshops focused on a single topic. For instance, ISTA plans a couple of multi topic workshops in Hyderabad, India later this year, which will be focused on basic seed testing and molecular methodologies. That way we will improve local expertise and skills." India is the largest member of ISTA, with 22 Member Laboratories and 6 Accredited Laboratories.

'During the ISTA Congress there will be three days dedicated to the topic Progress in seed testing and seed quality improvement through science and technology', says Beni Kaufman

Seed sampling certificate

Last year, ISTA introduced the idea of a Seed Sampling Certificate, which will allow to separate seed sampling – that could be done by an ISTA accredited sampling entity, and sequentially, sending the sample to one of the Accredited Seed Laboratories for testing. "That provides a higher operational flexibility while maintaining a traceable 'chain of custody' of the samples. All stages of the process will have to follow our guidelines regarding seed sampling methodology, lot and sample identification and quality testing, but can do so with higher operational flexibility." The Seed Sampling Entity will execute the sampling and issue an ISTA Seed Sampling Certificate, while the accredited testing laboratories will test the samples and provide an ISTA Orange Certificate for the lot. "This new system will be put up for a vote during the 2016 ISTA Ordinary General Meeting (part of the ISTA Seed Congress), and if approved will initially be conducted as an experiment."

Breeders have to factor in the extra workload

Bert Visser

On 12 October 2014, the Nagoya Protocol on Access and Benefit Sharing came into force. This Protocol to the Convention on Biological Diversity (CBD) aims at sharing the benefits arising from the utilization of genetic resources in a fair and equitable way. The Protocol adds a new dimension to the CBD.

Whereas benefit sharing in response to access had already been formulated in 1992 as the third goal of the CBD, in practice only a limited number of access and benefit-sharing (ABS) agreements were realised in the next two decades. In evaluating the feasibility of ABS agreements, developing countries noted that it was impossible for them to monitor if agreements, once concluded, were followed up by users, whereas developed countries reported that conditions for access were either non-existent or not transparent in many developing countries.

All genetic resources

The Nagoya Protocol[1] is an attempt to improve the conditions for the realisation of ABS agreements by setting clear requirements for access[2] and introducing the monitoring of compliance of users by authorities in the countries where the users carry out their research and development[3]. Currently, 72 Parties have ratified the Nagoya Protocol, whereas a large number of countries are still preparing for ratification. It can therefore be expected that the number of Parties to the Protocol will continue to grow in the near future. The CBD and the Nagoya Protocol cover all genetic resources, except human. In other words, these agreements do not only address plant breeding, but also the development of products in the pharmaceutical, cosmetic, food and beverage, and biotechnology industries.

Providers and users need to negotiate the conditions for access and agree on the benefit sharing

Monitoring compliance

Like all international agreements, the Nagoya Protocol is an agreement between States. As such, it does not bind citizens in the Contracting Parties (i.e. member countries). National legislation (or regional legislation as in the case of the European Union) is required in order to oblige users to follow the rules set out in the Nagoya Protocol. Users should obtain Prior Informed Consent (PIC) from the country where access to genetic resources is sought. In addition, providers and users need to negotiate the conditions for access and agree on the benefit sharing in so called Mutually Agreed Terms (MAT). The outcome of such process (PIC and MAT) should be reported by the authorities of the providing country to a dedicated website, called the ABS Clearing House, administered by the CBD secretariat[4]. This set-up should provide more legal certainty to users. Authorities in the country where the user performs research and development on the obtained genetic resources have to monitor whether a user acts in agreement with the conditions contained in the MAT, the contract with the provider. To assist in reaching this goal, the EU Regulation for the implementation of the Nagoya Protocol in the European Union[5] has introduced the principle of due diligence as well as two checkpoints. The user has to prove to the authorities in EU member states that (s)he has made great efforts to come to an agreement with the providing country, and that (s)he fulfils the agreed obligations. In the EU, this also means that when obtaining a financial contribution (grant) or when making a product ready for placement on the market, the user needs to notify the national authorities and to declare that (s)he used the genetic resources in accordance with the agreement with the provider. These are the two checkpoints.

The other kid on the block

Already back in 2001, the International Treaty on Plant Genetic Resources for Food and Agriculture was concluded under the Auspices of the Food and Agriculture Organisation of the UN (FAO). The Treaty is a specialised instrument, and the Nagoya Protocol states that in such a case, the Nagoya Protocol does not apply, but rather the specialised instrument. The Treaty takes into account the specific features of plant breeding, and to this end has established a so-called Multilateral System for Access and Benefit-Sharing (MLS) with fixed rules. However, this MLS only applies to a limited set of crops, mainly the large staple

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Photograph: Quick Shot

It is no longer possible to buy seeds from a farmer in Bandiagara, Mali, without approval from the national authorities

crops and, for instance, only few vegetable crops. In practice it means a split field: for some crops, access and benefit-sharing comes under the Treaty, whereas for other crops the Nagoya Protocol applies. Plant breeders tend to strongly prefer the Treaty.

Consequences

Many plant breeding activities will fall under the scope of the Nagoya Protocol. This means that if a plant breeder wishes to access novel genetic resources from another country for incorporation in his/her plant breeding programmes, (s)he needs to acquire PIC and MAT from the provider country, and to report to the authorities the use of such genetic resources when obtaining a grant or when placing a product on the market. These legal requirements have clear consequences for plant breeders.

It will no longer be possible to buy seeds from farmers in a nice local market, or to take a sample from a botanical garden, nor to collect plants from the wild, and to export such material, without approval from the authorities (PIC). To allow export, the authorities may set conditions that need to be fulfilled (MAT). The breeder will subsequently have to document when and how these genetic resources are used and which genetic resources end up in new products. Finally, the breeder needs to report such use at the time a new product containing such resources reaches the market. The national authorities will monitor the use of genetic resources.

Obtaining permission, documenting the use of the obtained materials, reporting requirements and facilitating authorities in monitoring visits are likely to result in an increased administrative workload for the user. In particular the first step, i.e. obtaining permission and agreeing on access conditions (i.e. reaching PIC and MAT) may appear cumbersome, and require substantial time and capacity. In some cases, it may still turn out to be impossible to obtain PIC and MAT. In all cases, breeders will have to consciously decide whether the pros of access balance the cons of a higher workload and/or a risk of not being successful in reaching PIC and MAT.

It is hoped that over time, both providers and users will get used to working with the rules set in the Nagoya Protocol. In an increasing number of countries, the National Focal Point on Access and Benefit Sharing (NFP) can provide some assistance. The NFPs are listed on the CBD website.

Advice to plant breeders

Plant breeders are advised to carefully check whether they need to fulfil ABS conditions under the Nagoya Protocol and, if so, to contact the authorities in the providing country. When doing so, they are also advised to - if possible - approach the authorities in

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The farmer market in Phu Quoc, Vietnam is no longer a place to buy local seeds

the providing country, together with a local counterpart/partner, who often has better knowledge of the authorities, speaks the language, is located nearby, and knows the local government culture. In general, it is not sufficient to tell the authorities in his/her own user country that (s)he tried, but unfortunately was not successful; it is considered insufficient to check the ABS Clearing House.
In the EU, due diligence implies a substantial and demonstrable effort on the part of the user to reach the authorities in order to agree on PIC and MAT. Finally, the breeder is advised not to pursue the use of genetic resources in the case of doubt. The authorities in the country where the genetic resource is used can ultimately take sanctions and enforce penalties, including fines and even imprisonment.

Impact on genebanks

Accessing new plant materials is core business for plant collection holders, whether genebanks or botanical gardens, and whether public or private professionals. Plant collection holders are likely to invest more in a successful international transfer of plant genetic resources than an average breeder. Genebanks and botanical gardens may have better networks enabling them to obtain agreements on PIC and MAT. Still, also for plant collection holders, the administrative load has increased and is likely to further increase with the further implementation of the Nagoya Protocol.
Two aspects may be highlighted. Firstly, several genebanks have agreed, including a large number of European genebanks, to provide their genetic resources under the standard conditions of the International Treaty on Access and Benefit Sharing (SMTA), which forms an advantage for users since these standard conditions can be fulfilled more easily by users. Even more important is that the EU Regulation states that if a user obtains genetic resources under the conditions of a SMTA, that (s)he is then assumed to have fulfilled the due diligence obligations.
Secondly, the EU Regulation has provided the option for collection holders to have themselves officially registered by the European Commission. Users accessing genetic resources from such collections do not have to check which ABS conditions applying to them have been agreed with the provider country, since that is then the responsibility of the collection holder. It is obvious that this means that the collection holder will take over some of the work load of the actual user.

Conclusions

The implementation of the Nagoya Protocol brings substantial changes to the practice of using plant genetic resources. Not only is access no longer free, but users should expect to be monitored by the authorities at some time in the future, to see if they have agreed ABS conditions, and whether they have complied with this agreement. This implies an increased workload for the plant breeder, who will have to factor in when access to specific genetic resources is worth the increased effort, not only of accessing the genetic resources but also of carefully documenting the subsequent use of such resources in his/her breeding programmes.

1) Full title: The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity
2) See Article 6 of the Nagoya Protocol: https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf
3) See Article 15-18 of the Nagoya Protocol
4) See https://absch.cbd.int/
5) See Regulation EU/511/2014

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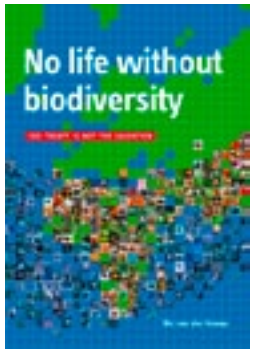
Drawbacks of the Nagoya Protocol

Sometimes it is better to **start all over**

Monique Krinkels

Will the Nagoya Protocol help the countries to reach the goal aimed at in 1992 during the Earth Summit 'The world in our hands' held in Rio de Janeiro? Nic van der Knaap clearly does not believe it, and he is not the only one to criticize the efficacy. He developed an alternative system and wrote a book on the topic.



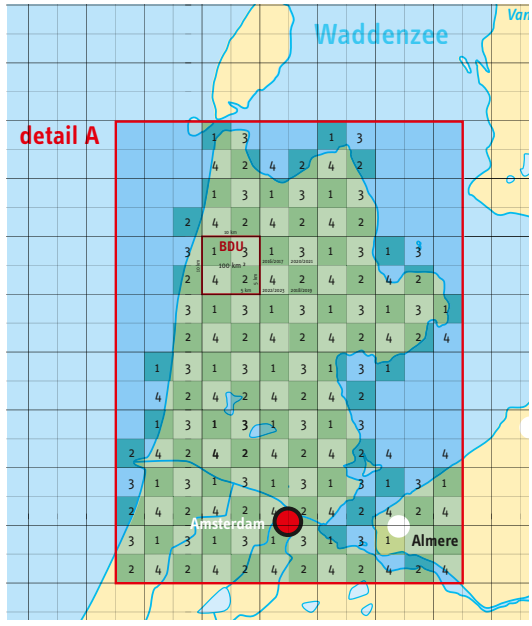
At the Earth Summit in 1992, 105 countries agreed that biodiversity on earth deserved protection. More than twenty years later they settled on the method that should ensure protection, the Nagoya Protocol. "It took so long as the participating countries kept discussing the matter. The end result is therefore based on compromises upon compromises", says Nic van der Knaap. "Everyone agrees that the protection of all life forms is of the utmost importance. The rapid loss of species we are seeing today is estimated by experts to be between 1,000 and 10,000 times higher than the natural extinction rate. Mankind has no future without biodiversity. But despite all the good intentions, the Convention on Biological Diversity has become a fiasco."

Paralysed

So far, many countries have ratified the CBD treaty. The United States of America is one of the few exceptions. "In itself it is not a bad idea to have a treaty on how plants, animals and other living organisms should be used for commercial purposes and who should receive compensation. Countries with a rich diversity of nature will get paid when a company wants to use its natural resources", explains Nic van der Knaap. "It might stimulate those countries to better protect their natural surroundings."
But there are several weak elements in the Nagoya Protocol, he points out. "For one thing, not everyone who profits from nature has to contribute. A tourist enjoying a safari in South Africa just has to pay for his holiday. Nor does the travel agency have to compensate for the protection of the wildlife. It is rather strange that only few parties, such as pharmaceutical companies and plant breeders, pay the price for the conservation of biodiversity."
Furthermore, Van der Knaap doubts that the system will work. "So far, it has been almost impossible to obtain plants for commercial use. The countries with which plant breeders have to negotiate have no idea what their nature is worth. They are paralysed by the chronic fear of asking too little."

Alternative system

Van der Knaap developed a system that counters the drawbacks of the Nagoya Protocol – the so-called DABB-system. It does justice to the most important



principle of the protection of biodiversity: it is vital for everyone. He proposes to create a database of all life forms and where they can be found, and let the use of these life forms be free.
"In my plan, the world will be divided into squares of equal size. In a cycle of eight years, bioscouts make an inventory of the life forms present. These bioscouts are local people who, armed with a camera, record metre by metre what nature has to offer. Whether it is tropical rainforests, deserts or cities, everything is captured on camera. The photographs are saved in an open-access database."
The costs for the database and the bioscouts are covered by the compensation every country has to pay. "And as the compensation depends on the Gross National Product, countries that are financially wealthy pay more than developing countries." Van der Knaap worked out his DABB-system with great detail, including an overview of the costs per country. It seems to be a good starting point – keeping the failure of the CBD treaty in mind – to rethink how we can prevent the extinction of life forms in a very short time.

'No life without biodiversity: CBD treaty is not the salvation' by Nic van der Knaap (ISBN/EAN 978-90-79598-06-9) is available at www.bol.com and costs 14,95 euro.

A **rose** by any other name would smell as sweet

Judith de Roos

For the last number of years, there have been heated discussions in the EU about the collision between plant breeders' rights and patent rights, but these are not the only IP rights that come into conflict. When it comes to variety denominations and trademarks, there are also some battles to be fought.

Recently, the Board of Appeal of the OHIM (Office for Harmonization in the Internal Market) gave its opinion about the possibility of registering a trademark if the same designation is already in use as a variety denomination in one or more species. Furthermore, both the EU legislation on trademarks and the rules on the denomination of plant breeders' rights are currently under revision.

Trademark legislation

The European trademark system is (comparable to the EU system of Plant Breeders' Rights) a dual system, made up of EU-granted trademarks and the national systems of EU Member States. A revised EU Trademark Directive (EU) 2015/2436 came into force mid-January 2016 and will be implemented in the national Trade Mark Laws before 15 January 2019. On 23 March 2016, the new EU Trademark Regulation (EU) 2015/2424 came into force. As of this date, the EU body in Alicante that grants EU-wide Trademarks which was called 'Office for Harmonization in the Internal Market' (OHIM) will now be called the 'European Union Intellectual Property Office' (EUIPO).

Both in the new Directive and new Regulation, the following absolute ground for refusal is introduced:

The following shall not be registered:
trademarks which consist of, or reproduce in their essential elements, an earlier plant variety denomination registered in accordance with Union legislation or the national law of the Member State concerned, or international agreements to which the Union or the Member State concerned is party, providing protection for plant variety rights, and which are in respect of plant varieties of the same or closely related species

In the past, such a trademark could already be refused, based on the fact that it consists exclusively of an indication to designate the type of the goods. A plant variety denomination is the generic designation of a specific variety, and needs to be used by everybody to indicate the identity of that variety. With this new ground for refusal, this has been made clear in a more specific way, at least when it comes to PBR-protected varieties.
The problem, in practice, is that a trademark will usually not be registered for one species, but for the whole of class 31[1] of the 'Nice Classification'. Within Class 31, the trademark registration can be specified to all living plants, which still includes all genera and species. The question now is what the effect will be of an identical, or very similar, name which is already in use as a variety denomination in one, or several, species. On this matter, the Board of Appeal of the OHIM (as it was still called at the time) gave its opinion on 15 October 2015 in six merged appeal cases[2]. Five applications were filed by Rosen Tantau and one by Kordes, both breeders of roses. In all cases, the trademark was originally applied for as follows:

Class 31 – Live plants and natural flowers, in particular roses and rose plants; propagation material for plants

Here, the wording 'in particular' is seen as clarifying example, but is not limiting the scope as such. During the appeal procedure, the applicants chose to restrict their applications significantly. In the case of 'Wasabi', the examiner had argued that consumers buying propagation material of this variety might be under the false impression that they were buying horseradish seeds. The application was brought back to: Class 31 – Roses and rose plants, and rose-propagating stock. For 'Goldrush', 'Geisha' and 'Silverado', where in all cases there were existing variety denominations, the application was brought back to: Class 31 – Live plants and natural flowers, namely roses and rose plants; rose propagation material. The wording 'namely' is limiting the registration to only roses and rose plants.
In the other two cases, the species in which there were existing variety denominations were specifically excluded. With 'Skyfire', where there was an Iris variety with that denomination, the application was amended as follows: Class 31 – Live plants and natural flowers, other than those of the botanical genus 'Iris'; propagation material for plants, other than those of the botanical genus 'Iris'.
With 'Ice Tea', where there were two variety denominations within the genus ('Dianthus L.' and the tea plant Camellia L. is a compound of the cool beverage Ice Tea), the application was brought back as follows: Class 31 – Live plants and natural flowers, in par-

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The CPVO only refuses a proposal for a denomination in case of a formal objection by a trademark owner

The examiner argued that consumers buying propagation material of 'Wasabi' might be under the false impression that they were buying horseradish seeds

ticular roses and rose plants, other than those of the botanical genus 'Dianthus L.' and 'Camellia L.' (tea plant); propagation material for plants, other than that for plants of the genus 'Dianthus L.' and 'Camellia L.' (tea plant).

To better understand the practice of variety denominations and the possible conflicts with trademarks, the Board of Appeal of the OHIM organised a public hearing in spring 2015, which is quite unique in the history of the OHIM. In addition to the lawyer of the applicants, representatives from the CPVO, ESA and Plantum were invited as experts. They argued that there is no need to refuse the trademark registration altogether in case of one of more already existing variety denominations. Restricting the trademark registration within Class 31 as much as possible is favourable. The experts were all of the opinion that the best way to decide whether the goods are closely related is to follow the system of UPOV classes. It was indicated that variety denominations are mostly used by professional users (growers), but often not by the end consumer. The risk of confusion in case of use of the same designation in different classes is therefore not so likely. In the decisions, this recommendation was followed. Further clarification will probably follow by means of an Implementing Act under the new EU Trademark Regulation.

Denomination Guidelines

The UPOV explanatory notes on variety denomination, explaining article 20 of the UPOV Convention 1991, are currently being revised. The aim is to streamline the practices of the UPOV Member States in order to prevent the use of synonyms (in case one country accepts a name and another refuses it). The CPVO will also carry out a similar exercise. With regard to the possible conflict between a variety denomination and trademark, the UPOV Convention states that an authority should not accept a variety denomination if a prior right (like a trademark) exists. Currently in the EU, the CPVO deals with this in a very pragmatic way, which is fully supported by the breeders. The CPVO does not refuse a proposal for a denomination in case of existence of a prior trademark, but informs the applicant about it and lets the applicant decide whether he wants to continue with the name. The name proposal is only refused in case of a formal objection by the trademark owner. This is because a trademark only has value if it is actually being used for the designated goods. If it has not been genuinely used within 5 years after its registration, it can be declared void or partially void for a particular category of goods.

Coming back to the six appeal cases before the Board of Appeal of the OHIM: if the original registration for the whole Class 31 would have been granted, but the trademark is only being used for roses, a breeder of tomatoes who would want to name his tomato Wasabi or Ice Tea would not have much to fear from the trademark owner, if the latter would start a legal case. Nonetheless, it is better to prevent such situations as much as possible by limited trademark registrations.

1 CLASS 31: (Natural agricultural products) Agricultural, horticultural and forestry products and grains not included in other classes; living animals; fresh fruits and vegetables; seeds, natural plants and flowers; foodstuffs for animals, malt.

2 R 279/2014-1 (Silverado), R 280/2014-1 (Goldrush), R 528/2014-1 (Geisha), R 691/2014-1 (Wasabi), R 894/2014-1 (Skyfire) and R 895/2014-1 (Ice Tea)

Filling the toolbox of **precision breeding** methods

Jan Schaart, Clemens van de Wiel, Bert Lotz and René Smulders

Plant breeding has resulted in numerous high-quality crop varieties being cultivated nowadays. Breeding based on crossing and selection remains an important and ongoing activity for crop improvement, but needs innovation to be able to address urgent future challenges, including increasing crop production in a rapidly changing environment.

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The efficiency of crossing and selection has already been improved for several crops by employing marker-assisted selection, but plant breeding still faces limitations in crops with complex genetics (e.g. due to polyploidy, heterozygosity, self-incompatibility) or a long generation time (e.g. fruit trees). In addition, the search for useful genetic variation remains laborious, even though next generation sequencing technologies have increased efficiency of screening, and introgressing such variation from crop-wild relatives into the cultivated germplasm is tedious.

'Traditional breeding would require much more effort and take considerably longer'

In the past two decades, new precision breeding methods have been developed to facilitate breeding of improved crop varieties. The toolbox of precision breeding methods now contains much more than just genetic modification and provides innovative opportunities, for example, to create novel genetic variation to transfer variation, or to circumvent specific breeding limitations. These methods increase the precision or efficiency of making changes in genomes and reduce the time and effort that is needed in selection for new varieties. Below, we describe examples of precision breeding to produce genetic variation, to speed up breeding and to develop F1 hybrid systems. The improved plants produced using precision breeding methods can also often be obtained through traditional breeding, but that would require much more effort and take considerably longer.
Breeders use mutagenesis by chemical mutagens or ionizing radiation to create new genetic variation in breeding programmes. Screening for mutants is time-consuming and once mutant plants have been selected, several breeding steps are required to achieve homozygous mutations and to remove undesired mutations. The advent of site-specific nucleases (SSNs), and especially the latest innovation of SSNs called CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR associated) is causing a revolution in mutation breeding. SSNs induce double stranded breaks in DNA at predefined locations. The subsequent repair of this lesion by the cells' native repair mechanism frequently results in small errors that comprise deletion or insertion of one or a few DNA nucleotides (see figure), or a nucleotide substitution. Such small mutations may result in knocking-out the function of the targeted gene. In addition to the precision of this novel mutation technology, the mutations are often induced in both (in diploid species) or in multiple (in polyploid species) alleles, so that cumbersome backcrossing for homozygosity or removal of unwanted mutations can be limited.

Genome editing

Recently, several interesting examples of SSN-applications have been published. In soybean, an enzyme called fatty acid desaturase (FAD) converts the mono-unsaturated (omega-9) fatty acid oleic acid into the polyunsaturated fatty acid linoleic acid, which is undesirable in oils for consumption. Mutation of the FAD gene resulted in soybean lines that produce oil low in polyunsaturated fat. The fact that these soybean lines were created in a single generation demonstrates the power of SSN technology as compared to traditional mutagenesis. In another example, knocking out all four alleles of the vacuolar invertase gene (Vlnv) in a commercial (tetraploid) potato variety prevented the accumulation of reducing sugars during cold storage. Tubers from these Vlnv-knockout plants had undetectable levels of reducing sugars, and processed chips contained reduced levels of acrylamide. This example underlines the potential of this and other new breeding techniques for the direct improvement of existing elite crop varieties with complex genetics.
In a final example, an elegant application of SSN technology was generating resistance to commonly occurring bacterial leaf blight in rice caused by Xanthomonas oryzae. X. oryzae exploits the rice OsiiN3 sugar transporter gene during pathogenesis and OsiiN3 is therefore called a susceptibility (S-)gene. To activate the OsiiN3 gene in rice, X. oryzae bacteria secrete effector molecules that bind to the promoter of the OsiiN3 gene. Partial (5-10 base pairs) deletion of that binding region in the OsiiN3 promoter using

Mutagenesis based on sequence-specific nucleases

SSNs stopped X. oryzae from inducing Os11N3 gene expression, resulting in a strong resistance to infection by some X. oryzae pathotypes in rice, while the functions of the Os11N3 gene for the plant remained unaffected. The novelty of this approach was the precision - as compared to inducing random mutations - by which variants of the promoter could be made and tested for their effectiveness against X. oryzae. At Wageningen UR Plant Breeding, we are currently investigating the application of SSNs in amongst others wheat for targeting gliadin genes with the aim of producing wheat that is safe for people with celiac disease, in the oilseed crops Crambe abyssinica and Camelina sativa for improved oil quality and in potato to produce tubers with modified starch composition.

Speeding up breeding

Fruit trees generally have a long (5-7 years) juvenile phase, which is a constraint for creating, for example, new apple cultivars through crossing and selection. Fruit breeding can now be sped up using backcrossing schemes, involving lines overexpressing recombinant early flowering genes. Plants with these genes flower within a few months, so that one breeding cycle can be performed in one year. In the final cross, only progeny is selected that does not inherit the recombinant early flowering genes. These varieties are thus indistinguishable from varieties that would have been obtained through conventional breeding, but decades later. The successful application of this new breeding technique has been demonstrated in plum in the US, and in an early flowering-based apple breeding programme in Europe, in which pyramiding of resistance genes from two different sources was achieved in two years.

Hybrid varieties

Several new precision breeding methods have been developed to facilitate hybrid seed production. Inducing haploids using a transgenic CENH3 mutant parental line, which contains a mutated centromere-specific histone (CENH3) gene and a transgenic rescue construct containing a variant of wild type CENH3, can speed up the creation of doubled haploid lines. When the CENH3 mutant line is crossed to another plant, the chromosomes from the mutant are selectively lost, producing haploid progeny that may convert into doubled haploids through occasional non-reduction during meiosis. These doubled haploid lines are devoid of the mutant CENH3 and transgenic rescue constructs and can directly be used as parental lines in hybrid breeding programmes. In cooperation with breeding companies, Wageningen UR Plant Breeding has started a research project to create CENH3-based haploid-inducer lines for genome reduction in hexaploid Chrysanthemums. Other new breeding methods have been applied to create GM maintainer lines for propagation of transgene-free male-sterile lines that are used in hybrid seed production. Pioneer has now commercialized this application in maize and they also intend to implement it in rice and wheat.

Outlook

The development of new breeding methods enables faster and more efficient creation of new crop varieties that are difficult (in terms of time and effort) to obtain through traditional breeding methods. Although these novel crop varieties have been made using GM techniques, their end products are free of transgenic sequences and are similar to and often indistinguishable from traditionally bred plants. The current legal status of products from precision breeding is still unclear in Europe and an appraisal of the current GM regulations is required to prevent unnecessary overregulation and to ensure that breeders, growers and consumers can take full advantage of new precision breeding methods and products produced using these methods.

Bees and butterflies threatened by **extinction**

Marianne Heselmans

In February, the international biodiversity platform IPBES hit the alarm button: things are getting worse for the wild bees, bumblebees, butterflies and other pollinators, which is endangering food production. Among other things, flowering borders, intercropping and less use of pesticides can bring wild pollinators back to the fields.

Cocoa, coffee, cherries, canola, tomatoes, pears, cashew nuts, cotton, soybeans. We have to thank an army of pollinators for these and many other crops in the world. As many as three-quarters of our food and oil crops need pollinators. The female plants do not get their pollen for fertilization from the wind, but from flying bees, bumblebees, hoverflies, butterflies, birds, bats and other animals.

Precisely this method of fertilizing crops has now become a problem worldwide, the biodiversity platform IPBES of the United Nations observed in February, in a report 'Thematic Assessment of Pollinators, Pollination and Food Production'. More and more species that help pollinate crops are dying off or are so rare that they are only able to visit small numbers of flowers. 16.5% of all birds, bats and other vertebrate pollinators are threatened with extinction, estimates the IPBES. For pollinating insects, including bees and butterflies, this could even reach 40%.

Millions lost

Among the causes are diseases and pests, climate changes and invasive alien species. But the main reason seems to be the intensification of agriculture. Monocultures only bloom for a few days. The rest of the year, there is hardly any food for pollinators on the bare, vast fields, not even weeds. Also, the often intensive use of pesticides does do the pollinators any good.

77 experts contributed to the report. Together they assessed 3,000 scientific articles. They also included non-scientific sources with indigenous knowledge about pollinators. "Too few pollinator species in a field appears to cost farmers and horticulturalists money," says Koos Biesmeijer, researcher at Naturalis and the University of Amsterdam and one of the authors of the report. "We looked, for example, at Gaia apples in England. In ten fields, we studied the number of bee species and the number of times they visited flowers. We then compared the number of apples and the quality of the apples - the better the pollination, the more round the apple. By comparing the well-pollinated orchards with the less well-pollinated orchards, we were able to calculate that, from a total value of £20 to £25 million for English Gaia apples per year, the sector was losing £6 million as a result of suboptimal pollination. For blueberries, strawberries, pears and other fruits, we find a similar importance of pollinators.»

Researchers have thus compared fields and orchards all over the world, including in the Netherlands, Ghana, Brazil and Nepal. The distance to a nature reserve was one of the determining factors. The closer to a nature reserve, the more pollination, whereas the number of species rapidly decreased from a distance of 300 to 400 metres.

Honeybee

Boxes with honeybees are being placed in more and more fields to aid pollination. The authors of the report welcome this, especially since all these boxes worldwide will also produce another 1.6 million tonnes of honey every year. But it is not enough according to them. For many crops, such as tomatoes and vanilla, the honeybee is unsuitable. Furthermore, it is dangerous to put all your eggs in one basket, says IPBES. A disease, such as the varroa mite currently to the honeybee, can cause considerable damage in certain years to the deployed pollinator. Furthermore, several studies thus show that more pollinator species provide better pollination.

According to one of the largest survey studies observed by a panel - published in Nature Communications on 16 June 2015 - wild bees make a substantial contribution to the production of about 20 surveyed crops, including rapeseed, sunflower, strawberry, broad bean, apple and pear. Even with deployed honeybees in those fields, wild bees still frequently accounted for half of the pollination. Its contribution averaged more than $3,000 per hectare per year.

But the authors also found, in those surveyed crops, that the number of wild pollinators was limited to a few dominant species present; rare species hardly played a part. Only 2% of the wild species in a region, supplied 80% of the pollination. Netherlands, for example, has 360 wild bee species, most of which are rare. The apple is then mainly pollinated by a group of about 7 wild bee species that are doing pretty well. Worldwide, there are about 20,000 species of wild bees. It is estimated that about 12% of wild bee species visit crops.

Flowering strips

Biesmeijer, however, does not consider this finding

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There are about 6,000 species of hoverflies who feed mainly on nectar and pollen

a reason not to protect rare species by establishing nature reserves. Rare species could play a greater role, if they are going to fair better again in nature. Furthermore, wild bees are also important for wild flowers - 90% of wild flowers depend on a pollinator. Solutions to protect the dominant wild pollinators on farmland are not that difficult as such, according to the IPBES report. More crop diversity and strips of flowers alongside and through fields already produce more wild bees in the following year. "It requires land," says Biesmeijer. "But that is also required for less fruit and lower quality fruit, so you will earn that back."

Once farmers and horticulturalists start to see the importance of wild pollinators, IPBES expects that they will become naturally more cautious with pesticides and find organic alternatives. That already happened, for example, in Dutch tomato cultivation. Up to 1998, workers pollinated tomato plants in Dutch greenhouses using a stick - which was time-consuming and expensive. Once the gardeners started to set out bumblebees, they eventually switched to natural enemies of pests, such as the parasitic wasp against white fly and ladybirds against aphids. Pesticides no longer worked, because they were found to kill the bumblebees.

Less harmful

One of the studies examined by the panel had found a negative effect of neonicotinoids on wild bees. Biesmeijer thinks that is an important study, but does not think that the abolition of neonicotinoids will solve the problem; neonicotinoids are, when used correctly, less harmful than many older pesticides, and you have to properly weigh up the alternatives.

In the long term, however, he feels that we really must find an alternative to monocultures with lots of pesticides and fertilizers. "We should use nature far more in the fight against pests and diseases."

Establishing nature has already proven to counteract the decline of species. In the Netherlands, Great Britain and Belgium, bees, hoverflies and butterflies declined radically between 1930 and 1990 as a result of construction and intensification of agriculture. Since 1990, this decline is less severe. In some places in nature reserves and in cities, there has even been some recovery of species diversity. Dutch and British researchers observed this in a publication in 2013 in Ecology Letters. As a basis, they used the observations of insects which amateurs and professionals made between 1930 and 2012.

Indigenous knowledge

It is now important to prevent further decline in the non-Western regions which are still rich in pollinators. Biesmeijer: "Countries must not make the same mistake as us, and unilaterally intensify agriculture. There is often much indigenous knowledge in non-Western regions about bio-diverse farming systems and the place of pollinators herein. We must ensure that it is not lost. "

Parliament Supports Proposals

New EU phytosa**regulation** is c

John van Ruiten

In 2016, new EU legislation will be finalized on Plant Health and on Official Controls. Last year, it was decided not to renew the existing rules on marketing and quality of seeds and planting material (the so-called Plant Reproductive Material = PRM proposals). The 12 existing EU marketing directives will be the basis of legislation in the 28 EU Member States for the next 4-5 years at least.

Plant health has become an area of increased importance. From mainly a 'professional issue' for growers, it has developed more and more during the last 15-20 years into a matter of concern within society in general, followed with great interest and also with political attention (as well as crop protection). Following the introduction of new plant pests and diseases, such as sudden oak death, forest beetles (like Asian longhorn beetles), invasive weeds/species, red palm weevil and recently olive die-back, it has become apparent that a better EU focus on prevention of new diseases from coming into the EU or establishing themselves in the EU, is absolutely necessary.

Strengthening the system

The old EU legislation and existing EU Plant health policy are obviously not effective enough to prevent imports of those diseases or to take adequate action to eradicate new diseases once they arrive. The existing phytosanitary legislation in the EU basically dates back to the 1970s and focused mainly on known diseases and European trade. Since then, the EU has enlarged from 9 to 28 Member States and global trade in seeds, plants and plant products has grown enormously.

After the evaluation of the existing legislation in the period 2011-3013, the EU Commission came up with a proposal in May 2014 for a new Plant Health Regulation. It was presented to the Member States, EU Parliament and all agricultural stakeholders. The bigger picture or 'common thread' in this proposal was broadly accepted in the EU. Both Parliament and Member States supported the proposals for strengthening the EU phytosanitary system.

And recently, in a so-called trialogue, political agreement was reached about the content of the new Regulation. It is expected that final publication will take place at the end of 2016. Then a transition period of 3 years will start, in which a great number of implementing regulations have to be formulated. It is now

nitary oming

Both Parliament and Member States supported the proposals for strengthening the EU phytosanitary system

foreseen that the new regulation will come into force at the end of 2019 or the beginning of 2020.

Practical consequences

The main issues with practical consequences in the Regulation are:

Improved traceability systems will be developed, amongst others, by a new obligation to register professional companies working with plants. In case of problems, direct/immediate trace-back actions must be possible. An international database (TRACES) with information on marketed material will be set up.

Nearly all seed/planting material of all species marketed will be plant-passported during trade between professionals. This will include all 'plants-for-planting', so also indoor pot plants, bedding plants, balcony plants, etc. Plant passports are relevant for both quarantine and quality diseases

The number of EU quarantine diseases is decreased. The focus of policy/regulations will be on a smaller number of global threatening diseases.

A new group of diseases, called EU Quality pests or RNQP (regulated non quarantine pests) is introduced. In this group, a great number of present-day quarantine diseases that occur regularly/frequently in the Union and can be transmitted via planting material, will be included. New protocols for controlling and (low) tolerances for marketing seeds and young plants will be formulated.

A new and improved 'early warning system' within the EU is created, with obligations for Member States to immediately report findings of infestations/interceptions of new pests /diseases to each other and to the EU Commission.

The requirements for import of plant material/products into the EU will be more severe and also the import inspection system at border points of the EU will be strengthened. The obligation of first making PRA (Pest Risk Analysis) reports for a number of products when coming in from new sources/countries will be introduced.

Next steps

Many details of the new Regulation have to be further studied and worked out during the next three years. It is expected that in the first year, all the relevant points related to plant passports (format/information on the document/modes of operation) will be formulated. In the second year, the list of priority quarantine organisms and the requirements for importing/marketing/checking material in respect of these diseases will be defined. And in the last year, the requirements for RNQP diseases (which diseases will be on that list) will be formulated and it has to be decided for each pest if a zero tolerance will remain or if a certain low tolerance for marketing seeds/planting material will be defined.

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HOLLAND STRAWBERRY HOUSE

Origin Not Yet Discovered

Brown rot shocks rose growers

The black colouring of stems is a sign of the presence of the bacterium Ralstonia solanacearum

John van Ruiten

It is not uncommon that growers of products encounter new diseases in their crops, but normally the problems that occur are already known in other regions/countries. But last year, cut flower rose growers in the Netherlands, but also in Poland, Belgium, France and Germany, were faced with severe problems with their crops.

After investigation by the Netherlands Plant Protection Organisation (NVWA), it turned out that the bacterial disease brown rot (Ralstonia solanacearum) was the cause of the problems. This organism is on the EU Quarantine list and is also listed as EPPO A2. Genetic analyses of the strain (R. solanacearum race 1) found in various locations with brown rot infestations in rose crops showed that they were all identical, but the strains were very different from earlier brown rot infestations during the last five years in ornamental crops (such as Anthurium or Curcuma, in which race 1 was identified) or other crops (like potato, in which in the EU infestations of race 3 have been found). The strain identified in roses up until now is not found anywhere. Research is ongoing in culture collections of bacteria to see if homology with other sources can be established. The finding of one specific isolate indicates that there must be a primary source of infection.

Origin

It became apparent from further investigations that problems in the cut flower crops in various cases were related to the propagation material used. This propagation material, however, has never shown any symptom and the material came to growers via a number of propagating nurseries. It has not yet been possible to trace back the origin of the problem that has occurred. Discussions and investigations are ongoing whether the occurrence of the disease is related to primary influx of infected material (eg. rootstocks/budwood) from an unknown origin, or if another source (insects/man) has created the problems.

The disease, causing severe wilting of young shoots and browning/black colouring of leaves and stems, is transferred via water (most growers grow their crops in substrate with water recirculation), via planting material or via knives/equipment used. Once an infection is present in a company, rigorous measures have to be taken to eliminate it from the production system. It is possible, however, and that has been shown by growers who, after cleaning/disinfecting their glasshouses, were able to start a new crop in the autumn of 2015, to become clean again and they have not seen symptoms of infection since then. Testing water and plant material can be done with a PCR test within a few days. If a positive result is found, however, it takes a number of weeks to verify and control the identity of the bacterium found and to be sure that it is the strain that is harmful to roseplants.

Photo's: Netherlands Plant Protection Organisation (NVWA)

Brown rot causes wilting and yellowing of the leaves

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NAL today and **tomorrow**

Adrie Molenaar

Naktuinbouw Authorized Laboratories (NAL) is an official system and the exclusive domain of seed companies: companies with production and marketing of seeds from their varieties as core business. Participation is internationally open and on a voluntary basis.

Today, the scope of NAL is about sampling and laboratory testing for seed analysis (such as germination, usable plug tests and purity) and seed/plant health (for bacteria, fungi, viroids and viruses). When companies are working in compliance with the NAL Conditions, it will give them the confidence that their clients will receive seeds meeting their high standards because of reliable test results, reflecting the actual quality and health status of the seeds.

Monitoring results

Naktuinbouw monitors the performance of the NAL-authorized companies by annual external audits and NAL proficiency tests. Especially the latter is valued as most important by the participants, because it often leads to further improvement. NAL proficiency tests are available for voluntary participation by other labs as well. Indirect monitoring takes place by following official international notifications by National Plant Protection Organizations (NPPOs). There were, however, no notifications related to an authorization for NAL during 2015. The monitoring as a whole provides confirmation that the companies are in control regarding the processes which they have brought under NAL-authorization.

NAL-authorized companies (2016)

Bejo Zaden, Warmenhuizen (NL)
Enza Zaden Seed Operations, Enkhuizen (NL)
Germains Seed Technology Group, Aalten (NL)
Hazera Seeds, Berurim (IL)
Hazera Seeds, Made (NL)
Incotec Europe, Enkhuizen (NL)
Monsanto Holland, Bergschenhoek (NL)
Monsanto Vegetable Seeds (a division of Monsanto Company), Woodland CA (USA)
Nunhems Netherlands, Haelen (NL)
Nunhems USA, Brooks ID and Parma OR (USA)
Rijk Zwaan Production, De Lier (NL)
Sakata Seed Corporation, Yokohama (J)
Sakata Vegetables Europe, Uchaud (F)
Syngenta Seeds, Enkhuizen (NL)
Takii Europe, De Kwakel (NL)
Vilmorin, La Ménitré (F)
Another two companies will have an initial audit this spring.

Verification Programme

Since it is likely that the European Union will formulate new plant health and control laws, the NAL-system will be adapted in such a way that it will remain fit for the future. Therefore, new modules (extensions) have been / are going to be developed, together with the companies. The umbrella for all these modules is what one might call a 'verification programme for seed production and market access'.
New conditions in this case will also be based upon the drafts of the EU PRM laws that were voted down in 2014/2015, because it is likely that the relevant and important elements therein will come into force somehow, sooner or later. Aspects that are regarded as important are seed/plant health (quarantine, as well as quality diseases/pathogens), varietal trueness, varietal purity, quality aspects (like noxious weeds) and tracking and tracing. Companies will remain responsible themselves, as they are today.
Because NPPO's want results of field inspections to be taken into account more and more for issuing phytosanitary certificates, the module 'Naktuinbouw Authorized Field Inspection' (NAFI) has been set up. Representatives of almost all NAL participants in the Netherlands were present during a second meeting in december 2015. The introduction, the draft standard (with as important aspects: risk-based control of the process, the inspector (training, qualification, back up, proficiency test) and the inspection, the draft audit regulations (how often checked, by whom, when, consequences if out of tolerances) and the guidelines were discussed.
The draft, still in development, was introduced in 2015. 2016 will be a probationary year, in which participants can try to implement this on a voluntary basis, to see whether it has added value and is feasible for compliance. However, Naktuinbouw will offer training as well - this will not be a mandatory truck system. If there are also good alternatives which we can rely on, these will be evaluated to see if they can be approved.
An evaluation will be held at the end of 2016. The final

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[Table 1] Programme of NAL Proficiency tests 2016-2018

	Pathogen	Crop	Method
2016-1	Tobamo	Tomato/pepper	Bioassay
2016-2	Alternaria	Carrot	Blotter
2016-3	LMV	Lettuce seedlings	ELISA
2016-4	Pspor	Leek	Dilution plating
2016-5	To be determined	-	-
2017-1	Xcc	Cabbage treated	Dilution plating
2017-2	Acidovorax citrulli	Cucurbits	Sweatbox
2017-3	PepMV	Tomato	ELISA
2017-4	Fungus (to be determined)	-	-
2017-5	LMV	Lettuce seeds	ELISA
2018-1	Acidovorax citrulli	Cucurbits	Direct PCR
2018-2	CGMMV	Cucurbits	ELISA
2018-3	Tobamo	Tomato/pepper	ELISA
2018-4	Cmm	Tomato	Dilution plating
2018-5	Phoma lingam	Cabbage	Blotter

NAFI standard will then be submitted to the Board of Naktuinbouw for approval. For the moment, NAFI will be the domain of seed companies. But in future it might be that it will be available to and extended to other branches as well (e.g. arboriculture, plant nurseries, etc.).

NAFI

There are four arguments for choosing NAFI:
Firstly, because it must be of interest for the participant: ensuring that monitoring of seed production is state-of-the-art and reports thereof will give reliable information, reflecting the true quality.
Secondly, it must develop into a system where competent authorities can rely upon results concerning official inspection. Therefore, we will start in the Netherlands. It fits perfectly with the philosophy of Naktuinbouw, where companies can run quality controls very well for themselves.
Thirdly, the aim is to create a system for improved market access, where also the National Plant Protection Organization (NPPO) can rely upon results regarding issuing phytosanitary certificates.
Fourthly, it is the aim to make it globally applicable, making it possible to deal with foreign productions as well.

Other modules

Starting this year, other modules in NAL are also going to be developed, such as:
Naktuinbouw Verification Programme Identity
Naktuinbouw Authorized Maintenance
Naktuinbouw Authorized Processing
Naktuinbouw Authorized Sampling
Naktuinbouw Authorized Shipping
In the end, it will not be each individual module that counts, but the total will be more than the sum of the parts.

Harmonisation

At the moment, the Quality Management System (QMS)-requirements for our international systems are harmonized in a way that the requirements will become the same for ASLN, NAL and Naktuinbouw Elite ornamental crops. This will make auditing more efficient and cost-effective.

Cooperation

The NAL programme tries to establish working and equivalence relationships with the Netherlands PPO (NVWA), and other plant protection organizations, like USDA-APHIS (as well as the NSHS) and the Australian DAWR.

Platform

In 2015, a platform meeting was organized in Roelofarendsveen Netherlands, with all kind of workshops related to important elements of international systems such as ASLN, NAL and Naktuinbouw Elite. This was valued very highly by the participants. On 9 June 2016, a Platform meeting will be held once again, to enable participants to discuss various items with their counterparts from other companies.

Probiotics Protect Roots

Bacteria bodyguard fruits and vegetables

Marianne Heselmans

Brown rot is a plant disease caused by the soil bacterium Ralstonia. It causes dirty, brown spots in potatoes and bananas; tomatoes become soft and yellowish. If these plants are likely to rot due to the soil bacterium, Chinese farmers rinse the soil clean with water and methyl bromide. But this poison kills all soil organisms. The Chinese government is therefore searching for more environmentally friendly control methods.

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That desire gave ecologist Alexandre Jousset, guest lecturer at the Faculty of Agriculture at Nanjing (southern China), a new idea a few years ago: he wanted to protect tomato plants by providing the roots with 'good' root bacteria or probiotics. Jousset and colleagues from the universities of Utrecht, Nanjing and York decided to find out how to build a bacteria buffer around tomato roots which can keep Ralstonia under control. To this end, they started to cultivate tomato plants in sterilized soil. They systematically added fertilizer, 'good' soil bacteria and Ralstonia to that soil.

'Good' root bacteria may prevent crops from disease. Provided of course they have enough nutrients in the soil

Last year they published the results in Nature Communications: merely one bacterial species added to the root barely protected the tomato plant. But with five good soil bacteria around the root, the plant continued, even when Ralstonia was added quite liberally, to yield solid red tomatoes.
The secret of this strong defence team, according to the Nature article? Together they consume the same as the pathogen: one 'good' bacteria consumes the sugar, the next one the amino acids (building blocks of proteins), and the next one again the organic acids. "The team members must not like the same nutrients", says Jousset. "Because then they will kill each other in competition and Ralstonia will conquer."

Soil improvers

Jousset's trial illustrates the increased interest in the 'good' microorganisms around the roots. A little section of the garden centre now has a separate shelf for 'soil improvers'. Such as Gazon (= lawn) AZ by Ecostyle, a coconut fibre enriched with root bacteria. Or 'root activator' RhizaMax, enriched with fungi. Multinationals are also investing. Thus, in 2013, seed and chemical company Monsanto and biotechnology company Novozyme AgBio, started a company for agricultural root organisms. Last season it carried out 500,000 field trials in the US and Canada with 2,000 different bacterial and fungal species.
As a result of ever-cheaper chemical techniques such as sequencing, it is possible to 'see' ever more clearly into the soil and what all is happening around a root. And then the micro life on and around roots turns out to be naturally much richer than that in our guts. Some 10,000 bacterial species live in a healthy intestine. One gram of healthy soil may contain as many as 30,000 bacterial species, in addition to thousands of fungal and small organisms (see box).
Also, this micro life appears to play an important role in the resilience of plants against diseases. Nestled in the small pores of sand, clay or root, the soil bacteria and fungi help 'their' organism with nutrients and protection. Already it has been shown in a few bacteria that they start to emit semiochemicals in the vicinity of a pathogen, which stimulate the plant to react defensively. Other bacteria produce antibiotics against a pathogen, or they protect the plant by simply taking up space.

Back2Roots

Hundreds of laboratories worldwide are searching for good species. In the Netherlands, among others, there is the Back2Roots programme financed by STW-NWO (Technology Foundation STW & Netherlands Organisation for Scientific Research). The researchers paid by this programme will, for example, travel to Peru. There they observe which bacterial species in the soil are attracted by the wild potato to protect itself against pathogens.
Pius Floris, director of the Dutch company Plant Health Cure, is an adviser for Back2Roots. In 1995, he was one of the first in Europe who supplied microorganisms for orchards. Twenty years later, he also advises farmers and golf course managers and his company delivers several tonnes of bacteria and fungi annually. His soil improvers contain six types of bacteria.
One bacterium is 'fantastic' at releasing the phosphates in the soil, says Floris, the next one binds atmospheric nitrogen, and another one again produces antibiotics or the root growth promoting chemical indolebutyric acid. That such a team can work has been demonstrated by field trials funded by the EU in

The experiment in China made clear that plants benefit from 'good' bacteria with on the right tomato plants grown in soil enriched with probiotics

Photo: Tianjie Yang

an eroded, infertile agricultural area near León. Those trials are being conducted by Floris along with the Universidad de Valladolid and five other partners. Three types of field trials are being studied by the partners: two where oats, vetches and other crops are given organic fertilizer and some 'good' fungi and bacteria, and one where they are only given the usual amount of fertilizer. Last summer, the first two fields yielded noticeably more oats and vetches, says Floris. The plants look better. And the roots also go deeper, so that they are able to absorb groundwater and irrigation is not necessary. (Not yet published in a peer-reviewed article)

Competition

In monocultures, treated with fertilizers and pesticides, the diversity of micro-organisms is much lower than in organically managed fields or natural grasslands, such are the results of the first comparative studies of the bacterial DNA. Traditional deep ploughing also reduces soil life. But perhaps cultivated plants do not need such a huge variety to be protected, and much has already been gained with a few species.

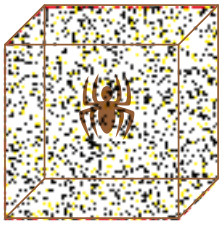
"That is why we also examined for that Nature article how many species are now the minimum requirement to protect the tomato against brown rot", says Jousset. To this end, his PhD student, the Chinese researcher Tianjie Jang, filled tens of thousands of plastic cups, each measuring a few cubic millimetres, at the University of Utrecht. In each cup, she allowed two or more bacteria to compete for nutrients with each other and with the pathogen Ralstonia. In total she tested 50 nutrients and 40 combinations of soil bacteria – plant-friendly relatives of Ralstonia, but for example also species of Bacillus and Pseudomonas.

Teams that suppressed Ralstonia remained. Those were tested, successfully, in pots with further sterilized soil by a Chinese tomato grower.

No additional bacterial nutrients are added to the tomatoes and bacteria which are tested by the researchers from Utrecht and China. The soil improvers in the trade do often have that, because modern fields and orchards are often lacking in organic matter. Plant Health Cure, for example, adds humic acids, derived from grape press waste, amongst other things to its bacteria. The company also adds so-called mycorrhizal fungi. That is also often lacking in orchards and cultivated fields. With their fine network of strands (their 'hyphae'), these soil-borne fungi help the root hairs to absorb the nutrients. Jousset can imagine that his 'team' of bacteria, once on the market, will also absorb extra nutrients.

EU regulations

1.5 billion euros are being spent globally each year on soil improvers, compared with over 200 billion euros on fertilizers and pesticides. Ecologist Jousset would be delighted if this ratio shifted toward the soil improvers. But according to Floris, the regulation in the EU would have to be amended to that end. Soil improvers may now only make vague claims such as 'helps soil life' or 'protects the plant'. Specific claims which can compete with those of pesticides, such as 'protects tomato plants against brown rot', must be supported by expensive studies - pathways which large companies can more easily navigate than small firms. Floris: "Hence, our products continue to be placed in the alternative section."



In a cubic metre of soil, dug under natural grassland, you might find the following:

100,000,000,000,000 bacteria
10,000,000,000,000 fungi
100,000,000 algae
1,000,000 nematodes
70,000 springtails
70,000 mites
70,000 annelids
200 millipedes and centipedes
100 fly larvae
100 beetle larvae
100 earthworms
70 spiders
70 aphids

Source: Global Soil Atlas 2015

'There's so much more to it than **blowing hot air** through seeds'

Monique Krinkels

Whether the location of the seed treatment plant is in a cold dry region, a hot humid one, or anything in between, the equipment of Agratechniek is able to dry seeds to the desired level. The software combines the local air conditions with the crop-based requirements and comes up with tailor-made solutions.

"A mistake that is often made is drying seeds with air that is too dry", says Jan Appelman, CEO of Agratechniek. His company specialises in drying technologies. "The result with a basic thermostat is that the seeds underneath are far too dry and warm, while the upper layer is still too moist. The solution: automatically increase the temperature of the process-air and keep the whole batch in balance."

Laws of physics

In his office in Anna Paulowna, a small town amidst a flourishing agricultural area in the Netherlands, Jan Appelman shows his elaborate documentation. Screenshots support the explanation of the sophisticated software that controls the drying systems. A long list of flow charts guides you to a proper understanding of the different types of installations. But there is no installation to demonstrate the drying process. "I cannot show you anything here, because we build custom-made. No condition is the same, so you'll only find our installations at our customers' sites", he explains. "That is our main selling point. Nothing is standard, everything is according to the client's criteria."

It is all based on the laws of physics, says Jan Appelman. "If you use outside air for drying seeds, it means of course that the air has to be dryer than the humidity of the seeds. Moisture will stay in the air because

'We are able to steer the equipment towards specific local conditions, thanks to our advanced air driers and ABC software', says Jan Appelman

water molecules move, thanks to the energy in the air. Warm air has more energy and can therefore hold more moisture than cold air. When the air is fully saturated with moisture, we have a 100% moisture content; the maximum Absolute Humidity at that temperature (AH maximum).

It is important to measure the Relative Humidity (RH) in combination with the temperature and calculate the Absolute moisture content. The moisture content of the air output of the seed is an indicator for the drying process. At some point, the moisture content of the seed will find an equilibrium with the air; equilibrium moisture content.

Reaching the desired (low) moisture content of the seed can be a problem when ambient air is not dry enough. This can happen with all types of drying installations where air from outside is heated and forced through the seeds with a ventilation system. At that point, you can no longer use the outside air if you need to dry the seeds further."

Advanced systems

"This is where our advanced systems kick in", says Jan Appelman. "Of course other manufacturers also use internal and external air circulation systems, but we are able to steer the equipment towards specific local conditions, thanks to our advanced air driers and ABC software. Our ABC processor can be set to dry seeds automatically to the required equilibrium moisture content by programming the corresponding absolute humidity (or RH at 25°C) of the air coming out of the seed. There is a big difference if you want to dry seeds in tropical areas with a high air-humidity or in more moderate climate zones; often outside air can be used for the first drying phase, but this air is not always suitable to reach the final (low) moisture content of the seed Then dehydrated air is needed; the drying time will be reduced significantly and you're always sure you'll reach the desired moisture content. To regulate the process, you have to measure the real (absolute) humidity of the outside air, the processing air and the air coming out of the seeds. In the algorithm, the temperature and the relative humidity are taken into account by the ABC processor for calculating the Absolute Humidity. By mixing outside or inside air with dehydrated air, the desired moisture content of the process air is being reached.

The 'Central Air Dehydration System' makes it possible to have all your (existing) drying installations, drying sections and conditioned rooms individually provided with dehydrated air by one central air drier.

"The finishing touch is the fine-tuning of the equipment in using the exact right airflow, temperature and desired moisture content with the very specific properties of the seeds, which vary per crop. To make it easy for the operator, the ABC processor is equipped with more than 30 menus with pre-settings for the different crops. The result: all seeds have a perfect moisture balance."

No quality seeds without **skilled staff**

Steven Groot and Henk Hilhorst

The seed industry in the Netherlands is the largest exporter of horticultural seeds, reaching farmers in every country of the world. High seed quality is one of the key factors of this success. Maintaining and increasing the level of seed quality requires skilled staff, innovation and collaboration.

Seed companies all over the world share one concern: how to provide farmers with the highest-quality seeds? The right genetic constitution is the first thing and much effort is put into breeding of high yielding varieties with resistance against pathogens or abiotic stress. Seeds are most often the vehicle to deliver varieties to the farmer and produce a crop. High seed quality is essential for the start of a good crop, as poor emergence or a seed-borne disease can result in a considerable reduction of potential yield. Farmers are more and more aware of that and their expectations are increasing.
Innovations in seed quality are based on two pillars. One is continuous investment in seed research infrastructure by the industry, with an increasing number of staff involved in applied research into seed quality and seed treatments. The other pillar is the long tradition of cutting-edge seed research at Wageningen University and Research Centre (Wageningen UR), which provides fundamental knowledge, innovation and training.

Training in seed technology

An example of the current training activities is the Master Class in Seed Technology, organised by Wageningen Seed Centre, a virtual centre which bundles Wageningen UR seed-related research. In 2016, the 14th edition of the Master Class will be held from 26-30 September in Wageningen. The Master Class offers professional seed technologists the opportunity to further deepen, broaden and update their knowledge and expertise on an academic level. The course has an intensive in-depth seminar programme and informal discussions, along with several hands-on sessions and demonstrations of recent technological developments.
During the Master Class, the participants will start thinking from a 'seed's perspective' through modern techniques, such as metabolomics and transcriptomics, as well as studies about the ecological relations of seeds with environmental factors, such as temperature, oxygen and pathogens. The course has a limit of 16 participants to allow maximum interaction and attracts participants from all over the globe. Time after time, the participants were very positive about having attended the course, as it enabled them to think more broadly in relation to their expertise than before attending the Master Class.

Seed quality, when used for propagation material, is optimal at natural shedding, but for most crops it is unpractical to harvest seeds at that time. Thus, seed producers have to harvest seeds before full maturity and, consequently, obtaining a mixture of immature to (almost) mature seeds. Some of the seeds may still mature during slow drying, but the near-mature seeds may have a lower vigour as compared to the most mature seeds. Although very immature seeds can often be removed by size sorting, the less mature seeds cannot. Also conventional colour sorters are often ineffective.
Making use of the observation that chlorophyll is degraded during seed maturation, a method was developed in Wageningen to measure, in a very precise manner, the fluorescence of residual chlorophyll levels. Incorporating this technique into spectral seed quality analysers and sorters allows seed companies to upgrade seed lots to a very high level by selecting only the most mature seeds. The method is commer-

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The Master Class offers professional seed technologists the opportunity to further deepen, broaden and update their knowledge and expertise on an academic level

cially available and both seed chlorophyll fluorescence analysers and sorters are used to optimize seed production and cleaning. The technique is also used in research to understand why the less mature seeds have a low seed vigour and, for instance, a shorter shelf life.

Extending shelf life

As with all living organisms, seeds are prone to ageing and it is well known that seeds age faster at higher temperatures and humidities. This is why vegetable seed companies in the Netherlands use climate-controlled warehouses with 30% relative humidity and temperatures between 15-20°C. Nevertheless, for crops such as onion or lettuce, shelf life may still be too short. This inspired Wageningen UR researchers to look for solutions. After noticing that all damage accumulating in seeds during dry storage is related to oxidation, they tested if seed shelf life can be extended under anoxia. This type of storage is used by the food industry, for instance to preserve the taste of nuts or roasted coffee beans.
Primed seeds from celery or celeriac are known for their very short shelf life of only a few weeks. However, when such seeds were stored for three weeks with the inclusion of an oxygen absorber, they survived very well, even at 35°C, whereas seeds from the same batch failed to provide normal seedlings when stored at that temperature in ambient air. These findings by researchers at Wageningen UR provide new opportunities to prolong the shelf life of seeds, especially in the tropics where cool storage is often too expensive.

Fast dry ageing test

Seed companies want to predict the shelf life of their seed lots. It is important for their clients and to fine-tune recipes for seed treatments, such as priming, which may reduce shelf life. Companies use so-called controlled deterioration tests or accelerated ageing tests as fast methods to predict shelf life, but in practice these tests frequently fail to estimate correctly the shelf life in the warehouse. The most likely reason is that in the warehouse the seeds are stored dry, whereas the fast ageing tests are performed under moist conditions.
Now we know that under moist conditions, the physiology of the seeds is different, there is a need for a better fast but dry ageing test. This led us in Wageningen to the idea of using elevated concentrations of oxygen, using high pressure air. Indeed, such elevated oxygen levels turned out to accelerate ageing under dry conditions. Now it offers a new method to study seed ageing, without the need to increase seed moisture levels.

Participation in research

Seed research at Wageningen UR is aimed at solving social and economic problems. Therefore, seed companies are often actively involved in our research projects. Not only do they participate in the project committees, but they also execute part of the experiments in the company labs. In this way, the research methods and the results are actively transferred to applications in the seed industry.

Quick **phenotyping** speeds up breeding process

Gerrit Polder, Gerard van der Linden and Rick van de Zedde

High throughput plant phenotyping facilities are rapidly becoming essential for significant advancements in plant breeding. Wageningen UR combines all of the necessary expertises in PhenomicsNL and is involved in several (inter) national phenotyping initiatives to lead and explore the field of plant phenomics.

Wageningen UR is partner in EU-funded research projects like SPICY, Phenomen-All (COST FA1306) and the European Plant Phenotyping Network (EPPN). Very recently, partners from several countries, involved in these projects, aligned and shared their facilities more thoroughly. On March 10th, the new ESFRI roadmap was presented at the KNAW in Amsterdam for representing initiatives for large-scale research facilities within Europe. One of the new projects is EMPHASIS, the European Infrastructure for multi-scale Plant Phenomics and Simulation for food security in a changing climate. EMPHASIS aims to address the technological and organizational limits of European Phenomics, for a full exploitation of genetic and genomic resources available for crop improvement in changing climate, known as the genotype-to-phenotype gap.

Combining disciplines

High throughput plant phenotyping demands integration of technical disciplines such as image analysis, pattern recognition, automation and robotics, combined with biological disciplines like plant physiology, including crop growth models, quantitative and molecular genetic poses several scientific challenges. Within Wageningen UR, sixteen research groups are working together on plant phenomics. In total, more than 200 Wageningen researchers contribute in order to better understand and predict plant behaviour and quality of plants. This research operates under the name of PhenomicsNL, the entry point to develop and use facilities and tools for large-scale field and greenhouse plant phenotyping.
The phenotyping techniques currently developed at Wageningen UR include 2D and 3D plant reconstruction modules, hyperspectral cameras, X-ray tomography for root analysis, chlorophyll fluorescence setups and other novel sensors. Vision-guided robotics is used to automate manual labour, and recently developed software for modelling growth and yield in 3D, facilitates predictions of plant performance based on the measured phenotypic features. Currently, several research projects are exploring the potential of these technologies, spanning the full spectrum from long-term academic research to industrial applications. The future perspective of research and development in this field is illustrated in a number of examples that are discussed in this article.
Plant breeding has achieved major advances in agricultural production in the past, but yield increase is levelling off and new approaches are required. With new techniques, the generation of (molecular) data is not limiting anymore, but efficiently linking this information from genomics, phenotyping and the genome to the phenotype is the bottleneck. To improve breeding even further, we need to characterise huge numbers of genotypes by objective, reliable and informative measurements, preferably automated by the use of robots.
Phenomics is a new research area that has gained a lot of interest and has grown enormously in recent years. It is a broad field, which comprises on the one hand of fields of expertise summarized as 'omics', such as metabolomics, genomics, and transcriptomics. Research in these areas has led to better understanding of the functioning of the plant in relation to its genetic make-up and, on the other hand, the development of methods and devices that quantify what the plants look like in a non-destructive way. This can be done at multiple developmental stages, and includes information on whole plant architecture and growth, flower or fruit development, maturity and aging, but also physiological traits.
This generates huge amounts of data that need to be managed and mined efficiently. Therefore, also experts in the field of (bio) informatics, mathematics and artificial intelligence (AI) are essential in order to draw useful conclusions from the mountain of data and find the genetic factors that control plant growth and yield under varying conditions.

The future greenhouse

Most plant phenotyping technologies are based on the approach that plants are brought to a special image- recording unit, e.g., through conveyer belt and/or robotic arm systems. Such a transportable pot plant system is suitable for a range of plants and offers the possibility of high throughput in an automated way. The use of a closed recording unit with individual plants in pots offers the clear advantages of a controlled environment. However, this type of phenotyping technology cannot be applied to (large) plants that are not transportable or to crops that need

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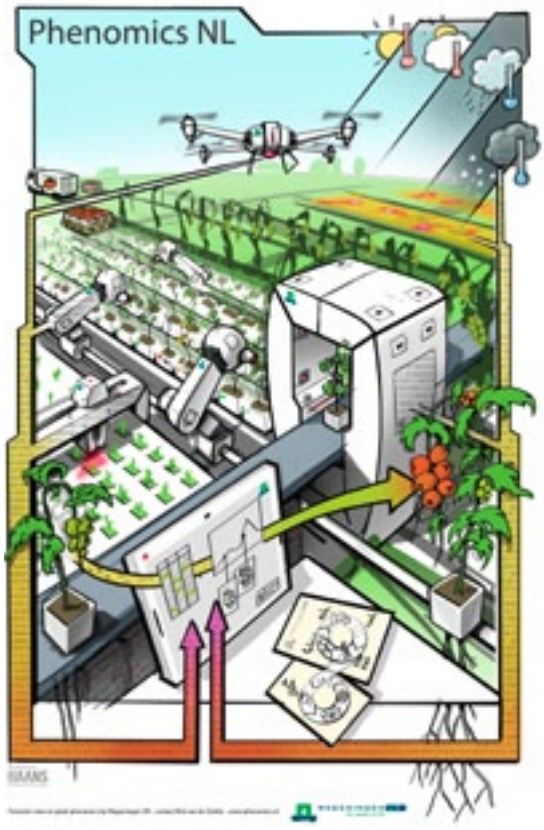

Futuristic view of phenotyping techniques currently developed at Wageningen UR

'Yield increase is levelling off and new approaches are required'

to be recorded under their cultivation conditions, for example tomatoes in a greenhouse.
An alternative plant phenotyping approach brings the camera-system to the plants creating great flexibility in observing plants under practical growing conditions.
One of the challenging aspects of plant phenotyping is to translate experimental results from controlled conditions in climate room tests to actual full-scale field experiments, in which the plants perform in their natural habitat. PhenoBot is an automated platform which moves on the heating pipes in the greenhouse, and images the plant in detail (such as flower and fruit growth, diseases and stress). PhenoBot uses a sophisticated 3D camera based on lightfield technology. The lightfield camera produces pixel-registered colour and depth (RGB-D) information, which makes it possible to measure plant and fruit morphology, independent from the distance to the camera.
Wageningen UR has developed MARVIN™: a machine that builds 3D images within 50 milliseconds using multiple cameras, and on that basis assesses quality. This system is utilised to sort young plants based on their 3D shape. The system can also be used to assess seed quality automatically and at high speed. MARVIN can also monitor the development of the seedlings and select the offspring that exactly meets the breeder's requirements. The machine operates more objectively and more rapidly than humans.
Postharvest shelf-life is another important phenomics parameter that is linked to loss of fresh and processed fruit and vegetable products. A major challenge in ensuring global food security is to avoid that up to 50% of our produce is discarded, therefore improving shelf-life is a very important breeding goal.
Shelf-life is determined by quality traits such as firmness, mealiness, colour, flavour and antioxidant levels, but also by the resistance to fungal and bacterial diseases. Postharvest quality traits are traditionally measured using invasive technologies, using for instance a potentiometer, a brix meter, taste panels, or chemical analysis of extracts. In this case, every sample (for instance a fruit) can be measured only once during its postharvest storage life and it is therefore very difficult to study the dynamics of the ongoing physiological processes during storage and their effects on final product quality. Therefore, there is a need to measure postharvest quality traits by non-invasive technologies, such as for example VIS-NIR imaging. This greatly increases the throughput of postharvest measurements and allows the dynamics of various postharvest traits during storage to be included in the analysis.
The PTR-TOF-MS can 'smell' volatiles and can be used in both invasive and non-invasive experiments. It enables monitoring the ripening of fruit and vegetables but can also detect stress. Plants under stress from, for instance, fungi or insects produce signalling molecules that can be detected by the machine.
This system will be implemented as part of the instrumental flavour models developed for tomato and strawberry. The HTP platform allows us to screen a large collection of diverse tomato and strawberry varieties for variation in postharvest quality traits. These traits may form the basis of novel breeding programmes geared towards the development of novel varieties with improved postharvest shelf-life.

Seeking cooperation

We have given a taste of the vast phenomics techniques and platforms available for research and breeding, with several but certainly not all applications. We invite the industry, as well as researchers from other disciplines, to join forces with us to help develop new techniques and think of novel applications, and develop phenomics-based solutions for cultivation and breeding challenges in the near future.

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Quality system gives plants an optimal start

Adrie Bohm

Naktuinbouw Elite is a quality standard and certification system, especially created over the last 25 years for vegetatively propagated ornamental crops. It is based on the principle of 'start clean and stay clean' which is secured in several ways.

Adrie Bohm is coordinator Elite programme of Naktuinbouw, Roelofarendsveen, the Netherlands, a.bohm@naktuinbouw.nl

The Elite certification system is a three step process. Firstly, by checking the entire propagation process and related processes which affect the propagation by auditing the quality management system of the company. Secondly, by sampling and testing crops involved in the programme for all relevant pathogens by the company itself. And last but not least, control sampling and testing by Naktuinbouw as a final check before the company can be granted the trademark Elite and plant material can be certified.

Clean material

Certification standards have been developed for more than 260 species now included in the programme. The Quality Management System (QMS) covers all processes within the company related to propagation. At the end of the process, propagating material is certified and a certificate can be issued on request. Elite certification stands for clean stock material, tested on pathogens according to the latest global phytosanitary requirements.

For global players in the market, 'Naktuinbouw Elite' is a very useful tool to achieve the goal of clean propagation material. The required QMS helps a company to be organized and keep all processes organized, while the sampling and testing of the mother plants gives the ultimate certainty of a good start. In addition, it can be helpful to fulfil phytosanitary border requirements or to cut down obstacles for practical market access.

During the annual meeting of the participants of Naktuinbouw Elite in 2015, a certificate of 'Authorized Participant Elite' was handed over to Selecta Klemm GmbH for the production location 'Juja' in Kenya for the production of Dianthus

Synchronized

Recently, the Elite requirement for the Quality Management System has been synchronized with other schemes such as NAL (Naktuinbouw Authorized Laboratories) and ASLN (Authorized Service Laboratories Naktuinbouw). This has been done to allow the audits for different schemes to be carried out by one auditor, in order to efficiently combine several audits and schemes. Because those programmes run worldwide, this can be very helpful.

In order to keep market access and acceptation as high as possible, the protocols for sampling and testing of the species are regularly (at least annually) updated to the state of the art. New species can be included rapidly. If new pathogens occur, they will be implemented in the protocols with immediate effect or with retrospective effect. During the annual meeting, all relevant issues are discussed and achievements are celebrated.

More information about the Naktuinbouw Elite certification scheme can be found on the website of Naktuinbouw, www.naktuinbouw.nl/en/topic/naktuinbouw-elite

Shared information platform strengthens the efforts

Evert Keuken

With ever-growing competition and the availability of more market-related information, companies ask themselves how they can improve this process of product introduction. And also, how can we manage this process.

It may well be one of the most challenging jobs in a seed company; managing the products (varieties) that you put out in the market. When do we promote a variety? Where do we focus our R&D efforts? How will it affect my market position? Often these questions are asked; and answered during product management sessions based on partial information and intuition.

Product management

Product management is typically organized around three main areas of expertise in a seed company; production, sales and R&D. The job of a product manager is to create an optimal effort by the three departments. Important tasks are:
– Identify potential in the market
– Identify potential technology
– Match sales with demand and production
– Supply R&D with market information
In the end, Product Management is typically responsible for the end result.

Segmentation adds to complexity

To effectively carry an interesting portfolio of products, most seed companies define so called market segments. This helps them to put focus on specific areas and helps to connect to specific areas that R&D is working on. The given Tomato example in figure 2 simplifies a typical segmentation in a vegetable seed company. For every market (for example Sauces), one or multiple products can be identified; the so-called Product Market Combination (PMC). Typically, this segmentation also includes regions to account for variation in culture and climate.
This means a typical product manager is working on a long list of varieties that are included in one or multiple PMC's. How do you make sure product management is keeping focus and is not overlooking the overall opportunities? What would be a structure to allow for this process to be managed properly?

Structures to support introduction

The current trend in many industries nowadays is working in small, committed teams. This 'project- or subject-based' organization tends to be agile in its setup. The struggle is often how to define those teams. PMC's tend to become smaller and smaller, in some industries to the point where one variety is developed for one exclusive customer. In management terms, it is considered good practice to divide work based on the aspects of greatest variation. In seed companies, this typically leads to segmenting on a functional basis, for example 'production' and 'R&D'. The day-to-day activities differ on a functional level. However, when one assesses the total process flow of a product from initiation through to its sale, there are of course processes in which the departments are both involved (or even more departments). To account for this, companies introduce coordination mechanisms. A 'product introduction meeting' is an example, during which topics are discussed that affect both the production and sales, as well as the R&D department. The goal of this meeting is to align processes between the departments. This organizational design strives to optimize the processes between production, sales and R&D as much as possible. By drawing a boundary around the department, people tend to be more able to focus on their work and optimize the internal process. The question then is, however, does this enable you to manage the processes that do cross the boundary you introduced?

Hug your customer

The current trend for seed companies shows an increased attention to the customer. The definition of a customer varies; but it is mostly the retail, and with them the consumers, that have an increased voice in the process of R&D. This 'customer intimacy' approach tends to conflict with the functional division of work described above. The functional division is driven by more internal factors, such as efficiency and quality; not so much by specific customer demands. This leads companies to a situation where they are willing to compromise on factors such as efficiency within production, or R&D, if it would enable them to better satisfy their customer.

Break the walls

One approach to become more customer intimate and allow products to be created more smoothly throughout the organization is to introduce multidisciplinary teams. In an ideal situation, working with people from different departments will simplify the process steps that involve more than one department. The team needs to consist of people who represent every

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For every market (for example sauces), one or multiple products can be identified; the so-called Product Market Combination (PMC)

step in the process. When traditional, line-oriented companies introduce multidisciplinary teams, they tend to struggle with topics such as responsibility and accountability. When a sales person sees an issue within the R&D phase, is he able and willing to step up and tackle the problem? Or will he sit back and think this is not his (primary) responsibility? This example shows that not only employees but also management need to rethink their way of working. When spring comes and production work peaks, what will the production team member do? Will he keep on working on the new to-be-developed tomato variety or will he get back in production to help get the seeds shipped? And how is he being held accountable for the choice he makes?

Share people and information

Figure 4 shows the process of variety development that is common in most seed companies in a nutshell. Within this simple scheme, almost every department in a seed company is involved. This process repeats itself over and over. When the new variety has been developed, it enters the next phase of product management. One could draw a similar figure for the process of introducing a variety in the market. The input tends to be a bit more strategic; it often raises questions like when do we introduce, or what is a good mix of new variety introduction.

For every company, the situation will be different; however, what remains of the utmost importance is the availability of reliable information. In every process there are key information components that influence decision making. A good example is the segmenting of crops. Typically, marketing creates other (more market-oriented) product segments. For example, tomatoes for the fresh market in northern Italy. Because this region has very specific needs, they defined a separate PMC. Within R&D, however, they tend to work with a more botanical segmentation. This example might fall into the 'fresh market tomato – Mediterranean region'.

When it comes to assessing the need for new varieties for the PMC in northern Italy, the team has to translate between the two different information sources. What is their basis for decision-making? This underlines the need for a shared information platform that supplies information that is comparable between the different departments to support decision-making. That enables management to discuss based on facts, rather than intuition and hidden information, to ultimately improve the process of product introduction.

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Biotech without modification of genes

Monique Krinkels

Armed with more than 26 years of experience in plant breeding and agricultural biotechnology, KeyGene's team of more than 130 employees spread around three continents are more excited than ever about how their proprietary technologies and non-GM approaches towards crop improvement can help address the needs of global agriculture.

"We use our expertise in plant-omics and molecular breeding to develop and deliver crop improvement opportunities for our partners along with tools and techniques essential for accelerated breeding of better non-GM varieties", says Dr. Fayaz Khazi, CEO of KeyGene USA. Last year the company expanded its presence in the Americas and established its presence at the University of Maryland's Institute for Bioscience and Biotechnology Research (IBBR) campus. Besides brand new offices and molecular biology labs, KeyGene USA also has a state-of-the-art greenhouse and access to modern growth chambers, tissue culture facilities, microscopy, NMRs and other resources.

Expanding into new crops

KeyGene, which marked its presence in the USA with a small business development effort is on a growth trajectory and has engaged multiple partners in the US, Canada, Mexico, Brazil and Argentina in deals and discussions on long-term strategic partnerships. "KeyGene adds value to its partners' R&D efforts through its knowledge on crops spanning a diverse array of species and traits", says Fayaz Khazi. "Further, our proprietary technologies, tools and trade-secrets quickly establish a unique competitive advantage equally applicable in maize as it is in eucalyptus or oil palm."

International collaboration

KeyGene was founded on 22 June, 1989 by several Dutch seed companies. Goal was to create synergy and higher efficiency in the molecular genetic research programmes of these companies and thus improve their seed breeding efforts. Today, KeyGene has four strategic shareholders active in the field of vegetable breeding: Enza Zaden, Rijk Zwaan, Vilmorin & Cie and Takii & Co.
As a leader in an plant molecular genetics, KeyGene is involved in improvement of Food, Feed Fiber, Flower, Fuel and Fun crops. The company is headquartered in Wageningen, the Netherlands, has a subsidiary in Rockville, Maryland, USA and a Joint Lab at the Shanghai Institute of Biological Sciences in Shanghai, China.

Sequence Based Genotyping

The company credited for developing the Amplified Fragment Length Polymorphism (AFLP) technology that revolutionized plant breeding has maintained the innovation streak with proprietary technologies like SNP Select and Sequence Based Genotyping or SBG. The SBG technology patent claims which were upheld by the USPTO after an ex parte re-examination request is a testament to the strength of the technology and its significance in molecular breeding. The SBG technology enables rapid and cost effective discovery and scoring of genetic variation for improvement of crops without prior knowledge of their genomic sequences.
"Our business model thrives on our ability to add value to our partners' product portfolio. Since its inception KeyGene focused on the practical and sustainable use of biotechnology. Value creation is at the intersection of deep knowledge of crop breeding systems and cutting-edge technologies We have shown over the years how this combination can be harnessed to benefit our collaborators", says Fayaz Khazi, who brings a highly interdisciplinary background to his current role at KeyGene USA-ranging from plant molecular biology, gene therapy, synthetic biology and business development.

Fayaz Khazi: 'Since its inception KeyGene focused on the practical and sustainable use of biotechnology'

Sustainable agriculture

Businesses and federal agencies in the Americas see the advantages of KeyGene's approaches and its portfolio of non-GM technologies for crop improvement, as KeyGene positions itself to become a responsible partner with both industry and academia.

Youth and adulthood in plants

Geert-Jan de Klerk and Mehdi Massoumi

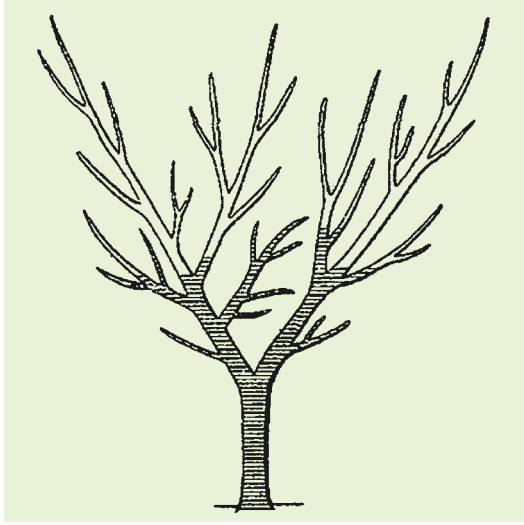
In plant research, there has been much attention to the transition from juvenile to adult. This is brought about by the rooting ability of juvenile cuttings (thus their suitability for vegetative propagation), and the flowering ability of adult plants. Recently, considerable progress has been made in influencing the transition.

During their lifetime, plants progress through a number of developmental phases, just as animals do. These include the embryonic, juvenile vegetative, adult vegetative, adult reproductive and senescence phases. The successive steps in the life of plants have been examined for some 100 to 150 years. It was soon recognized that plants do not merely increase in size (biomass, height) but that their morphology and physiology also change.

There was particular interest in the transition from juvenile to adult vegetative, a transition of great practical importance. The juvenile form is able to produce roots, but is unable to flower. The adult vegetative form responds to flowering inducing signals, but cannot root. So, by the time breeders have evaluated the flowering characteristics of new cultivars, the tree cannot be propagated anymore since it has lost the capability to root. This is more or less a Tantalus' torment for the breeders. There is a second complication: one of the main drawbacks in the breeding of many, especially woody, crops is the lengthy juvenile period before the flowering and fruiting characteristics can be evaluated and before crossings can be made. Some bamboos only flower after some 120 years!

Ways to root adult plants

With regard to the loss of the capability to root, the situation is, however, not so bad as it may seem. Fully reproductive plants are mature, but this holds only for the most recently generated tissues in the individual plant: tissues of the same plant generated in the period before the phase change maintain the juvenile characteristics. Thus, plants display a gradient of juvenile to mature tissues in the above-ground portion (Fig 1a). Maturation is highest near the apical meristems. The region near the base of the tree has the youngest ontogenetic age. The axillary meristems formed early in the life of the tree, but blocked in outgrowth by apical dominance, are usually still present and have the characteristics associated with juvenility. When these meristems grow out by removal of the apex (Fig. 1b), they carry leaves having a similar morphology as juvenile seedlings. More Important from the practical point of view is that they are often capable of rooting after an auxin treatment. Thus, a



[Fig. 1] Left: Schematic representation of an adult tree. In this tree, the striped parts appear to be still juvenile when excised from the tree, whereas the white parts are truly adult. (redrawn from F. Passecker, der Züchter 1952). Right: A trunk from which the larger part has been removed, with epicormics shoots originating from the outgrowth of axillary buds that had been dormant after formation during the juvenile phase

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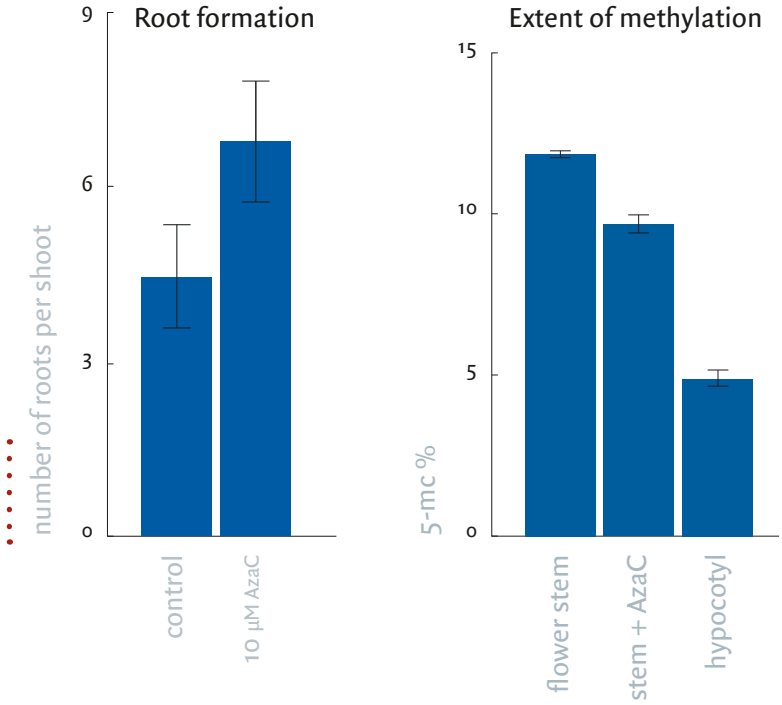


[Fig. 2] Left: The formation of adventitious roots from flower stem segments of Arabidopsis cut from plants that had or had not been treated with 10 µM of the demethylation agent 5-azaC. Right: The extent of methylation of the flower stems used in the rooting experiment and for comparison of hypocotyls. Unpublished results by M. Massoumi and G.J. de Klerk

[Table 1] Overview of various factors on phase change

Decrease of methylation	Inhibits transition juvenile to adult	arabidopsis
microRNA-156 (miR156)	Inhibits transition juvenile to adult	arabidopsis
Sucrose	Promotes transition juvenile to adult	arabidopsis and lily
Low temperature (15 instead of 25)	Promotes transition juvenile to adult	lily
Inorganics (in particular phosphate?)	Inhibits transition juvenile to adult	lily
Cytokinin	Promotes transition juvenile to adult	lily

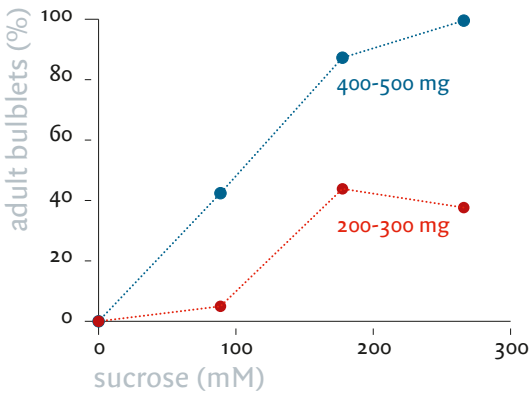
common practice in tree breeding is to allow plants to advance to flowering. Then, the tree is cut shortly above the soil. Soon, shoots develop from the stool. These shoots are called epicormics shoots.
But there are more possibilities. Plants can be rejuvenated by repeated subculturing in tissue culture. In apple, rooting increased from close to 0% directly after initiation to close to 100% after some 10 subculture cycles. Ex vitro, rejuvenation may be achieved by repeated pruning or repeated grafting.
Much research has been done on shortening the juvenile phase. The transition to the adult phase is achieved earlier when growth has been enhanced. Many reported treatments seem not to endure after critical examinations.

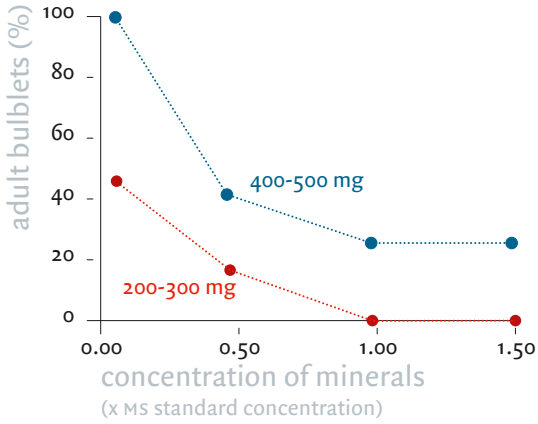
Biochemistry and molecular biology

Initial research on the backgrounds of phase change was at the anatomical and morphological levels. Later, it was attempted to find biochemical and physiological characteristics, both to increase understanding and to develop novel tools to influence the phase change. However, researchers did not find consistent differences between juvenile and adult tissues, although in a number of species gibberellins seemed to be involved. With the advent of molecular studies, striking differences between juvenile and adult tissues became apparent with respect to DNA-methylation and expression of microRNAs. The experiments on methylation and microRNA shown below were done with Arabidopsis plants. Arabidopsis plants show a typical gradient from juvenile to adult in their above-ground parts with respect to morphological features. We illustrated that rootability showed a similar gradient.

(De)methylation of DNA

The step from juvenile to adult coincides with increased methylation of DNA. Although not absolutely consistent, increased DNA methylation (hypermethylation) at a locus correlates with a reduction in expression, possibly resulting in complete silencing. This may be the cause of the loss of rootability. It should be noted that the extent of methylation is not a reliable characteristic of the ontogenetic state, as various developmental and environmental conditions have vast effects on methylation. Dormant tissues, for example, are highly methylated. The same holds for tissues that suffer from environmental stress. More conclusive results were obtained when methylation was inhibited. This may be achieved by 5-azacytidine,





[Fig. 3] Left: A juvenile and an adult bulb after 5 months' growth in soil. At planting, the bulblets had the same physiological age. Middle and right show the effect of sucrose and mineral concentration in the tissue culture medium during bulblet regeneration. At harvest, per condition, the bulblets were divided into weight groups. Redrawn from Langens-Gerrits et al., Physiol Plant. 119, 2003.

a drug that is also used in medicines. For proper use of 5-azaC, its mode of action should be considered. 5-AzaC, a hypomethylating agent, does not attack existing methylated DNA, but acts during DNA duplication: when methylated DNA is duplicated, the newly produced DNA is methylated in the same way, unless the drug 5-azaC has been added. So, when 5-azaC is added at seed germination, all tissues in the seedling are hypomethylated and explants also contain hypomethylated DNA. 5-AzaC may also be added to explants of non-treated plantlets and, in this case, only DNA synthesized after excision is hypomethylated.

We added 5-AzaC in both methods, during explant generation and during the rooting treatment. 5-AzaC did not influence rooting in hypocotyl tissue, probably because this tissue is very juvenile. In flower stems, 5-azaC promoted rooting (Fig. 2), especially when it had been added during the generation of the shoots before the rooting treatment (that is during the propagation step). The experiments on methylation were also done with apple shoots growing in vitro and similar results were obtained.

MicroRNAs

More recently, small RNAs (19–24-nucleotide RNAs) have received much attention. In particular, microRNA156 (miR156) has been identified as key component of the genetic control mechanisms that underlie plant phase changes. In the juvenile phase, miR156 is highly expressed and decreases dramatically during vegetative phase change. This small RNA, which is conserved throughout the plant kingdom, controls the expression of transcription factors. An increase of miR156-expression by transformation delays flowering and strongly increases rooting in non-juvenile tissues.

Experiments with lily

Phase change is usually associated with woody plants. It seems, however, that all higher plants have a similar development process from embryonic to flowering. We carried out some experiments with lily. Juvenile lily bulblets sprout with a rosette and adult ones with an elongated stem (Fig. 3). The lily system is very convenient for such study, as the developmental process occurs within a few months in the culture room in tissue culture conditions. The latter facilitates strict control of physical and nutritional conditions.

We observed that the size of the bulblets is decisive for phase change. Large bulblets make the change much more often than small ones (Fig. 3). Moreover, high sugar concentration in the medium promotes and high inorganics inhibit phase change (Fig. 3). With respect to the inorganics, there are strong indications that especially phosphate is crucial. These results were repeated by a Japanese group. They also report that cytokinin and low temperature promote phase change. Lily bulblets regenerating in tissue culture are a very convenient system and should be used for an in-depth study.

Conclusive remarks

From a practical point of view, it is very important to be able to influence phase change. It is essential for the speed of the breeding process and for the rooting in vegetative propagation. Some 30 year ago, knowledge about influencing factors was almost absent. Now, we know a lot more due to studies in arabidopsis and lily. These studies should be extended and the response of other species should be examined.

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