

THE ANNUAL 2020

Journal for breeders and producers of plant material

Prophyta

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- Plant Passport
- Quality-plus systems
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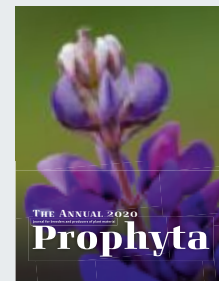
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On the cover: Andean lupine forms a strong plant with a lot of leaf mass which increases the agricultural competitiveness

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- 4 In short
- 5 Editorial
- 6 Plant Protein Production
Andean lupine - an emerging crop
- 8 Nigeria-Netherlands Seed Partnership
Exploring ways to enhance Nigeria's seed sector
- 12 Healthy Seeds for Tanzanian Farmers
Smartphone boosts vegetable production
- 14 Biodiversity
Tomato diversified by modern plant breeding
- 20 Corporate Social Responsibility
Sending children to school, not to work
- 25 Plant Health in the EU
Safeguarding a sound phytosanitary status
- 26 Digi Dutch Spring Trials 2020
There is more to strawberries than meets the eye
- 28 CRISPR-Cas
New era of precision plant breeding
- 32 Plant Variety Protection
PVP toolbox helps to make a country's system UPOV-proof



- 34 The Salt Doctors
Increasing salinity: problem or opportunity?
- 38 Phased Genome Sequencing
Opportunities for more effective crop breeding
- 40 Naktuinbouw Authorized Field Inspection
Quality programmes extended
- 43 The Future of Seed Health Testing
Going back to basics



- 47 Fries4All
Amazing quartet invents healthy fries
- 48 Plant Tissue Culture
Contamination remains a never-ending challenge

Microplastic-free coatings



Up to 2018, a total of 86 million tonnes of plastic has ended up in the sea. Some 0.5% of this floats on the surface, while 23 million tonnes (23%) ended up in coastal waters

INCOTEC WILL LAUNCH microplastic-free film coatings later this year. The company has prepared a series of new products for sunflower, maize and vegetables seeds that are microplastic-free. These new and innovative products address all the most important

aspects of conventional seed treatment, making sure that all the features of advanced seed treatment have not been lost. Incotec will make sure that its full portfolio is microplastic-free and sustainable to the max, which is their strategic direction of Mission Zero.

Microplastics in the seed industry became a hot topic two years ago. These polymers are resistant to normal environmental degradation and thus will be present in the environment for a long time after their initial release. They are considered to be a potential health risk. In

2018, the European Chemical Agency (ECHA) published their first draft restriction proposal, in which seed treatment featured as one of the industries that contributes to intentionally added microplastics in mixtures.

Number of starving people doubled

WORLDWIDE, THE NUMBER of people suffering from acute hunger may have doubled by the end of this year, as a result of COVID-19. That is what the World Food Programme (WFP) of the United Nations foresees. WFP expects the number of people in acute food insecurity to rise to 265 million by the end of 2020. An economic specialist from the WFP says people who can only eat if they earn a salary are the most vulnerable group. "It only takes one shock, like COVID-19, to push them over the edge. We need to act together to mitigate the global catastrophe," he adds.

According to the WFP, current programmes must be maintained in order to counter such an increase. WFP also released global hunger statistics for 2019. In South Sudan, more than 60% of the population was in dire straits last year. And in another six countries, at least 35% of its inhabitants were starving, namely Yemen, the Central African Republic, Zimbabwe, Afghanistan, Syria and Haiti. More than 130 million people worldwide live in food insecurity.

ABS introduces Growers Online

AGRO BUSINESS SOLUTIONS presents a new addition to their software: ABS Growers Online. It makes it possible for a seed company to collect data from growers on their crops on a regular basis and in an easy way. Using ABS Growers Online growers can enter their findings on their own fields. Updates such as status of the field and product, diseases, off-types, yield estimations, etc. can be provided with the simple click of a mouse. ABS Growers Online is connected to the ABS Seed

software, making the growers information available in real-time. User-friendliness and multi-language are the keys to get growers online. Agro Business Solutions has created software solutions that have been developed for companies that specialize in plant breeding, seed multiplication and fresh produce. It provides companies with the analytical tools needed to gain insight into business activities and can be customized to meet a company's needs. More information: www.agrosolutions.nl.

Takii Europe develops Phytorich

AT FRUIT LOGISTICA 2020, Takii Europe introduced Phytorich tasty vegetables containing exceptionally high levels of healthful phytonutrients. These are natural compounds that help protect plants from insects and diseases, but their protective qualities can be just as beneficial to human health. Scientific experts have identified over 25,000 phytonutrients in plant food, of which thousands are contained in fruits and vegetables. The flagship variety of the Phytorich concept is called Goshiso PR-7 tomato and is part of an extensive assortment composed of other vegetables such as onion, carrot, squash, collard, mizuna, okra, spinach, Chinese cabbage and pepper. Goshiso PR-7 contains twice as much Lycopene as regular tomatoes. The richness of Lycopene goes hand in hand with a very high level of glutamic acid, a compound that triggers the characteristic 'Umami' taste.



Longevity and healthy habits have long been at the top of consumers' minds in Japan and the Phytorich concept has successfully built on this approach since its launch in Japan, 25 years ago. The company is working on science-based data to prove the positive health impact of certain varieties with high phytonutrients. More information: www.phytorich.eu.

Harsh punishment for illegal tomato reproduction

LAST YEAR, THE OWNER of the Dezio farm in Vittoria, Italy, was convicted to a year in prison and had to pay 70,000 euros in civil damages. Salvatore Dezio illegally reproduced tomato plants of a variety protected by Plant Breeders' Rights in four of his greenhouses. In addition, he received a fine of 15,000 euros. The process began with a complaint filed by the AIB (Anti-Infringement Bureau for Intellectual Property Rights in Plant Material). During the investigation, DNA analysis was carried out on the

Dezio company's crops, which confirmed the presence of the PBR protected tomato variety in its greenhouses. The financial police confirmed that the company was unable to provide purchase invoices for seedlings of the protected variety or other documentation demonstrating the legitimate origin of the seeds used. It is the first criminal sentence regarding new protected plant varieties, which creates a precedent in the seed market.

Strange times

For the first time in about 80 years, the World Seed Congress has had to be cancelled. Only the Second World War was able to prevent seedsmen and -women from all over the world meeting each other. The outbreak of coronavirus is therefore unprecedented. It is generations ago (the Spanish flu in 1918-1919 infected 500 million people) that a pandemic like this happened. Such scenarios are usually described in the film section of papers. Now it's creepy non-fiction, with no guarantee of a predictable Hollywood ending.

For those living in developed countries, coronavirus caused a higher death rate than normal, with many people suffering from the after-effects of the disease. And, of course, the economic effects will be felt by many. The consequences for developing countries are even more dramatic. The World Food Programme of the United Nations expects the number of people in acute food insecurity to double to 265 million by the end of this year due to COVID-19.

In this edition of PropHYTA, attention is given to several ways seed companies, governments and NGO's are willing to improve food security in Africa, the original destination of this year's congress. It shows how many people are involved in reaching out to the less fortunate. The subjects vary from coping with salinity, to breeding hybrids of local crops, to knowledge transfer on plant breeders' rights to strengthening the seed sector in developing countries. COVID-19 brought one important message to the world: the food chain is of vital importance. It is a message ISF and other organisations have been trying to communicate to the world for years, but was never fully understood by the masses till now. Where most people had to stay at home and avoid contact, governments made an exception to companies in the food chain. The world not only needs doctors and nurses to survive, but also farmers and seed companies. It showed that professions, such as politicians, lawyers, businessmen and, yes, even journalists, are less important than they often regard themselves.

Monique Krinkels

Andean lupine - an emerging crop

Monique Krinkels

6 It tastes like a hamburger, has the bite of a hamburger and yet no meat has been involved in the making. The secret behind this animal-friendly novelty: lupine flour. It is one of the many advantages of this multi-purpose crop. In the LIBBIO Project, research institutes and companies work together to develop food, feed, non-food and bio-energy products from Andean lupine adapted to European farming conditions.

• The demand for plant-based proteins is increasing. While the number of vegetarians is still relatively small in the Western world, the amount of people who regularly use meat substitutes - the so-called flexitarians - is growing quickly. Only in the last three years, have sales of alternative products in the Netherlands been increasing by 51%, while meat sales have decreased by 9%. Scientists and politicians agree that a shift from animal to vegetable proteins has multiple benefits. Not only to combat climate change, but also to stimulate sustainability, circularity and human health. But a product such as soya beans has considerable disadvantages. Nearly 80% of all soya that is grown is genetically modified, which Europeans are not fond of. The cultivation of soya also causes all kinds of problems: tropical forests are cut down, soil is exhausted, water is polluted by pesticides and fertilizers and field

The LIBBIO Project

LIBBIO, a European Horizon 2020 project, started on 1 October 2016 and is expected to end on 30 September 2020. The estimated project costs amount to 5 million euros. The aim is to develop consumer food, feed, non-food and bio-energy products from Andean lupine



varieties (*Lupinus mutabilis*), adapted to European farming conditions by applying bio-refinery cascading principles for crop value creation and modern crop breeding technologies. In addition, to increase crop yield and harvest index and accelerate supply chain development, via a consumer-driven approach, in order to develop high value-added food and non-food products by applying state-of-the-art solvent-free technology for raw material processing. In the project, 14 partners from 8 countries are participating: Project Leader: Pall Arnason, Nyskopunarmidstod, Iceland; Project partners: Netherlands: Hanzehogeschool Groningen, Wageningen University, Louis Bolk Instituut, Color & Brain BV, Vandinter Semo BV; Iceland: Landgraedska Ríkissins; Germany: German Institute for Food Technology; Austria: Bio-Institut HBLFA Raumberg-Gumpenstein; Spain: Agencia Estatal Consjo Superior de Investigaciones Cientificas; Portugal: Instituto Superior de Agronomia, Lusosem - Produtos para Agricultura; Greece: Agricultural University of Athens; Romania: Universitatea de stiinte Agricole si Medicina Veterinara ion Ionescu de la Brad

workers are exploited. Consequently, there is room for ‘new’ agricultural products. Andean lupine could play a role in reducing soya imports and making the EU more independent from foreign imports.

History

The history of Andean lupine (*Lupinus mutabilis*) goes far back in time. The plant was domesticated by pre-Incan civilizations in the Andes thousands of years ago. Remains of seeds are present in tombs of the pre-Inca Nazca culture (about 500 AD) in the Peruvian coastal desert, and the plant is represented in stylized paintings on large pots of the Tiahuanaco culture (500 BC to 1,000 AD) of the Andean highlands. At present, farmers in Peru, Ecuador and Bolivia grow tarwi or chocho, as the crop is locally known. For the Indians, it is a staple food.

One of the interim results of LIBBIO is that it will be profitable for farmers to grow Andean lupine in Europe. The yield of dry biomass and lupine beans appears to be very high. Thanks to the good fatty acid composition, the lupine oil is perfectly suited for food applications such as margarine and mayonnaise. But it can also be used as feed, or even in cosmetics, and (wood)glues. The bean is very rich in proteins and oils, which can be used as functional food ingredients or as cosmetic ingredients. Lupine alkaloids can be used as natural and biodegradable biocide.

Undemanding nature

Due to the low nutritional requirements and its undemanding nature, the crop grows well on ‘poor’ agricultural land, that would otherwise be excluded from food production. Even on the volcanic ash in Iceland or the bone-dry Greek soil, Andean lupine will flourish. A profitable by-effect is that it works as a soil improver. The plant is able to fix nitrogen and mobilise soil phosphorous. Hence, among the fourteen participants in the LIBBIO project, one can find the Innovation Center Iceland, the Soil



Andean lupine can be used as a food ingredient, feed, biomass, for oil refineries but would also suit well as an ornamental

Conservation Service of Iceland and the Agricultural University of Athens in Greece. Rob van Haren, professor of transition bioeconomy at the Hanze University, Groningen, the Netherlands, is developing a pilot multi-purpose biorefinery. The Andean lupine contains alkaloids that could become very useful as for instance biodegradable crop protection products. The components are extracted from the plant in an integrated supercritical biorefinery. Extraction takes place under high pressure. “This allows us to extract the oil as well as the alkaloids from the lupine without using solvents. With soya beans, on the other hand, the oil is extracted using hexane, a toxic substance which always leaves a residue behind. With supercritical extraction the hexane is not necessary.”

Breeding

The Dutch Product Board Arable Products and Innovation Network of the Dutch Ministry of Agriculture have initiated pre-breeding cultivation trials in advance of this project, together with the Julius Kühn Institute, the Federal Research Centre for Cultivated Plants in Groß Lüsewitz, Germany. The potential grain yield of individual plants has been extrapolated for initial yield estimates. The researchers concluded that, with the right choice of varieties, bean yields of 3 to 7 tonnes per hectare are possible. Wageningen Economic Research compared the economic return on growing Andean lupine and winter wheat. The conclusion: in Germany or Poland, the cultivation of Andean lupine from 3 t/ha is competitive with winter wheat. Assessment of *Lupinus mutabilis* genetic material in European conditions started almost 100 years ago, in the 1930s, characterizing and breeding with accessions from the Andean region. This was done

by the legendary German plant scientist and breeder, Reinhold von Sengbusch, who developed ‘sweet’ low alkaloid varieties for all the bitter agricultural lupins, including the Andean lupine. The breeding and selection work continued in the 1970s, which eventually resulted in sweet Andean lupine variety ‘Inti’. Three years ago, one of the participants in LIBBIO started a breeding programme. The first variety by Vandinter Semo is now being tested in Poland. “But DUS-testing is difficult,” says director Bert-Jan van Dinter. “There is no protocol, nor are there comparable varieties. It is without question distinct.” The same goes for the recommended list of varieties: as there are no Andean lupines on the list so far, the first variety will always be an improvement. Vandinter Semo took over the Andean lupine collection from the Dutch Product Board Arable Products and the Julius Kühn Institute. They added landraces and species from Ecuador, Bolivia and Peru. “We have about three hundred different Andean lupines to select and cross breed with. Our main breeding goal is increasing the yield. We select new varieties for the northern European climate as summer crop and the southern European climate as winter crop. Our climate obviously places different demands on crops than the South American mountain region. Testing on diseases and yield are central. Hundreds of crossings are sown repeatedly. Farmers in Western Europe need a return of 2,500 euro per hectare, any less and winter wheat would be more profitable.” On the face of it, it is surprising that the Indian tarwi has, so far, not been developed as an international crop. Its beans contain more than 40% protein and more than 20% oil. It can therefore compete with peas, beans, soya beans and peanuts, as well as with other oilseed crops. Andean lupine might have a bright future.

Exploring ways to enhance Nigeria's seed sector

Walter de Boef and Marja Thijssen

8 Government, industry, knowledge, regulatory and civil society organizations in both Nigeria and the Netherlands started to work together in strengthening the performance of the seed sector in Nigeria, contributing to the country's tremendous potential in agricultural development, promoting private sector development for income generation and employment, enhancing food and nutrition security, while also addressing major challenges in the climate crisis.

In 2019, SeedNL organized a delegation, including various stakeholders from the Netherlands, to Nigeria. During a visit to the National Agricultural Seed Council, the seed apex body of the Federal Ministry for Agriculture and Rural Development, its director, Dr. Ojo, being aware of the status of the Dutch seed industry, expressed considerable interest in exploring collaboration. The conversation quickly turned to a question of Nigeria's strategy for seed sector development. The country has an overarching strategy for agricultural transformation, but Dr. Ojo indicated that his country did not yet have one specific to the seed sector. In shaping the collaboration in the seed sector, it was subsequently agreed to conduct a seed sector review, with the aim of supporting the development of a multi-year seed sector development strategy, or Seed Road Map. It was agreed that, based on this National Seed Road Map, Nigerian and Dutch stakeholders would engage in the development of a multi-annual collaborative programme.

Seed road map

A team of Dutch and Nigerian consultants facilitated a consultative process, involving more than 100



At a Field Day in Akufo, Oyo State, Nigeria, organized by the National Agricultural Seed Council, NASC, seed scientist Dr. Julius Olasoji shows farmers newly released improved varieties of maize (Photo: NASC)

domestic and international stakeholders. The document was formulated and compiled through a participatory and iterative process, which included a desk study, crop and crop group-based focus group discussions, expert consultations, a multi-stakeholder workshop, and a review by national and international resource persons. Within the context

impact. SeedNL was officially launched during World Food Day on 16 October 2019, and a covenant between the key partners, i.e. the Dutch Ministry of Agriculture, Nature and Food Quality, the Ministry of Foreign Affairs and Plantum, the seed trade association of the Netherlands, was signed in February 2020. The partnership's objective is to strengthen the seed sector in developing countries and emerging markets to increase crop productivity and thereby enhance farmers' income and improve access to safe and healthy food. SeedNL has decided that it will develop pilot Seed Partnerships in which seed sector stakeholders from both the Netherlands and a partner country join and contribute to this objective. Ethiopia and Nigeria were selected for the development of the first Seed Partnerships. Partners join in activities that will improve the seed business enabling environment, support domestic and globally operating seed companies in their promotion and marketing activities, and enhance the professional and institutional capacity of stakeholders.

East-West Seeds-Knowledge Transfer instructs Nigerian farmers on proper practices for vegetable growing using quality seed as a starting point

(Photo: East-West Seed)



Federal Republic of Nigeria

With 206 million inhabitants, Nigeria is the most populous country in Africa and the seventh most populous country in the world. Despite its wealth owing to the oil resources, agriculture remains the base of the Nigerian economy, providing the main source of livelihood for most Nigerians. Agriculture is the largest sector of the Nigerian economy and employs two-thirds of the entire work force, however, its production hurdles significantly stifle performance. Food (crop) production increases have not kept pace with population growth, resulting in rising food imports, declining levels of national food self-sufficiency, unemployment amongst the rural youth and consequent impact on rural society. The main factors undermining production include reliance on rainfed agriculture, smallholder landholding, low productivity due to poor planting material, low fertilizer application, and a weak agricultural extension system. The Federal Government of Nigeria aims to boost the contribution of the agricultural sector to its economy. Crop yields remain very low in a country where potentially highly productive farmland is abundant and the potential of a productivity boost is tremendous; therefore, the agricultural sector can be considered a sleeping giant of regional and global dimensions. This potential, combined with demographic growth, rapid urbanization, and an increased demand for agricultural products in neighbouring countries, creates a major opportunity for the agricultural sector, which has not gone unnoticed amongst the global and domestic agribusiness sector, including both the national and international seed industry.

of SeedNL, the consultant team had two consultative meetings with relevant stakeholders, including several seed companies in the Netherlands.

Structure

The National Seed Road Map document (NASC & SEEDAN, 2020) is structured by three primary questions on seed sector performance: (i) Where are we now? (ii) Where do we want to go? and (iii) How will we get there? No single response exists to the question, "Where are we now?". The structure of the seed sector is complex, with its multiple stakeholders, each with their own interests, and the diversity of crops. Creating some structure to the complexity of more than 80 challenges mentioned by stakeholders, the consultants' team structured them into six functions, being (i) service provision; (ii) seed production; (iii) market development; (iv) revenue generation and reinvestment, (v) coordination and governance, and (vi) regulation and management. Challenges were synthesized and transformed into ambitions groups within these six functions. Thus, from the problems, positive points on the horizon were defined. The collection of ambitions shapes a future vision, in which the seed sector becomes high-performing by ensuring access to and availability and use of quality seed of improved varieties of all crops for farmers.

An assembly of 22 prioritized topics shapes the response to the question, "Where do we want to go?". Examples of such topics include the following: decentralization of seed quality assurance; supporting the development of a sustainable structure for revenue generation for seed quality assurance services; promotion of commercial and sustainable

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10 Nigerian farmers proudly showing the result of their efforts on improved practices for tomato cultivation after being trained by East-West Seeds-Knowledge Transfer
(Photo: East-West Seed)



Seed sector in Nigeria

The major market for domestic seed companies in Nigeria is maize seed, including primarily open-pollinated varieties. It is estimated that approximately 50% of company seed sales target institutional markets, e.g. government subsidy programmes, development organizations and NGOs. Another important grain crop is rice, for which grain production is increasing and a more professional seed industry is gradually developing. In the more semi-arid northern part of the country, sorghum and cowpea are major crops, for which seed supply is primarily informal. Vegetatively propagated crops are very important for the agricultural economy; they include the crops yam, cassava, potato and sweet potato, each with their distinct and mostly constrained seed system. The horticultural sector is gradually developing from a nascent stage, and quality vegetable seed for most crops is imported and sold primarily through local seed companies and agro-dealers. Stakeholders in the seed sector include a high number and diversity of government organizations, industry, knowledge, regulatory bodies, civil society and farmer organizations. 314 formally registered seed companies, of which the majority annually processes

less than 1,000 metric tonnes of grain crop seed, together with agro-dealers shape the industry. The Seed Entrepreneurs Association of Nigeria (seedan) is the country’s seed trade association, with 72 seed companies registered as members in 2019. National Agricultural Research Institutes and various cgiar Research Centres are important for the development of new crop varieties for most food crops, as well as for the production of early generation seed. The nasc is the government body responsible for promoting and stimulating the development of a dependable seed industry, regulating and controlling seed registration and release, protecting farmers from the sale of poor-quality seed, and facilitating the production and marketing of high-quality seed. Despite the operations, infrastructure and economies of scale of this diversity of seed sector stakeholders, farmers’ access to and use of quality seed of new and improved varieties remains limited, which contributes to crop productivity in Nigeria continuing to be low. The gap between demand and supply of quality seed of improved varieties was estimated to be more than 90-95% for all crops, except for open-pollinated maize varieties, for which the market is saturated.



At the laboratories of the National Agricultural Seed Council in Sheda, Abuja, Nigeria the Physical Purity Test of maize seed is evaluated
(Photo: NASC)

More to read

NASC & SEEDAN, 2020. National Seed Road Map for Nigeria; Document submitted to the National Agricultural Seeds Council (NASC) and Seed Entrepreneurs Association of Nigeria (SEEDAN) in conclusion of a seed sector review in Nigeria (September 2019–February 2020). National Agricultural Seeds Council, Abuja, Federal Capital Territory: https://seedcouncil.gov.ng/wp-content/uploads/2020/03/National-Seed-Road-Map_Nigeria-NASC-Adopted.pdf

supply of early generation seed strengthening extension on seed and advanced cultivation practices as means to boost the seed market, for example in potato and vegetables; tackling of counterfeit seed; enhancing the capacity of seed companies in marketing and variety promotion; supporting sector governance and coordination; strengthening seed information management; strengthening the seed trade association; supporting operationalization of plant variety protection; and increasing efficiency and effectiveness in variety release. The core of the seed road map responds to the final question, “How will we get there?”. For each of the 22 topics, the document provides a detailed description of the ambitions and associated challenges, based on stakeholder inputs. A strategic innovation pathway elaborates a maximum of five steps for each topic, describing what can be done to achieve the ambition in a sustainable manner. The stakeholders, who are critical to achieving each ambition, are mentioned. A catalyst, i.e. an organization that is in the best position to initiate and facilitate the change process, is proposed. Furthermore, each pathway provides reference to the primary policy documents of the Nigerian government and relevant institutions. To make changes tangible for each of the pathways, a two-, five- and/or ten-year horizon is described. To illustrate that the proposed pathways are not isolated from developments in Nigeria, Africa and other regions of the world, the description of the pathway concludes with illustrative examples of systemic change processes or situations in which similar ambitions have been achieved.

Partnership

The Nigeria-Netherlands Seed Partnership proposes a number of areas of intervention for collaboration, based upon selected topics and strategic innovation pathways of the National Seed Road Map. Contribution to their implementation ensures that the partnership takes a systemic approach for sustainably addressing key challenges in the seed sector. The pathways were selected based on the priorities of Nigerian and Dutch stakeholders; promoting Nigerian and Dutch business opportunities; building upon expertise in other Dutch-funded projects, such as in Ethiopia, Uganda and Myanmar; and ensuring that topics and activities are not yet addressed by other donor agencies and development partners. Pathways were identified that will generate immediate results on the ground and achieve high impact with limited investment. Eight pathways are proposed that vary from seed sector governance and coordination, decentralized quality assurance, strengthening extension on seed and cultivation practices, to more legal and regulatory topics, such as supporting the implementation of plant variety protection and enhancing the capacity in variety release. The partnership will involve seed sector stakeholders in Nigeria and the Netherlands through both governments, but also through their industry, knowledge, regulatory and civil society organizations. Together, they will engage in strengthening the seed sector in Nigeria, contributing to the country’s capacity in fostering the development of the agricultural sector, contributing to food and nutrition security and economic development, while also addressing major challenges in the climate crisis.

Smartphone boosts vegetable production

Monique Krinkels

12 In 2008, Rijk Zwaan founded a subsidiary in Arusha, Tanzania. The aim was to provide the farmers in Eastern Africa with F1 hybrid seeds of local crops to improve yield and income security. One of the difficulties the company foresaw was how to communicate with farmers who live in hard to reach villages. Luckily, at the same time, the mobile phone gained ground in Africa.



Afrisem trained almost 3,000 families in using hybrid seeds, improved growing techniques and integrated pest management

• **Smallholder farmers in East Africa** who want to produce African eggplants, African tomato or Chinense peppers used to be dependent on land races. That all changed when Rijk Zwaan’s daughter company, Afrisem, started to breed hybrid varieties of these locally popular crops. Furthermore, the company tested varieties from its international assortment to determine whether these were suitable for cultivation in the area. Marketing specialist, Heleen Bos, supports the introduction of the state-of-the-art seeds.

Exploring

When Rijk Zwaan started its seed production farm in Arusha, Tanzania, in 2002, it soon became obvious that local farmers faced hardship. The seeds they used were of questionable quality and would often not even germinate. If they did, it regularly resulted in disappointing yields, followed by food shortages in the villages and financial setbacks. Together with East-West Seed Company, the company explored whether it would be possible to improve the lives of the smallholder farmers and their families. Hybrid

seeds of high quality, education to adjust cultivation techniques and support for farmers by crop advisors were the key elements that could change the future of small farmers.

But would these farmers adopt innovation? Rijk Zwaan and East-West Seed asked two employees to inventory the

wishes of local farmers. Both companies knew, of course, beforehand that starting a breeding programme for local crops is an undertaking that would not be remunerative in the short to medium term. But the main question was: could the local farmers be persuaded to buy hybrid seeds and listen to crop advisors? One of the worries the former managing director, Anton van Doormalen, faced was simply how to contact the farmers. After ample discussions, the companies decided in 2007 to give it a go and started Afrisem. It meant the first hybrid breeding

programme in East Africa focussed on local crops. That was the moment Heleen Bos joined the project. She had comprehensive experience with farming projects in third world countries. For GiZ, the German based organisation for international cooperation, she carried out a rice programme in Bangladesh. In Nicaragua, she was active for SNV, a Dutch development organisation, and supported the maize, beans and rice growers. “To introduce the new vegetable varieties, we cooperate with several international development organisations,” she explains. “Combining our strengths, the local contacts of the development organisations and the crop knowledge of our staff seems to be the smartest way to start. The people of local NGO’s are located all over the country.”

Major change

The focus on hybrids meant a huge change for the smallholder farmers. The price of F1 hybrid seeds is higher than what they paid for locally grown seeds, it entails a costly investment from the growers. But the local farmers soon discovered that the return on investment was well worth it. “On our demo field, we showcase and discuss our assortment and growing methods all year round,” says Heleen Bos. “In Arusha, Afrisem employs a staff of 70 people and we have 20 hectares of farmland, including trial fields, greenhouse facilities and isolation blocks that we combine with modern facilities from within the Rijk Zwaan group.”

As luck would have it, the start of Afrisem coincided with the upswing of mobile telephony. At the turn of the century, there were virtually no mobile phones anywhere in Africa, now they are everywhere. Today, no one is amazed when encountering a simple shack in the middle of nowhere with a solar panel next to it to recharge the mobile phone. The apparatus conquered the continent and that changed people’s lives. “Mobile phones are not produced anywhere in Africa, but they are fully repaired and adapted. That sometimes provides knowledge that manufacturers can use to improve their phones. Repairers who come up with good tips will have access to a variety of technical secrets, so they can do their jobs better. So, both parties profit,” says Mirjam de Bruijn, Professor of Citizenship and Identities in Africa at the University

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Thanks to mobile phones, the farmers can form groups, even if they live far apart

of Leiden, the Netherlands. She coordinated the research programme ‘Mobile Africa Revisited’, a comparative study of the interrelationship between Information and Communication Technologies (ICTs), agency, marginality and mobility patterns in Africa.

The mobile phone plays an important role in the introduction of the new varieties. “People who have attended a course use Facebook and WhatsApp to create groups to share information. Thanks to mobile phones, growers who followed one of our courses can maintain close contact, even if they do not live in each other’s neighbourhood. They can take a photograph of a plant that has a fungus and ask the crop advisor for the best solution. And, of course, they use it to order seeds. As payment with a mobile phone is common practice in Africa, we can send the package of seeds immediately,” says Heleen Bos.

Vegetables for all

Since 2014, Rijk Zwaan has participated in the Vegetables for All programme in Tanzania, part of the Amsterdam Initiative against Malnutrition (AIM). In this project, our Rijk Zwaan Afrisem colleagues have cooperated with ICCO, Rabobank Foundation and its Tanzanian partner, NMB Foundation, the Tanzanian Horticulture Association (TAHA), the Wageningen Centre for Development Innovation, the World Vegetable Centre and the Global Alliance for Improved Nutrition (GAIN).

“In the northern Tanzanian regions of Arusha, Tanga, Kilimanjaro and Mayara, we succeeded in training almost 3,000 families in using better quality (hybrid

seeds, improved techniques, such as drip irrigation, and general Good Agricultural Practices (GAP), with an emphasis on integrated pest management. This training was provided on demo fields, created especially for this project. Within the scope of Vegetables for All, four professional nurseries were established as well, to supply growers with good quality seedlings,” explains Heleen Bos.

Part of the programme was the introduction of small-scale dryers, to dry vegetables by means of solar heat (to increase shelf life), the formation of farmer groups (‘strength in unity’) and training in entrepreneurship and financial skills. Rabobank Foundation, through its partner, NMB Foundation, provided loans to both individual growers and these farmer groups.

Cooking programme

The involvement of Rijk Zwaan in the region goes beyond merely farmers. It helped whole communities to improve wellbeing. “About a third of all kids under five in Tanzania is suffering from chronic malnutrition. More than 50% of all children in the same age category suffer from anaemia, caused by a lack of iron (e.g. legumes, dried fruits and leafy vegetables contain iron).” Root cause: a one-sided diet, consisting mainly of corn porridge, cassava or rice. Vegetables for All, as the name indicates, was focused on improving access to affordable vegetables on the one hand, and increasing awareness about healthy and varied consumption on the other.

“An important element of the Vegetables for All project was providing education on healthy consumption to Tanzanian families. In total, 122 cooking demonstrations were held, reaching almost 8,000 families. Moreover, 47,000 pupils were reached by campaigns designed for schools.

Vegetables for All produced impressive results. Abel Kuley, Product Development Specialist at Rijk Zwaan Afrisem, who was overseeing the local implementation of the project, is happy. “Many of the growers we trained have become our customers, which allows us to continue supplying them with our seeds and services!”

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Tomato diversified by modern plant breeding

Henk Schouten, Yury Tikunov, Richard Visser

14 The diversity among glasshouse tomatoes in NW Europe was low in the 1950s, and even lower in the 1960s, due to inbreeding and selection. But from the 1970s onwards the diversity increased considerably because of introgressions of disease resistances from wild donors, and introgressions for fruit quality since the 1990s in response to the ‘Wasserbombe’ crisis.

• A paper in the *Nature Biotechnology* journal on domestication of tomato voiced the general belief that “breeding of crops over millennia for yield and productivity has led to reduced genetic diversity. As a result, beneficial traits of wild species, such as disease resistance and stress tolerance, have been lost (...). Despite the increases in yield conferred by domestication, the breeding focus on yield has been accompanied by a loss of genetic diversity and reduced nutritional value and taste” (Nat. Biotech. 36, 1211-1216, 2018). The loss of genetic variation in crops has been termed genetic erosion. During domestication preferred genotypes were selected, leading to loss of alleles and a decrease in genetic diversity of landraces, compared to wild accessions. Breeding can further reduce genetic diversity by continued selection or, in contrast, may broaden genetic variation through the introgression of alleles from wild relatives. The question remains whether the increase in diversity because of introgression has compensated for the reduction of genetic diversity as a result of inbreeding and selection. We will answer this question, using tomato as an example. We have studied the diversity of commercial tomato varieties for glasshouses in North West Europe since

the 1950s. We randomly selected about 12 varieties per decade and investigated these on two levels: 1. Genetic diversity, so at the DNA level; and 2. Phenotypic diversity of the fruits.

Genetic diversity

We isolated DNA from 90 tomato varieties that were commercially released between 1950 and 2016 in North West Europe. All varieties were for greenhouse production of tomatoes for the fresh market. The DNA was analyzed by means of a SNP array, yielding 7720 SNP-marker scores per variety. These SNP scores allowed us to estimate the genetic diversity between the varieties within a decade. Figure 1A shows that the genetic diversity was very low initially, in the 1950s and the 1960s. However, from the 1970s onwards, the diversity increased markedly. Apparently, the increase in diversity caused by introgressions from wild accessions was far more pronounced than the decrease in diversity by selection. Nowadays, approximately 28% of the genome of modern glasshouse tomato varieties is ‘novel’, compared to the 1950s and 1960s. That ‘novel’ DNA consists of chromosomal fragments from wild relatives, introgressed by plant breeders.

There is a tremendous increase in diversity, both at the genotypic and phenotypic level



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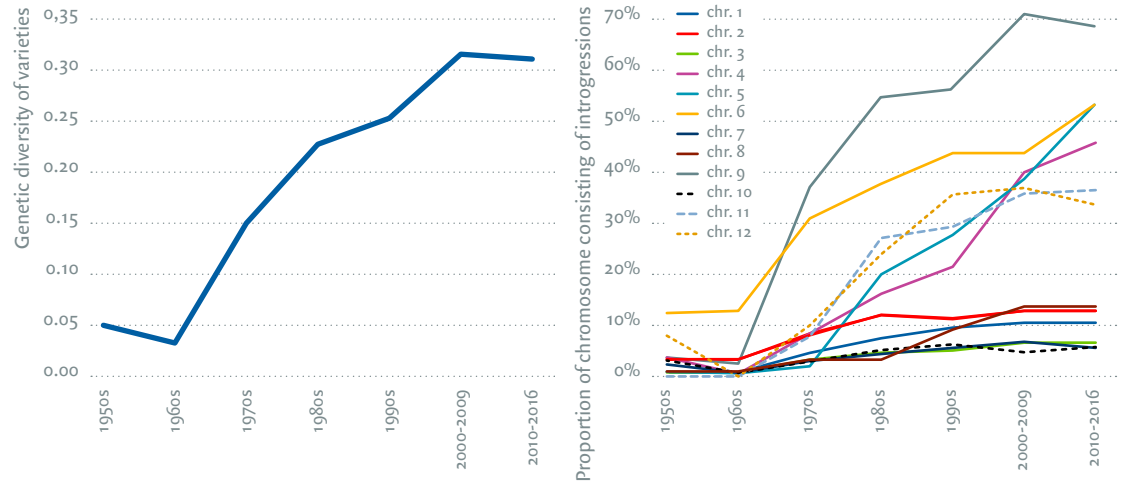


Figure 1. Genetic diversity of tomato varieties that were commercially introduced from 1950 through to 2016. (A) The diversity between varieties within a decade; (B) Proportions of the chromosomes of commercial tomato varieties consisting of introgressions, compared to the prevailing tomato genome in the 1950s and 1960s (modified from <https://doi.org/10.3389/fpls.2019.01606>)

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The introgressions have occurred in all tomato chromosomes, but some chromosomes had far higher proportions of DNA from wild relatives than other chromosomes (Fig. 1B). These differences in quantity of ‘wild DNA’ between the chromosomes are not caused only by the number of introgressed traits per chromosome, but also by the linkage drag that comes along with the introgressed alleles.

First diversity boost

The Dutch Descriptive Variety Lists (‘Beschrijvende rassenlijst voor groentegewassen’) provide insight into the phenotypic traits of tomato varieties since the 1940s. These lists show that at the phenotypic level, too, the diversity among varieties was very small in the 1950s and 1960s, but from the 1970s onwards, the diversity increased because of an increasing number of resistances to diseases and pests. In the 1940s, 1950s and early 1960s, minor differences in fruit sizes were mentioned, being associated with the number of locules per fruit, however, no beef or cherry types were mentioned yet. No other differences were mentioned for the commercial tomatoes in these decades. As the diversity in traits was very

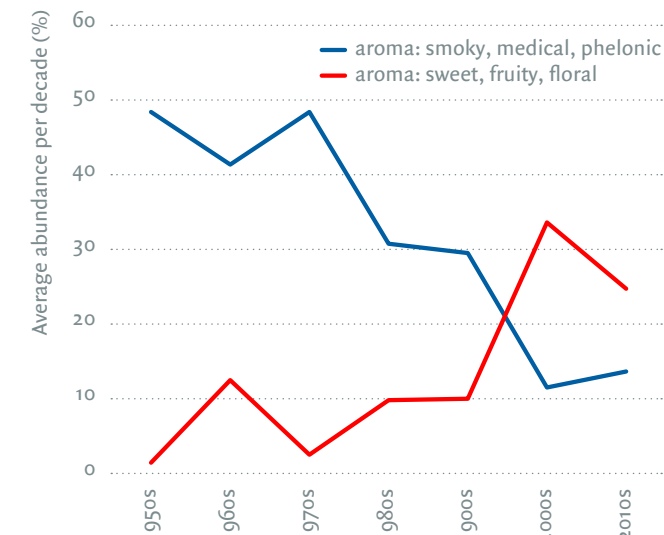
low, one would expect that the number of varieties would be low too. However, that was not the case. In the Descriptive List of 1951, 66 greenhouse tomato varieties were mentioned. This List mentioned that, sometimes, hardly any or no differences could be observed between varieties. In spite of that, these varieties were still released as separate commercial varieties or selections. In these decades, all varieties were still ‘open pollinated’ inbred lines, so no hybrids yet. No disease resistances were mentioned in the Descriptive Variety Lists until the late 1960s. Remarkably, in the Descriptive List of 1951, ornamental tomato types were also mentioned (‘siertomaten’), with special colours (yellow), sizes (cherry, currant) or shapes (pear shape). These tomatoes were not meant for commercial tomato production, nor for consumption, but just as ornamentals, in gardens, for example. In later Descriptive Lists, these ornamental tomatoes were no longer mentioned. However, the special fruit colours, sizes and shapes would become very important at the end of the 20th century and in the 21st century, albeit not for ornamental purposes, but rather for consumption! From the late 1960s onwards, more and more

SOWING A VIBRANT FUTURE



The International Seed Federation, Asociación Nacional de Obtentores Vegetales (ANOVE) and Asociación de Empresas Productoras de Semillas Selectas (APROSE) warmly invite you to the ISF World Seed Congress 2021 in Barcelona, Spain from 17-19 May. Barcelona, the cosmopolitan capital of the Spanish region of Catalonia, is a melting pot of cultures and famous for its art and architecture. Enjoy its dynamic spirit while living the ISF World Seed Congress experience.

Figure 2. Average abundance (%) of guaiacol (smoky, medical, phenolic aroma) and 2-phenyl-ethanol (sweet, fruity, floral aroma) over the seven decades since 1950s



resistances to pests and diseases were introgressed from wild relatives, leading to a sharp increase in the genetic diversity of commercial varieties. These introgressed resistances are the reason for the increase in diversity in the 1970s and 1980s, shown in Fig. 1. An additional factor was the replacement of homozygous tomatoes by F1 hybrids during the 1970s, but that was not the main reason.

Sometimes, resistance genes were accompanied by large chromosomal fragments that were genetically tightly linked to the resistance genes, leading to linkage drag. A rather extreme example is a huge introgression fragment from *Solanum peruvianum* in chromosome 9. This introgression carries the tomato mosaic virus (ToMV) resistance gene Tm2 (derived from *S. peruvianum* PI 126926) or its allele Tm22 (derived from *S. peruvianum* PI 18650). The exotic fragment encompasses about 79% (53 Mb) of Chr 9 in this modern variety, leading to the enormous change in the composition of Chr 9, shown in Fig. 1B. Although breeding companies already started selling tomato varieties with this introgression in the 1970s, and although more than 90% of the modern greenhouse varieties carry one of these *S. peruvianum* introgressions, the introgression size has remained very large.

From the 1970s onwards, the genes Cf-genes were introgressed to provide resistance to leaf mould disease, caused by *Cladosporium fulvum*. Another resistance gene introgressed during the same period is the Mi-1 gene from *S. peruvianum*, conferring resistance to southern root-knot nematode (*Meloidogyne incognita*). This introgression fragment has also remained very large since its introgression, occupying nearly 60% of chromosome 6. Many more resistance genes have been introgressed since the 1970s, on nearly all chromosomes of tomato.

Therefore, we can conclude that from the 1970s onwards, there has been a pronounced boost in diversity, caused by introgression of resistances to pests and diseases. The introgression of new resistances

has not stopped since the 1970s, and is still ongoing.

'Wasserbomben' crisis

In the meantime, the area occupied by glasshouses for tomato production in the Netherlands increased threefold from 713 ha in 1955 to 2150 ha in 1968. The older open-pollinated tomato types were replaced by new hybrid cultivars. However, the large beef tomatoes increased in importance. In the Descriptive Lists, relevant traits were yield, fruit size, fruit shape, disease resistances and suitability for different growing seasons, with or without heating. Remarkably, taste and sweetness were not mentioned as relevant traits... But in the late 1980s and early 1990s, German popular news media described Dutch tomatoes as watery-tasting, naming them 'Wasserbomben' (German for water bombs). In 1985, 80% to 85% of these Dutch tomatoes were still being exported to Germany. However, because of the serious damage to their reputation, the Dutch tomato exports to Germany collapsed shortly after, reducing the area occupied by round tomatoes dramatically from ~1,700 ha in 1985 to ~275 ha ten years later. This became an important milestone in tomato breeding in North West Europe, marking the need for consumer quality traits, such as flavour. The 'Wasserbomben' crisis fuelled the second boost of diversity, namely a diversity in fruit types and improved flavours.

Second diversity boost

The most obvious phenotypic diversification that occurred from the 1980s onwards has been fruit size. In the 1980s, the large beef tomatoes emerged already, but since the 1990s, the small cherry and cocktail tomatoes became more and more important. Analysis of fruit traits showed that the cherry tomatoes were sweeter and contained more soluble acids, compared to the standard round tomatoes. Moreover, they were firmer and juicier, all leading to a higher fruit quality. Ripe tomato fruits produce a few hundred different volatile organic compounds, which are responsible for what we recognize as tomato fruit aroma. Different compounds have different sensorial characteristics, or odours. We observed that the average abundance of phenolic volatiles, such as guaiacol,



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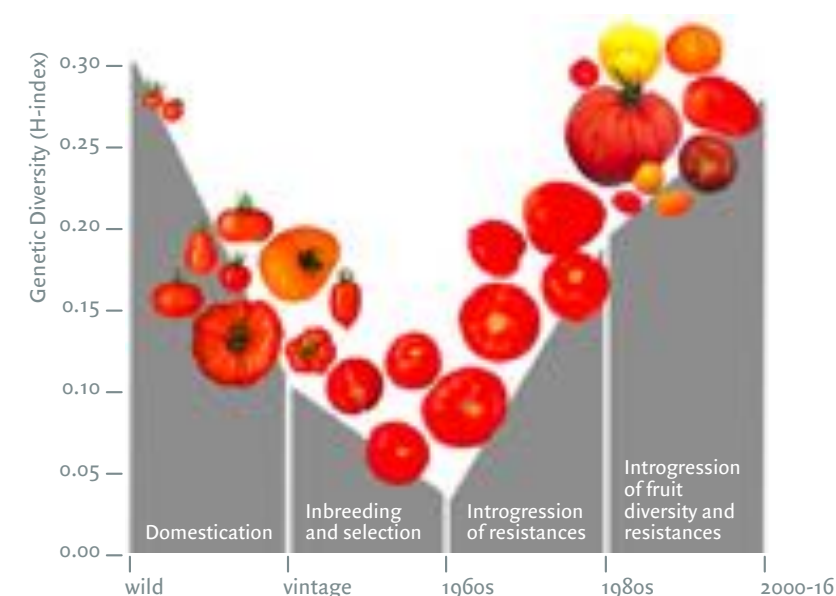


Figure 3. The genetic diversity of tomato over the course of time. The upper left group represents the ancestors (*S. lycopersicum* var. *lycopersicum*, *S. lycopersicum* var. *cerasiforme*, and *S. pimpinellifolium*), which led to the vintage types, including landraces through the process of domestication. Inbreeding and selection among these vintage tomatoes gave rise to commercial varieties in the 1960s with a very low genetic and phenotypic diversity. From the 1970s onwards, resistances to diseases and pests were introgressed from distant species, including *S. peruvianum*, *S. pennellii*, *S. chilense* and *S. habrochaites*, considerably increasing genetic diversity among commercial tomato varieties. After the 1980s, fruit size, colour and flavour started to vary substantially, further increasing the genetic diversity of modern varieties (modified from <https://doi.org/10.3389/fpls.2019.01606>)

19

which has a smoky, medical and phenolic odour and is considered as an undesirable off-flavour, reduced over 60 years of breeding (Figure 2). In contrast, abundance of another group of volatile compounds, represented by 2-phenylethanol, which has a sweet floral and fruity odour, increased over the same period of time. Apparently, several undesired flavours were gradually removed during breeding, whereas desired flavours increased.

The second conclusion is that, on top of the boost of diversity caused by introgression of disease resistances, a second diversity boost was triggered by the ‘Wasserbomben’ crisis, leading to a clear diversification of fruit quality traits from the 1990s onwards.

Domestication and breeding

The data shown in Fig. 1 represents varieties from the 1950s through to the present time, reflecting the period of modern commercial tomato breeding. In order to put this diversity in a wider context, we estimated the diversity of 385 ancestors (*S. lycopersicum* var. *lycopersicum*, *S. lycopersicum* var. *cerasiforme*, and *S. pimpinellifolium*), and 129 vintage accessions including landraces, using data from Blanca et al. (BMC Genomics. 16, 257; 2015). They used the same SNP-markers as we did for Fig. 1.

Fig. 3 shows the relatively high diversity among ancestors belonging to *S. lycopersicum* and *S. pimpinellifolium*. Domestication of these species and genetic bottlenecks due to transport led to vintage types. According to this figure, only one third of the variation in the ancestors was still present in the vintage accessions. Further inbreeding and selection led to the commercial varieties in the 1960s, representing only 10% of the diversity of the ancestors. However, introgressions of resistances to diseases and pests in the 1970s and 1980s increased the genetic diversity considerably, even above the level of the vintage types. The second boost of diversity, i.e. the breeding for fruit size diversity, colour differences, improved taste,

and additional resistances, further increased the diversity, nearly to the level of the ancestors. We have to keep in mind here that the introgressions in the modern varieties not only descend from *S. lycopersicum* and *S. pimpinellifolium*, i.e. the species from which the domesticated tomatoes have been derived, but also from more distant relatives, including *S. peruvianum*, *S. pennellii*, *S. chilense* and *S. habrochaites*. This explains the high level of diversity of the modern varieties compared to the ancestors *S. lycopersicum* and *S. pimpinellifolium*. Furthermore, we have to bear in mind that absolute levels of diversity depend on the applied DNA-markers.

Other crops

Wouw et al. (Theor. Appl. Genet. 120, 1241–1252, 2010) performed a meta-analysis on genetic diversity of eight field crops in the 20th century, based on worldwide data from 44 other papers. Wheat was the most represented crop in these papers. Wheat showed the lowest diversity from the 1960s through to the 1980s. This decrease was 6%, compared to the first half of the century, and was significant. However, a recovery of diversity was observed in the 1990s. For seven other major crops (barley, maize, oat, flax, soybean, pea, rice), a diversity dip also occurred in the 1960s, but the recovery was earlier compared to wheat.

Increase in diversity

We can conclude that the concern about decreasing diversity among tomato varieties due to modern breeding, as voiced in recent papers, is not supported by our study. In contrast, we have observed a tremendous increase in diversity, both at the genotypic and phenotypic level since the 1970s. Other crops have shown similar trends, albeit less pronounced, compared to glasshouse tomatoes in North West Europe.

Sending children to school, not to work

Anke van den Hurk

20 Since the early 2000s, the problem of child labour has received significant attention. Even though reports still mention child labour, especially after 2015, the percentage of child labour has significantly decreased in the seed sector. Most companies prefer children to go to school, instead of the seed production fields.

• **The seed sector is an international sector,**
• many companies have offices and research stations in different countries to develop plant varieties for all different types of ecosystems and different farmers.
• Seed production occurs in many more locations, under own management, by seed producers or by seed farmers. In this way, the sector provides support to the development of many economies. By working in those different economies, it is recognized that social compliance measures need attention and may need to be improved. Several reports, such as the ones by the India Committee of the Netherlands, now named ARISA, demonstrate that children are part of the workforce in seed production of cotton, maize and vegetable seeds.

Actions of the companies

As companies became more aware about child labour in seed production, especially in cotton and vegetable seed production, they started to examine the status in their own company and introduced prevention measures. Some companies started this process seriously as early as 2004, while others followed later. The actions are mostly done at company level and in some cases, they work with small groups of companies. The measures relate to different due diligence steps, such as commitment, assessment of risks and impact, integration in the business processes, external monitoring and communication, as well as the improvement of the livelihood of children and their families.

More and more seed companies are developing human rights strategies, which require the support of and action by all relevant managers and employees, as well as suppliers. This means that action needs to be taken both within and outside the company. Training of both staff and suppliers helps to achieve this. In order to prevent child labour, one must have an insight into the seed production chain; where do the risks of child labour occur, where are the risks highest and where do they have the highest impact? Different methods may be used to carry out risk assessment. One should work with a broad range of stakeholders of the whole supply chain and be persistent to get the right information. Child labour may occur in different countries and at different stages of the supply chain. Companies must prioritize risks

Family farm culture

The majority of children in farming work on their parents' farm as unpaid family labour. The work that children perform is often invisible and unacknowledged because they assist their parents. Participating in some farm activities can give children an opportunity to develop skills and a sense of belonging to the community. However, this becomes a problem when farm tasks interfere with schooling and when they are hazardous in nature.

Source: United Nations' International Labour Organization

and start with activities with the highest risk and the highest chance of success.

Integration

Several companies have developed contracts or clauses in contracts for seed producers in which seed producers must approve the banning of any child labour in their activities and which are often combined with sanctions or incentives. In some cases, more than one moment in the development chain is chosen to sign agreements on clauses that forbid to have child labour. These contracts are often combined with sanctions and or incentive measures. Some companies are examining the procurement system to see what effect it may have on child labour. Recognizing the impact is one thing, but actively changing a system is something else, especially if it is not a combined effort by the whole sector. Most companies that have developed human rights policies have an internal monitoring system in place, which clearly identifies who is responsible for monitoring and how frequently and where it happens. This monitoring may be announced or unannounced, and external monitoring may also take place. Most companies learn from their systems and therefore continuously aim for improvement.

Projects

Several companies are involved in specific projects with other stakeholders in order to stop child labour and change policies and culture in production areas. Bayer, for example, has actively worked to implement a comprehensive multilevel Child Care Program (CCP) in five states in India - Andhra Pradesh, Karnataka,

During field days farmers are told that seed production is no child's business, learning is



Maharashtra, Gujarat and Tamil Nadu - where the company has contract cotton seed production. They want to get children off their contract farmers' fields and into school instead. The project included next to monitoring and back to school programs, training of pollinators, vegetable growers and backyard farming training to improve farmer's livelihood. Another example is the work by Syngenta together with the Fair Labour Association, which has already been happening for many years. East-West Seeds carried out a project named 'Towards vegetable seed production without child labour'. Many others are also directly or indirectly involved with organizations to stop child labour. Earlier this year, the Enabling Child and Human Rights with Seed Organizations (ECHO) previously named CCP was founded by the seed industry and NGOs. Several companies delegated members on the ECHO Executive Committee: Bayer, Syngenta, East-West Seed India, UPL Advanta, HM. Clause and BASF Vegetable Seeds. ECHO, a multi-stakeholder forum, was established to share experiences and consult organizations on various social compliance issues, such as child rights, minimum wages, and healthy and safe working conditions in the production of hybrid seeds.

External monitoring

In addition to internal monitoring systems, several

companies and company groups work with auditors who monitor their systems, including any aspects of child labour. The frequency of monitoring differs and is mostly unannounced. SGS, the Child Care Programme India, the Fair Labour Association, Ernst & Young and others are among the auditing organizations involved. Four Dutch vegetable companies work together with SGS in India. The Child Care Programme India entails a comprehensive field monitoring programme. Cotton production staff with the programme carry out frequent unannounced assessment visits, in addition to internal monitoring. To keep the monitoring as objective as possible, teams are rotated between different locations. The Fair Labour Association also offers monitoring programmes that are also used in the seed sector. Although most of the auditing has initially been focused on India, other countries are now also being audited, or plans for audits are being made.

Communication

Several companies have established human rights policies and communicate those on their websites, as well as the specific activities in which they are involved. They communicate not only to be transparent about their process, but also have communication activities to create awareness within the company, as well as outside. Raising awareness outside the

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<https://www.cropsience.bayer.in/Social-Commitment/Rural-Development/Child-Care-Program.aspx>

<https://english.rvo.nl/sites/default/files/2020/02/Lessons%20learned%20and%20practical%20steps.pdf>

https://www.fairlabor.org/sites/default/files/documents/reports/seeds_of_change_report-final_o.pdf

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company focuses, on the one hand, on people directly involved in the seed production chain and, on the other hand, on the local communities where the seed production takes place. The latter helps to explain that a child's education is important and will have a positive impact on the future of that child and society, as a whole. Several of the projects where companies are involved in direct improvement of the children and their families lives. Subsequently, different education programmes and schools have been developed.

Improvement of livelihood

Several of the projects where companies are involved in direct improvement of the children and their families lives. Different education programmes and schools have been developed. Not only the education is supported, but also the buildings for schooling, furniture for schools, pure cold-water for the children and vegetable gardens. School attendance of children is monitored and stimulated by creating incentives for pupils (presents like school materials) and staff (classroom and facility improvement). Training of pollinators improves their productivity and skills and thereby enhances their affordability compared to untrained children. Training of farmers in vegetable cultivation and backyard farming resulting in more income which can support sending children to school and direct support for better nutrition.

Role of seed associations

As indicated above, companies felt it is their own responsibility to deal with human rights policies, rather than as consortium. In recent years, this view has changed and a role for seed associations is also recognized. The International Seed Federation, the Asia and Pacific Seed Association and Plantum, in the

Seed companies not only support the education of children, but also school buildings, class furniture, pure cold water and vegetable gardens

Netherlands, have agreed statements that aim for a seed sector without child labour. In addition to the statements, ideas are provided on how to stop child labour.

During the last couple of years, further collaborative actions have been considered. They are mostly focused on awareness raising and exchanging

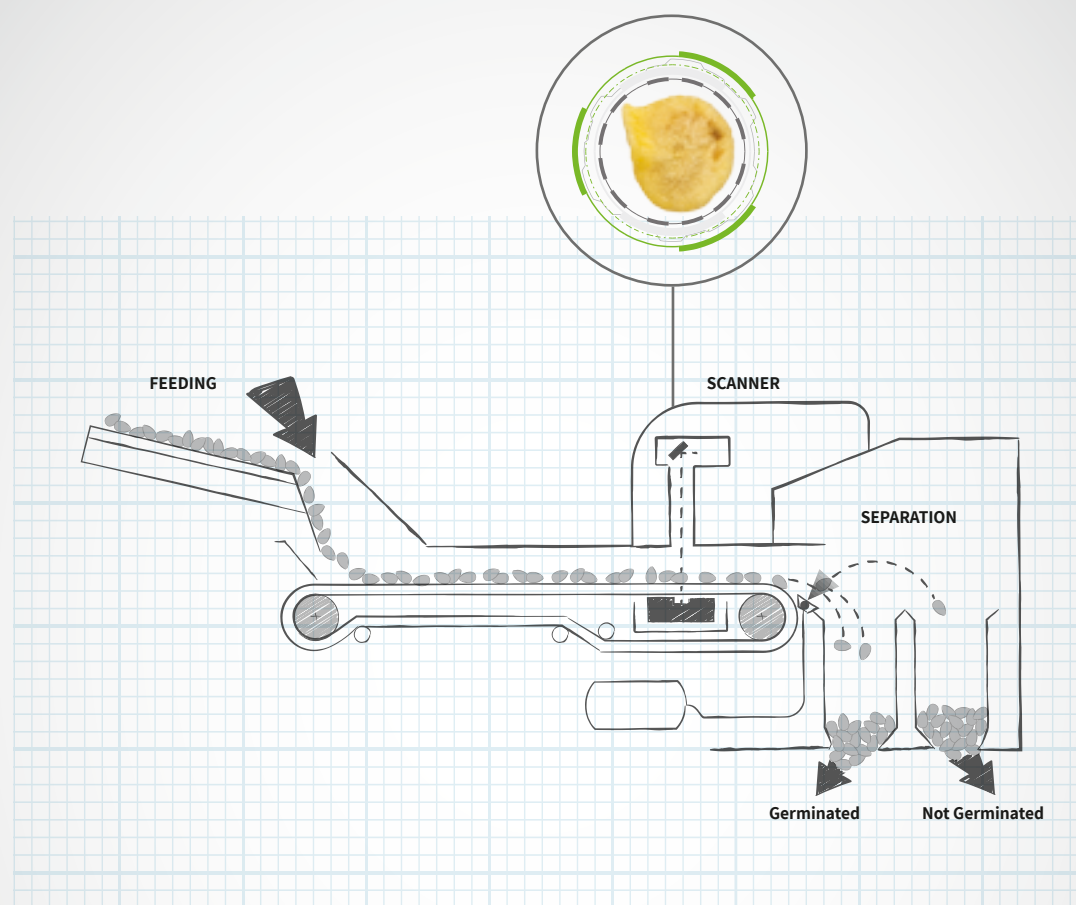
experiences. Plantum, for example, regularly writes about corporate social responsibility in its newsletter, has developed some information material and had an information meeting for its members on prevention of child labour and living wages. During this meeting, visions of these topics were shared by the government and NGOs. Also, experiences on the prevention of child labour and the payment of living wages in practice, within and without the sector, were shared.

Future activities

Future activities of seed associations probably will be focused on further awareness raising, and possible collaborative projects on the topic. Even though the contributions of the individual companies to prevent child labour and, to a lesser extent, to the payment of minimum wages and/or living wages are very positive, in some cases it is required that collaborative actions take place to make a next step. This requires huge commitment which is challenging if at all possible for a sector as a whole with companies of different size, from different countries. Also collaborative action may not be sufficient without commitment of local seed companies, and legal enforcement by local governments.

As more and more policies, guidance documents, rules and regulations on corporate social responsibility are developed nationally and internationally, the seed associations may become more involved in advocacy on the topics and also in informing members of the consequences of those. Currently, for example, the Netherlands is finalizing the implementation rules of a law on due diligence obligations to prevent child labour. Plantum, together with the Chamber of Commerce, is following this process and is keeping the members informed.

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Plant Health in the EU

Safeguarding a sound phytosanitary status

John van Ruiten

After a long period of preparation and finalizing many implementation rules, since 14 December 2019, the new Plant Health Regulation ('PHR'; 2016/2031/EU) has come into force, together with the renewed Official Control Regulation ('OCR'; 2017/625/EU). These two regulations now form the core of obligations that have to be fulfilled in order to safeguard a sound phytosanitary healthy status in agriculture, horticulture and nature/landscaping in the EU.

• **With the new regulations**, the EU wants to further harmonize both policy and execution of phytosanitary standards. The Union also wants to better protect itself against incoming pests and plagues and wants to limit the possible spread of harmful organisms within its territory. A more stringent approach towards imported material (notably the import ban of plants of high-risk species/commodities) is part of this legislation. But also, the internal plant passport system has undergone some changes. Plant passports, when marketing material within the EU, are now obligatory to use for all plants for planting and for a number of seeds. And the plant passports must have a uniform layout in all EU member countries.

Quarantine organisms

And last but not least: the list of quarantine organisms has been reduced enormously. Now on this list, only the pathogens are listed that are not present in the EU or are being eradicated. A strict zero tolerance for these organisms is in place. All other harmful pathogens (many of them being quarantine diseases in the past) now have a new status: they are so-called Regulated Non-Quarantine Pests (RNQP). For most of these pathogens and pests, the norm is that material marketed must be free of these organisms. If they occur during production, measures can be taken to clean the crop.

An important EU document in which all these organisms and their status/norms can be found was published by the EU Commission on 28 November 2019 (Regulation 2019/2072). Recently, also the marketing directives (for vegetable seeds, ornamental plants, seed potatoes and fruit plants, for example) have been changed (Implementing Directive 2020/177/EU). The disease requirements that were in the past an integral part of these directives have been transferred to the RNQP status in the new PHR, but the inspection rules are still in these directives, however adjusted. Regarding plant health, the focus in the EU at this moment is on several procedural points (documents for moving material within the Union after import; passenger information for travellers; creation of awareness, release material from quarantine) and on the two diseases *Xylella fastidiosa* and Tomato Brown Rugose Fruit Virus (ToBRFV). *Xylella* has been found in various regions in southern



Xylella fastidiosa is transmitted by 0.5 cm cicadas, such as the black-and-red froghopper (*Cercopis vulnerata*) and can be found in olive trees, lavender but also in weeds

Italy, Corsica, Spain, France and Portugal. Not only in crops like *Olea* (olive), *Prunus* (almonds) and *Lavandula* (lavender), but also in weeds. There is great concern in the Union that the bacteria will move further north. The Commission wants to introduce new adjusted emergency measures to deal with controlling the spread of the disease. More testing, the use of better test protocols and systematic testing of imported plant material of host plants from third countries.

With regard to ToBRFV in tomato and sweet pepper, it can be seen that the virus now occurs in the EU (officially notified) in Germany, France, the Netherlands, Spain and Italy. It is thought that infestations are also present in other countries. The virus was first discovered in the EU in 2018 and most probably arrived from the Middle East (route unknown). It has a devastating effect on most tomato cultivation, whereas in sweet pepper, most varieties seem to be resistant/tolerant against this very infectious virus. Also, transmission by seeds is possible. The EU Commission proposes intensification of testing seed and plant material, both from EU and non-EU sources. Also, the preferred and possible test methods (PCR, ELISA or bioassay) are discussed.

There is more to strawberries than meets the eye

Monique Krinkels

26 One of the effects of the lockdown caused by COVID-19 is that the Dutch Spring Trials 2020 had to be cancelled. Not a major drawback compared to the havoc the virus instigated, but still a disappointment for those eager to learn more about the latest novelties in flowers and bedding plants. To compensate, ABZ Seeds invited guests to a virtual tour on their premises.

Many Dutch ornamental breeding companies organize spring trials. Among them is ABZ Seeds, as the strawberry varieties are as well praised for their quality as bedding plants as for the fruit production. In the demo greenhouse at the Holland Strawberry House, ABZ Seeds displayed all their latest varieties and experimentals. The Holland Strawberry House is a replica of South African buildings in the Cape Dutch style. It underlines the bond between the Netherlands and South Africa as the grand, ornately rounded gables are reminiscent of features of townhouses in the Dutch capital, Amsterdam. It would have been a fitting concept if the World Seed Congress did not have to be postponed due to COVID-19. It remains, however, a very beautiful head office.

Diversity

It is amazing to see how diverse strawberries can be, with many different forms and colours. At ABZ Seeds, the flowers vary from white to pink, to deep rose and even red. Some have single flowers, others double, the shape of the plants vary from compact to plants with many runners and the fruits from conical shaped to almost round. Via a live video chat connection,

The happiest fruits

Scientists found that just thinking about strawberries makes people happier, a unique characteristic compared to other fruits. Its appeal is so strong that 86% of people feel more relaxed just by imagining putting a strawberry in their mouth, a study by the University of London's Centre for the Study of the Senses revealed. Fruits, such as apples, bring back less pleasant memories, while bananas and plums were the least likely to improve mood, concluded



Professor Barry Smith, who investigated the link between emotions, taste and smell. Strawberries proved to be the happiest fruits. The smell of strawberries is difficult to create artificially. Flavouring companies have failed at it, by and large, and yet it is so powerful as a reminder of happy summer days.

ABZ Seeds' director/breeder, Gé Bentvelsen, was able to provide his guests with information about the many varieties the company produces and those that are still in an earlier stage of trials. And, of course, the bright red fruits were on display as well. A virtual tour makes it possible for all distributors worldwide to visit the trials. The advantage (besides complying to the safety measures made compulsory by the corona virus): people are able to visit without time-consuming travelling. But it had a noticeable disadvantage: with video chatting, only two senses are used: ears and eyes and, especially with strawberries, smell and taste are of the utmost importance. "It is the distinctive aroma of strawberries that evokes happy memories," explains Gé Bentvelsen. The fragrance centre is close to the emotion centre in the brain, so the neural lines are short. That is why smell plays an important role in the triggering of memory tracks. And at ABZ Seeds, it is crucial comparing the different scents, as the company is highly focused on smell and taste. Think, for instance, of the series of delicious gourmet strawberries ABZ Seeds created. More than 35 years ago, Gé Bentvelsen became involved in breeding strawberries at S&G (nowadays known as Syngenta). His goal at the time: to redirect and intensify a small breeding programme for bedding plants towards commercial fruit production. But, as strawberries are octoploids, it was far from easy to reach that goal. "When I studied at Wageningen University, Professor J. Sneep always reminded us that 'Patience and perseverance is what a breeder needs to possess. And when you have an initial success, you should never rest on your laurels', he always added," says Gé Bentvelsen.

A few years later, when the company was forced to set priorities, it was decided to sell the breeding programme altogether. Gé Bentvelsen continued the strawberry breeding programme with his newly formed company ABZ Seeds. The abbreviation stands for strawberry from seeds. The first F1 hybrid strawberry was introduced on the market in 1996.

Sustainability

For a long time, ABZ Seeds was the only company involved in hybrid strawberry breeding. "Today, Japan has one hybrid variety and, in Canada, a governmental institution has started a hybrid breed-



Gé Bentvelsen belongs to the twenty most innovative plant breeders in the European seed sector

ing programme, as did two other Dutch breeding companies. In the Netherlands, common practice is still strawberry breeding for cutting varieties. At least six companies are present in the market." Meanwhile, ABZ has over twenty F1 hybrid varieties, mostly designated for the bedding plant market. "For commercial fruit production, we have chosen to aim at a special segment: covered cultivation in north-west Europe. For these companies, the reliability of the plants is of the utmost importance. Our varieties have a germination percentage of over 90%, which makes them very reliable for plantlet producers." Bentvelsen hopes he will, in due course, convince the strawberry fruit producers to choose his varieties. "It is a more sustainable alternative," he claims. The Dutch minister of Agriculture, Carola Schouten, has instigated a research programme as strawberry is a problem crop. Environmentalists accuse growers that their strawberries are six times more 'toxic' than other fruits in the supermarket. That might be a bit exaggerated, as the European standards for these harmful substances are very strict. But growers do have to use high levels of chemicals, as cuttings are more contaminated with diseases and pests.

Huge experience

The minister has formed a commission that is looking into alternatives. "So far, there are several scenarios that will be worked out. Production in greenhouses, for instance, where the water is re-circulated and strict hygiene measures can be maintained. The use of rock wool, instead of peat or coconut coir, would also reduce environmental pres-

sure. And last, but not least, the use of seeds instead of cuttings," explains Gé Bentvelsen. "We have 25 years of experience with strawberry plants from seed worldwide," says Gé Bentvelsen. The varieties for fruit growers are suitable for year-round production. At the Proeftuin Zwaagdijk, strawberry varieties are tested in a greenhouse with assimilation (LED) lighting for cultivation from November through to April. "We use everbearing strawberry varieties, such as Delizzimo. In South Africa, a project with this variety has been very successful." For today's strawberry growers, it would, however, mean a huge change in cultivation techniques. It would be comparable to tomato growing. Even vertical farming will be possible, according to Gé Bentvelsen, as some varieties have compact plants that are suitable to this relatively new cultivation system. It would open up a whole new market.

Praise

The ABZ Seeds varieties have received many rewards. This year, Gé Bentvelsen received the IPM 'Nieuwheid Des Jahres' (the innovation of the year) Prize with the Fragaria x ananassa Duch F1 'Summer Breeze Snow' in the Tub Plants category. The jury called it "a jack of all trades". "As an attractive flowering plant and as a tasty treat for patio and balcony, it is ideally suited for city gardening. Its unusually large, semi-double flowers form a proper inflorescence and, therefore, stand attractively above the foliage. This is what makes the ornamental value of this strawberry so interesting." It is one of many ABZ-varieties that definitely has the wow-factor, especially if you use all your senses.

New era of precision plant breeding

William de Martines, Daniel Monino Lopez, Jan Schaart, Richard Visser

28 Genome editing gives breeders an incredibly accurate tool to facilitate breeding. In this article, the researchers explore some specific CRISPR-Cas based genome editing techniques, including where and how they complement traditional breeding, giving examples that are researched at Plant Breeding at Wageningen University & Research.

In plant breeding, everything boils down to enlarging the genetic variation of traits and crops. Several ways are available to increase genetic variation, ranging from looking at wild relatives to inducing mutations by various means. However, all these methods do have in common that they take a lot of time, are often directionless and cause side effects (varying from linkage drag to many extra mutations, besides the one in the target gene). In recent years, genome editing techniques became available (Zinc Finger nuclease technology, TALENs and CRISPR-Cas). Recently, CRISPR-Cas has become a buzz word, it is billed as revolutionary, promising to change the field of plant breeding as we know it. This revolution comes down to CRISPR-Cas' ease of use. CRISPR-Cas is a tool that allows us to easily cut DNA at very specific locations in the plant genome. Subsequent repair of the cut allows targeted changes to the DNA sequence to be made. Cas enzymes use a guide RNA, which matches a target DNA sequence to direct its cutting; by switching between guide RNAs, you can easily target new sequences. Previous techniques for targeting DNA sequences were complex and time consuming, but with CRISPR-Cas, we can easily make targeted DNA cuts, simplifying genome editing.

Pushing for change

The GeneSprout Initiative was formed in the wake of the European Court of Justice ruling as a voice for change. It was formed to create an open dialogue and to represent young researchers in public outreach and policy making in relation to genome editing and other new plant breeding techniques (NPBTs). As part of both public and political outreach, the GeneSprout Initiative organizes and attends various events, such as information evenings and conferences. Presently, they are expanding their website to serve as an open source of information on NPBTs. Members are currently located in both the Netherlands and Belgium, but the initiative is looking to expand internationally. If you would like to get involved please visit their website, <https://www.genesproutinitiative.com>



Ir. W. de Martines, Dr. D. Monino Lopez and Dr. J.G. Schaart are researchers and Prof.dr. R.G.F. Visser is Chair & Head of Plant Breeding, Wageningen University & Research, Wageningen University & Research, Wageningen, the Netherlands, email Richard.visser@wur.nl

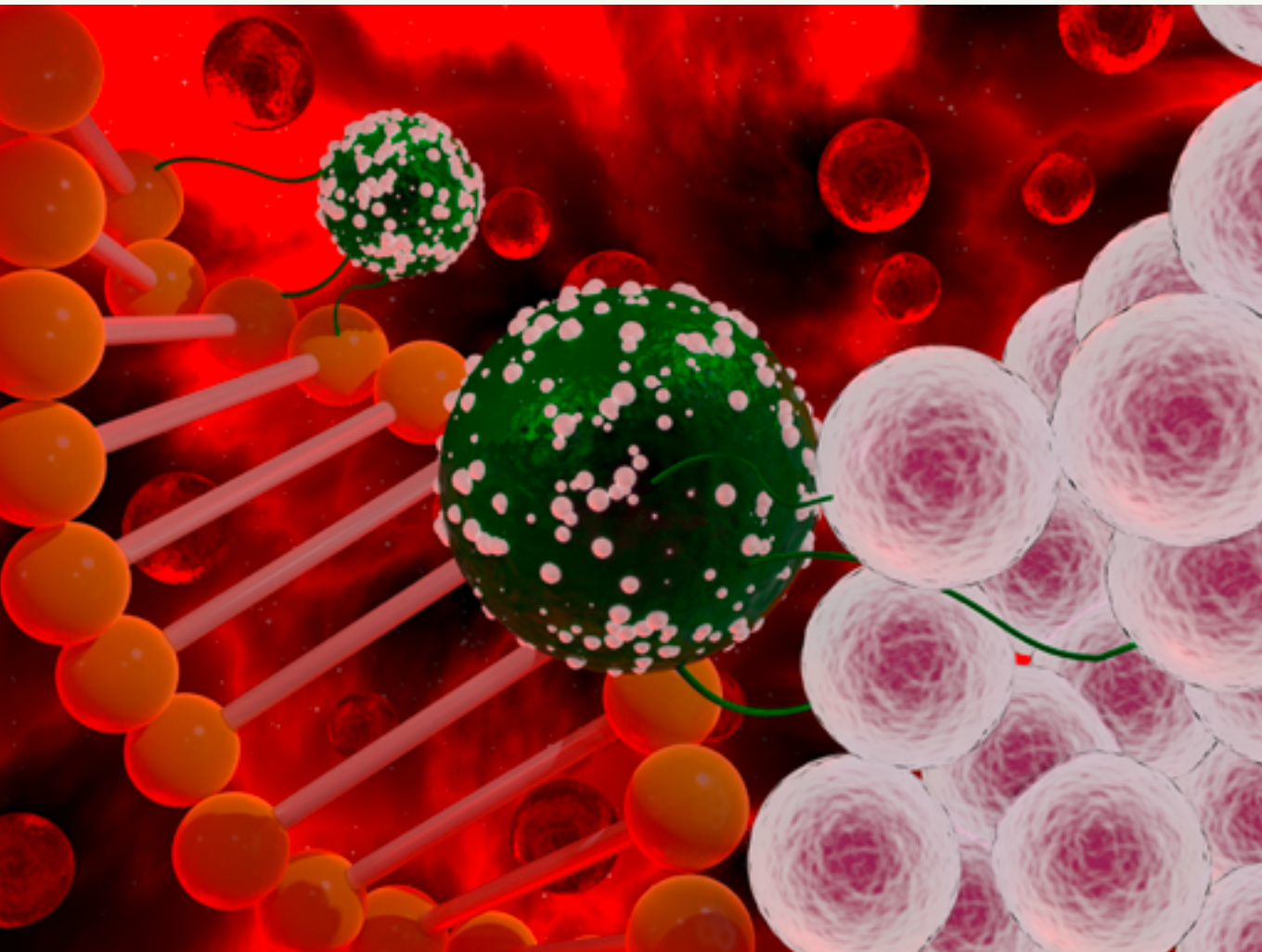
In traditional plant breeding, beneficial genes are introduced by many years of crossing and selection into a new variety. With genome editing, you can quickly change detrimental or non-functional genes to beneficial ones. This is not a replacement of traditional breeding. Making crosses and selecting for improved progeny will remain an important activity, but genome editing gives us an incredibly accurate tool to facilitate breeding.

Knock-out application

The most explored application of CRISPR-Cas is the generation of mutant plants in which specific genes have been knocked-out. When the Cas enzyme is directed by the guide RNA to a specific target sequence, it cuts the DNA at that location. In plants, the predominant DNA repair mechanism is non-homologous end-joining (NHEJ), which leads to mutations or disruption of the targeted gene. Traditionally, we have used chemical or radiation-based mutagenesis to create random knockouts, hoping to find desirable traits, which requires screening of thousands of plants. Traditional mutagenesis has been sufficient in many crops with simple genomes and has resulted in many plant varieties with improved characteristics. However, many of our cultivated crops are polyploids or contain gene duplications, meaning that they carry more than the normal two copies of a gene. To get a complete knockout you need to mutate every copy of a specific gene, and each additional copy makes it more difficult to get a complete knockout. A good example is the creation of hypoallergenic wheat, which requires knocking-out hundreds of gluten genes, which are present as multiple copies in the wheat genome. Using traditional techniques, it is nearly impossible to achieve such a goal, but with CRISPR-Cas you can target and knockout all these genes virtually at once. This application could benefit millions of people living with coeliac disease, who currently must avoid products containing wheat. At Plant Breeding, we have produced prototype wheat lines in which a large number of gluten genes have been deleted.

Gene inversions

With traditional breeding, when trying to bring together beneficial genes, chromosomal inversions that are present in many plant genomes can limit the



movement of individual genes. In such a situation, the beneficial gene carries along multiple detrimental genes, which are located on the same chromosomal segment. It is, however, possible to flip back the inversion, using CRISPR-Cas to cut on both sides of the inversion, freeing the beneficial genes from the detrimental ones. A clear example of this situation is the Ty-2 resistance gene in tomato against the TYLCV virus. Here, breeders cannot free this gene from detrimental ones, making it difficult to develop a good cultivar carrying this resistance. However, this inversion can be flipped using CRISPR-Cas. At Plant Breeding, we are now exploring methods for flipping chromosomal inversions. This allows us to leave behind the detrimental genes, letting us develop a good cultivar with viral resistance requiring less pesticides to control whiteflies, which transmit the virus.

Homology Directed Repair

Above, we described that cut DNA can become mutated through NHEJ. Alongside NHEJ, there is another repair mechanism called homology-directed repair (HDR). HDR uses matching DNA to guide the repair, and essentially copies the matching strand to get a precise and error-free repair. Since plants have multiple copies of their DNA, it can use one of

these copies to guide the repair if another copy is damaged. However, we can also introduce a novel matching DNA piece into the plant to guide the HDR to make specific changes. In this way, we can change a non-functional gene into a functional one directly, instead of having to do multiple crosses to introduce the functional gene. This can speed up the breeding process enormously in situations where plants have complex genomes or are vegetatively propagated, as explained below.

An example of applying CRISPR-Cas and HDR to speed up the breeding process is the development of disease resistant crops. Most crop varieties carry non-functional versions of resistance genes. Converting non-functional resistance genes to functional ones can be done using HDR, with small pieces of DNA from functional resistance genes. Such an application would be great for a crop like potato, which has a complex genome and is vegetatively propagated. Potatoes are very prone to getting late blight disease, which requires potato plants to be sprayed with pesticides throughout the growing season. Many wild potato species carry late blight resistance genes, which have become non-functional in cultivated potatoes. With CRISPR-Cas and HDR, we can directly replace the non-functional genes with functional genes from

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The CRISPR-Cas system works like a molecular pair of scissors. The Cas enzyme is guided by a guide RNA that matches the target DNA sequence. Once the Cas enzyme finds its target, it binds and cuts the DNA. The DNA is repaired via one of two main mechanisms, non-homologous end-joining or homology-directed repair. Non-homologous end-joining is by far the most common repair mechanism but is error-prone, often leading to gene disruption or knock-out. Homology-directed repair occurs far less often in plants, but it does result in error-free repair by using a matching DNA strand to guide repair

wild species, without having to do complex crossing and selection. We are currently investigating this approach at Plant Breeding. Developing resistant potatoes this way will give us much more sustainable potatoes, requiring much less pesticides.

Not just a pair of DNA scissors

We have also disabled Cas enzymes that do not cut DNA, instead these can just target and bind specific DNA sequences. We can use these disabled Cas enzymes to carry other enzymes that make targeted modifications to the DNA. For instance, we can use modified Cas-enzymes, called base editors, which change individual bases in DNA. So, these are perfect to make subtle changes to improve a gene. For example, if a beneficial gene has been turned off through a small mutation, you can turn it back on by changing the DNA base underlying this mutation. Again, a nice example here is late blight resistance in potato. With a few base changes, we can turn a non-functional resistance gene into a functional one. This approach has two advantages in that: 1) the original varieties which are appreciated by industry and consumers because of their attributes remain available, and 2) they are now resistant to late blight instead of susceptible, thus requiring no or less use of chemicals, leading to a more sustainable potato cultivation.

Major impediments to use

Currently, the largest impediment to adoption of genome edited crops is regulation. In the European Union, we have Directive 2001/18/EC, which is legislation that has essentially stopped the commercialization of GMO crops in most of the EU for nearly

20 years. This legislation also promises the same fate for plants obtained through genome editing. This directive vaguely defines GMOs and GMO exemptions. Its vagueness was interpreted by the European Court of Justice, which ruled that genome edited plants are GMO and are not exempted from the same strict regulation as classical GMOs. Even with better regulation, we can still run into limited consumer acceptance of genome editing. Everyone has the right to ask questions about their food, and many people feel a strong connection with food. This makes genome editing prone to outright rejection by consumers. Without consumer acceptance, you no longer have a market for genome edited crops, plain and simple. However, the benefits of this technology are so great that we all need to work together to ensure smart regulation, while at the same time reassuring consumers that acceptance is to their benefit as well.

PROPHYTA ANNUAL 2020

PVP toolbox helps to make a country's system UPOV-proof

Marien Valstar

32 The Netherlands has a programme to support countries to develop a plant variety protection system. The so-called PVP development programme strengthens the cooperation between countries and promotes the exchange of expertise between possible new and current UPOV members and the Dutch authorities on the implementation of plant variety protection and on DUS testing.

• **Plant breeding is essential** to adapt agriculture to a changing climate and to meet the changing needs of society. By improving variety characteristics, such as disease resistance and yield potential, plant breeders make agriculture more sustainable. Plant breeding requires a big investment of time, knowledge and money. It may take on average 8-10 years for a breeding programme to create a successful variety. Without adequate protection of the intellectual property of this new variety, few breeders would invest in plant breeding and this would slow down agricultural development. The UPOV Convention, promoting an effective system of plant variety protection, is essential to promote investment in plant breeding.

Development programme

Since its inception in 1961, the Netherlands has been a strong promoter of the UPOV Convention. Countries that become members of UPOV have legislation in place that protects the rights of the breeders of new varieties. The Netherlands has

UPOV

The purpose of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants for the benefit of society. The members of UPOV are: African Intellectual Property Organization, Albania, Argentina, Australia, Austria, Azerbaijan, Belarus, Belgium, Bolivia, Bosnia and Herzegovina, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Czech Republic, Denmark, Dominican Republic, Ecuador, Egypt, Estonia, European Union, Finland, France, Georgia, Germany, Hungary, Iceland, Ireland, Israel, Italy, Japan, Jordan, Kenya, Kyrgyzstan, Latvia, Lithuania, Mexico, Montenegro, Morocco, Netherlands, New Zealand, Nicaragua, North Macedonia, Norway, Oman, Panama, Paraguay, Peru, Poland, Portugal, Republic of Korea, Republic of Moldova, Romania, Russian Federation, Serbia, Singapore, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Trinidad and Tobago, Tunisia, Turkey, Ukraine, United Kingdom, United Republic of Tanzania, United States of America, Uruguay, Uzbekistan and Vietnam.

a programme to promote UPOV membership, called the PVP development programme. In this programme, the Netherlands provides a structured assistance to governments, PBR offices and DUS testing stations in the field of Plant Breeders' Rights systems around the world. There is an annual call for proposals. The programme provides specific and continuing responses to requests from countries worldwide. Together with Dutch experts, they can develop and implement Plant Variety Protection systems or improve existing procedures. With relatively small amounts of funds, projects can be carried out that are very helpful for countries to become acquainted with UPOV and PVP, provide very practical, technical and administrative training and support. Some projects can lead to more long-term relationships with counterparts in other countries which can also be funded from other sources.

The fact that most of the activities are carried out by staff from examination offices, who themselves work in the field of PVP, is a great advantage. Through the good cooperation between the Dutch Government, the agricultural counsellors in the various countries, the Dutch Board for Plant Varieties, the Dutch breeding sector (Plantum) and the Inspection Service/ Examination Office Naktuinbouw, all elements are in place to contribute to successful projects with a positive effect in the receiving countries. International cooperation takes place with CPVO and UPOV.

PVP toolbox

There is a large range of tools available in the programme, such as:

- awareness missions in the Netherlands for groups of decision makers
- local experts participating in the PVP course in Wageningen
- training for crop experts on DUS programmes and testing
- help in the establishment of an Office for Plant Variety Protection in a country
- advice on organisational and administrative matters
- supporting the use of the UPOV electronic PVP-filing system



Naktuinbouw supports countries worldwide to set up legislation and implement a plant breeders' rights system

- tailor-made technical training in the country
 - awareness programmes for farmers and traders
- In 2019, the execution of the Toolbox programme was evaluated by an independent team of experts. They concluded that the PVP Toolbox is a very useful instrument that helps the countries, together with

International course

One of the PVP toolbox instruments is to support the participation of local experts in the course 'Plant Breeders' Rights for Food Security and Economic Development'. The main learning objectives are to obtain knowledge and practical skills in legal aspects, institutional aspects, technical aspects and about the exploitation of plant breeders' rights. All subjects are approached from both theoretical and practical viewpoints. The course is organized by CDI (Wageningen University and Research) in cooperation with Naktuinbouw.

the Dutch institutions and companies, to stay on top of development of protecting plant variety rights. Projects have been executed in many parts of the world, ranging from Tanzania, Egypt, India, Mexico, Japan, Russia, Malaysia, Kazakhstan, Rwanda, Uganda, Mongolia, Jordan and Nigeria. Stakeholders say that the activities performed and the information provided have been used to increase technical knowledge, setting up a plant variety bureau, drafting operational guidelines, improving DUS knowledge and making the plant variety rights system EU-proof. Legal and practical knowledge about enforcement and infringement of plant breeders' rights was greatly appreciated. It is the intention of the Netherlands to continue the programme in the coming years.

Ir. M. Valstar is President of the UPOV Council and Senior Policy Officer, Seeds and Plant Propagation Material, Ministry of Agriculture, Nature and Food Quality, DG AGRO, The Hague, the Netherlands, email m.valstar@minlnv.nl

Increasing salinity: problem or opportunity?

Arjen de Vos

34 The salinity levels of soil and water are increasing in many areas around the world. Often, this leads to reduced crop yields and sometimes farmers completely stop cultivating crops. But what if salt-affected land can become highly productive? What if we can use saline water for irrigation? Can these saline resources be used to improve the livelihood of farmers and can it be applied on a large scale, so it can contribute to global food security?

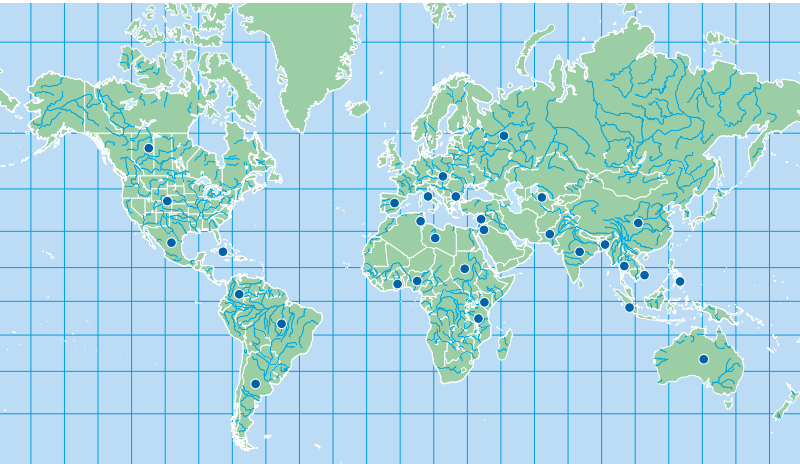
Globally around 400 million hectares are salt-affected and 20%, or 63 million hectares, of all irrigated agriculture is salt-affected

Dr. A.C. de Vos, founder and director of The Salt Doctors, Den Burg, the Netherlands, arjen@thesaltDoctors.com

It is generally assumed that food production has to increase by at least 70% by 2050 to feed the growing world population. This seems far away, but that's only 30 years from now! New arable land and fresh water are scarce and increasing the yield per hectare in many commercial farms will not be enough to reach this 70% production increase. One of the overlooked resources in the world are the salt-affected areas. According to the FAO, globally around 400 million hectares are salt-affected and 20%, or 63 million hectares, of all irrigated agriculture is salt-affected. Also, there is a similar amount of saline water readily available as there is fresh water in the world. If this saline water and salt-affected soils can be used for crop production, then the impact can be enormous!

Salt tolerant crops

Using these saline resources begins with salt tolerant crops. Actually, we live on a saline planet with 97.5% of all water consisting of seawater. Apart from the coastal ecosystems like salt marshes, natural salt lakes and saline land can be found far inland, well away from oceans. In these environments, thousands and thousands of plant species have evolved and adapted to these saline conditions and become salt tolerant. Some of these plants are known as halophytes, these are plant species that have developed a very high level of salt tolerance and can often survive with seawater as the main water source. There are several examples of halophyte plant species that are being adapted into agricultural crops.



Salinized areas	area (in million hectares)
Africa	69.5
Near and Middle East	53.1
Asia and Far East	19.5
Latin America	59.4
Australia	84.7
North America	16.0
Europe	20.7

Source: FAO/UNESCO Soil Map of the World

However, in most cases, the plants need further domestication and a solid business case for upscaling is still lacking. Conventional crops can also be salt tolerant. For instance, all modern-day beet varieties (beetroot, sugar beet, fodder beet, chard) were domesticated from a common wild ancestor, the sea beet. Research shows that most of the commercial beet varieties are highly salt tolerant, thanks to their wild ancestor.

Breeding for salt tolerance

So, the different beets are, more or less, all salt tolerant. This also implies that despite the domestication and the development of the various varieties, the level of salt tolerance was not lost. In fact, it is well known that salt tolerance is a multi-genetic trait and, in the case of the beets, is not something that is easily lost. This also implies that salt tolerance is a trait that is not easily incorporated into a crop. So, it could be difficult to turn a highly salt sensitive crop into a highly tolerant crop. Also, the mechanisms that contribute to salt tolerance are diverse and often differ between plant families. In short, salt tolerance consists of two stages. The first stage is an osmotic effect caused by the dissolved salts in the pore water in the root zone. This causes a smaller osmotic difference between the



Salts can affect nutrient uptake and - in case of clay soils - negatively affect the soil structure (Photo: Arjen de Vos)

soil and the plant which makes it harder for the plants to take up water. This stage resembles drought stress and wilting of salt sensitive plants can be observed. Plants that are able to continue to take up water also need to adjust to the ionic stage (stage 2) of salt tolerance. At this stage, plants have to deal with the salts (especially sodium) and do that by either keeping the salts out of the plant at root level (exclusion), storing the salts inside the plant (in vacuoles, often associated with succulence) or by excreting the salts after uptake (often with special glands). In general, plants have adapted to salinity by specializing in one of the three basic strategies. All strategies are complex and not easily incorporated into plants that lack these characteristics. Also, marker-assisted breeding is difficult since multiple genes and plant activities are linked to these traits.

The Salt Doctors

The Salt Doctors (www.thesaltDoctors.com) is a social enterprise from the Netherlands that specializes in improving crop yield under saline conditions and putting the solutions into the hands of farmers. We do this through research and advice, often by providing practical training in the field. In addition, we can quickly diagnose the local situation and develop tailor-made solutions, help with the implementation by setting up demonstration trials or introduce salt tolerant crop varieties in combination with specific cultivation strategies. In short, The Salt Doctors work as a service provider to make crop cultivation under saline conditions not only possible, but also profitable.

35 However, many present-day crops have wild relatives that thrive in saline environments. For instance, wild carrot and wild cabbage are plants that can be found in saline habitats and even potato and tomato have wild relatives that have evolved under saline conditions. Often, these wild types have already been used for breeding and by screening many different varieties of a single crop, it is often possible to identify varieties with higher levels of salt tolerance. These selection trials give quick results and directly identify crop varieties that can be used under saline conditions. Also, by identifying several tolerant varieties, cross breeding can take place that could further enhance the level of salt tolerance and still provide new varieties for commercial use in the relative short term, in comparison with cross breeding with wild types. So, there are already possibilities to introduce more tolerant varieties to farmers in salt-affected areas and selective breeding, with salt tolerance as a new trait, can deliver even more promising varieties in the mid- and long term.

Crop cultivation

In Pakistan, our method delivered up to 42% higher potato yield compared to local conventional methods (during the 2016-2017 season). In 2018, we were able to double the carrot yield under saline conditions in Kenya. In Bangladesh, currently over 5,000 farmers are trained in saline agriculture practices and their income increased by 34% after two years into the project. The salt tolerant varieties of the crops used are the foundation of the success, but crop cultivation under saline conditions can be challenging. The salts can affect nutrient uptake and negatively affect the soil structure (in the case of clay soils) and good irri-

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More to read

A.C. de Vos, B. Bruning, G. van Straten, R. Oosterbaan, J. Rozema, P. van Bodegom: Crop salt tolerance under controlled field conditions in the Netherlands, based on field trials conducted by Salt Farm Texel (2016). Report 39 pages. G. van Straten, B. Bruning, A.C. de Vos, A. Parra Gonzales, J. Rozema, P.M. van Bodegom: Distinguishing potato varieties by salt tolerance through novel analysis of multiple-year field tests (2020). Submitted. M. Qadir, E. Quillerou, V. Nangia, G. Murtaza, M. Singh, R.J. Thomas, P. Crechsel, A.D. Noble: Economics of salt-induced land degradation and restoration (2014). National Resources Forum, United Nations. DOI: 10.1111/1477-8947.12054

Increasing salinity

gation management is vital. In short, saline agriculture makes use of salt tolerant crops in combination with improved management practices. Also, the level of the salinity is vital, of course; at moderate salinity levels, there are many options for crop cultivation, but at very high salinity levels, the crop options are limited. Many salt-affected arable lands show moderate saline conditions (salinity level of 4-10 dS/m in the soil) and variety selection and breeding can provide varieties of many different conventional crops at these salinity levels.

Opportunities

The immense area of salt-affected land was mentioned previously and this number is increasing by 2,000 ha every day; crop damage in the irrigated areas is estimated at US\$ 27.3 billion every year. This crop damage can also be seen as a market opportunity, if

salt tolerant crops and improved management practices are developed and used, then at least US\$ 27.3 billion can be saved. So, this is a huge market already. Moreover, salinity is expected to increase even further under current climate change predictions. With a growing human world population and climatic changes on a global scale, salinity is an issue that will only grow in importance and urgently requires a solution. If salt-affected soils are put into production (again), then millions of hectares of new arable land do not have to be developed and natural ecosystems and the associated biodiversity can be saved. So, salt tolerant crops can contribute to global food security, now and in the near future, but it can and should also be seen as a new market opportunity and crop salt tolerance should be embraced as a new trait for breeding.



We share Jose's eagerness to grow and develop

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

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

Sharing a healthy future



Opportunities for more effective crop breeding

Erwin Datema

38

Recent advances in single-molecule sequencing, physical mapping and long-range chromosome conformation capture have resulted in one, or sometimes multiple, high-quality genome sequences for almost every conceivable crop species. For more than a decade now, the cost of sequence data generation has continued to decrease, and evermore sophisticated computational approaches have been developed in order to produce increasingly accurate and complete genome sequences.

Genome sequencing has become a ubiquitous and indispensable tool in plant breeding research, and we can now routinely elucidate the complete genetic composition of individual varieties and the differences between varieties at nucleotide precision. Whereas, as recently as five years ago, the genome sequencing and assembly technologies were still limited to resolving diploid, homozygous genomes, such as those commonly found among self-pollinators, these tools have developed to a point where it is now feasible to resolve the distinct haplotypes of heterozygous and even polyploid genomes at chromosome scale. These so-called ‘phased’ genomes present new opportunities for more effective crop breeding strategies, in particular for outcrossers and polyploids.

What makes a phased genome superior to the haploid reference genome we have been using during the past two decades?

Remember that DNA is (in its simplest form) a linear polymer chain of nucleic acids, and that genes and other functional elements can be thought of as intervals on this linear chain. Each gene encodes a protein, and variations in the DNA sequence of that gene result in amino acid changes that alter the function or specificity of that protein, or may cause the protein to lose its function altogether. In the one-dimensional world of the haploid reference genome, the impact of all variants within a gene are implicitly considered together – that is, the sum of all variants is assumed to determine the final protein sequence. In reality, adjacent variants may, in fact, be found on different copies of the chromosome, resulting in multiple distinct (functional or non-functional) alleles – or haplotypes – of that gene. In addition to providing the true functional haplotypes of each gene, a phased genome contains the connection between the alleles of adjacent genes on each chromosome, or in other words, which set of alleles are in linkage disequilibrium (LD) and therefore are likely to inherit together to the progeny.

How does this benefit the breeding practice?

First and foremost, a more accurate representation of the genome affects all sequence-based analyses and experiments, such as marker development and genotyping, but also targeted genome editing. It also

positively affects the accuracy of gene annotation, the elucidation of allele-specific gene expression, and all downstream genome analyses for candidate gene identification. Perhaps one of the most crucial analyses in this respect is the mapping of phenotypic variation, such as increased yield and resistances to biotic and abiotic stresses, to specific genomic regions that underlie this variation. This process is known as Genome-Wide Association Studies, or GWAS, and has become a commonly used tool in the repertoire of plant breeders with the introduction of high-throughput genotyping platforms.

Accurate separation of haplotypes at the gene level results in higher resolution and precision in GWAS analyses, as it connects the observed trait values to the true underlying alleles, rather than to an individual sequence variant that only controls part of the phenotype. Many extreme phenotypes – those often targeted by plant breeders – are explained to a large degree by rare alleles that are often absent from bi-parental mapping populations. Thus, the bi-allelic single nucleotide polymorphisms (SNPs), commonly identified from GWAS analyses, do not always represent the causal variants, but instead point to loci that are in LD with a gene or a regulatory sequence that affects the trait of interest.

It has already been demonstrated in both animal and plant breeding that the use of haplotype information results in higher accuracy in both traditional QTL identification and GWAS alike, compared to the use of individual SNPs. For example, take the rice gene *OsSPL13*. Through GWAS analysis, an individual DNA implicated this gene to determine grain size; but only by sequencing the candidate gene region, it became clear that not this single variant, but in fact two distinct haplotypes corresponded directly to small or long grain phenotypes. Haplotype analysis of three cell wall invertase genes in wheat revealed that strong allelic selection for increased grain yield over the course of domestication and breeding has narrowed the genetic diversity of these genes in modern cultivars, compared to wild germplasm.

On the chromosome scale, a haplotype-phased genome contains hitherto hidden information that may revolutionize the current breeding practice. The added value of haplotypes over individual SNPs was recently demonstrated in relation to rice. A total of



Revolution

With the advent of high-throughput genotyping technologies during last two decades, marker-assisted breeding has revolutionized the plant breeding process. Today’s DNA sequencing technologies allow us to look beyond the single marker and generate phased genome sequences, which enable breeders to design novel genotypes that contain ideal combinations of haplotypes.

120 previously functionally characterized grain yield and quality genes were screened against the 300 Rice Genomes panel, resulting in the identification of superior haplotypes of these genes having the most net positive impact on these traits. Such a collection of haplotypes forms the basis for the next logical step in marker-assisted selection and breeding: haplotype-based breeding, in which superior haplotypes of genes – and in fact, entire genomic regions – can be combined into an ideal variety.

Nonetheless, breeding for highly quantitative traits with low heritability, such as yield, remains challenging in marker-assisted selection, even when exploiting haplotypes. Such traits are generally polygenic and span many loci on multiple chromosomes. To overcome these challenges, plant breeders have adopted the genomic selection paradigm from the animal breeding domain. Breeding pools of most crops contain strong genome structure as a result of narrow (and often artificial) selection bottlenecks, implying that haplotype information can be a powerful tool to further improve accuracy and efficiency of not only marker-assisted breeding, but also genomic selection. A phased genome improves the predictive power of genomic selection methods, either directly through the haplotype information itself, or indirectly by requiring a smaller number of markers.

What does it take to achieve a phased genome?

With today’s long read sequencing and chromosome-level scaffolding technologies, high-quality phased genome sequences are within reach for almost every crop. With the onset of third-generation, long-read sequencing technologies, efficient assembly algorithms have been realized that produce highly

contiguous genome sequences in a matter of days on commodity hardware. This holds true even for large, repetitive, heterozygous and polyploid genomes. During the last year, novel approaches have been developed for haplotype-specific scaffolding – that is, anchoring the assembled sequence contigs to their correct chromosomal position and linkage phase. Combined, these approaches have already culminated in the elucidation of phased reference genomes for e.g. raspberry, strawberry and grape.

Outlook

As sequencing costs and computational requirements continue to go down, long-read de novo genome sequencing is set to complement, or even replace, short-read re-sequencing. Taken together, a collection of high-quality, phased genome sequences – also known as a pan-genome - represents the true extent of genetic variation within a crop, not only on the nucleotide level, but also on a structural level. In the bacterial world, this pan-genome concept has been around since the turn of the century, but in crop genomics, this currently remains mostly unexplored territory. Since these types of data are still relatively new, computational tools for the analysis of crop pan-genomes, and even individual phased genomes – which in essence are a minimal pan-genome comprising all the haplotypes within that individual – are in active development. The first results, so far, are highly promising and there is no doubt that, in the coming years, we will move away from the simplified world of the individual, haploid reference genome, towards the use of haplotype-phased, population-scale pan-genome in plant breeding.

Last year, KeyGene delivered a high-quality phased raspberry genome to BerryWorld Plus Ltd. “Plant breeding is a numbers game,” commented raspberry molecular breeder, Dr. Guillaume Daverdin. “By using molecular tools and genomic insight, we will be able to significantly increase the number of plants screened each year and enhance the chance of identifying plants that present the ideal combination of traits we are looking for”

39

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Quality programmes extended

Adrie Molenaar

40 The system of Nak-tuinbouw Authorized Laboratories (NAL) has proven a worthwhile instrument to guarantee the quality of seeds. Recently the Naktuinbouw extended the programme with the Naktuinbouw Authorized Field Inspection (NAFI) and a draft Naktuinbouw Authorized Processing (NAP) will be introduced shortly.

• In the October edition of Prophyta an article appeared on the disaster that struck Almeria, Spain in 1991: the outbreak of Pepper Mild Mottle Virus. And how that resulted in the founding of both ISHI and NAL. Recently Naktuinbouw initiated a Verification Program for seed production and market access. This year it is already 25 years ago that the first NAL participant was welcomed. Since then, the number of NAL participants has steadily grown to twenty, spread around the globe. From California to Japan. Laboratories with testing (parts of) plants/seeds, for plant/seed health and germination in their scope.

Proper testing

Good sampling and good testing are tremendously important. That is why countries often require proper laboratory testing, before seeds can be shipped to their countries. And that is why Naktuinbouw has established NAL. The level of control within the seed companies is high. And at this moment, there is no single case

80% of the vegetables seeds is produced by a company that has a NAL-certificate

known of a notification by a NPPO, related to seeds that have been tested under NAL. That is however a part of the story. The seed production process starts with breeding of new varieties, maintenance of OPs / parent lines, production of seeds and processing of seeds (cleaning, enhancement and packing), before shipment to customers can take place. At Naktuinbouw, we believe that every aspect of the seed production chain is important. That monitoring the production (field inspection) and processing (tracking & tracing and avoiding cross contamination) are crucial too. And that a keen and integral eye on the whole seed production process does pay off. In other words: the total will be more than the sum of its parts. You can do a perfect test and declare the seed lot negative. But if the seed lot did become contaminated later in the process, the result still will be poor. This is why Naktuinbouw did initiate a Verification

Program for seed production and market access. We believe we need to work on this pro-actively. To demonstrate the several stake holders (customers, authorities and ourselves), the seed production process is controlled well enough and that the produce in question can be accepted, without hesitation. At the moment, we have (next to NAL) established Naktuinbouw Authorized Field Inspection (at the moment three participants) and a draft Naktuinbouw Authorized Processing, that we are rolling out now. If you want more information, please visit www.naktuinbouw.com/vegetable/inspections/inspection/nafi-naktuinbouw-authorized-field-inspection or ask bureau Team International Systems of Naktuinbouw.

Daily practice

The true value of the new Authorized systems is best reviewed by the people who work with it. Antoine Artz, Head of Hygiene at BASF Vegetable Seeds and John Potters, Head of Vegetable Plants at Vreugdenhil Young Plants both have a longtime experience with NAL and have started to work with the other systems.

Did the NAL initiative bring what you had hoped for?

Antoine Artz: “NAL has become an international standard that instils trust among PPO’s and customers.” John Potters: “Absolutely, we are very glad there is a standard, but it should go further than the laboratories. Sometimes tests result in false positives or, worse, false negatives, which can lead to huge consequential damage.”

What do you think of the NAFI initiative?

Antoine Artz: “First of all, we want to supply healthy seeds to our customers, wherever they are located. We embrace the ideas that have led to NAFI. We are implementing the Plant health scope for field inspections. Globally we are confronted with an ever-growing set of phytosanitary requirements with an increasing complexity. We have to work with a matrix of crop, destination country, country of origin, warehouse country, using pest names without standardization and demands ranging from seed health tests to field inspections amongst others to understand which steps we need to take. If NAFI will



Marcel Kortekaas, production specialist at Rijk Zwaan, and Ronald Boer, inspector at Naktuinbouw, monitor seed production

Photo Naktuinbouw

build trust among NPPO’s that may in due time lead to a less complicated situation. “We believe NAFI can lead to a higher level of sustainability by increasing the quality of field inspections. No matter how high the competency level of the external field inspector, our internal field inspectors will visit the crops much more often. We register information to make sure our seed batches will not carry pests and to keep our seed production sites clean. We share this in the NAFI program to bring clarity on the phytosanitary status of our crops.” John Potters: “Field inspections are certainly a worthwhile extension. It gives more certainty that the plants are healthy. As long as the inspection is carried out by the right people who have had the right training, that is a prerequisite.”

Do you recognize the need for a verification programme, such as that of Naktuinbouw?

Antoine Artz: “The added value of the field inspection module for plant health is clear. We do not yet see the same for the other modules. Most companies already implement programmes or standards to reach the right level for the other aspects.” John Potters: “NAFI and NAP is the last step and it is crucial to a healthy start. It takes a lot of money and energy to implement the programmes. And then it will take time before it has gained trust and the customers recognize its value. I would like to

see that the modules are being organized like a standard as Good Seed and Plant Practices (GSPP) that was developed ten years ago for clavicbacter in tomatoes. But with a broader scope on pathogens of course. The auditors and/or inspectors should have a practical view though. Not ticking off a checklist but talk with people who do the job. It should not become a paper trail resulting in the umpteenth certificate.”

What tips do you have?

Antoine Artz: “We hope that other companies will take part in NAFI. If we can show under authorization of Naktuinbouw that we are in control and do the right thing we hope this will lead to acceptance by NPPO’s. We believe also that this initiative could be an interesting model for other countries, eventually reducing the risk of pest spread. If more countries will adopt this method, we hope there will be a good harmonization.” John Potters: “It is vital that the Naktuinbouw communicates to the stakeholders what the programmes entail and how the inspections are carried out. The customers should be aware that it is an additional quality measure that they should trust and value.”

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The Future of Seed Health Testing

Going back to basics

Radha Ranganathan, Merel Langens, Joyce Woudenberg and Gerbert Hiddink

Companies go to great lengths to produce and supply healthy seeds to their customers. Seed health testing to detect seed-borne pathogens is an important step in their quality management systems that range from reliable production locations, preventive and hygiene measures, disease inspections and pathogen control during seed production to applying best practices for sorting, sanitation and seed treatments. These systems are continuously optimized based on risk assessments.

Seed health testing is also necessary to fulfill country specific import requirements. National Plant Protection Organizations (NPPO) wish to prevent the entry and spread of exotic pests to protect domestic plant industries and native biodiversity. Testing seed lots for seed-borne pathogens is an important element of most national phytosanitary regulations. The seed industry has always looked to science to provide tools to generate and deliver a product that meets customer needs - at the right time, at the right cost and of the right quality. While new techniques have brought speed and efficiency to seed health testing, their use for regulatory purposes has also brought new challenges. This article outlines developments in seed health testing, interpretation of results obtained and how the use of new technologies could affect seed trade in the future.

From past to present

Traditionally, methods for detecting a seed-borne pathogen are based on demonstrating the presence of a living organism in or on the seed. They generally

ISHI-Veg and Method Development

Since its early years, ISHI Veg has developed seed health test methods based on the needs generated by disease outbreaks and for the detection of seed-transmitted pathogens that could cause them. Over time, methods have evolved because of the need for faster, simpler and more sensitive seed health tests.

pathogenicity of the isolate by inoculation onto assay plants.

Examples of widely used tests include growing seed under disease-conducive conditions (grow-out or sweatbox), spreading seed extracts on a medium known to stimulate growth of bacteria (dilution plating), incubating seed on a moist blotter paper for fungal growth (blotter test) or applying plant tissue (e.g. seed or leaves) suspected of being infected with a pathogen to an 'indicator' plant to assess its

infectivity (bio-assay). By isolating the target pathogen and showing its viability and pathogenicity, these methods provide the final determination of the health status of a seed lot. The biological relevance of these methods, commonly called direct tests, is in their results: confirmation of the presence of the target pathogen, and proof of its viability and pathogenicity. Despite their biological relevance, the methods described above can be laborious, time-consuming and expensive for routine analysis of a large number of samples.

Speed and efficiency

The need for speed and cost reduction has played an important role in the development and application of techniques in seed health testing that permit a rapid analysis of a large number of samples at a relatively low cost. These techniques also differ from the older ones in the meaning and interpretation of their results.

Enzyme Linked Immunosorbent Assay (ELISA) is an example of such a technique. It is an 'indirect' test as it detects proteins associated with a target pathogen, without discriminating whether the source of the protein detected is pathogenic, viable or even intact. An ELISA positive is, therefore, a putative positive while a negative result indicates a healthy lot. In combination with the direct tests mentioned earlier, ELISA provides valuable use as a pre-screen. A pre-screen rapidly separates healthy lots from suspect ones that require further investigation, with a follow-up test that confirms the viability and pathogenicity of the target pathogen in the seed lot.

Sensitivity and specificity

Although ELISA is commonly used in seed health testing, another indirect test, Polymerase Chain Reaction (PCR), has gained importance. PCRs are sensitive, specific, fast and allow simultaneous detection of multiple pathogens.

PCR-based methods detect the presence of nucleic acid of the target pathogen. In PCR, a 'signal' is produced when DNA or RNA of the target pathogen is detected. However, the signal does not indicate whether the source of the DNA or RNA is pathogenic, viable or even intact. Factors other than target DNA or RNA detection may also trigger a signal, and these



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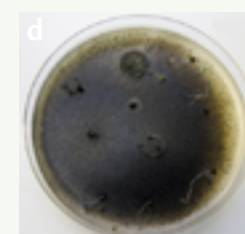
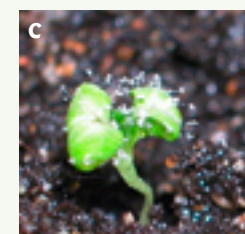
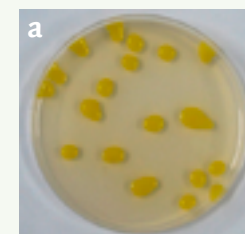


Figure 1.

- a) Seed extract on a medium to promote growth of the bacterium;
- b) Lesions on tobacco leaves, the indicator plant, in a bio-assay;
- c) Plantlets of corn salad showing fungal spores in a grow-out;
- d) Black roots caused by a fungus pathogenic on corn salad

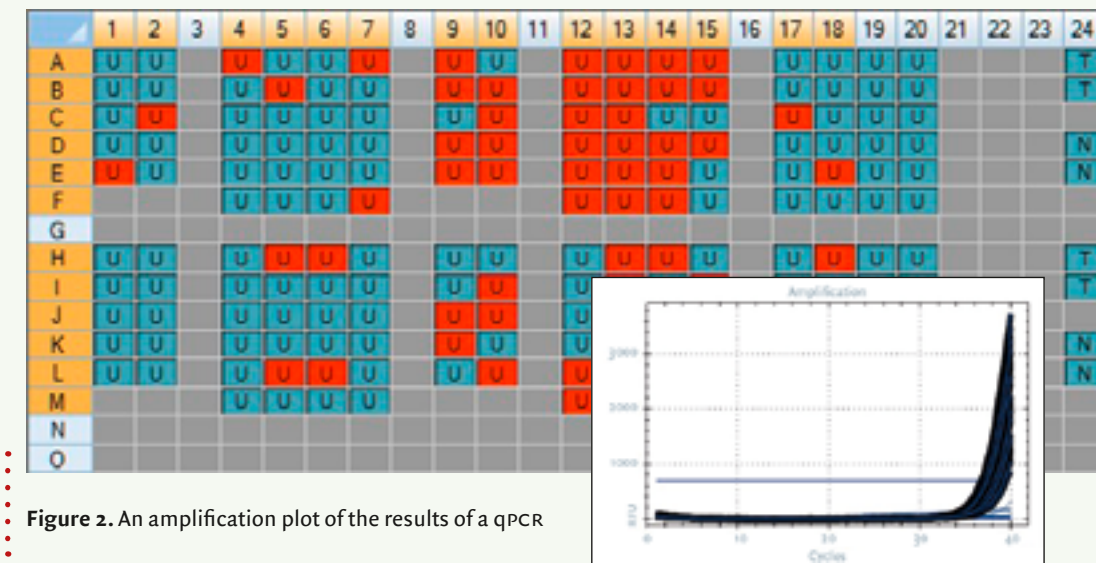


Figure 2. An amplification plot of the results of a qPCR

are difficult to distinguish from the signal produced by the target.

Despite this drawback, PCR is very useful as a pre-screen because it identifies healthy seed lots more rapidly than direct testing.

Similar to the ELISA test, a PCR positive is a suspect lot that requires further investigation for a final conclusion about the health status of the seed lot. High Throughput Sequencing (HTS), also termed Next Generation Sequencing (NGS), is an emerging technology in plant pathogen detection. HTS can generate massive amounts of DNA sequence data at very low cost. Publicly available reference databases of sequences associated with seed transmitted pests, essential for interpreting data generated using HTS assays, are being developed. Although HTS is still not widely used routinely in seed health testing, as an indirect method it might be useful as a pre-screen in future.

Back to basics

Both the seed industry and NPPOs have embraced the use of nucleic-acid based assays in seed health testing. The industry uses them as a pre-screen to identify healthy seed lots, and in combination with direct tests to confirm viability of the target pathogen and demonstrate its pathogenicity. NPPOs employ PCRs, some as a pre-screen, but more often as a stand-alone test that is the basis for a final decision on whether to allow the entry of a seed lot, or to either reject or destroy the consignment.

The way forward is to use new technologies for the advantages they bring to seed health testing

The increased sensitivity of methods developed based on nucleic-acid detection has led to a growing number of seed lots being rejected, even if there was probably no risk of introducing a seed-borne patho-

gen to importing countries. The lack of a clear link between the so-called positive result in PCR and the real presence of the target pathogen in the seed lot is a weakness of nucleic acid-based detection methods. The difficulty of discerning a 'true' signal from an artefact also contributes to seed lot rejections. An additional challenge to the seed industry is that NPPOs also independently develop their own testing protocols, which leads to different results making correct interpretation of the health status difficult. If the trend of using pre-screen methods to take a final decision on the health status of seed lots continues, the prospects for the seed industry to be able to move seed internationally are uncertain.

How can the seed sector meet the challenge posed by NPPO requirements to use highly sensitive PCR methods to test seed lots to prevent the entry and establishment of a seed-borne pathogen?

Producing seeds in 'sterile' conditions could help, but it is not a realistic option because of the expense and logistics, and even this will not reduce the impact of nucleic acid test signals that are artefacts. Producing seeds exclusively for domestic markets is not an option either. It will slow down the development of new, innovative varieties for any other than home markets. Today, there is no country that could fully supply farmers with seed of their choice, solely from their own domestic seed production. Seed companies produce and trial seed in different countries all over the world to mitigate the risk of crop failures due to adverse weather and growing conditions. By finding optimal locations for seed production, and by using appropriate preventive hygiene measures, disease inspections and pathogen control during seed production, the seed sector ensures the steady supply of healthy seed for farmers everywhere.

The way forward is to use new technologies for the advantages they bring to seed health testing, but in combination with the conventional methods that establish biological relevance of the organism detected. The future lies in going back to basics.

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Fries4All

Amazing quartet invents healthy fries

Monique Krinkels

When an author, a cardiologist, a breeder and a coating specialist team up, the result is bound to be surprising. And it is! Together, they came up with the french fries Leon & León, which are both low in carbs and in calories. It took them nearly eight years to complete the creation. Since a few months ago, the healthy fries have been available in the supermarket.

It all started when Leon de Winter, the well-known author of, amongst others, ‘Looking for Eileen W.’, was given a serious warning by his doctor: he should follow a strict diet to lose weight in order to prevent type 2 diabetes. Doctor’s orders sounded simple: a low carb diet, no more bread, no potato, no pizza, nothing. A terrifying future loomed for this bon vivant. Instead of succumbing to his fate, he started wondering about solutions. Is it possible to make fries and pizza with the same taste, the same experience, but without the calories and carbohydrates that give us so much trouble? He called his cousin, León Eijnsman, a cardiologist. In no time, he was studying ‘all night long’. What is frying, what happens to starch in a potato, he wanted to know it all. And although a carbs-free potato sounds like a sea without water, they approached the Dutch potato breeder, HZPC, to see if they could help.

Laughing stock

In Metslawier, the research facilities of HZPC, Leon and León met Hans van Doorn and his colleagues. “When we asked for a low-carb potato variety to make healthy fries, they had to laugh heartily,” says Eijnsman. “The HZPC biochemist explained what happens when you use potatoes with these properties for fries: an enhanced Maillard reaction. Sugars and amino acids react to the heat of the oil, producing the possibly carcinogenic acrylamide in high concentration. The potato burns in fifteen seconds.” But as the cousins would not give up easily, the HZPC team provided them with two large bags of Carrera, a variety in the SunLite series, to experiment. “We have been selecting and breeding for low-carbohydrate potatoes as a nutritional characteristic for about 15 years and it is becoming increasingly relevant in modern diets and lifestyle variants,” explains Hans van Doorn, programme manager at HZPC. The SunLite potatoes contain 30% less of both amylose and amylopectin. The latter is the problem-carb, as it is quickly digestible, causing a spike in blood sugar that is eventually converted into subcutaneous fat. “The ratio of both forms is virtually the same for most potato varieties. The total amount of starch is genetically determined and has a spread (measured as dry matter content) between 14.5% to more than 30%,” says Hans van Doorn.

Rocket science

On the advice of HZPC, the idea was quickly patented. A new company, Fries4All, was established with equal shares for HZPC, the Rocket Science Kitchen belonging to the two cousins and De Korrel. The Belgian fries manufacturer, Mydibel, takes care of production, sales and distribution of the Leon & León fries. The Leon & León fries are available in a Dutch supermarket chain and an English restaurant chain. “We are working hard to roll out our concept further, both nationally and internationally, and both towards retail and food service,” says Gert Jan Feddes, business development manager at HZPC. “The consumer has embraced the Leon & León fries, sales are developing well. Besides the fact that our fries contain less carbohydrates, calories and fat, the taste of our fries is also very much appreciated by the consumer, especially the crispy outside and the creamy inside.” In retrospect, the question remains: why did these companies not run away with the concept themselves? Leon de Winter and León Eijnsman are convinced: because they are nice people. “Straightforward Frisians, people from the Bible Belt. Our word is our bond.”

Contamination remains a never-ending challenge

Geert-Jan de Klerk

48 Many microorganisms flourish better on plant nutrient media than plant tissues themselves. Because of that they may overgrow the culture. A main source of contamination is the plant tissues that are transferred from the ex vitro environment to the nutrient medium. This paper focuses on this plant-derived contamination.

For plant tissue culture, there are various major prerequisites: appropriate organic and inorganic nutrition, adequate steering of developmental processes by plant hormones and a proper physical environment. Besides that, sterility is also a main issue. Sterility concerns the decontamination of media, containers, etc. and prevention of contamination during the handling and the culture of plant material.

Growth of bacteria

A huge number of microorganisms live in close association with plants. They include bacteria, fungi, viruses, yeast, algae and phytoplasmas. It should be mentioned first that when plant tissues are transferred to tissue culture, only the associated microorganisms that flourish well on the nutrient media cause difficulties for the tissue culturalist, because they overgrow the plant tissues. This likely concerns only a very small percentage of the associated microorganisms. The ones that do not grow on plant tissue culture medium may very well be harmful to the plants' wellbeing, but they are not insurmountable for successful tissue culture.

Biologists have been using bacterial nutrient medium to culture microbes from environmental samples, allowing each cell to form a colony. It has been reported more recently, however, that only a small percentage of bacteria is culturable on the common bacterial nutrient media. This finding has been referred to as 'The Great Plate Count Anomaly'. So, when a sample from the open field is being examined, the number of microbes under the microscope and the number of colonies after microbiological examination using a Petri dish with nutrient medium is extremely different: about 100 (!) times more cells are observed microscopically than colonies counted on the Petri dish. This observation was already made more than 100 years ago.

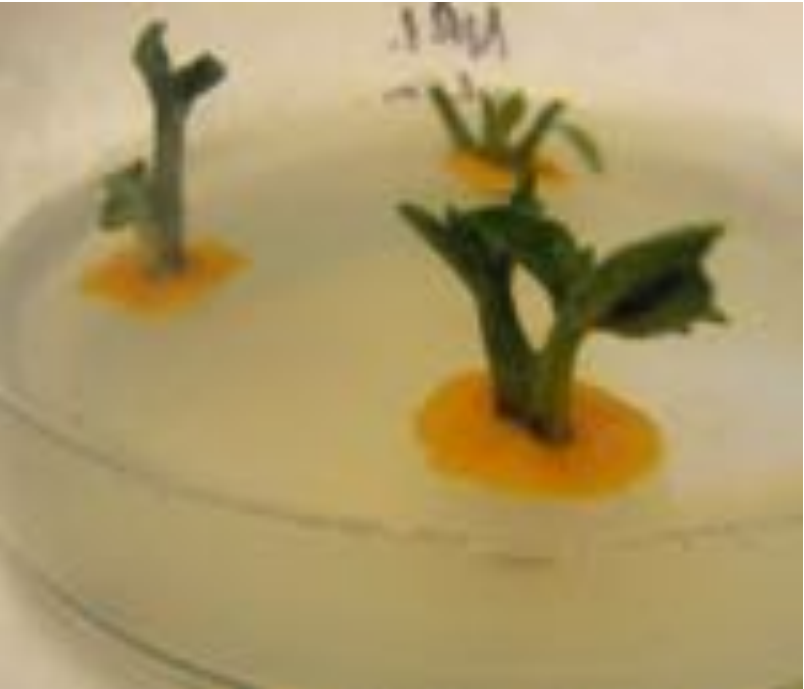
The rRNA approach developed in the 1980s provided insight into the microbial world missed by traditional cultivation in Petri dishes. So, many microorganisms cannot be cultured in a common bacterial medium. We know about their existence from microscopical observation, corroborated by the occurrence of (bacteria-specific) 16S rDNA. The reason for poor growth on the artificial medium is likely that during evolution, the microorganisms have adapted to a

specific environment and have lost the capacity to grow on the very 'rich' manmade nutrient media. On plant tissue culture media, even far more bacteria fail to grow, probably because bacterial media also contain amino acids, in addition to carbon and minerals, which are often added using unidentified compounds, e.g., beef or yeast extract. These additions meet as yet unidentified demands of the bacteria. These additions are usually omitted from plant tissue culture media. In research carried out in the early 1990s, ten bacterial strains derived from tissue-cultured plants and grown in bacterial nutrient medium supplemented with broth were transferred to common plant nutrient medium with MS and 3% sucrose only. Just one strain (*Bacillus subtilis*) showed significant growth, whereas the other nine died off. In agreement with this, molecular analysis (16S rDNA sequencing) has shown the ubiquitous presence of endogenous bacteria in micropropagated plants. So, the commonly-held belief that micropropagated plants are free from endogenous microbes does not hold at all.

Epiphytes

From the microorganisms associated with plants, those that live within plants are a minor fraction: most inhabit the plant surfaces or are living close to the plants, the so-called epiphytes. When plant tissues are transferred to tissue culture, the microbes that inhabit the surface are removed by short treatment (usually 15-20 min) with a sterilizing agent. There are only few reports that compare sterilizing agents, but the commonly used solution of sodium hypochlorite is often the best. Another chemical, HgCl₂, that has a similar efficiency should be avoided because it is very toxic and involves an environmental hazard. For proper action of the sterilant, it should be able to reach the microbe in a sufficiently high concentration. So, all soil particles should be removed, tissues should not be folded up and a low concentration of detergent should be added to the sterilant solution. It should be noted that the epidermis is not a smooth surface. Stomata, hydathodes, and hairs may protect the microbes. The microbes may, for example, enter the cavities of the stomata to some extent. Such is indeed the case as shown by the increase of the

Contaminated culture of Skimmia



Contamination of cultures of lily scale-explants that had not been (control) or had been treated with plant hormone ABA before the surface-sterilization with NaClO. ABA closed the stomata so that during the sterilization treatment the microbes were protected from the chlorine. The closure was temporary so that during tissue culture, the microbes could move to the nutrient medium

contamination percentage when tissues have been treated before sterilization with ABA, the plant hormone that closes the stomata (see bar graph). Another potential pitfall is that, during rinsing to remove the remainders of the NaOCl, sterile explants may be cross-contaminated by heavily contaminated explants. This can be easily overcome by rinsing with a low concentration of NaOCl (0.03%). This concentration kills the microbes and has no or little negative effect on the plant tissues. Here it should also be mentioned that NaOCl is not very stable, so it disappears automatically from the cultures. Finally, it is important to note that surface contamination can be reduced by the proper precultural growth of the donor plants, in particular by avoiding wetting of the plants and by lowering RH.

Endophytes

Microbes in the tissue are referred to as endophytes. The term 'endophyte' is ambiguous as many authors restrict the term to microbes that are advantageous to the host, even though the term literally only means 'within plants'. In this article, the term is used for all microbes living within tissues. The microbes may be living for a very long period within the tissue and plants may benefit from their presence. Microbes may also be parasites. Usually, endophytes occur in all tissues, but have a higher incidence in roots. They may occur in between cells, but also in cells. In the supplement to an article on endophytes in banana, an excellent movie is presented on bacteria living in banana cells (Movie 05 in supporting information of the article in <https://academic.oup.com/aobpla/article/doi/10.1093/aobpla/plu002/156142#78433120>, free access). These bacteria belong to the group of non-cultivable bacteria. The bacteria seem to 'swim' freely in the cytoplasm. The integral intracellular association suggests a

Contaminated culture of Skimmia

mutualistic relationship between endophytes and the host. Rhizobia (bacteria that fix nitrogen after becoming established inside the root nodules of legumes), on the other hand, are intracellular but surrounded by a host-derived membrane that keeps them separate from the host cytoplasm.

Entering tissue

Despite the long-held belief that reproductive and disseminative organs of plants are sterile, it is now well established that seeds host diverse microbial assemblages occurring on and in the seed coat, root, hypocotyl, cotyledons and the perisperm/endosperm. So, microbes are transferred via seeds. Microbes also enter the plants via wounds that may be inflicted by mechanical damage or originate by rupture of the epidermis when side roots emerge. From the area of entrance, they slowly spread through the plant body. For movement, the microbes depend greatly on the movement of fluids in the vascular tissues. In stagnant liquid, *E. coli* cells have a mean speed of roughly 30 µm/s. When moving in the plant body, microbes meet many obstacles, in particular cell walls, so the actual velocity will be much lower and will often be zero.

When transferred to tissue culture, plant tissues contain these endogenous microbes and more of them when the donor plants have been wounded during culture in the glasshouse or the field, prior to taking the explants. The microbes that have entered by the inflicted wounds are not highly adapted to life within the plant body and more likely to grow on artificial nutrient media.

For tissue culture, an additional source of infection is the wounding that occurs when taking the explant. It should be remembered initially that within the plants, a negative hydraulic pressure occurs, caused by transpiration of water. In lily scales, when an explant is taken, liquid close to the cut surface was sucked up and penetrated at least 1 cm into the vascular tissue. Bacteria are 1-2 µm in diameter and easily move into and through the xylem. In an experiment with lily, we found that this accounted for 20% contamination. In lily, this type of contamination was dealt with by taking explants when scales were submerged in 0.03% NaClO. In an experiment with tea, bamboo, and rose, shoots of ca. 30 cm in length were taken,

49

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Types of tissue-culture contamination and possible remedies

Time of contamination	Location of contamination	Treatment
During cultivation of the donor plant	Epidermis highly contaminated	<ul style="list-style-type: none">▶ When watering the donor plants, make sure that leaves are not wetted▶ Cultivate donor plants at low RH
During cultivation of the donor plant	Endogenously contaminated	<ul style="list-style-type: none">▶ Donor plants should not be damaged during cultivation in the glasshouse or in the field, or during harvest▶ Detached shoots may be precultured for some days with mild sterilant
During cutting the explant	Endogenously contaminated in particular in xylem	<ul style="list-style-type: none">▶ When cutting the explants, submerge tissue in 0.03% NaClO▶ Surface-sterilise long shoots (ca. 30 cm) and cut the desirable small explant in the laminar flow
During processing of the plant material in the laminar flow cabinet	Epidermis contaminated	<ul style="list-style-type: none">▶ Operators should take care taking into account, e.g. the airflows in the laminar flow cabinet▶ Tools should be properly decontaminated
During cultivation in vitro, contaminants leave the explant	Both epidermal and endogenous contamination	<ul style="list-style-type: none">▶ Add mild sterilant to the culture, e.g. PPM or 0.0003% NaClO

surface-sterilized in 1-litre measuring cylinders, and after that, the usual explants were cut from the surface-sterilized shoots in a laminar flow cabinet. These were transferred to the in vitro environment. Contamination was reduced from ca. 50% to ca. 20 % in all three crops. So, taking explants without precautions may result in increased levels of microbes in the plants and many of these 'extra' microbes are located in the vascular tissue, in particular in the xylem. During the first days in culture, the wounds are healed and, as a consequence, the vascular tissues are closed. Thus, new infections decrease with time because the contaminants are being locked up in the tissue. These explants are small microbe bombs; when the explants are cut at subculturing, seemingly clean cultures display infection.

Remove endophytes

To kill surface-microbes, a high concentration of disinfectant is needed but (1) such high concentration is difficult to achieve within a plant tissue because of the very slow movement of solutes inside plants and (2) disinfectants at a high concentration are toxic for plant tissue. So, the treatment of explants with disinfectant does not work for endophytes. This may be worked around to some (likely a limited) extent by pre-treatment of shoots, keeping them for a prolonged time (hours or rather days) in a beaker with their base in the mildly sterilizing solution, increasing the transpiration so more of the sterilant is moved into the shoots, using as high a concentration of disinfectant as possible and choosing the most appropriate disinfectant. Such treatment has been done with shoots of trees using 8-hydroxyquinoline citrate and/or mixtures of antibiotics, but no careful evaluation was done.

The only reported alternative to removing endophytes is a hot-water treatment (HWT). This method originates from the 1870s (!) and was developed to remove pathogens from seeds. In the 1910s, this method was used by Van Slogteren in the Netherlands to remove pathogenic nematodes from daffodil bulbs, and later to remove fungi and bacteria from hyacinth bulbs. It was introduced to disinfect plant material for tissue culture in the 1990s, using organs that can survive severe stress imposed by the treatment, namely bulbs and dormant buds from trees. Unfortunately, the underlying mechanisms of the HWT have not attracted a great deal of attention: how are the pathogens killed by the treatment? In the case of nematodes, they probably cannot survive the high temperature. However, in the case of bacteria, this seems unlikely: a few hours at the high temperature (depending on the crop from 42–54°C), it is unlikely to be lethal for bacteria knowing their resistance to pasteurization. Alternatively, the microbes may be killed by the response of the plant tissue to high temperatures. It is tempting to speculate that the stress treatment evokes a burst of ROS (reactive oxygen species) and it is known that ROS kills microbes. This hypothesis is corroborated by the finding that a stress-resistant cultivar of Narcissus, 'Golden Harvest', requires a HWT of 1h at 54°C and the stress-sensitive Narcissus tazetta var. Italicus requires 30 mins at 40°C. So, the microbes are killed when the plant tissue displays a stress reaction: 'Golden Harvest' is heavily stressed and displays a ROS burst (but still survives) at 54°C, whereas tazetta already displays this response at 40°C. If this holds, it may be the basis of novel methods to reduce endogenous contamination.

Seed drying & equipment

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Individual open box dryer



Drying of seed (A) in boxes (B) per box individual. Per box a fan (C) and heating source (D). Extracting outside air (E), dehumidified air (F) or inside air (G).

Individual closed box dryer



Individual closed drying units for conditioned drying of seed in boxes.

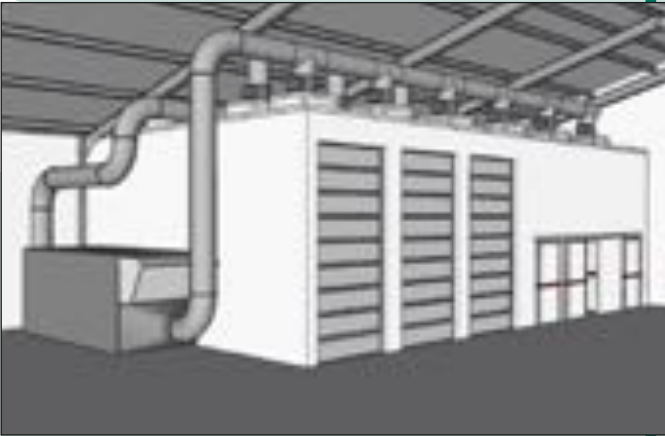


Optimal use of dried air

Central hybrid air dryer (left) to be connected to different drying installations, drying units or drying rooms; optimal and economic use of dried air.

Humidification of too dry seed

Humidification unit (A) to increase moisture content of too dry seed without making the seed wet. Damp air will be distributed through the seed by any kind of aeration system. The safest way for automatically humidification of your seed.



Drying seeds in closed rooms and Seed vault for storage

Central hybrid air dryer for drying rooms (left) and seed vault (right). Storage of previous seed at 15°C and 20% RH or 10°C and 25% RH.



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