

THE ANNUAL 2017
Journal for breeders and producers of plant material
Prophyta

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- Import inspections
- Plant Passport
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THE ANNUAL 2017

Journal for breeders and producers of plant material

Prophyta

Prophyta – The Annual 2017

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Photo: Sacha de Boer

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In Short

First true potato seed receives PBR

THIS APRIL, BEJO ANNOUNCED the introduction of the first true potato seed (TPS) cultivar Oliver F1, that is protected by breeders' rights. "The advantages of TPS are especially important for small farmers in Africa, Asia and

Central America," says Rien van Bruchem, Crop Manager TPS. "In these developing regions, long distribution times for seed potatoes often have very harmful effects on the quality of the starting material. In contrast, the quality of TPS remains outstanding during the distribution process." Lysbeth Hof, Scientific Researcher DUS agricultural crops, at Naktuinbouw in the Netherlands, has supervised the



DUS-tests. Naktuinbouw carries out all DUS tests on behalf of the Board for Plant Varieties. Especially the uniformity demanded attention, as a hybrid is bound to be less homogenous than a clone. "We had applications from two companies, Bejo and Solynta," she explains. The latter was a parent line and the application was meant only as a way to help drafting the DUS procedures for TPS. Naktuinbouw expects to receive applications for TPS hybrids

from Solynta in the near future. "We had to draft two protocols for true potato seed, as the two breeders use completely different methods to come to a parent line. This has a large impact on the uniformity that may be expected from the respective hybrids, and that has to be reflected in the protocols. It is hard to inbreed potato, as the crop is by nature not tolerant to inbreeding. Bejo therefore used tetraploids which were inbred only few generations. Solynta,

on the other hand, uses diploid material which contains a gene discovered in Japan that makes inbreeding possible. In theory, that should produce a more homogenous crop. We have introduced the names SIP-hybrid (Slightly Inbred Parent Line hybrid) and HIP-hybrid (Highly Inbred Parent Line hybrid) to separate both types of hybrid. After carefully having studied the new SIP-hybrid for two years, we concluded that it was sufficiently uniform to be granted PBR."

Testing for Distinctness was, however, simple compared to Uniformity: "there is nothing that looks like Oliver, as it is the first European hybrid potato," she claims. "And Stability was also no problem. We compared two generations of Oliver and they looked very similar."

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AIB brings pirates to their knees

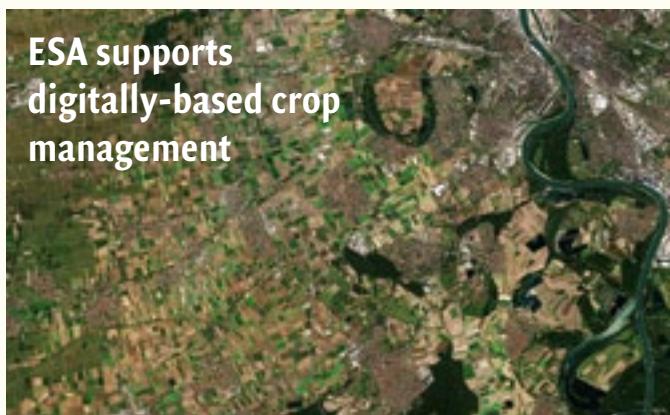
THANKS TO THE ANTI-Infringement Bureau for IP Rights on Plant Material in Brussels, two Spanish plant raisers in Almeria Province have been convicted of piracy. It was proven that both companies vegetatively propagated (PVP) protected tomato varieties and sold the plants to growers. The owners were sentenced to three months in prison. "We started investigating these infringement cases in August 2012," says AIB-director Casper van Kempen. "These cases take quite some time with regard to research and collecting evidence, followed by penal investigations." In Italy, two cases went to penal courts last March for the same offence, one in Nocera Inferiore (near Naples) and one in Ragusa, Sicily. While the decisions of these courts are as yet unknown, the prosecutors are convinced that the cases are straightforward. The efforts of the Anti-Infringement Bureau are acknowledged by the international crime fighters at Interpol. In September last year, the International Intellectual Property Crime Investigators College (IIPCIC), operated under Interpol and Underwriters Laboratories (UL), awarded the organisation with the prize for Outstanding Public and Private Partnership in the Fight against Intellectual Property Piracy.

Through an effective public-private partnership, the AIB and the technical law enforcement agencies in the main vegetable producing countries, monitor the piracy and counterfeiting situation in the market and formulate strategic plans. AIB also educates law enforcement agents and seed companies' field staff about the importance of IPR protection and how to detect illegal activities.

Hybrid African aubergine introduced

RIJK ZWAAN HAS INTRODUCED its first hybrid varieties of African aubergine. The new varieties were developed by Afrisem, the breeding station in Arusha, Tanzania, where Rijk Zwaan started breeding in 2008. The African aubergine varieties are Kazinga rz, Kerio rz and rz Limpopo, named after African rivers which symbolize the lifelines of the continent. The varieties have been tested over the past two years, while at the same time the company launched large-scale seed production. In January, the three varieties were presented to farmers at the demo days in Arusha.

The breeders at this station also develop hybrid varieties of other African vegetables, such as African kale, Chinese pepper and determinate tomato. The goal is to help small-scale growers to play a key role in building a sustainable food supply in Africa.



ESA supports digitally-based crop management

THE EUROPEAN SPACE AGENCY (ESA) and BASF have joined forces to see how data from satellites can best serve the agricultural community. The aim is to translate satellite imagery and data into digital tools and services. Satellites, in particular the fleet of Copernicus Sentinels, offer a valuable source of information for digitally-based crop management. It will be possible to provide farmers with more targeted agronomic advice on the use of crop-protection products and machinery. They

will also have information for a variety of agriculture decisions, such as optimal planting and harvesting times.

“Together with BASF and farmers, we will focus on delivering real-time satellite-based information to optimise fertiliser use and reduce water demand for irrigation systems,” says Josef Aschbacher, Director of ESA’s Earth Observation Programmes. “Data from our satellites can also be used to improve yield prediction, ensuring a return on European investments in space.”

Scientists discover early Solanaceae

FOSSILISED PLANT REMAINS from the Argentine region Laguna del Hunco, in Patagonia, have dated the ancestor of modern day nightshades back to about 52.2 million years BP. The fossils are so well preserved that even the embranchment of the veins in the leaves were visible in the stone. According to the scientists, the fruits looked similar to Mexican tomatillos. The Eocene forbearer of tomato, paprika, potato and lantern fruits grew in the remainders of the super-continent Gondwana, which had a tropical climate at the time.

Back to the future

Things never went smoothly between the UK and Europe. For a long time, the British were doubtful about membership of the European Community. Not until 1973, after negotiating a special position, did they become a member. But for almost everyone, it came as a shock when, on 23 June 2016, the majority of British voters said ‘nay’ to the European Union. We now find ourselves at the start of a two-year negotiation period on how to split up after forty years of cooperation.

Brexit will undoubtedly have many consequences, also for the seed business. Take, for instance, Community Plant Breeders’ Rights. Will these rights remain valid in the UK? Will Britain adopt the reports of the CPVO or will they demand new DUS-tests? What will happen with the applications that are in the middle of variety testing procedures? These are some of the questions that spring to mind. And what about the DUS-testing of grasses in Northern Ireland? Will the EU want to find another location within its boundaries? And then science. When British researchers are to continue to be partners in EU-research projects, the British government must contribute financially.

The phytosanitary ‘divorce’ may cause additional headaches. The automatic access to the common market will be terminated. The UK will have no say in the EU phytosanitary demands when they export goods to the EU. But the other way around will also become more complicated. Will Britain draft their own regulations and will companies based in the EU need certificates for export or will a Plant Passport do? And how fast will British customs operate if seeds or plants are exported to Britain? For potatoes, the arrangement is that a shipment is released within 48 hours. But two days is much too long for a vulnerable crop, such as young tomato plants. And the manner in which the UK will deal with GMO regulations may affect trade of seed across the Channel as well.

Questions, questions, questions and no answers in the near future, as the British government has not even started yet to systematically consult the agricultural organisations in their country. It may take years before we can make any real conclusions about whether Brexit will be profitable for the British, for Europe or for neither. For now, it seems that the agricultural community will lose. The relationship with the UK goes back to the future. What a waste.

Monique Krinkels

Welcome to Magyar Köztársaság

Monique Krinkels

6 “Organizing the World Seed Congress is the peak of my personal career and herewith I express my thanks to all participants and cooperating partners for the participation and for the teamwork during the past 33 years,” says Arpad Pavelka, Chair of the National Organising Committee. “I am sure that we are facing an event which will be full of business results and pleasant experiences, topped up by the Hungarian atmosphere and beauty of Budapest.”

It is sometimes said that Hungary could feed the whole of Europe. This is thanks to the Pannonian Basin, a plain that covers most of Hungary and is a major agricultural area. It consists of the bottom of the Pannonian Sea, which dried out in the Pliocene era 4,000 million years ago, on which thick layers of sediment were deposited. Later in history, a layer of rich loamy loess was added. An ideal soil for plants. And although rain is not plentiful, it usually falls when necessary. The strong seed sector forms an important basis for the success of the Hungarian farmers. All the more reason for Arpad Pavelka to be proud of his country.

How many members does the Hungarian Seed Federation and Interprofessional Organization (vszt) have?

“The Hungarian Seed Federation and Interprofessional Organization (vszt) has 892 members. Compared to the size of the country and of the market, this is a very high number of members. In Hungary, the membership is obligatory by law for all companies working in the seed industry.”

Are seed companies in your country mainly seed trade, seed producing or plant breeding companies?

“vszt has members dealing with seed production, seed business and also plant breeding companies. Membership is very colourful. Almost all of the companies dealing with seed production are Hungarian local companies and private entities. Among seed business companies, there are international operating companies with Hungarian subsidiaries or agencies - in smaller number, but bigger significance. Regarding plant breeding companies, there are state-owned research institutes, Hungarian private companies and also Hungarian organizations of international companies.”

Wheat and maize form the main part of breeding and seed producing activities in Hungary. How about vegetable production? And ornamentals?

“Naturally, the largest volume is represented by arable crops. However, the vegetable and ornamentals seed sector has been present in Hungary traditionally for more than 100 years. At the beginning of the 1900s, when the world was not as accessible and transparent as today, Hungary was the centre of the European



‘The volume and quality of hybrid sweet corn seed production has been acknowledged worldwide since the 1960,’ says Arpad Pavelka, Chair of the National Organising Committee

vegetable and flower seed production. Premium quality seeds were produced on large numbers of small farms, in excellent natural conditions by many well-prepared growers. This is well represented by the vegetable and flower seed catalogue published in 1910, of which we re-printed a facsimile, in original form, and we will offer it to the participants of the Congress as a present. The professionalism and assortment of this catalogue is amazing.”

Has the seed business in Hungary profited from the international collaboration in the EU? Has the membership led to major changes?

“Certainly. It is important to emphasize that the Hungarian seed sector remained open and maintained wide connections with companies from all over the world, even during the closed system of



Viticulture is an important sector in Hungary, with the Tokay wines as the most famous specialty. This sweet wine is made from grapes affected by noble rot, the beneficial form of *Botrytis cinerea*. This style of wine has a long history and is even mentioned in the national anthem

socialism. Proof of this is the previous ISF World Seed Congress in Budapest which was held in 1983. International seed companies already made important business relations with Hungarian seed companies in the 1970s and 80s. The volume and quality of hybrid sweet corn seed production has been acknowledged worldwide since the 1960s. “Joining the EU created big opportunities with free trade of products, and using the EU database of registered plant varieties is also very advantageous. At the same time, it created stronger and more strict competition for the Hungarian companies. Keeping domestic market positions was not simple, and in many areas Hungarian companies’ market share decreased significantly.”

Hungary is one of the strongest opponents of agricultural biotechnology in the European Union, promoting its position to initiate a joint alliance for genetically engineered (GE)-free agriculture, for GE-free crops, livestock and food production and feeding.

“Yes, in Hungary, using GMO-free products in the agricultural production is obligatory by the constitution and by the seed law. In favour of conscious food consumption, government and authorities have recently introduced strict controls.”

Does organic production of seeds have an important market share?

“No. For the time being, use and production of organic seeds is not significant in Hungary. The Seed Federation, in collaboration with Bio Control organisations, has prepared the conditions of organic seed production. Within the framework of VSZT, the bio seed sub-section was organised.”

You are the director of ZKI Zöldségtermesztési Kutató Intézet Zrt. Do you have a background in plant breeding or in another field?

“I have been director of sales at ZKI since 1995. My personal career in the seed business started in 1984, after the previous ISF Congress in Budapest, when I worked as director of seed production at Royal Sluis Magrovet Kft. My professional knowledge was significantly developed during the cooperation with the Dutch company. In the past 22 years, we have succeeded in completing the activities of ZKI Zrt and to transfer the company from a research institute, dealing only with plant breeding, to an active participant of the seeds business.”

Can you tell me more about your company?

“ZKI Zöldségtermesztési Kutató Intézet Zrt. is a state-owned company, limited by shares (Co. Ltd.). It has been running as a research institute since 1943. During the mid-90s, after the change of the political system, subventions ceased, therefore changes were needed. This was when we decided to transfer the research institute into a seed company. Today, the



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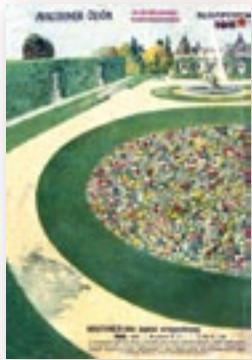
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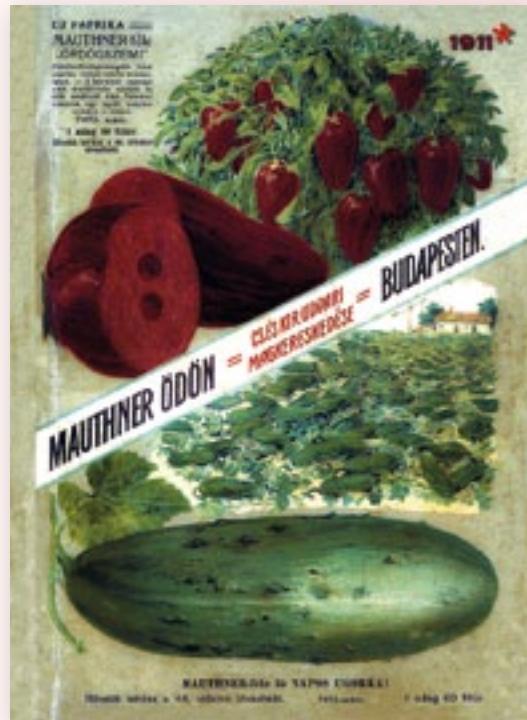


Australian Seed Federation
SOWING SEEDS





The Hungarian hosts prepared a special gift for the delegates of the ISF Congress: a facsimile of a vegetable and flower seed catalogue published in 1910



9

Arable crops	Production in tonnes
Cereals	16,661,503
Wheat	5,592,136
Maize	8,806,893
Barley	1,608,160
Rye	80,777
Oats	101,439
Triticale	472,098
Potatoes	402,853
Sun flower seed	1,892,509
Rape seed	881,961
Sugar beet	1,075,625
Silage/green maize	2,154,426
Lucerne	971,883
Vegetables	
Onions	60,339
Carrots	78,164
Tomatoes	200,351
Watermelon	195,998
White cabbage	64,701
Sweet corn	478,992
Hungarian red paprika	21,513
Fruit	
Fruit	777,716
Apples	511,460
Pears	36,793
Sour cherries	76,792
Plums and greengages	46,023
Apricots	19,855
Peaches	37,372
Viticulture	
Viticulture	472,348

Source: Központi Statisztikai Hivatal

whole seed product line is present at the company. Breeding and development of new varieties, seed production, marketing activities with the aim of introducing new varieties, quality control, seed packaging and sales – not only in Hungary, but also in the surrounding countries.

“During the changes, we decided to decrease the number of species we breed and to concentrate on those which are important for the professional seed market. Today, the most important species which we deal with in the breeding and in the whole product line, and where we have continuously new output, are: peppers and paprika for spice, green pea for processing, watermelon, cucumbers (pickling and beith-alpha).

"With all of its activities, ZKI Zrt. concentrates on the professional market. More than half of the company's income is from exports. Our seeds are used by processing plants and professional fresh market growers in more than forty countries."

How many delegates do you expect to attend the World Seed Congress this year?

“In Uruguay, I asked the participating 1,050 delegates to bring one person with them. Let’s have 2,000 participants in Budapest! Of course, this vision is too optimistic. I would like to and I hope to exceed the number of participants at the Krakow Congress (1650 participants) and to achieve the honorary title and the ambitious goal of hosting the largest ever congress.”

How a Mexican crop became a Hungarian specialty

John van Ruiten

10 Hungary, paprika and goulash, these three words are very closely connected in the minds of many people. Mention one of them and the others will follow. The interesting question is when and how this connection came into being. In other words: what is the history behind the use of paprika as an iconic ingredient in a traditional Hungarian dish? Going through books and articles on the internet, the story starts to unfold and I gladly share my findings with the Prophyla reader.

It is well known that all peppers from the Capsicum genus (not to be confused of course with peppers from the Piper family) originate from the new world, from Latin America. Capsicum annuum, the species to which nowadays almost all bell pepper/paprika varieties belong, was domesticated in the region of south east Mexico around 8,000 years ago. And these peppers ('indian pepper/piemento de las indias'), both the pungent/sharp as well as the non-pungent/sweet forms, have been grown there ever since and became an important crop. Local people used peppers for healing and for seasoning meals. Peppers are easy to use (even fresh/uncooked) and can also be dried/stored easily for a longer time.

Columbus

That the name pepper (peperi, piper, peper, papar,) was used for this spicy vegetable had an obvious reason: at the end of the 15th century, (black) pepper (*Piper nigrum*) had become an expensive spice that arrived in Europa from the East Indies. But in 1453, Constantinople became the centre of the Ottoman empire and at that time it became much more difficult for the Europeans to travel and trade via the old spice and silk

routes. In their search for a new route to this source via the west, the 'discoverers' came to land in the Americas. And they found out that Capsicums were abundantly used for flavouring meals. They called them peppers.

It was dr. Diego Alvarez Chanca of Sevilla that brought back home with him seeds of these new peppers on the second voyage of Columbus in 1496. This is believed to be the first introduction of the species *Capsicum annuum* in Europe. In the following years, many parallel introductions must have taken place and records show that already in

1498, Vasco da Gama took capsicum seeds with him on journeys to India/Goa and to the East Indies islands like Timor. And it is clear that there are official descriptions that in Japan, Korea and China capsicums were grown around 1550, probably brought there via Portuguese missionaries. They were grown successfully everywhere and became part of the local food.

Botanical garden

In Hungary, the first records of growing Capsicum go back to 1529, and plants were successfully grown in the second half of the 16th century in a baroque botanical garden of Margit Széchy in Buda. The plants were called 'red Turkish pepper'. It is assumed that the seeds used came in from Turkey. Big parts of Hungary, as it is today, became part of the Ottoman empire in 1526 after the battle of Mohacs. The plants grown then in Hungary were not meant for eating, but as an ornamental plant first and later as a medicinal plant. But how did most of these capsicum plants reach Turkey? There are two theories, which could both be true. One of them states that these seeds/spices came to Turkey via traditional trade routes from Persia and India (as a new product grown there). The other explanation states that seeds came in via Turkish trade relations with western European countries, like Belgium, Netherlands and Germany which was of considerable size in the 16th century. The plants/seeds coming from these sources came ultimately from Spain. The Ottoman empire did not have trade relations with the Kingdom of Spain itself, as they were fierce (religious) enemies at that time. So that was the reason for this indirect route.

Dominant spice

The use of this vegetable in the kitchen, as an ingredient in meals, was not yet important in Hungary however in the 16th and 17th centuries. The use as a medicine for curing fever, typhus and to relieve pains was predominant. Nice to know that much later, vitamin C was discovered in paprika by the 1937 Hungarian Nobel prize winner, Albert Szent Györgyi. Fresh paprika contains 5-6 times more of this vitamin than, for example, oranges or lemons. In rural areas in the south of Hungary, the use of



Hungarians used paprika as a medicine for curing fever, typhus and to relieve pains



In the central market of Budapest, long strings of paprika pods are hanging out to dry

paprika by peasants, shepherds and herdsmen, most accustomed to Turkish culture, as a spice and a herb in their stew food must have been gradually integrated into their habits of preparing meals in the 17th century. But it took till long after the defeat of the Ottomans in 1699, before growing paprika (probably also with other/new varieties) started to become more important in the mid-18th century in the southern part of Hungary. The crop was possibly reintroduced at that time. The Hungarian name 'paprika' is said to go back to the beginning of the 18th century (originating from the Slavish word 'paparke', meaning little pepper). Even then, it took almost more than a hundred years (till the mid-19th century) before paprika was widely known as the most dominant spice used in Hungarian cuisine. And at the time it was known as the 'red gold' that gives character and spirit to Hungarian dishes. It was from then onwards that in autumn one could see long strings of paprika pods, traditionally hanging out to dry under the roofs and perches of almost every local farm. Also in that period, pörkölt and goulash were identified as interesting meals

and started to become popular, also for upper-class citizens of the cities in Hungary.

Home of cultivation

The climatically very favourable conditions of sunny areas of Szeged and Kalocsa have ever since that time been the 'home' of traditional Hungarian paprika cultivation. And, today, they are still important production areas. Ference Horvath and Jeno bermayer, from Kalocsa, bred/selected the first non-pungent pepper variety in the world. This paprika was sweet and there was no need to remove seeds/veins. Kalocsa is still home to the biggest paprika museum in the world. Cultivation, drying and preparation techniques of paprika powder have been modernized, of course. Drying is done slowly in ovens, and many new varieties, ranging from sharp to sweet, have been developed. But still, many farmers' families make their own paprika powder! Nowadays, eight types of mixtures of paprika powder can be found on the markets, ranging from very mild (Különleges, with a bright red colour) to very hot (erös, brown/orange coloured).



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Why validation matters

Radha Ranganathan, Merel Langens and Wayne Wiebe

In some cases, diseases can be associated with the seed, and if conditions are appropriate, transmit to the seedlings that emerge from the seed, causing economic loss. Diseases that are new to an area may be introduced with seed as the pathway. Therefore, the seed industry takes its responsibility to deliver healthy seed very seriously and actively engages in developing systems and procedures to help prevent the movement of pests and diseases with the seed.

- **The vegetable seed industry** has an increasingly global scope, involving movement of seeds across many international borders. Seed of breeder lines may be developed in a particular country, increased in another and sent to a different one to produce hybrid seed. This hybrid seed could then be sent to the country of origin or another for processing, evaluation and packaging, before being shipped all over the world to commercial markets. Such multinational movement of seed over long distances and across multiple borders is now common in the industry.

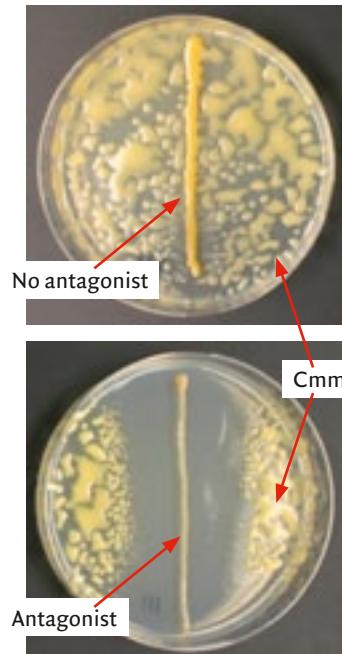
Seed health tests

Field inspections (phytosanitary inspections) in the country of seed production for the presence of specified diseases that importing countries want to keep out of their territories is one way to help assure healthy seed. When inspection may not be sufficient or is not available to determine if a pest may be present in or on seed, it may be detected by a laboratory test. The methods, such as freeze-blotter, seed wash/plating, serology and molecular among others used to test seed, may vary according to host and target pest.

Although important, the intent of this paper is not to describe these methods but rather to discuss the importance of verifying if the method is fit for purpose, a process called method validation. The seed health test has to be meaningful and, to fulfil its purpose, it must detect the target pest – if it is present – without giving any ‘false positive’ or ‘false negative’ results. A false positive result can occur when closely related organisms that do not cause disease are also detected by the test or other factors interfere with the test. A false negative can occur when the test gives a negative result when, in reality, the target pest is present. A false positive can lead to further processing or disinfection of seed, which can be damaging to germination or shelf life, or discarding valuable pest free seed. A false negative could end up in releasing a seed lot for sale that contains a transmissible pest. Therefore, the outcome of a seed health test has to be reliable and reflect the real disease status of the seed lot.

Method validation

Newly developed seed health tests must be validated before they are routinely used. There are many details



Non-pathogenic organisms may suppress target pathogenic ones, such as the Cmm pictured here. This interaction is called antagonism. (Photo: Naktuinbouw)

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that need to be checked and the performance criteria of the test must be evaluated before its use. These performance criteria include:

Specificity: The ability of the test to differentiate between the target pest and other closely related organisms or contaminants is an important feature of a good test. In some types of tests, for instance, it is hard to distinguish bacteria that are morphologically similar to the target pest but are not pathogenic. Some examples of the so-called look-alikes are common to *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) and *Xanthomonas campestris* pv. *campestris*, which respectively cause the diseases bacterial canker of tomato and black rot of crucifers. So if, for example, a test intended to detect Cmm in tomato seed also detects close relatives that are not pathogenic to tomato, a false positive may occur. However, if the test is not specific enough to detect all known relevant strains of Cmm, the outcome could be a false negative result with potentially serious consequences.

Sensitivity: It determines the minimum amount of the target pest that the test detects. Seed tests need to detect the target pest at and above what is known as the disease threshold, below which the pest is at too low a level to cause disease.

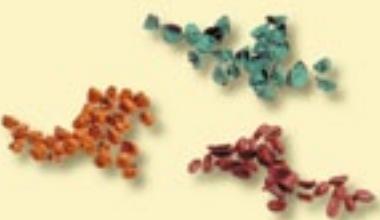
The sensitivity of a test may be influenced by the presence of antagonistic organisms that are not

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Wilting and lesion caused by a suspected Cmm isolate during a test. The bacterium Cmm causes the disease bacterial canker of tomato



Photos: Syngenta Seeds



pathogenic to the host but inhibit the growth of the pest, making it difficult to detect. For some diseases, positive seed lots can be disinfected through physical or chemical processes. Ideally, a test should distinguish between a viable and non-viable pest as, after disinfection, it is possible that inactivated particles of the pest (such as viral coat proteins or genetic material) may still be present on the seed.

Selectivity: This criterion determines if a method developed for, say, leaves is suitable for testing seed. Seeds contain starch and storage proteins that inhibit or adversely affect test performance. Or, for instance, determine if a method developed for, say, melon is also as good for seed of bitter gourd, both cucurbit species.

Reproducibility: How consistent are the results of a method and what effect do differences in equipment,

personnel and other factors have on whether similar results are obtained? The test should give the same result in different laboratories over time, and reproducibility is evaluated by doing what is called a ring test, where a number of laboratories perform the test in the manner described and using the same reference seed lots. Labs must detect all infected samples and healthy samples must be identified as such.

Repeatability: The test should give the same result when performed several times under similar conditions in the same lab.

In addition to demonstrating that a seed health test method is fit for purpose, method validation provides a template to record protocol modifications and the corresponding explanations. As technology progresses, new techniques that are more sensitive, faster, simpler and less expensive are being employed in seed health testing. All technical modifications to a method need to be evaluated to determine if the changes affect test performance. This is done by monitoring the performance criteria of the method and re-validating it, if necessary.

The seed industry

The International Seed Health Initiative for Vegetable Crops (ISHI-Veg) is a seed industry platform under the aegis of ISF that, since its formation in 1993, develops reference methods for vegetable crops for a consistent evaluation of seed health (see <http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg/#protocols>). ISHI-Veg has currently developed 23 seed health test methods of which 14 are ISTA Rules or NSHS (US National Seed Health System) Standards.

The first step in method development is to define the method's purpose or objective. Many factors are taken into consideration for making this decision, including whether the seed is known to be a pathway for the pest. To help clarify whether seed is a pathway for the entry or spread of disease or not, ISF initiated its Regulated Pest List Initiative in 2007 (see <http://www.worldseed.org/our-work/phytosanitary-matters/pest-lists/#isf-regulated-pest-list-initiative>). The assessment of whether seed is a pathway is based on reviews of scientific literature, and seed company expertise and risk assessment built over many years of practical experience.

The combined expertise and experience of its members allows ISHI-Veg to develop methods that best meet the needs of the laboratory conducting the test, the seed producers and their customers. In an era of increasing phytosanitary requirements for importation of seed, ISHI-Veg ensures its methods are properly validated. The methods undergo a thorough review every few years, so they keep or obtain national and international recognition. Overall, the seed health test result is only meaningful if it is a reliable reflection of the risk that is present when the seed lot is brought into the market.

Desperate need for basic research

Geert-Jan de Klerk

16 **Applied tissue culture desperately needs basic research to understand the underlying mechanisms. Only in this way, will many instances of recalcitrance be solved and present applications may be boosted. This is the take-home message from dr. Geert-Jan de Klerk who will retire soon after more than 40 years of research in plant physiology, of which 30 years were in tissue culture.**

Tissue culture has become a major pillar of horticulture and agriculture. But has its further development come to a standstill? For a number of decades, no major breakthroughs have been achieved. The lack of progress is for a large part due to the orientation of research. A shift of emphasis in research from the use of tissue culture as a tool in breeding and vegetative propagation to the underlying mechanisms of growth and development in tissue culture is required. A major research item should be how plants succeed in growing in vitro.

The start

The birth of plant tissue culture was at the start of the 20th century. Haberlandt attempted to culture somatic cells in an artificial medium, initially in hanging drops and later in small glass dishes. The medium contained mineral salts (Knop-formulation), sucrose, glucose, glycine, asparagine and peptone. His attempts failed: he observed cell-stretching and wall-thickening, but cell division never occurred. During the next five decades, the various components of the tissue-culture procedure were specified. Agar was added to obtain a semi-solid medium convenient to culture explants. Haberlandt had used the Knop formulation for inorganics. More advanced formulations used in horticulture were tested in tissue culture, and finally, MS was developed. MS is now being used in most formulations. Sucrose appeared to be suitable for organic nutrition, a choice later corroborated by the finding that sucrose is the natural transport carbohydrate in the plant kingdom. How to work steriley was picked up from microbiology. The remaining component for successful tissue culture, the last obstacle to be dealt with, was adequate hormonal regulation to steer growth.

In his experimentation, Haberlandt had noticed the importance of obtaining cell division, the milestone he was unable to pass: "It will be the problem of future culture experiments to discover the conditions under which isolated cells undergo division." In 1913, he reported that phloem exudates from various plant species did stimulate cell division in wounded potato tubers. Now we know that these exudates may contain high levels of cytokinins. In the 1940s, it was found that coconut milk in concert with auxin, promoted cell division in plant cells. Auxin had been

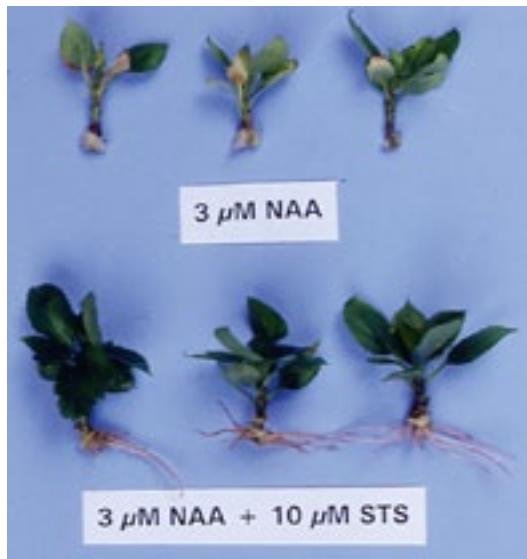
discovered in the 1930s. In the 1950s, Skoog and colleagues started a search for the active ingredient of coconut milk, but were unsuccessful. Autoclaved herring sperm also stimulated the cultures. Skoog et al. purified the fractions from the autoclaved sperm until they had identified the active chemical. It was named kinetin. Letham was the first to isolate a native cytokinin, zeatin, from immature maize grains. This was in 1963, half-a-century after Haberlandt had experimentally demonstrated the hormonal regulation of cell division in plants.

Primarily a tool

Thus, by the 1950s, all components of the tissue culture technique had been developed. Just after that, practical applications were discovered. The first was the freeing of plants from diseases by meristem culture. Various applications for breeding were developed, including embryo-rescue, haploids, cryopreservation and genetic engineering. A major usage was in vegetative propagation. In tissue culture, tiny vulnerable tissues can be grown to a size sufficiently robust for growing in soil. Moreover, processes like axillary branching and rooting can be relatively easily induced by adding hormones. The key step in the development of micropropagation was the introduction of subculturing. This enabled all-year-round propagation with a number of propagation cycles in one year, instead of only one. This discovery is nicely demonstrated in lily. At first, it was attempted to make as many bulbils as possible in one step, e.g., by Stimart and Ascher who reported, in 1978, the production of 8000 or more bulbils in one step from an average bulb with 100 scales (so without subculturing). A few years later, Takayama reported that higher propagation is achieved when bulbils produced in the first step of tissue culture are subcultured, being used as starting material for a next growing cycle. In animal physiology, Ross Harrison published a short article at the very beginning of the 20th century that successfully introduced tissue culture in animal physiology to settle the argument how nerve fibres originate. He solved basic tissue culture problems of medium composition and culture vessel. He was able to deal with the problem of contamination (which he did encounter) because his cultures were relatively short term. It should be noted that for both Haber-

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Figure 1. The complex role of hormones in rooting. Auxin promotes rooting but it also stimulates the synthesis of ethylene, another hormone. Ethylene in turn blocks the growth of roots. The ethylene inhibitor STS therefore promotes rooting



landt and Harrison, tissue culture was actually only a tool used for solving a scientific problem. For Harrison, the problem was how nerve fibres originated; for Haberlandt, tissue culture would give insights into the properties and potentialities of individual cells and it would also provide information about the interrelationships of cells within a multicellular body. So, tissue culture has been seen from the very beginning primarily as a tool and not as a subject of examinations by itself. It will be argued that this omission is responsible for the many setbacks in the use of tissue culture. In present-day research, researchers frequently pull some hormone matrices out of their hat to achieve success in one specific crop only.

Present-day definition

Tissue culture has never come apart from this status as tool. This may also explain that even the term 'plant tissue culture' is used in different ways. It has been equated with micropropagation, its most used application: "Plant tissue culture, also called micropropagation, is a practice used to propagate plants under sterile conditions or in a controlled environment" (review in 2009). By others, it has been equated with cell and callus culture. This becomes most apparent from the statement in many reviews that tissue culture takes its roots from an essential property of plant cells, totipotency (in many reviews) and the reference to the Schleiden and Schwann cell theory (1838) as a necessary step for the development of tissue culture (in most, if not all, historical accounts of plant tissue culture).

These opinions are at odds with the present use of the term, even though they are very often found in present-day literature. They find their origin in the early days of plant tissue culture, when White maintained (in 1934) "that a tissue culture should be of a single type of cell and should undergo only limited differentiation." He explicitly stated that cultures of spores, embryos and cuttings do not belong to tissue culture. An adequate present-day definition is "Culture of

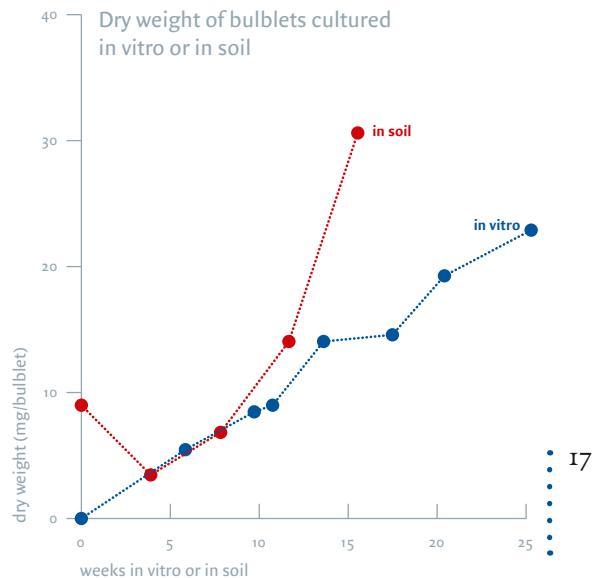


Figure 2. Growth of lily bulblets in soil (sucrose from photosynthesis only) and in vitro (sucrose both from the medium and from photosynthesis). The light intensities are similar

Redrawn from Acta Hort. 325: 513-520.

plant tissues excised from the parent body, under sterile conditions on an artificial nutrient medium containing inorganic and organic nutrients."

Central problems

The problems in tissue culture can be simply categorized. Firstly, they concern the failure of somatic cells to regenerate a new shoot, root or embryo, and so the incapability of cells to switch to a new developmental programme. The normal tissue culture approach is to test different tissue culture conditions, very often the types and concentrations of hormones (Figure 1). A number of problems have been solved in this way, to such extent that users can live with it. But there are also many persistent problems. Solving these problems is becoming increasingly urgent. Recent genome-editing techniques, such as CRISPR-Cas9, enable sophisticated crop engineering but still require regeneration of genetically modified cells into plants and here regeneration is the weakest link. In addition to spray-and-pray research, there is also fundamental research. The applied research on recalcitrance to regenerate will undoubtedly benefit and is likely fully dependent on the fundamental research, e.g., with *Arabidopsis* mutants.

The second central problem concerns off-type plants. These may be genetic or epigenetic ones. Why they occur frequently in tissue cultured plants is not known, but they possibly vanish when the stress imposed on the plantlets is reduced.

The third central problem is the disappointing poor growth in vitro. One would expect that growth in vitro is fast: at first sight, the conditions seem to be very beneficial with respect to nutrition, water availability, and temperature. Nevertheless, growth is slow.

We share Johan's ambition

to surprise consumers time after time



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

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

Sharing a healthy future



Figure 3. Tulip shoots in vitro. They grow very slowly



Figure 2 shows the gain in weight of lily bulblets in vitro and in soil. It should be noted that the ex-vitro condition was in a growth cabinet with about the same light intensity as in vitro. Surprisingly, 'soil' outperforms 'tissue culture'. Why is growth in vitro disappointing? Here, we again come across a lack of genuine scientific research in plant tissue culture. Unfortunately, Fick's second law on diffusion has escaped the attention of almost all tissue culture researchers. This law demonstrates that to diffuse 50 μm (the distance across a typical leaf cell) takes 0.6 sec. One metre, though, takes 8 years! So, diffusion is often adequate for the movement of solutes within leaf cells and especially inside organelles such as chloroplasts and mitochondria. On the other hand, diffusion is too slow to deliver building stones and energy for growth in multicellular organisms. Xylem and phloem have therefore an inextricable role in long-distance transport in plants: solutes hitch along with the water flow in the vascular bundles. And what about shoots in tissue culture? The distances are on average 0.5-2 cm, with sucrose taking between 17 hours and 210 hours of diffusion respectively, which is far too long for adequate growth. Therefore, the vascular tissues are most likely also accountable for the translocation of nutrients in tissue-cultured plants. However, the functioning in vitro of both xylem and phloem is problematic because the driving forces – transpiration by the leaves (xylem) and accumulation of photosynthetate in the vessels (phloem) – are decimated by the tissue culture conditions.

Transpiration

Nevertheless, many crops do grow in vitro, but how they achieve this is not known. The most likely explanation is that the strongly reduced transpira-

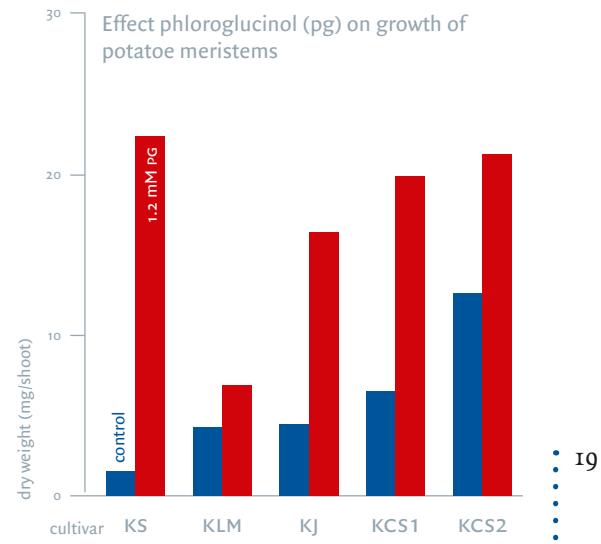


Figure 4. The effect of Phloroglucinol (PG) on the growth of excised potato shoot tips (300–500 μm long dissected from aseptically grown in vitro plantlets)
Redrawn from PCTOC 60: 139–149

tion in vitro nonetheless enables a flow in the xylem adequate to pipe sufficient nutrients. If so, the extent of transpiration is a major determinant of growth. In preliminary experiments, increased growth was indeed observed when transpiration was increased by stimulating the opening of the stomata or by reducing the wax-layer on leaves. Moreover, evidence was found that in tulip shoots that grow very slow in tissue culture (Figure 3), transpiration is much reduced by the very thick wax layer and by low stomatal density.

In addition to transport problems, uptake from the medium is also problematical because of the formation of wound tissue with a suberin layer, which is difficult to permeate. In a similar situation, with detached flower stems, such a layer reduces uptake of water within a few days. Unfortunately, for in vitro culture, such research has been done only once (ca. 1990), again demonstrating the frustrating lack of scientific knowledge.

The decimation of transport in the vascular bundles is caused by the exchange of ex vitro for in vitro conditions, but an inclusive view of all consequences of this exchange is difficult to achieve. The effect on photosynthesis may be drastic. When photosynthesis is inhibited, e.g., by low CO_2 or by sucrose from the medium, electrons may still be formed and oxygen may act as electron acceptor, leading to the formation of Reactive Oxygen Species (ROS). Because of this, plants may be severely damaged. ROS may also be formed when plants are wounded and when the wound surface is relatively large (e.g. when meristems are prepared), and cause severe damage. Damage by ROS may be solved by adding antioxidants to the medium and this may explain why phloroglucinol, a phenolic compound that is also an antioxidant, enhances growth in tissue culture (Figure 4).

Organic market is booming business

Monique Krinkels

20 Some consumers assume organic products are healthier because no chemicals are used. Others believe they taste better as they are grown in soil. Still other people argue that the benefit of organic production is the sustainable use of resources. One thing is certain, the organic market in Europe is booming. According to a supermarket manager: 'Just put an 'O' on a product and it sells.'

It is estimated that the compound annual growth rate of the global organic market will be around 14.3% over the next decade. In the USA the total organic market is worth 40 billion dollars and in Europe 25.8 billion euro. In the Netherlands, 10% of all vegetables sold in supermarkets are organic, in France it is as much as 18%. So far, 11.2 million hectares in the EU is used for organic food production and it is rapidly increasing in area, as well as in number of growers. The motive might as well be idealistic as economic. More and more people are willing to pay extra for food that is regarded to be superior to the mainstream products. And the whole chain, from seed companies and farmers to supermarkets, profits.

History

The organic movement began at the turn of the 20th century, when in the early days of industrial agriculture a small group of farmers became worried about the shift towards synthetic nitrogen fertilizers and pesticides. They started associations such as Demeter (Germany), the Australian Organic Farming and Gardening Association (Australia), the Soil Association (United Kingdom) and Rodale Press (United States) encouraging organic farming. Since the early seventies, organic food became popular among a larger group of people that were concerned with environmental pollution caused by persistent agrochemicals, such as DDT. At the time, organic vegetables were mainly sold in specialised shops, the so-called health food stores. The products were often biodynamic, i.e. a form of organic production based on the anthroposophical principles of philosopher Rudolf Steiner, who had a holistic view on nature and farming. In 1972, the International Federation of Organic Agriculture Movements (IFOAM) was founded in Versailles, France. In the decades thereafter, the popularity of organic products steadily increased. Governments started to introduce certification schemes to ensure standards of production.

From the start of this century, organic products have made a leap forward. The product range widened from food to beauty, health, body care, household products and fabrics. Supermarkets introduced organic vegetables as part of their 'normal' fresh product range, but also processed foods took over the

shelves in the shops. From meat to wine and from coffee to cookies, for almost every product there is today an organic alternative. "Bejo started in 1996 with organics," says Bart Kuin, business manager organics. "We were not the only ones entering that market; Enza, Rijk Zwaan and other conventional seed companies did the same. Besides, there were companies that specialised in organic seed production. It took us four years before we could organise our first organic open days. In 2001, we started selling our first organic seeds." Today Bejo produces organic seeds of forty different vegetable crops; more than 150 varieties in total.

Different mind-set

Since 2004, all seeds used in organic agriculture should be organically propagated. Even some breeding techniques are not wanted by part of the organic sector. "That means that, for instance, in cabbages in our organic program we don't use cytoplasmic male sterility derived by protoplast fusion," says Bejo's CEO John-Pieter Schipper. "Only conventional breeding techniques can be used. Our varieties are nonetheless hybrids, which is permitted. While some of the biological dynamic growers prefer open pollinated varieties, bred by local breeders, we focus on organic hybrid seeds."

	Area x 1,000 ha	Grassland %
Belgium	69	67.3
Bulgaria	111	26.8
Croatia	76	44.3
Cyprus	5	7.5
Czech Republic	478	84.9
Denmark	167	18.8
Estonia	156	53.1
Finland	225	1.7
France	1,375	36.8
Germany	1,089	55.1
Greece	407	62
Hungary	130	49.9
Ireland	73	93.7
Italy	1,493	28.6
Latvia	232	45.6
Lithuania	214	29.8
Luxembourg	4	52.7
Malta	n.a.	n.a.
Netherlands	49	57.7
Poland	581	27.2
Portugal	241	69.5
Romania	246	30.8
Slovakia	182	66.1
Slovenia	42	82.1
Spain	1,969	51.5
Sweden	519	23.2
United Kingdom	496	70.0



Certified organic area in hectares per crop type in the EU Member States, 2015

Source: IFOAM

"Most varieties that are used in organic growing can also be obtained as conventional seeds, but we do look in our breeding programmes with a special focus on organics," explains Mr. Kuin. "To improve, for instance, the root system, to make sure plants can cope well with the available nutrients in the soil. These are sometimes less available or slower released than in conventional growing systems. We have greenhouses and trial fields specifically dedicated to organic research. But we see that conventional growers are also interested in the varieties bred and selected for organic production." Mr. Schipper adds: "There is much to learn from organic cultivation. We applied some of the lessons learned in organic seed production also to our conventional processes to make these more sustainable."

It takes a while before a grower is used to producing organic seeds. It needs a completely different mind-set. "You have to think ahead, as whatever happens during the growing period, less can be done to cure the problem. It all amounts to prevention," explains Mr. Schipper. "For instance, organic growers tend to use a different spacing, as a little more distance between the plants prevents the occurrence of mildew." "And you need to take very good care of the soil," adds Mr. Kuin. "Crop rotation and soil preparation has to be well planned, as the condition of the soil should be perfectly fitted to the crop."

Not combined

The need for a different mind-set also means that seed producers cannot combine production of organic seeds and regular seeds. It would be too confusing. "And besides that, for organic production the soil should be free of any remains of fertilizers or crop protection chemicals. It takes at least two years

before a soil is sufficiently recovered and the grower can obtain the mandatory certificate."

When the organic seeds arrive at the premises of Bejo in Warmenhuizen, the Netherlands, the cleaning, coating and packaging lines are also physically separated. There is no chance whatsoever that the seeds become mixed up with conventional seeds. Even the people working with the seeds are completely focused on either organic or conventional seeds. "In our view, you cannot be responsible for both type of products, that is why we have a separate team that handles the organic products," according to Mr. Schipper.

Varying regulations

The increasing market for organics is not limited to Europe and North America. Africa has 1.3 million hectares of organic agricultural land as of 2014, mainly in East Africa. This is largely due to production for the European market. "But it attracts the attention of local farmers as they see that, with an adapted growing technique, it is possible to save on costly chemicals," says Mr. Kuin. According to the United Nations, there is also a growing recognition among policymakers that organic agricultural has a significant role to play in addressing food security issues, land degradation impacts, poverty alleviation and climate change in Africa.

"We also export seeds to South America, where organic products are gaining a market share." In major cities, such as Buenos Aires, Mexico City and São Paulo, more and more consumers choose for organics. "It is, however, not easy to meet the demands for organic seed production for these markets, as the regulations vary from country to country. But we still aim to have organic seeds for growers in all countries available," concludes Mr. Schipper.

The impact of the viruses' transmission strategies

Martin Verbeek, Ineke Stijger and René van der Vlugt

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New diagnostic and rapid 'next-generation sequencing' methods facilitate the discovery of new plant viruses. However, virus detection or elucidation of the sequence of its DNA/ RNA is no guarantee for understanding its epidemiology. In order to develop effective control measures in field crops or in greenhouses, knowledge of how viruses move from one plant to another is essential.

Virus infections in plants are of major concern in agriculture. These tiny pathogens, only consisting of nucleic acid (RNA or DNA) protected by a shield of protein, the virus coat, can cause considerable damage to plants and crops. For their replication, viruses depend completely on their host cell machinery, which they manipulate to multiply themselves. Doing so, they alter the plants metabolism which can lead to all sorts of symptoms such as mosaic, chlorosis, malformation, etc. These often result in lower production or even unmarketable plants and fruits. But even when they do not cause noticeable symptoms, plant viruses can become a problem, for instance when certain viruses hamper export as they are quarantine organisms in other countries.

How do viruses spread?

Virus particles cannot move on their own and depend on other organisms for their transmission. Such virus-transmitting organisms are also known as vectors. The majority of plant viruses are spread by insects. Aphids are major virus vectors but also whiteflies, thrips, plant- and leafhoppers and beetles are known for their role in virus transmission. Other vectors of plant viruses are mites, nematodes and soil fungi. Larger animals (e.g. cattle) and humans can spread plant viruses too. Viruses, plants and vectors closely interact, often influenced by the surrounding environment. These interactions are very specific, not only between virus and host, but also between virus and vector. For instance, viruses transmitted by aphids are not transmitted by whiteflies. Understanding these interactions forms the basis of control measures that help growers to prevent virus infections or keep virus diseases under damage thresholds. Of utmost importance is the virus status of the planting material. Virus-free starting material – plantlets or seeds – will give the grower a head start. For this reason, planting materials and seeds are tested and certified by inspection bodies before market release.

However, virus-free planting material is not a guarantee for a virus-free crop. The grower still needs hygiene measures and to be alert for possible virus sources (such as weeds) and different virus vectors which could bring unwanted diseases into the crop.

Viruses use various strategies to get themselves from a source plant to a new host plant. Each strategy implicates a different means of control. To illustrate this, we will discuss three examples: the transmission of viruses by contact, by soil and by aphids.

Contact-transmitted viruses

Some groups of viruses are transmitted via contact by animals wandering through the field or by humans during crop handling. Examples of such groups are the tobamoviruses, like Tobacco mosaic virus, and the potexviruses, like Potato virus X or Pepino mosaic virus. These viruses multiply to very high concentrations and are present in every epidermis cell, including the tiny trichomes ('hairs') which will break upon touching. A small amount of virus-containing plant sap will then contaminate skin, clothes or tools. These viruses are extremely stable and stay infectious for weeks. Coats worn by a person walking through a greenhouse with tomatoes infected with Pepino mosaic virus still contained infectious virus particles after two weeks. These viruses can also stay infectious on seeds and are able to infect the seedling during germination. The percentage of seed transmission in this way is low, but only one infected seedling may be a source from which the virus spreads through an entire greenhouse.

For contact-transmitted viruses, very strict hygienic and disinfection measures are appropriate. Growers, and visitors to a field or greenhouse, have to be constantly aware of washing hands, wearing disposable coats and disinfecting tools and materials.

Soil-borne viruses

Soil-borne viruses can also be of great concern to growers. These viruses can easily infect plant material from a source that is difficult to control. Soil-borne viruses can be very diverse and do not necessarily share vectors or transmission strategies. Some viruses are nematode-transmitted (e.g. nepoviruses and tobraviruses), some are transmitted by soil fungi (e.g. ophioviruses and benyviruses) and some do not need a vector to move through soil from plant to plant (e.g. tombusviruses and some potexviruses). Especially fungi-transmitted viruses can make a soil useless for growing certain crops, as they can survive in very persistent resting spores of their vector for

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Many plant viruses are transmitted by aphids; here the lettuce-currant aphid (*Nasonovia ribisnigri*) on a lettuce plant

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for potyviruses to attach themselves to the inside of the aphid's stylets. When the aphid decides to leave the first plant and to probe another still healthy plant, potyviruses are already released from the stylet and will infect the new plant. Poleroviruses (e.g. Potato leafroll virus) use the aphid in a completely different way: these viruses are only taken up by the aphid during actual feeding on the plant's phloem. The virus particles then travel through the aphid's intestine to its salivary glands. When feeding on a new plant, virus particles are released with the aphid's saliva into this plant. Using this strategy, the virus will be transmitted throughout the entire life of the aphid (persistent transmission). Control of aphid-borne viruses is essentially controlling the aphid vectors. However, preventing aphids from colonizing in a crop will help in the control of the persistent poleroviruses, but hardly in the non-persistently transmitted potyviruses. Using an incorrect aphid control strategy may even promote the spread of virus diseases.

more than 20 years (this was shown for ophioviruses transmitted by the chytrid fungus *Olpidium virulentus*). Current control measures focus on sterilising the soil (using steam or solarisation) and chemical control of nematodes. However, the current trend to ban more and more chemicals will not only affect soil-borne pests and pathogens, but therefore also soil-borne virus diseases.

Aphid-borne viruses

Many well-known plant viruses are transmitted by aphids by a variety of different strategies. Potyviruses (e.g. Potato virus Y), one of the largest group of plant viruses, are transmitted non-persistently on the stylets of an aphid. After landing on a visually attractive plant, the aphid probes this plant with its stylets to test if it is a good host for feeding and colonizing. This probing lasts just seconds, but it is long enough

Understanding and acting

Virus diseases need special attention when it comes to control, since it is simply not possible to cure plants from a virus infection. Nowadays, ever more emphasis is put on development of very sensitive detection tools at the expense of epidemiological research.

Indeed, detection and diagnosis of the viruses present is important; one needs to know the enemy. However, knowing the presence of the enemy, does not defeat it. Understanding the enemy's tactics and its allies is equally important.

When it comes to plant viruses, these tiny pathogens use a wide variety of transmission strategies and vectors as allies. Research and development of knowledge about which virus uses which strategy and on its vector's behaviour, will enable the application of more efficient control measures, which will help the grower to keep his crops healthy.

Concentration of DUS-testing stations inevitable

Monique Krinkels

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The ways to establish distinctness have evolved little over time. For over forty years, Kees van Ettekoven was responsible for variety testing at Naktuinbouw. The Chairman of the Technical Committee of UPOV foresees few changes in the near future. “Reading a report on the laboratory analysis will not do. We will keep looking at the morphological characteristics of a plant.”

“When I started in 1975 at what is now called Naktuinbouw, the obligation to register vegetable varieties had only existed a few years. In the Netherlands, we preferred to organise the necessary DUS-tests separately from Plant Breeders’ Rights testing. Instead, we developed a system where part of the testing was done on the premises of the seed companies,” explains Kees van Ettekoven. “We have always been a very industry-oriented organisation.” Today, Naktuinbouw tests vegetable varieties for registration as well as for Plant Breeders’ Rights. “And besides vegetables, we also do the DUS-tests for arable crops and ornamentals. Every year, about 2,000 new varieties are tested. And now we do most of the work ourselves, as it appears that we can do it more efficiently than the seed companies.”

Discerning differences

With thousands upon thousands of varieties on the market, the chance that a newly bred variety is hardly distinguishable seems more than likely. After all, how many red colours could possibly exist in tomatoes? And there are already over 3,000 tomato varieties registered in the EU. “Enough,” reassures Kees van Ettekoven. “Less than 2% is insufficiently distinct. The people in the variety testing department have trained eyes and can discern existing differences.” He notes that the genetic variation has widened over the years. “Especially with tomatoes, the work has become easier, compared to the seventies and eighties.” Since 2005, Naktuinbouw has been responsible for DUS-tests in all crops, arable, ornamental and vegetable. “Adding ornamental crops to the workload was not that difficult, compared to the vegetable and arable crops.” The latter takes place at another location. The DUS test of potatoes and grasses is done in the control fields of the NAK, the inspection service for arable crops. Flower bulbs are tested on the premises of the BKD in Lisse. The only exception is fruits. “We only have one or two application per year. To maintain an orchard as a reference collection would be far too expensive. In the Netherlands, the breeder has to pay the full costs of the testing, without any compensation by the government. That is why DUS-testing on, for instance, apples is done in Germany.” A complication with arable crops is that they not only

need to be registered, they should also be an improvement to existing varieties. Therefore, the value for cultivation and use should be determined before they can be added to the Recommended List. This is done by the CSAR, an independent organisation. The supervision on the trials is done by Naktuinbouw on behalf of the Board for Plant Varieties.

Only about 40% of all new vegetable varieties are registered in the Common Catalogue of the EU, as some breeders believe that their vegetable is well-protected because it is a hybrid. The remainder also has Plant Breeders’ Rights.

Distinctness

The fundamental definition by UPOV of what distinctness is, remains rather general. Finding out whether a variety is ‘new’ by merely seeing whether it is ‘clearly distinguishable’ would not be very practical. Therefore, UPOV developed ‘test guidelines’ with morphological characteristics. In CPVO, following UPOV, the EU member countries have agreed on a fixed, obligatory set of morphological characteristics that the inspectors have to check. In, for instance, lettuce the inspectors look for about forty identifying characteristics.

There are three types of characteristics: qualitative, quantitative and pseudo-qualitative. Qualitative characteristics (either/or characteristics) give clear undisputed minimum distances. The quantitative characteristics are the most sensitive to growing conditions and observation techniques. Therefore, example varieties (standards) must be incorporated in the examination. The pseudo-qualitative characteristics are, for instance, colour tones and shapes.

There are several drawbacks to identification based solely on morphological traits. For one, it is subjective. However, hardly anyone tries to make it more objective; in the end, it is a person’s eyes that note the differences and similarities. A second drawback is that plants of the same variety may look different due to growing conditions. DNA is objective, can be repeated and does not depend on a person’s experience. “DNA tests are, however, not used to establish distinctness. The problem is that a large part of the plant genome consists of DNA with no or unknown effects on the morphology, the so-called junk DNA. Lily has probably the largest genome in the living



Photo Naktuinbouw

‘To establish distinctness, DNA alone does not yet help us at all,’ says Kees van Ettekoven

world, with 10^6 billion base pairs. No less than 90% of this genome seems to have no effect on the morphological appearance of the plant at all. On the other hand, a similar DNA band pattern in tulips may belong to two clearly different varieties, for example, because of a point mutation that changes the colour. So, to establish distinctness, DNA does not yet help us at all in this particular discipline,’ explains Kees van Ettekoven. ‘The other way around is, however, very effective. If a breeder believes someone illegally propagates his variety, a DNA test can either confirm or negate that fact to a great degree of certainty.’

Valuable addition

There are more situations where DNA fingerprinting has proven to be useful. The 1991 UPOV Convention gives breeders the right to seize end products, but this is only possible if the identity of the product can be established. In these circumstances, DNA fingerprinting is the only viable way to identify a variety.

‘How many red colours could possibly exist in tomatoes?’

A DNA fingerprint has other advantages, for instance, when the plants involved take a long time to mature, such as trees. You cannot wait years until an oak tree produces acorns to compare it with a suspected acorn, whereas with DNA fingerprinting you can do it in days, or at most a few weeks.

And then there is the human factor: judges have a weak spot for DNA. They are easily convinced that the simple stripes on a sheet are the irrefutable proof of an identity. They are used to forensic investigations by the police, where the identity of individuals is also established by DNA fingerprinting. And as Naktuinbouw is an independent institute, it can provide valuable expert witnesses and judges trust their opinions. With morphological tests, it is possible to create doubt, as it is hard for judges to fully comprehend the concept.

Future

But, in the end, it will come down to the morphological characteristics of the phenotype. ‘Don’t forget that the UPOV Convention is based on the state of science in 1960, when the initial convention was established,’ says Kees van Ettekoven. ‘We have to develop improved protocols within the existing regulations.’ An exception is China. ‘That country prefers DNA, as it does not have large living reference collections. The challenge will be to find a good balance between the use of DNA fingerprints and results of morphology in the field, so we can reduce testing time and costs without weakening the strength of the protection.’ One of the main problems DUS-testing is facing is the lack of funding. Especially in the Netherlands, where the seed industry bears the full financial burden. ‘We used to have Community Test Fields for harmonization of characteristics, but the EU has stopped funding these. And we do not have the manpower to continue this, however important these fields were.’ One of the solutions is to reduce the number of DUS-stations. So far, there are more than twenty in the EU. ‘I believe, in the end, between three and ten will remain.’

What will we eat in 2030

Tim Benton

26

The future of food is eagerly contested, with multiple competing ideas about how the future will evolve. The growing human population, with a significantly increasing global middle class, will be the engine of increasing global demand. Historically, increasing wealth has led to changing consumption patterns, particularly more meat and other resource-intensive foods like cheese and eggs. The question is the extent to which historical trends will play out in the future.

On a global basis, more people are now of an unhealthy weight than a healthy weight. At the same time, the historical ‘hunger challenge’ is slowly receding, while malnourishment is increasingly associated with excessive weight and obesity, creating a new challenge for food systems. This is creating a new policy interest in ‘food for health’ which has the potential to help shape diets and thus food systems. Another reason historical trends might not be used to forecast the future is the Paris climate agreement. It pledges to keep climate change to well below 2°C. Given that food systems – growing food and feed, making and transporting food, cooking, eating and throwing food away – account for just under a third of greenhouse gas emissions, food alone has the potential to use up the entire Paris agreement’s carbon

budget. As many people have written, the most potent way to ‘decarbonise’ the food system is to reduce the

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Freeze-dried fruits will replace candy, expects food trend watcher Anneke Ammerlaan. Freeze-dried fruits are crispy and have the structure of a meringue. As sugar consumption should be reduced, these are a tasty alternative.

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amount of greenhouse-intensive food we produce – notably meat.

Thus, on the one hand, projections of demand are growing, but on the other hand, important policy drivers that may constrain demand growth – or increase demand for ‘sustainable nutrition’ – are also growing.

Wrong sort of food

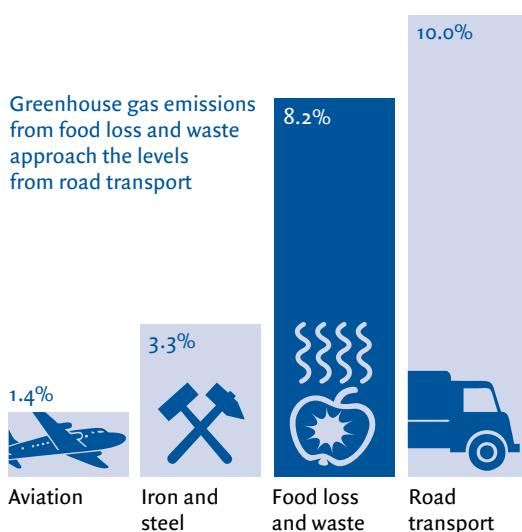
It is undoubtedly true that some areas of the world need access to radically more food, but equally, other areas of the world are suffering from eating too much

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Last year, the American company, Habit, introduced the personalised diet, which is adapted on cell level to an individual. The system is based on nutrigenetics, the relationship between food, genotype, phenotype and health. It uses over 60 biomarkers and the changes of these markers due to a metabolic challenge test. Besides, self-reported data on for example length, weight and blood pressure are used. With the Nutrition Intelligence Engine, Habit can establish the optimal food ingredients a person needs. Habit offers USA-citizens a data-driven personalised nutrition service, with which the client receives personalized diet advice and online coaching from a dietitian. It is also possible to have home-delivered customised meals made by the Habit chefs.

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of the wrong sort of food, and filling landfills with discarded food waste. Many commentators agree we are likely to need a ‘contract and converge’ model, or as Tim Lang, a Professor of Food Policy at City University in London, has eloquently put it: “the rich



In restaurants, the phenomenon ‘botanical dining’ is introduced. Vegetables are presented as a bouquet on a plate. The variation in the plant world is the central theme in this type of food consumption, explains food designer Marielle Bordewijk. Meat is not forbidden; the chefs use the 80/20-rule – 80 percent vegetables and 20 percent meat. The emphasis lies on the richness of tastes that vegetables have to offer.

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Tim Benton is Professor of Population Ecology, School of Biology at University of Leeds, t.g.benton@leeds.ac.uk. He is the expert in global food security from a system’s perspective: from production and its impacts on the environment to trade, manufacturing and retail, and impacts on nutrition and health. This article is based on his publication on World Economic Forum



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Markets will grow for both local 'real food' as well as nutritious 'convenience food'

need to eat less, and differently, so the poor can eat more and differently."

As well as uncertainty over how global demand will evolve, there is uncertainty about how production will evolve. Since the green revolution, the global focus has been to produce a relatively small handful of commodity crops – maize, wheat, rice, soy, palm oil – in ever larger quantities and ever greater technical efficiencies. This means that 'large scale' agriculture can produce calories very cheaply.

For many, food is cheaper than it has ever been (relative to income) and this allows us both to eat a

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Seaweed is the food of the future, predicts food designer Marielle Bordewijk. It grows fast, does not need fresh water and is filled with proteins, vitamins and minerals. There are many types of seaweed and it is an all-round crop that can be used in many ways. The Czech company, Foodlife, which produces many healthy raw food snacks has developed seaweed crisps in a package similar to that of Pringles. The Japanese company, Sakura, has introduced seaweed salad kits on the market and the Swedish company, Nourimare, even makes seaweed chocolate.
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The future diet will consist of more vegetables, says food trend watcher Anneke Ammerlaan. She forecasts a star role for cauliflowers, a vegetable that is gaining popularity in the USA. Besides veggie burgers, she believes in the veggie steak, a slice of for instance aubergine or cauliflower prepared as if it were meat.
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lot and waste a lot. But, however efficient 'big ag' is, it creates significant environmental costs – particularly in eroding soil health, water quality and reducing biodiversity. It also supports few livelihoods, with capital investment increasing, and labour requirements falling.

Healthy diet

One can imagine a different food system. If we lived in a world where demand was different – perhaps because people wanted to eat healthily and sustainably – it is possible to imagine a much greater mix of big and small farms, producing a larger range of produce, employing more people and creating a more local and circular economy.

So what might we eat in 2030? I think demand will be shifting and more people will want to eat a

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What will we eat in 2030

- healthy diet, one that is less intensive (and wasteful) of resources. The increasing emergence of localism, wholefoods, organic, artisanal and 'real food' movements is a sign of this – at least for the rich and dedicated. So our diets may be more veg and fruit, whole grains and vegetarian food or new alternatives (soya products, or perhaps insects or artificial meat),

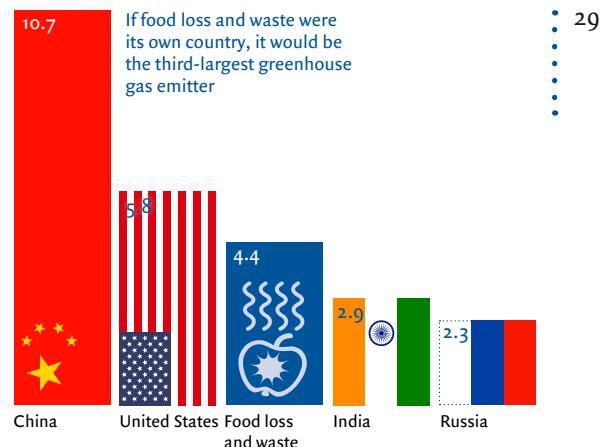
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Common duckweed (*Lemna minor L.*) might well become the main source of protein on our future dinner plate. It is easy to grow, can be harvested year round, is highly productive and it consists of about 45% protein. One hectare of duckweed produces as much protein as ten hectares of soybean. So far, eight varieties of common duckweed is granted European Plant Breeders' Rights.

Using bio-refinement, it is possible to obtain pure and high quality protein that can be used directly in human nutrition, from a veggie burger to an energy drink. A blueprint has been designed for a pilot plant that will process 10,000 tonnes of duckweed into protein and fibres.

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and less fried and sugary things. We'll still eat meat, but, perhaps more like our parents and grandparents, see it as a treat to savour every few days. For the world's poor, the cost of a nutritious diet is so much greater than the cost of a calorie-dense one. It is therefore likely that commodity crops (maize, wheat, sugar, oil) will continue to underpin the global food system – but will be processed in ways that are better for our health. This may include fortification (or biofortification) – where nutrients are engineered in, either in the biology or manufacture of food – and the significant reformulation of current foods for fewer calories and more nutrients. 'Ultra-processed' foods need not be unhealthy.

Diversification

Although there are signs of a push-back against globalisation, its many benefits suggest that increasingly the historical divide between the 'developed' and 'developing' world will break down, and the issues, for every country, will be how to ensure access to cul-



turally acceptable, healthy diets, that are affordable by the poor. This will involve both locally produced food and food traded from afar. Food systems are likely to

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Wageningen University and Research has developed a novel method to produce meat-like products from protein found in soybean, wheat, rapeseed, maize or peas: the shear-cell technology. The university has started a public-private partnership with amongst others Unilever, to enable large-scale production. It is expected that the new products will be marketed in four years' time. Unlike other meat substitutes which resemble either chicken or minced meat, the new products have the same structure as, for example, steak or cutlet.

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diversify as markets simultaneously grow for local 'real food' as well as nutritious 'convenience food'. We won't have an 'organic world' or a 'big ag' world, we'll have both. But we must have better nutrition, less waste, and more sustainability – otherwise we simply stack up ever more problems for the future.

Fresh and healthy from the lettuce factory

Monique Krinkels

30 Later this year, the first commercial 'vertical farm' in Europe will be opened. The Staay Food Group, a fresh fruit and vegetables company, will produce lettuce in a new production plant, in Dronten, the Netherlands. The facility will serve one of Europe's largest supermarket chains, Aldi.

It took almost three years of preparation. The Staay Food Group, Philips Lighting and vegetable breeder Rijk Zwaan collaborated and undertook intensive research to determine the best combination of lettuce varieties and growth recipes in order to improve crop quality and yields. Having the right growth recipe ready prior to the start of operations at the vertical farm will help Staay achieve a faster return on investment. "Yes, it is an expensive system of growing lettuce," says Marc Celis, crop consultant at Rijk Zwaan and specialist in hydroponics. "But it has many advantages."

Benefits

In Europe, vertical farming is a new phenomenon. So far, only research institutes held trials. In South East Asia, however, it is the only way to produce lettuce. "The high temperatures in the tropics are no obstacle for growing lettuce, if you do it in a closed system, where the climate is regulated. Before, the lettuce had to be imported from Australia and that made it

a very expensive vegetable," explains Marc Celis.

North America also has some vertical

farms, mostly to avoid the long-distance transport from California to the East Coast. But there are more benefits than the local for local production. "In a vertical farm, the products don't need any plant protection measures, but strict hygiene. It means you can avoid all pesticides, and therefore no residues, but it also means much lower bacterial count as there is no contamination."

An extra advantage at the Staay production plant in Dronten is that packaging is done on site. "We are building a new plant for FreshCare with an in-house vertical farm," says Rien Panneman, CEO of Staay Food Group. "Our customer, Aldi, has stimulated this development." At FreshCare, potatoes, fruit and vegetables are cut and processed into a limited range of fresh produce that makes its way to the supermarkets in Europe each day. Aldi has over 10,000 stores in 18 countries. Together with the low bacterial count, the in-house production ensures that the lettuce has a longer shelf life at the retailers.

"Also, by avoiding weather fluctuations, we maintain

'You can avoid all pesticides'

an optimized and stable production environment to guarantee consistent and optimal product quality." He is convinced that vertical farming will become profitable in due course. "The difference in cost price will decrease. And besides the quality aspects, it is also environmentally friendly. In winter, we have to import lettuce from Southern Europe, but with a vertical farm we can produce it here. It also helps that the fresh convenience market share is growing rapidly; the supermarkets carry ever more fresh salads and salad meals. We hope that our vertical farm will become a catalyst to local-for-local production of vegetables."

Indoor farm

The 900 m² indoor vertical farm will have over 3,000 m² of growing space. "Compared to open field growing, the yields are remarkably high. On 1 hectare of land, you can grow 100,000 lettuce plants per year. On the same surface of gutters in a vertical farm, 3 million lettuce plants can be produced. And they can be precisely programmed to be ready for harvest at a preset date, preventing both waste as well as shortages." It is also a sustainable technique, as there is minimal water loss and the use of electricity is limited. The light source is from Philips Lighting, whose researchers trial a variety of crops at its research centre GrowWise under different LED lighting and climate conditions to help establish their economic potential. "And the varieties come from Rijk Zwaan. We had to determine which varieties would flourish in the special conditions in a vertical farm and also which varieties offer the best taste and texture. The varieties should not be susceptible to tipburn or bolting. The temperatures in a vertical farm are relatively high compared to the amount of light. Furthermore, the light is less strong than sunlight and the day-length longer. Not all varieties are suited to an 18-hour LED-day." Most lettuce types react well to vertical farming, romaine and iceberg lettuce are the only exceptions.

Hydroponics

Growing on water has become fairly common. "I spend 90% of my time consulting lettuce growers on hydroponic systems. In Scandinavia and Russia, 95% of the lettuce is grown on water. In Belgium, that is 15% to 20% and in the Netherlands the area is



A new phenomenon: growing crops in a 'factory', instead of on a field or in a greenhouse, lettuce stacked layer after layer in water filled gutters under candy-coloured lights

increasing in greenhouses as well as in the open air." There are three hydroponic systems:

N.F.T System: The Nutrient Film Technique (N.F.T.) uses a constant shallow stream of nutrient solution, which is pumped from a reservoir into the growing tray. The plants require no growing medium and the roots draw up the nutrients directly from the flowing solution.

Deep Floating: This system is the simplest of all hydroponic techniques. The roots of the plant are totally immersed in water which can be as deep as 30 cm. The water contains the necessary nutrients and an air pump oxygenates the water to allow the roots to breathe.

Aeroponic System: In the aeroponic systems, the growing medium is primarily air. The roots hang in the air and are misted for a few seconds every couple of minutes with nutrient solution.

For several years, Proeftuin Zwaagdijk has been testing several crops in hydroponic systems. Not only lettuce, but also rocket salad, spinach, herbs, cauliflower, Chinese cabbage and endive. "Together with the growers involved we have opted for the deep floating technique," says researcher Matthijs Blind. "As that is, in our experience, a very reliable and mobile growing system. With lettuce, it is not necessary to oxygenate the nutrient solution actively, so not every grower does it. It has, however, advantages as the lettuce matures more quickly and evenly if air is applied to the nutrient solution."

Hydroponics is gaining popularity in the Netherlands. Problems with Fusarium in lettuce grown in soil make growers search for alternatives such as the deep floating system. "We started a research project on the risks of Fusarium in this growing system last year and it looks promising. At first sight, it seems that the system is less vulnerable to this fungus. When we added Fusarium spores to the water, all plants remained unaffected. However, further research is necessary."

No hype

Although the advantages are obvious, Marc Celis warns against hype. "We believe that vertical farms will become increasingly important, because in the future we will see more economic and environmental pressure to produce fruit and vegetables, such as lettuce, closer to where end-customers are located. But, on the other hand, it is often more expensive than regular lettuce growing. And, yes, it is sustainable, pesticide-free, harvest secure and completely controlled, but you have to consider whether it will bring return on investment. Be realistic and think hard before you start."

How to keep them out!

John van Ruiten

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Recently, the EU decided to start the process of implementing the new Plant Health Regulation 2016/2031. The member states of the Union are bound to carry out the requirements of this Regulation with effect from 14 December 2019. Two and a half year to go for the EU to come up with formats for harmonized plant passports, decisions about Q-organisms, introducing RNQPs and the inspection requirements/tolerances for these diseases.



Xylella on Nerium oleander (Photo NVWA)

Diseases in ornamentals are causing great concern worldwide. In a recent presentation for the members of Fleuroselect, John van Ruiten, director of Naktuinbouw Netherlands, presented an overview of various actual threats that are causing headaches, both for producers (young plants/flowers/pot plants) but also for inspection agencies and phytosanitary services.

1. The main concern in very many crops is the relatively new disease *Xylella fastidiosa*. Various strains (3 up till now) of this bacterial disease have been identified in olives, fruit plants and ornamental plants in the southern part of the EU (South Italy, South of France, Corsica, Mallorca, Ibiza). The disease is also known in the Americas (both North and Latin America). In grape vine, it is known as Pierce Disease. Very strict protection rules have been taken by the EU phytosanitary authorities to prevent the introduction (host plants are only allowed to enter the EU from countries, areas or production sites that are disease-free) and the spread within the EU (a large number of species has to be inspected officially during the growing season before

marketing with an official plant passport is allowed). In regions/locations in the EU where the disease is occurring, a zone of 10 km around the infected area is 'blocked' and no plants for planting are allowed to be moved outside this zone. Extensive research programmes have started to learn more about the epidemiology, susceptibility and detection possibilities.

2. Since 2015, in the cultivation of roses (first in the Netherlands, later also in other countries), there has been great damage to crops because of the presence of *Ralstonia solanacearum* ('brown rot'). A specific strain (not yet known to occur in other crops) has infected both propagating material (rose plants) and cut flower crops. The devastating bacteria can be easily transmitted through water or mechanically. Up to now, the origin of the rose-infections has not yet been identified, but it is assumed that it must have entered professional production systems in Europe via infected plant material from elsewhere. To combat this disease and also investigate the risk of the introduction of other diseases, a group of professional propagators and breeders has started with the implementation of a prevention/hygiene programme,

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Ralstonia on rose (Photo Naktuinbouw)



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Bemisia



Rhodococcus on Poinsettia (Photo Naktuinbouw)

Select Plant. This system is run by Naktuinbouw in the Netherlands.

3. *Bemisia tabaci* (tobacco white fly) is an insect causing more and more problems. Difficult to control and with a broad host plant range, not only in ornamentals, but especially also in vegetable crops, it is widely occurring in the EU. Particularly, there is a fear that various viruses (TYLCV, TCV, CVVV and many others) are going to spread and can cause big losses to tomato, cucumber and other crops. Especially from the UK, requests for intensifying control programs and low (to zero) tolerances for *Bemisia* on plants have been advocated.

4. Following outbreaks of pospiviroids ten years ago in ornamental solanaceous plants (*Brugmansia*, *S. jaasminoides*), these viroids have received a lot of attention worldwide. In the 'family of pospiviroids', nine different viroids are identified in both ornamental plants, vegetable species and potatoes. In setting up propagation programmes, it is strongly advised to thoroughly screen and test the pre-basic plants for absence of all these viroids. In production circum-

stances of seeds and cuttings of ornamental and vegetables, awareness and control measures should be applied.

5. Bacterial diseases like *Curtobacterium* in Poinsettia, *Agrobacterium* and *Rhodococcus* (*Pelargonium*, *Kalanchoe*, *Impatiens*) are of increasing incidence. Some of them are 'old' diseases, had almost disappeared, but have reoccurred in production. They are easily spread, become latent after a time and, if present in the production system, they are very difficult to control. Great care and attention is required.

Measures for staying disease-free and keeping crops healthy against all these pathogens cannot be found in applying crop protection. For most of them, there are no effective compounds available. Hygiene, prevention, screening selected material, regular control sampling and breeding towards resistance/tolerance are the routes that we have to follow to keep nurseries free of these harmful pathogens and to produce healthy plants for consumers.

CO₂ starvation causes major stress

Geert-Jan de Klerk and Naser Askari

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At a cursory glance, tissue culture seems like paradise, a land of Cockaigne for plants. Ample amounts of water and carefully chosen nutrients are available for consumption and the temperature is always 'pleasant'. However, researchers have noted several instances of severe stress. This article deals with an as yet unnoticed, devastating peril: CO₂ starvation.

In-vitro conditions have a huge effect on the physiology of plants. They dramatically alter their water balance and hormone household. For several reasons, there is also a vast, negative effect on photosynthesis. First, the low level of light allows only little photosynthesis. Furthermore, the disordered regulation of the opening of the stomata affects photosynthesis. Moreover, sucrose taken up from the nutrient medium inhibits photosynthesis substantially. Some researchers even believe that in tissue culture photosynthesis is virtually absent. However, since the observed circadian changes in the levels of CO₂ and O₂ are as expected for active photosynthesis, this is obviously not true. It is surprising that, even though the occurrence of photosynthesis in vitro is evident, the relative contributions of photosynthesis and added sucrose to plant growth are still not known. It seems though that the contribution of photosynthesis is much less than the contribution of sucrose added in the nutrient medium, since in sucrose-free medium, growth is very much reduced, usually by far more than two thirds. Some tissue culture researchers have 'returned' to photosynthesis as the sole source of carbohydrates by omitting sucrose from the nutrient medium. The photoautotrophic culture conditions included increased light and forced ventilation with humidity and CO₂ control. Such photoautotrophic tissue culture was developed by T. Kozaï's group in Japan. Larger, leafier, faster growing shoots were obtained than with conventional mixotrophic conditions. The costs and complexity of the growth chambers, though, turned out to be large obstacles to wide scale commercial use. Furthermore, the comparison between autotrophic and mixotrophic performance was made using conventional tissue culture conditions that are very likely suboptimal.

Reactive Oxygen Species (ROS)

In a tissue culture container with green plantlets cultured under conventional tissue culture conditions, photosynthesis lowers the CO₂-level during the light period, often to less than 100 µmol.mol⁻¹ (0.1 %), so much lower than the atmospheric CO₂ concentration of 400 µmol.mol⁻¹ (0.4 %). Even in loosely capped vessels or vessels capped with gas-permeable film, the concentration is frequently lower than 200

µmol.mol⁻¹. CO₂ can be completely removed from the headspace by placing a small vial with concentrated KOH solution on top of the medium. In the course of CO₂ starvation, all endogenous electron acceptors become reduced and oxygen is the main available electron acceptor. Oxygen can serve as electron acceptor in the Mehler reaction. The products of the Mehler reaction include superoxide, hydroxyl radicals and hydrogen peroxide (Reactive Oxygen Species, ROS). ROS are frequently produced by plants. In stressful conditions, ROS rapidly increase (oxidative burst). ROS are very harmful to cells because of their ability to oxidize cell constituents such as DNA, proteins, and lipids. ROS are removed by antioxidants and antioxidative enzymes. In recent years, it has also become apparent that ROS have an important signalling role in plants.

Culture in a CO₂-free atmosphere

We studied growth of *Arabidopsis* seedlings under four conditions (Fig. 1): Under standard conditions with or without 3% sucrose in the medium with or without removal of CO₂. Removal of CO₂ was achieved by placing a small vial with saturated KOH

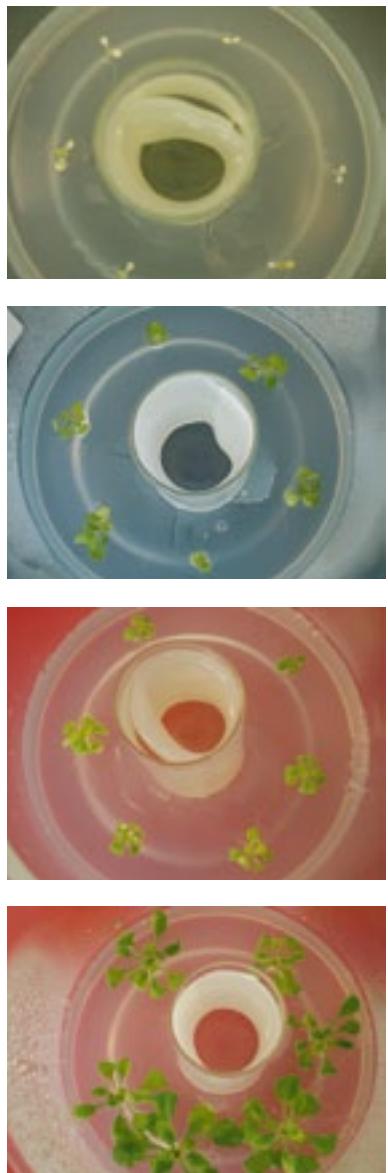


Figure 1. *Arabidopsis* seedlings were cultured for 7 days on medium with (3%) or without (0%) sucrose. In addition, in half of the containers a small vial was placed with 20% KOH to remove CO₂. As a control for the drop of the RH by the 20% KOH solution, in the other containers a small vial was placed with saturated KCl

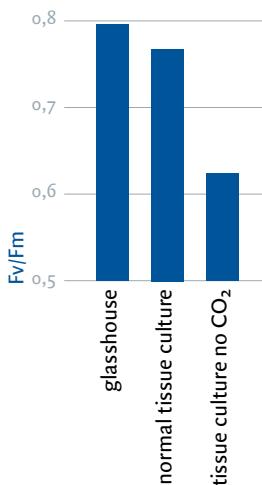


Figure 2. Chlorophyll fluorescence of plants from the glasshouse, from normal tissue culture and from tissue culture in the absence of CO₂



solution on top of the medium. When CO₂ is not eliminated, the CO₂ level in the headspace expectedly fluctuates being (very) low when the lights are on and high in darkness.

Omitting sucrose resulted in significantly reduced growth, evidently brought about by a lack of sucrose as supplier of energy and building blocks for macromolecules like cellulose. Moreover, because the light intensity was low, photosynthesis was insufficient. When all photosynthesis was stopped by the removal of CO₂, no sucrose could be supplied via this pathway. The remarkable result of this experiment was that growth was almost fully inhibited when the seedling was provided with sucrose via the medium and CO₂ was eliminated. This result indicates either that sucrose in the medium cannot replace sucrose produced in photosynthesis (not because it is chemically different but, for example, because it is transported via another route) or that some other factor is involved. Our research indicated that the latter explanation holds: another factor turned out to be crucial. To identify this factor, we did some more experimentation. It should be noted first, that the CO₂-starved seedlings looked bleached. This usually points to the activity of ROS. We also examined whether the photosynthesizing apparatus was damaged, another indication of ROS. For this, we used chlorophyll fluorescence (PSII efficiency). This is a simple and widespread method to establish photosynthesis performance. The ratio of variable (Fv) to maximal (Fm) chlorophyll fluorescence (Fv/Fm) in photosystem II of the photosynthesis apparatus is believed to be an index for maximum photon yield. Fv/Fm has been used to assess damage to photosystem II. As a control, we used glasshouse-grown Arabidopsis



Figure 3. In plantlets grown in the glasshouse, in tissue culture under normal conditions or in tissue culture without CO₂ in the headspace, ROS was stained with nitroblue tetrazolium. Note that for easy handling for the condition without CO₂ large seedlings had been selected

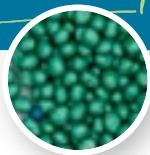
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seedlings. The bar diagram (Fig. 2) shows that the tissue culture condition itself reduced the ratio Fv/Fm a little, but a very strong reduction occurred when CO₂ had been removed. So it seems that ROS produced in the Mehler reaction had damaged the photosynthesizing machinery.

We examined the endogenous levels of ROS by staining 3-week old *Arabidopsis* seedlings with nitroblue tetrazolium (NBT) solution. The presence of ROS is shown by an intense blue colour. Glasshouse-grown seedlings showed hardly any staining, whereas seedlings cultured in vitro without CO₂ were very blue (Fig. 3). Seedlings cultured in vitro under normal conditions show little but unmistakably blue staining, indicating the occurrence of low levels of ROS.

ROS under normal conditions

Plantlets grown in vitro in the absence of CO₂ suffer from severe stress as shown by the great quantities of ROS. Under glasshouse conditions, CO₂ is the receptor for electrons produced by excited chlorophyll but in its absence O₂ acts as receptor leading to massive production of ROS. The NBT staining also indicated the occurrence of ROS under normal tissue culture conditions (Fig. 3). These ROS may originate in the same way as in the absence of CO₂ because in normal tissue culture conditions, CO₂ levels in the headspace can be very low, often less than 100 µmol.mol⁻¹. Occurrence of ROS may, however, also have been caused by other stresses imposed by the tissue culture conditions. It is likely that at low levels, ROS inhibits growth to some extent. The frequently observed increase of growth in tissue culture after adding antioxidants like phloroglucinol may be related to this.



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SEED & SERVICES

Smart Seeds

Stop fooling the consumers

Rosanne Hertzberger

According to the advertisements of supermarkets, our food is produced by weather-beaten farmers who raise their crops by (muddy) hand. This romantic notion is supposed to give consumers a safe and almost cosy feeling. But it is time to stop fooling people: the plants we eat do not grow from grandma's seeds, but from high tech seeds that are developed in a laboratory. And that fact should be the number one selling point.

No one in their right mind would consider buying a new car built by the mechanic at the local garage, or a mobile phone produced by a nifty neighbour. We want things to be smart: smartphones, smart-cars, smart-television and smart-houses, created by smart people in high-tech surroundings. But when it comes to food, the consumer seems to be a present-day Luddite: agriculture should be natural and farmers craftsmen who produce healthy products with seeds selected by hand, based on intuition, without genetic profiling or bioinformatics. Simple, straightforward and old-fashioned. It is time for them to learn the truth: there is as much technology, knowledge and science in an onion as in an iPhone or a Tesla.

The Age of Man

We live in a new era: the Anthropocene. For 10,000 years, man has been the most important factor on Earth. We pollute, move, irrigate, heat and acidify our natural surroundings. We decide who is to live and who is to die. Even species that once were main causes of death – HIV, malaria, tuberculosis – are regularly defeated by humankind. For plants, animals, bacteria, fungi and other living things, there is but one strategy that guarantees success: please humans.

For a plant, the best option is to genetically lay belly up and let the people in laboratories rebuild your characteristics. The reward is big: the offspring will populate hundreds of millions of hectares. Take a mandarin, nothing is more annoying and inelegant than to have to remove dozens of pips from your mouth because your fruit has decided to be fertile. As this is the Anthropocene, we decide when, how and

Rosanne Hertzberger is molecular microbiologist and author of the book, 'Ode aan de E-nummers' (Ode to the E-numbers), on how food and agricultural technology make our lives better



If fruits and vegetables were labelled in the same way as processed foods, it would dishearten consumers as all fruits are, in fact, filled with chemicals and E-numbers. According to chemistry teacher, James Kennedy, an all-natural blueberry contains: **AQUA** (84%), **SUGARS** (10%) (fructose (48%), glucose (40%), sucrose (2%)), **FIBRE** (2.4%) (E460, E461, E462, E464, E467), **AMINO ACIDS** (< 1%) (glutamic acid (23%), aspartic acid (18%), leucine (17%), arginine (8%), alanine (4%), valine (4%),

glycine (4%), proline (4%), isoleucine (3%), serine (3%), threonine (3%), phenylalanine (2%), lysine (2%), methionine (2%), tyrosine (1%), histidine (1%), cystine (1%), tryptophan (<1%)), **FATTY ACIDS** (< 1%) (omega-6 fatty acid: linoleic acid (30%), omega-3 fatty acid: linolenic acid (19%), oleic acid (18%), palmitic acid (6%), stearic acid (2%), palmitoleic acid (< 1%), ash (< 1%), phytosterols, oxalic acid, E300, E306 (tocopherol), thiamin), **COLOURS** (E163a, E163b, E163e, E163f, E160a), **FLAVOURS**

(ethyl ethanoate, 3-methyl butyraldehyde, 2-methyl butyraldehyde, pentanal, methylbutyrate, octene, hexanal, decanal, 3-carene, limonene, styrene, nonane, ethyl-3-methylbutanoate, non-1-ene, hexan-2-one, hydroxylinalool, linalool, terpinyl acetate, caryophyllene, alpha-terpineol, alphaterpinene, 1,8-cineole, citral, benzaldehyde), methylparaben, 1510, E300, E440, E421 and **FRESH AIR** (E941, E948, E290)

with whom a mandarin will propagate. And, therefore, we consume sterile mandarins.

Proud

I believe we should be proud that we have reached the Anthropocene era. It is the ultimate victory, something every species aspires. If, in a hundred thousand years, people look back, they will see our fingerprints. And it is our duty to make sure this era will continue. To do so, it is necessary to end the technophobia, the sentimental longing for the old ways and the romanticism about agriculture, as it blocks innovation. We need smart solutions. The toolbox of a plant breeder

contains CMS, protoplast fusion, embryo rescue and many more technologies. A modern vegetable is a highly productive F1 hybrid plant that will grow uniformly, is resistant to pathogens, is cheaper and has a longer shelf life. It is partly responsible for the fact that there are 7.5 billion people on Earth, of which fewer and fewer go to bed hungry.

We do not have to tell people what they want to hear, to feed their sentiments about food. It is time to outrightly and proudly sell smart seeds. To tell people to wake up and get used to it. We humans adapt nature to our wishes and not the other way around. We do not have time to monkey about.

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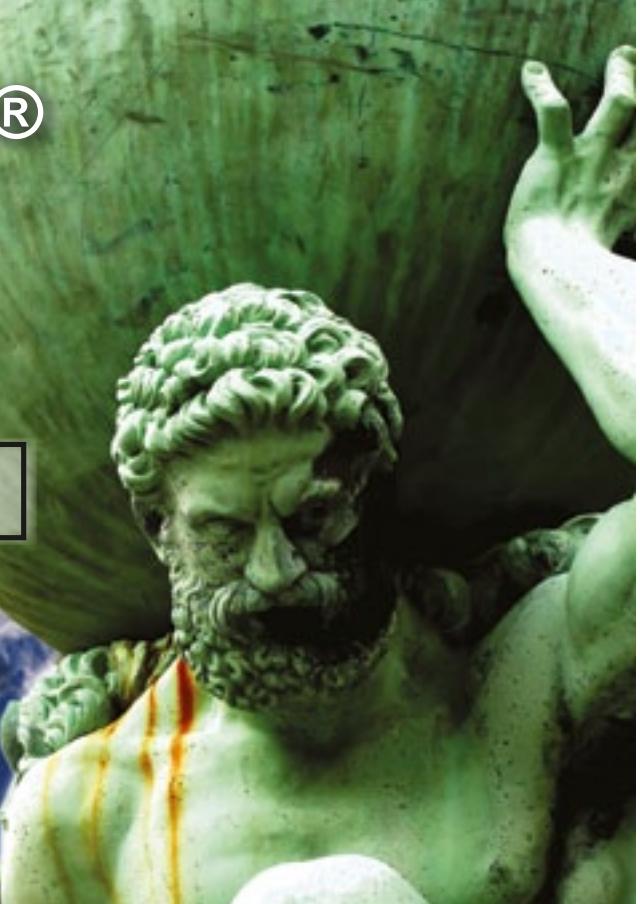
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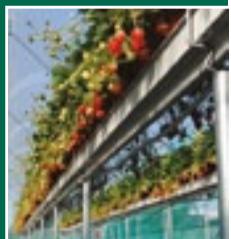
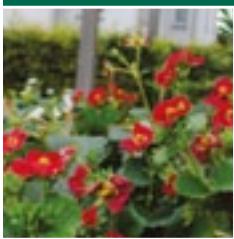
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HOLLAND STRAWBERRY HOUSE

Marloes Oost

Seed Processing Holland is committed to co-operating with seed companies in developing and manufacturing suitable solutions for their needs and challenges. They have been fulfilling this mission for 50 years, beginning with the introduction of small, simple manual machines in 1967 right through to their latest fully automated and integrated seed processing installations.



Innovation of seed processing equipment never stops

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Climate change creates the need for companies to continuously adapt their seeds. At the same time, the world population is expected to grow from 7 to 9 billion (according to the United Nations) by 2040, which means the demand for food is set to increase by 70%. That is why, now more than ever, we need to get the utmost percentage of harvest out of the crop. In addition to this, seed companies are focused on optimizing their internal operations and automating more and more processes via methodologies, such as Lean and Six Sigma.

Partners

Seed Processing Holland is at the forefront of the development of new machines and they are true partners throughout the full process. By adding more automation, flexibility and accuracy to the machines, they are quicker and easier to operate. This results in higher productivity for their customers. One of their customers described their dedication as 'Truly your staff have saved many seed companies from pulling their hair out, over how to process various types of seeds.'

An example of such a partnership project is the design of a complete new facility for one of their American customers. The goal of this customer was primarily to improve seed quality by finishing processes such as sizing (calibration), seed treatment and gravity separation processing. Seed Processing Holland helped

them with every step of the process in designing an efficient, self-cleaning production flow and ensuring a safe, dust-free and low noise working environment. From initial discussions for matching their needs, through the delivery & installation process, the training process with employees and lastly the fine-tuning of the equipment to ensuring it functioned properly. This resulted in a high-quality, durable facility with all types of seed processing equipment.

Robotica

In the coming years, the focus will be even more on improving the service level to meet our customers' needs and expectations, says Arjan Kunst, CEO at Seed Processing Holland. The company will be offering worldwide services and maintenance for their machines. They will keep on developing new machines and processes for all the steps in seed processing, seed breeding and research. Robotica and optical sorting are two important techniques on the R&D roadmap. These will be used to focus on keeping the seed quality as high as possible and increase, where needed, the total process flow from the extraction of the seeds through to the packaging.

Nowadays, at the beginning of the seed extraction process, the different quality of seeds is often spread, which causes the overall quality of the seed batch to drop more than necessary. This results in the need for a longer priming process to upgrade the batch to high quality. By sorting the vegetables in quality earlier in the process, before extraction, the number of seeds that need priming is smaller and thus the total priming process is shorter. This obviously saves time and resources in the process.

"Our continuous drive for innovation and partnering with customers has made the company so successful over the past 50 years and will have an even stronger impact in the years to come," he concludes.

Seed Processing Holland

Seed Processing Holland is a leading equipment manufacturer for the seed processing industry. It offers over 150 standard machines in 10 product groups and develops custom-built, special machines and turnkey projects. The company has supplied to over 135 countries.

Oxfam pleads for more recognition of Farmers' Rights in UPOV

Bram de Jonge

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Smallholder farmers in developing countries access 91% of their seeds from informal systems. They also obtain new improved varieties mainly through seed exchange and local trade, because they have limited access to retailers or cannot afford the price of their seeds. Oxfam hopes this practice will find approval by the UPOV-members.

Oxfam's vision is a just world without poverty. We believe that people can build and strengthen their own livelihoods, provided their rights are respected and implemented. The Sowing Diversity = Harvesting Security (SD=HS) programme (www.sdhsp.org) supports the rights of local and indigenous peoples and smallholder farmers, largely women, by enabling them to access and develop plant genetic resources for food and agriculture suited to their needs. Oxfam works with national partners, e.g. the Community Technology Development Trust (CTDT) in Zimbabwe.

Creating agro-biodiversity

Farmers are the original plant breeders who created the basis of agrobiodiversity that exists today. Nowadays, farmers continue to improve food crop diversity through continuous adaptation to diverse and changing socio-economic, cultural and political conditions, as well as changes in agro-ecological circumstances, where adverse conditions are intensified due to climate change. Local and indigenous peoples and

smallholder farmers manage their seeds and actively utilize Plant Genetic Resources for Food and Agriculture (PGRFA), subjecting cultivars under various selection pressures, testing, selecting and evaluating seeds, encouraging introgression and even actively crossing varieties to create new diversity. Seeds are saved for replanting, exchanged as public goods with family members and neighbours, or sold in the local markets. This is the system by which smallholder farmers maintain and create crop genetic diversity, in the process using agrobiodiversity for adaptation to opportunities and pressures related to markets, on- and off-farm livelihoods and climate change.

Smallholder farmers cannot be seen as mere end-users of innovations from the formal system, since this would not recognize their practices and traditional role in the management of PGRFA. Such reduction to the role of end-user would create undesirable dependency and disempowerment, and would result in a major loss of innovation in agriculture as smallholder farmers continue to provide the world with new, diverse and adapted cultivars.

In remote, marginal areas farmers have very limited access to retailers. Furthermore, the quality and the timely supply of these seeds are often unreliable. By not allowing the practice to freely save, exchange and sell farm-saved seed/propagating material with regard to varieties protected by plant breeders' rights, smallholder farmers can be criminalized and their main channel to access and fully utilize new varieties produced by the formal sector is blocked.

According to the International Treaty on Plant Genetic Resources for Food and Agriculture, a legally binding treaty with over 140 Contracting Parties, farmers have the right to participate in making decisions, at the national level, on matters related to the conservation and sustainable use of plant genetic resources for food and agriculture. The constructive and active participation of farmers should be facilitated and accessible and unrestricted information provided. In particular, participation by farmer organisations and other stakeholders from developing countries should be promoted and financially supported. In practice, however, this is often not the case.

Oxfam's Sowing Diversity = Harvesting Security programme aims to develop farmers' capacities

Oxfam's recommendations

In order to support the implementation and realization of Farmers' Rights, in particular regarding local and indigenous communities and smallholder farmers in developing countries, Oxfam urges UPOV to consider and follow-up the following recommendations:

- Allow and support smallholder farmers the full execution of their right to save, exchange and sell farm-saved seed, including seed of protected varieties.
- Allow and support Member States to implement measures in their PVP law that secure compliance with relevant (inter)national obligations dealing with the protection of traditional knowledge and benefit-sharing relevant to PGRFA.
- Improve transparency and democratic accountability in decision-making processes on PVP laws at the national and international levels, including by promoting and ensuring the full and active participation of farmers and their organizations in the further implementation of UPOV at the national and international levels.

Dr. Bram de Jonge is researcher at the Law and Governance Group of Wageningen University and Research and seed policy advisor at Oxfam, The Hague, the Netherlands, bram.de.jonge@oxfamnovib.nl, www.sdhsp.org

Oxfam is an international confederation of 19 organisations, networked together in more than 90 countries, as part of a global movement for change, to build a future free from the injustice of poverty. It was formed in 1995 by a group of independent non-governmental organisations. Oxfam uses a combination of rights-based sustainable development programmes, public education, campaigns, advocacy and humanitarian assistance in disasters and conflicts. The name 'Oxfam' comes from the Oxford Committee for Famine Relief, founded in Britain in 1942. The group campaigned for food supplies to be sent through an allied naval blockade to starving women and children in enemy-occupied Greece during the Second World War. The member organizations of the Oxfam International confederation are based in: Australia, Belgium, Canada, Denmark, France, Germany, Great Britain, Hong Kong, Ireland, India, Italy, Japan, Mexico, Netherlands, New Zealand, Quebec, South Africa, Spain and the United States. More information: www.oxfam.org.

Photo: Sacha de Boer



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Women preparing for a Seed Fair at the Chibika Seed Bank, Zimbabwe

in managing their genetic diversity and supports communities to claim their rights to do so effectively. It encourages communities to participate in decision-making processes related to their seed and food security, and aims for structural changes by engaging in necessary policy reform.

Solution

UPOV 1991 contains an optional 'farmers' privilege', through which countries may allow farmers to save and reuse seed of a protected variety 'on their own holding' and 'within reasonable limits and subject to the safeguarding of the legitimate interests of the breeder'. UPOV Contracting Parties have the flexibility to consider, where the legitimate interests of the breeders are not significantly affected, in the occasional case of propagating material of protected varieties, allowing subsistence farmers to exchange this against other vital goods within the local community. This interpretation of UPOV's private and non-commercial use exemption is too narrow, as any person farming for subsistence needs, will aim to sell some of his or her harvest after a good season, or exchange seed with, for example, family members living outside the local community. Furthermore, the term 'vital good' is unclear and leaves unanswered if cash would qualify as vital good.

Whereas UPOV's interpretation of the private and non-commercial use exemption has been notably narrow and restrictive, the European Seed Associa-

tion holds that 'Subsistence farmers in developing countries however are not prohibited to exchange seed with or sell seeds to other subsistence farmers as according to the UPOV 1991 Convention the protection conferred by a plant breeders' right does not extend to acts done privately and for non-commercial purposes.' Oxfam urges UPOV to establish a proper and explicit balance between Farmers' Rights and Plant Breeders' Rights in order not to obstruct the practice of seed exchange and trade amongst smallholder farmers, thus enhancing seed and food security, as well as continuous innovation of the plant genetic resources used by smallholder farmers. This should be done by providing a clear interpretation of the private and non-commercial use exemption, allowing smallholder farmers to freely save, exchange and sell farm-saved seed of protected crop varieties amongst themselves and in local markets, and to assist (prospective) member states to include such interpretation in their national legislation. In order to make such broadened exemption to the breeders' right legally and practically operational, a more precise definition of the targeted category of farmers is necessary. For example, the Ethiopian draft Plant Variety Protection bill defines a smallholder farmer as someone whose total earnings from sales of crops produced do not exceed the average household income. Oxfam is currently discussing possible definitions with policymakers and representatives of various seed companies.

Next Generation Sequence Technology reveals bacteria and viruses

Theo van der Lee and Peter Bonants

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The application of Next Generation Sequence (NGS) Technology for diagnostic purposes is a new development. Within the EU project 'TESTA', this technology together with TaqMan PCR has been applied on clean, spiked and contaminated tomato seed lots. Using DNA seq and RNA seq performed on the Illumina sequencing platforms, different seed lots have successfully been analysed for the presence of plant pathogenic bacteria and viruses.

Different plant pathogens can be transmitted by seed (Prophyta Annual 2016:10-11). Therefore, seed-borne pathogens pose a serious threat to modern agricultural cropping systems, as they can be rapidly disseminated to many geographical regions around the world. With trends of increasing global seed production and trade, seed-health testing, including detection of pathogens, is an important quality control step to prevent the introduction and spread of these harmful pathogens. The risk for world-wide dissemination of the pathogen was shown by Van Brunschot (2014), in which an outbreak of Potato Spindle Tuber Viroid (PSTVd) in tomato was linked to imported seed.

Seed transmitted pathogens

Current methods for detection of seed-borne plant pathogens include:

Fungi: Blottertest: freeze, incubate, examine based upon morphology, PCR

Bacteria: Dilute plating, (semi-)selective media, confirmation by PCR and/or Immunofluorescence (IF), pathogenicity assays

Viruses: ELISA, indicator plants, RT-PCR

Viroids: RT-PAGE, RT-PCR

ISHI (International Seed Health Initiative), ISTA (International Seed Testing Association) and EPPO (European Plant Protection Organization) have developed, validated and published procedures within their seed health committees and diagnostic panels. All these methods are based on detecting a single pathogen. In the last decades, molecular methods have been developed, validated and implemented in modern seed technology. These methods are fast, specific and sensitive. In our study, we investigated several important seed-borne pathogens of tomato:

- 1) Pepino mosaic virus (PepMV), a positive, single stranded RNA virus, member in the genus Potexivirus, family Flexiviridae and is pathogenic to tomato (*Solanum lycopersicum* L.).

- 2) Potato spindle tuber viroid (PSTVd) is a quarantine pathogen (A2) in the European Union and causes damaging diseases of solanaceous crops. PSTVd is the type member of the genus Pospiviroid (family Pospiviroidae) and consists of a single-stranded, circular RNA molecule, measuring approx. 360 nucleotides.
- 3) *Clavibacter michiganensis* subsp. *michiganensis*, a

seed-borne bacterial pathogen, is the causal agent of bacterial canker of tomato.

4) A complex of four *Xanthomonas* species responsible for bacterial spot disease on tomato and pepper: *X. euvesicatoria*, *X. perforans*, *X. vesicatoria* and *X. gardneri*.

5) *Pseudomonas syringae* pv. *tomato*, the causal agent of bacterial speck of tomato plants. This bacterium produces angular necrotic spots on leaves and fruits, followed by the appearance of a chlorotic halo. The disease causes serious economic losses especially on fruits of susceptible genotypes.

Molecular detection

For all these tomato pathogens, molecular detection methods are available. DNA/RNA extraction is an essential part of most molecular methods. Extraction of nucleic acid is a challenge for routine diagnostic testing, based on molecular biology procedures, because the robustness of subsequent steps often relies on the quality of extracted nucleic acids. For seeds, many methods have been described, but they are versatile in nature. Current limitations of DNA/RNA extraction for seeds:

- Treatments and/or coating of seeds with fungicides, dyes, pelleting materials, etc.

- Too many different extraction technologies are used, dependent on where the pathogen is: on or in the seeds and also the plant seeds vary dramatically in size and composition.

- The presence of inhibitors of PCR.

- Internal controls are often not integrated in the assays to screen for these inhibitors.

Different commercially available DNA/RNA extraction kits were evaluated in a generic approach, using also internal controls to check the extraction procedure. One of the preferred methods for detection at the moment is real-time PCR (TaqMan or SYBR Green). For many seed-transmitted plant pathogens (bacteria, viroids, viruses and fungi), (real-time) PCR assays have been developed and sequences are available from different sequence databases. In the EU project TESTA (Development of seed testing methods for pests and pathogens of plant health concern), we validated and implemented a generic method for detection of pathogens in tomato seeds using real-time TaqMan PCR, so that detection can be performed

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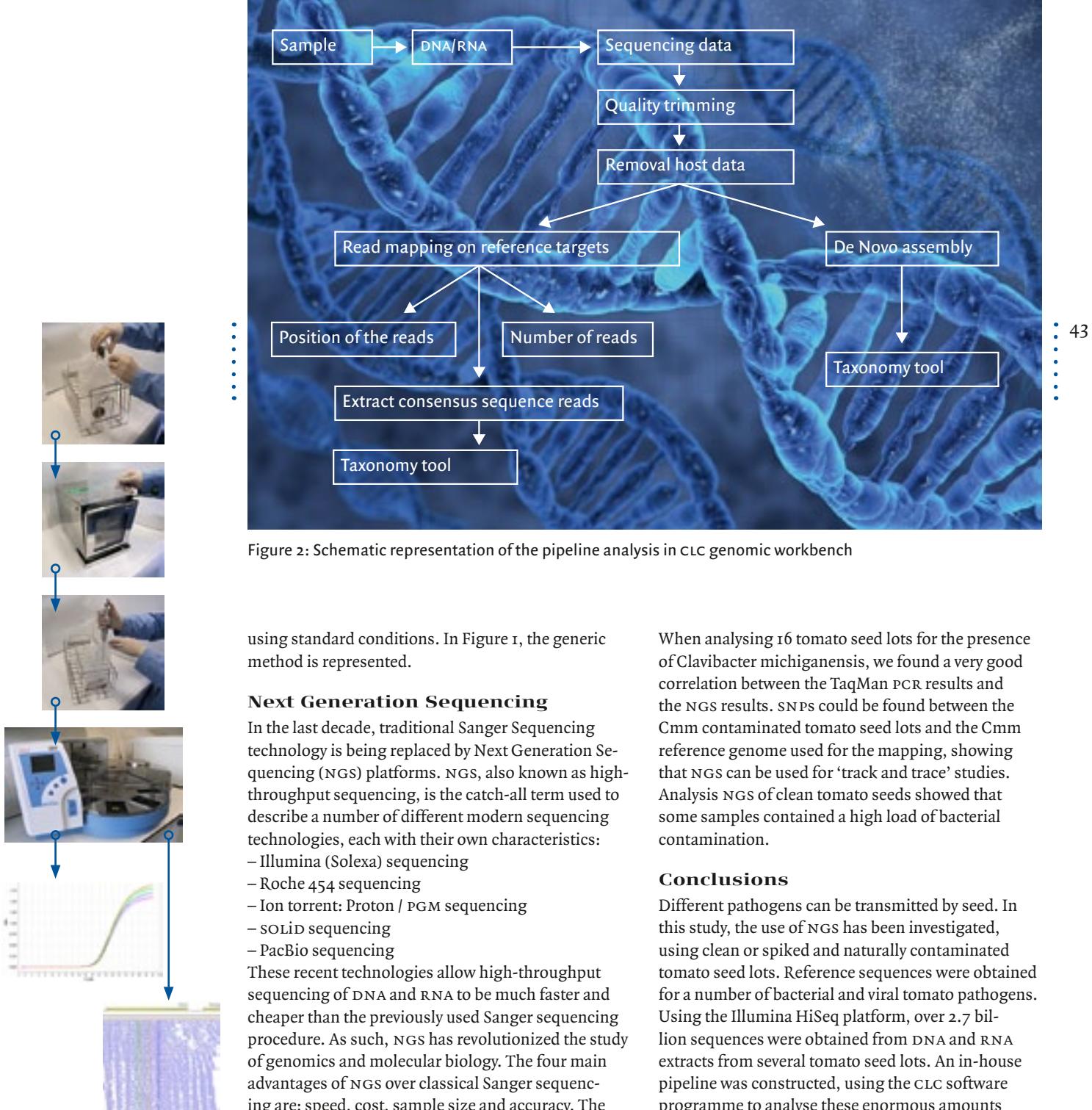


Figure 2: Schematic representation of the pipeline analysis in CLC genomic workbench

using standard conditions. In Figure 1, the generic method is represented.

Next Generation Sequencing

In the last decade, traditional Sanger Sequencing technology is being replaced by Next Generation Sequencing (NGS) platforms. NGS, also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies, each with their own characteristics:

- Illumina (Solexa) sequencing
- Roche 454 sequencing
- Ion torrent: Proton / PGM sequencing
- SOLiD sequencing
- PacBio sequencing

These recent technologies allow high-throughput sequencing of DNA and RNA to be much faster and cheaper than the previously used Sanger sequencing procedure. As such, NGS has revolutionized the study of genomics and molecular biology. The four main advantages of NGS over classical Sanger sequencing are: speed, cost, sample size and accuracy. The Illumina HiSeq 2500 platform has been used in our study to investigate the possibilities for the detection of the tomato pathogens in the different tomato seed lots, using NGS on DNA- and RNA extracts. However, analysis of the obtained sequences needs extensive bioinformatics. To analyse the millions of sequences obtained from seed tomato seed extracts, we built a so-called pipeline (Figure 2).

Figure 1: Schematic representation of the DNA extraction procedure from tomato seeds followed by TaqMan PCR and NGS analysis

In the TESTA project, DNA extracts of different tomato seed lots were analysed following this pipeline.

When analysing 16 tomato seed lots for the presence of *Clavibacter michiganensis*, we found a very good correlation between the TaqMan PCR results and the NGS results. SNPs could be found between the Cmm contaminated tomato seed lots and the Cmm reference genome used for the mapping, showing that NGS can be used for 'track and trace' studies. Analysis NGS of clean tomato seeds showed that some samples contained a high load of bacterial contamination.

Conclusions

Different pathogens can be transmitted by seed. In this study, the use of NGS has been investigated, using clean or spiked and naturally contaminated tomato seed lots. Reference sequences were obtained for a number of bacterial and viral tomato pathogens. Using the Illumina HiSeq platform, over 2.7 billion sequences were obtained from DNA and RNA extracts from several tomato seed lots. An in-house pipeline was constructed, using the CLC software programme to analyse these enormous amounts of sequence reads. Next to the identification of new targets derived from validated databases for pathogens, such as Q-bank (www.q-bank.eu), a new feature (Taxonomy Tool) was included in the pipeline to analyse sequences available in NCBI and other reference taxonomy databases. Using this approach, we showed that there was a good correlation between TaqMan PCR and NGS data for the known pathogens, but we also demonstrated the added value of NGS to detect the presence of previously unknown bacteria that are associated with tomato seeds.

Obtaining disease resistance by changing S-genes

Henk J. Schouten, Yuling Bai

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Plant pathogens exploit plant genes in the interest of the pathogens. These plant genes are called 'susceptibility genes', abbreviated as S-genes. Knocking down or knocking out S-genes may hamper the ability of the pathogen to infect the plant, leading to durable resistance.

In cases where people are allergic, their immune system is hypersensitive to something in the environment that usually causes little or no problem to most people. This hypersensitivity leads to exaggerated defence reactions. Similarly, plants can overreact, giving hypersensitive responses, even when pathogens are absent. In order to prevent such overreactions and autoimmune syndromes, the plant has genes that prevent or pacify unnecessary defence responses. These genes are called 'defence suppressor genes'. Plant pathogens, however, may abuse these defence suppressors, by activating them during infection, thus preventing an adequate defence by the plant. In that case, activated defence suppressor genes lead to susceptibility of the host. The largest group of known S-genes belongs to these defence suppressor genes. Knocking down (silencing) or knocking out defence suppressor genes by using RNAi or mutation techniques can prevent suppression of defence, thereby leading to enhanced disease resistance. However, knocking out defence suppressors can also lead to hypersensitive responses during absence of pathogens, and thereby to negative fitness effects. This is discussed at the end of this paper.

Early pathogenesis

Another group of S-genes is involved in the very early stages of pathogenesis. The most well-known examples belonging to this group of S-genes are the MLO-genes that support powdery mildew fungi. Already in 1942, it was discovered that mutations caused by Rontgen irradiation of barley led to loss of susceptibility to powdery mildew, and therefore to increase in resistance. Later, it was discovered that in this barley mutant, the Mildew Locus O (MLO) gene was knocked-out. The MLO-gene appeared to be an S-gene. The term 'susceptibility gene', however, was not used yet, and it was only launched in 2002 in view of the S-gene Powdery Mildew Resistance 6 (PMR6). By then, the mlo-resistance in barley had already been shown to be very durable, and still it has not been overcome yet by the pathogen. MLO-like S-genes for powdery mildew are not limited to barley, but are omnipresent in plant species, both in monocots and dicots. Usually, plants have more than 10 MLO-genes, but only up to three or four of them are S-genes.

Typically, one of these MLO-genes shows the highest transcript (mRNA) levels in the leaves, and therefore plays the major role as S-gene for powdery mildew. We have shown this in our lab for tomato and for cucumber.

The transcription of MLO S-genes is already induced by the fungus about 6 hours after inoculation, before fungal penetration of plant epidermal cells, indicating that these S-genes are involved in early phases of pathogenesis. The coded protein is anchored in the plasma membrane, so close to the outside of the plant cell.

Feeding the pathogen

A group of S-genes that plays a role during later phases of pathogenesis, are the plant genes that help to feed the pathogen, thus allowing growth of that pathogen. An interesting example of an S-gene belonging to this group is the Ossweet14 gene in rice. This gene encodes a protein that pumps sucrose from the plant cell into the apoplast. The pathogen *Xanthomonas oryzae* pv. *oryzae* has an effector that binds to the promoter of this SWEET-gene, thus activating this gene, and thereby stimulating sucrose transport to the apoplast where the pathogen resides. By doing so, the pathogen hijacks a plant gene, for its own nutrition.

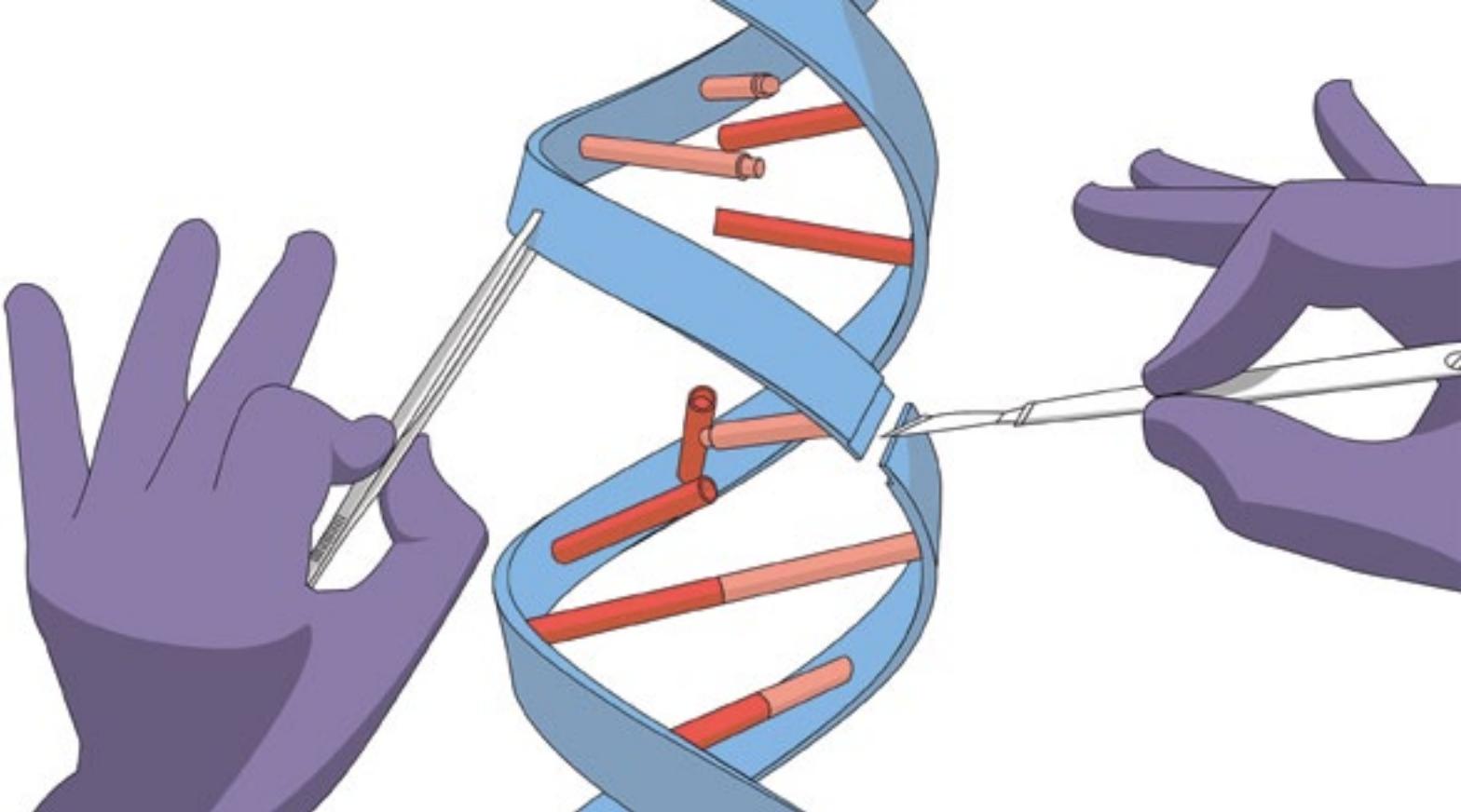
Discovery of many S-genes

Since the launch of the term and concept of 'susceptibility genes' in 2002, the number of discovered S-genes has increased rapidly. Schie and Takken (Ann. Rev. Phytopath. 52 (2014): 551–581) listed more than a hundred S-genes, subdivided into the three mentioned groups. S-genes have been described for very different types of parasites and pathogens, including viruses, bacteria, fungi, oomycetes, insects and nematodes.

Resistance

Resistance based on the presence of resistance genes (R-genes) is usually inherited dominantly: the presence of one functional allele of the R-gene usually suffices for acquiring resistance. For S-genes, all functional alleles of the S-gene should be absent or inactivated in order to acquire resistance. Therefore, in the case of S-genes, resistance inherits recessively.

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Another contrast with R-genes is that resistance caused by the absence of a functional S-gene is usually very durable. The *mlo*-resistance to powdery mildew in barley, described in 1942, has not been overcome, despite large scale commercial application in the crop for many decades. The durability is thought to reflect the great difficulties for the pathogen to 'invent' alternative pathways to manipulate the plant. Durability of disease resistance is the major advantage of impaired S-genes, compared to R-genes.

Pleiotropic effects

The evolutionary reason for the presence of S-genes in plants is not their support of pathogens. These genes have important roles for the plants themselves. Therefore, knocking down or knocking out S-genes can have negative effects on these plants. We already mentioned that knocking out defence suppressor genes can have negative effects, such as auto-necrosis. Enhanced resistance to biotrophic pathogens may lead to increased susceptibility to necrotrophic pathogens, and vice versa. The previously mentioned *SWEET*-gene in rice is involved in sugar transport. Reduced sugar transport by knocking out the *SWEET*-gene can lead to reduced growth of the plant. Such undesired pleiotropic effects in crops usually vary with the genetic background and plant species. Using the right genetic background may prevent undesired effects. Further, negative effects may be prevented by not completely knocking out the gene, but by silencing it, or by adding a mild mutation that does not completely stop the gene's effect, but reduces it. Any loss of the crop's value due to pleiotropic effects should be offset by the valuable gain of durable resistance. Fortunately, there are now various examples of impaired S-genes, leading to enhanced disease

resistance, but without observed negative pleiotropic effects. We have seen this for instance in *mlo* resistance in tomato and cucumber.

Applications in breeding

The recessive nature of the resistance and the possible pleiotropic effects in some genetic backgrounds may hamper the application of impaired S-genes in plant breeding. However, the durability of resistance is an important advantage, which stimulates finding ways to overcome these hurdles.

S-genes usually have a conserved sequence, which can be helpful when looking for candidate S-genes in a wide range of plant species. Impaired S-genes can be detected in wild germplasm by means of disease tests, possibly combined with allele mining, using, for example, DNA re-sequencing data and bioinformatics, as we have shown for cucumber.

Impaired S-genes can be created using untargeted mutagenesis, such as EMS. Very promising tools are targeted mutation techniques, such as CRISPR-Cas9 or ODM (oligo-directed mutagenesis). We mentioned the *OSSWEET14* gene for sucrose transport in rice. An effector protein of the bacterial pathogen *X. oryzae* can upregulate that gene by binding to a promoter region of that gene. Li et al. (Nature Biotech. 30 (2012):390-392) were able to remove that binding site from the promoter by means of targeted mutations, thus preventing upregulation of the *SWEET* gene by the pathogen, leading to enhanced resistance, while preserving the plant's other promotor elements and the coding sequence, and thus the gene's physiological function for the plant. Similarly, other S-genes may be edited in a targeted manner, reducing its S-gene function, while maintaining its function for the plant.

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Trapping nematodes with catch crops

Monique Krinkels

For more and more farmers, improving soil quality is at the top of the to-do list. No wonder as prices for arable land are extremely high. In the Northwest of the Netherlands, prices of 125,000 euro per hectare are not uncommon. Green manures and catch crops against nematodes are gaining popularity.

- Since 2010, regulations in Europe concerning soil health have become increasingly stricter. Soil disinfection with methyl bromide has been banned completely in the Netherlands and there is no chemical alternative that will protect against nematodes and harmful fungi as efficiently. Besides, the use of the soil has intensified. On average, a hectare of land in the Netherlands costs 56,500 euro, but can be as high as 125,000 euro in regions close to the sea, where viruses are less of a problem. In West Germany, the price of farmland is 40,000 euro and in East Germany, France and Great Britain 10,000 euro (prices in December 2016). Yields of 80 tonnes of potatoes or 100 tonnes of onions per hectare are needed to obtain a return on investment. Furthermore, policy makers promote soil quality improvement as a healthy soil is better able to cope with the effects of climate change, such as fierce rain showers, gives off less greenhouse gasses and contributes to a better quality of ground water.

Sustainable agriculture has therefore become a focal point for farmers.

Few options

In a few countries granular nematicides are allowed and there are some resistant varieties of sugar beet and potato. But most farmers are left with only a few options in their battle against nematodes: fallowing and crop rotation, leasing grassland or using catch crops. Fallowing has several disadvantages. With the high land prices, it is economically uninviting, but even more importantly, nematodes can survive for many years without host plants. If a farmer applies fallowing every four years, it will reduce only half of the nematodes. If the potato cyst nematode has been found, potato cultivation is forbidden for a decade. Moreover, nematodes spread easily as farmers in the

Netherlands discovered when they started to cultivate the reclaimed polders in the IJsselmeer. A study in the late seventies proved that it took the parasitic roundworms only a few years to infest the virgin soil. They arrived with plants from elsewhere which had soil between the roots, but also directly via the water through the ditches.

Leasing grassland is not always as good an alternative as one might believe. Some nematodes thrive

on grass, such as the oats cyst nematode, the grass root-knot nematode, the maize root-knot nematode, root lesion nematodes, *Trichodorus* and *Paratrichodorus*. Besides, the grassland may contain fungi and harmful insects that will hamper cultivation of arable crops.

Catching nematodes

At Vandinter Semo, the battle against nematodes has intensified over the years. The best-known nematodes are cyst nematodes, such as the potato cyst nematode,

which is responsible for potato senescence and the beet cyst nematode which does the same thing with beets. Other infamous nematodes are the root-knot nematode and the root lesion nematode. "We breed varieties that attract nematodes, such as fodder radish (*Raphanus sativus* subsp. *Oleiferus*) and yellow mustard (*Sinapis alba*). A beet cyst nematode will settle on the roots of the fodder radish and mistakenly take it for a host plant, so the eggs will never hatch," explains Bert-Jan van Dinter, CEO at Vandinter Semo.

"But it is very specific. There are no crops that attract the full range of harmful nematodes. So far, we have only a few varieties of fodder radish that catch both beet cyst nematodes (*Heterodera schachtii*) and root-knot nematodes (*Meloidogyne chitwoodi*), but it is a rare characteristic." Another catch crop is bristle oats (*Avena strigosa*), a cereal which is specially used for



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Root-knot nematodes feed on the roots, causing large galls or 'knots' throughout the root system of infected plants. It reduces the yield, as well as the quality of the surviving plants

combating root lesion nematodes (*Pratylenchus penetrans*) in potato, but also in carrot and lilies. Bristle oats combine that with a resistance to *Meloidogyne hapla*, a root-knot nematode.

"And sixteen years ago, we introduced sticky nightshade (*Solanum sisymbriifolium*). We have managed to make this crop more suitable as a killer of cyst nematodes (*Globodera pallida* and *G. rostochiensis*) in potato. Sticky nightshade reduces the nematode population by about 70%, significantly higher than fallowing with 50%. And at the same time, it is a valuable green manure which improves the soil structure."

The latter is more important than it would seem at first sight. A recent study showed that the amount of organic material in the soil increases the yield significantly. An increase of 1% organic material resulted in a 10% higher yield of both sugar beet and potato. The researchers expect that prevention of soil degradation will add another 10% of efficiency improvement. Furthermore, the soil is better capable of retaining water; 6.8 mm in sandy soil and 9.3 mm in clay, which means that irrigation can be postponed for a fortnight.

Specific

Some companies advise farmers to use a mixture of catch crops. It would help to combat a variety of nematodes in one cycle. "But that does not work," says Bert-Jan van Dinter. "Catch crops in a mixture compete with each other, so much of the beneficial

effect is lost. We believe that the catch crop should specifically target a certain nematode. Firstly, it has to be established which nematode causes the problem the farmer observes in his crop. And secondly, the optimum matching catch crop should be found." Vandinter Semo has many varieties of fodder radish, brown and yellow mustard, bristle oats and sticky nightshade to choose from. "And we constantly work on improvements. That is why we have built our own in vitro laboratory, where we can test plants on their ability to catch nematodes. Besides, a catch crop/green manure should have certain characteristics, such as quick development, late flowering, firmness and resistance against fungi such as *Alternaria* and *Botrytis*. And, of course, they should not survive winter to prevent regrowth."

Exploding demand

In the Netherlands, the market for green manure has tripled over the last few years and the same goes for Germany. France is an upcoming market and farmers in the UK are experimenting with this alternative for fertilization and pest management. Even in the USA, although at a slow pace, farmers are looking at catch crops. "Soybeans fields are crawling with cyst nematodes. Fodder radish could greatly improve that situation. The outlook was positive, as former President Obama introduced a subsidy to stimulate sustainability thinking. But we are not sure whether this land policy will continue."

Formation of new modules for the NAL system

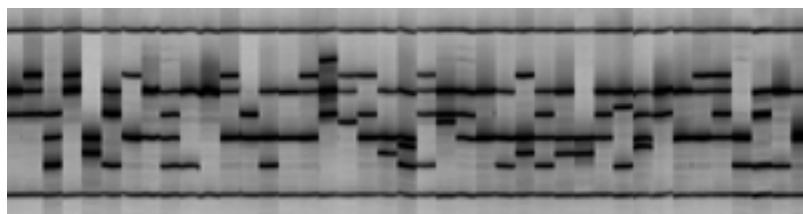
Adrie Molenaar

50 Since it is likely that the European Union will formulate new plant health and control laws, Naktuinbouw wants to further develop the NAL-system in such a way that it will remain fit for the future. Therefore, the organisation is busy creating new modules (extensions), together with the participating companies.

• **The umbrella for all the new modules** is what one might call a 'Verification Programme for Seed Production and Market Access'; a monitoring programme with a process-oriented and risk-based approach. For the moment, this will be the domain of seed companies, companies with production and marketing of seeds from their varieties as core business.

Important aspects

The conditions for this monitoring programme will be based upon all aspects that are regarded as important: seed-/plant health (quarantine as well as quality diseases/pathogens), varietal trueness/identity,



Variety identity analysis using SSR markers

varietal purity, quality aspects (like noxious weeds), tracking & tracing and compliance. Companies will remain responsible for carrying out the proper checks and balances themselves, as they are today. You can imagine the modules Maintenance, Field Inspection (NAFI), Varietal Identity & Purity, Processing, Sampling, Laboratory testing (NAL) and Shipping. Participation is voluntary. It is possible for companies to choose in which modules they participate. Of course, in the end, it will not be each individual module that counts, but the total will be more than the sum of its parts.

Why these modules

Firstly, out of interest for the participant: ensuring that monitoring of seed production is state-of-the-art and reports thereof will give reliable information, reflecting the true quality of the seeds.

Secondly, to create a system where Naktuinbouw can rely upon results of company checks concerning official inspection. This fits with its philosophy that if companies can responsibly carry out the checks themselves, this is the adequate basis for certification. Therefore, the start is in the Netherlands.

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Thirdly, to realize that NAL is a system for supporting worldwide market access, where also the Dutch National Plant Protection Organization (NPPO), the NVWA, can rely upon results with regard to issuing phytosanitary certificates.

Fourthly, the aim is to make it global, making it possible to deal with foreign productions as well.

Therefore, a secure NAL system is needed, with the right/sufficient checks and balances, in which it is demonstrated that it is justified to have confidence, also for NPPOs and inspection bodies.

Last December, the conditions for the module field inspection (NAFI) were approved by the Board of Naktuinbouw. Since then, it has become possible to become authorized. The first application for participation is expected shortly.

In September 2017, a 4-day training course for company field inspectors will be organized. Besides theoretical background information, practical examples will be discussed on the trial field. Information will be provided upon request.

For 2017, another target is to practically discuss and develop a new module for varietal identity and purity (NAVIP).

Cooperation

Establishing and maintaining good relationships with the Dutch NPPO (NVWA) and foreign phytosanitary organizations (like USDA-APHIS/NSHS and the Australian DAWR) is regarded as an important aspect of the programme. This year, auditors/policy makers from NSHS and Naktuinbouw will participate in each other's audits (laboratory and field inspection). Next point of attention will be cooperation in the approval of protocols and the joint organization of proficiency testing.

In the past three years, Naktuinbouw has organized a platform meeting with various workshops regarding Quality Assurance (awareness, training, harmonisation, validation of protocols, auditing, improvement and developments), related to the field of its international systems: ASLN, NAL and Elite. This was valued very highly by the participants. On 12 October this year, there will be another platform meeting to enable participants to discuss various items with their counterparts from other companies. Information will be provided upon request.

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