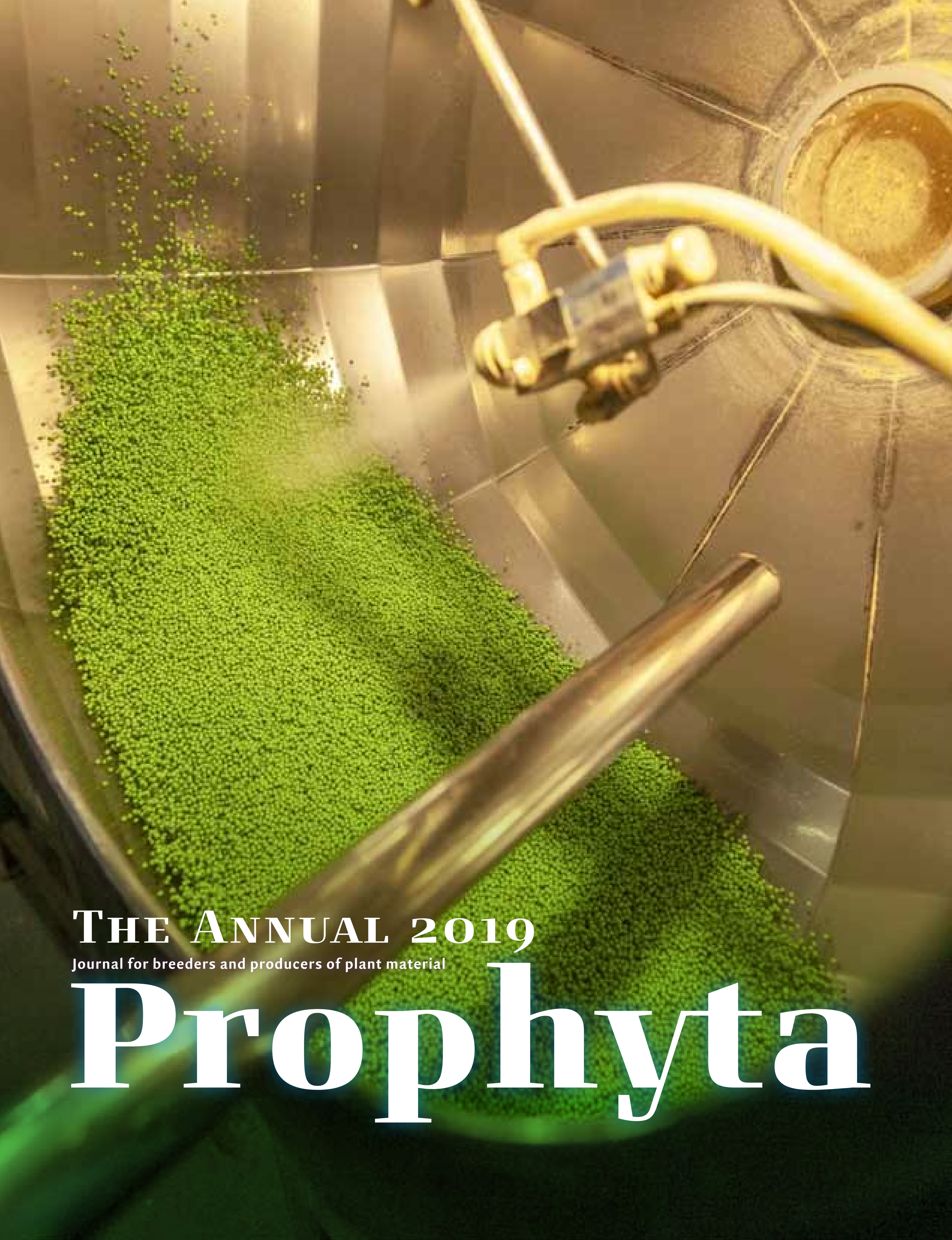

THE ANNUAL 2019

Journal for breeders and producers of plant material

Prophyta

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The Annual 2019

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Prophyta

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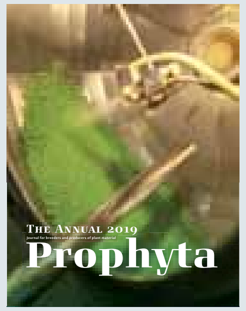

On the cover: Pelleting chicory seeds

Plants are fascinating, don't you think?

There are many stories to tell about what plants do and can do. And we told these stories during the international Fascination of Plants Day. From 16 to 18 May, there was a large greenhouse on the Plein (main square) in The Hague with fascinating stories about plants and seeds. Visitors could come and listen, examine, experience, taste, learn, admire, encounter, discover and feel why plants and seeds are so fascinating and important.
After a sporty and spectacular opening of the greenhouse on Thursday 16 May, four PhD students presented their research during the Science Battle. Who has the best story about his or her plant research? At which presentation does a world open up for you? Do you understand what it is about? Do you feel the passion? Can you retell it?

The many visitors at Fascination of Plants Day were fascinated by the fantastic stories that plants have to tell

During the EU agricultural and horticultural debate on Thursday evening, various Dutch candidate MEPs from the parties VVD, CDA, FvD, SGP, D66 and PvdA were presented with statements from the agricultural and horticultural sector. On the basis of these statements, the parties were able to express their vision of our sector. A great opportunity to find out what the various parties stand for and how they represent our sector within Europe.
The importance of plants must also be passed on to the new generations. That is why, on Friday, schools were invited to visit the greenhouse. In this way, students could see how important plants are to our society and that this sector is not boring at all, but rather innovative and international. On this day, biology teachers received additional training on saline agriculture and salt tolerance. A topic that they can also include in biology lessons in a fun and practical way.

What's in a name?

Three years ago, the EU started a process of clarifying and actualising the list of scientific (Latin) names and common names for vegetable species. The problem was that certain varieties of vegetable species belong to the species listed in Directive 2002/55 under their Latin name, but not to the type/variety group listed under their common names. Variety testing specialists of the Member States have evaluated the list and the EU Commission has now presented a proposal (draft implementing directive) that is indeed improving this situation. It is expected that the proposed list of scientific and common names will be published shortly and that it will be effective from 1st October 2019.
Most of the varieties/species are marketed throughout the whole EU and the changes are therefore relevant to all countries. But the clarification also takes into account that sometimes the marketing of seed and plants of varieties of certain species only has regional or national relevance.
The International Code of Nomenclature for Cultivated Plants (ICNCP) has in the past introduced the concept of 'cultivar groups' to classify varieties of cultivated species. The EU regards this as a suitable instrument and will also use this group approach. The use of proper scientific names followed by cultivar groups (instead of common names) is clarifying the situation considerably.
A third point is that it is also incorporated that inter-specific and intra-specific hybridisation can lead to varieties not belonging to a group. In that case, it is now explicitly regulated that these hybrids also fall under the scope of the vegetable seeds and vegetable plant directive 2002/55 and 2008/72.
Finally, a taxonomical update of scientific botanical names has been done.

Some of the changes are:
- *Allium ascalonicum* is now listed as Allium cepa Aggregatum Group
- *Allium cepa* is replaced by Allium cepa Group
- *Brassica pekinensis* is replaced by Brassica rapa Chinese Cabbage Group
- *Lycopersicon lycopersicum* is replaced by Solanum lycopersicum
- The species *Brassica oleracea* is now divided into nine cultivar groups
- *Chicorium intybus* has three cultivar groups (chicory, leaf chicory and industrial chicory)

Gene bank makes salt tolerant plants available

The gene bank of the International Centre for Biosaline Agriculture (ICBA) will make their genetic material available to breeders worldwide. This gene bank, based in Dubai, UAE, contains over 14,000 accessions of around 240 plant species from more than 150 countries and territories around the world, in addition to around 250 seed samples of 70 wild plant species from the UAE. It is the world's largest collection of genetic material of heat- and salt-tolerant plant species. The move is part of an agreement signed by the Director-General of the Food and Agriculture Organization (FAO) of the United Nations, Dr. José Graziano da Silva, and Director-General of the International Centre for Biosaline Agriculture (ICBA), Dr. Ismahane Elouafi, to expand existing cooperation between the two instituti ons on plant genetic resources, biosaline agriculture and climate change adaptation in the world's marginal environments.
Under Article 15 of the agreement, the crop germplasm collection stored in ICBA's gene bank will officially become part of the Multilateral System of Access and Benefit-sharing. This multilateral system currently comprises over 2.6 million samples of crop germplasm.
Dr. Kent Nnadozie, Secretary of the International Treaty, said the inclusion of ICBA's valuable germplasm collection will make it "more accessible to a broader range of users and ultimately farmers, while affording ICBA new partnerships and involvement in the global governance framework."

Seeds of the golden age

The Sow to Grow museum in Enkhuizen, the Netherlands, has set up an exhibition about the golden age. It was a period in the history of the Dutch Republic, roughly spanning the 17th century, in which trade, science, military, and art were among the most acclaimed in the world. The exposition provides information on ancient vegetables, spices and flowers which were pictured in paintings and cookbooks. In addition, archeological finds from the surroundings of Enkhuizen are displayed, among others, several seeds.

Thank you plants

For politicians, agriculture still harbours many secrets, it seems. Even though she is the daughter of a (dairy) farmer, the Dutch Minister for Agriculture, Carola Schouten, stated that we need a radical change of direction. She stated last April that she no longer wants to focus on discussions about which pesticide should be allowed and which should not. Instead of chemical solutions against pests and pathogens, she wants companies to strengthen plants to be able to withstand diseases and climate changes. Surprise, surprise, that is just what plant breeders have been doing for decades. Hopefully, a visit to the large greenhouse, Plantum, set up close to the parliament building in The Hague, made that clear to her. And firms which supply seed companies support the efforts.
Innovations in plants create great interest in society, as was shown by the huge attention for the international Fascination of Plants Day. It is celebrated in over forty countries worldwide. And objective information works. This January, a group of students from our local grammar school was given a short biology course centred around the potato. At the start, the six graders did not know much more about potato than that it can be food, as well as a snack. They learned about the history of the crop, the problematic diseases, but also about modern production methods and new technological developments, such as CRISPR-Cas. In the beginning, most of the class were quite firm in their opinion that 'genetic manipulation' was a danger. At the end of the course, they had discovered the beneficial aspects of GMOs. However, they made the restriction that no foreign genes should be introduced in a crop.
Last winter, the Dutch Flower Council introduced a clip to promote the use of houseplants by consumers. In 'Thank you plants' (see https://bit.ly/2DyD4md), they praise the contribution plants make to the quality of life. From the oxygen in the air that we breathe, to the mental boost it gives us. Unfortunately, it only promotes ornamentals and not the food, feed, fibre and fuel plants granted to us. Hopefully, the Fascination of Plants Day teaches people that plants are something to be grateful for.

Monique Krinkels

New campaign to stop illegal propagation

Pot and bedding plant breeders, united in Fleuroselect, launch a new campaign against the illegal reproduction of legally protected varieties. Grower controls are executed at random and a tip-off hotline has been created to report suspected illegal reproduction. Within the framework of this annual campaign, Royalty Administration International (RAI) visits growers to check if the number of finished plants matches the quantities of the original cuttings delivered. A whistle blower tip-off hotline has been created in order to report suspicions about illegal reproduction. Anyone active in plant production and distribution is welcome to use this tip-off hotline.

Suspicions about illegal reproduction can be reported in three ways:
By calling our dedicated tip-off hotline on 0031 (0)85 0645251
By sending an email to tips@fleuroselect.com
By completing the dedicated form on the Fleuroselect website.

All tips-offs are considered highly confidential; the name of the person providing the tip-off is never revealed. If upon inspection, accompanied by the relevant delivery information, it appears that the reported potential offender has indeed reproduced material illegally, then they will be charged the royalty fees plus a fine. Karol Pawlak, Fleuroselect President and owner of Vitroflora: “Growers respecting intellectual property already welcome the campaign as it creates a fair playing field. Information activities to raise awareness among international distributors and retailers are scheduled.”

RAI carries out a visit to the reported offender as part of the regular campaign. RAI is entitled to visit the production location or nursery at any time

Land of Grapes, Land of Wines

How France gave the world its **noblest drink**

John van Ruiten

Ask any person what France is world famous for and 10 to 1 you will get the answer: 'wine'. But how did this so well-known product, the tradition of grapevine growing and wine production came into this country? And, interesting for breeders and horticulturists, how did grapevine varieties develop?

In the old times - long before the ice ages 12 millennia ago and long before mankind started with agriculture and domestication of crops - wild grapes were growing everywhere in Europe. During the ice ages grapevines were pushed back to minor Asia, close to the region that we know as the fertile crescent (one of the cradles of agriculture). Based on archaeological findings, historians believe that around 5000 BC the fundament of cultivation of grapevines and the production and storage of wine took place in the region that we know as Georgia (on the south side of the Caucasus mountains).The 'oldest winery of the world' Areni was excavated in Armenia quite recently (2007). The archaeologists unearthed an old wine press, fermentation vats, storage jars, potteries and even labels.

Domestication

Wild populations of *Vitis vinifera*, the most widely used *Vitis* species for wine production, can be found in the Zagros Mountains of North Iran. The domestication process of grapevines probably started there. Wild species of grapes were mainly dioecious, whereas nowadays cultivated grapes are monoecious. It is thought that at that time seed propagation was the method of propagation. And that has led to an enormous wide diversity of cultivated grapes. Hybrids can be created with all 60 worldwide known *Vitis* species; they are all crossable. In Babylon (2200 BC) already 'wine legislation' was made.

In ancient Egypt, according to findings in the pyramids, around 2500 BC in the Nile valley grapes were also grown and used to make wine with. From the Caucasus area grapevine plants travelled eastwards to Persia, India and China, but not in an extent as compared to the movement westwards. From the very beginning, wine was not just a liquid to drink. It has always been closely connected with religion, ceremonies, culture, art and festivals. The drink of the gods (Egypt Osiris; Greek Dionysos, Roman Bacchus). A sacral drink. In the bible, drinking wine is positively valued. The blood of Christ.

In Europe the Greeks were the first ones to grow grapes along the Mediterranean coast, and they brought wines and plants to their colonies, widespread around the Mediterranean Sea (Sicily, Italy, Cartago, Spain and Massilia (now Marseille/Provence). They used the technique of vegetative production and grafting plants. It is assumed that in the 6th century BC producing and selling wine was already an important source of income/trade product from Massilia. The Romans, in later times, spread grape cultivation everywhere in their vast empire. So even in northern parts of their occupied territory (Britannia, The low countries) grapes for wine production arrived and were grown there for a number of centuries. The classic and still important European wine regions in Bordeaux, Languedoc but also in Romania, date back to that Roman era.

Also the characteristic tastes and types of some wines, like the use of honey, resin (inhibiting the growth of vinegar bacteria) and spices, originate from that time. Basically because hygiene and conservation/sterilization methods were not to a knowledge level that allowed a long life, these additions were necessary to make acceptable wines that could be transported and stored for some time.

Monks

At the end of the Roman Empire until the early middle ages, grapevine cultivation decreased. It mainly survived only in cloisters and abbeys. It were monks that produced wines that was drunk by the clergy and the aristocrats. The ordinary people had beer as the regular drink. It was Charles the Great (around 800 AD) who brought grape cultivation back on a larger scale in Europe. Great wine regions in Burgundy and Germany developed. It is estimated that hundreds of years later (around 1400/1500 AD) the amount of hectares of vineyards in Germany was three times bigger than it is today.

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The great wines that we know now are not made with the varieties that arrived in France long ago

France produces around 8 billion bottles of wine a year. Global production of wine is estimated to an amount of 3 billion hectolitre

The Pinot Noir grape dates back to the 14th century in Burgundy, and many (at least over 60 clones/mutants) have been derived from this variety ever since.

In 1600, the golden age of the Dutch, they marketed wine around the globe and a huge quantities of white wine were distilled to make brandy ('brandewijn'). They also brought wine cultivation to various parts of the world like South Africa (in 1655).

It is probably in Cistercian monasteries in France that selection and development of varieties started to play a more important role in wine production from 1100 AD onwards. If we look at the most important varieties (variety groups), they all were created in the 16th/17th century: the blue cabernet sauvignon (a crossing of older varieties cabernet franc and sauvignon blanc) is surely the most important one, followed by blue merlot and the white chardonnay (a crossing of pinot and gouais blanc. The latter is a 13th century variety created in the Burgundy area. DNA-research has shown that this variety has more than 80 offspring, hence the nickname Casanova among the grapes.

Devastating diseases

A very important period for grapevine cultivation development and innovation was the second half of the nineteenth century. It was the time of big devastating diseases in mainly European wine growing areas. Both heavy infestations with powdery mildew (*Oidium*) in 1847 and followed by the great French wine blight caused by the grape aphid (*Phylloxera*) from 1865 devastated the wine production in France. On top of that downy mildew (Peronospora) arrived around 1880. These three diseases were called 'the American plagues' as they were introduced from the USA (probably through imported material via England). And they all three spread at lightning speed throughout Europe.

One of the solutions for overcoming these diseases was found at the end of the 19th century. Grafting old noble varieties on resistant rootstocks, originating from the USA, turned out to be very successful. This idea was both developed in France (Jules Planchon, a botanist from Montpellier) and in the USA (Charles Riley, an entomologist from Missouri). Species such as *Vitis riparia*, *V. rotundifolia*, *V. labrusca* and *V. rupestris*) were used to create these resistant rootstocks.

Thomas Munson, a breeder from Texas, was one of the pioneers in the creation of resistant varieties. So this technique did not only save grape cultivation, it also saved many old (although not all) traditional 'noble' varieties with their characteristic taste. Nowadays, in legislation in wine producing countries in the EU and the USA, the use of resistant rootstocks is obligatory. And the use of copper to combat mildew is used till today.

Resistance

In the last 40 years it is resistance breeding that has created new grapevine varieties that have a high resistance to mildew. And because of that resistance, the leaves of plants of those varieties stay green longer on the plants, making the grapes on those plants ripening longer and making a higher sugar content possible. These varieties can be grown on more northern latitudes. so it can be seen that that gives an impulse to grapevine cultivation in countries like UK, the Netherlands, Northern Germany and Denmark.

Tomato Brown Rugose Fruit Virus

ISHI-veg develops new, specific **detection** method

ISHI-Veg

ISHI-veg's test for *Tobamoviruses* in tomato and capsicum is in use by the seed industry for many years and it detects all *Tobamoviruses*, including ToBRFV. However, to facilitate trade a new test that provides an immediate conclusion on the identity of ToBRFV, if present in the seed lot, has now been developed.

Tomato and capsicum (fresh and chilli peppers) are grown worldwide and are among the world's most consumed vegetables. According to FAO global production of tomatoes in 2017 was 182 million tons and another 36 million tons of capsicum. Asia has the highest production share of tomatoes by region at 61.1%, followed by Europe (13.5%) and the Americas (13.4%)(1). It is estimated that the market value of tomato fruits is roughly US$ 510 billion.

The *Tobamovirus* genus comprises multiple economically important and contagious pathogens that infect solanaceous crops. They are considered to be the most stable and infectious viruses known, and are readily transmitted mechanically by workers, tools and equipment during plant handling. *Tobamoviruses* can also be spread via fruits and insects. They survive in soil, water and infested debris from previous crops. They can be seed borne, however, infection and spread most likely occurs mechanically.

To protect their investment in fresh produce production, producers and plant raisers have access to a toolbox of mitigation measures, such as clean seed, resistant varieties and hygiene measures during crop growth. The use of resistant varieties has been the most effective control strategy in last decades for growers. Non-resistant varieties when accompanied with strict hygiene management have also allowed growers to produce healthy solanaceous crops.

The seed industry plays an important role in providing resistant varieties and healthy seed for the start of a clean and profitable fresh produce production cycle. As incorporation of disease resistance genes in new vegetable varieties is a long multiyear process, the industry uses more direct mitigation measures at every stage of seed production until shipping to end user, to mitigate the effects of seed borne diseases. Measures include application of hygiene protocols, field and crop inspection, seed health testing and (preventive) seed sanitation treatments.

A new virus

Tomato brown rugose fruit virus (ToBRFV) is a new *Tobamovirus* isolated from tomato plants grown in greenhouses in Jordan in 2015 (2). An outbreak of a new disease infecting resistant tomato cultivars grown in net houses observed in 2014 in Southern Israel was caused by an Israeli isolate of ToBRFV that had a high genomic sequence identity to the Jordan isolate. More recently, ToBRFV was detected in tomato plants in production fields in Mexico, Germany, USA and Italy according to EPPO(3) but wider spread is likely.

Tobamoviruses infecting tomato crops are of great concern in general, but ToBRFV outbreaks are particularly worrisome because of its ability to overcome resistance rendered by the Tm-2^2 gene.

Disease symptoms on tomato include chlorosis, mosaic and mottling accompanied occasionally by narrowing of leaves and yellow spotted rugose fruit, making fruit unmarketable. Fruit may also mature irregularly. On susceptible capsicum, EPPO describes symptoms including foliar deformation, yellowing and mosaic while fruits are deformed with yellow or-brown areas, or green stripes.

Table 1a. Sequences of ToBRFV-specific and the Positive Extraction Control (PEC) primers

Primer target	Primer Name	Sequence
ToBRFV	CaTa28-Fw	5' – GGT GGT GTC AGT GTC TGT TT – 3'
ToBRFV	CaTa28-Probe	5' – 6FAM – AGA GAA TGG AGA GAG CGG ACG AGG – BHQ1 – 3'
ToBRFV	CaTa28-Rv	5' – GCG TCC TTG GTA GTG ATG TT – 3'
ToBRFV	CSP1325-Fw	5' – CAT TTG AAA GTG CAT CCG GTT T – 3'
ToBRFV	CSP1325-Probe	5' – VIC – ATG GTC CTC TGC ACC TGC ATC TTG AGA – BHQ1 – 3'
ToBRFV	CSP1325-Rv	5' – GTA CCA CGT GTG TTT GCA GAC A – 3'
PEC	BaCV-Fw (PEC)	5' – CGA TGG GAA TTC ACT TTC GT – 3'
PEC	BaCV-Rv (PEC)	5' – AAT CCA CAT CGC ACA CAA GA – 3'
PEC	BaCV-Probe (PEC)	5' – TxR – CAA TCC TCA CAT GAT GAG ATG CCG – BHQ2 – 3'

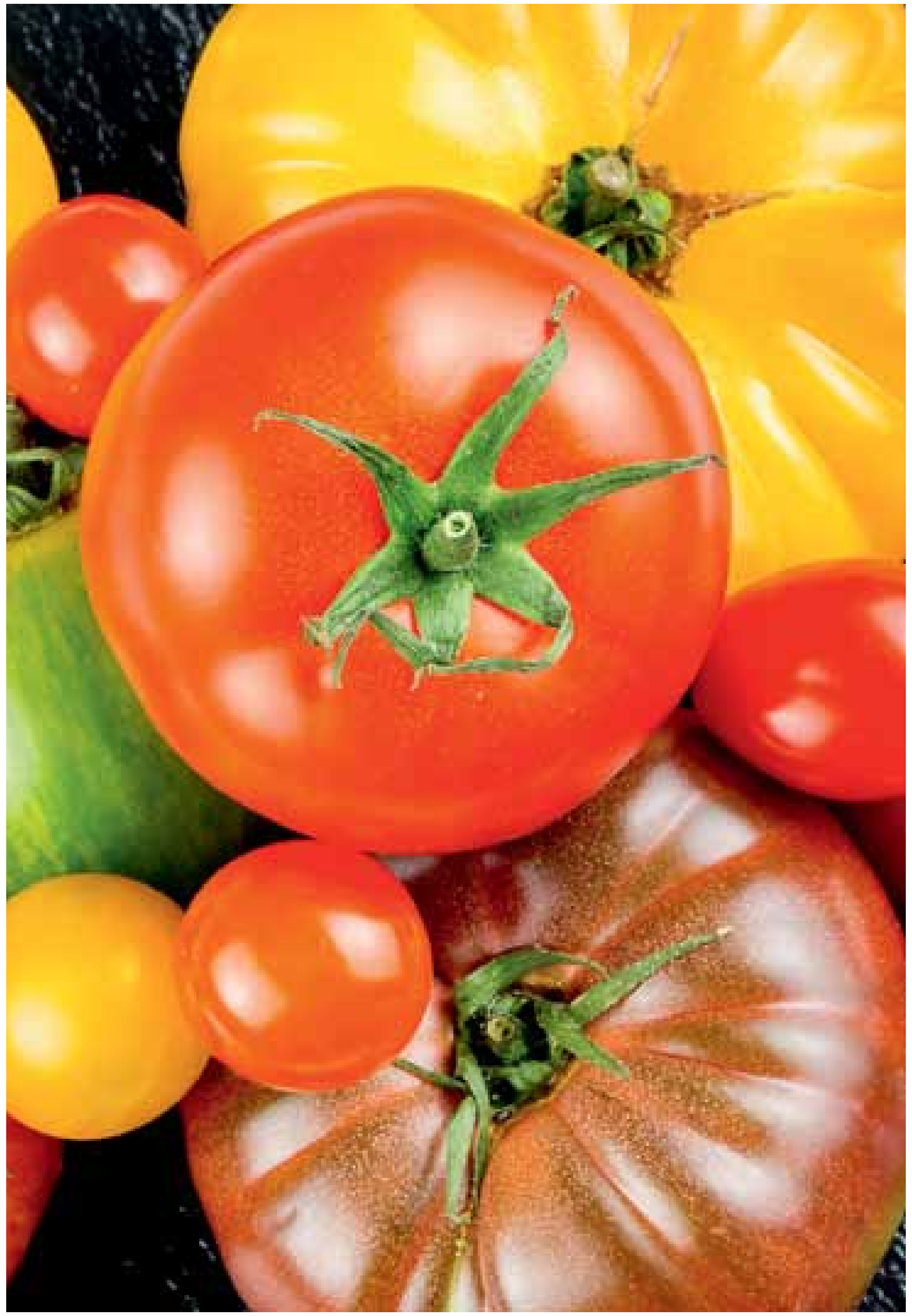


Table 1b. Thermal cycler run conditions for the amplification of RNA-fragments of ToBRFV

RT reaction	10 min 50°C
Denaturation	180 sec 95°C
Cycling	10 sec 95°C 60 sec 60°C 40x

Once the virus is introduced in an area, infected plants should be eliminated and strict hygiene protocols implemented. Inoculation experiments show that besides its main hosts, tomato and non-resistant capsicum, *Nicotiana benthamiana*, *N. glutinosa*, *N. sylvestris* and some *N. tabacum* (tobacco) were susceptible and developed initially necrotic lesions and later on mosaic symptoms. Weeds such as *Chenopodium murale* and *Solanum nigrum* may act as reservoirs for ToBRFV. Eggplant and potato did not show symptoms after inoculation of the virus and ToBRFV was not detected when the plants were subsequently tested by ELISA(4).

Call for cooperation

ISHI-Veg invites researchers seeking to develop ToBRFV-specific detection methods to use its primer sets and harmonise methods used for phytosanitary certification.

Seed health test for Tobamoviruses

An outbreak of disease caused by PMMOV in Spain in 1992 in capsicum and the need to resolve the issue of diverging seed health test results based on different types of assays used by different labs led to establishment of the International Seed Health Initiative for Vegetable Crops (ISHI-veg). It is an industry led platform for development of seed health test methods (5).

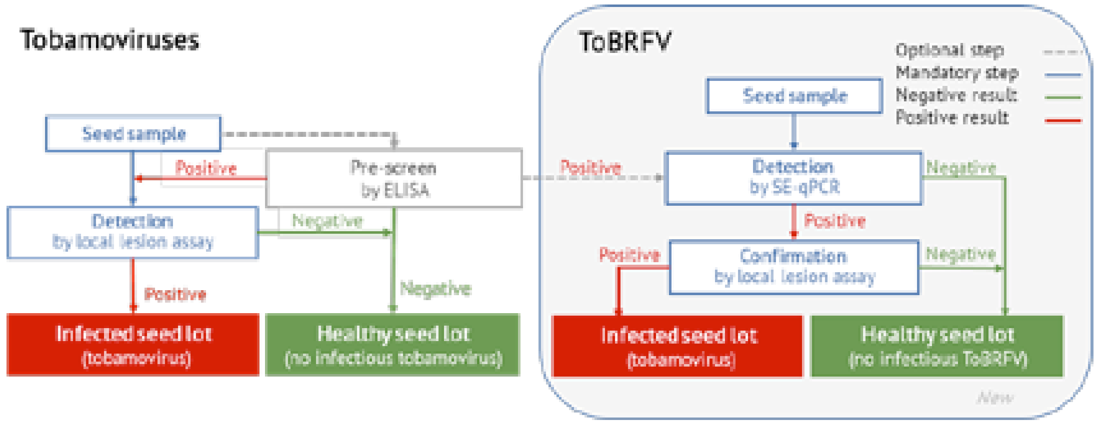
The current ISHI-veg method for detecting *Tobamoviruses* in tomato seed, an industry standard, is a local lesion assay that provides conclusive evidence of presence of viable and infectious *Tobamoviruses*, in other words a 'direct' test (6). In the assay, leaves of indicator plant species *Nicotiana tabacum* cv. Xanthi NN or *Nicotiana glutinosa* are inoculated with an extract from seed of solanaceous crops. If the seed extract contains infectious virus they provoke development of small, necrotic local lesions on tobacco leaves. These clear necrotic lesions are typical for *Tobamoviruses*. For higher throughput, an ELISA can be used to pre-screen seed lots; a negative test result indicates that the tested seed lot is free from *Tobamoviruses*.

An ELISA detects proteins that are specific to the target pathogen but does not demonstrate the presence of infectious virus. An ELISA, for instance, may also give a positive result when disinfected seed is tested. Inactivated virus fragments may still be present on or in seed. ELISA is, therefore, considered an 'indirect' test and is used as pre-screen; a positive ELISA result is confirmed by a direct test – the local lesion assay – to confirm viability and pathogenicity of *Tobamoviruses* (see ISF's Viewpoint on Indirect Seed Health Tests(7)).

Detection of ToBRFV

ISHI-veg's local lesion assay for *Tobamoviruses* in

Seed health test flow for detection of tobamoviruses and ToBRFV



Seed health test flow for detection of Tobamoviruses and ToBRFV

tomato and capsicum is in use by the seed industry for many years, and found to be fit for purpose as it detects all *Tobamoviruses*, including ToBRFV. It does not, however, provide information about the specific *Tobamovirus(es)* that caused local lesions on the leaves of indicator plants. This is no problem for seed trade as a negative test, i.e. no lesions on leaves of the indicator plants, indicates no infectious virus present in the seed lot and that it can be traded safely.

Several countries, such as Mexico, Turkey, South Korea and Australia, have put in place import restrictions on tomato, capsicum and eggplant seed to contain or prevent the spread of ToBRFV through infected seeds. These import requirements vary from country to country with some accepting results obtained with the local lesion assay with ELISA as a pre-screen, but others require ToBRFV-specific PCR tests.

To facilitate trade, a ToBRFV-specific PCR has now been added to ISHI-Veg's current method. It can be used to identify ToBRFV after a positive ELISA or to detect ToBRFV by running the PCR test directly on a seed extract. For positive results from the ELISA and TaqMan tests, the local lesion assay should be run to confirm viability and pathogenicity of tobamovirus in the seed lot. The final conclusion on the status of the seed lot is based on the result of the local lesion assay (see the flow chart).

The advantage of the TaqMan PCR is that it provides an immediate conclusion on the identity of the virus present. In addition the test is a 'one-tube assay' which reduces not only labour costs but also the risk of cross contamination. Cross contamination is of serious concern in molecular tests when run using a multi-tube system; PCR tests are very sensitive and are able to react positively to miniscule aerosol particles containing ToBRFV present in the lab. Such false positive results may lead to rejected seed lots even though no infectious virus is present.

TaqMan PCR also allows multiplex assays to be developed for simultaneously detecting viroids and other viruses, like specific *Tobamoviruses* and Pepino Mosaic Virus. ISHI-veg is already engaged in such a project.

ToBRFV TaqMan PCR test

Primers are an important feature of PCR assays for identification and detection, as they are designed on specific genetic targets. ToBRFV-specific primer sequences developed by ISHI-veg are presented in Table 1. Within *Tobamoviruses* there is genetic variability (8) that may cause false negative results. To prevent that, ISHI-veg always aims to have at least two primer sets based on different areas of the pathogen genome in molecular assays.

Another essential component in PCR assays on seed extract is the ability to verify proper execution of the test. This is done by adding a known amount of another virus to the seed extract, the so-called Positive Extraction Control (PEC). The use of a PEC is essential and the user is free to choose any PEC provided it is shown that the one selected does not influence test results. ISHI-veg used a sequence from *Bacopa chlorosis virus* (BaCV) but other PECs such as *Squash mosaic virus* (SqMV) and *Dahlia latent viroid* (DLVd) have also been used and are compatible with the ToBRFV-specific primers.

Table 2. Specificity of ToBRFV TaqMan PCR in combination with different PEC tested on several plant species (*source: ISHI-Veg members*)

Virus tested	Specific-PCR		PEC-PCR	
	CSP1325	CaTa28	BacV / DLVd / SqMV with *Tobamo virus*	BacV / DLVd / SqMV with PEC spike
ToBRFV	Detected	Detected	No cross reaction	PEC detected
ToMMV	Not detected	Not detected	No cross reaction	PEC detected
TMV	Not detected	Not detected	No cross reaction	PEC detected
BePMV	Not detected	Not detected	No cross reaction	PEC detected
TMGMV	Not detected	Not detected	No cross reaction	PEC detected
ToMV	Not detected	Not detected	No cross reaction	PEC detected
PMMoV	Not detected	Not detected	No cross reaction	PEC detected
PaMMV	Not detected	Not detected	No cross reaction	PEC detected
TSAMV	Not detected	Not detected	No cross reaction	PEC detected
CGMMV	Not detected	Not detected	No cross reaction	PEC detected
TSWV	Not detected	Not detected	No cross reaction	PEC detected
PepMV	Not detected	Not detected	No cross reaction	PEC detected
Negative Control tomato	Not detected	Not detected	Not applicable	PEC detected
Neg. Control capsicum	Not detected	Not detected	Not applicable	PEC detected
Neg. Control N. glutinosa	Not detected	Not detected	Not applicable	PEC detected
Neg. Control N. bentamiana	Not detected	Not detected	Not applicable	PEC detected

Photo 2a and 2b: ToBRFV infection of tomato: Mild mosaic on leaves and uneven ripening and necrotic lesions on fruit (Courtesy of David Levy, Hazera seed, Ltd)

Before ToBRFV-specific primers can be used in routine testing, it is essential to verify that they give reliable and consistent results when detecting ToBRFV. They should give a positive reaction with the target pathogen but not with, for example, closely related non-pathogenic organisms, in other words the test should not give false positive results. False negative results are even more worrisome as infected or contaminated seed may potentially cause a disease outbreak. To minimize the risk of false negative results during testing, pathogen isolates collected from different geographical regions, time periods and hosts are tested to verify if primers detect the complete range of known target isolates. Forty-five different virus strains tested showed no cross reactivity and ToBRFV strains were always detected. The specificity of the ToBRFV primers was thus demonstrated (see Table 2)

Validation

As leaf and seed components are known to interfere with PCR reactions or degrade target material, the assay was tested on tomato, capsicum and tobacco leaves and on seeds of tomato and capsicum. Leaves of ToBRFV-infected tomato and tobacco plants tested positive and non-infected leaf material gave a negative result. For capsicum no infected leaf was available but in tests on non-infected leaves a negative result was obtained.

To test suitability of the PCR test on seeds, tests were carried out in multiple labs. Tomato and capsicum seed extracts were subjected to RNA extraction on the ELISA extract (250 seeds). ToBRFV was detected in ToBRFV infected tomato seeds. As no ToBRFV-infected capsicum seeds were available, infected tomato leaf material was added to healthy seed extracts. The PCR test was as sensitive as the ELISA on all the positive seed extracts showing suitability of the PCR assay for identification and detection of ToBRFV.

The local lesion assay is sufficiently sensitive to

Photo 3a and 3b: Lesions on a tobacco leaf inoculated with ToBRFV-infected tomato leaf (left) and Tobamovirus-infected seed extract (right) (Courtesy of Gerbert Hiddink, Enza Zaden)

Table 3. ToBRFV TaqMan PCR assay on a sample of one ToBRFV infected seed in 999 healthy tomato seed
(source: Naktuinbouw)

Sample and controls	Triplex ToBRVF + BaCV PCR		
	ToBRFV-CSP1325	ToBRFV-CaTa28	BaCV
1	Detected	Detected	Detected
2	Detected	Detected	Detected
3	Detected	Detected	Detected
4	Detected	Detected	Detected
5	Detected	Detected	Detected
6	Detected	Detected	Detected
7	Detected	Detected	Detected
8	Detected	Detected	Detected
Positive Isolation Control	Detected	Detected	Detected
Negative Control Seed	Not detected	Not detected	Detected
Buffer control	Not detected	Not detected	Detected
Negative Process Control	Not detected	Not detected	Not detected
Positive Amplification Control	Detected	Detected	Detected

prevent disease outbreaks from infected seeds in the past two to three decades. The ToBRFV TaqMan PCR has been developed to be more sensitive than the local lesion assay to exclude any false negative test results from the TaqMan PCR assay. This is in line with ISHI-Veg's technical paper on real-time PCR pre-screening (see 9).

There are studies ongoing on the sensitivity of the PCR to enable a bigger subsamples size, such as 1,000 seeds instead of 250 (see Table 3).

Summary

A new *Tobamovirus*, ToBRFV, has recently become a threat to the tomato industry, as it overcomes the Tm-2^2 resistance in tomato. *Tobamoviruses* have been a burden for growers and seed industry alike for decades. Easy mechanical transmission of the virus is a threat to modern fresh produce farming. Good and appropriate management of the disease has contributed greatly to current levels of production. The seed industry has had a leading role in the management of these *Tobamoviruses* through the development of resistant varieties and seed health testing.

The fact that there have been no outbreaks of *Tobamoviruses* in tomato, capsicum and eggplant linked to a failure of the seed health test, shows that the current method is fit for purpose and disease management strategies are effective. Like other *Tobamoviruses* ToBRFV can also be managed by carrying out the correct seed health tests and implementing strict hygiene management.

ISHI-veg aims to secure the delivery of sufficiently healthy seed to customers by developing methods for seed health testing that are internationally recognized as reference methods and accepted as industry standards. The current test for Tobamoviruses, a local lesion assay in combination with pre-screen ELISA on 3000 seeds, is able to detect the new Tobamovirus ToBRFV.

To facilitate the international movement of seed and help companies comply with import requirements for ToBRFV, ISHI-veg has developed a new method for specifically detecting ToBRFV. This TaqMan assay subjected to a seed extract can be used either to detect seed lots for ToBRFV or to identify ToBRFV after a positive ELISA.

References

1. FAO (2019). http://www.fao.org/faostat/en/#data/QC/visualize
2. EPPO (2019). https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_viruses/tomato_brown_rugose_fruit_virus
3. Sela, N., Abu-Ras, A. and Ezra, N. (2017). A new Israeli Tobamovirus isolate infects tomato plants harboring Tm-22 resistance genes. PloS one, 12 (1), p.e0170429. Salem, N., Mansour, A., Ciuffo, M., Falk, B.W. and Turina, M. (2016). A new Tobamovirus infecting tomato crops in Jordan. Archives of virology, 161 (2), pp. 503-506. doi: 10.1007/s00705-015-2677-7.
4. Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., Elad, N., Tam, Y., Salem, N., Mansour, A., Ciuffo, M., Falk, B.W. and Turina, M. (2016). A new Tobamovirus infecting tomato crops in Jordan. Archives of virology, 161 (2), pp. 503-506. doi: 10.1007/s00705-015-2677-7.
5. ISF (2019). https://www.worldseed.org/our-work/phytosanitary-matters/seed-health/.
6. ISF (2019). https://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg-protocols/.
7. ISF (2019). http://www.worldseed.org/wp-content/uploads/2015/10/Indirect_Seed_Health_Tests_2013.pdf.
8. Maayan, Y., Pandaranayaka, E.P.J., Srivastava, D.A., Lapidot, M., Levin, I., Dombrovsky, A. and Harel, A. (2018). Using genomic analysis to identify tomato Tm2 resistance breaking mutations and their underlying evolutionary path in a new and emerging Tobamovirus. Archives of virology, 163 (7), pp. 1863-1875. doi: 10.1007/s00705-018-3819-5.
9. ISF (2019). http://www.worldseed.org/wp-content/uploads/2018/03/Real-time_PCR_pre-screens_2018.pdf.

On the brink of PHR-day

John van Ruiten

The date of 14 December 2019 is rapidly approaching. This 'PHR-day' is the coming date in the European Union that the new phytosanitary legislation will come into force. This change will have great impact on both EU companies and on companies exporting seeds and planting materials to the Union. In this article the major changes that are going to occur are described.

After more than 7 years of preparation, the EU decided in 2016 to replace the existing Phytosanitary Directive (2000/29) on pathogens and plant passports with a new Plant Health Regulation (2016/2031).

There were a number of reasons for the Union to decide that the phytosanitary policy had to change. The most important one was that the EU, after a series of new outbreaks of plant pests and pathogens in the last 20 years, concluded that the existing rules and the system of import inspections were not providing enough certainty that not yet occurring pests and plagues could be kept out as intended.

Too many pathogens

As became clear from the evaluation, one of the reasons for that was that the focus of phytosanitary authorities was not specifically set on a number of threatening pests, but that there were too many pathogens that had to be taken into consideration and had to be inspected/tested/supervised. Including a great number of quarantine diseases that were relevant in the past, but that nowadays occur on a wider scale in various parts of the Union. In fact, the long list of quarantine pathogens was fundamentally still the same as it was back in 1977, when the first EU directive was created.

Table 1. Priority pests

pathogen	common name	crop
Xylella fastidiosa	olive die back	many species
Bactericera cockerelli	vector zebrachip disease	potato
Ralstonia solanacearum	brown rot	various crops
Candidatus liberibacter	citrus greening	citrus spp
Agrilus planipennis	emerald ash borer	wide host range
Popilla japonica	Japanese beetle	wide host range
Thrips palmi	melon thrips	very wide host range
Thaumatotibia leucotreta	false codling moth	wide host range

A second reason is that the Union with 28 Member States showed considerable differences between both national legislation and the implemented inspection approaches of 28 competent authorities. Through harmonization of procedures, more prescribed inspection/testing protocols, standardization of documents (plant passports) and more intensive monitoring and supervision of the EU Commission, the Union wants to overcome this issue. A Regulation is a piece of legislation that is directly binding in the whole EU territory. And at the same time, a new electronic management information system is being introduced (IMSOC/TRACES) that will help to keep much better track of materials imported and marketed.

Quarantine pests

Probably the biggest change in legislation is the separation of current quarantine pests into two categories: Quarantine pests (QP) and so-called Regulated Non-Quarantine Pests (RNQP). The basic difference is that QP diseases are absent in the Union or have a very limited presence and eradication programmes are active. In the category RNQP, a huge number of pests and diseases are taken up that can be or are mainly spread with seeds and/or plants for planting and that occur frequently or sometimes, even widespread in the Union. "Their presence on the seeds/plants would have an unacceptable economic impact and should therefore be prevented," according to the EU Commission

Within the category Quarantine pests, there are so-called Priority Q pests, other Q pests and Zona Protecta Q pest, for which it has been identified that they are not present in certain regions of the Union. Irrespective of the way that testing was or can be transmitted, these Q pests have an absolute zero tolerance and immediate official measures have to be taken if their presence is recorded. Also, immediate notification to the whole EU and other Member States has to take place. A list of a number of important Priority pests can be seen in table 1. The final list will be published in September 2019.

Pragmatic approach

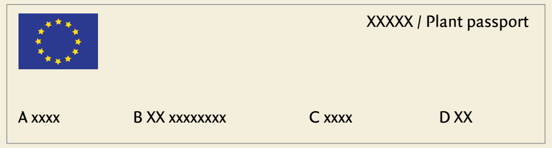
Within the category RNQP, a very long list of harmful pathogens, that can be spread via propagating material and plants, has been compiled. By means of EPPO expert groups and through consultation processes with authorities in EU Member States, it has been assessed whether the spread of these diseases can be controlled by regulatory measures and inspection/marketing requirements. Although the definite list of RNQPs still has to be finalized and officialised in the next months, it can be seen that many former day quarantine pathogens and many important quality pathogens (that used to be regulated through the system of Marketing Directives in the Union) are on the list.

In general, the rule or requirement will be that the marketed seeds and plants must be completely free of those pathogens at the time of marketing. But during production, methods of roguing and/or cleaning up can be applied, as long as it leads to a situation in which the material that is going to be marketed is free of the pathogen. For these RNQPs, there will not be a policy that the company or production location or immediate vicinity have to be free of the pests. So to speak: a more pragmatic approach, in which strict hygiene and testing and inspections are still necessary, is going to be in place for RNQPs. The risk of spreading (compared with Q pathogens) is however somewhat larger, but that is accepted. Some examples of RNQP pests are mentioned in table 2.

Table 2. Important diseases in the RNQP list

pathogen	crop
BACTERIAL DISEASES	
Xanthomonas spp	tomato, pepper, strawberry, beans
Erwinia amylovora (fireblight)	woody host plants
Clavibacter michiganensis	tomato
VIRUS/VIROID DISEASES	
Plum pox virus	plums
Tomato spotted wilt virus	many crops
Potato spindle tuber viroid	potato, tomato, pepper
Chrysanthemum stunt viroid	chrysanthemum
Pepino mosaic virus	tomato
PHYTOPLASMA DISEASES	
Apple proliferation	*Malus*
Pear decline	*Pyrus*
European stone fruit yellows	*Prunus*
FUNGAL DISEASES	
Plasmopara halstedii	sunflower
Puccinia horiana (white rust)	chrysanthemum
INSECTS	
Opogona sacchari	banana moth
Red palm weevil	many palm species
Bemisia tabaci	many pot plants and vegetable crops
NEMATODES	
Ditylenchus dipsaci (stem nematodes)	wide host range

New plant passport / layout



- For trade within EU
- Regulation 2017 / 2313 (details plant passport
- A = botanical name
  B = country code – official registration number (of operator)
  C = traceability code (by companies)
  D = country of origin (in EU or 3rd country)

Another very important change is the further implementation of a system of import bans. A list of so-called high-risk species has been created, with species of which the importation on plants will be forbidden in the Union, unless the exporting country has obtained permission from the EU to export plants. It is up to the exporting country to file a PRA to the EU first. This detailed PRA will be examined by EFSA and if advised as positive, the EU can decide to grant permission.

The import ban will also start on 14 December, so the first requests from third countries to have the opportunity to continue exports to the EU are at this moment being evaluated. It is not yet clear how long this process will take and how strict the requirements will be. Seeds and tissue-cultured plants are excluded from this import prohibition. The list of relevant species (among them are important crops such as *Acer*, *Ficus carica*, *Malus*, *Nerium*, *Persea*, and *Pyrus*) can be found in EU Regulation 2018/2019.

The third item for attention coming from the new Regulation is the introduction of new uniform plant passports in the EU. Since 1993, the system of plant passports has been in operation, and the evaluation has shown that it is working quite well. There are, however, two points that will be changed. Firstly, a new harmonized layout of passports will be introduced. All passports (attached to the smallest unit of marketing - bag, box or bundle) have the same layout, as shown in the example. The blue EU flag, the botanical name, the registration number of the company, a traceability code and the country of production/origin (in or outside the EU) have to be put on the passport.

The number of species for which a passport has to be issued (by or under the authorization of the competent authority) is increasing. Basically, all vegetatively propagated species and a number of seed propagated species has to be plant-passported. Also, pot plants and bedding plants and all other rooted plants marketed professionally (also if sold through garden centres) must be passported. Professional companies should keep records of buying and selling of passported plants/seeds on their records for three years.

Health standards

In the coming 6 months, the Union will decide on the last number of implementing Regulations that will together form the set of rules that will be applicable with effect from 14 December. In the Netherlands, Naktuinbouw and other official inspection bodies are very active in preparing companies for the new rules to enable them to market inspected material from that date with the correct documents. Thus, they can make clear to their customers that the material fulfils all relevant health standards (as plant-passporting is combined with certification programmes).

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Polyploid breeding in the 21st century

Peter Bourke and René Smulders

Even though there are important crops among the polyploids, such as potato, wheat, cotton, coffee, sugarcane, rose and strawberry, they have remained relatively unaffected by the application of genotyping technologies. Until recently that is, because a public-private partnership has started work on the use of genotyping technologies in polyploid breeding.

The basis of all breeding work is selection – choosing plants with improved characteristics for further propagation and discarding those that do not make the grade. In the past, selection was for physical attributes only (what we now term 'phenotyping') – be it the size of a plant, the colour of its flowers, or the sweetness of its fruit. Although phenotypes are the ultimate proof of a new variety's performance, they can be costly and time-consuming to score, and may involve phytosanitary growth conditions if screening for certain quarantine pathogens.

Parallel to the development of better and smarter phenotyping technologies (like image analysis software or robotics), are the so-called 'genotyping' technologies that characterise a plant's DNA using molecular markers. In one application, these markers can be used to directly substitute a phenotypic measurement and they are becoming increasingly embedded in modern breeding programmes.

Polyploids

Not so for polyploid crops, at least not until recently. Polyploids are a large and diverse group of organisms that are characterised by having more than two copies of each chromosome. Well-known crops like potato, wheat, cotton, coffee, sugarcane, rose and strawberry all fall under the heading of 'polyploid', and many more besides. Despite their agricultural and economic importance, polyploids have remained relatively unaffected by the application of genotyping technologies, not least because of the difficulties encountered when using genotyping technologies on material with multiple chromosomes. It is also far more complicated to make sense of the data that is generated because the plants may harbour multiple functional alleles (gene variants that may have different effects), while the number of copies of an allele may also affect the phenotype.

To address this issue, a group of researchers led by Plant Breeding, Wageningen University & Research, have been working collaboratively on the use of genotyping technologies in polyploid breeding as part of a public-private partnership (http://tinyurl.com/yyb9gre3).

The first step in correctly interpreting DNA marker information in polyploids involves the estimation of marker dosage, which are the counts of different DNA marker alleles, most often SNPs, carried by an individual. In a diploid, a marker may be absent (0 copies), present in heterozygous form (1) or homozygously (2). In a tetraploid, these counts range from 0 to 4, meaning that there are multiple possible heterozygous classes to be distinguished. Separating these different classes is a key challenge in polyploid genotyping, and this process can easily be affected by noise or bias in the data. The 'fitTetra' package in R was developed to perform this task in tetraploids; this has since been updated to 'fitPoly' which can estimate dosage scores across all possible ploidy levels. Both packages are open-source.

Genetic analysis

Once marker data has been generated and scored, it can be used in the genetic analysis of agricultural traits, helping to identify markers that are located close to genes of interest for breeders. One of the key bridges between markers and traits in the past have been genetic maps (also called linkage maps), allowing a breeder to plot the location of genes, rather than selecting on markers alone. Up until recently, there was only a single software available ('TetraploidMap') to produce these maps (developed by researchers from BioSS at the James Hutton Institute in Scotland), but it was restricted to tetraploids and could only deal with small numbers of markers and individuals.

Thanks to the work of the Dutch consortium, it is now possible to generate such maps across a range of different ploidy levels using the 'polymapR' software. This has been demonstrated in polyploid crops such as potato (4x), rose (4x), chrysanthemum (6x), kiwifruit (in this case 4x) and blueberry (4x). 'polymapR' is also open-source software.

Apart from the increased computational complexity, one of the main differences between traditional diploid (2x) maps and polyploid maps is the need to assign markers to particular parental chromosomes. A marker located close to such a locus will co-segregate with the trait, but a marker on one of the other homologues will also show a statistical correlation. As can be imagined, the more homologous chromosomes that are present (e.g six per parent in a hexaploid, thus 12 in total in a biparental cross), the more possible assignments there are. Finding the most plausible location of a marker on a chromosome is not the only objective in polyploid mapping – but rather *phasing* that marker across parental homologues is the critical step, particularly for downstream applications, such as teasing apart the genetic make-up of important trait-controlling loci.

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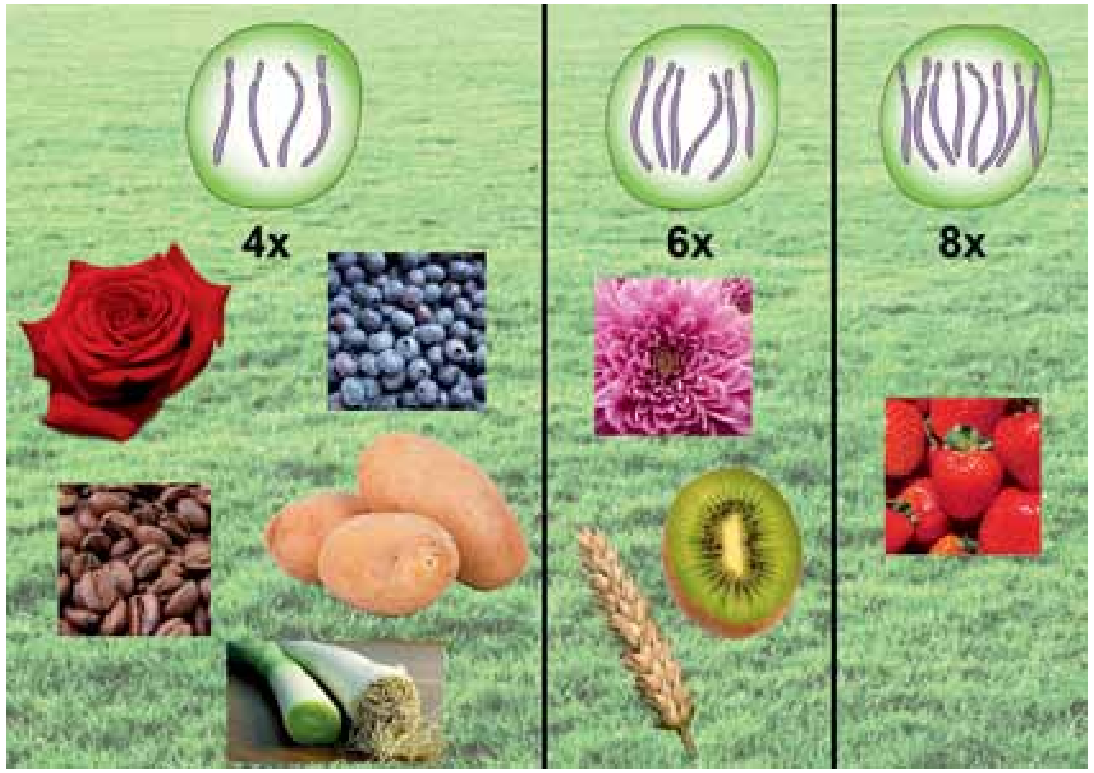
It is far more complicated to make sense of the data that is generated on polyploids



As a further application, genetic maps are also useful in assembling reference genome sequences (a reference DNA sequence of an organism that represents the species). The tetraploid rose maps produced by the team in Wageningen were recently used to help piece together the reference sequence of *Rosa chinensis*, one of the most important founder species of modern roses. These maps can also help provide a snap-shot of the parental meiosis (the process of cell replication that leads to the production of gametes), which remains a poorly-understood phenomenon in many polyploid crops. As seed-production and reproduction are at the heart of the breeding cycle, better understanding of what happens during meiosis can help inform breeding and selection decisions.

Interestingly, the pairing of chromosomes in rose was found to be somewhat intermediate between the two most commonly-observed types – being neither fully random (like we see in potato, for example) nor fully separated into subgenomes (as in wheat, for example), but something in between. It is likely that as more data is generated on polyploid breeding populations, these sorts of phenomena will become more widely diagnosed and understood.

One further novel aspect of 'polymapR' is its applicability to cross-ploidy populations, such as triploids (3x). Crosses between, for example, tetraploids and diploids are possible, although the offspring from such crosses are usually sterile. However, such crosses are often used in fruit and ornamental breeding, as they can lead to seedlessness or the suppression of (unwanted) pollen production.

Tailoring tools

The next step for polyploid breeders is to tailor these tools for their specific crops. From our experiences to date, it appears that each new species requires a slightly different treatment when it comes to generating high-quality genotyping data, and these protocols and technologies have yet to be fully optimised. However, as more polyploid breeders begin to apply these technologies, it is also likely that genotyping service-providers will come on board to further optimise these technologies on a per-crop basis.

All the potential benefits of genotyping, from batch identification, parental selection or identifying variety infringement, to marker-assisted and genomics-assisted breeding programmes will soon be available to polyploid breeders. However, how to best make use of these opportunities remains, as yet, unknown.

As with most other aspects of polyploidy, the application of marker technologies is complicated by the more complex inheritance patterns observed, and the possibility of multiple allelic copies of genes of interest being present, each with potentially different effects on the crop (both singly and combined). Also, the effect of allele dosage (how many copies of an allele are present) can be very prominent, as was shown for petal number in rose. Therefore, although we are a step closer to knowing how to generate genotypes in polyploids, we are still far from sure about how to best deploy them for maximum impact in breeding programmes. There are, therefore, many exciting and challenging questions that have yet to be answered in the years ahead.

Mergers structurally changed seed industry

Koen Deconinck

In March 2017, activists of Friends of the Earth staged a strange wedding ceremony in front of the European Commission offices in Brussels to protest against what they saw as a 'marriage made in hell' – the merger of Bayer and Monsanto. Together with the earlier merger of Dow and DuPont and ChemChina's acquisition of Syngenta, the Bayer-Monsanto merger (which has since been approved) has reorganised the global seed industry.

As the mergers were unfolding, it stimulated a public debate around competition in the seed industry and the potential effects of the mergers on prices, innovation and product choice. To inform this debate, the Organisation for Economic Co-operation and Development (OECD) conducted a study on 'Concentration in Seed Markets: Potential Effects and Policy Responses', published in December 2018. The report includes new, detailed evidence on market concentration in seed and GM technology across a broad range of crops and countries, along with a discussion of the structural changes occurring in the sector. The full OECD study is freely available online.

From Big Six to Big Four

A first important insight is that consolidation in seed markets has been going on for a long time. Over the past three decades, a series of mergers and acquisitions created the 'Big Six' (Monsanto, Bayer, BASF, Syngenta, Dow and DuPont), companies with strong positions in crop protection chemicals, as well as in seed and biotechnology (with the exception of BASF). Especially after the development of herbicide-tolerant GM traits, it made sense for firms to try to bring activities on pesticides, seed and GM technology in-house, to facilitate R&D and marketing. And the same forces are at work today: for all three recent transactions, the result is a more 'balanced' portfolio of crop protection chemicals, seeds, and GM technology.

Competition

In the jargon of competition economics, a 'horizontal' merger is one where the two firms are active in the same markets and sell competing products, as when two banks merge. Competition authorities are especially vigilant for such mergers, as they remove a competitor and could therefore reduce competition and lead to higher prices for consumers. By contrast, a 'non-horizontal' merger combines firms active in different industries, as when a car manufacturer buys one of its suppliers. These mergers are typically considered to carry fewer risks of negative outcomes, in part because the number of competitors in the market does not change and in part because the merger may have important efficiency benefits, which could end up benefiting consumers in the long run. But those mergers are not harmless: the car manufacturer could buy a sole supplier of essential parts in order to shut down deliveries to competing car companies, for instance. The recent mergers had important 'horizontal' aspects - Bayer's Liberty herbicide was considered the main alternative to Monsanto's Roundup, for instance. But the intertwining of pesticides, seeds and GM traits means the mergers also had important 'non-horizontal' aspects. How could these affect competition? The OECD study provides a detailed case study of the US cotton seed industry to illustrate these dynamics.

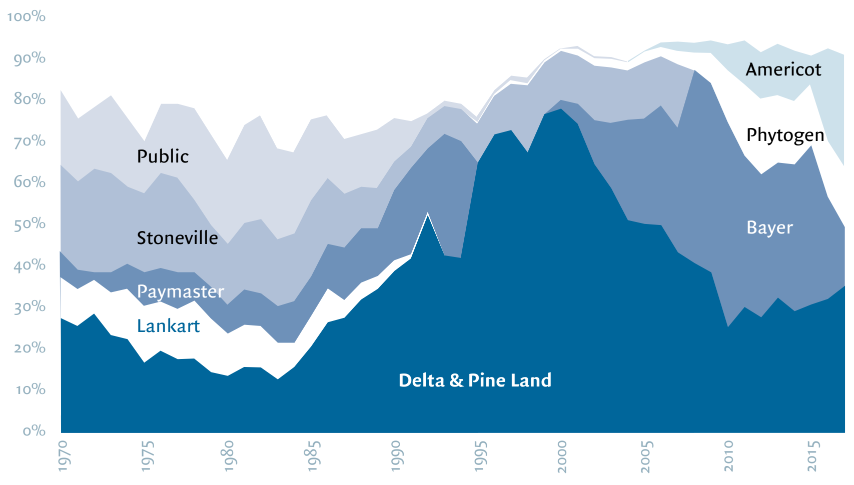


Figure 1: Market shares in US upland cotton seed, 1970-2017
Note: See the full OECD study for details on data sources and ownership changes over time

US cotton seed

Starting in the late 1980s, Delta & Pine Land grew to become the undisputed market leader in US cotton seed, accounting for more than 70% of the market at its peak between 1995 and 2000. During this period, Delta & Pine Land collaborated with Monsanto to supply insect-resistant cotton varieties. As Monsanto followed its strategy of acquiring seed firms, it also eyed Delta & Pine Land, which it finally acquired in 2007. By then, Monsanto's BollGard (insect-resistant) trait was used on more than 70% of cotton acres, while its RoundupReady trait was found on more than 80% of cotton acres. Strictly speaking, a 'non-horizontal' merger of Monsanto and Delta & Pine Land would not have reduced the number of competitors in either cotton seed or GM traits. But the risk of anti-competitive outcomes was still considerable. After the merger, Monsanto could in theory refuse to license its GM technology to competitors of Delta & Pine Land,

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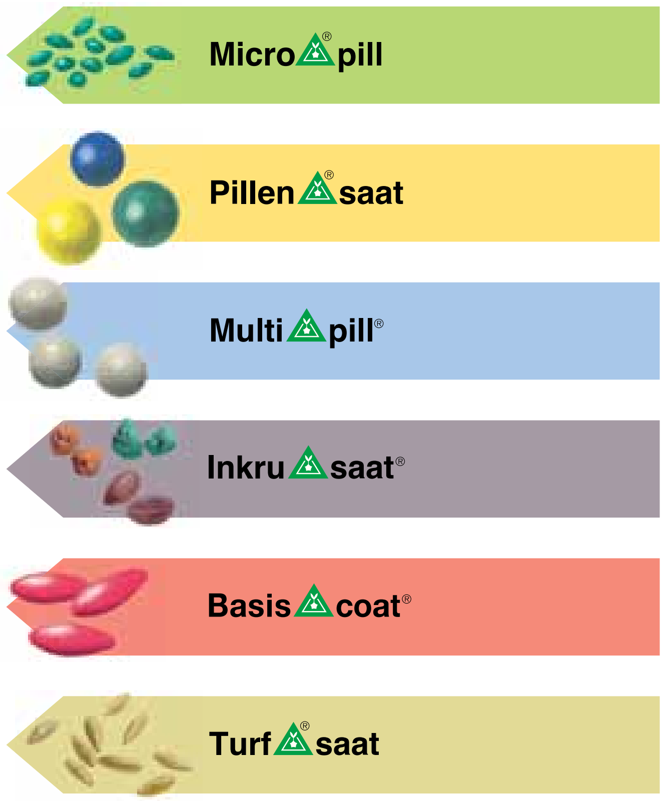
The USA is ranked third in cotton production behind China and India

New data on market concentration

As part of its analysis, the OECD study provides new, detailed information on the degree of market concentration in a large number of seed markets (see Figure 3). To measure how concentrated a market is, the OECD study uses a standard tool of competition analysis known as the Hirschman-Herfindahl Index (HHI), which ranges from 0 (perfect competition) to 10,000 (perfect monopoly). The data shows considerable variation in concentration levels across countries and crops.
A statistical analysis confirms that seed markets for sugar beet, sunflower and cotton tend to be much more concentrated than seed markets for wheat and barley, although the underlying mechanism is not clear.
For some markets, for instance for vegetable seed, it was harder to find market share information to estimate market concentration levels. However, data on the ownership of plant breeders' rights can provide a rough approximation, as shown in Table 2 for cucumber, carrot, lettuce and tomato in the EU. In all segments, the four largest firms account for most plant breeders' rights: 72% in tomato, 79% in lettuce, 94% in cucumber, and 95% in carrot. Prior to the mergers, Monsanto held a strong position in cucumber (46%), while Bayer (through its Nunhems brand) held a strong position in carrots (52%).
Several specialised firms have strong positions in vegetable seed, such as Rijk Zwaan and Enza Zaden, both privately held family-owned businesses from the Netherlands.
It should be pointed out, however, plant breeders' rights are not a perfect measure of market shares. A single 'star' variety may have a disproportionate market share while many other varieties may have little or no sales. Many successful varieties were introduced before the period under consideration (2013-2017), including some no longer covered by a plant breeders' right. Moreover, firms may differ in their strategy regarding protection of their varieties. For these reasons, the correlation between market shares and plant breeders' rights is far from perfect.



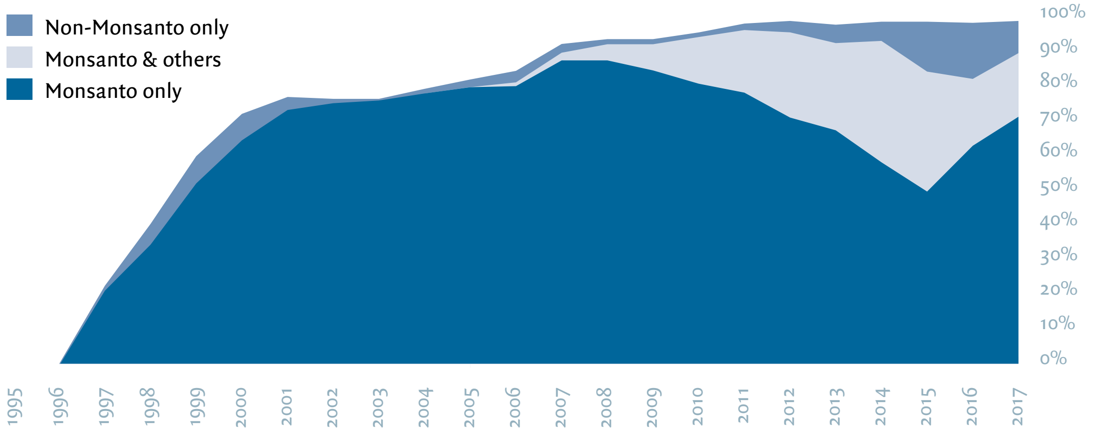


Figure 2. Cotton GM traits by owner, as a share of total cotton acres
Note: Figure shows share of US cotton acreage by owner of GM traits. See the full OECD study for details on data sources and methodology

while Delta & Pine Land could refuse to incorporate GM technology of Monsanto's competitors. In other words, the strong positions in GM and cotton seed could in theory be used to reinforce each other, stifling competition in both markets.

To avoid this, the Department of Justice only allowed the merger conditional on several measures. For instance, the firms had to sell several Delta & Pine Land cotton varieties, additional Monsanto germplasm, and Monsanto molecular technology.

Some of the divested assets were acquired by Bayer; others ended up with Americot, at the time a minor player. Another condition imposed was that Monsanto had to revise its GM licenses to allow the stacking of Monsanto and non-Monsanto GM traits.

Did this work? In the seed market, the measures indeed prevented Delta & Pine from maintaining a dominant position. Its market share fell from 50% in 2006 to 36% today. Americot, a firm with negligible market share in 2007, quickly rose to prominence and in recent years had 27% market share, thanks to the varieties it acquired from Delta & Pine. Hence, the measures in the seed market clearly worked.

Results were more mixed in the market for GM traits, however. Before 2007, nearly 100% of the GM traits in the US cotton market were 'Monsanto-only'.

After 2007, Monsanto was required to allow other firms to combine Monsanto and non-Monsanto GM traits, and these combinations indeed became more popular. In 2014 and 2015, such combinations were planted on 35% of the total cotton area. However, Monsanto clearly maintains a strong position in this market. This is especially clear when we look at varieties offering only non-Monsanto GM traits. These were practically non-existent in 2007, and have seen some growth since, but still only reached 17% of the market in 2016. In other words, the remedies may have prevented a double monopoly on seed and GM traits, but they did not succeed in fully opening up the market for GM traits to other firms.

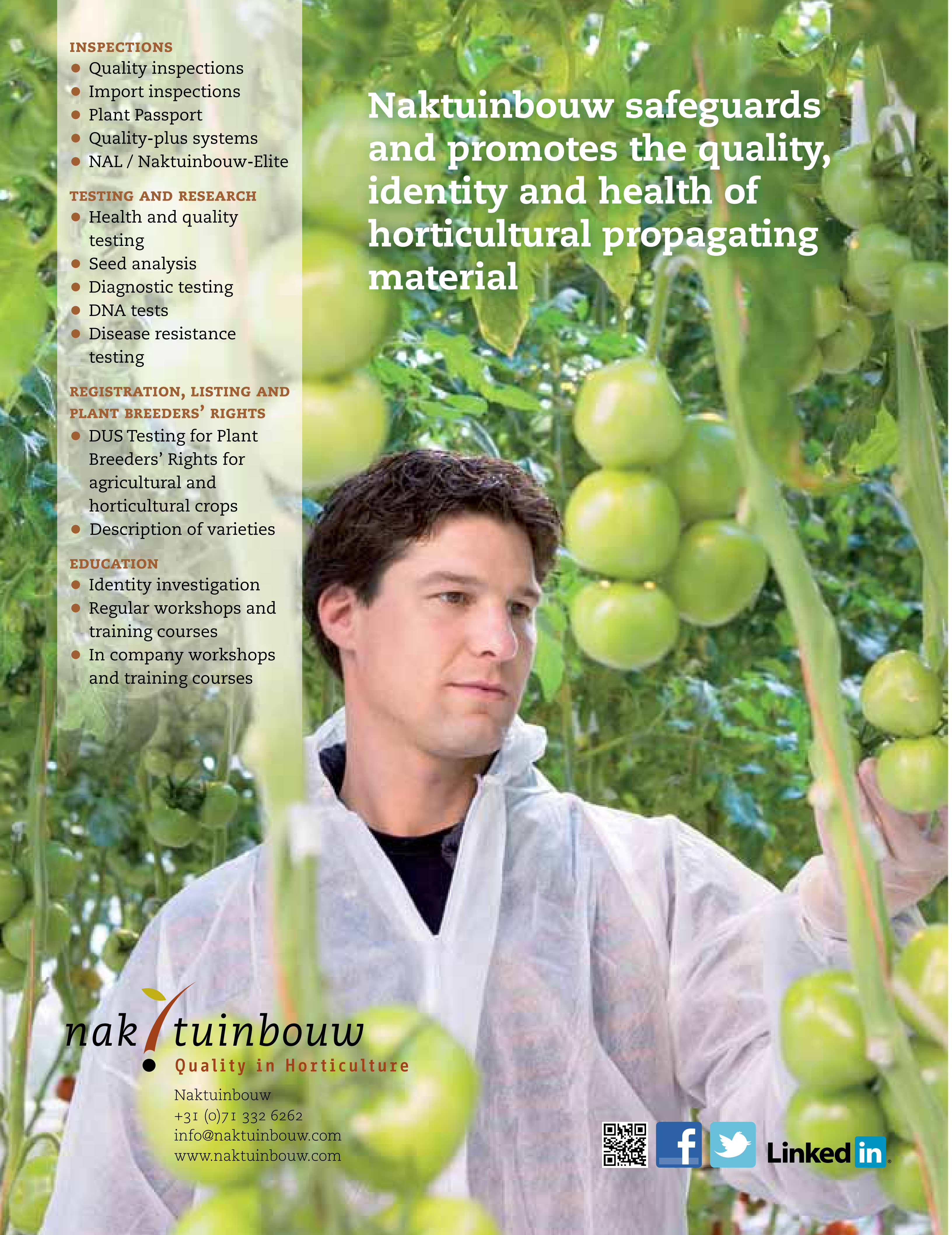
Responses by policymakers

The example of the US cotton seed industry illustrates the challenges of evaluating 'non-horizontal' mergers: to avoid a reduction in competition, it is important to understand the dynamics in the market. Competition authorities took these lessons to heart as they evaluated the recent mergers. In scrutinising the mergers, they did not only look at possible direct effects on prices, but also considered

Table 1. Bayer assets divested to BASF

Crop Protection	Seeds & Traits	Digital Farming
Global glufosinate-ammonium herbicide business (Liberty, Basta, Finale) Selected glyphosate-based herbicides in Europe 3 non-selective herbicides under development Selected seed treatment products	Essentially the entire soybean business Essentially the entire canola/ rapeseed business Cotton business (except India, South Africa) Global vegetable seeds business LibertyLink technology R&D capabilities for divested crops R&D platform for hybrid wheat	Digital Farming business (Bayer receives a non-exclusive license for certain applications outside North America)

Source: Bayer investor presentation: 'Monsanto Acquisition Update', June 2018



Source: OECD (2018) using the Kleffmann amis®AgriGlobe® database

Figure 3. Concentration in seed markets

Table 2. Ownership of plant breeders' rights for vegetables in Europe

EU-wide plant breeders' rights only, 2013-2017

	Cucumber		Carrot		Lettuce		Tomato	
Rijk Zwaan	9	11%	-	-	130	32%	63	20%
Monsanto	39	46%	4	0	25	6%	77	24%
Enza Zaden	5	6%	-	-	106	26%	18	6%
Nunhems (Bayer)	27	32%	11	1	26	6%	47	15%
Syngenta	5	6%	-	-	47	11%	43	14%
Limagrain	-	0%	3	0	44	11%	44	14%
Bejo	-	0%	2	0	12	3%	-	0%
Other	-	0%	1	0	19	5%	26	8%
Total	85	100%	21	1	409	100%	318	100%
Four-firm concentration ratio		94%		95%		80%		73%

Note: Table shows total number of approved EU-wide plant breeders' rights applied for in the period 2013-2017 by applying party using the following uc-codes in the PLUTO database: CUCUM_SAT (Cucumber), DAUCU_CAR (Carrot), LACTU_SAT (Lettuce) and SOLAN_LYC (Tomato)

Source: OECD analysis using the UPOV PLUTO database (version 16 February 2018)

indirect effects on innovation and whether there was a risk of one firm 'locking out' other firms, as in the Delta Pine case. As a result, firms were required to divest themselves of several businesses to safeguard competition in the industry. For instance, Bayer agreed to sell nearly its entire seed business to BASF, as well as several other businesses including products under development as well as its Digital Farming platform (see Table 1). Of course, only time will tell whether these divestitures were sufficient to protect competition.

Finally, while most of the discussion on the mergers has focused on decisions by competition authorities, several other policy options exist to stimulate competition and innovation in the seed industry. The OECD study identifies opportunities in three areas. First, although proper regulations are necessary to create trust in markets, excessive regulation could create barriers to entry (which could in turn contribute to higher levels of market concentration) and could slow down innovation in plant breeding. Policy makers should therefore make sure to avoid unnecessary regulatory barriers. This point is of course especially important given the new plant breeding techniques, which could present opportunities to smaller enterprises.

OECD (2018) Concentration in Seed Markets: Potential Effects and Policy Responses, Paris: OECD Publishing. Available online at https://doi.org/10.1787/9789264308367-en

Two hundred **types of green**

Monique Krinkels

Two Austrian brothers, Franz and Ferdinand Bauer, rank among the very best artists that made botanical watercolours which, up to the present day, still help botanists identify plants. They invented a numerical system which represents exactly the colour shade to be used in the final painting. It meant a revolution in scientifically correct botanical illustrations.

Even today, a field guide for identifying plants still uses botanical illustrations, rather than photographs. Of course, photography is a valuable supplement, but details can only be clearly displayed in a drawing, damaged parts repaired, and precisely those details brought forward that the botanist finds most important. Botany is not unique in that respect: the same goes for field guides for birds and medical illustrations.

From decorative to accurate

The art of drawing scientifically correct botanical illustrations stems from the 16th century, when the first artists emerged who wanted to display plants accurately and who were able to produce such refined wood carvings so that very detailed images of plants could be produced. Most of these early plates were used in herbal books for medicinal purposes. So, it was not initially the botanists for whom the books were intended, but for doctors and pharmacists. Earlier wood carvings were used more as decorative illustrations, than to give a correct representation of the plant being described, for instance, the Latin Herbarius (1484) and Ortus Sanitatis (1500).

Eye-catching exhibition

This spring, an exhibition in Teylers Museum in Haarlem, the Netherlands, displayed the works of Franz and Ferdinand Bauer. It showcased the rare and precious volumes containing the Bauers' work, which were purchased for the natural history library at Teylers Museum immediately upon publication in the early 1800s. In addition, the Naturhistorisches Museum in Vienna has contributed to the exhibition with field drawings made by Ferdinand. The Natural History Museum in London added elaborate watercolours painted by the brothers.

Teylers Museum, the oldest museum in the Netherlands, was founded in 1784 as Pieter Teyler van der Hulst left his considerable fortune of two million guilders (equivalent to about 80 million euros today) to promote theology, the sciences and the arts. As a typical representative of the Enlightenment, Teyler valued the arts and sciences as ways of enriching society and humanity, in general. The collection in his former residence reflects his broad interests: from drawings by Rembrandt, Michelangelo, Raphael and Goltzius, to rare minerals, dinosaur bones, fossils and machines that generate electricity.

Aloe vulgaris by Ferdinand Bauer

Franz and Ferdinand Bauer are among the most prominent botanical artists of all time. Their watercolours are among the very best and most beautiful produced during the so-called 'golden age' of botanical art, around 1800. Ferdinand Bauer (1760-1826) travelled as a botanical artist on dangerous expeditions to territories unknown to Europeans, in the Ottoman Empire and Australia. This last voyage, the first circumnavigation of Australia, was on behalf of the British admiralty. The objective was to identify useful plants, either for food or feed, for fibres or as ornamentals. Botany was a pivotal science in the rivalry between European powers. Whoever was the first to discover a new, useful crop could make a huge profit. On the 4-year trip with the survey ship, HMS Investigator, Ferdinand recorded almost 2,000 plants and animals, that is more than one drawing per day.

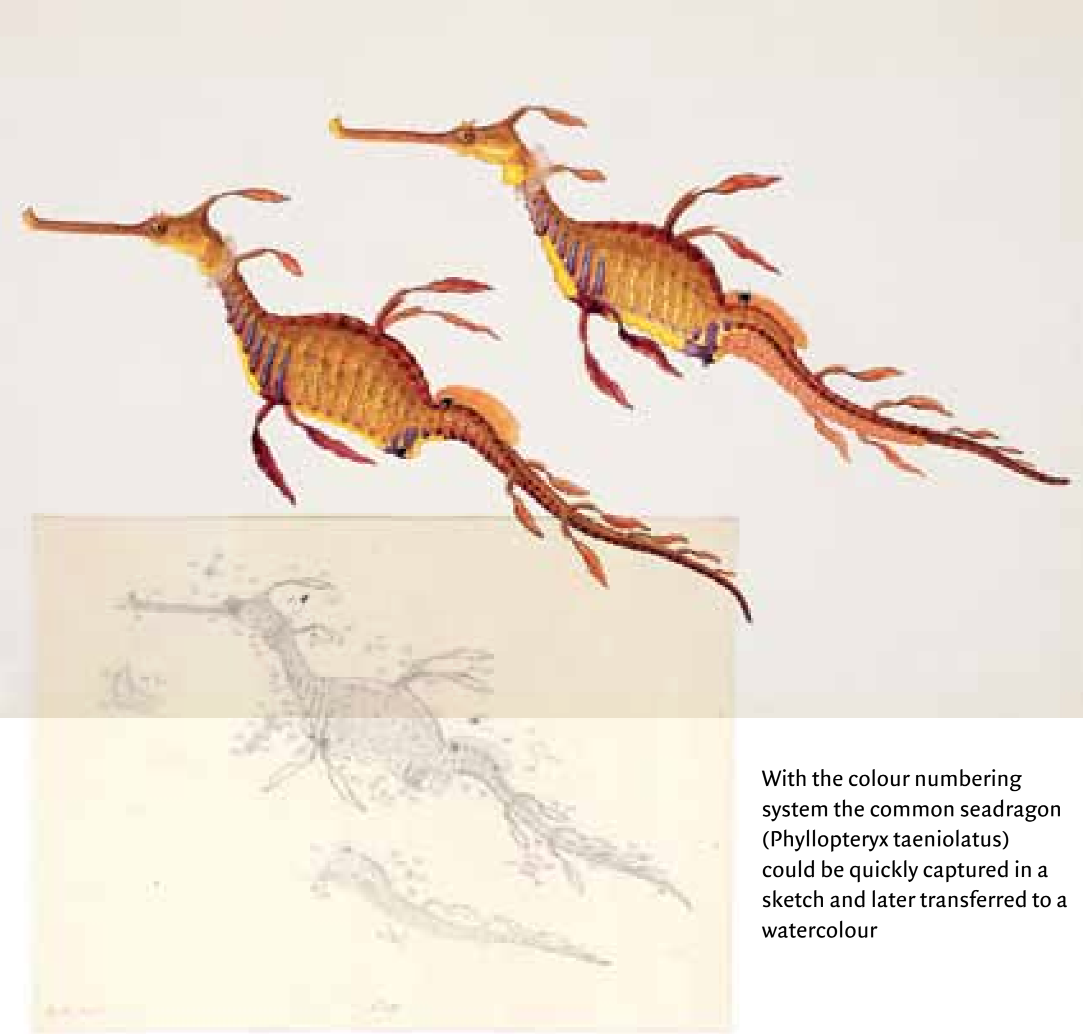
Thanks to the influential President of the Royal Society, Joseph Banks, his brother, Franz Bauer (1758-1840), became the first 'Botanick Painter to His Majesty' at Kew Gardens, producing delicate watercolours of the plants that were brought to Kew Gardens from all corners of the globe. These played an important role in research of species that could prove useful to the British Empire. In Kew, he also collaborated closely with scientists who were studying the anatomy of human beings and animals. Franz was a pioneer in the use of the microscope, of which he owned as many as fifteen, along with a large set of lenses. He recorded his observations, down to the smallest detail, in relation to plant diseases, plant reproduction, and the anatomy of animals. The first scientific description of the cell nucleus was based on his drawings of orchid cells.

Colour by numbers

In their youth, the brothers developed a system of making a drawing in the field, using numbers representing the colours. In their early teens, they were placed in the custody of Dr. Norbert Boccius, the subprior of the monastery of the Brothers of Mercy in Feldsberg and professor of anatomy, surgery and nursing. As a passionate amateur botanist, Boccius collected new species for the monastery garden. He started to assemble a collection of botanical drawings, the Liber Regni Vegetabilis (book of the plant kingdom). Presumably, his objective was to bring together all known plant species, as an enlightened encyclopaedia of nature and useful for imparting botanical knowledge to his students.

Today, the book consists of 14 volumes, known as the Codex Liechtenstein, with 2,750 watercolours depicting around 3,100 plant species.

At the time, the usual process was to paint the watercolours in the studio, copying the examples of others. The watercolour technique was too cumbersome and too sensitive to use in the field. But, for Liber Regni Vegetabilis, it was necessary to make watercolours of plants, as yet unrecorded. That is why the brothers developed a revolutionary working method. During the growing season, they quickly captured images of plants outside with graphite marker, using a numbering system to register 140 different shades of colour. Those drawings, on thin and cheap sketch paper, were later transferred to more expensive and durable paper and developed in colour with watercolour paint. Painting such a watercolour was a labour-intensive job. It has been calculated that producing a single completed watercolour took about a week. Not only did the brothers paint the watercolour, but they also had to prepare the paper, grind the pigments and so on.



With the colour numbering system the common seadragon (Phyllopteryx taeniolatus) could be quickly captured in a sketch and later transferred to a watercolour

Nerium oleander

Nerium oleander

Enhanced system

Later, Ferdinand enhanced the system to over 1,000 numbers to capture colour nuances. By comparison, to describe new varieties for plant breeders' rights applications, only 884 colours are currently used. It is unknown whether Ferdinand was able to identify colours without tools, but no colour card has survived the ages. He created a series of colour nuances: red (1-100), purple to pink (101-200), pink to mauve (201-300) and lilac and violet to purple (301-400), 200 shades of green (401-600), and another 100 colours of yellow (601-700), orange (701-800), brown (801-900) and finally 100 shades of white through grey to black.

With alchemical symbols, he described sheen: the moon (☽) for silver, the sun (☉) for gold, Venus (♀) for copper, Mars (♂) for iron, Jupiter (♃) for tin, Mercury (☿) for mercury and Saturn (♄) for lead.

The exhibition in Teylers Museum was the first time that the numbered field sketches and the elaborated watercolours were reunited and exhibited together.

All-New Software for Plant Breeding

Genovix combines power with **simplicity**

Chris Leonard

For 29 years Agronomix Software has developed plant breeding software helping plant breeders, crop researchers and agronomists with yield increases, data management, data analysis, seed inventory and increased research productivity. Their latest achievement is the development of Genovix, a revolutionary software tool for breeders.

Development of Genovix began over five years ago using Microsoft's latest SQL Server and .NET technologies. The aim was simple: To create a powerful database and intuitive interface, suitable for all in breeding and testing research. The biggest challenge was how best to integrate hundreds of practical capabilities and tools into an efficient and easy to use interface for all breeders and variety testers with very diverse needs. However, developing scientific software which is both easy to use with inherent 'simplicity' yet powerful at the same time is not easily done.

Challenge

After consulting with breeders, testers and researchers over many years, they are clearly facing pressures and have requirements like everyone else. The needs of researchers for field and row crops are different from vegetable breeders and again different for horticultural crops. Perennial and clonal crops, with their breeding systems, need to store and process data easily across years, multiple cuts or picks, often with seasons or months. Database structure requirements become more challenging. All the software development must be done with a solid, practical understanding of plant breeding and variety testing.

Some companies and government institutions have failed in developing such a system, despite spending millions of dollars.

Having talked to breeders over the years, many wished that their organization – including some large companies – would have simply licensed proven plant breeding and variety testing software instead of having to use a system that they see as inferior. Indeed, this contrast will be even larger when Genovix is released. There is no shortcut or substitute for the practical knowledge of such requirements accumulated over time.

Highly relational

The interface is fresh and modern, easy to navigate, yet powerful at the same time. This has been confirmed by all who have seen the system to date and their request for an as early release date as possible. But that is only part of the story – the underlying architecture of the software must be 'highly relational'. Data, genotypes, experiments, traits and more must be inter-linked to permit the necessary tasks for the breeder or variety tester. You may know the old saying about buying a car: 'look under the hood before you buy'. The car might look quite appealing, but the performance is just not there.

For example, breeders should be able to link genotype data across generations, trace or query all the data or plots or seed lots of one or more genotypes, connect genetic information for legal compliance to a genotype everywhere in the database, rename a genotype or trait instantly with a few clicks across hundreds of experiments or nurseries, show all the experiments where a genotype has been planted – to list just a few such important capabilities.

Don't forget the import of past breeding data, maybe over twenty years as some of our clients have done, wherein all the genotype connections and histories are created and readily appear in the database. This cannot be overlooked, especially if royalty payments are due for use of some germplasm that was used in the breeding program years ago. Legal issues can become costly.

A simpler database architecture simply 'will not deliver the results', not even close. This all goes towards saving time and further empowering the critical decision-making tools for breeders.

Over the last 30 years Agronomix Software has gained recognition and a growing user base in over 40 countries. During those years, the company has interacted closely with breeders of many crops with diverse

Easy to customize

"The most remarkable feature of the new plant breeding software from Agronomix is the ease by which one can adapt the programme to one's needs and preferences. Besides looking more modern, the user interface has really been improved. Breeders are a quirky lot, in my experience. It definitely is a new generation tool. Another aspect that will come in handy is that you can share part of the available information. When tests are run elsewhere, you only send the need-to-know data. And when the results come back they are easily integrated in the databank." *Tim Spoor, assistant potato breeder at Solynta, Wageningen, the Netherlands*

Use of graphics improved

"The visual aspects of the new software is much improved. Especially the use of graphics has been upgraded. I am looking forward to the transition in June. We have worked with Agronomix Software since 2007. It can cope easily with the large numbers that are involved in breeding grasses and fodder crops. Another consideration for choosing AGROBASE is that the excellent helpdesk of Agronomix can be easily reached. As our breeding stations are spread all over the world, and thus time zones, it is important that the users of AGROBASE can request assistance 24/7. That truly is an important asset." – *Piet Arts, global research director, Royal Barenbrug Group, Nijmegen, the Netherlands*

Chris Leonard MBCS is President of Agronomix Software Inc., Winnipeg, Canada, leonard@agronomix.com

CEO and founder, Dieter Mulitze PhD, is quite excited about the contribution that Genovix will make to plant breeding and variety testing globally

Crisp and modern

"As a leading canola hybrid breeding institution in Canada, Nutrien Ag Solutions (and legacy institutions) we have used the AGROBASE software system for more than 22 years including its updated version Generation II. All of our breeding activities including generation of the crosses, development of the double haploid inbred lines, molecular marker data, field nursery and yield trial set ups, harvesting, oil chemistry analysis data base, hybrid tent creations in Chile and all the components in the hybrid program are accurately and efficiently handled by AGROBASE.

We are quite impressed with the new capabilities the new version offers. Some of the key attributes I really liked are; crisp and modern interface, more icons & visuals, handles up to 1,024 fields at a time and more accessible tools for direct interaction with R for clustering. Also, I liked the greatly enhanced graphics including 3D & dendrograms with images and data views, mobile data manager, more powerful labels & reports, audit trail (to see changes that happened), cross combinations with data decisions to easily identify winners and quick data access and also GPS coordinates for locations for easy presentations of yield data." – *Andy Andrahennadi, hybrid crops breeder, Nutrien, Saskatoon, Canada*

Choosing security level

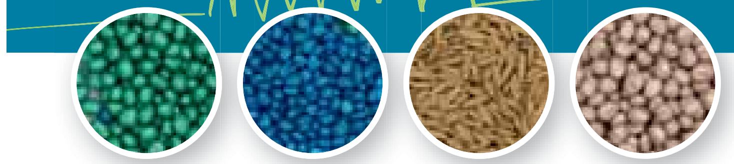
"The new software also comes in a cloud version. Breeders can access data from different locations. With the security levels we can set up exactly who has access and who can enter data. This improved security system is important to us as we have breeding stations for wheat and barley all over Europe. A handy extra is the link to Google Maps, so you can see exactly where the trials are carried out. Another helpful feature is that we can define the order in which the columns are displayed. The new software offers more fields: 1,024 columns. Not enough for storing all datapoints used with high density genotyping, but much better than it was. Furthermore, it is possible to display the data in graphs with just one click." – *Charles Snijders, Ph.D., Director Plant Breeding, Asur Plant Breeding, Estrées-Saint-Denis, France*

> **Data, genotypes, experiments, traits and more must be interlinked to permit the necessary tasks for the breeder or variety tester**

breeding systems reflecting each crop's biology. With breeders in the company directing the software development, this has led to a software system that many current and potential clients have said '... that software functions how a breeder thinks'. But even breeders of the same crop within the same company 'do things differently'. That has presented a 'perennial' challenge to the ongoing software development with the need for customization and flexibility. Reflecting on the vast changes in software technology and leaning on the knowledge gathered over 30 years of interaction with researchers, Agronomix Software decided to build an entirely new software system. Agronomix Software made the conscious decision to invest a lot of time and effort in a developing powerful database architecture with efficient algorithms well beyond their current software. That resulted in increasing the software development team and in 2017 moving to a new office with double the space. In the last decade especially, more researchers have embraced open source R for both data analysis of trials data as well as a multitude of bioinformatics analyses for genomic predication and much more. In addition to Agronomix Software's proven proprietary analytics including spatial analyses of yield trials, Genovix users can utilize R for advanced data analysis and visualization. Given current trends and input from users, there will be even more such development in Genovix.

Security

In response to the larger corporate clients requiring more security and control with offline data acquisition, the Genovix Mobile data manager will be first released for the android operating system. This permits data capture via third parties who then can send encrypted data back to the client for validation, synchronize with the database, and maintain a history of such data acquisition. A new data audit tool in Genovix will also keep track and report on a multitude of database changes done by any or all users accessing the software.



Plant breeding enables a **circular** bioeconomy

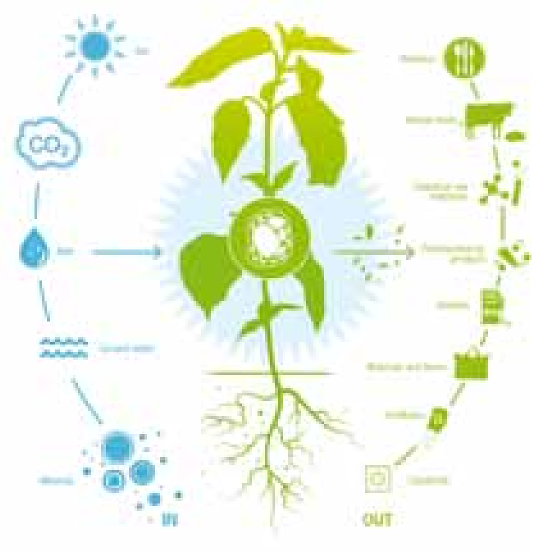
Luisa M. Trindade

Climate change, energy transitions, sustainable production of food and non-food, and preservation of biodiversity are among the most important global challenges in the near future. To address these challenges, profound changes in our societal and economic models are required in parallel with technological developments.

Dr. Luisa M. Trindade, Plant Breeding, Wageningen University & Research, Wageningen, the Netherlands, luisa.trindade@wur.nl

A transition from an obsolescence-driven economy to a circular and sustainable economy is needed. The efficient use of (natural) resources for food, feed, fuels, chemicals and materials is at the basis of these global challenges. A more sustainable and efficient production system implies replacing fossil carbon by sustainable and renewable carbon-based raw materials; it comprises developing a circular economy in which the essential resources of carbon, water and nutrients are recovered and reused. This points toward a switch from a fossil-based to a renewable resources society, where sunlight is the primary energy source for photosynthesis for optimal growth of (new) crops, both terrestrial and aquatic.

Efficient use of feedstocks, alongside new technologies for producing, recycling and reusing of carbon-based materials, will form the basis for this transition. To ensure a resilient system, the transition has to go beyond simply the closure of cycles, the circular economy needs to be heterogeneous and diverse. The shift from petrochemical to circular green industries will foster the development of new business models for regional and global economic development. Plant breeding plays a central role in the transition towards a circular biobased economy.



The plant as a green factory (illustration Kay Coenen)

Biobased economy

For many centuries, growers, and later breeders, have focused their efforts on improving yields (and, more recently, also quality) of edible parts of the plant. Most crops cultivated currently produce one (or few) main product(s) in addition to (large) waste streams with limited economic value. The plants of the future will be tailored to optimal feedstocks for biorefineries, where all components have been optimized to be used or processed in an efficient manner, while complying with sustainability criteria and preserving soil properties and natural resources. To develop such new plants, where the plant is considered as a factory of different 'building blocks' for multiple products, new breeding tools are required. The development of such tools is the focus of the plant breeding Biobased Economy group, at Wageningen University & Research.

A biobased economy demands feedstocks with novel properties and the crops currently available, selected and bred during the last centuries, are thus far from optimal, as they have been optimized for different purposes. Important targets for plant breeding towards a biobased economy are the improvement of: i) yield and quality of the different plant components, ii) suitability for downstream processing and iii) resource use efficiency (low input and high output).

Apart from better crops, where more biomass can be efficiently used, a larger agricultural production area is needed in order to fulfil all (future) needs. One way is to develop crops able to grow economically in areas that are currently not being used for agriculture, such as poor-, hedge- and marginal-soils. Under such marginal conditions, the most common constraints for plant production are heat, cold, drought, salinity, low amounts of essential nutrients and high concentrations of (toxic) metals; and these are important targets to remedy by plant breeding.

There are currently only few crops for which biomass is being fully used. These include fibre crops such as hemp or miscanthus, but also food crops such as wheat, where besides the grains for food, we use the straw for non-food applications, for example horse bedding.

Miscanthus sinensis

Great potential

Miscanthus is a crop with a great potential in a circular bioeconomy, owing to its high yields, efficient use of resources such as light (C4 photosynthesis), water and nutrients, and tolerance to cold and salt. This barely breed crop produces biomass of good quality for many applications, including biomaterials (e.g. paper, concrete, textile, bioplastics) bioenergy, biofuels and, more recently, a good source of fibre for ruminants.

For many food crops, like tomatoes, bell peppers and cucumbers, only a fraction of the biomass production, the fruits, is being efficiently used. The stems and leaves contain many valuable components, such as proteins, fibres and bioactive compounds (secondary metabolites) but are currently not used because they are difficult to extract and/or process. Breeding these crops for a whole-crop use would empower the use of such compounds for different applications. These include applications in food, such as new resources of protein, bioactive compounds for pharmaceutical industry and fibres for textiles, a sustainable and local alternative to cotton. And, who knows, Calvin Klein cucumber-jeans or Dolce & Gabbana bell-pepper-jackets might soon be in the stores!

Seed Treatment

On the lookout for **alternatives**

Monique Krinkels

The European Commission is rapidly reducing the number of crop protection products that may be applied on seeds. After 30 January 2020, the widely-used fungicide Thiram will no longer be allowed in the EU. In response, researchers at Germains Seed Technology are actively working on Thiram alternatives including chemical, physical and biological seed treatments.

"It did not come as a total surprise," says Ab Aarnoudse, Germains European Head of R&D. "Over 30 years ago, when I began my career in the seed industry, the potential ban of Thiram was a topic of discussion. At the time, the Dutch government was seriously considering prohibiting the use of Thiram as a fungicide, because of its claimed toxicity."

Finding alternatives

In contrast to its application on plants, the EU seed industry argues that Thiram as a seed treatment poses no dangers. The sector also underlines the importance of Thiram as a seed treatment, because there are limited effective, broad-spectrum and widely-accepted alternatives available. Because of the Thiram ban in the EU, growers will suffer yield losses due to soil fungi, they warn; and seed companies will encounter problems with exports where fungicide seed treatment is often a phytosanitary requirement. It also distorts international competition, because the use of Thiram is still allowed in many important export regions.

Research for other chemical treatments the industry may use for vegetable seeds is complicated. "The crop chemical industry tends to concentrate its R&D activities on large arable crops. Finding an alternative product for crops like soybean, rice or cotton is much more profitable than vegetables. In the latter, it is a difficult business case. So far, few new chemicals are approved for vegetable seeds. However, I am confident that if we remain persistent with our research, Germains or the industry will come up with alternative solutions," Ab Aarnoudse declares.

Biologicals can also be an alternative to chemicals. Aarnoudse shares: "Our R&D team is actively researching biologicals to add to our health programmes. One of the obstacles is that in the EU biologicals must follow the same registration process as chemicals. Of course, biologicals must also be safe for applicators and the environment which requires extensive field trials with a plethora of registration paperwork for any new treatment. It takes time, but we have succeeded before."

Alongside traditional seed treatments, Germains provides innovative seed hygiene methods. Recently, Germains introduced a new hygiene process Exilis to the EU market. Exilis is a seed hygiene process that removes or reduces seedborne contaminants such as *Pseudomonas* from Swiss chard and red beet or *Xanthomonas campestris* from brassica seeds with minimal impact on the seed's vitality.

ABF subsidiary

Germains Seed Technology is a subsidiary of AB Sugar which is a part of Associated British Foods plc (ABF). Headquartered in London, ABF is a leading, multinational food, ingredients and retail group with an annual revenue of £ 15.6 bn (€ 18 bn) and 137,000 employees in 50 countries operating under the direction of George Weston, CEO. ABF is the world's second largest producer of both sugar and baker's yeast. It has brands ranging from Twinings, Patak's and Ovaltine to Primark.

USA

In Gilroy, California, Germains operates two research facilities that focus on finding new solutions for the North American sugar beet and vegetable industries. Germains North America has a robust seed health program with both conventional and organic options. For example, gopure is a conventional seed treatment for spinach that uses a combination of fungicides, which inhibits the growth of seedborne fungal pathogens, such as *Verticillium dahliae*, *Stemphylium botryosum*, *Cladosporium*, and *Alternaria* with protection for seeds from soilborne pathogens such as *Pythium*, *Rhizoctonia*, and *Fusarium*. "Gopure's efficacy at removing these seedborne pathogens, without impacting seed vitality, is a proven technology," says Dale Krolikowski, Head of Business Development and R&D for Germains, North America.

Germains North America also pays much attention to the sizable organic market segment and its specific needs. Another innovative organic seed disinfection option for crops such as Swiss chard, red beet, kale, and coriander, is ProBio gopure. According to Krolikowski, "the idea for ProBio gopure began with a single customer inquiry. The customer had 100,000 pounds of organic Swiss chard seed that was contaminated with a seedborne bacterial disease. Rather than discard or sell the seed at a reduced cost, Germains formulated an effective certified organic disinfection process to clean the seed. ProBio gopure removes seedborne pathogens such as *Pseudomonas syringae* pv. *aptata* from Swiss chard and beet seeds, and *Pseudomonas syringae* pv. *coriandricola* from coriander seeds."

Victoria Lawrence, Managing Director, Dale Krolikowski, Head of Business Development and R&D North America (left) and Ab Aarnoudse, European Head of R&D (right)

In 2018, Germains USA launched ProBio SafeGuard, an organic seed treatment for spinach that focuses on early plant protection against *Pythium*. "ProBio SafeGuard establishes a barrier around the emerging plant and root system, helping to protect spinach plants during germination until the first true leaf stage," explains Dale Krolikowski.

American ancestry

In 1871, Swiss immigrant Eugene Germains started a horticultural supply company in California, USA. For years it was one of the leading seed companies in the western USA. Over the years, it diversified its activities into a wide variety of market segments including rose breeding, producing agriculture paper products, and seed pelleting. In the 1950s Germains became the first company in the USA to launch pelleted seed for sugar beet, which was a leap forward for the entire seed industry. The success of the pellet attracted the attention of UK sugar beet growers and British Sugar. In 1990, the company was acquired by Associated British Foods.

Today, Germains' sole focus is on the supply of industry leading seed treatment services with five technology categories: Prime, Pellet, Filmcoat, Health, and Polymers. The companyemploys some 200 employees across nine locations in the UK, USA, the Netherlands, and Spain. Of the nine facilities, four are dedicated to Research and Development and five are processing plants. Headquartered in Kings Lynn, UK, the processing facility there treats the majority of sugar beet seeds sown in the UK. In addition, the company has two R&D facilities in the UK: one in Kings Lynn and the other in Norwich. In the USA the company has R&D and processing facilities in California along with a processing plant in North Dakota where they treat vegetables, sugar beet, and other alternative crops. In the Netherlands and Spain, there is a clear focus on vegetable and flower seed processing in both sites with an additional R&D facility in Enkhuizen.

In 2018, Victoria Lawrence was appointed as Germains' new Managing Director. "I am confident that my roles in general management, global strategy and business development within AB Agri, the agricultural division of ABF, will stand me in good stead to lead the global Germains team," she states. "I am delighted to be a part of this fast-moving industry, especially in a forward-thinking company such as Germains Seed Technology." Victoria is very enthusiastic about the seed industry and looks forward to meeting customers and suppliers at the ISF in Nice, France.

New Tool for Tulip Breeders

Mind-boggling sized **genome** unravelled

Ron Dirks

For a long time, genome sequencing of plants was restricted to food crops. However, floriculture is quickly catching up, as flower breeders recognise the benefits of using molecular markers. This February, scientists succeeded in identifying the DNA sequence of the tulip genome, one of the biggest genomes ever sequenced.

For competitive reasons, tulip farmers are continuously looking for new flower variants and other beneficial properties, such as resistance against diseases and speed of growth. The latter property is of particular importance, since breeding new tulip variants is a very time-consuming process. Developing seed into a new flower variant takes at least five years and the route from tulip seed to bulb sale can even have a multi-decade trajectory.

Although agriculture, in general, has already been using molecular markers since the start of DNA sequencing some 40 years ago, this was initially mostly focused on animal husbandry (livestock) and food crops; however, floriculture is now quickly catching up and most flower farmers recognise the great benefit of using molecular markers in plant breeding programmes or other applications, such as fingerprinting of variant specimens and measuring genetic diversity. In comparison with traditional morphological markers (phenotypic traits, such as flower colour) and biochemical markers (e.g. enzyme variants), the main advantage of molecular DNA markers is that they are available in much higher numbers and that they can be used independently of the plant's developmental stage and environmental status.

Genome	Chromosomes	Million basepairs
Human	2 x 23	~ 3,235
Chicken	2 x 39	~ 1,000
Rice	2 x 12	~ 375
Maize	2 x 10	~ 2,300
Tomato	2 x 12	~ 950
Onion	2 x 8	~ 16,000
Rose	2 x 7	~ 710
Tulip	2 x 12	~ 34,000

Genome size

Once the decision is made to use molecular markers for the aforementioned applications in floriculture, one of the first steps is to determine the full genome sequence of the plant in question. Unfortunately, the tulip turns out to be on the wrong side of the spectrum with respect to genome size. Although it is a diploid species and has only twelve chromosome pairs, the average size of each individual chromosome equals the size of the human genome. In total, the tulip has a mind-boggling estimated genome size of 34 billion base pairs (34 giga base pairs; 34 Gb). Until very recently, people did not even consider sequencing this genome, because sequencing technology and assembly software were simply not ready for such a challenging task.

Historically, three generations of DNA sequencing can be distinguished: first-generation sequencing, or 'Sanger sequencing', started around 40 years ago (late 70s) and was the method of choice for unravelling the human genome sequence of more than 3 billion base pairs. Second-generation sequencing, or 'massive parallel sequencing', was introduced around 2006 and the Giant Panda genome sequence was the first vertebrate genome that was de novo assembled from second-generation (Illumina) short read sequences only. De novo assembly of these genome sequences was always based on the same strategy, namely finding identical words (k-mers) in the (partial) sentences (sequencing reads) and, by overlapping those words, reconstitute at least paragraphs or chapters (contigs, or contiguous sequences) of the complete book (genome).

The maximum read length that can be obtained with first-generation sequencing (~1000 nucleotides) and second-generation sequencing (~250 nucleotides) is most often the limiting step in the de novo assembly process, because a considerable percentage of larger genomes is composed of repetitive elements. If the size of the sequence read is smaller than the size of the repeat element, the assembly software cannot decide which unique sequences are located on both sides of the repeat and as a result the genome assembly will become more fragmented (more contigs).

This problem was partially solved by longer sequence reads with the introduction of third-generation sequencing, also known as 'single molecule sequencing', initially by Pacific Biosciences (~2011) and quickly followed by Oxford Nanopore Technologies (~2014). The major advantage of third-generation sequencing is that it allows mass production of ultralong sequence reads of tens to hundreds of kilobases (10-100 kb) with extreme examples reaching more than 2 million base pairs (>2 Mb long Nanopore reads). Thanks to long read sequencing, most repeat regions can now be solved and placed in the proper context of the unique part of a genome.

The genome of Tulipa gesneriana 'Orange Sherpa' consists of 34 billion basepairs

Revolutionary tool

With this knowledge in mind, bioinformatician Christiaan Henkel from Leiden University became interested in accepting the challenge of assembling the huge genome sequence of the tulip. In 2014, the Leiden based company ZF-screens BV (partially continued as Future Genomics Technologies BV) had just joined the early access programme of Oxford Nanopore's MinION technology and was eager to be part of this prestigious plan. Financial support for a pilot project was provided by Hans van den Heuvel, R&D director of Dümmen Orange, one of the main floriculture companies in the Netherlands, and the project was embedded in a small consortium that also included Leiden University, University of Applied Sciences Leiden and the genomics company BaseClear. Initial issues related to extraction of pure and high molecular weight DNA from tulip tissue were successfully solved by Hans Jansen and his team members at ZF-screens/Future Genomics and, subsequently, enough Nanopore long reads could be generated to cover about 6 times the estimated size of the tulip genome (6x 34 Gb = ~200 Gb of raw Nanopore sequence reads).

In the meantime, Christiaan Henkel had begun to develop a revolutionary new de novo assembly tool, which he named TULIP, an acronym for The Uncorrected Long-read Integration Process. This was mainly triggered by the limitations of existing de novo assembly tools, which used the strategy of comparing every individual long sequence read with every other individual long sequence read in the complete pool of raw sequence data. This approach works perfectly well for small genomes of up to tens of millions of base pairs, but becomes extremely demanding with respect to computing power when the genome size is much bigger than a billion base pairs, not to mention the extreme size of the tulip genome.

Jigsaw puzzle

Instead of comparing complete Nanopore read sequences with each other, the TULIP assembler uses only the short informative parts of the long reads in the assembly process. A simple metaphor is a jigsaw puzzle, where large areas of 'water' and 'sky' that are difficult to assemble are ignored and only unique pieces that contain a 'boat' or a 'tree' are used in the assembly process. A proof-of-principle version of the TULIP programme was then used to assemble the first vertebrate genome sequence from Nanopore reads alone, namely that of the European eel (Anguilla anguilla), which was published in Scientific Reports in 2017.

When the TULIP software was first used for de novo assembly of the tulip Nanopore reads, it resulted in a total assembly length corresponding to approximately 15% of the estimated genome size. This indicated that the software was working; however, based on 6-fold coverage (~200 Gb) of raw Nanopore data, one would expect a theoretical maximum assembly length of at least 60% of the estimated 34 Gb genome. In the past year, a massive effort was made by Christiaan Henkel and Hans Jansen and his team to further optimise the TULIP software, resulting in the much improved Tulipa julia de novo assembly pipeline.

The major breakthrough was reached in February of this year, when 65% of the tulip genome sequence could be successfully assembled from the same set of ~200 Gb raw Nanopore data. Astonishing detail: the whole assembly process took only a few days on a single CPU!

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Conquering Varroa Mites

Breeding a perfect **Bejo bee**

Monique Krinkels

Most honeybee colonies that contain varroa mites are severely weakened if untreated. But there are hives where bees and mites live in (not overly friendly) co-existence. It is a result of a behaviour that is based on genetic characteristics. Reason enough for some employees at plant breeding company Bejo to extend their work to something completely different: bee breeding.

The 1,700 employees at Bejo are supported by a diligent team of co-workers. About a billion bees make sure all plants are pollinated. They are indispensable assistants for seed production. “Our 20 beekeepers take care of the 10,000 hives at Bejo, each inhabited by about 50,000 honeybees. In addition, we hire hives off private beekeepers,” explains Youri Draaijer, international seed production research manager.

For Bejo, investing in beekeeping is of course a matter of business interest. “In our international Bee-group we discuss how best to keep our bees healthy and productive. We select bees based on several characteristics such as no tendency to swarming, eagerness to collect nectar and, not to forget, a placid character.”

Natural behaviour

The Varroa destructor mite is the largest threat to apiculture worldwide and has been responsible for devastating losses of wild honeybee populations in Europe and North America. The appearance of the Varroa mites outside Asia is relatively recent. Wild populations of the Asian *A. mellifera* are well adapted to these pests. They clean themselves and each other regularly by rubbing, removing mites and biting off their legs. In addition, they carefully watch over the larvae as the mites can only reproduce in honeybee brood. If a mite has entered a cell of a brood box, they react immediately, removing the infested larvae out of the hive. “The idea is that they are able to somehow sense infected larvae,” says Youri Draaijer.

Productive insects

100 grams of honey requires 256 bees to travel 40,055 km and visit 1 million flowers. That is 156.5 km per bee.

This behaviour, called VSH (Varroa Sensitive Hygiene), is not limited to Asian bees. Some European honeybee colonies display the same activities.

“It must have been five years ago that our former CEO, Ger Beemsterboer, suggested we should start breeding Varroa resistant bees. Healthy bee colonies safeguard the natural pollination of the seed production fields. But Ger Beemsterboer was also motivated by the social importance, as bees have a crucial role in food production and nature.” A third of global food production is dependent on animal pollination. The large majority, 80-90% of this pollination, is done by honeybees.

Queens

Cross breeding is something new for beekeepers. Hitherto, beekeepers were limited to selecting the best colonies and induce the worker bees to produce queens. “That is fairly simple. As soon as a young larva is put into a large cell, the workers start to feed it the so-called ‘royal jelly’, instead of the pollen and honey fed to the future worker bees and drones. Hereby all these larvae will develop into queens,” adds Youri Draaijer.

Beekeeping began about 9,000 years ago, but the level of domestication of the bees remains limited, even today. “In fact, honeybees are still wild animals. We can provide them with a sugar solution as feed, but that does not match the natural nectar and pollen. As for royal jelly, it is secreted from the glands in the hypopharynx (a part of the mouth) of nurse bees.” As a consequence, people can enforce a colony to produce queens, but only in the summer when there are sufficient flowers to provide the necessary nutrients. The breeding period is therefore restricted to a few months.

Markers

The fast route to obtain honeybee colonies with VSH is first to determine which genes are involved in that behaviour. “That is what we have started to do,” says Henk Huits, research manager Marker Technology & Genomics. “We have just decided how to approach this project. We have selected 150 hives with bees that don’t show the VSH-behaviour at all and 50 hives with colonies that are 100% clean. Researchers will sequence the pools of bees from the hives. We then hope to identify the regions involved in this trait by looking at changes in allele frequencies. It is, however, uncertain whether the behaviour is based on the same genes in all colonies. What we hope and expect to find, however, are markers that are unique for chromosome fragments involved in VSH.”

Varroa mite

The Korean varroa mite (*Varroa destructor*) was first discovered in South East Asia in western honeybee colonies in about 1904 but is now present on all continents, except Australia. During the 1980s, the mite quickly expanded in Europe, the USA and Southern America. The mite can only reproduce on honeybee brood. It directly weakens the bee by sucking fat body tissue and haemolymph (‘insect blood’), but also causes severe damage to the bees by spreading viruses and bacteria. For example, the Deformed Wing Virus (DWV) which causes deformed wings and shortened abdomen.

With markers it will become possible in due course to select for VSH-colonies. “But it will not be as easy as it sounds. The differences between plant and bee breeding are enormous. There are, for instance, no pedigrees of colonies available and there are no uniform lines as in plants.” The common practice is to select a queen and have her fertilized by ten to twenty drones. To prevent foreign drones from participating in the mating party, this is often done on islands or peninsulas, as water forms a natural barrier for bees that they tend not to cross.

“It will take at least four years to define the regions in which the VSH-genes can be found. After that, we have to validate these regions and the markers involved. And only then can we start cross breeding bees. To complicate matters, bee genetics is special. The drones are haploid and they stem from the queen’s unfertilised eggs. While worker bees, as well as the queen herself, have 2 x 16 chromosomes, the drones only have 1 x 16. But artificial insemination is possible. We can fertilise the queen with a single or even with multiple drones,” according to Henk Huits.

One of the advantages of plant breeders applying their knowledge to select for new bee varieties is that they are used to work with large numbers. “We produce thousands of seeds that we never plant, if we decide the line does not have the proper characteristics. We might try the same with bees, producing a lot of queens from which we will select the best ones to continue the breeding programme with.”

Cooperation

Honeybees are in the news almost on a weekly basis. The increased losses of colonies have the attention of governments, companies and the public in general. There are several initiatives in Europe and the USA to create Varroa resistant honeybees. The problem is that most of these projects are run by volunteer beekeepers. While they are very motivated, their options are limited. Most of them have no more than twenty hives, they lack a scientific background in genetics and breeding and they do not have adequate financial support.

In the Varroa-project, Bejo closely cooperates with Arista Bee Research. This foundation combines the efforts of beekeepers, institutes, universities and other stakeholders in a Varroa resistance breeding programme. “It is not simply crossing a VSH-queen with a VSH-drone. The other characteristics such as health, eagerness to collect nectar, efficiently pollinating crops and a gentleness are at least as important to the future Bejo bee,” concludes Youri Draaijer.

Increasing yields

Super-plant promises new agricultural revolution

Maarten Keulemans

Plants that grow much faster, fields that yield crops several times per season. Man is about to hack photosynthesis. It will, according to everyone involved, turn agriculture on its head.

At Nijmegen station, out of a neglected piece of pavement near a sandy shunting yard, grows a small plant that can change the world. Not that Hirschfeldia incana is so distinguishable. It is no more than a flat clump of weed from which slender flowers sometimes grow. Yet, ‘hoary mustard’ (due to grey green foliage), as experts call it, possesses a secret so special that PhD student, Pádraic Flood, has harvested some of his seeds with the greatest care and taken them to a greenhouse in Wageningen. The plant is now there, while professor of plant genetics, Mark Aarts, and associate professor, Jeremy Harbinson, look at him with great delight and say: “This is our champion. He is doing exceptionally well.”

The art of photosynthesis

Because let hoary mustard go its own way, and within, it comes to life in a way from which other green plants can still learn. Light particles stimulate its chloroplasts, enzymes pluck carbon dioxide from the air, atoms click together to form sugars and proteins; it all goes much more smoothly with hoary mustard than with other plants. The weed masters the art of photosynthesis - the conversion of water, carbon dioxide and light into green matter - much better than other plants.

Discovered in Australia by chance, says Harbinson, by a biologist blown over from Ireland, with a round face and a busy patterned shirt. “A photosynthesis researcher picked one from the garden to test his equipment. By pure chance, he seems to have chosen a superstar in the field of photosynthesis.”

Something strange is happening with photosynthesis. The process, evolved at a time when there was hundreds of times more CO_2 in the atmosphere than now, only works at a very low level. A green plant uses only 0.5% of incoming sunlight for photosynthesis, and that is similar to a restaurant customer who orders an entire meal and then only takes a few bites. Shame, inefficient, lazy, biotechnologists always say. Although that is of course just how you view it, emphasizes professor of plant genetics, Mark Aarts: “It is undoubtedly good enough for nature.”

But behind the scenes, things are happening. In the past few years, several experiments have succeeded in increasing the photosynthesis of green test plants, such as thale cress and the tobacco plant, by tens of percent, resulting in plants that grow up to one and a half times as fast as the unaltered ones. Futuristic situations: for example, a London research group led by biochemist Bill Rutherford grew loose purple and yellow chloroplasts that also absorb energy from infrared light - which provides insight into purple plants growing at night.

First experiment

And more is coming. Experts eagerly await the results of the world’s first experiment with enhanced grain, in a closely guarded test field at the University of Essex near London. Meanwhile, in the American state of Illinois, another team is testing super-photosynthesis in African yardlong beans and soy. “The pieces of the puzzle are starting to fall into place,” points out Wanne Kromdijk, a Dutch plant physiologist who researched photosynthesis in Illinois. “The next major breakthrough is that we are finally going to see if this also works in crops that make us happy, instead of test plants such as tobacco. Will we really be able to produce more food as a result of this?”

This will be the next big revolution, says almost everyone to whom you talk about it. The sensation which will turn agriculture completely on its head. “You have to think about crops that will grow much faster and larger than they are now,” says Harbinson. “And about fields that will yield crops several times a season.”

And not only that. Super-photosynthesis will also give the plant more and deeper roots, expects Harbinson, and therefore the plant can store more carbon underground. The result: healthier, better nourished soils, more soil life and more CO_2 captured from the air. According to some calculations, a few extra percent of photosynthesis would be enough to make half of all the carbon emitted by humans disappear into the soil. Part of it is released again later when the plant dies, in the form of fermentation gas. But gigaton or thereabouts trickles down, petrifies, disappears from circulation.

And as if CO_2-guzzling fields with metre-high corn stalks are not exciting enough – wait till you see the ‘bycatch’. Larger and fuller crops mean less land, leaving more room for nature and energy crops such as sugar cane, oilseed, elephant grass and firewood, which are desperately needed to achieve the climate targets. Certainly, an area as large as India full of organic crops will be required for this, an analysis showed last autumn.

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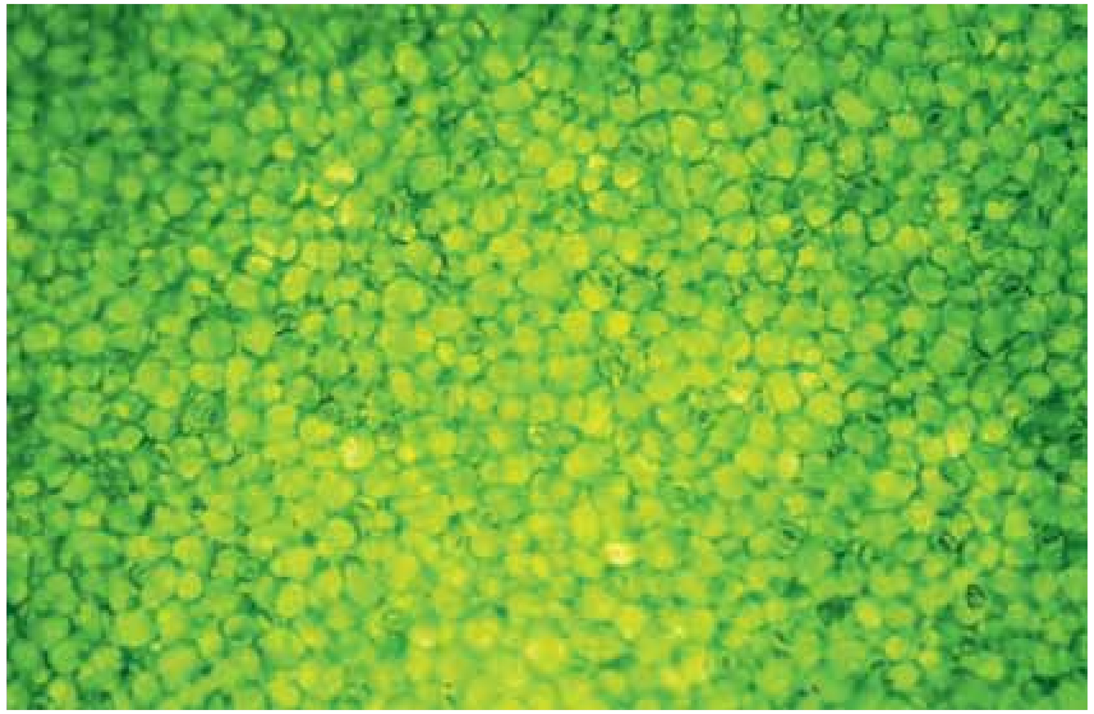


Photosynthesis consists of two systems: a ‘light-dependent reaction’ in which sunlight converts water into oxygen and energy, and a ‘light-independent reaction’ in which the chloroplast uses the energy to convert CO_2 into sugars

Environmental benefits

Moreover, the super-crops have unexpected environmental benefits, says Kromdijk, skyping from Cambridge, where he now works. He discovered, for example, that a tobacco variant whose photosynthesis he had enhanced, uses a quarter less water. “That was a complete surprise,” he says. It is also expected that crops that have been enhanced will use nitrogen more efficiently, which would mean that the plant would need less fertilizer and emit less laughing gas (N_2O), a greenhouse gas. “We do not want to give the plant a larger engine, but an engine that runs more efficiently,” says Kromdijk. “Then these are the benefits that you get.”

Just need to see that it works. The chain that makes up photosynthesis consists of approximately 140 stages. An infinitely complex sequence of chemical reactions, side steps, accelerations and feedbacks, whereby waste is now released again which the plant has to dispose of, then the re-emergence of hormone signals that halt everything and the next time suddenly the energy supply is interrupted again because the electrons have run out.

“Initially we thought: we will just do this by breeding existing varieties,” says Harbinson, who was already working on photosynthesis when Abba’s ‘The winner takes it all’ was still in the charts. “But it turned out to be much more complicated than that.”

A major catalyst of the new spring is an influential state-of-the-art article that appeared in 2010 in the trade journal Annual Review of Plant Biology. Turning photosynthesis on its head in one fell swoop is not possible yet, conclude Don Ort, Steve Long and Xin-Guang Zhu from the University of Illinois. But don’t worry: the darn process has at least nine weak points that can definitely be improved.

Placing the leaves better in the sun will yield a gain of 30%, outlines Ort, among other things. More lightly tinted leaves through which sunlight can penetrate deeper into the foliage: an additional 30%. Better processing of CO_2 inside the chloroplasts: again 30%. “All the changes together have the potential to more than double the yield of our most important crops,” thus says Ort.

Exhausted revolution

And no wonder, writes the group. So far, agriculture has pretty much been able to keep up with population growth, thanks to the tsunami of improvements in cultivation, fertilization and varieties that together are called the Green Revolution. But the revolution is starting to become exhausted. For example, the yield of rice per hectare of paddy field has not increased for some ten years. And that while the population is becoming more prosperous, is eating more and more luxuriously and is also growing up to an expected 10 billion mouths in 2050. That means that productivity must increase by 60% to as much as 120%, another team calculated.

That was exactly the pep talk that was needed. The charity foundation of Bill and Melinda Gates kicked off a major research project to improve the photosynthesis of crops such as cassava, soy and yardlong bean, and in Wageningen, chairwoman Louise Fresco declared hacking photosynthesis as a priority: the ‘holy grail of our profession’.

Meanwhile, the research is starting to yield more striking results. Take ‘Rubisco’, a crucial enzyme that is responsible for processing CO_2. Great, were it not for the fact that Rubisco works extremely

Pimp your plant

There are countless ways to boost photosynthesis. Here some of the most extraordinary.

1. Use a better dimmer

The problem: in bright sunlight, the plant slows its photosynthesis. Once in the shade again, it takes a while for the plant to ‘restart’ again.
Solution: incorporate a faster gearing-down. Or ensure more upright leaves that are less exposed to the sun.
Status: the first solution works with tobacco: 15% more biomass. The second solution is already widely used in distribution programmes for, among other things, corn and rice.

2. Become translucent

The problem: the light-processing components (‘thylakoids’) are packed so tightly together that light can hardly penetrate the leaf.
Solution: make these ‘antennas’ less tall, so that the leaves become more translucent and light is better distributed throughout the leaf.
Status: is currently being investigated for, amongst others, tobacco and soy, with hopeful results.

3. Colour your granules

The problem: plants use only a limited part of the light spectrum. In addition, plants have two photosynthesis systems that compete for the same light particles.
Solution: build the purple algae system into the plant so that it can also ‘receive’ infrared.
Status: successfully tested in test tubes, ongoing research.

4. Get a CO_2 pump

The problem: the supply of CO_2 is inadequate, because the gas gets stuck between the cells and because the Rubisco processing enzyme is slow and negligent.
Solution: provide the chloroplasts with ‘carboxysomes’, balloons that gather CO_2, originating from cyanobacteria or certain primitive plants.
Status: under investigation with tobacco.

5. Clean up your mess

The problem: waste products are produced when CO_2 is processed, after which the plant spends a lot of energy breaking it down.
Solution: give the plant a system that breaks down the waste more efficiently.
Status: tested with tobacco, 40% more biomass.

6. Accelerate the supply of raw material

The problem: CO_2 is processed in the plant by binding it to a sugar called RUBP. But after that, the RUBP has to be recreated again and that requires a lot of energy.
Solution: by allowing the plant to produce more of a certain protein, the RUBP replenishment is faster.
Status: provided 30% more revenue from tobacco, now in research on food crops

carelessly: one in five times the enzyme grasps the wrong molecule, oxygen instead of CO_2. After which a mess is created which the plant has to clean up - which delays the photosynthesis. Orts’ team put a few more efficient ‘clean-up’ molecules to work, from the pumpkin and from algae: his tobacco plants promptly grew by 40%. “We see that all the time,” Aarts explains. “Improve the process slightly, and you will immediately see a very striking advancement. Very encouraging.”

Or take the discovery with which Wanne Kromdijk made the news around the world a year and a half ago. Working for the Bill and Melinda Gates Foundation project, Kromdijk tightened a chemical spring in photosynthesis, whereby a plant jumps back to full photosynthesis more quickly when it comes into the shade from bright light. Voila: another additional 15% biomass. In Australia, in the meantime, others have embedded a type of CO_2-catching balloons from algae into plants: in theory, good for another 25% more green.

Ethics

But such striking results also create concerns. Because all that technical tinkering, as if such a plant is an old moped, is that appropriate? “There is something wrong with nature and we will just quickly fix it”, ethicist Bernice Bovenkerk (University of Wageningen) summarizes the underlying idea.

No misunderstanding though: “If we can develop a plant that uses fewer raw materials and produces more, then I really don’t necessarily have any objections,” emphasizes Bovenkerk. “But are we going to use that extra production capacity to unburden nature and the environment, or just to meet even more human needs?”

After all, a lesson from the Green Revolution is that agriculture became indeed more efficient, but also that the population could continue to grow, that new environmental problems such as over-fertilization arose, and that the proceeds disappeared mainly into the pockets of the rich, Bovenkerk explains. “I am not saying that it will be like this again. But you have to keep in mind: which problem do we actually want to solve? And is such a technofix then the correct solution?” Hunger is primarily a distribution issue, she emphasizes. “People living in a conflict area do not have enough food because crops do not grow fast enough, but because they live in a conflict area.”

On the other hand, Aarts says: “I know many people who would like to see their little field with yardlong beans yield twice as much.” The answer lies somewhere in the middle, Bovenkerk believes. “In any case, it has to be contemplated. Let us not forget that every new gimmick also has new consequences for

Hirschfeldia incana (hoary or short pod mustard) is the only species in the monotypic genus Hirschfeldia which is closely related to Brassica

the structure of agriculture.”

Genetic modification

And then there is the technology itself. The new super plants are generally genetically modified - the genes of a bacterium or of another crop have been screwed in - and that technology is heavily restricted in Europe and is controversial elsewhere, to say the least. “We think it is possible without genetic modification,” says Aarts. “But it would be very handy if we had it as well.”

By the way: can it do any harm, such a genetically enhanced boaster in the field? Can it not escape, repress other plants, outgrow nature? Take the dioecious amaranth (Amaranthus palmeri), another natural powerhouse when it comes to photosynthesis. The plant, edible and once an agricultural crop of the North American Indians, is considered a nuisance herb in Asia, Europe, Africa and North America itself. And that is not least because it grows so fast. Except that is a desert plant, hardened by millennia of drought and severity on the prairie. A case apart. That is different with food crops. “Corn is not overrunning the Netherlands either,” says Harbinson. Perhaps that applies even more to the new varieties. The other plants see it coming, with its finely tuned photosynthesis and semi-translucent leaves. “They will make mincemeat of it,” says Aarts.

That does not alter the fact that much can be learned from natural tough things like the dioecious amaranth or the Hirschfeldia incana plant from Nijmegen. In a long cellar corridor, PhD student Roel van Bezouw opens a large, iron door to show how that learning is working. We see rows of small green seedlings, 1,440 in total, of the thale cress Arabidopsis thaliana, the laboratory mouse among plants. Van Bezouw is on the trail of a gene that slightly speeds up photosynthesis, he says. And even if it only concerns 1% improvement, the gene must be found. “It must be somewhere on chromosome 5,” he says, pointing at the plants. So, the thale cress is cross-bred and cultivated here in the basement and carefully compared to each other, in the same way you select the largest from a bowl of fruit.

Radical changes

“Because don’t think we’re already there,” says Harbinson. “No matter how visionary and beautiful the American and British work is, it does not provide the doubling in photosynthesis that we do see with some photosynthetic top athletes in nature,” he says. “More is needed for the radical changes that we require. By studying extreme photosynthesis champions that we find in nature, we hope to detect something very new. “

Plenty of indicators. Take the experiment by plant technologist Tom Theeuwen, one of Aarts’ PhD students, with the thale cress. With the test plant, Theeuwen decided to shuffle the chloroplasts of one plant with the cells of another. With insane results: suddenly one of his test plants yielded up to 30% more biomass, for reasons he does not yet fully understand. Photosynthesis is sometimes like a faltering old TV: give it a big blow, and suddenly it is working again, for no apparent reason.

The photosynthesis of the future will not have one recipe, Wanne Kromdijk expects. “Rather I’m thinking of an array of techniques and methods. And which is the most effective will differ per climate zone and per crop.”

The farmer in Africa with an enhanced bean that requires little water, the European who wants to achieve his climate goals with an oil crop with an insatiable appetite for CO_2, the American soy producer who wants to produce plenty of food with a brown-purple crop that does not look good but that rapidly produces soy and corn.

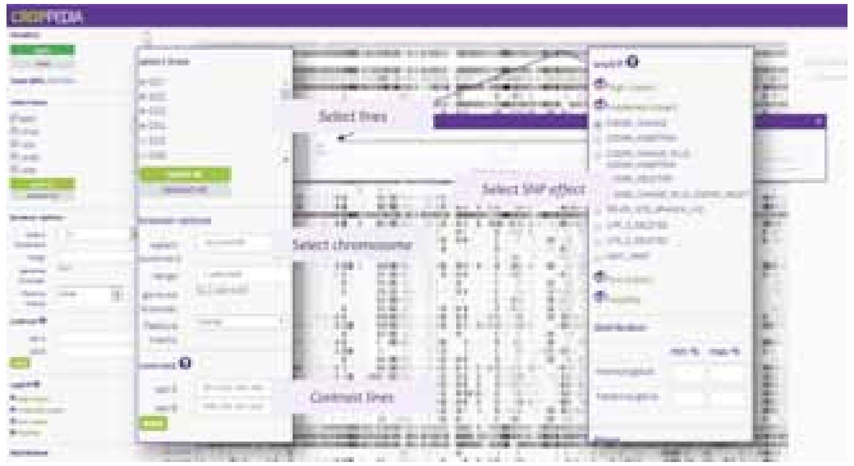
It is a strange, somewhat unsettling future that is looming. “I always say thus,” muses Harbinson. “If nature was already perfect, I wouldn’t need my glasses.”

Easy Access to Information

CropPedia discloses the **needle in the haystack**

Monique Krinkels

Imagine you are a lettuce breeder and you want to improve the taste of a butterhead in your new breeding lines. Optimising the biosynthesis of volatile X seems the best option. Wouldn't life be easier if you could identify the best genotypes within your germplasm in just a few seconds? Well, that is now possible: just look it up in CropPedia.



CropPedia is a user-friendly tool to visualise genome-wide genetic variation. It can handle hundreds of genotypes, billions of SNPs and convert the data into interpretable visualisations

Research on genetics and genomics generally yields huge piles of unorganized data from genetic and genomic research. Finding your way through these data, or worse, trying to find a specific lead, is like looking for a needle in a haystack. To make life easier, KeyGene data scientists have developed CropPedia, a software package that provides combined access to and use of genetic and genomic databases and scientific papers. CropPedia visualizes genome-wide genetic variation. It can handle hundreds of genotypes, hundreds of millions of genomic features, billions of SNPs and convert the data into interpretable visualisations. With it, users are able to better direct plant and animal breeding and other research and to find lead genes more quickly, thus yielding time gain and faster market introductions.

Fast and easy

KeyGene's research for crop innovation is one of the sources of the huge piles of data on genetic resources. Its data scientists know how to handle this data overload to find clues to improve varieties. To make the information available to breeders, KeyGene developed CropPedia - an intelligent interface that allows people to really make use of the data provided by KeyGene, data generated in other ways and scientific papers available worldwide. CropPedia has been especially developed for two types of users: scientists involved in biological research and bioinformaticians. Biological scientists can easily find and visualise relevant information, bioinformaticians can easily extract relevant data for further analysis.

CropPedia is as easily assessable as its famous namesake, Wikipedia. Just type in the name of the trait and, in a matter of seconds, CropPedia shows which genes are known to be involved in its biosynthesis, what variation in the genes is available in your germplasm, what variation in gene expression is known and which scientific papers deal with the genetics of this volatile. All of these are 'clickable', allowing you to get access to the abstracts of the scientific papers and details about the genes and genotypes.

Zillion data

Or if you know the location of some important QTL's of an important trait on the plant's chromosomes, CropPedia will identify a list of genes in those regions for you. CropPedia couples those possibly involved genes with available knowledge about those genes. This will tell you which genes are most promising, what the genetic variation is within those genes within his or her germplasm, what gene expression data is available, which other genes have the same expression scheme and what papers have described the genes. Handling the DNA sequence data of hundreds of genotypes, hundreds of million genomic features, gene expression data of more than 100 k gene/environment combinations, biosynthesis pathway data encompassing thousands of compounds and PubMed indexed scientific papers - CropPedia users can query and extract useful information in just a few seconds.

An overview

Organic agriculture is growing steadily

Daniël Ende

Within the complete spectrum of farming, the organic sector is a well-established and respected sector. Although relatively small in size compared to conventional farming, the area under organic management has shown steady growth throughout the years. Eurostat data demonstrates an increase in area and share of the total agricultural area under organic management.

The common goal in plant breeding: to create diversity and choice in varieties. All farmers have to be able to choose the varieties best suited to their farming conditions, production goals and personal wishes. Whether they cultivate GMO maize, flowers under conventional conditions or organic carrots, growers need a wide palette of varieties to be able to choose the variety that best suits their needs. The plant breeding sector provides diversity to choose from, in varieties of trusted quality.

Most of the professional plant breeding companies are focused on conventional farming. However, several see a growing market in providing high quality seeds to the organic sector. Some companies exclusively produce for the organic market, others produce for conventional as well as organic farming. The development of varieties suited for the organic sector also provides valuable information for the development of varieties for conventional farming. After all, all forms of farming benefit from robust varieties, with a lower need for artificial fertilizers and plant protection products, while remaining on par in quality and quantity of harvests. Testing varieties under organic growing conditions helps plant breeding in general to contribute to the sustainability of all forms of farming.

Supply and demand

Today, an estimated 60-70% of seeds used in organic production in the EU are produced under conventional farming conditions, the only difference with conventional being that they do not receive any chemical treatments before selling. In part, this is due to the farmers' lack of knowledge of availability of organic seeds, but cost difference compared to organically produced seed may play a role as well.

	2012	2013	2014	2015	2016	2017
European Union 28 organic area x 1,000 ha	10,048	10,071	10,315	11,106	11,932	12,560
Percentage organic of total agricultural area	5.64%	5.65%	5.78%	6.20%	6.67%	7.03%

Data source: Eurostat

The European Union is currently investigating the availability of organic seeds.

At the same time, efforts are being made to ensure that Member States have regularly updated databases with quantities of seed for organic farming available in the market. This will help organic farmers find the best suited varieties for their situation. It will also boost demand for seeds originating from seed companies that develop varieties for the organic market and that produce seed under organic conditions.

Heterogeneous material

Farmers already have a lot to choose from in varieties of different types, but more options are on the way. Many professional growers like to use F1 hybrid varieties for their 'hybrid vigour', often leading to strong characteristics such as better disease resistance, uniformity and often a higher yield. Others prefer open pollinated varieties that show some diversity in the field but are still defined as a variety. In some crops, varietal mixtures may be marketed. A new option has been created, as organic production regulation 2018/848 opens the market to heterogeneous material. Heterogeneous material can be described as a population with a high level of genetic diversity, not being a mixture of varieties.

The regulation stipulates that European marketing conditions that apply to varieties do not apply to heterogeneous material. However, there will be specific conditions for marketing in order to ensure only quality seeds reach the market and to ensure there is no possibility of infringement of plant breeders' rights. One of the restrictions that has already been specified in the regulation is that the heterogeneous material will have to be bred and produced under organic conditions.

In the second half of 2019, the Directorate-General Santé will establish the conditions under which heterogeneous materials may be marketed.

Breeding for Organic vs. Organic Plant Breeding

Most professional plant breeding companies develop new varieties under conventional management, i.e. with the use of synthetic fertilizers and chemical crop protection. Plant breeding companies that select cultivars suited to organic farming will then test new varieties for performance under organic management. Certified seeds of the successful varieties are then produced under organic management.

EU Regulation 2018/848 mentions organic plant breeding. This means plant breeding activities where the entire development of new varieties is carried out under organic management, i.e. without the use of synthetic fertilizers and chemical crop protection. There are hardly any commercial plant breeders that develop new varieties under organic management at this time. The suboptimal field conditions create great risks, such as a loss of (promising) varieties and/or seed productions that do not meet set quality standards.

A third option still available to farmers today is the use of non-chemically treated (NCT) seeds produced under conventional conditions. Often, these are varieties that have been developed for the conventional sector. According to EU Regulation 2018/848 this option will no longer be available after 2035. The EU is currently investigating availability of seeds for organic farming in order to see when the derogations for use of NCT-seeds can end without ending organic farming of the crops involved.

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Gene Pool Erosion

Cut rose breeding **in peril**

Dik de Vries and Lidwien Dubois

Despite a flow of newly introduced varieties, the genetic base of cut roses is too narrow to use them successfully in future breeding programmes. Breeders should therefore reduce inbreeding effects and breed more vigorous varieties. To that aim, a number of practices scarcely applied in rose breeding, may be successfully used.

Economically, cut roses are the most important of all ornamental crops. A rough estimate is that all over the world, there are about 20,000 ha cut roses. Varieties are grown on their own roots or grafted on mainly vegetatively propagated rootstocks. Currently, about 70,000 plants/ha (7 pl/m^2) is a standard. Depending on the grower's skill and cultivation under various environments, there is a considerable variation in the shoot production per m^2, however, 200 shoots per year yielding €30-150 is a good average.

Present situation

Rose producers are provided with new cultivars by no more than ten prominent breeders. These breeders maintain batches of 200-500 progenitors, to be used in cross breeding. New progenitors are chosen among recently introduced varieties, on the basis of flower colour, shape, size, number of prickles, shoot length, shoot yield and fruit set after experimental pollination. Perfume and disease resistance play minor roles only. The descent of these varieties is known by their breeder only.

Depending on the previous year's results, the composition of these batches changes continuously, resulting in genotypes that are rarely older than ten years. From the mentioned 200-500 progenitors, breeders raise about 1,000,000 seedlings, resulting in 100-150 new cultivars annually. In the current assortment, varieties are rarely older than 15 years. Most new cultivars are protected by breeders' rights or plant patent, which is the basis for breeders to maintain their business. Important growers, however, increasingly try to influence the level of royalties on plants.

Large and medium-sized flowered cut rose varieties belong to the Hybrid Tea (HT) class, which are allo-tetraploids (2n=4x=28), and vegetatively propagated. Except for the nulliplex character 'perpetual flowering', seedling populations show large segregation for all plant properties, the combination of which enables the breeders to select. HT roses are bred in the glasshouse in 2-year cycles. *First year*: crossing (2-3 months), fruit ripening (5-6 months), seed stratification (3-4 months). *Second year*: seed germination, transplanting of seedlings in e.g. 7x7 cm pots, 1st flowering of seedlings, first, second and clonal selections.

More than 10,000 hybrid tea roses have entered the market since 'La France' was introduced in 1867

HT seedlings grow fast: under glasshouse conditions at 20°C, plants develop a 20 cm long shoot with 6 alternate leaves and an apically placed flower in 50 days. In breeding practice, first selection is made at 1st flowering of the seedlings, where 15-20% of the plants is retained and replanted in larger containers for second selection. A few of these plants become 'own selections' for further breeding, but all individuals that are not chosen are definitively discarded. In the subsequent years, the 10% plants that survive second selection, will be cloned and tested under various environments over the course of several years.

Not really new

It is a fact that the results of crossing and selection do yield new varieties, but on close inspection 'new' is 'different', rather than really new. It should be mentioned here that, although breeders design crossing schemes each year, at the same time there is some scepticism about the results of 'aimed crosses', because the best varieties often came as a surprise as they were selected from insignificant populations. Owing to lack of registration of plant data, the descent of several important varieties was lost.

Comparing the composition of individual batches of progenitors at breeders, shows a gigantic overlap of genotypes; a rough estimate is up to 50%. The other 50%, the so-called 'own selections' are descendants of the original 50%. The total number of progenitors actually in use can only be estimated.

Considering the present narrow gene pool, the continuous erosion of this pool, as well as the way new cultivars are created, it was no surprise that, doubtless due to a slow inbreeding process, the yield of new cultivars would slowly decrease. Although in the practice of rose growing, yield reduction of cultivars has hardly been observed, an analysis of the flower yield in the 2nd year of more than 200 newly introduced HT varieties, tested over a range of 17 years (1968-84), certainly showed that a slow yield reduction had already begun in the eighties of last century. A similar situation, but from a different angle and with much more reliable data, has been reported with regard to the gene pools of apple and cherry, where breeders have been operating with greatly reduced genetic diversity since the end of the 20th century.

In HT seedling populations, the effects of inbreeding should be perceptible as an increase of the percentage of similar plants, a reduction of the mean plant height at first flowering and an increase of the percentage of rogues. Now, it is a known fact that despite the availability of breeding software, rose breeders hardly make or record plant observations, so that exact data about populations is lacking. However, when asked whether there are more similar seedlings per population than, for instance, 20 years ago, the breeders' answers are affirmative, but populations must be larger to select outstanding plants. In addition, it has been noted that several, initially good-looking seedlings had shoots that were too short when cloned.

Equally old hybrid tea rose seedlings differing in length at 1st flowering

It is thus seen that, despite a flow of newly introduced cut rose varieties, their genetic base is apparently too narrow to use them successfully in future breeding programmes. If so, that situation seems a challenge and an opportunity for breeders to reduce inbreeding effects and breed more vigorous varieties. To that aim, a number of practices, scarcely applied in rose breeding thus far, may be successfully used to raise such genotypes.

How to proceed

Considering the above situation, it is clear that although science may play a role in showing the way how to proceed, that in addition to their normal breeding activities, the practical execution is primarily a matter for the breeders.

Genetic modification. Over the last ten years, research has made tremendous progress. The rose genome was unravelled and genes mapped. Indirect and direct plant regeneration of rose has been realized so that GM, Cis-genesis or CRISPR-Cas may be applied. Great hope was drawn to absolute disease resistance, different flower pigments or higher yield. Owing to scarce plant data, the development of molecular markers has lagged. However, despite these important developments, it is feared that, due to the wise decision of the Court of Justice EU: (Case C-528/16 of 25 July 2018), the highly desired application of modern techniques in plants and animals has effectively been postponed for years.

Mutation Breeding. Spontaneous mutations, 'sports', seem rather frequent in the rose. Although

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climbing mutants from bush roses are known, a remarkably large percentage of sports relate to flower colour. From several varieties, complete families of colour sports have been selected e.g. from the once-famous 'Koster' roses, from 'Frisco' and recently, from 'Avalanche'. Colours tend to shift from white to pink, from red to pink, or from deep red to orange. Evidently, mutations occur in the genes that are responsible for anthocyanidins and leucoanthocyanidins, rather than in the ß-carotenoids, which are responsible for yellow flower colour. As mutagens X- and g-rays have been used, while so far, the use of chemicals is unknown. Ideally, for each new cultivar, a dose response curve should be established, but in practice doses of 35-50 Gy X-rays have been applied, aiming at LD 50. Due to the recommended use of *in vitro* plants, that cannot be produced in own management by breeders, mutation breeding in the rose has not been popular. Much more suitable and cheaper starting material than *vitro* plants, are just-rooted softwood cuttings of cultivars. The selection process of mutants from both irradiated cuttings and *ex vitro* plants remains laborious, since it involves repeated cutting-back to lateral buds. This does not mean that mutation breeding should be discouraged. On the contrary, cross breeding with mutants may be highly promising.

Hybrid tea rose softwood cuttings treated with 45 or 50 Gy X-rays

Interspecific crosses. As mentioned, HT roses are allotetraploids, produced over a range of centuries, initially by open-pollination and later by aimed crosses. Despite the aversion of breeders to use *species* roses in crosses with cut roses, the reintroduction of genes from a careful choice of known species like *R.chinensis* Jacquin, *R.multiflora* Thunberg or *R.foetida* Herrmann, offers promising opportunities to broaden genetic diversity again.

Reuse of important former varieties. Genes from all important cut rose varieties of the late nineties have largely contributed to the properties of the current assortment. Reuse of those varieties in crosses with modern ones, seems another simple and not too drastic way to broaden genetic diversity, simultaneously meeting inbreeding depression. Although there is no known gene bank for HT varieties available, there is 'The Historic Garden' Aalsmeer, the Netherlands, where a large number of former HT cut rose varieties are being conserved and are available for propagation.

Selection for shoot length and yield. This highly effective method of selection, developed at the former Institute of Horticultural Plant Breeding, Wageningen, has rarely been applied since it requires counting and registration of all seedlings in populations and, regrettably, sometimes discarding of good-looking seedlings. HT-seedlings of the same age (= day of seed germination) differ in both the time to 1st flowering and in plant height at that time. It was shown that plants with a short time to 1st flowering, yield the highest number of shoots when adult. Simultaneous selection for long shoots among 'early' seedlings, has proven highly effective. In several populations, however, the combination 'early-long' just fails to occur.

Deliberate inbreeding. Deliberate inbreeding in any crop always aims at greater uniformity and/or heterosis or 'hybrid vigour'. While in seed propagated crops inbreeding is a common practice, leading to desired uniformity, in vegetatively propagated crops (apple, cherry, rose), inbreeding seems to be prevented by self-incompatible-like symptoms. It was shown that selfing the HT varieties 'Baccara', 'Dr.Verhage', 'Hadley' and 'White Weekend', induced viable seeds, giving 'normal' seedlings in the latter only. The other three cultivars seemingly were self-incompatible. Since selfing in rose is obviously too strict a method for deliberate inbreeding, a 'simpler' form, e.g. backcrossing to a recurrent parent, may be applied. It should be clear to rose breeders that, next to their normal activities, an inbreeding programme is a new discipline, intricate, highly time-consuming (5-6 years) and requiring consequent and accurate recording of plant observations in, for example, Excel.

In the backcross method, one very successful parent, e.g. the red flowered HT variety A, is the 'recurrent' variety, meaning that it is repeatedly used as a female parent. Hence, variety A is pollinated with another red variety, for example variety B, that has a highly desirable character that is missing in A. In the AxB F1 population, selection is primarily for A-like seedlings, that carry the desired character of B. In the next generation, A will be pollinated by, either a pollen mixture of selected F1 seedlings, or by individually selected F1 plants, thus creating an AAB generation. This procedure may be repeated, creating AAAB etc., still with A-like plants, carrying the desirable B character.

Proof of the pudding

It cannot be predicted how many generations of recurrent crossing may continue without the breeding of populations that completely consist of rogues or, worse, sterility. Selection should aim at small but still fertile plants. Assuming now that, from *different* inbred lines, a number of plants is ready for crossing, the proof of the pudding are crosses between individuals of different lines, among which heterosis should occur.

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