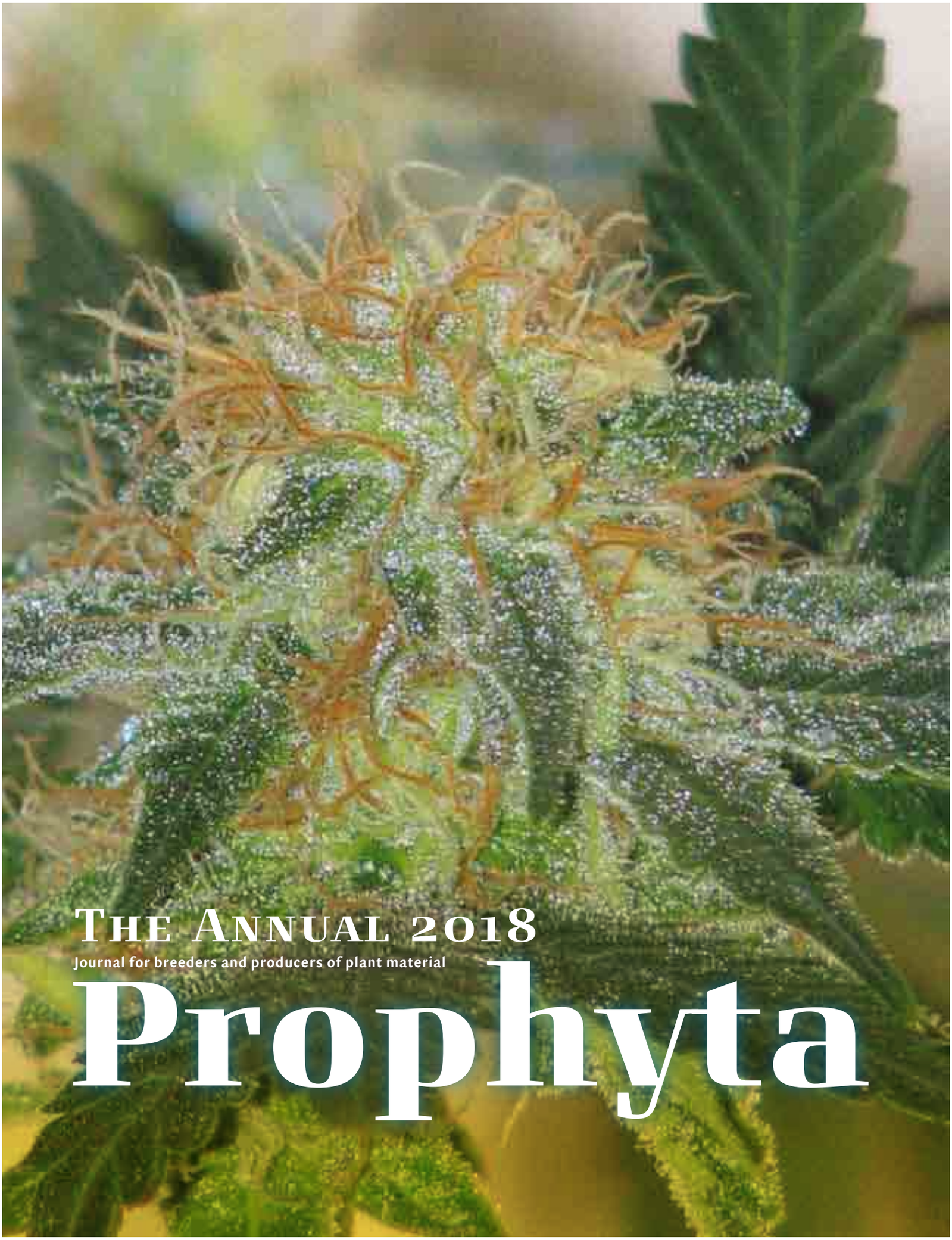

The Annual 2018

Journal for breeders and producers of plant material

Prophyta

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

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

REGISTRATION, LISTING AND PLANT BREEDERS' RIGHTS
- DUS Testing for Plant Breeders' Rights for agricultural and horticultural crops
- Description of varieties

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- Identity investigation
- Regular workshops and training courses
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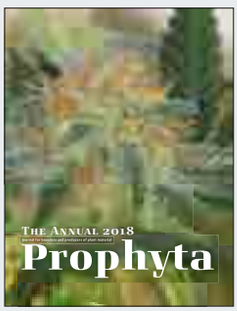

On the cover: At the Italian research institute CREA-CI Cannabis sativa L. varieties were developed the medical world was waiting for

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Climate change threatens doomsday vault

The Svalbard International Seed Vault on the Norwegian island of Spitsbergen, which was founded 10 years ago, will receive a new entrance. It should provide better protection for the approximately 1 million seeds from 234 countries that are now stored there. At the end of 2016, water was discovered near the entrance. Because of the exceptionally high temperatures and rain at the end of that year, water had entered the 100-metre long tunnel to the seed vault. The moisture did not get any further because it was frozen, but scientists started to worry. It has now been decided to build a new watertight entrance. Norway will spend 100 million Norwegian Crowns (€ 10.4 million) to upgrade the doomsday seed vault. The work is expected to last until May next year. The seed vault was constructed in an abandoned Arctic coal mine at a depth of 120 meters in a mountain in the vicinity of Lonyearbyen, the capital of Spitsbergen. It has the capacity to store 4.5 million crop varieties. The aim is to store and safeguard the world's crops and plants against global natural or man-made disasters. If nuclear war or global warming kills certain crops, governments will be able to request seeds from the vault to restart their agricultural industries.

Record in Nanopore Sequencing

This year, KeyGene researchers generated 76 gigabases of DNA sequence of a lettuce (Lactuca sativa) genome with a single Oxford Nanopore Technologies (ONT) PromethION flow cell. This is a new record in the plant genome sequencing area for long reads. As the PromethION has the capacity to run 48 such flow cells, large-scale de novo whole genome sequencing will soon be feasible. KeyGene expects that PromethION's long reads and low costs per base will disrupt the current sequencing field, accelerating crop innovation. The nanopore sequencing of the lettuce genome was completed for KeyGene's partners Rijk Zwaan, Enza Zaden, Limagrain Vegetable Seeds and Takii & Co, which use the genome sequence to support the development of new varieties. Lettuce has a complex genome of approximately 2.7 gigabase pairs (Gbp). The output of 76 gigabases generated by KeyGene with a single PromethION flow cell thus represents approximately 30-fold genome coverage. The N50 reads lengths were 29 kb with a fair amount of reads longer than 100 kb. With these very long DNA sequences, the assembly of complex genomes becomes much easier.

Workshop on seed identification

This autumn, Naktuinbouw (Netherlands Inspection Service for Horticulture) will again organise a Seed Identification Workshop for international participants. The workshop will take place from 1-3 October 2018 in Roelofarendsveen, the Netherlands. The aim is to improve identification of seeds found as impurities in seed samples. The participants learn how to identify seeds and fruits from the seventeen most important wild plant families occurring in European arable landscapes. These plant families are responsible for frequent impurities.
The workshop is specifically developed for seed analysts and those in industry who are involved in seed purity analysis and/or seed identification. Specific knowledge of seed identification is not a prerequisite to attending the workshop.

Content of the workshop

Over the three days, participants are given lectures, excursions and plenty of practical assignments in which they have to identify seeds under the microscope themselves. A first major step in seed identification is the recognition of the plant family to which a particular seed/fruit belongs, making it possible to consult reference collections efficiently and seed atlases for a final identification. In this workshop, a selection of families has been introduced that represent the majority of the seeds from the ISTA universal list of plant species.
More information available at: www.naktuinbouw.eu

100th Dies Natalis

In 2018, Wageningen University & Research celebrates its 100th anniversary. A century in which the organisation has proved its worth as a worldwide expert in the field of healthy food and living environment. In these hundred years, it has acquired a wealth of valuable knowledge and developed high-quality education. The unique combination of teaching and research has enabled the organisation to improve quality of life.
A hundred years ago, in 1918, the university was awarded academic status. During the past hundred years, it has developed into a knowledge institution of worldwide renown. The organisation's strength lies in the responsible way it conducts research and applies the knowledge it acquires.
During this year, there will be a number of activities inspired by 100 years of Wageningen Wisdom & Wonder. More information available at: www.wur.nl

Short circuit in communication

Nowadays, people suffer from an information overload. Apart from the daily newspaper, television and radio, they also receive e-mails, e-newsletters, e-magazines, read Facebook, Instagram and Twitter, and search the Internet for the latest developments in scientific, economic and business-related topics. The drawback of the rapid advances made in information and communication technology. At the same time, the paradoxical situation is that, although there is an abundance of information available, it is often difficult to obtain useful, relevant information when it is needed. No wonder that managers sometimes feel overwhelmed and productivity falters, as reading and sifting takes too much time of the individual.
Nevertheless, the seed industry wants the general public to learn more about seeds and plant breeding. There is a need to open the 'black box' in the food chain, as many people hardly know what happens to food before it is displayed in the supermarket. And even if they are aware that there are farmers involved in food manufacturing, the processes of plant breeding and seed production remain hidden from sight. 'Unknown is unloved', as a Dutch proverb goes, and it makes the sector an easy target for unfounded criticism.
Seed organisations have recognised the need and have taken action. ISF and ESA are active on Twitter and Facebook, ASTA offers, among others, blogs by plant breeders. But what is needed is smart action. Not worsen the information overload, but introduce new, innovative ways to get the message through to the public. For instance, last year Plantum built a temporary greenhouse, 'the green machine', just across from the Dutch parliament buildings in The Hague. Passers-by could learn what plants mean for humanity and how plant breeding can help secure food supplies. This year, the organisation started with a series of films where ten people, varying from chef to farmer, to economist, to activist, explain their view on the importance of seeds.
A new approach is the 'Sow to Grow experience' that has started in Enkhuizen, the Netherlands. It combines educational elements with, for instance, computer games. The key element is that it must be fun to visit. And what could be more fun for the digital native generation than to play plant breeder on a tablet?

Monique Krinkels

Innovative atmosphere offers **fertile ground** for seed industry

'Where innovation shines' is the motto of this year's World Seed Congress. And that goes for the seed industry as well as for the agricultural sector of Australia as a whole. On average, each Australian farmer produces enough food to feed 600 people at home and 450 overseas, the National Farmers' Federation proudly states. And it all starts with a strong seed sector.

The numbers underline the importance of agriculture in Australia. There are approximately 85,681 farms, 304,200 people are directly employed in the sector and, if you include the food and fibre industries, agriculture provides 1.6 million jobs. The gross value of farm production last year was 60 billion Australian dollars (37.5 billion euro).

Australian Seed Federation

Together with the ISF, the Australian Seed Federation is organising the World Seed Congress 2018 in Brisbane from 3-6 June. Its 120 members are delighted to welcome the overseas guests once more. They look forward to meeting the expected 1,200-1,300 delegates from all over the world. Steve Brill, President of the Australian Seed Federation: "Our members cover the whole spectrum of the seed industry within Australia and within the Australian Seed Federation. It does depend upon which market segment the company is operating in as to their involvement. For example, in tropical seeds the companies are generally involved in production, processing, domestic sales and export. There is very little breeding within this sector of the industry. However, if we look at the temperate crop and pasture seed, there are seed companies involved in each of seed trade, breeding, seed production, seed processing and export, either individually or vertically integrated through each of the sectors. Most of the larger companies have head-offices overseas, but there are a significant number of our members who have their base in Australia. These tend to be smaller companies but no less important to the flow of seed throughout Australia."

Seed trade

	Export		Import	
	Quantity (tonnes)	Value (million US$)	Quantity (tonnes)	Value (million US$)
Vegetable crops	1,254	20	2,675	56
Flowers	51	1	10	3
Field crops	12,458	85	33,870	60
Total	13,763	106	36,555	119

Source: ISF compilation based on official statistics and international seed trade reports

Broadacre crops*

	Production ('000 tonnes)	Area ('000 ha)
Barley	12,920	4,624
Canola	4,073	2,539
Grain sorghum	991	371
Oats	2,126	955
Wheat	30,363	11,720

*land suitable for farms practicing large-scale crop operations. Source: Australian Bureau of Statistics

Australia owes its name to the alleged continent terra australis incognita ('unknown southern country')

In the eyes of Europeans, Australian agriculture is focused mainly on sheep. There are over 70 million of them, nearly three times as many as there are people. What are the most important crops besides grasses?
"Sheep are a significant part of Australian agriculture and will remain so. That said, Australian agriculture is very diverse, given our climatic zones running from tropical zones in the north of the country to temperate zones in the south, from regions that receive 1200mm+ annual rainfall to regions with rainfall as low as 250mm annually. In the cropping regions, the major crops produced in Australia are wheat, barley, canola, chickpeas, mung beans, cotton and sugarcane with many, many other crops grown across the country."

How about vegetable production and ornamentals?
"The vegetable sector of our industry is small by world standards and, as such, is highly reliant on

The use of biotechnology in cotton has made a significant contribution in the dramatic reduction in insecticides applied to Australian cotton crops

With over 70 million sheep to feed, grasses form an important part of the Australian Seed Federation. A Pasture Seed Database helps farmers to choose the optimal varieties

Vegetables

Production ('000 tonnes)

Potatoes	1,171
Oranges	350
Tomatoes	326
Apples	267
Onions	256
Bananas	254
Carrots	243

Source: Department of Agriculture and Water Resources

seed imports for the local production of vegetables. Ornamental production in Australia follows a similar path as the vegetable industry, where it is mostly for domestic consumption, with some producers taking advantage of the Asian markets and their proximity to Australia."

Does organic production of seeds represent an important market share? "Yes, it is important to the organic sector of the production cycle within Australia. It is not a large sector of the industry, but if impediments are put in the way of the organic industry to access seed that is suitable for their production, then we discover the importance of the general seed industry to supply seed that is suitable for organic production."

Are people in Australia generally opposed to GMO-crops? "The population of Australia have all been exposed to the negative publicity surrounding GMO's, however the regional/agricultural areas of Australia have also seen the benefits available to them on-farm and across the region from using GMO crops and, in general, are very accepting of crops produced by GMO means. Many of the urban population without the same exposure to the benefits will still be opposed to the concept of GMO's - whether they actually understand which products are produced using GMO crops is another story."

Livestock

Number on holding ('000)

Dairy cattle	2,620
Meat cattle	23,323
Sheep and lambs	70,156

Source: Australian Bureau of Statistics

Do you anticipate that Brexit will cause major changes in trade? Will the seed business in Australia profit from Brexit or is international collaboration with the EU more important? "My exposure to the export markets and the changes that are occurring as a result of Brexit are limited, but I would have thought the majority of Australian seed companies that trade throughout Europe will adapt to whatever changes that occur with Brexit and will trade with the EU and/or Britain without any significant effect on the profitability of their business."

You are the director of Seedtech. Can you tell me more about this company? "Yes, I am a director and shareholder of Seedtech Pty Ltd. My expertise in the industry is as a seed cleaner, with some involvement in seed production and seeds sales. Seedtech was established in July 2000 as a service provision company. We are now a small independent company specialising in seed cleaning, seed treatment and distribution of seeds. There have been times during these years where our majority shareholder was a multi-national company, which by default made us a subsidiary of these multinationals."
"We are predominately processing for the domestic market, in particular the seed market domestically, with commodity export products processed during our quieter months of the year. Seed cleaning and seed treatment are the major income streams of our business. We do fill in some of our production time slots by processing commodity products, predominately for export, such as chickpeas, mung beans, azuki beans and coriander."

The ongoing debate on the **history** of agriculture

John van Ruiten

There is an intense scientific debate raging about the early development of agriculture in Australia. The core of that debate: did the original early inhabitants of this huge continent practice 'agriculture' or did they always remain hunter-gatherers? And was agriculture, in fact, only introduced in European 'colonial times'.

Most scientists agree that hardly any results/findings have been made in excavations that make it plausible for them to have been any pre-colonial Australian agriculture on any scale. Very different from, for example, on/in the northern Indonesian archipelago. If there are any signs of management of natural resources of plants and animals, then it is to do with re-planting small murnong tubers (a kind of yam-like plant, Microseris lanceolata) after collecting/excavating the larger tubers. And the harvesting and use of native millet (a kind of grain, Panicum decompositum) and then sowing it again at the same spot from the remaining seeds on unprocessed soil. Remnants or evidence of food storage structures were never found anywhere. These activities are rather called proto-agriculture. There was indeed a food production system, but as far as we know, no real crop cultivation practices with its associated management, soil cultivation, tools, etc.

Harsh climate

A very interesting question, of course, is why the original inhabitants down under, so differently from large parts of the rest of the world, did not focus on agriculture. The most commonly heard explanation for this is: because of the low population density around the location of the first people in Australia some 55,000-60,000 years ago and the availability of sufficient food in nature, it was not necessary to implement that innovation (to develop agriculture). The very difficult, often very dry climate and also the few fertile soils will also have contributed to this. The incredibly large country apparently provided enough for the few inhabitants to live on; a wide variety of fruits, seeds, tubers and nuts, in combination with meat. The population never became dependent on staple crops. If there were shortages, people simply moved to other regions.

In other parts of the world, according to current leading theories, agricultural development started in the first instance by keeping cattle. This cattle was apparently suitable for domestication/livestock farming and this livestock needed to be fed. In particular, this provided the impetus to sowing grasses/grains and further selection in order to be able to harvest it better and increase the yield through cultivation measures. That process already existed well before those crops were actually (made) suitable for direct human food use. In a combined process, livestock and crops and people were 'domesticated'. Sheep and goats (and dogs for keeping them together) proved to be the first very suitable animal species. Later came pigs, cattle and horses. Apparently, Australia also lacked animals readily suitable for domestication.

The larger animals indigenously present on this southern continent (the so-called megafauna) that occurred in large numbers 40,000-50,000 years ago, are believed to have completely disappeared, mainly as a result of human intervention (especially hunting). From archaeological findings, it is evident that more than 45 species of larger animals disappeared

Large animals such as the Diprodoton or giant wombat disappeared between 5,000 and 10,000 years ago due to hunting

(Illustration: Anne Musser, Australian Museum, Sydney)

Ir. John van Ruiten is director of Naktuinbouw, Roelofarendsveen, the Netherlands, j.v.ruiten@naktuinbouw.nl

Aboriginal people used a variety of cooking methods based on the particular food being prepared. Their most common cooking methods included cooking in the ashes of their fires, boiling, steaming in a ground oven and roasting on the coals

some 5,000-10,000 years ago. There is a long list of large mammals, birds and reptiles that have become extinct. Species include eloquent names such as Diprodotons, Zygomaturus, large carnivorous kangaroos, ratites/ostriches, lizards, reptiles, lions and tigers. As a result of, among other things, burning down areas of jungle (and of course the forest fires that were prevalent there at the time), hunting was also made easier because the animals came together to feed in those areas.

Sophisticated gathering

Hunting and gathering in a sophisticated way became and remained the basis upon which hundreds of generations of Aboriginals were able to survive as tribes/societies. In a society that was stable and relatively sustainable and never knew the collapses that became the rule rather than the exception in structured societies elsewhere in the world. As far as can be ascertained, there were also never long periods of abundant availability of food, and therefore never a large population growth associated with that. Hence, a balance existed between the size of the human population and what nature had to offer.
Another remarkable point: in other parts of the world where the introduction of agriculture also saw the development of woven clothing and textiles from wool and fibres from plants, this is not evident in Australia. Apparently, that was also not 'necessary'.

The famous explorer, James Cook, already noticed in 1770 when he landed in Australia that "the inhabitants are scarcely dressed in animal skins and that the given clothing/fabrics are left lying on the beach." In fact, the clothing of the original inhabitants consisted entirely of animal skins (of kangaroos and possums) particularly in order to protect themselves against the nocturnal cold. Nor are there any remnants of 'material' goods from those pre-colonial societies that were of importance or that indicated status or wealth. The belongings were functional and suitably portable. Also, housing was always temporary shelters.

Acclimatisation society

Agriculture as we define it actually started in the country after the arrival of Europeans. With crops that they brought with them. Also, with varieties that initially accompanied the English and Dutch farmers. Varieties of grains and legumes of which it quickly became apparent that they were not very suitable for growing in the very different and difficult climate there (heat, drought). Very often, a good harvest was not ultimately achieved. Also, because diseases (grain rust) brought along at the same time could spread freely and massively and had a dramatic effect on the harvest.
In 1861, the first 'Victoria State Acclimatization Society' was created, which aimed to adapt seeds

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Quandong seems to be the best candidate to become a 'new native crop'. It contains twice the vitamin C of an orange

and plants to the local conditions (we are talking about pre-Darwin and pre-Mendel times). Knowledge of photoperiodism, genetics, vernalisation, disease/epidemiology did not exist yet. But that would change quickly in the subsequent 50 years. Breeding and selection and distribution of the results on the continent started at the end of the 19th century. The 'godfather' of the Australian breeders' society was William Farrer (1845-1906), who came from England. Educated in Cambridge, he emigrated in 1870 and began to build a large collection of wheat varieties from around the world. And started selection, carried out crossbreeding and eventually developed the famous rust resistant 'Federation Strain' of wheat. The cultivation of this crop exploded and within 20 years Australia became a world exporter of grain.

Nowadays, the agricultural and horticultural production system in Australia consists for 99% of crops that have been introduced there during the last 150 years. There are actually very few examples of crops that originally appeared here and have successfully developed into a global crop (perhaps due to the absence of those thousands of years of domestication). Only a few ornamental plants and the very important tree species Eucalyptus (which is cultivated worldwide for, among other things, paper production) started to spread from these southern regions after the age of the explorers.

The most famous crop from Australia is probably the Macadamia. The aboriginals gathered the nuts/fruits from these trees but did not cultivate them. Only from 1858 onwards did any kind of cultivation begin and in 1880 the first larger plantation was planted. And this also started the improvement of the crop. Currently, several dozen varieties of this fruit crop are in circulation and, given its short history, many improvements will undoubtedly still be possible.

New native crops

Currently, work is also being done on the development of 'new native crops'. There is a lot of interest in this (especially amongst foodies). The best candidate seems to be the fruit crop quandong (Santalum acuminatum). They are shrubs with deep red, tasty fruits. Breeding programmes are running and currently there are three varieties with breeders' rights. Another crop is Acacia victoriana, bardibush, which yields protein-rich legume seeds that can be used for bread additives, among other things. Here too, it is true that the original inhabitants already ate these seeds. In addition, the many types of 'bush tomatoes' can be mentioned. In Australia, as in South America, there are a lot of wild Solanaceae, some of which have edible fruit such as Solanum centrale, the 'bush raisin-kutjera'. Also, the so-called 'mountain pepper', which is not peppery Solanaceae, but from the species Tasmannia lanceolata, seems to have a lot of potential for human use. The dried leaves and berries are good to use as pepper. And to conclude: various Citrus species such as Citrus australis, Citrus glauca and Citrus papeda seem promising as consumption fruits. This is of course only a small summary of the many different plant species that are currently being worked upon. The rich Australian flora also has many dozens of species that offer beautiful global possibilities in the world of ornamentals!

Digital Sequence Information (DSI) not the Solution

Trapped by biodiversity **legislation**

Anke van den Hurk

International biodiversity legislation on access and benefit sharing has resulted in a decrease of the use of genetic resources maintained in gene banks and in the wild. This is an undesired direction according to many, including gene bank managers. Moreover, it is not in line with the Sustainable Development Goals (SDGs).

National legislations based on the international biodiversity legislation have often not been formed yet, or are so complex, that it does not result in access and benefit sharing agreements. Some countries think that benefit sharing is not taking place because users are circumventing the use of genetic resources. It is believed that they only use the Digital Sequence Information (DSI) of genetic resources to develop new products and avoid benefit sharing. Therefore, the governments of these countries would now like to also regulate DSI. In this article, it is argued that DSI is not in the scope of all the international legislation. Moreover, regulating it would limit access, and thus benefit-sharing, even more. This is not good for conservation and sustainable use of genetic resources.

Using genetic resources

Plant breeders use elite materials as parent lines, unless the required characteristics are not present in this advanced material. Then they may use landraces and/or crop wild relatives mostly from gene bank collections. The importance of maintaining biodiversity has already been recognized for decades. Gene banks have been established and they continue to maintain their collections. As their work is no longer fashionable, it is hard to obtain the right funding. Nowadays, not only is more funding for maintenance requested, but also the importance for more intensive use is advocated. Gene bank managers are of the opinion that the value of the accessions in their collections is not sufficiently explored by plant breeders.
According to the Sustainable Development Goals (SDGs), it is also desired that plant breeders use more genetic diversity. Plant breeders should develop new crops, place more focus on underutilized crops and simply broaden the gene pool of the existing crops with which they are working.
The momentum of using more genetic resources seems right. Several new biotechnological tools have been developed to make this possible. Those tools on DNA identification and exploration may be used to improve breeding and also the characterization and evaluation of genetic resources in gene banks. For breeders this means, on the one hand, that the gene pool is broadened, as further distant materials can be used in the breeding. On the other hand, those technologies make breeding more efficient, which could make breeders more enthusiastic to use genetic resources that have the desired characteristic, but also many that should be bred out.
Gene banks could use the technologies to better describe the genetic resources, link phenotypic and genotypic data and with that create value to the genetic resources for further use. Well-evaluated and well-described genetic resources will be used more often.
Even though the momentum seems to be there, the exchange and use of genetic resources is decreasing. First of all, this is due to the lack of clarity on legislation with regard the biotechnology tools, what is allowed and what is not allowed without additional obligations. Secondly, the legislation around access to genetic resources and fair and equitable benefit sharing has a negative impact on the use of new genetic resources. While the legislation is aimed at stimulating the facilitation of access to genetic resources, it has led to a standstill in their exchange, resulting in limited benefit-sharing arrangements.
In this article, the trap of access and benefit sharing legislation for use is described, and it is also explained why further regulation on Digital Sequence Information (DSI) will harm the use of new genetic resources even more.

International framework

In 1992, the Convention on Biological Diversity (CBD) was internationally agreed upon. Sovereign rights over genetic resources were established and, among other elements, it was agreed that access to genetic resources should be facilitated, through a bilateral arrangement of Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) as required. As the CBD remained very general, a more specific protocol on access and benefit sharing was finalized in 2010, namely the Nagoya Protocol.
The CBD and the Nagoya Protocol are not the only international agreements dealing with access and benefit sharing. Another relevant agreement for the plant breeding sector is the International Treaty on Plant Genetic Resources for Food and Agriculture (IT PGRFA). This treaty has the same objectives as the CBD, however, it only deals with genetic resources for food and agriculture. It made specific simplified rules for the exchange of genetic resources for 64 crops by

Anke van den Hurk is deputy director at Plantum, Gouda, the Netherlands

It will still take decades, if not longer, to be able to generate new plants merely from information

means of a standard exchange contract dealing with access and benefit sharing, the Standard Material Transfer Agreement.

National legislations

Even though countries already ratified the CBD, the Nagoya Protocol and/or the IT PGRFA, national legislations were only developed later or are still being developed. And in cases where the countries have ratified the Nagoya protocol and the IT PGRFA, often the IT PGRFA is not really implemented, especially in countries in South America, Africa and Asia.
The development of legislation has not led to access and benefit sharing in practice. Processes to obtain PIC and MAT for the access to a genetic resource are unclear and officials are uncomfortable to make agreements in practice. They would probably rather not sign any agreement, than have an agreement that is heavily criticised because benefit sharing expectations are not fulfilled.
On behalf of some members, Plantum had previously been involved in bilateral discussions with Ecuador on the exchange of tomato crop wild relatives and, more recently, with the Chinese Government on the exchange of Chrysanthemum. In both cases, it was not possible to reach an agreement, as the countries could not clarify their benefit sharing needs.
The fact that exchange of genetic resources does not really take place, was also understood from the monitoring authority on the Nagoya Protocol in the Netherlands. Based on the European legislation on the Nagoya Protocol, they checked tens of Dutch breeding companies on their compliance with the Nagoya Protocol and also with the IT PGRFA. The conclusions of the authorities were that companies are aware of the legislation but would rather not collect new materials because of all the complexity. They do not do this as, on the one hand, it is complex legislation as described above and, on the other hand, it is the fact that the origin of materials bought in the market or obtained freely is unclear. Is it the country where you received it, or do you have to trace it back and, if so, how far back?

Lack of benefit-sharing

Based on the above, it is understandable that very little benefit sharing takes place. If access is denied, or countries are not able to clarify their expectations, nothing will happen. Various people from Africa, Asia and South America think that users are trying to avoid making benefit-sharing arrangements, because they already took a lot of materials in the past (some think benefit-sharing of those materials should also still take place). Furthermore, those people think that the users of genetic resources avoid use, because they only make use of the digital sequence information

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Plantum has been involved in bilateral discussions with Ecuador on the exchange of tomato crop wild relatives. Unfortunately, it was not possible to reach an agreement

of those resources, in order to avoid benefit-sharing agreements.
So those countries now defend DSI being part of the scope of the CBD and the Nagoya Protocol and also of other agreements, like the IT PGRFA. Those countries, therefore, also think that benefit-sharing should also take place on the basis of DSI and they are currently discussing this at international level. No discussion has taken place yet on what DSI is, who owns it and on what basis.
In the meantime, several countries already include DSI in the scope of their national access and benefit-sharing legislation or proposals for legislation, e.g. Brazil, Malaysia and Indonesia.

DSI limits use

Regulating DSI with regard to benefit-sharing will further limit use of genetic resources and thus limit innovation, at least in those countries that are members of the treaties. Especially smaller companies, national research institutes and particularly those in developing countries will be affected most, as they have no access to lawyers.
Secondly, information sharing on genetic resources will most probably decrease. People will be afraid to share, as this may have benefit-sharing implications. This could immediately impact on collaboration in any form, hence also on public private partnerships. So, the further capitalization of gene bank materials will probably become more complicated.
Moreover, people will reinvent the wheel. In that case, they are not threatened by possible illegal use of DSI. This is, of course, a waste of resources. It again should be realized that those who are smaller and have less to spend will not be able to gather the information themselves and will be dependent on those 'owning' the DSI.
This leads to the third point why DSI should not be regulated. Who 'owns' DSI? Is it the person/country who developed the DSI? Is it the country where the genetic resource upon which the DSI is based originates? As indicated above, it is already difficult to determine where a genetic resource originates. Moreover, this assumes that one genetic resource can be linked to one source of DSI, which is most probably not the case.
Another difficult issue in the discussion on DSI is the value of the information. Who is going to determine this and on what basis? As it is already very difficult to estimate the value of a genetic resource, the value of DSI is probably more difficult to estimate and may differ, based on the value of that information for the market at a certain time.
Therefore, it remains the question if countries regulating access and benefit-sharing now are ready to specify their needs in order to make a benefit-sharing agreement.
Lastly, it should be realized that those theories of loopholes are not very probable for the plant breeding sector. It will still take decades, if not longer, to be able to generate new plants merely from information. Synthesizing new organisms is still a long way off, if not completely impossible.

Counter-productive

It is the question whether remaining constructive in the current negotiations will contribute to the objectives of the CBD, or whether we continue to be further caught in a trap, a deadlock of any exchange of genetic resources. Extending the scope will probably not lead to any further benefit-sharing to those countries that do not clarify their expectations and/or have expectations that do not meet the users' needs. Moreover, it may complicate rules and legislation in those countries and institutes where exchange is working. And what is worse, with all the complexity of access and benefit-sharing legislation, biodiversity is often no longer conserved and will be used less. This is contrary to the objectives of the CBD, Nagoya Protocol and the IT PGRFA.

What you **need to know** when using a PCR assay

ISHI-Veg Technical Group

Germinating beet seed for a grow-out assay

Seed health testing is a procedure by which a seed lot can be determined to be healthy or infected. A seed health test should be specific to the target pathogen, sensitive, reliable and reproducible. Ideally, it should distinguish between the presence of viable and non-viable organisms and/or inactivated genetic material that may be left on the seed after a disinfection treatment and assist companies in their risk management practices. It is, therefore, essential to understand the meaning of different test results and their consequence for the risk of disease transmission, pathogen establishment and risk mitigation.

Healthy seeds, free from known seed transmitted pathogens, are a prerequisite for sustainable food production. Seed companies contribute to the availability of healthy seeds by routinely testing seed to prevent or control plant pests and pathogens that may affect seed quality, seed movement, and their introduction into new territories.

Management practices that prevent seeds from being exposed to pests and diseases at all stages of seed development, production and commercialization significantly reduce overall pest risk, and seed health testing is often a final check. A seed health test is also frequently a phytosanitary requirement imposed by national plant protection authorities, before allowing seed to enter their territory.

Direct and indirect tests

Plants are checked for signs and symptoms to determine if they are healthy or diseased. For seeds this is more complex, as the pathogen and disease symptoms are usually not visible. To detect pathogens in seeds, tools other than looking for symptoms on young plants grown in conditions conducive to disease development may be required. Proof that the pathogen in question is causing the observed disease symptoms is key, as seeds can carry a wide variety of microorganisms, of which the majority are usually non-pathogenic.

A seed health test to detect the presence of a pathogen known to be seed-transmitted generally consists of three primary steps: i) isolating/extracting the

Definition

In seed health testing, an assay is an investigative procedure for qualitatively assessing or quantitatively measuring the presence, amount or functional activity of a target pathogen. Assays are developed based on different technologies, such as visual examination, selective media, seedling grow-out, serological techniques and PCR.

A method is a collection of one or more assays that together show the presence, viability and pathogenicity of a pathogen. A method is a description of how a test is conducted.

PCR reactions in a 96-well plate

(Photograph: GEVES)

Corn salad seedlings grown in a sweat box to determine the viability of the target pathogen and its pathogenicity

(Photograph: G. Hiddink, Enza Zaden Seed Operations bv)

disease-causing pathogen from seeds, ii) detecting and identifying it and, lastly, iii) confirming the viability and pathogenicity of the isolate. With such a direct method, the living presence of pathogen on and/or in the seed is demonstrated, and its pathogenicity is confirmed by inoculating young plants grown from healthy seed or indicator plants with a seed extract from infected seed and checking for disease symptoms.

Over the last few decades, direct methods have been complemented with faster, simpler and relatively inexpensive indirect methods that detect the presence of proteins or nucleic acids specific to the pathogen in seed, but without demonstrating pathogen viability and pathogenicity.

PCR assays

Although ELISA and immunofluorescence assays are commonly used in seed health testing, nucleic acid-based assays are gaining importance. Polymerase Chain Reaction (PCR) is the foundation of indirect methods that detects the presence of a targeted pathogen by its nucleic acid. PCR assays can be performed by anyone with good general laboratory skills, in contrast to other techniques that require specialist skills to identify the morphological characteristics of fungi, bacteria and nematodes.

A drawback of PCR assays in seed health testing is that a positive PCR result only shows an association of the suspect microorganism with the host tissue and association is not necessarily causality! The mere presence of a known pathogen does not mean that it can or will cause disease. Multiple pathogens and secondary organisms, such as saprophytes that have morphological or genetic similarities to the target pathogen, may be present on the seed.

To fulfil its purpose, a seed health test must detect the target pathogen(s) in the seed lot – if present – without giving any 'false negative' or 'false positive' results. A false negative can occur when the test gives a negative result even though the target pest is present. A false positive result can occur when closely related organisms that do not cause disease are also detected by the test.

An example is the species *Clavibacter michiganensis* that contains several subspecies that include important plant pathogens and also non-pathogenic bacteria. *Clavibacter michiganensis subsp. michiganensis* (or Cmm) is the causal agent of bacterial canker of tomato and is well-known in the vegetable seed industry. Cmm has many close relatives, the so-called look-alikes that are non-pathogenic to tomato. A PCR test to detect Cmm in tomato seed that also detects such close relatives, giving a 'false positive' result, could have serious consequences for seed companies, such as destroying valuable seed lots.

Another example is that of seed treatments applied directly to the seed and play a significant role in improving the establishment of healthy crops. Effective seed disinfection treatments that kill all the target pathogens can leave traces of intact DNA/RNA on the seed. This DNA/RNA can remain stable for long durations in the storage conditions used for seed for sowing. PCR assays can detect such nucleic acids, despite a complete absence of a viable pathogen, leading to a false positive result.

Despite these drawbacks, PCR offers several advan-

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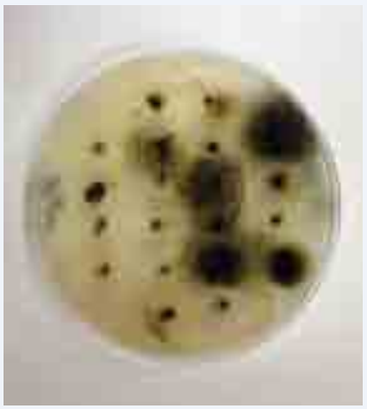
The importance of healthy seeds for sustainable food production should not be underestimated
(Photograph: Enza Zaden)



The fungus *Phoma valerianellae* growing on Malt Agar demonstrating its viability

(Photograph: G. Hiddink, Enza Zaden Seed Operations BV)

tages in seed health testing and the seed industry has embraced them and is also actively involved in their development and use. Importantly, they can identify non-infected seed lots more rapidly than direct tests. With the combination of available sequence data and simple low-cost assay design, specific and sensitive 'seed extract' PCR tests can be developed rapidly in response to emerging or existing pathogens.

Interpretation

For a correct interpretation of an indirect test result, it is essential to know and take into consideration specific factors, such as variability in the proteins and/or genetic material of the pathogen and its similarity to closely-related non-pathogenic organisms. This underlines the importance of method development and validation to ensure that a seed health test can detect all target pathogens, with minimal off-target false positives.

To raise awareness of such considerations in the use of indirect tests the seed industry adopted a position paper ISF Viewpoint on Indirect Seed Health Tests in Athens in 2013 (see http://www.worldseed.org/resources/papers/phytosanitary/) in the wake of the International Standard on Phytosanitary Measures on the international movement of seeds (ISPM on seed) being drafted. The paper was influential in raising awareness among NPPOs and policymakers on the use of indirect tests during preparation and finalization of the ISPM on seed. It remains relevant in the implementation phase of the ISPM on seed.

According to the position paper, a 'negative' PCR result indicates that the seed lot is not infected with target pathogen(s). On the other hand, a 'positive' pre-screen PCR result indicates the seed lot is suspected of being infected with the target pathogen(s) and a confirmatory test must show the target pathogen(s) to be viable and pathogenic.

A technical paper, recently developed by ISHI-Veg, provides guidance on the development of real-time PCR assays as a pre-screen in seed health testing.

Method development

ISHI-Veg aims to secure the delivery of sufficiently healthy seed to customers by developing methods for seed health testing that are internationally recognized as reference methods and accepted as industry standards (see http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg-method-development/).

It strives to develop seed health methods that are fit for purpose. Method validation is a critical aspect of method development in ISHI-Veg and performance criteria such as analytical specificity, analytical sensitivity, repeatability and reproducibility are measured to demonstrate that the method is fit for purpose. ISHI-Veg has also developed best practices for the use of several assays, among them PCR, in seed health testing. They identify the number and types of controls necessary to ensure accurate and reliable results and serve as a guide in method use and development of seed health methods.

In conclusion, nucleic acid-based assays, such as PCR, are a relatively simple, quick and inexpensive way of testing seeds and are a useful addition to the toolbox of techniques used by ISHI-Veg to develop seed health methods. Nevertheless, the results of PCR assays need to be interpreted with care: a negative result means the target pathogen is not detected and the seed lot is healthy; a positive result indicates the target pathogen is suspected of being present in the seed lot and a test to determine pathogen viability and pathogenicity is necessary to draw a conclusive result on the status of the seed lot.

Cannabidiol opens **new market** for breeders

Monique Krinkels

There is a faint cannabis scent in the greenhouse of the Naktuinbouw but, no, there is no illegal weed plantation in the neighbourhood. The fragrance is derived from the two hemp varieties registered for European plant variety rights. The Italian Institute, CREA (Council for Agricultural Research and Agricultural Economic Analysis), has bred the two.

So far, varieties of *Cannabis sativa L.* are mostly meant for production of hemp fibre derived from the stems of the plant. The fibre can be used either as bast fibre, a raw material for paper and textile industries, or as woody core. It is even used as automotive composites used for car interiors, but it can also be added to animal feed and even food products. And of course there is the – in most countries illegal – production of cannabis for recreational purposes. But a new application is gaining ground as the plant offers valuable consituents that can be applied as medicins. This is the reason for the Italian research institute, CREA-CI, to breed varieties with improved pharmaceutical characteristics.

Hemp plants contain a non-hallucinogenous cannabinoid named cannabidiol (CBD). This oil has been demonstrated as an effective treatment for epilepsy in several clinical trials. There is also evidence that CBD may be a useful treatment for a number of other medical conditions. Besides CBD, Cannabis varieties contain tetrahydrocannabinol (THC), a hallucinogenous component that has however medical benefits.

Current international controls

Cannabidiol is not listed in the schedules of the 1961,1971 or 1988 United Nations International Drug Control Conventions. However, cannabidiol is being produced for pharmaceutical purposes as an extract of cannabis by the British company, GW Pharmaceuticals. Cannabidiol that is produced as an extract of cannabis is currently included in Schedule I of the 1961 Convention.

Australia In 2015, CBD in preparations for therapeutic use containing 2% or less of other cannabinoids found in cannabis was placed in Schedule 4 as a 'Prescription Only Medicine or Prescription Animal Remedy'. Previous to this, it was described in Schedule 9 as a prohibited substance.

Canada CBD is specifically listed in 'Cannabis, its preparations and derivatives' as a controlled substance listed in Schedule II Controlled Drugs and Substances Act. However, in 2016 Canada's Access to Cannabis for Medical Purposes Regulations came into effect. These regulations improve access to cannabis used for medicinal purposes, including CBD.

New Zealand CBD is a controlled drug, however many of the restrictions were removed at the end of 2017. The changes will mean that CBD products, where the level of other naturally occurring cannabinoids is less than 2% of the cannabinoid content, will be easier to access for medical use.

Switzerland CBD is not subject to the Narcotics Act because it does not produce a psychoactive effect. It is still subject to standard Swiss legislation.

United Kingdom A statement was issued by the Medicines and Healthcare products Regulatory Agency (MHRA) in 2016 that products containing CBD used for medical purposes are considered as a medicine subject to standard licensing requirements.

United States CBD is one of many cannabinoids present in cannabis, and as such is in schedule I of the Controlled Substances Act. However, in December 2015, the FDA eased the regulatory requirements to allow researchers to conduct CBD trials. The Drug Enforcement Agency (DEA) stated that these modifications are intended to streamline the research process regarding CBD's possible medicinal value and help foster ongoing scientific studies.

Source: CANNABIDIOL (CBD) - *Pre-Review Report by the World Health Organization's Expert Committee on Drug Dependence*

Naktuinbouw performed the technical DUS tests for EU Plant Breeders' Rights on the new hemp varieties

Dr. Gianpaolo Grassi is chief researcher at CREA-CI, in the northern Italian city of Rovigo. For over twenty years he has been successfully breeding fibre hemp varieties. He selected for instance the only Italian monoecious varieties of hemp. His vast experience of improving the agricultural characteristics of fibre hemp helped him to develop the *Cannabis sativa L.* varieties the medical world was waiting for.
Where traditional fibre hemp may contain no more than 0.20% of the psychoactive ingredient THC in order to be registered in the EU Common Catalogue of Agricultural Plants, the Cannabis varieties he was looking for needed to have a far higher percentage. "We launched a national programme for the production of medicinal cannabis in Italy in 2014," Dr. Grassi says. "We developed CINRO (8% CBD and 7% THC) and CINBOL (20% THC). DUS tests of a third variety, named CIN-FE, which produces 18% CBD are under way. For the production of the oil, only the female inflorescences are used," explains Dr. Gianpaolo Grassi. The special characteristics of the two Italian cannabis varieties: they produce more oil, as well as an increased CBD and THC content and are therefore very suitable for the production of medicinal cannabis oil.

It all began in 2005, when he demonstrated at a conference on Cannabinoids in Medicine at Leiden University that CBD has a beneficial effect on children with untreatable epilepsy. "On June 2002 we started to treat an eleven year-old girl affected with a highly drug-resistant Lennox-Gastaut syndrome - a form of epilepsy - with CBD, administered at gradually increasing doses up to 20 drops daily. Results were encouraging: the girl, since she assumed CBD, did not need any longer to be admitted to hospital for her epileptic seizures, while her attacks decreased both in frequency and intensity. In addition, her awareness, postural tone and speaking ability improved, as to allow us to gradually decrease her barbiturate intake." It is just one example of the many children that participated in the trial.

Many uses

In recent years, it has become clear that there are a whole range of conditions that can be combated with cannabidiol. Oncologists prescribe it to prevent nausea during chemotherapy, it helps against muscle cramps for MS patients, reduces tics in Tourette's syndrome and can be used in therapy-resistant glaucoma. In psychiatry, it is used to combat psychoses, but it is also a good painkiller. "Which variety should be used depends on the disease. For example, glaucoma is more controlled by THC, while some other diseases need the combination of THC and CBD."
Another possible therapeutic application which is being investigated is the use of CBD to treat cannabis and tobacco addiction.
Several countries have modified their national controls to accommodate CBD as a medicinal product. The CBD oil available at the pharmacy has a THC content of less than 0.20%, otherwise it has to be prescribed by a doctor. In Italy, medicinal cannabis production takes place under the responsibility of the Ministries of both Defence and Health to ensure no illegal cannabis growing takes place. To date, there is no evidence of recreational use of CBD or any public health related problems associated with the use of pure CBD. "Cannabis oil has become a popular medicine in my country in recent years," concludes Dr. Grassi. And as the number of possible applications is growing rapidly, the new varieties will have a bright future.

The Idealists

Sowing **relief** in Africa

Christiaan Weijts

Last January, '*The Idealists*' was published, the last book in a romantic trilogy about world improvers written by Professor Louise O. Fresco, agricultural and food expert, and chairperson of the Executive Board of Wageningen University & Research. In this novel, she is looking for the answer to the question what science can really achieve in a world where superstition prevails.

You start each book with prejudice, or to say it in a nicer way: with expectations. Author's name, cover, title, the thickness of the book, the stories you have already heard about it in interviews... it all circles around that bundle of printed sheets and colours them. The best reading experiences are often those where things unexpectedly turn out very differently. For me, that was the case with the new novel by Louise O. Fresco.

Louise O. Fresco occasionally reminds one of Coetzee

I had quite a few prejudices. The author had also created a little of this herself. For example, by being a professor in the field of food and agriculture, and dealing with world food issues and development aid from all kinds of top positions. For example, by calling her novel '*The Idealists*', and – according to the blurb – setting it around a mission in Africa, where Marcus, a doctor, looks after a young, disabled patient Ndidi. It has all the elements of a book that once again impresses upon us how awful the situation in the world is.

The first surprise was immediate in chapter one, which introduces the characters and particularly describes the African landscape. A classic *Natureingang*, just 'not done' according to hair-splitters, but here it happens extraordinarily. Magnificently, sensorily, you are thrown into a universe in such a way that nature documentaries cannot manage to do: "There, on the rocky ridge above the village, where the plateau starts, they sat quietly listening to the lappet-faced vultures and the white-backed vultures, which swept just above their heads, like a gust of wind, before they let themselves glide over the edge of the valley, on their way to a carcass."

Whoever can write like that has grabbed my attention. The second surprise was the story and the characters. Oh yes, that doctor is a passionate idealist, but one

Fifty years after Biafra, there has been little improvement in our humanitarian ethic. Europe is scarcely concerned about the hunger in Africa, only about how aid can stop emigrants, according to Louise Fresco

'De Idealisten' (The Idealist) by Louise Fresco is being published by Prometheus, ISBN 9789044634969, price € 19.99 (hardcover), or ISBN 9789044634976, price € 11.99 (ebook).

who increasingly questions his idealism and doubts both the purpose and the motives of his undertaking. "He regularly played the role of the motivated reliever, even though he had the feeling that he was becoming complacent." And why did Marcus actually get started? Was it really just to save a poor continent? In conversation with a priest, Father Armand, he states that as a child of Jewish refugees you have two choices: "Either you seize the silence around you in order to forget everything and build a nice life for yourself, or you search for a new war, to banish the previous one."

In the same conversation he confesses that Ndidi's 'undeveloped intelligence' appeals to him, and he sees it "as an in vivo thought experiment. An attempt at sowing the Enlightenment, among a sample of one."

This book is also a thought experiment in many ways, and the extensive discussions, often part essays in monologue form, make up the core of it. Scientist Marcus, the faithful Armand, the disabled Ndidi, and later an American couple join them, with a Clinton-like Foundation, who delude themselves as being very involved but are hilariously and painfully unmasked.

The roles are thus divided, and they give this book an allegorical character, which occasionally reminded me of Coetzee's work, such as '*The Childhood of Jesus*'.

You can view it as a contemporary variation on Diderot, philosophical dialogues in the tradition of '*Jacques le fataliste et son maître*'. The danger therein is that the whole thing becomes static – especially if little happens at story level for a relatively long time – but those objections will disappear once you have come to terms with this genre. Although that sounds too negative for the stirring mental exercises where those different voices take you.

What can science really achieve in a world where superstition prevails and every disease is a poisonous bite by spirits and gods? If twins are born, then that brings bad luck and that must be concealed. What do freedom of choice, fate and happiness really represent from a biological perspective in an environment of permanent hunger?

Especially the fact of idealism, putting yourself in the service of something greater, is being investigated, upturned, exposed in all possible ways. Does idealism not always come forth from spurious motives – the swanky fake-idealism of the American benefactors, Marcus' dead-end attempt to banish the war from his parents – and does it actually make the world better? "Even now he did not have an answer to that question", it says at the end. "Every choice, no matter how good, also meant loss, every belief carried a shadow. It comforted him – finally – that all this was already so old and would continue."

This brings me to something that is not good to recount and what is ultimately the strongest aspect about this book. That is in its undercurrent: subcutaneously, all kinds of forces are working within, experiences and insights that the author must have gained from her international positions. In the absence of a better word: the wisdom of this novel.
You would want more people, such as Fresco, with huge international positions, to write novels – because they can convey ideas and experiences that go beyond the unambiguousness of opinion pieces or political position – especially if they can do so with such perceptive clarity.

Christiaan Weijts is a Dutch author, who has been awarded various prizes for his novels.

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

RIJK ZWAAN

International Seed Identification Training
From 1-3 October 2018 in the Netherlands

university of groningen | nak tuinbouw

The aim of this training course is to improve identification of seeds found as impurities in seed samples. Two experts in the field of seed identification, Renée Bekker and René Cappers of the University of Groningen are the trainers of the training course.

Recognition of the plant family
A selection of families will be introduced that represent the majority of the seeds from the ISTA universal list of species. Participants understand and can correctly apply the terminology that is often used in seed identification literature.
The practical assignments focus on the specific features that distinguish seeds/fruits of closely related species from each other. Participants are challenged to identify seeds to the species level.
In addition the workshop contains outings such as Aalsmeer Flower Auction and the Botanical Garden in Leiden.

Location and registration
Naktuinbouw, Roelofarendsveen, the Netherlands.
Detailed information and a registration form at www.naktuinbouw.eu (search: Seed identification).
Registration Fee: € 895,– (excl. VAT)

Registration is possible until 1st of July 2018.

Energy **efficiency** becomes more important

Monique Krinkels

Seed Processing Holland recognizes the importance of the operator's safety and health and continuously innovates the equipment to ensure safe machines

With prices of electricity rising continuously, the energy consumption of seed dryers is becoming more important. A heat pump based drying system is three to four times more energy efficient than ventilation based dryers. Simply reclaiming the energy released from the wet seed may reduce energy consumption for the drying process up to 50-70%.

"The condensation principle means that humid air is cooled down. And as colder air cannot contain as much water as warm air, the water will condensate on the cooler of the heat pump. The cooled dry air from the cooler will then be heated with the heat pump. Subsequently, when the dry heated air is blown through the seeds it will take up the moisture from the seeds. The heat pump uses the energy released from the drying process to evaporate the water," explains Willem van Dok, area sales manager Europe at Seed Processing Holland.

Improving results

"All our drying equipment is designed to help seed companies achieve uniform drying results with the desired air temperature and relative humidity. We have been at it for over fifty years and still we find ways to improve the results of our machinery. Our latest introduction is a seed dryer that enables multiple drying options: seed drums and fluidised beds. Drums from small 144 ml cups that can be used to dry a few grams of seeds to drums that may contain 22 kilos and everything in between."
Also important is that the recipe is the same wherever you are in the world, making it easy for multicentre companies to obtain the same results. Whether in a hot, humid climate or in a dry, cold region, the drying process is the same as it is a closed system, independent of weather conditions outside. "If the drying process takes twelve hours during the rainy season in Brazil, it will take twelve hours during winter in Canada. The recipes for the diverse crops are stored in the dryer, making it easy to start the drying process again. And when there is a hiccup in the system, the responsible manager will be automatically alerted with a text message on his mobile telephone. With valuable vegetable seeds, you cannot be too careful."
One of the things people have to cope with when drying seeds is dust in the air. When drying tomato or courgette seeds, for instance, a kilo of dust might be spread in the surrounding area. This particulate matter consists of fine dust, but also of endotoxins (the remains of dead bacteria) and is believed to cause severe health problems. "We have solved that problem by adding air filters to our machines that intercept the dust so that people can work in a healthy environment."

New office in Vietnam

Seed Processing Holland has opened a new office in Ho Chi Minh, Vietnam, fall last year to strengthen the service and sales in the Asia Pacific region. The region is one of the geographical focus areas for SPH. A dedicated sales team has been assigned to cover the region led by Paul Koomen, area sales manager. "The decision to expand our presence into Asia was a logical step in our business growth strategy as it has always been an important market for SPH. After almost 20 years of travelling from the Netherlands to different locations in the APAC region, it is time to be close to our important relations in this region. With this new step we want to offer our relations a quicker response on the information they require. Due to the dedicated APAC team, we are certain we will improve our services. We look forward to enhancing our presence in the region," says Paul Koomen.

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Half a Century of Seed Enhancement

Innovation never stops

Monique Krinkels

In the summer of 1968, David Dirkse, one of the colleagues at a Dutch seed company, finally succeeded in developing a split pill for lettuce. It took him five years of research to overcome the obstacles, but the end result was a uniformly germinating crop. That autumn, a new department was opened: the Royal Sluis Coating Department, which is today internationally renowned under the name Incotec.

The history of Incotec is a success story few other companies can match. Starting as a small department that took care of pelleting the company's lettuce seeds, it soon started to work for other vegetable seed companies as well. And the number of vegetable crops that were treated exploded, as did the applied technologies. Not only for the Dutch colleagues, as foreign companies were also interested. Within a decade, the first subsidiary was opened in the USA, soon followed by Brazil, Australia, China, just to name a few. To acknowledge the nature of the expanding activities, a new name was created in 1989, based on a description of the work: INtegrated COating and seed TEChnology, Incotec.

Innovations

"The man who developed the first Split Pill did so in his own time," says Mariska Wever, Product Manager. "Reason was that transplanting the geminated plants was labour intensive. Mechanically sowing was not possible as the shape of the seeds was irregular." She has worked at Incotec since 1990 and knows a lot about the history of the company. "He first tried it on violets, but there was too much variation in that crop. With lettuce, however, is worked out perfectly. The pelleted seeds could be planted mechanically, saving costly labour."

The highlights of the past half a century: in 1993 Incotec introduced film coating with DISCO, in 1996 the company started with disinfection, in 2008 X-ray was first used to recognise viable tomato seeds and in 2011 biological plant growth stimulators were added to the list of treatments the company offers. "In general we handle all vegetable and flower seeds in our own production plants, while for agricultural crops we deliver our own (film) coating materials."

Treasure trove

While Royal Sluis was taken over by a series of companies, ending up in 2005 as a part of Monsanto, Incotec became an independent company in 2002. That lasted until 2015, when Incotec was incorporated within Croda. This British company started out in 1925 as a refinery of wool grease into lanolin or wool wax used in salves. Today, it develops speciality chemicals that can be used (besides in crop care products) in health care, personal care, engine lubricants and many more applications.

"Our development teams have greatly benefited from the knowledge of our British colleagues," Mariska Wever explains. "The company has developed several excipients that improve the effectivity of the (film) coating materials we develop and market. For us, Croda is a treasure trove we can explore."

"We will celebrate our fiftieth anniversary on several occasions this year," says Mariska Wever

Monique Krinkels

28 **With the utmost concentration, a young 'digital native' tries to decide which plant to choose to become a parental line. Her friend is frantically pushing the buttons on the tablet screen to quickly separate the less uniform seedlings from healthy young plants, while another attentively listens to a mini-lecture on biotechnology. Meet the new exhibit 'Sow to Grow'.**

Photographs Ruben van Vlie

Let's **play** plan

"The intention is to open up the black box in the food chain," says Gerard Meijerink, implementation manager of the Sow to Grow experience, an initiative of the Saet & Cruyt Museum. "Most people hardly know what happens to food before it is displayed in the supermarket. But even if they realise that farmers have something to do with it, the step before – plant breeding and seed production – is something completely hidden from sight. We hope to educate people with the Sow to Grow experience. Plant breeding provides a significant contribution to sufficient and healthy food of the right quality in the right place at acceptable prices, now and in the future, and they should know that."

From looking to experiencing

For years, seed companies took their (mostly foreign) colleagues to the Saet & Cruyt Museum. It was based in Andijk, a small village close to Enkhuizen in the Netherlands. It shared the attic of the former polder pumping station with the polder museum. For people who love to see old machinery, ancient books, historical textbooks, plaster models or watercolours of vegetables, etc. it was a perfect exhibit, but most youngsters were less interested. And it was a bit out of the way, so passers-by were rare. In short: it was time for a complete make-over.

"The World Seed Experience is focused on the present and future of plant breeding, rather than on the past. The building in which Sow to Grow is established is the former City Orphanage, right in the centre of Enkhuizen, the Netherlands. An enormous seed cleaning machine at the forecourt is an attractive way to arouse curiosity for the unexpected visitor," explains Gerard Meijerink. Once inside, everyone receives a tablet which helps them to explore the exhibition. While there are many objects to illustrate the history of seed breeding, the focus is on the present and the future.

The official opening of the Sow to Grow experience in the former City Orphanage in the centre of Enkhuizen, the Netherlands, will take place on 14 and 15 September 2018. But from this spring, the public will be able to get a sneak preview of Sow to Grow

Using modern equipment, people learn amazing facts about seeds

For people who like to learn more, QR codes activate the tablets to produce extra explanations on, for instance, the plaster models of vegetables, and short films

While focused on the present and future, some eye-catching historical objects have found a place in the display

t breeder

Explaining and entertaining

The interactive experience provides insight into seed cultivation and seed trade. From collecting and breeding to multiplication, improvement and marketing. The visitor also learns about the companies that are active in the Netherlands and the diversity of functions that these companies have to offer. But also socially relevant topics, such as biodiversity, biotechnology, protection of intellectual property, seed disinfection, etc. are highlighted.
With the help of QR codes, people will learn more about certain objects, but the tablet also shows photographs and short animated films to explain the backgrounds. Other films allow people who work in the seed sector to tell the stories of their daily work. There is a laboratory, in which people can interactively experiment with DNA, and a cinema room where a documentary is shown. Mini-lectures ensure that the visitor who prefers in-depth information is taken care of and the smallest guests can turn to the cuddly toy vegetables. And, of course, there are interactive games for the youngsters. So, there is much to see and experience.
"We hope to show the importance of healthy seeds to cope with the world food problem. But at the same time, we want to entertain everyone from young to old. The visitor to this experience is introduced to plant breeding in an interactive way and experiences the enormous impact this has on our existence and its importance for the future. We hope that tourists will visit the Sow to Grow experience, but it is also an informative destination for horticultural students and school groups. And, of course, people who work within the seed industry are most welcome too," concludes Gerard Meijerink.

Sow to Grow can be found in the City Orphanage, Westerstraat 111, 1601 AD Enkhuizen, the Netherlands, info@sowtogrow.nl, www.sowtogrow.nl

The Very First Hybrid Plant

Oxheart cabbage (just) beat maize

Jan Velema

Many people believe that the first hybrid plant was a maize variety named 'Copper Cross' introduced by Henry A. Wallace in Iowa, USA in 1923. Others point out 'Single Cross', the tomato introduced by the Dutch company Bruinsma in 1946 as the first F1 hybrid vegetable. In fact, the very first hybrid plant variety was the oxheart cabbage 'Vroege van Sappemeer'.

The history of hybridization started in the early 1900s in Europe, as well as in the USA, when the laws of Mendel were rediscovered. The Dutch professor of plant physiology, Hugo de Vries, (1848-1935) was among the first to publish about this in a French summary: 'Sur la loi de disjonction des hybrides'. Soon afterwards, a horticultural consultant, Jan Heemstra, realized the potential of this discovery to create improved plant varieties. He started his breeding activities at the municipal horticultural experimental station in Sappemeer, a tiny village in one of the northern provinces of the Netherlands.

Theoretical case

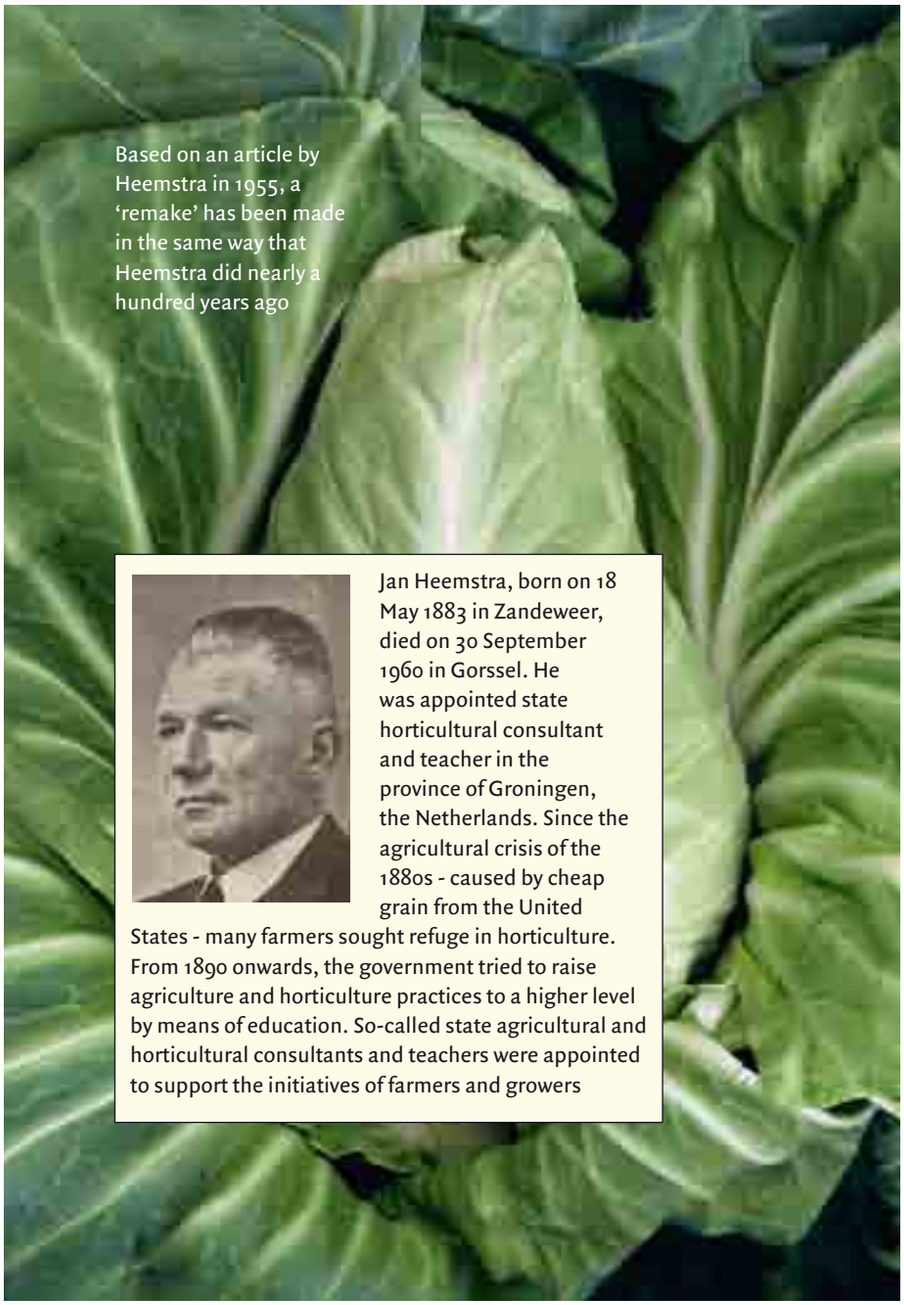
Heemstra theorised that the first generation after crossing two plants from a cross-fertilizing crop is much more uniform than an open-pollinated population. He calculated the case for ten splitting genes that an F1 hybrid has four times (in dominant genes) to ten times (in intermediary genes) less phenotypic variation compared to an open-pollinated variety. In reality, more than ten genes will differ in a not too uniform open-pollinated variety.
This phenomenon of better uniformity is also easy to understand when you consider that children from one family are much more similar than children of different parents. Full brothers and sisters are genetically less different from each other than all members of a large family, or a local population of people.
Heemstra started his experiments with oxheart cabbage, a popular vegetable at the time. By propagating the parent plants vegetatively by growing cuttings, he could produce F1 seed, year after year, on a larger scale that was not only relatively uniform, but also very stable over time. He thus obtained the uniform parental lines for his F1 hybrid not by breeding inbred lines, but by propagating them vegetatively. He let two clones bloom together and harvested the seeds from both clones. From various combinations, he selected the hybrid that performed best and was the most uniform. He did not know that cabbage shows self-incompatibility (which was not known in 1920, only discovered in 1955), but apparently he was lucky enough to choose two self-incompatible and cross compatible clones. Self-incompatibility prevents self-pollination within a clone, therefore the harvested seed (largely) consists of crosses between the two clones.

'Vroege van Sappemeer'

The variety 'Vroege van Sappemeer' (Early from Sappemeer) came into being in 1921, just one year before the first F1 hybrid maize was produced by George S. Carter of Clinton, Connecticut, USA. The popularity of the hybrid maize was however slowed down until the 1930s, as the yield improvement compared to the 'regular' varieties remained modest at best. 'Vroege van Sappemeer', on the other hand, was an immediate success. For 35 years it was the most grown oxheart cabbage variety in the Netherlands. 'Vroege van Sappemeer' was included in the very first edition of the Variety List - which appeared in 1943 - as a recommended variety and remained the undisputed number one until the mid-50s.
In the Variety List of 1951, it is recorded that 'Vroege van Sappemeer' is universally recommended. According to the description:

The variety is an F1 hybrid won from the same parents over and over again.
It is a small, slightly pointed cabbage, with rather coarse veins. Short stalk. Very little outside leafs. Plant distance of 50 x 50 cm is sufficient in open field growing. Will weigh approx. 1.25 kg. Suitable for early cultivation under flat glass and for all early combinations in the open field. The variety is harvestable late May, early June.

Jan Velema is breeder and co-founder of the cooperative De Zaderij and former director (and founder) of Vitalis, the Netherlands' first organic seed company.



Based on an article by Heemstra in 1955, a 'remake' has been made in the same way that Heemstra did nearly a hundred years ago

Jan Heemstra, born on 18 May 1883 in Zandeweer, died on 30 September 1960 in Gorssel. He was appointed state horticultural consultant and teacher in the province of Groningen, the Netherlands. Since the agricultural crisis of the 1880s - caused by cheap grain from the United States - many farmers sought refuge in horticulture. From 1890 onwards, the government tried to raise agriculture and horticulture practices to a higher level by means of education. So-called state agricultural and horticultural consultants and teachers were appointed to support the initiatives of farmers and growers

In 1911, Jan Heemstra initiated the founding of the horticultural experimental station in Sappemeer, which was at the time a local centre for vegetable growing

Attention should be drawn to the fact that sometimes there are selections on the market under the name 'Vroege van Sappemeer', which deviate considerably from the type of the experimental station described above. (Apparently imitations were launched on the market; the variety was being propagated by others who did not have access to the parental lines.)

When Heemstra published an article in Euphitica in 1955, he reported that the variety was still being maintained. The variety disappeared shortly afterwards and has fallen into oblivion. The variety did, however, not vanish altogether. 'Vroege van Sappemeer' has ended up in the gene bank of the Centre for Genetic Resources (CGN) in Wageningen, the Netherlands. That happened without people realizing that it was an F1 hybrid and thus the variety was simply maintained with seed. The data of the gene bank do not mention that it is an F1; only that the variety is not very uniform... Since F1 hybrids cannot be generatively propagated, and seed companies do not donate their parental lines to a gene bank, all old F1 hybrids have disappeared (except for 'Vroege van Sappemeer', which was not recognized as an F1 hybrid).

Introduction of F1 hybrid vegetables

Oxheart cabbage	1921
Tomato	1946
Cucumber	1958
Brussels sprouts	1966
Spinach	1966

Between 1970 to 2000 many F1 hybrids of other vegetables became available, for example white cabbage, cauliflower, onion, leek and beetroot

Rebirth

Only in 1955, when Heemstra was probably already retired, did he publish about his breeding activities during the period 1916-1923. Based on the article by Heemstra, a 'remake' has been made in the same way that Heemstra did nearly a hundred years ago.
De Zaderij is a cooperative of farmers and growers who select, propagate and sell organic seeds of traditional types of plants. Early 2014, CGN made seeds of 'Vroege van Sappemeer', 'Express' and 'Vroege Groningen' available, which were sown in the field at De Zaderij in Voorst, the Netherlands. A number of selected plants of these varieties were placed in an insect-free cage and bloomed in 2015.
The self-incompatibility of each plant has been tested by making self-pollinations and paying attention to the lack of setting. For a number of self-incompatible plants, test combinations have been made by hand. Heemstra was not yet aware of self-incompatibility, which was only discovered and applied much later. Heemstra has apparently been lucky enough to select two incompatible and cross-compatible plants. After harvesting the seeds, cuttings of the plants have been made for maintenance. In the spring of 2016, the test combinations were sown and assessed. The parental lines of the most beautiful combination were planted together for seed production and further vegetative propagation of the parental lines.
In 2017, this early cabbage was grown successfully for the first time at Nursery De Keukentuin (The Kitchen Garden) in Zuidbroek (near Sappemeer). Seed production has also taken place here. This has recreated the oldest F1 hybrid oxheart cabbage again, after almost 100 years. Whether the 'remake' of 'Vroege van Sappemeer' is good enough to be grown for a number of years, will have to be proven. In any case, it has been demonstrated that, without a lot of technical aids, an F1 hybrid oxheart cabbage can be made on the basis of vegetatively propagated parents. Moreover, this 'remake' is a tribute to Jan Heemstra who has not yet received the recognition for his extraordinary breeding work. He was far ahead of his time.

25-27 September 2018

Seed meets Technology celebrates five-year anniversary

Marlies Reus

It has become an unmissable event at the start of autumn: Seed meets Technology. An event which is totally focused on the seed sector, and results in visitors from all around the world. Seed meets Technology started in 2014 with 15 participating Dutch companies. In 2017, 43 companies, from Germany to Israel, also joined the exhibition. The expectation is that this number will increase even further in 2018.

Seed meets Technology is a central 'meeting point' in the Seed Valley area for different open field days during the same week.

In week 39, in September, the Seed Valley area again opens its doors to its customers. Besides the open field days at a number of seed companies, Seed meets Technology is an unmissable event. It is now possible for companies to register for the exhibition and Vegetable Trials (demo fields).

High-quality varieties

Expectation is that more than 45 companies from the Netherlands and abroad will display their high-quality varieties, new cultivation systems and seed technology. The fact that Seed Valley is the international centre for plant breeding and seed technology, is demonstrated by the quality and quantity of visitors coming to the Netherlands during that period. Seed meets Technology reached 1,700 visitors last year, of which almost half came from abroad. The target group consists of growers, seed technologists, and resellers of seeds and propagation material. By seed technologists, we mean employees of seed companies, such as laboratory technicians, operators, breeders and researchers.
The fifth edition of Seed meets Technology will again be held in the research centre buildings at Proeftuin Zwaagdijk. Incotec, Leba Metalworks and Seed Processing Holland are also initiators.

Practical information

The concept that the organization pursues is displaying innovation in practice. Seed processing machines are actually demonstrated and the results of new seed treatments are displayed in the greenhouse. An important element is also the vegetable trials. At the demo fields outside, several seed companies display their new range of varieties. It also includes hydroponics, the cultivation of crops on water basins. Besides the technology exhibition and the vegetable trials, there will be symposia and presentations organized by, amongst others, participating companies. The programme will be announced on the website by the summer.

Would you like to exhibit at Seed meets Technology? Feel free to contact Marlies Reus by email: marlies-reus@proeftuinzwaagdijk.nl or by telephone: +31 (0)228 56 31 64.
Keep in contact via social media and the webpage for more info and updates.

Marlies Reus is communication officer and event organiser at Proeftuin Zwaagdijk.

ISTA 2018 Annual Meeting in Japan

Flourishing **Asian market** inviting

Fabiola Rivera

The Asian seed industry has undergone constant and important changes, it has a great potential within the seed industry that is not yet internationally recognized, even though they have a blooming market valued at billions of dollars. The International Seed Testing Association has therefore decided to hold their 2018 Annual Meeting in Sapporo, Japan.

The Asian demand for seeds has expanded as their economies have flourished in recent years and are now considered one of the most important economies in the world. The main factors limiting the market growth include the difficulty of obtaining quality seeds and the domination of the informal seed sector. Despite this, the increasing share of the private sector and the government initiatives in seed policies are driving the market growth and development.

International standards

ISTA decided some years ago to put more effort into the Asian seed market, as the association recognizes the importance of endorsing the regional development and encouraging the use of quality seeds. The association would like to show its commitment to the Asian industry and support it, by facilitating the international seed trade of the region and contributing to the harmonisation of regulatory seed procedures toward internationally accepted standards.

The association has been working for a long time with some laboratories in the area, and only in Japan does ISTA have five accredited laboratories from 66 members in the Asian market. It is only natural that ISTA welcomes an opportunity to get closer to our Asian members and also to let the industry become aware of the association. The organisation has therefore decided to hold their Annual Meeting – which is the most important event for the association – in Sapporo, Japan.

From 11-14 June, ISTA-delegates will meet on Japan's northernmost island of Hokkaido. Sapporo is highly recognized as the best food city and for being the fifth metropolis of the country with 1.9 million citizens. It has also, for a long time, been a local production and consumption leader with an overwhelming food sufficiency rate of 208%, which is five times higher than the country's average.

Governmental support

The Executive Committee members, Masatoshi Sato and Keshavulu Kunusoth, have actively represented the Asian area for several years and made ISTA aware of the difficulties and opportunities of the seed industry market. Dr. Sato, originally from Japan, has enthusiastically supported the organization of this event. He recognises the relevance to the industry that ISTA will be holding the 2018 Annual Meeting in his country. Dr. Sato is a plant pathologist at the Seed Health Laboratory in the National Centre for Seeds and Seedlings (NCSS) in Japan, which is an ISTA accredited laboratory.

It is expected that delegates from more than 50 countries will gather in the 4-day event, where they will mainly discuss and decide upon proposals for changes to the International Rules for Seed Testing in 2019. The Japanese Ministry of Agriculture, Forestry and Fisheries, which is also the national ISTA Designated Authority in the country, will be supporting the 2018 Annual Meeting, for which ISTA is very thankful and pleased. The event will be held at the Hotel Emissa Sapporo, located at Shin Sapporo Station.

The meeting highlight will be the Technical Commit-

More information

The registration fee varies depending on early or late registration, as well as for members and non-members, and we have a special rate for students and for an accompanying person. ISTA also has a one-day option price for the seminar held on 11 July for members and non-members. Review our special rates on our website www.seedtest.org

It is very important for participants to review any visa requirements with the local consular authorities, as certain visitors need to follow special immigration procedures. For more information regarding your visit, please check the website of the Ministry of Foreign Affairs of Japan. Also, an official invitation letter can be provided by the National Organization Committee (NOC) for your visit to the country by emailing: ista_sapporo_visa@sakata-seed.co.jp. On behalf of the Centre for Seeds and Seedlings, Sakata Seed Corporation will take care of the paperwork needed for the visa application. The Sakata Seed Corporation is a member of NOC (National Organizing Committee) for the ISTA Annual Meeting in Sapporo 2018.

The exhibitor registration fee includes one person for the duration of the event, as well as the welcome reception, coffees, lunches and official dinner.

Fabiola Rivera is marketing and communications specialist at the International Seed Testing Association, Bassersdorf, Switzerland, email fabiola.rivera@ista.ch

The ISTA Annual Meeting is the perfect opportunity to learn, discuss and exchange new scientific advancements

tee's work presentation, as it is an opportunity to learn, discuss and exchange new scientific advancements, as well as to meet other world seed experts. The annual meeting programme further includes a seminar on 'Validated Vigour Tests and the Scientific Basis of their Development', which is mainly coordinated by ISTA Vigour Technical Committee. This committee aims to encourage the development and validation of vigour tests, but also to ensure the repeatability within laboratories.

Accreditation for beginners

A workshop on 'Quality Assurance and ISTA Accreditation for Beginners' will follow the meeting between 19-21 June and will be presented by Rita Zecchinelli, Executive Committee Member, and Florina Palada, the Head of Accreditation & Technical Department. The local organiser is Hiroyuki Fujiwara, from the Centre for Seeds and Seedlings NARO (NCSS), and it will take place in Tsukuba city, Ibaraki. The local organiser and sponsor is Food and Fertilizer Technology Centre (FFTC) for the Asian and Pacific Region. The aim of the workshop is to introduce and discuss the basic principles of quality management and focuses on the needs of seed testing laboratories that wish to comply with the ISTA Accreditation Standard and prepare for attaining and maintaining the ISTA Accreditation. The theoretical background will be given by means of English lectures and the participants will be actively involved in group work, discussions and presentations. The system part includes 'how to build quality documentation, document control and internal audits' and 'how to prepare a checklist, corrective actions and non-conforming work'. While the technical part will include how to deal with the equipment calibration, seed dividers and statistical aspects in seed testing.

We invite you to attend the Annual Meeting 2018, the association's most important event of the year, which will allow you to learn more about ISTA's work, committees and community. Finally, looking ahead, we would ask you to save the date for the 32nd ISTA Congress, which will be held in Hyderabad, India, between 26 June and 3 July 2019.

An Overview

Mineral **nutrition** in plant tissue culture

Geert-Jan de Klerk

There is a vast interest in inorganic nutrition in tissue culture. In Researchgate, a chapter on mineral nutrition in tissue culture by the present author received over 20,000 views within a few years. This paper provides a short overview.

Plants require carbohydrates, water and inorganic compounds to sustain survival and growth. Inorganics are supplied by the soil under natural conditions and by the nutrient medium in tissue culture. Tissue culture offers the possibilities to adapt inorganic nutrition relatively, but to do this is still complex and researchers have to be alert to potential pitfalls.

Inorganic nutrition

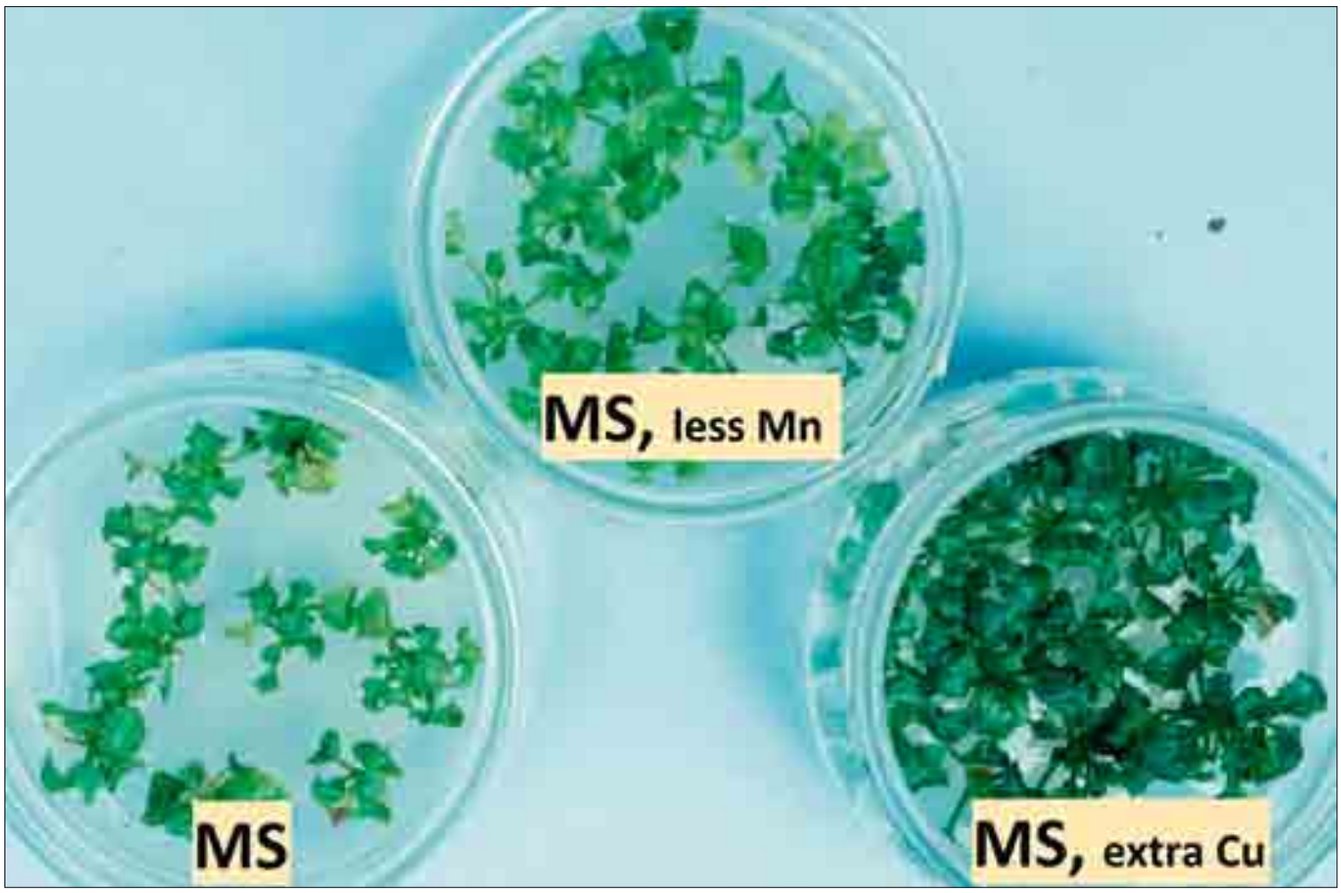
Plants require large amounts of ions of some inorganic elements (macronutrients), viz. nitrogen (N), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) and sulphur (S). They have need of small quantities of ions of some other elements (micronutrients), viz. iron (Fe), nickel (Ni), chlorine (Cl), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo). Together with carbon (C), oxygen (O) and hydrogen (H), these elements constitute the 17 essential elements. Certain other elements, such as cobalt (Co), aluminium (Al), sodium (Na) and iodine (I), are essential or beneficial for some species, but their widespread essentiality has not been established. In the first half of the 19th century, it was still a matter of dispute whether mineral elements do function as nutrients. It was Justus von Liebig (1803–1873) who showed that inorganic materials could provide inorganic nutrients as effectively as organic sources, like humus. Von Liebig established mineral nutrition of plants as a scientific discipline. This led to a rapid increase in the use of mineral fertilizers in agriculture. By the end of the 19th century, especially in Europe, large amounts of potash, super-phosphate and, later, inorganic N were used in agriculture and horticulture to improve crop growth and production. Liebig's conclusion that the elements N, S, P, K, Ca, Mg, Si, Na and Fe are essential for plant growth, was reached by observation and speculation rather than by precise experimentation. The need for microelements was only discovered some 50–100 years later. Since plants in tissue culture depend entirely on added nutrients, discovery of the essentiality of microelements was crucial for successful growth in vitro.

The early tissue culturalists used mineral formulations developed in horticulture, such as Pfeffer's and Hoagland's. The most commonly used formulation in tissue culture is now the one by Murashige and Skoog ('MS'). This medium was developed in 1962 to obtain optimal growth of tobacco callus. Apart from a large number of dose-response curves, they also used knowledge about essential elements and the composition of plant tissue. With respect to the essential element, at the time Murashige and Skoog developed their medium, nickel was not yet considered an essential element. This was only proposed in 1966. As a consequence, there is no nickel in MS. However, nickel is present in agar yielding concentrations of up to 1.1 µM, whereas a concentration of 0.1 µM is reported to be sufficient.

Table 1 shows the composition of MS compared to the mineral composition of healthy plants and compared to modified Hoagland, a modern formulation for a nutrient solution. Major differences between the compositions of MS and plants are the high levels of Cl and Mo and the low levels of Cu, Ca, P and Mg in MS. Interestingly, Hoagland is more similar to plants. MS is used for a very wide range of crops. Experimentation to improve the nutrient formulation for each crop by dose-response studies is very time-consuming because of the large number of elements

	Plant tissue (mmol.kgDW-1)	MS (mmol.l-1)	Modified Hoagland (mmol l-1)	Plant tissue (mol%)	MS (mol%)	Modified Hoagland (mol%)
N	1000	60	16.0	64.4	64.0	53 0
K	250	20	6.0	16.1	21.3	19.9
Ca	125	3	4.0	**8.0**	**3.2**	13.3
Mg	80	1.5	1.0	**5.1**	**1.6**	3.3
P	60	1.25	2.0	**3.9**	**1.3**	6.6
S	30	1.5	1.0	1.9	1.6	3.3
Cl	3	6	0.05	**0.19**	**6.4**	0.17
Fe	2	0.1	0.05	0.13	0.11	0.17
Mn	1	0.1	0.002	0.06	0.11	0.007
B	2	0.1	0.025	0.13	0.11	0.08
Zn	0.3	0.03	0.002	0.02	0.03	0.007
Cu	0.1	0.0001	0.0005	**0.0060**	**0.0001**	0.002
Mo	0.001	0.001	0.0005	**0.0001**	**0.0011**	0.002
Ni	0.001	0	0.0005	0.0001	0	0.002
Na		0.1	0.05		0.11	0.17
total	**1550**	**93.7**	**30.2**	**100**	**100**	**100**

Table 1 The levels of elements in shoot tissue of well-growing plants, in MS and in a modified Hoagland formulation used in horticulture. The major differences between MS and plant tissue are highlighted.



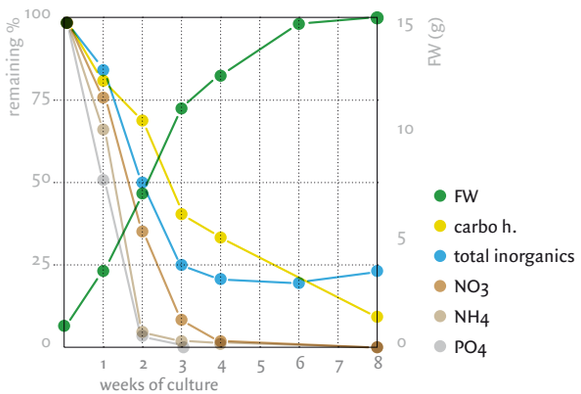
Effect of the micronutrients Mn and Cu on gerbera

and the interactions between elements. In addition, various media have developed that have gained some popularity, such as woody plant medium, Gamborg B5, and several others.

Nutrients, especially micronutrients, are also added via impurities, in particular via agar, as mentioned for nickel. Table 2 shows major inorganic impurities of various agar brands and their relative contribution to MS. Gelrite also contains inorganic contaminations in high concentrations. In addition to inorganic impurities, agar contains many organic impurities that may determine the performance of plants in vitro.

Uptake and transport

Inorganic nutrients are added to plant culture media as salts. In aqueous solutions, salts dissociate into cations and anions. An ion is an atom or a group of atoms, with a positive charge (a cation) or a negative charge (an anion). Roots absorb inorganic nutrients from soil almost entirely as ions. The ions are taken up passively or through active mechanisms involving the expenditure of energy. Both systems are influenced by the concentration of other elements, pH, temperature, and the biochemical or physiological status of the plant tissues. There is competition for uptake amongst the elements. For example, high K+ or Ca2+ concentrations may lead to Mg deficiency, and vice versa.



No extensive studies have been carried out on uptake of nutrients by in-vitro shoot cultures. This is a deficiency in tissue-culture research: as shoot cultures usually have no roots, how are medium components taken up in this case? Shoot clusters in tissue culture usually do have callus and it may well be that this callus functions as an organ for uptake. In tissue culture, uptake is generally proportional to the medium concentration up to a concentration of twice MS. For the plant hormone IAA, it has been shown that most uptake is via the cut surface and that only a small fraction is taken up via the epidermis. The same most likely holds for minerals.

But before being taken up, the nutrients must move to the interface of plant tissue and medium. There are two ways of movement of solutes (compounds dissolved in water): (1) via diffusion and (2) via water flow. There must be some water flow in semi-solid medium because shoots in tissue culture display

	Agar 1	Agar 2	Agar 3	Agar 4	Agar 5	Agar 7	Agar 7	Agar 8	Gelr.
Na	1212	336	3312	1980	2562	3804	684	313	591
S	69	29	29	111	77	98	25	69	0.8
Cu	90	204	108	144	24	96	nd	28	91

Table 2. Increase of the Na, S and Cu concentration in the nutrient medium brought about by contamination of agar (0.6%, originating from 8 companies) or Gelrite (0.2%). Increases are shown as percentage of the concentration of MS. The increase of other elements is maximally 20%.

transpiration albeit very little, but the contribution of both ways of movement to translocation of solutes is not known. Measurements of diffusion of minerals in semisolid medium showed that this movement was, as expected, very slow over relatively long distances (> 1 cm). Contrary to general belief, the speed of movement was essentially the same for the various inorganics and was also not affected by the agar or Gelrite concentration. In liquid medium, the medium becomes exhausted but at different rates for the various inorganics, with PO43- being exhausted at the fastest rate. In semisolid medium, the total medium in a container will hardly be exhausted during a subculture cycle because of the very slow movement of solutes to the explant. Close to the explant though, most components will be taken up within a few days and the rate of uptake after that will depend on the speed of movement in the medium towards the explant.

After uptake, the solutes find their way to vascular bundles that bring them upwards into the shoots using the water flow in these bundles. How this occurs has not been examined and perhaps transport occurs not only in the xylem but also in the phloem. Water flow in the xylem is driven by transpiration. In vitro the atmosphere is very humid, so transpiration is most likely much reduced, and it is not known whether the water flow is sufficient to provide growing tissues with enough nutrients. As the rate of transpiration determines supply of nutrients, it may be a major factor in the determination of the speed of growth. We have found indications that very low transpiration underlies slow growth of tulip shoots in vitro.

Table 3. Summary of the functions of inorganic compounds

Nutrients that are part of carbon compounds, like amino acids, proteins and nucleic acids	N, S
Nutrients that are important in energy storage (ATP) or structural integrity (contribute, e.g., to cell wall properties)	P, Si, B
Nutrients that remain in ionic form, e.g., as cofactors of enzymes or in establishing cell turgor	K, Ca, Mg, Cl. Mn, Na
Nutrients that are involved in redox reactions, e.g., constituents of cytochromes, alcohol dehydrogenase, urease and nitrogenase	Fe, Zn, Cu, Ni, Mo

The functions of the various inorganics

Inorganic compounds have numerous functions in plants, summarized in Table 3. Detailed and excellent overviews are provided in several books on inorganic nutrition.

Adaptation of the mineral formulation

As mentioned previously, MS is the universal medium and seems to be satisfactory for many genotypes and all types of tissue (shoots, roots, callus, etc). Even so, MS will usually will be far from optimal. It is difficult to optimize the mineral formulation by making dose-response curves for the various components. (1) The minerals are either a cation or an anion. When the concentration of a cation is altered, the concentration of an anion should also be changed. (2) Care must be taken for precipitation. Calcium phosphate is often a problem. (3) There are many elements required for fertilization and they often interact.

There are two ways to easily do research on this. Researchers have identified groups of elements and do not alter the concentration of a single compound, but of a group. These groups are: $NH4NO_3$, KNO_3, mesos ($CaCl_2$, KH_2PO_4, and $MgSO_4$), micronutrients (B, Cu, Co, I, Mn, Mo, and Zn), and Fe-EDTA.

A second way is to use of the composition of a healthy plant as the medium: supposedly, each species has its own characteristic elementary composition which can be used to adapt the medium formulation. Such media frequently result in improved growth - in our experience, in up to half of the crops that have been examined. An example of stimulation is gerbera

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