

THE ANNUAL 2014

Journal for breeders and producers of plant material

Prophyta

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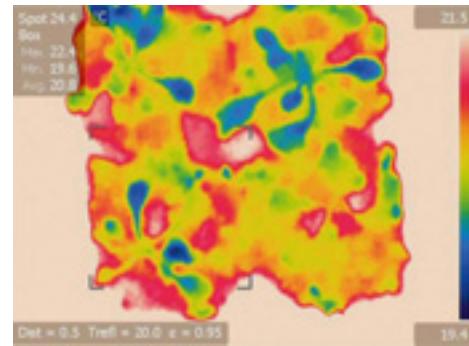
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In Short

Nunhems renamed Bayer CropScience Vegetable Seeds

THIS SPRING, the name Nunhems will no longer be used to indicate the vegetable business unit of Bayer. The name will, however, continue as the product brand. The aim is to clarify that it belongs to the same global company as Bayer CropScience, in which chemical and biological crop protection, biotechnology and consumer products are united. Bayer Crop-
Science Vegetable Seeds will be responsible for all vegetable seed activities worldwide, from research to after-sales. “By displaying being part of Bayer, its long standing history, its large global footprint in life sciences, its innovation power, its great reputation and brand recognition, its commitment to agriculture and its proud support of our vegetable seed business will make this an even stronger promise,” says Joachim Schneider, Head of Vegetable Seeds. The new visual identity for the brand Nunhems reflects the overall company strategy of Bayer - to significantly increase the value of its portfolio in fruit and vegetables. With a yearly turnover of 8.4 billion euro, Bayer CropScience is one of the leading and most innovative companies in these fields.

Fleuroselect announces Gold Medal Winners 2015

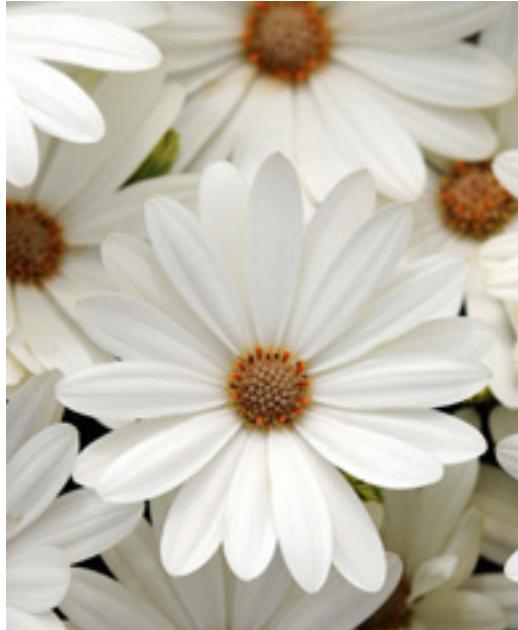
FLEUROSELECT, the international organisation for the ornamental plants industry, has awarded four Gold Medals to varieties that supersede existing ones in terms of breeding innovation and beauty. Fleuroselect's expert and independent judges tested the varieties at trial grounds across Europe. The main criteria are innovation, beauty and use.



‘Florific Sweet Orange’ from Syngenta Flowers is the first bicolor in seed-raised New Guinea Impatiens. Thanks to its excellent branching habit, this *Impatiens hawkerii* is a showy, full plant with huge flowers presenting splendidly above the foliage.



Begonia cultivar ‘California Sunlight’ from Selecta Klemm is a semi-upright, bushy plant with great garden performance all summer long. The jury highly appreciated this Begonia’s improvements at grower level and is convinced that growers and retailers will be equally enthusiastic.



‘Akila Daisy White’ from PanAmerican Seed is the first F1 hybrid seed-raised *Osteospermum ecklonis* with white flowers and yellow discs. Moreover, this new variety flowers earlier and more freely from early spring to late autumn.



‘Bandera Purple’ from Kieft Seed is the first commercially compact *Lavandula stoechas* raised from seed. The judges were particularly impressed with Bandera Purple’s compact plant habit and floriferousness.



Seed meets technology

A GROUP OF COMPANIES in Seed Valley in the Netherlands has organised an event to display the latest developments in seed technology, seed treatment, seed machinery, crops protection and horticultural growing techniques. 'Seed meets technology' will take place at the agricultural research centre Proeftuin Zwaagdijk, from 23-26 September 2014. It will be a platform where companies can display their products in a period when

many so-called 'open days' are organized. Besides innovative new growing techniques, new varieties will be displayed and workshops and masterclasses held. Among the visitors will be retailers of seeds, machinery and crop protection chemicals, large growers and professionals from the seed industry from Europe and abroad.

More information: Ronald Hand, ronaldhand@proeftuinzwaaagdijk.nl

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Reference book on plant breeders' rights

THIS SUMMER, a reference book on the implementation of plant breeders' rights will be published. It is meant to support those countries who

are considering adopting a breeders' rights system based on the International Union



for the Protection of New Varieties of Plants (UPOV), or who have recently became a member of UPOV. The authors, Dr. A.J.P. van Wijk and Dr. N.P. Louwaars, are very experienced in the implementation of plant breeders' rights in countries that lacked a system. The book examines the

worldwide UPOV-system, the development of a variety, how to set up, implement and create acceptance of a breeders' rights system and the costs that are involved. In addition, the book offers insight into the relationship between plant breeders' rights and the international obligations concerning biodiversity.

'Framework for the introduction of plant breeders' rights: Guidance for practical implementation' will be available from June 2014. The book can be ordered from Naktuinbouw. An order form is available on the website www.naktuinbouw.com. The price is € 24.95 (excluding shipping costs, excluding VAT).

More information: info@naktuinbouw.nl

Editorial

A very, very hot China

• **China is hot**, and not only because of its summer temperatures (+31°C in Beijing) or the spicy dishes (especially those of the 'heavenly country' Sichuan). China is 'hot' as it has a thriving economy. According to the IMF, the Chinese economy will increase by a staggering 7.5% this year. And that is the lowest rate for the last 14 years, so it surely has the 'wow factor'. Compare its growth to the meagre 3.6% economic growth worldwide, 1.2% in Europe, 2.8% in the USA, 4.9% in developing countries, 5.4% in India and 1.8% in Brazil, and it is easy to understand that companies worldwide are focusing their attention on this Eastern giant. Economists expect that, within a few years, China will have thrown Europe from its position as second trading region and that its GDP will be higher too. The only reservation: the Chinese government should continue their economic reforms.

The enthusiasm to be part of this success is brimming. Foreign politicians chase each other away, organising trade missions to introduce the companies from their home country to this 'promised land'. As a trade nation by nature, the Dutch government will even send their Prime Minister, Mark Rutte, twice in one year, just to give an example of the eagerness. It has become the prevalent market for many companies.

The agricultural and horticultural sector has known this for years and started to invest in China a long time ago, with the seed sector taking a leading role. Sharing knowledge on, for instance, plant breeders' rights, making better varieties available and displaying modern agronomic technology, helped to promote the rapid development of the rural economy. And with success. China has become a large agricultural trading nation and has a huge impact on the international market. It also led and promoted the reform of China's economic system in full swing and supported the rapid growth in China's economy.

When the Chinese President, Xi Jinping, recently visited the Netherlands, he wrote a letter to one of the newspapers. In it he said: "According to an old Chinese proverb, 'a suitable moment is less useful than a favourable geographical location, while a favourable geographical location is not as important as a harmonious relationship'. The world has need of development and development requires peace. The Chinese people, just as the population of other countries, have need of peaceful international surroundings to develop their country." Hopefully the ISF congress will contribute to this harmonious relationship.

Monique Krinkels

SHARING PASSION FOR NATURE



ISF World Seed Congress 2015

Kraków | Poland | 25 - 27 May



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We warmly invite you to Kraków to share our passion for nature and agriculture.

Organisers



Support the Foundation

The Prophyta Foundation is an independent non-profit organisation, aiming at informing interested parties worldwide about developments in e.g. plant breeder's rights, breeding techniques, genetics, biodiversity, technology, regulations, phytosanitary matters and more. Our communication methods include at present our Prophyta Annual and our website www.prophyta.org. The Foundation primarily works with volunteers, but in order to recover costs for these activities we need advertisements for our annual magazine and/or direct financial support to the Prophyta Foundation.



We greatly acknowledge the companies mentioned below for supporting Prophyta, by either advertising or donating. Please feel free to contact our secretariat (p.o. Box 40, 2370 AA Roelofarendsveen, the Netherlands, email: foundation@prophyta.org) to join membership of our distinguished group of agricultural and horticultural companies, both for further information or for donations.



ABZ Seeds	Bovenkarspel, the Netherlands	www.abz-strawberry.nl	F1-hybrid strawberries
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Agronomix	Winnipeg, Manitoba, Canada	www.agronomix.com	Computer software
Bejo Seeds	Warmenhuizen, the Netherlands	www.bejo.nl	Vegetable seeds
Corn. Bak	Assendelft, the Netherlands	www.bromelia.com	Bromeliads
De Groot en Slot	Broek op Langedijk, the Netherlands	www.degrootenslot.nl	Allium
Enza Zaden	Enkhuizen, the Netherlands	www.enzazaden.com	Vegetable seeds
Fleuroselect	Noordwijk, the Netherlands	www.fleuroselect.com	Organisation for ornamentals
Naktuinbouw	Roelofarendsveen, the Netherlands	www.naktuinbouw.nl	Inspection, varieties, testing
Nunhems	Haelen, the Netherlands	www.nunhems.com	Vegetable seeds
Rijk Zwaan	De Lier, the Netherlands	www.rijkzwaan.nl	Vegetable seeds
Suet Saat- und Erntetechnik	Eschwege, Germany	www.suet.de	Pelleting and seed coating
Syngenta Crop Protection	Basel, Switzerland	www.syngenta.com	Seed protection
Takii Europe	De Kwakel, the Netherlands	www.takii.nl	Vegetable and flower seeds
Takii & Co., Ltd.	Kyoto, Japan	www.takii.co.jp	Vegetable and flower seeds
American Takii, Inc.	Salinas, CA, USA	www.takii.com	Vegetable and flower seeds
Vandijke Semco	Scheemda, The Netherlands	www.vandijksemco.nl	Seeds and services

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'A platform for exchanges and cooperation'

Zou Dan and Monique Krinkels

8 **Usually, the president of the National Organizing Committee is a director of one of the leading seed companies of a country. Not so this year. Mr. Lin Keqing is one of the eight Vice-Mayors of China's capital city Beijing. It reflects the importance the host country ascribes to the World Seed Congress.**

With 1.36 billion people, China is by far the largest country in the world when it comes to population. Measured in surface, only Russia, Antarctica, Canada and the USA are larger. The cultivated land area in China is 133 million hectares, thus per capita less than 0.1 hectare is used for food production. That means that 9% of the world's arable land feeds 21% of the world's population.

It is the first time the World Seed Congress will be held in your country. Is there any special reason for the event taking place in Beijing this year?

"Yes, this is China's first time ever to host the World Seed Congress. China is a large agricultural country, but also a country demanding large amounts of seeds. The Chinese government attaches great importance to the development of the seed industry, providing guidance to the planning of the industry, innovating institutions, improving laws and regulations, strengthening technological innovation, nurturing and promoting improved varieties. Zhongguancun National Innovation Demonstration Zone in Beijing has gathered a large number of domestic and foreign high-tech seed companies. We hope to create a platform for trade, industry exchanges and cooperation, learn from the positive experiences of the international seed industry, strengthen industry exchanges and cooperation between China and the world and promote the development of China's seed industry by hosting this World Seed Congress."

When did you start as President of the National Organizing Committee?

"I serve as Vice-Mayor of Beijing. In early 2013, I started as President of the NOC for the 2014 World Seed Congress. I am in charge of the organization and coordination work during the preparation under the commission of the Mayor of Beijing, Mr. Wang Anshun. We hope the world will come to know Beijing and China's seed industry through this opportunity. We expect approximately 1,400 delegates, who will discuss, amongst other things, international trade, revision of the International Seed trade rules and have academic exchanges. We will serve as we promised to the delegates and guests from all over the world."



Lin Keqing (born 1966) is a native of Xiantao City, Hubei Province. He entered the work force in 1988, and joined the Communist Party of China (CPC) in 1990. From 2008 to 2013, he was Secretary of CPC Beijing Municipality Daxing District Party Committee. Since January 2013, he is Vice-Mayor, People's Government, Beijing Municipality.

Were you involved in agriculture before?

"I graduated from the School of Philosophy at Renmin University of China and received my PhD in philosophy. As Vice-Mayor of Beijing, I am in charge of rural areas, agriculture, water and landscape work of the city. Before that I was governor of the Daxing District in the South of Beijing. Daxing District is an agricultural region growing maize, wheat, vegetables and fruits. Especially the watermelons from the town of Panggezhuang are nationally famous. That is why I am quite concerned about the development of China's seed industry and the trends of the world seed industry."

China is a vast country with a great diversity in climates and soils and it is rich in biodiversity. How do Chinese breeders use that abundance?

China, as a vast country with a long history, is occupied by many different types of soil, which is favourable for a good diversity of plants. Our ancestors learned a long time ago to collect germplasm and used it in agricultural production. Currently, there are about 400,000 crop germplasm copies reserved in the National Genebank. Each province, municipality and autonomous region of China also reserves crop germplasm resources according to their soil type and climatic characteristics. These resources are public resources that can be provided to seed companies and breeders without payment. In addition, seed companies also have their own germplasm reservation where the ownerships belong to the enterprise. Taking advantage of the rich germplasm, Chinese breeders have bred an abundance of improved crop

Zhongguancun Street in Beijing is also known as China's Silicon Valley, one of the most important technology centres throughout the world



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Zhongguancun

Zhongguancun National Innovation Demonstration Zone is the most intensive scientific, education and talent resource base in China. It boasts almost 40 colleges and universities such as Peking University and Tsinghua University, more than 200 national (municipal) scientific institutions such as the Chinese Academy of Social Sciences and the Chinese Academy of Engineering, 67 state-level laboratories, 27 national engineering research centres, 28 national engineering and technological research centres, 24 university S&T parks and 29 overseas student pioneer parks.

varieties. Totally 1,632 crop varieties - including corn, rice, wheat, cotton, soybeans and others - were bred nationwide in 2013. These varieties contribute greatly to the 60 million tonnes of grain production in China."

What has been the most important breakthrough in plant breeding that China has offered the world?

"The most important breakthrough that China has brought to the world in plant breeding is hybrid rice. It greatly improves the yield. In 2002, Yuan Longping's Super Rice exceeded 13,500 kg per hectare. If these new hybrid rice varieties and new technologies are promoted to the world, they will certainly play an important role in food production and contribute to the solution to hunger and poverty in parts of the world. In addition, maize, wheat and other crop yields and total production also increases year on year, the role of breeding new varieties cannot be ignored."

China faces the challenge of feeding a huge population. How are breeders/the seed industry meeting this endeavour?

"China is a large agricultural country, and agriculture plays a very important role in the national economy. China has achieved an increase in grain yield over ten consecutive years from 2004 to 2013 through the efforts of all parties. Grain production in 2013 is more than 60 million tonnes.

The Chinese government has always attached importance to the development of the seed industry, providing support in terms of policy and funding. Through the joint efforts of breeders and seed men, China has now built up a modern seed chain with integrated

'breeding, propagation, promotion'. Particularly, the promotion of hybrid rice plays a very significant role in the increase in China's grain production. Hybrid rice is not only cultivated in China now, but also exported to Asia, Africa, South America and many other countries, which increases local grain production, and to some extent protects food security."

Has China accepted biotechnology as a means to boost agricultural production?

"China's attitude towards the application of genetically modified crops is clear and consistent. That is: the research must be positively supported and adhere to independent innovation, while promotion and application of GMO should be cautious to ensure safety. Currently, cultivation of GM crops is mainly in cotton in China, considering consumers' concerns about the safety of GM foods. Due to the use of cotton as a fibre and not for consumption, GM cotton has been used in China for over a decade. Other GM crops such as maize, wheat, rice are currently in the research stage, not in promotion and application.

GM crops have also been discussed in China. The government has set up a strict evaluation system and management system. Research institutes and seed companies also follow the two principles above. The Chinese government also attaches great importance to the views and suggestions of seed companies, would take measures regularly to listen to their views, including the reasonable opinions and suggestions about the relevant rules and regulations. Seed associations at each level are open to the views of their seed members, the constructive advice is forwarded to governments at corresponding level to ensure they are adopted in policy formulation."

Governments should encourage collaboration

Zhen Liu

10 For her thesis, Zhen Liu compared two similar business sectors in two different parts of the world, China and the Netherlands. She investigated the influence of innovation networks, absorptive capacity and other key factors on innovation and business performance of vegetable breeding companies.

• **The seed business** plays a crucial role at the basis of the food supply chain, and companies which are active in plant breeding, production and sales of seeds are embedded in a competitive environment. They continuously face challenges to develop higher yielding varieties with better or new qualities, optimized for sustainable production under a wide variety of growing conditions. To meet those challenges, innovation through R&D is extremely important.

Fast development

In China, the vegetable breeding industry is developing fast, has access to an exceptionally large internal market and is experiencing a transition from a planned to a market economy. In 2013, the total number of licensed seed companies was approximately 6,500. Most of them, however, are only active in seed trade. There were 112 integrated vegetable breeding companies (VBC), active in breeding new varieties, seed production and sales. These companies were divided into public VBCs, often originating from vegetable research institutes; domestic private VBCs; and foreign private VBCs, including wholly foreign-owned subsidiaries and joint ventures.

In contrast to the situation in China, the vegetable breeding industry in the Netherlands is an established industry, which has developed into one of the most innovative and outstanding in the world. It accounts for one third of the world's vegetable seed exports and one eighth of the world's vegetable seed imports. Due to a period of consolidation, the top ten vegetable breeding companies in the world account for over 85% of the global vegetable seed market. Many of these companies originated in the Netherlands or have important R&D facilities in the Netherlands. Innovation is important for the vegetable breeding industries in both countries, although they are at different stages of development.

Knowledge is the most important source of a company's sustainable competitive advantage, and it is the basis for its core competencies, especially the capability of innovation. In this study, the knowledge-based view was, therefore, used as a framework to analyse the factors influencing innovation and business performance. The Sectorial Innovation System (SIS) was further developed with emphasis on analysing the knowledge flow between different

domains (business, research & education, and intermediate organizations) and the institutional aspects that affect knowledge stocks and knowledge flow (market demand and the infrastructure & framework conditions).

Knowledge flow

One of the major results of this study was the observation that lack of interaction and knowledge flow between different domains constrains the innovation of the vegetable breeding industry in China. In contrast to this, it was shown that the exceptional innovation level of the vegetable breeding industry in the Netherlands is not only based on an outstanding performance of each domain within SIS separately, but also on good interactions between different domains. So both studies in China and the Netherlands indicated the importance of interaction between different domains for innovation in the sector, but the approaches to stimulating innovation at the sector level were quite different.

In the past, the Chinese government played multiple roles, with public organizations being active as important players in all three relevant domains within the sector: business domain, research & education domain and intermediate domain. Large governmental investments in research organizations and state-owned companies have discouraged investments by private companies. Besides, there were only a limited number of intermediate organizations that aim to stimulate and facilitate collaborations in R&D. So the innovation network has been constrained and this has led to the limited knowledge flow between and within the different domains.

By contrast, the players in the different domains of the Dutch vegetable breeding industry are specialized within their domain and intensively collaborate with players in the other domains. The government plays a supportive and facilitating role to encourage different players to innovate in their own domains through favourable policies and stimulate initiatives to build collaboration platforms of players across domains. The R&D activities of organizations within the different domains have built knowledge stocks and absorptive capacities, while innovation networks are organized to facilitate the knowledge flow between different domains.

Zhen Liu, PhD, Business Developer for the Food and Biobased Research group of Wageningen UR, Shanghai, China.

The article is based on her PhD research 'Vegetable breeding in China and the Netherlands: a study at sectoral company and project level'.



Interaction and knowledge flow between different domains promote innovation

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Therefore, key drivers for a successful innovation system are the specialization of players within their own domains and collaboration of players across different domains. Constraints in knowledge transfer between the domains can be considered one of the main barriers to innovation.

Two strategies

Taking into account the technological and managerial complexity of product development, companies are no longer able to do all innovation activities within their own premises, but are embedded in dense networks of contacts and collaborations with external innovation partners, such as supply chain partners, universities and research institutes, intermediate organisations, consultants, governmental organizations, and even competitors. It requires companies to have the absorptive capacity to recognize the value of new, external information, assimilate it, and apply it to commercial ends. This part of the study led to the conclusion that VBCS can achieve improved business performance using two main strategies. The first is an absorptive capacity strategy, by which a company improves its absorptive capacity, then achieves a higher innovation level and increases its competitive strength. The second is an innovation network strategy, by which a company achieves better business performance by improving its innovation network. It was shown that the quality of the external network mediates the effect of potential absorptive capacity on innovation output; the internal innovation network was shown to be positively related to innovation output. So the innovation network strategy could enlarge the effect of absorptive capacity by accessing and making effective use of external knowledge. It is recommended that VBCS combine these two strategies to extend and improve their external network and to improve their absorptive capacity in order to be able to make effec-

tive use of this external knowledge. Another important aspect of innovation is the conclusion that the four stages of innovation processes (acquisition, assimilation, transformation and application) dynamically interact with each other, and communication capabilities play an essential role.

Team communication proved to be important in the acquisition and assimilation stages to identify, discuss and interpret external information for use in an innovation project. Cross-functional communication was especially important at the transformation and application stage, where it is known to help overcome barriers created by the novelty of an innovation project.

Implications

In general, this thesis provides more insight into the role of innovation network and absorptive capacity, two important parameters in understanding innovation and business performance at different levels. The implications of this study for the vegetable breeding industry can be summarized as follows. Governments should encourage both specialization and collaboration of players in the different domains of an industrial sector to achieve better sectorial innovation performance. It is important for the players in the different domains of the vegetable breeding industry to accumulate knowledge by their own investment in R&D and gain external knowledge by collaboration. Companies should invest in both innovation networks and absorptive capacity, because the innovation network will enlarge the effect of absorptive capacity on innovation output and business performance. Furthermore, companies should encourage both team and cross-functional communication to ensure better project performance. Team communication plays an important role in the acquisition and assimilation stage of an innovation project and could help the project team to develop new ideas and to identify market potential. Cross-functional communication is important at the transformation and application stage and will help to develop related functional capabilities for supporting the development and marketing of new products.



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New project strengthens bond between China and the Netherlands

Chris van Winden

Dutch companies in the field of propagating material have been active in China since the 1990's. By doing so, China gets better access to high-quality varieties and propagating material and the market for their own varieties increases.

- Since several years, a close cooperation between the Netherlands and China in this field takes place.
- This cooperation is supported by both governments.
- From Dutch side, Naktuinbouw, the Centre for Genetic Resources Netherlands (CGN) and Plantum are involved in the project activities. Furthermore, the Dutch breeding companies active in China, are very cooperative in the various activities.

Since China has joined the UPOV Convention 1978, relatively recent (in 1999), many activities were initially focused on setting up a good system of Plant Variety Protection (PVP). This included also lateral aspects like creating awareness among Chinese stakeholders, enlargement of the list of crops for which plant variety protection may be requested, and enforcement of plant breeders' rights. Besides plant variety protection, also the conditions of establishment for foreign companies in China and the exchange of genetic material between the two countries have been discussed in previous projects.

New project

In a new collaborative project, initiated in 2013 and funded by the Dutch government, a further elaboration of the topics mentioned above is given. In the field of plant variety protection, attention is now focused on improvement of the PVP-system. A number of issues has been identified by private companies, based on their recent experiences in China. These issues will be discussed with the Chinese plant variety office to improve efficiency.

A key point is the length of time between application and granting of plant breeders' rights. Also in the field of communication and the supply of plant material, improvements can be made. The Netherlands is also willing to train Chinese experts in DUS testing in order to facilitate the Chinese plant variety office to enlarge the list of crops that can be protected. Furthermore, China and the Netherlands can offer each other much support in the field of genetic resources. In an earlier project, the laws of both countries were examined. The continuation is now focused on the ABS (Access and Benefit Sharing) conditions by conducting a pilot project. For this pilot project, the crop chrysanthemum is chosen. A third issue is the establishment of conditions

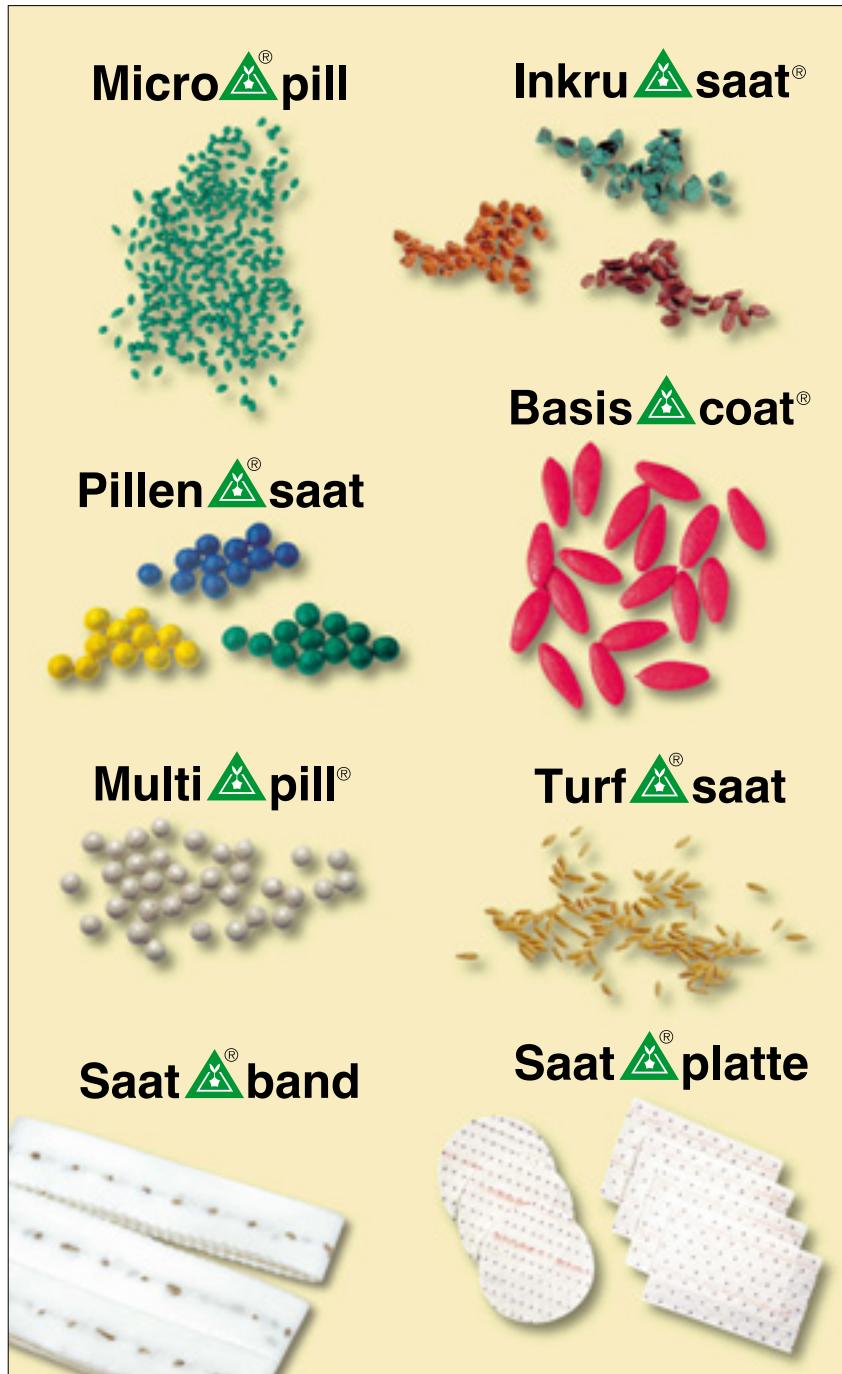


Chinese growers would benefit from the availability of modern varieties

for foreign (breeding) companies in China. These conditions should be established in such a way that foreign companies can perform their operations in China while maintaining their own independence as such. Changing Chinese legislation could hamper the activities of foreign companies in China. And this again could also have consequences on the availability of modern varieties for Chinese farmers and growers. That is an important point to discuss with the Chinese government.

The activities in the new cooperative project will be realized over a period of 3 years.

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Looking back at 7,000 years of farming

John van Ruiten

Being one of the eight Vavilovian centres of origin of cultivated plants and being an area in which there is a long tradition of domestication of species and development of agriculture, it is worthwhile to look at some historical aspects of Chinese agriculture and food production.



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Hosting the ISF World Seed Congress, China is not only offering opportunities to get acquainted with its nowadays rapid developments in agriculture, it also gives the delegates an opportunity to get in touch with the enormous historical background of food production in this huge country. Currently, China as a country is both the biggest producer and also the greatest consumer of agricultural products in the world. Almost 1.4 billion people (20% of the world population) have to be fed with produce coming from 10% of the world's available arable land. And historically those percentages have over many hundreds of years been the case! Combined with insecure water supply and catastrophes (big droughts interspersed with heavy rainfall and flooding), this has throughout the centuries very regularly led to chronic food shortages. Therefore it is not surprising that intensifying agricultural production has been a topic of interest for ages, for both farmers and also emperors.

The change from 'hunting, fishing and gathering' by

nomadic tribes towards cultivating plants, gardening and later on farming in China occurred around 6000-7000 BC, in fact shortly after the last Ice Age. As in other parts of the world, it was mostly women that took care of sowing and nursing plants. Gardening was predominantly a female activity; certainly in the period that villages were not permanent places to live, but only temporary settings for 5-6 years. Only after the moment that animals like cows were domesticated in China and horses came in from other regions (Middle East), were the first 'permanent villages' created around 4000 BC, and from that moment onwards, man started to be active in farming due to heavy work, such as ploughing and bringing manure onto the fields. It is also the time that the use of tools (mainly made from bamboo and iron) started.

It is believed that agriculture in China developed separately in two different areas, in the north (north of the Yellow River) with a cool and dry season and in the south (the Yangtze region) with a warm and wet season. The most important basic food crops were

16 'There must have already been contacts with China in the time of the Egyptians'

- millet (*Panicum miliaceum*) in the north and rice (*Oryza sativa*) in the south. For these crops, China is in the middle of the genetic centre of origin, and
- there is still great variation in these genera and species to be found.

Centre of origin

China is also known to be the centre of origin for crops such as tea (the most popular beverage of all time), bamboo, cherries, almonds, walnuts, but also vegetables such as radish, carrots, Chinese cabbage, cucumbers, garlic and onion originate from this region (and were domesticated there). Hemp (*Cannabis sativa*) was grown for eating seeds, fibres for clothing and also the anaesthetic properties were known thousands of years ago.

And although the name *Prunus persica* (peach) suggests differently, this tasteful fruit also has its development roots in China. Findings in archaeological sites show evidence of its presence and use already in 3000 BC. The peach got its name when it was imported into Rome around 300-200 BC and was thought to come from Persia. It was Alexander the Great who, through various conquered countries, stood at the basis of the development of the so-called 'Silk Road', which remained the most important trade route between Europe and China for over 1,500 years (Marco Polo being one of its most well-known travellers).

Interestingly enough, it is known that there must have already been contacts with China in the time of the Egyptians, because silk elements were found in mummies. Mulberry (*Morus alba*) and silkworm culture (the first known domestication of insects!) date back to at least 4000 BC. In biblical times, spices common in China, like cinnamon and cassia, were transported to the Middle East.

Kung fu-tzu

Remaining written evidence about plants in China goes back as far as 500 BC. The well-known Confucius ('kung fu-tzu', kong master) wrote three of the most important books in the history of China: the book of history, the book of changes (I Ching) and the book of songs. In these three books, Confucius described China in the ages of the Zhou Dynasty. In the book of songs, there are hundreds of references



to food and drinks, giving an 'agricultural picture' of that time period.

But not only food was important. Floriculture, considered as one of the seven arts, was already practiced before 200 BC. Still today, the four seasons in China are visualized and identified with flowers: peach blossoms in spring, lotus flowers mark summer time, chrysanthemums announce the autumn and the winter period is pictured with narcissus.

Confucius described the use of 44 food plants in his books, but for agricultural and technical descriptions, we had to wait several hundred years. It was in the 1st century AD that an agricultural manual was written by Fan Sheng-chih. In this series of books, descriptions on among others irrigation, crops scheduling, composting techniques, use of iron tools, biological control of insects (in litchi/orange cultivation) and pre-treatment of seeds were given. The pre-treatment was soaking seeds before sowing in a 'fertilizing substance' made of cooked bones, manure and plant poisons, like Aconitum.

The period between 200 BC and 200 AD was, all in all, an interesting period and very important for the development of agriculture in China. New crops were imported, pickling and salting techniques to preserve food were developed, fermentation was perfected,



Over time, the Chinese developed an agricultural system of 'organic' production with recycling of all possible nutrients. Everything organic, including bones, leather, manure and straw was brought back on the land. But as populations continued to increase, the lack of innovation (and with that the lack of modern agriculture with fertilizers, crop protection, technology) eventually resulted in stagnation. Combined with massive erosion, deforestation and bad weather conditions, this led to very regular periods of famine and great poverty in rural areas.

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noodle producing technology was developed. Cooking transformed from a technique for preparing food into an art, using animal parts like 'shark fins', water snakes, bird nests, grasshoppers and seaweed. In fact, this Han Dynasty period shaped China and its food behaviour as it is today.

While around 50 AD, 75% of China's population lived north of the river Yangtze, by 1250 some 75% of the population lived south of the river. Such large scale migration was possible due to the introduction of quick ripening strains of rice from Vietnam, suitable for multi-cropping.

Colombian exchange

A big change occurred when the so-called Colombian exchange started after 1492, when the old and new worlds were connected. Also, very soon after 1500 AD, trade started between China and the new world (directly but mostly through trade posts in the Spanish Philippines, Manila) and new world crops found their way to China at the end of the Ming Dynasty period. These new crops were very much favoured and liked in China. Peanuts (1538), maize (1555), tobacco, sweet potato, potato, tomatoes and hot peppers are amongst the crops that nowadays are inseparable from the Chinese kitchen.

Close connection

The last big change in the agricultural system of China came with and after the Civil War and the Cultural Revolution. In 1952, land was taken from landlords and given to 300 million small, peasant family farmers. Some years later (1958), Mao declared the Great Leap Forward which also had its great effect on agriculture. Farmers were forced into collectivity (communes and cooperatives) and an industrial approach of agriculture was advocated. It was not a success at all. Already soon after the Great Chinese Famine in 1961, it became clear that close connection and adhesion to land and crop for a farmer is a necessity to be responsible and to work hard. Since the end of the 1960s, gradually further economic and agricultural reforms have been made, to give agriculture and horticulture the form that it has today. Migration of many peasants to industrial areas on the east coast, pollution of the soil and shortages of food led to new problems.

Still, feeding its own population is (as it always has been) one of the great challenges for China. Sustainable intensification, introducing and breeding new varieties, new technology and other market mechanisms must support the Chinese government in reaching that goal.

Seed companies extend a helping hand

Monique Krinkels

18 **Where farmers and growers in Western Europe have seen their harvests increase by double digits, smallholder farmers in developing countries still have to struggle to make a living from the land. The causes: no knowledge of up-to-date agronomical technology, lack of adequate plant protection measures and fertilizers, and no access to high quality seeds of modern plant varieties. Seed companies extend a helping hand.**

• **Westland, the Netherlands, 1954:** in a simple greenhouse, a grower picks 6 kilos of tomatoes per square metre per year. Sixty years later, the greenhouse is a high-tech, fully automated building with LED-lights where his grandson or granddaughter harvests 80 kilos of tomatoes on that same square metre. On top of that, only half the amount of water is needed and diseases and insects are controlled by biological means, so the impact on the environment is minimalized. Most of the tomatoes are meant for export, as growers in the Netherlands produce an abundance of vegetables. Far more than his or her countrymen are able to consume.

How different is the story in developing countries? The differences between 1954 and 2014 are minimal and farmers still have a hard time making a living. Using age-old self-reproduced landraces might sound romantic to laypersons, but in truth the productivity is far lower, the need for water is larger and the susceptibility to diseases is greater compared to modern varieties. That combined with the fact that smallholders are typically farming in the less fertile grounds and do not have access to modern agronomical techniques, threatens their livelihood. And while we can see the effects of climate change in Europe in slightly warmer, wetter weather conditions, in the tropics it causes either droughts or torrential rains, wreaking havoc with the crops in the fields.

The quality of the seeds is but one element in this dramatic picture. Seed companies, however, have felt since way back that they should lend a hand to these farmers. It is not by chance that food security plays an important role in their external communication. Time after time, they warn that the world faces a serious endeavour if we are to provide nine billion people with sufficient, safe and healthy food by the year 2050. And the one source of food is plants. Besides that, more and more plants are needed for clothing, energy and industrial raw materials.

There are several ways in which the problems of smallholder farmers can be tackled. A few examples.

Breeders without borders

The latest initiative to make a difference to smallholders comes from Anthony Leddin, a research manager and plant breeder at Valley Seeds, Victoria, Australia. He developed the concept of Breeders

Without Borders, a plan similar to the successful Doctors Without Borders.

The idea is that experienced plant breeders, undergraduate and graduate students volunteer and are then matched with overseas projects that are looking for plant breeders. These projects would mainly deal with underutilized species where no breeding work is being done to avoid conflicts of interest. The volunteer plant breeder along with the undergraduate and graduate students would spend a period of time in the country, working with local farmers/agronomists to acquaint themselves with common plant breeding methodology.

The projects will stimulate community based breeding where the farmers will eventually run the programme and can be supported by the volunteer plant breeder back in their home country. This will start the new generation of plant breeders in the developed and the developing world as young people will see how interesting plant breeding can be. Young plant breeders will gain experience from more senior plant breeders and their knowledge will not be lost. These young people will come back to their countries being more practical and resilient breeders, a trait that is needed with the many cutbacks seen in plant



The latest initiative to help smallholder farmers comes from Australia, where Anthony Leddin initiated Breeders Without Borders



In Sudan, farmers have to transport water to their fields. The country remains one of the least developed nations in the world with water supply problems

breeding projects around the world.

It is all about knowledge transfer, according to Anthony Leddin. "There are a lot of plant breeders from the green revolution time retiring and if we don't transfer their knowledge to a new generation of plant breeders, it will be lost."

So far, four universities in Australia have shown an interest in undergraduate mentoring by a senior plant breeder at a project, and those universities are willing to fund the students' travel expenses. FAO (Food and Agriculture Organization of the United Nations) is interested in the undergraduate mentoring aspect as well. There are a number of volunteer aid organizations around the world that are willing to fund plant breeders to travel to the project sites.

The present phase of the project is that Anthony Leddin is looking for 'champions' from around the world who can promote Breeders Without Borders and also a major sponsor that could get the ball rolling with funding support.

More information: the International Seed Federation has invited Anthony Leddin to explain his project during the plant breeders meeting at the ISF congress in Beijing, China. Furthermore, he has a stand on Level 1 in the Orient MGM International Conventional Hotel.

Ranking a top ten

An approach to stimulate seed companies to do more for smallholder farmers is the Access to Seeds Index, an organisation initiated and financed by the Dutch government. Following the example of the Access to Medicine Index, the plan is to publish every two years

a ranking of companies that support commercially sustainable ways that provide farmers with access to good seeds. The list will identify positive actions and best practices. It is hoped that peers will want to emulate. The goal is to enable farmers to grow more diverse and healthier crops, achieve superior yields and build a successful business. This may contribute to food security and help to reduce poverty.

Last year, it received a positive reaction in Addis Ababa, Ethiopia, from representatives of farmers' organisations. In October 2013, seed companies and other stakeholders met in Washington D.C. to discuss the matter. So far, the seed companies show mixed feelings. They wonder how the ranking will be made, and whether the ranking will be a pat on the back of those who top the list, or a disgrace to those who end lower. Furthermore, the competition is felt to be unfair, as not all companies are involved in global markets or have knowledge or products that are suitable to smallholder farms in Africa. On the other hand, for multinationals with a wide range of products it might turn out to stimulate them to show their corporate social responsibility.

The idea is to create two indexes: one for field crops and one for vegetables. The methodology of the index, the technical areas and indicators, are all based on a multi-stakeholder agenda developed in a round-table dialogue with all the relevant stakeholders.

Seed companies will be asked to fill in an elaborate questionnaire and these data will be combined with publicly-available information on those companies that decide not to participate voluntarily.

More information: www.accesstoseeds.org

Cowpeas are rich in quality protein and have almost the equivalent energy content to that of cereal grains, are a good source of quality fodder for livestock and also provide cash income



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- The African Agricultural Technology Foundation (AATF) is a non-profit foundation, managed and led by Africans to foster public-private partnership. The aim is to give smallholder farmers access to appropriate agricultural technologies. The organisation was incorporated in the United Kingdom in January 2003 and registered in Kenya, its host country, in April 2003. The projects are focused on mechanisation, but also on producing better varieties, for instance the cowpea project. Cowpea (*Vigna unguiculata* L. Walp) is considered the most important food grain legume in the dry savannas of tropical Africa, where it is grown on more than 12.8 million hectares of land. It is rich in quality protein and has almost the equivalent energy content to that of cereal grains, is

a good source of quality fodder for livestock and also provides cash income. Nearly 200 million people in Africa consume the crop. Many biotic and abiotic factors greatly reduce cowpea productivity in the traditional African farming systems.

Among these constraints is the pod borer, *Maruca vitrata*, which perennially damages cowpea pods on farmers' fields. In severe infestations, yield losses of between 70-80% have been reported. Control through spraying with insecticide has not been adopted by farmers due to the prohibitive costs, causing resource-poor farmers to opt for cheaper but more toxic alternatives that impact their health. The project aims to create GM varieties that are resistant to this harmful butterfly, with amongst others the help of

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Vegetable production provides a way out of poverty for smallholder farmers and the landless in Tanzania. It generates more income and jobs per hectare than most other agricultural enterprises



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Monsanto, which has vast experience with modifying crops with the cry1Ab gene, which can protect cowpea against the Maruca pod borer.

More information: www.aatf-africa.org

Founding a subsidiary

The most far-reaching help of a seed company to smallholder farmers is to create a subsidiary solely devoted to breed varieties of local crops. That is exactly what Rijk Zwaan and East West Seed did in 2008 in Arusha, Tanzania. Afrisem breeds hybrid vegetable varieties, that are resistant against local diseases and pests, and fit in with the wishes of the local consumer market. The seeds are reliable and affordable and, furthermore, the company shares its knowledge on growing techniques.

In Africa, the consumption of vegetables is less than 50 kilos per person per year, while it is the most sustainable strategy to overcome micronutrient deficiencies. Improving vegetable varieties creates a win-win situation for local growers, by providing income as well as healthy food. Afrisem concentrates on four crops, combining local germplasm with the breeding knowledge of the two parent companies.

African eggplant, *Solanum aethiopicum*, is an indigenous and very popular vegetable in Tanzania. The open pollination (OP) varieties are low in production, have a limited shelf life and are susceptible to red spider mite, a major pest in Tanzania. Afrisem will introduce varieties of the bitter and the sweet type, with increased yield, improved shelf life and a better level of resistance to red spider mite.

The extremely hot chinense pepper, *Capsicum chinense*, is not indigenous, but locally very much preferred because of its unique aroma. The Afrisem chinense pepper varieties will offer high early yield,

improved shelf life and increased disease resistance. African kale, *Brassica oleracea*, is a non-heading cabbage. Individual leaves are harvested on a weekly basis and leaves are bunched to be sold. Currently, the varieties grown are OPs which are often very non-uniform. Afrisem develops hybrid varieties with superior yield and leaf characteristics. Tomato, *Solanum lycopersicum*, is by far the most important vegetable crop in Africa. The tomato breeding programme at Afrisem is a Rijk Zwaan activity. Local varieties are very susceptible to a wide range of pests and diseases and farmers need to spray often to control diseases. Therefore the breeding programme focuses on creating varieties with superior resistance characteristics. Improved shelf life and high early yield are the main breeding goals.

This January, Afrisem opened the field days showcasing selected Rijk Zwaan varieties, as well as African Eggplant hybrids that are not yet commercially available. Guests from Senegal, Rwanda, Burundi, Kenya, Uganda and Tanzania, as well as RZ colleagues from France and the Netherlands, were present. The demonstration plots on 0.6 ha on the lower part of Afrisem's 20 ha were well laid out and the crops were at full maturity, showing off the high potential that improved seeds can bring in African growing conditions. Different growing systems, in steel and wooden greenhouses, steel tunnel, shade net structure, and outdoors were clearly displayed. Drip irrigation, plastic mulching, and varying crop support systems further showed how technology can assist farmers achieve better yields. It holds a promise for African smallholder farmers that, in the near future, they too can see their harvest grow in double digits.

More information: www.afrisem.com

From technological to organizational challenges

Evert Keuken

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Since the introduction of biotechnology in the late 70s, new tools and techniques have gradually entered the R&D departments of seed companies. These technological developments not only speed up the breeding process, but they also have an impact on the organization of the R&D departments.

For decades, the seed industry has answered the question of increasing food demand by innovation; new varieties were bred that are better accustomed to specific environments and provide higher yield. The introduction of new technology to assist in the development of new varieties creates a challenge in the R&D departments of seed companies. Researchers are able to predict characteristics of a plant before it is fully grown. Potentially, this causes the lead time for variety introduction to be shortened significantly which is the key to success for a seed company.

Breeding 2.0

The traditional process of plant breeding is being challenged by new possibilities for research - molecular plant breeding. Today, the use of molecular tools in plant breeding is broadly adopted by (the larger) vegetable seed companies, where it serves as an extra element in the traditional R&D process of developing new varieties. The next step is the integration into the existing process of breeding. The R&D managers that were interviewed for this article claimed that the success of a seed company lies mostly in their ability to integrate the different disciplines. The process of innovation for the introduction of a new variety has shifted from one discipline (plant breeding) to multiple disciplines (molecular biology, cell biology and quantitative genomics). The breeder is depending on different disciplines in his process of innovation.

Structure

Typical departments that are distinguished in seed companies are: research, breeding, logistics, sales & marketing and production. This functional division of work leads to a need for coordination between the different entities. The research department (at least) depends on input from marketing and breeding. The challenge here is that the processes handled within research are becoming more complex and dependent on data from other sources within the company. Most companies put breeding in the lead when it comes to the innovation process of new varieties; research then facilitates on demand to aid in the process of innovation.

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The challenge of the plant breeder. The cloud indicates the areas of work the plant breeder is involved in

Process

Seed companies tend to see molecular research as an addition to the existing innovation process. Both molecular researchers and plant researchers have the same goal on a general level: new variety development. However, both work from a different angle. Plant breeders typically work based on incremental improvement of certain existing varieties. Molecular researchers aim to shorten this process by applying tools and techniques. Molecular researchers rely on publicly available research and their own creativity to develop new tools and techniques to apply in the innovation process. The area of work that the plant breeder is involved in is visualized in figure 1. Typically, breeding management sets the goal and priorities. The breeder is then in charge of selecting whichever method he assumes appropriate for the development of the new variety.

Data

Molecular research poses new demands upon the quality of data. In order to link a phenotypical trait to a DNA marker, researchers need data that are both reliable as well as comparable over different crops. Plant breeders typically work on one main crop. If, however, a new DNA marker is being developed, researchers need as much phenotypical data as pos-



sible. This would mean research would demand not only the phenotypical data of one crop, but rather its entire family.

Knowledge

Molecular plant breeding requires specifically trained academics. Seed companies that have introduced molecular plant breeding in-house indicate they are facing challenges to place new personnel and knowledge in the existing company structure. The average

plant breeder has limited knowledge of molecular plant breeding, yet he is often the person to decide whether or not to apply

certain techniques in his breeding process. Some companies aim to close this gap in knowledge by introducing new function profiles, a liaison function. For example, a pre-breeder who is responsible for selecting the right techniques that are available and are fit for practical application. But still, the different background and type of people make integration a challenge.

All the above is assuming the knowledge and application of molecular tools and knowledge is in house. But what about outsourcing? Smaller companies have no option but to outsource application and development of molecular techniques. The clear client-customer relationship solves several challenges which

the in-house adoption of application and knowledge creates.

Pharmaceutical industry

In the pharmaceutical industry, companies are in a comparable challenge. During the 80s and 90s, pharmaceutical companies were faced with a tremendous increase in R&D expenditure and new technological approaches. At the same time, the annual number of newly introduced pharmaceutical substances was decreasing. On top of that, there was extreme competition on a global level which led to aggressive marketing strategies, but also to new ways of organizing R&D departments.

Process

Pharmaceutical companies have witnessed an important shift towards more fundamental science. Experiments have shifted from a craft based incremental improvement to a high throughput process of finding genes and their function. This has significantly increased the level of automation and scale of the research projects. The availability of large amounts of data led to new work processes to analyze data and translate them into information for the primary process, the discovery of new compounds.

Knowledge

Adopting new molecular techniques in pharmaceutical research basically required a move to a more random drug design. Molecular researchers were

'We need to organize our R&D to let knowledge flow in'
MERCK PHARMACEUTICALS (2000)



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'Outsourcing application and development of molecular breeding solves several organizational challenges'

required to perform more fundamental research and made drug discovery less predictable. That said, companies that did not have a reputation for investing in basic science and publications struggled to recruit adequate scientists for their research programmes. With a growing importance of molecular research in the competitive advantage of the companies, a gap occurred between companies that successfully adopted molecular research and the companies that struggled to do this.

Structure

In a situation where R&D productivity is decreasing and cost is increasing, pharmaceutical companies have been searching for new organizational structures. A case study on Novartis showed that pharmaceutical companies are no longer capable of dividing their R&D work simply by hierarchical functional structures and/or geographical structures. Projects is one of the organizational forms these companies now apply. Project organizations allow companies to selectively take over work from several existing entities. This enables them to cut through complexity because a project is able to be rationalized and choices made. The temporary aspect, however, did lead to conflicts in balance between strategic and operational activities. Novartis chose to organize their research around 'centres of excellence' with extensive project teams with a long term character. Most companies in the pharmaceutical industry have conceded that fundamental breakthroughs in technology or science are increasingly likely to happen outside of their own R&D department. The diversity and complexity of new sciences and new

technologies is simply too complex for any company to handle on its own. Merck has concluded in their annual report of 2000, that their company accounted for 1% of the global biomedical research. In order to tap into the remaining 99%, the company aims to actively reach out to universities and research institutes. Seeking input from outside the company is supported by the fact that developing new drugs is still considered a serendipity. The more knowledge and creativity that is combined, the higher the chance of a new discovery.

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Recommendations

The introduction of molecular plant breeding changes the working process of the breeding department. Breeders face the challenge of managing the entire innovation project and this includes the decision whether or not to include molecular tools in the process. It seems seed companies are in the midst of what is referred to as disruptive technologies. New technology that changes the innovation process and possibly allows (new) competitors to enter the market.

The introduction of new steps in the innovation process can be viewed as a significant change in the way breeders perform their day-to-day work. The seed companies that have introduced molecular plant breeding seem to have given significant thought to the rationality of this change. However, there seems to be a lack of the 'effect' component: what will this change mean for my day-to-day operation? What will I be doing differently next Monday morning? The plant breeder needs to alter his daily work.

The challenge larger seed companies face, with regard to molecular plant breeding, is shifting from a technical challenge towards an organizational challenge. Alignment within the different functional departments is a challenge that potentially leads attention away from the important, strategic research projects. Approaches such as cross-functional teams and competency-centres are solutions worthy of consideration. In the meantime, molecular plant breeding is a relatively new area of research and is evolving quickly. Seed companies need to assess what is the best way to gain access to newly created knowledge.

Starting up and growing stronger

Geert Vissers and Ben Dankbaar

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KeyGene is not what you might call the 'typical' biotechnology company. It was not set up by university researchers with a patented invention, it was not supported by 'business angels' and venture capitalists, it is not part of a local biotech cluster, and, despite its success, it was not taken over by a large multinational company.

KeyGene was founded in 1989 and located in Wageningen, home town of the internationally renowned Dutch agricultural university, but it was not a university spin-off. Following an initiative by Cebeco in 1984, it was created by five Dutch seed breeding firms and a bank-owned Biotech Venture Fund. The founding firms were potato breeders Ropta-ZPC and Cebeco, and vegetable breeders Royal Sluis, De Ruiter Seeds and Enza Seeds. It was an act of defense. The founding firms could not afford biotechnology R&D facilities of their own, but they wanted to have access to such research in response to the fact that many large firms were installing biotechnology research laboratories. KeyGene was founded under the agreement that research would be precompetitive and that shareholders would always be independent firms involved exclusively in seed breeding. Therefore, when Royal Sluis was acquired by Petoseed in 1994, it had to offer its shares. Ropta-ZPC started a research facility of its own (RZ Research) and left the consortium after a few years. Ropta-ZPC and Royal Sluis were succeeded by VanderHave (field crop seeds) and Rijk Zwaan (vegetable seeds). In 1996, VanderHave merged with Zeneca Seeds to form Advanta. Zeneca later became part of Syngenta and Advanta withdrew as shareholder. In 2001, the French company Vilmorin joined as the first foreign shareholder. In 2003, Cebeco withdrew, in 2005 Takii (Japan) joined, and in 2008 De Ruiter had to offer its shares after being acquired by Monsanto.

The result is that KeyGene, founded by Dutch firms involved in horticultural but also in field crop seeds, is now owned by an international consortium of firms involved in vegetable seeds – except that Takii is also in the business of flower seeds. As a result of shareholder changes, work on field crops was suspended. For several years, KeyGene would concentrate fully on vegetables. Today, vegetables are still the main focus, but work on field crops has been resumed.

AFLP, the first patent

It is plausible that at the time of its foundation, KeyGene's founders expected it to conduct research in the area of genetic modification (GM). However, KeyGene took a different route. Pieter Vos, one of



Founding Fathers of KeyGene: Jan Leegwater, Kees de Ruiter, Piet Mazereeuw, Rindert Dankert and Johan Prins

KeyGene's leading researchers, explained in 1996 that "all other biotechnology firms were focusing on genetic modification. We did not want to do that. There are other ways to enlarge the stock of genetic materials. Plants have a mass of attributes. If only you know how to search, you will find useful genes in plants that can be cross-bred." First intentions may be uncertain, but what followed is quite clear. On September 24, 1991 – only two years after KeyGene was founded – researchers Marc Zabeau and Pieter Vos filed a European patent application entitled 'Selective restriction fragment amplification: A general method for DNA Fingerprinting'. Further applications of the AFLP (Amplified fragment length polymorphism) technique followed, as well as scientific papers. The most notable is Vos et al. (1995), an article that in the period 1996-2012 was cited 6870 times according to ISI Web of Science. Zabeau worked in Ghent, in the firm Plant Genetic Systems, before he became the first managing director of KeyGene. Vos came from the department of biophysical chemistry of NIZO, the Dutch Institute for Dairy Dairy Research, located near Wageningen. There is no evidence of collaboration between the two previous prior to the founding of KeyGene. AFLP must have been new work. Not only was AFLP new, it was very innovative. Based on already existing techniques (the use of restriction enzymes, PCR, gel electrophoresis) it was a method for of 'DNA fingerprinting' that was faster and less laborious

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The Supervisory Board of KeyGene consists of (from left to right): Jaap Mazereeuw, Wim Nijssen, Arjen van Tunen, CEO, Leo Zwinkels, secretary of the Board, Joop Sisternmans, chairman of the Board, Hans Dons, Ben Tax and Jean-Christophe Gouache

than some, and more accurate than other available methods. And a very important quality was that it did not require a known DNA sequence.

External partners

There is no indication of early external collaboration on AFLP – which is hardly surprising considering that in biotechnology intellectual property protection means everything. Contacts with university groups can be gathered from joint publications, and the earliest of these were published in 1995, some years after the patent application: with the Max Planck Institut für Züchtungsforschung in Cologne, Germany; with the John Innes Centre for Plant Science Research, Norwich, UK; with the Departments of Plant Breeding and Nematology, and with the Graduate School of Experimental Plant Sciences, all Wageningen University. Of course, the early development of AFLP did require some external contacts. The article by Vos et al. (1995) provides a list of suppliers of materials used for the analysis. It is likely that most of these material suppliers were already known to KeyGene researchers, who

had a background in academic, semi-public, and commercial research.

Contacts with universities and institutes like Max Planck helped to give AFLP scientific recognition, necessary in a science-based field such as biotechnology. Scientific research was not limited to the examination of the new method as such, but also explored its use in many plant varieties, thus validating KeyGene's claim that AFLP has a broad range of applications. Between 1995 and 2000, 704 articles with AFLP in 'topic' were published in journals covered by the Science Citation Index. Of these, 27 were co-authored by KeyGene researchers, and 2 were written by KeyGene researchers only. The articles co-authored by KeyGene researchers were mainly in plant biotechnology. Most were collaborations with researchers from the Netherlands and the US, some with researchers from the UK, Belgium, Germany, and Italy. France and Japan, two countries that were also playing a significant role in biotechnology, were not included, possibly because KeyGene researchers lacked contacts with universities in these countries. From a strategic

We share Johan's ambition

to surprise consumers time after time



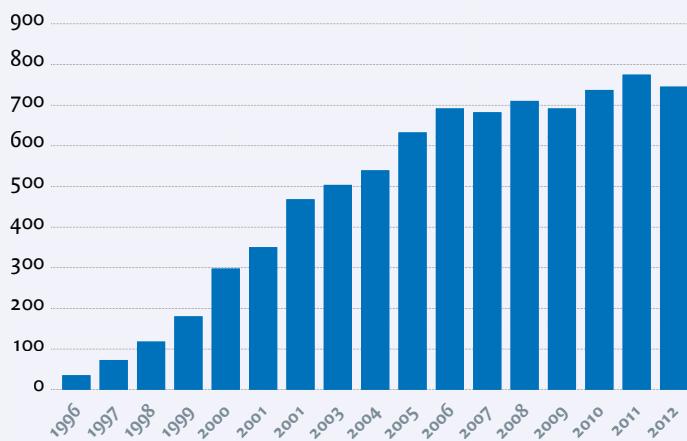
Johan Solleveld comes from a tomato-growing family and has been involved in variety development at Rijk Zwaan for over 30 years. Thanks to his extensive experience, and to the fact that he really speaks the growers' language, he knows exactly what to look out for when selecting new tomatoes. Over the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

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Sharing a healthy future



Vos et al. (1995) number of citations 1996-2012



point of view, then, the entry between 2000 and 2005 of a French and a Japanese firm as shareholders of KeyGene must have been attractive.

Commercial success

KeyGene was founded by plant breeding firms that wanted access to biotechnology research, but there is no indication that KeyGene functioned as their remote R&D laboratory. The shareholding firms had first access to KeyGene's research results, but they had to pay for it like any other firm. There are signs of research collaboration between KeyGene and its shareholders, but most of these are quite recent. Thus, KeyGene acted from the start as an independent firm, charged with the task to develop technology, bringing it to the market, and making a profit. Three options were created for selling AFLP-based products to other firms or to knowledge institutes: a service (KeyGene performs the analysis), a license, or a kit. Commercial licenses are to be negotiated on a case by case basis, but KeyGene prefers to offer services or limited licenses for commercial uses of AFLP. Limited licenses cannot be used by customers for commercial service activities. For research applications, two options are available. One is a project-based research license, granted for a specific project, a limited time period, and a maximum number of 'runs' per year. The second option is the use of an AFLP kit. Three such kits are available, offered by KeyGene's marketing partners.

By 2000, KeyGene had become a respected company and AFLP a respected technology, but KeyGene relied on this single product. The firm then started to extend its activities in several directions, trying to use established technologies in new ways (as had been the case with AFLP). An example is sequencing. Combining this with AFLP-based technology enabled KeyGene to increase the scope of its analyses. AFLP had been developed as a genotyping technology, to demonstrate similarities and differences between varieties of a plant.

Adding sequencing made it possible to use actual gene fragments for fingerprinting – showing not only that differences exist, but also which differences exist. For its work on sequencing, KeyGene uses equipment from suppliers such as Roche, Solexa, Applied Biosystems, and GE Healthcare. KeyGene collaborates with these suppliers, contributing knowledge of preparation and analysis of plant genetic materials that improves the performance of the equipment. Working with such suppliers is important because DNA sequencing is increasingly dominating the field of plant breeding, and 'sequence based breeding' may be combined with automated phenotyping.

Conclusion

Today, KeyGene is today one of the largest dedicated biotechnology firms in the area of plant biotechnology and it is quite successful. Part of this success derives from the continuous support of KeyGene's shareholders, which allowed for a long term strategy, but AFLP has been extremely important as well. It was AFLP that made it possible for KeyGene to take its particular position in the plant biotechnology field, a position that set the firm apart from most other newly founded biotechnology firms. AFLP was a smart blend of established technologies and principles from molecular biology. None of these were novel but the combination was new, and this new combination proved to be very useful, especially because it offered an alternative to genetic modification in at a time when the latter was under attack. But having a useful technology is one thing, turning it into a commercial success is another. KeyGene's success is based on its willingness and ability to go to great lengths to protect AFLP, to elaborate it, test it, explore its range of application, and bring it to the market. The same qualities will be required when working with new technologies in the which are no doubt equally competitive in the environment of the future.

Standard procedure contaminates lily

Geert-Jan de Klerk, Niels van der Rest and Naser Askari

30 In tissue culture, contamination is a persistent problem and frustration. In lily, a substantial portion of plant-related contamination is introduced by operators. There are major flaws in the standard initiation procedure. The same shortcomings are likely to occur in other crops.

- When they manage to enter the tissue-culture container, many microorganisms (bacteria, fungi and yeasts) flourish on the plant nutrient media (Fig. 1).
- They often overgrow the explants. There are four major sources: the operators, the plant material, faulty equipment and micro-arthropods. Usually, it is believed that the larger part of the contamination originates from the plant material.

Limiting factor

Microbial contamination has been identified as the most serious limiting factor in tissue cultures. Growth of microorganisms in the medium can be inhibited by antimicrobial agents. Antibiotics, for example, are often added to the nutrient medium when it is not possible to obtain explants free from bacteria. A major reason for their ineffectiveness, with respect to endogenous microorganisms, is that the disinfectants do not reach a sufficiently high concentration within the tissue to be successful. The ways in which to remove or avoid endogenous microorganisms are very limited. The first is a warm-water treatment that kills the microorganisms but not the plant tissues. This method can only be used in robust plant tissues, like bulbs and nonsprouted buds, and is often not successful among others because dormant spores survive and are even activated. The second way is to start with tissues that are free from microorganisms. In particular, meristems are free from microorganisms.

Standard disinfection procedure

Early in the history of plant tissue culture, tissues were excised from aseptic regions in the plant, in particular apical buds, and transferred under sterile conditions to sterilized nutrient medium but without surface sterilization. Tissue culturalists now always surface-sterilise plant material before transfer to tissue culture. For surface sterilization, a common standard protocol is used initially 'borrowed' from seed sterilization. The explant is excised from mother plant.

After pre-rinsing with water to remove dirt and occasionally a short submergence (30 sec) in 70% ethanol, the tissues are kept for 10-30 min in a concentrated solution of disinfectant. Often 1% NaClO is used. A few drops of detergent (Tween



Figure 1. Contaminants flourishing on the plant nutrient medium. The location of the contaminants indicates that the explant, a scale fragment of lily, is the source of the contamination

20) are added as wetting agent. Next, the excess of chlorine is removed by rinsing three times with sterile water. The cultures are stored in sterile water until use. Slight modifications have been made depending on the extent of contamination and the preferences of the researcher.

Leak one

Because it is not feasible to sterilize and rinse the explants individually, usually a batch of 5-30 explants is processed in one beaker ignoring cross-contamination. Remarkably though, after that, cross-contamination is considered as a major threat and the explants are cultured typically individually (one per container). Apparently, it is believed that the duration of rinsing is too short for cross-contamination whereas the alternative, processing each explant individually, is not a viable option because of the labour.

In lily, we examined the incidence of microorganisms

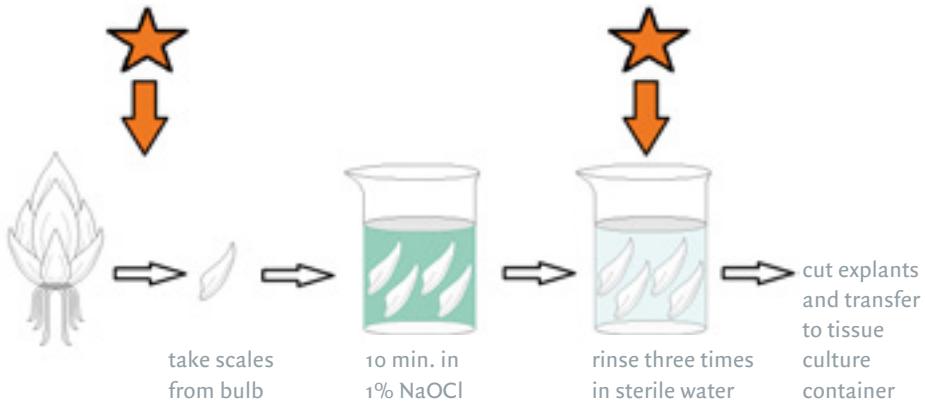


Figure 5. The procedure of initiation in lily. The hitherto unnoticed contamination events are indicated by red arrows

Table 1. Presence of microorganisms in rinsing fluids as detected with LB solid (SLB) and liquid (LLB) medium. The tests were done 3 and 2 times, respectively. (- not contaminated, ++ medium contaminated, +++ highly contaminated)

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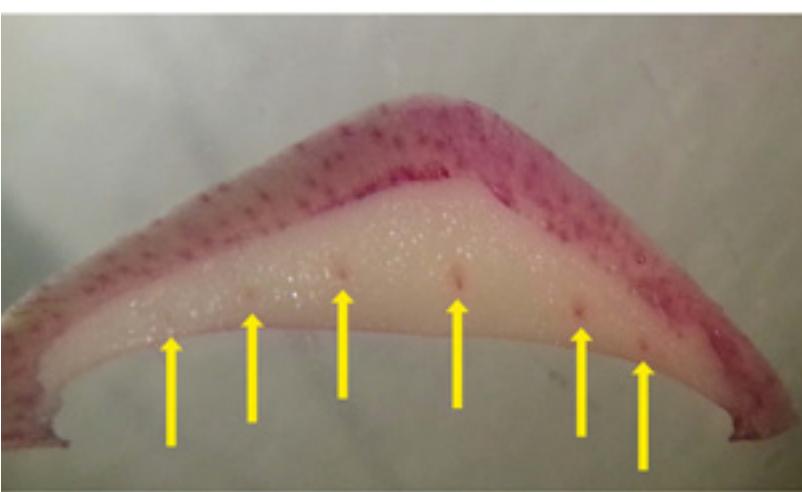


Figure 3. Uptake of the magenta dye acid fuchsin by vascular tissues after detaching a scale from a bulb submerged in a solution of acid fuchsin. After detachment, the scale was almost immediately (after a few seconds) rinsed and cut transversely ca. 1 cm from the base of the scale. The dye has entered the scale up to at least 1 cm within a few seconds

in the rinsing and storage water and found contamination already in water used for the second rinse (Table 1). Therefore, we decided to rinse with diluted NaClO. A concentration of 0.03% was found to remove all contaminants from a liquid medium. When the scales were rinsed in 0.03% NaClO instead of water, the rinsing fluid was aseptic (Table 1) and contamination of scales strongly decreased (Fig. 2). Tissue culturalists are very anxious that residues of NaClO are toxic and therefore extensively rinse with sterile water. It is very doubtful whether this anxiety is justified, as there are several reports on tissue culture with addition of low levels of NaClO. Rinsing with 0.03% was definitely not toxic in lily. Important parameters - weight per bulbule and number of the regenerated bulbules - were the same after the water and the 0.03% NaClO rinse.

Leak two

A critical analysis of the successive steps in the initiation process showed a second potential leak. Inside plants, especially in the xylem, a negative hydraulic pressure occurs caused by transpiration of water. When an explant is excised, the cavities of the xylem are in direct contact with the surroundings of the tissue and will suck up nearby fluid. When this fluid contains microorganisms, these will also enter the explant.

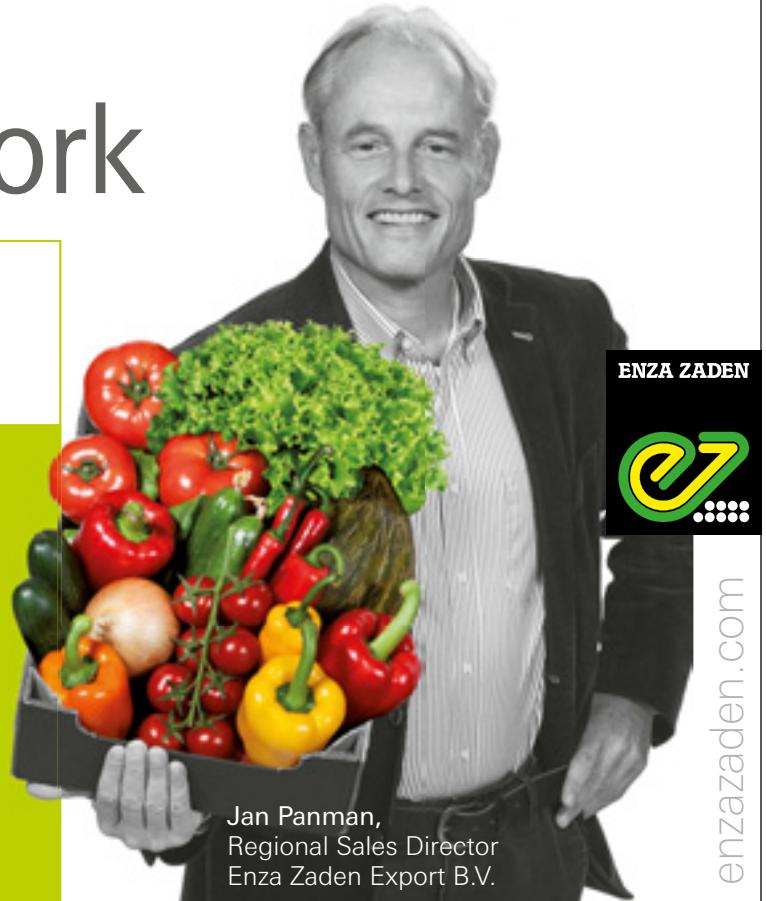
To examine whether this is indeed a source of contamination, first the occurrence of transpiration by lily scales was examined. The transpiration was found to be $10 \mu\text{l} \cdot \text{scale} \cdot \text{h}^{-1}$, about the rate of shrubs and grasses in the tundra. Microscopic examination

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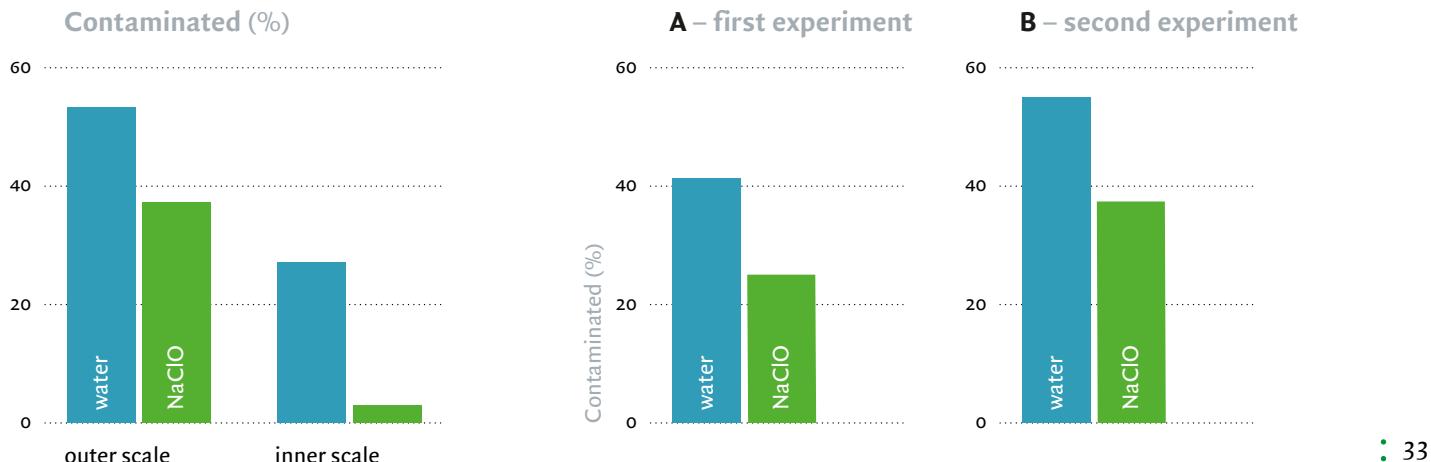


Figure 2. Contamination after rinsing scales in either sterile water or in 0.03% NaClO

Figure 4. Contamination after detaching scales from bulbs submerged in water or submerged in 0.03% NaClO

33

revealed the occurrence of stomata. Next, it was examined whether a negative pressure occurred in the xylem. A bulb was submerged in a 1% solution of the dye acid fuchsin and a scale was detached. Immediately after that (within a few seconds), the scale was rinsed to remove the excess of acid fuchsin and cut transversally at ca. 1 cm from the wound surface. Figure 3 shows that the fuchsin had penetrated at least 1 cm into the vascular tissue. It should be remembered that the diameter of a xylem vessel is 10 – 100 µm, depending on the position in the stem where the diameter is measured. Bacteria are 1-2 µm, so they can easily move into the xylem. There is some controversy as to whether bacteria move easily within the xylem because they must cross inter-vessel pit membranes through pores. The current opinion, based among others on translocation of latex particles linked to a dye, is that bacteria may move freely through the stem. The previous paragraph demonstrates that detachment of tissue itself may constitute a major source for endogenous contamination. To the best of our knowledge, this has been considered only once by tissue culture researchers. Diluted NaClO also deals successfully with this type of contamination. When the bulbs were submerged in 0.03% NaClO during detachment of the scale, contamination was much less (Fig. 4): the NaClO solution was sucked up and microorganisms were killed. The kinetics of the start of microbial growth on the medium indicated that the 'extra' contamination in scales detached in the normal way resided deep inside the explant and was apparently endogenous. This makes sense when the microorganisms have been sucked up into the xylem vessel. However, the chlorine that had entered the xylem may be toxic. This was examined and found not to be the case. The number of regenerated bulbules was not affected and the weight per bulbule was actually 20% higher than in the control,

indicating that the endogenous contaminants were inhibitory.

Bacteria associated with plants

As a concluding remark, it should be mentioned that the standard plant nutrition medium is not suitable for many bacterial species associated with plants. From 10 such species inoculated on MS medium with 3% sucrose, only one, *Bacillus subtilis*, showed significant growth. When plants were cultured on the same medium together with the bacteria, an additional five showed growth, indicating that the plants did excrete compounds vital for the bacteria. Some microbiologists go beyond this and believe that the conventional bacterial media are unsuitable to nourish most bacterial species, so that we are actually unaware of their existence. This has been referred to as the 'Great Plate Count Anomaly', referring to the discrepancy of the number of bacteria in a droplet seen with the microscope and the number as detected by bacterial plates. Recently, it has also been suggested that plants cultured *in vitro* almost ubiquitously harbour endophytic microorganisms. These uncultivable bacteria may be detected by molecular assessment.

Conclusion

The procedure for initiation of lily is shown in Figure 5 and invasion by microorganisms is indicated. Together, both leaks may result in large losses. It seems very likely that similar modes of contamination occur in other crops and experiments on this are underway. It is striking that the standard initiation process, that has been described in tissue culture books like the ones of Pierik and George, has these flaws.

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Number of accredited laboratories increases

Adrie Molenaar

In a meeting in March 2014, the Board of Naktuinbouw determined the new NAL Conditions 2014. They will come into force from July 2014. Most important changes are: review by a native speaker, there were more definitions added and further clarification, especially about proficiency testing (introducing categories).

Currently, 14 laboratories are accredited for NAL. It is expected, however, that seed companies with their testing laboratories from Japan, India, France and the USA can be added to this list in the coming year. Participants in the still growing system are: Bejo Zaden B.V. – Enza Zaden Seed Operations B.V. – Nunhems Netherlands B.V. – Rijk Zwaan Production B.V. – Syngenta Seeds B.V. – Monsanto Holland B.V. – Incotec Europe B.V. – Nickerson-Zwaan B.V. – Vilmorin S.A. – Germains Seed Technology – Sakata Vegetables Europe S.A.S. – Nunhems USA – Hazera Genetics Ltd. – Takii Europe B.V.

Proficiency tests

In 2014, the NAL bureau will organize the following proficiency tests: Seed analysis, Acidovorax, Xcc, CGMMV, SqMV, Phoma and Xap. If vegetable seed companies are interested in voluntary participation, please feel free to contact Naktuinbouw for more information about possibilities, planning and fees.

Verification programme

Naktuinbouw has started a new project under the working title 'Verification Programme for Seed Production'. In mid 2013, Naktuinbouw conducted a survey amongst the NAL accredited companies, to see whether there is support for a future extension of NAL with other modules (like accredited sampler, accredited field inspection, accredited risk analysis and a verification programme for varietal identity), partly as a reaction to the draft of the EU 'Better Regulations'. With this new programme, a tool for the seed companies is going to be developed to demonstrate that they comply with the requirements and control various critical parts of the seed production chain and their processes. Their own responsibility in this matter and control measures based upon a risk analysis are important pillars. And it is a logical thought that when companies do control their processes demonstrably, that this might influence the intensity of future quality and seed health inspections by authorities.

The idea is to have this embedded in the NAL system in a number of years, with a NAL Board of Experts, a participants meeting, conditions, a dedicated team of auditors, audit regulations, review of protocols

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The new conditions can be found on the Naktuinbouw website (www.naktuinbouw.com). In the last chapter, there is an overview of changes



by experts and proficiency testing.

USDA

In December 2013, a meeting was organized with the USDA Agricultural Policy Specialist from the Pest Permitting Branch and Senior National Seed Health System (NSHS),

also Accreditation Manager APHIS Plant Protection and Quarantine Plant Health Programmes. The meeting aimed to build a closer relationship between NAL and NSHS and explore options to possibly share expertise in the future regarding audits, proficiency testing and review of protocols. The number of NAL participating laboratories in the US (often also member of NSHS) is growing. Naktuinbouw's head of laboratories will visit the seed testing specialists of Iowa State University Seed Science Center in 2014 as a follow up of this meeting.

Australia

In total, three laboratories, Bejo Zaden, Rijk Zwaan and Monsanto are accredited now according the NAL-AQIS (now DAFF, Department of Agriculture, Fisheries and Forestry of Australia) protocol for seed export to Australia. ENZA Zaden is in the process of accreditation. With the approval letter, vegetable seed lots do not have to be sampled and tested again at the point of entry, but they can enter Australia directly with the NAL Quality certificate.

Financing Curious Scientists

The cradle of innovation is fundamental research, the so-called 'blue sky research'. It is research that is done by free spirits who are motivated by curiosity, rather than by a pre-set, practical goal. It is a field of research that is primarily focused on gaining knowledge and insights for no other sake than satisfying the inquisitive science-oriented mind. But who is to finance these scientists?

36 Monique Krinkels

• **If Albert Einstein** would have lived in the Netherlands of the 21st century, would he have come to the conclusion that $E = mc^2$? Probably not, as who would be willing to support a scientist who wants to know more about something that has, at first sight, no financial or strategic value at all. Both politicians as well as the business community would rather stimulate research that has some economic or societal potential. But to answer the questions that will arise tomorrow, it is essential to build a strong knowledge infrastructure today. Unfortunately, governments of some European countries, among which the Netherlands, are reluctant to take that responsibility.

Tight corner

There are three types of research: experimental development, industrial research and fundamental research. Whereas financing experimental development and industrial science might be a shared responsibility of governments and the business community, fundamental research and science is not. It is a basic prerequisite of a healthy, economically viable society. New findings are based on serendipity and there is no linear pathway from idea to innovation. According to The Young Academy, part of The Royal Netherlands Academy of Arts and Sciences, the influence of the business and political communities should not lead to loss of independence and quality of scientific research.

"A unidirectional attention for topics and sectors that seem to have, at this moment in time, a certain economic potential, leads to short-termism and less consideration of independent and curiosity-driven research that may offer unexpected solutions for the questions and societal problems of tomorrow. Other values of science, other than economic ones, should not get into a tight corner." The Young Academy concludes that only when universities can finance their own

Facts & figures

In 2012, 50 million euro was spent on experimental agricultural research in public academic institutions in the Netherlands. In total, 500 researchers, including PhD students and postdocs, were involved. The research was funded by the Ministry of Education, the Ministry of Economic Affairs, a governmental fund and the EU. Companies contributed 15 million euro in the period 2002-2012.

Because of the austerity policy, research funding has decreased by 20%. This trend stands in sharp contrast with the increasing budgets for private R&D by the Dutch seed companies. Four R&D intensive seed companies spent 214 million euro, of which 142 million in the Netherlands. According to a survey by the European Technology Platform 'Plants for the future' in 2012, a knowledge intensive sector also needs sufficient public research. This survey shows that companies in the Netherlands need at least 35 academically schooled plant scientists a year, most of whom with a PhD degree.

Blue sky



There is a need for people with wild, novel ideas that we cannot

research programmes, can they offer a stable, fruitful environment for independent and long term research covering the whole scientific spectrum.

In that light, the Dutch Centre for BioSystems Genomics (CBSG) initiated and published a white paper, in which the importance of independent, fundamental research is underlined. "The Dutch agricultural sector attaches great value to fundamental research. It has been proven that large trail-blazing innovations would not have been possible without it. And in addition to being a source for innovation, it is also the breeding ground for future employees, as after their PhD many researchers end up in a company. Furthermore, researchers function as sparring partners and the academic world is a vital part of the network of agricultural production. Hence, a good knowledge infrastructure is an important prerequisite for companies who are considering developing activities in a certain country."

research at risk



foresee today

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needs a strong
home market,
not only for sales,
but also for the
recruitment of
employees and
accumulation of
knowledge**
Gerard Backx,
President of Plantum,
the Netherlands

Both scientists as well as seed companies focus on long term projects. "Because the development of a new variety takes at least ten years, companies consider investments in projects shorter than five years as throwing money down the drain. Public-private partnerships can only exist if both parties are willing to commit themselves for a long term period. Both the scientific as well as the business communities feel the need for continuity in financing research."

Besides, critical mass is important. "Disconnected research projects are of little use. Coherent, integrated, interdisciplinary research programmes are needed to solve the challenges the sector is facing. In 2050, 9 billion people will depend on plants for their food, feed, fibre and energy supply." These problems will cause an increasing international mobility of science and education. And talent is scarce. To be able to compete internationally, it is important to offer young people an attractive sci-

tific environment. This will stimulate companies to set up in the Netherlands.

Responsibility

Innovation is a pipeline of knowledge, starting from fundamental research that works its way step by step up to more applied research and, in the end, to applicable innovations in companies. By focusing solely on the last part of the pipeline, companies might encounter the situation that there is a shortage of fundamental knowledge. It is feared that: 'we will squeeze the pipeline until it is empty.'

While the agricultural sector freely supports applied and strategic scientific research, the companies cannot and should not finance fundamental

research, according to Plantum. "Financing fundamental research is not a rational investment, as the economic return, if any, is uncertain. And scientists should not have to turn to companies to acquire the financial back-up for their independent research." Moreover, fundamental research is part of the knowledge infrastructure comparable to the traffic infrastructure, and therefore basically a task of the government. That does not mean that the business community does not support research. Whereas the government is responsible for maintaining the infrastructure, companies will pay for the use of the knowledge.

The Netherlands invests about 1.8% of its Gross National Product in science. That is a far cry from the 3% that is the goal set for 2020. "And even the latter might not suffice," concludes The Young Academy.

Political power play in Brussels

John van Ruiten

38 In May 2013 the European Commission launched proposals for a complete renewal of both the seed legislation and the plant health legislation of the European Union. The suggested renewal is being regarded as necessary because the seed legislation that now exists in the EU is built around twelve different directives for various crop groups and is in some respects outdated.

• **In the field of plant health**, it is considered that the present legislation in the EU is insufficient to adequately protect against incoming quarantine diseases and there is a lack of harmonization in phytosanitary approaches (e.g. plant passporting, inspections, notifications) in the 28 Member States. Furthermore, the number of regulated harmful organisms is enormously high (over 300) and that means that an evaluation on necessity and new prioritisation on these harmful organisms is needed to improve inspection and measurements. In addition to the two new regulation proposals, the Commission is also suggesting the adjustment of the so-called 'Control regulation' in such a way that also plant health and seed quality controls are brought under its umbrella and regulated at an EU-level. Until now, the planning, execution, financing and reporting of results of control systems for seed quality have been the responsibility of individual Member States.

Proposed changes

Currently in the European Union, twelve directives govern the requirements for production and marketing agricultural, forestry and horticultural seeds and young plants (including vine plants). The directives contain requirements to safeguard identity, quality and general health (non-quarantine) issues. For each crop (e.g. potato) or group of crops (e.g. vegetable seeds or vegetable plants), the requirements are specifically formulated. All individual Member States have to implement these directives into their own seed laws.

Some of the directives in the EU originate from the 1960s. The most recent ones came into force in the 1990s. The content and the approach of these twelve directives are different. The older ones (in agriculture and forestry) are built around the concept of allowing only registered varieties to be marketed. The newer ones (in floriculture, fruit crops and vegetable plants) are created around the concept of registered nurseries taking responsibility to produce young plants that fulfil minimum requirements.

In the proposal, the EU Commission now suggests three important changes:

- To change from directives into regulations. The

difference is that regulations do not have to be implemented in national legislation at the Member States anymore. After adoption, they will be 'automatically and immediately' relevant and applicable in all the Member States of the Union after a certain transition period.

- To use the same set of definitions, terminology and approaches, for example for variety registration and certification, for all crops for which this is relevant (the so-called listed species). For all other so-called non-listed species, a basic set of minimum requirements is proposed (comparable to what is now regulated in the ornamental sector)
- To further integrate phytosanitary and quality controls, to transfer all disease requirements to the plant health regulation and to convert the plant passport into a general health document

Besides these three main lines, proposals are also being made to increase the possibilities for marketing of non-traditional materials (e.g. for ecological production). It concerns the creation of possibilities to market heterogeneous material, to have specific requirements for niche markets and to make clear that the EU-seed legislation is only applicable if material is produced and marketed by professional operators (and not by amateurs). The proposed 'PRM (Plant Reproductive Material) Regulation' contains many points that have to be further elaborated and for which requirements have to be formulated in implementing or delegated acts.

Co-decision Parliament and Council

Since the Treaty of Lisbon 2009, new legislation in the EU has to go through both channels (European Parliament (EP) and Council of Ministers) before it can be finalized and decided. So there is not only the debate on new legislation in these two circuits, also the final decision and approval of legislation (always proposed by the Commission) has to be taken by voting with qualified majority (Council) and normal majority (European Parliament).

In the autumn of 2013, the discussion around the new proposed package of legislation started. To the surprise of many, it is not the plant health proposal, but mainly the PRM proposal that is causing an intensive and quite heated debate within the

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parliament. But also in various Member States there are intensive debates from NGOs, MPs of national parliaments, growers' organisations and others with their Ministers of Agriculture. The debate is in general not on technical points, but rather on a more fundamental level, mainly around the topics of 'ownership of genetic resources' and 'power of the EU Commission'.

This is new. For a long time, most of the responsible government/agencies and growers and their organisations discussed and (in the end) agreed on seed quality and plant health legislation as more technical affairs. Based on experience, agricultural scientific developments, trade policy etc. legislation was created, in relative quietness.

Fierce opposition

How different it is now. Fierce opposition,

newspaper articles, broad 'anti' coalitions and intensive lobbying with MEPs. The discussion is not a technical one for agricultural specialists, but a political and often emotional one. Seed issues have really been brought to society. The way in which that has happened, however, is causing some frowning. Many say that wrong or even false arguments, insufficient information and a completely wrong view of the agricultural and horticultural needs and developments are behind this debate of 'negative one-liners'. It resulted in mid-March in a vote in the EP in which a vast majority (almost unanimously) voted to reject the EU Commission proposals.

When we take a closer look at the over 1,400 EP amendments that were filed in the last months, there are three major points that are behind the reluctance against this PRM proposal.

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There is a great concern that strict and costly variety registration requirements will lead to fewer varieties on the market and therefore will harm biodiversity and will lead to more monocultures on agricultural fields, orchards and greenhouses.

There is a great concern that obligatory variety registration and certification will lead to stronger marketing positions for big agro industrial companies (including their intellectual property), since they are more capable of tackling regulatory hurdles. The fear is that food production will ultimately be in the hands of only a limited number of companies.

There is a concern that because of the great number of proposed delegated acts, many decisions will be made by the EU Commission. It is felt to be non-democratic, and it is also felt that with this approach more power will be in the hands of the bureaucracy in Brussels, and not in the parliaments of the Member States.

So, EP rejected the proposal. It is now the question as to what the Member States' Ministers in the Council will decide. If they also heavily criticize the proposal, it will be most probably taken from the table by the Commission and then they have to restart the process. Possibly, if the Council is not negative but critical, the Commission can try to adjust the proposal and come up with a new version, taking

into account many of the items raised during the last months.

41

Dialogue needed

But surely, it will be very difficult to come up with renewed PRM legislation that is to the satisfaction of all groups now involved in this societal debate on seeds. An open and continuous dialogue, organized by the Commission with the wide group of stakeholders, interested societal/environmental groups that have their concerns about seeds and food security, is certainly needed for that.

And what will happen if no new position and regulation can be found? The EU Member States will then have to continue with the old existing legislation that is less adjusted to the needs and desires of niche and other alternative markets and does not have specific features for preserving biodiversity. Not harmonized, not modernized, but traditional, solid, very differentiated for various crops and obviously fit for continuation of the general purpose of improving productivity and quality in agriculture.

Are there profits in removing epidermal wax

Geert-Jan de Klerk and Dewi Pramanik

42

The outer layer of the skin (epidermis) of plants is covered with wax. Wax protects plants from pathogens and uv irradiation. Moreover it prevents loss of water by acting as a barrier for diffusion. These qualities of wax are not required in tissue culture; are there any profits in removing the wax barrier?

Leaves of most plants from temperate conditions are covered with 8 to 40 µg wax per cm². Plant epicuticular waxes are complex mixtures of very long chain fatty acids (ranging from 20 to almost 40 carbons), hydrocarbons, alcohols, aldehydes, ketones, and esters, together with low levels of triterpenes, sterols and flavonoids. There are few studies on wax in plants grown in tissue culture. Tissue-cultured leek plants contain 10 and ex-vitro plants 30 µg wax per cm². In other crops, the amount of wax in tissue-cultured plants is also much lower (carnation). However, in many crops the amounts are similar (chrysanthemum, spathiphyllum).

Significance

Cuticular transpiration (loss of water from the surface of plants excluding loss via the stomata) is inhibited by wax. This type of transpiration is under normal conditions only a few percentage of total transpiration. The thin wax layer in vitro was thought to be a main reason for the desiccation of tissue-cultured plants after transfer to greenhouse conditions. Now it is generally believed that even though wax is often reduced, the main reason of the inferior water retention in tissue-cultured plants is the poor functioning of stomata.

The second possible significance of wax for tissue culture is that wax prevents uptake of medium ingredients, in particular during culture in liquid medium. When no or only little wax occurs on the surface of an explant all

of the epidermis will participate in uptake of medium components. The other main functions of wax in ex-vitro plants, protection from pathogens and irradiation are not relevant in tissue culture. In the present article, the role of wax as inhibitor of transpiration is dealt with.

It was surprising to find that the amount of wax can be reduced easily by adding synthesis inhibitors. We used trichloroacetic acid (TCA). In developing maize leaves, TCA inhibits wax synthesis in a very late step in the synthetic pathway, namely elongation by the incorporation of acetate in C₃₂ chains. TCA is actu-

ally well known in biochemistry for other reasons. It is used routinely to precipitate proteins and nucleic acids from extracts. The amounts of wax can be easily measured gravimetrically. Wax is rinsed off from the epidermis with chloroform, the chloroform is evaporated and the residue is weighed. The amounts of wax obtained with this method are the same as the amounts measured chemically.

Wilting in vitro

The RH (relative humidity) in the headspace of tissue culture containers is ca. 99%, but how high exactly depends on various factors, in particular the air exchange between the vessel and its environment. It is difficult to believe that leaves of tissue cultured plants do dry out at such high RH. However, apple shoots cultured with TCA do wilt (Fig.1). The photo shows wilting and not death by toxicity of TCA because there was no wilting when a layer of liquid medium (with the same concentration of TCA) had been added. Wilting occurs when water lost by transpiration is not replenished by water taken up from the medium. Apparently uptake of water from the double layer is much faster than from solidified medium.

Hyperhydricity

Hyperhydricity (HH) is a physiological disorder that often occurs in tissue-cultured plants. Hyperhydric tissues are characterized by a high water content caused by flooding of the intercellular spaces. In leaves of control *Arabidopsis* plants, 85% of the volume of the intercellular spaces is occupied by air and 15% by water. In hyperhydric plants it is the other way around: 85% is water and 15% air. Because of the flooding, gas exchange by cells is severely inhibited and as a result anaerobiosis and accumulation of gases like ethylene occur. This causes the "HH-syndrome". In *Arabidopsis* seedlings, severe HH leads to dying off three weeks after the first visible symptoms. Evidently, the excess of water in hyperhydric plants is brought about by too much entry and/or too little transpiration of water. Water transpires from plant tissues via the stomata and a little via the cuticle. The movement of water through the cuticle is strongly reduced by the wax layer that covers the epidermis. In plants growing ex vitro, stomatal transpiration usually makes up more than 95% of the total transpira-



Figure 1. Wilting in vitro of apple shoots after 3 weeks of culture on standard nutrient medium with 4 mM trichloroacetic acid (TCA). TCA is an inhibitor of the synthesis of epicuticular wax

Figure 3. *Arabidopsis* seedlings after 17d culture on the indicated media. Note the occurrence of hyperhydricity symptoms on Gelrite and their absence on agar and on Gelrite + 1 mM TCA

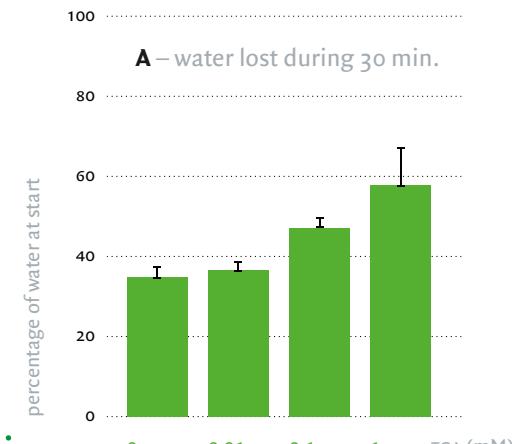


Figure 5. Amount of wax (left) and transpiration (right) of tulip shoots. Even though the amount of wax is reduced by 90%, the amount is still so high that transpiration is not enhanced

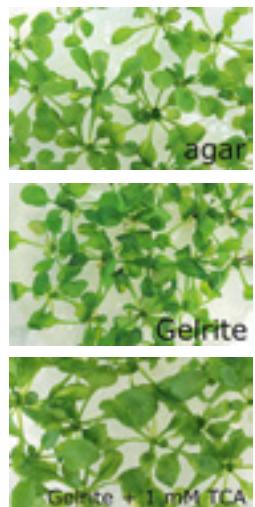
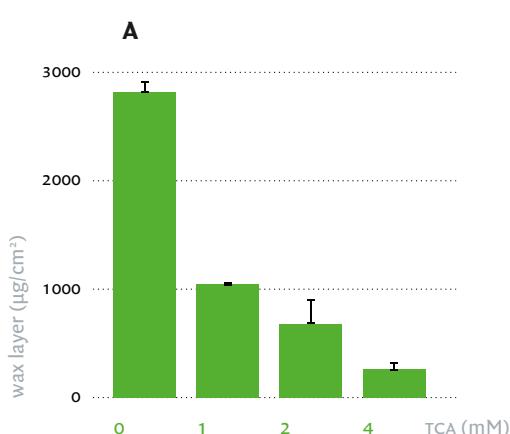
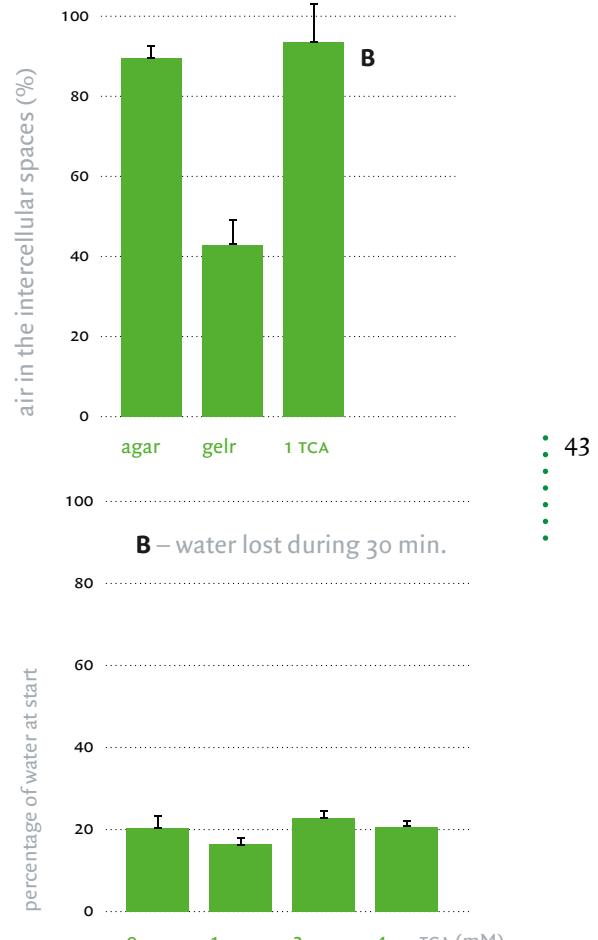


Figure 2. Transpiration (top) and air content of intercellular spaces (bottom) of *Arabidopsis* leaves grown on agar, gelrite or Gelrite + TCA. Note that with 1 mM TCA transpiration is high and the amount of air in the leaves is high

tion. It was interesting to examine whether HH could be reduced by enhancing cuticular transpiration. Reducing the wax layer enhanced transpiration of water by the leaves as shown by the rapid loss of water from detached leaves (Fig. 2a). This results in air-filled intercellular spaces (Fig 2b) and the absence of HH symptoms (Fig. 3). It is very remarkable that there are no unwanted side-effects of TCA. TCA is an interesting tool to prevent HH because its effect is temporary and in a cycle without TCA wax recovers. At the same time, it is likely difficult to establish the adequate concentration of TCA.

Tulip shoots

Tulip is propagated vegetatively in very large numbers in the field: each year some 4 billion tulip bulbs. In spite of this huge number, a critical analysis indicates that propagation is actually the major Achilles' heel for the tulip industry. Conventional propagation performs poorly with respect to the introduction of newly produced cultivars and with respect to the phytopathological and physiological quality of the bulbs. The obvious solution is vegetative propagation in tissue culture which produces very rapidly disease-free plants with high vigour. There are however various stumbling blocks which have hampered commercial micropropagation thus far. One of them



is the very poor growth of tulip in tissue culture. This may be related to the poor transpiration caused by the low density of stomata in tissue-cultured shoots, their closure and the thick wax layer. The wax layer is so thick that shoots get a whitish appearance (Fig. 4). We hoped to increase transpiration by adding TCA. TCA indeed diminished wax but at the highest concentration TCA the wax was still 250 μg/cm², 10 times higher than ex-vitro plants. Correspondingly, transpiration was not increased and growth remained poor (Fig. 5).

Final remarks

Reducing the wax layer may be used in tissue culture to reduce HH and to increase growth. We did find an increase of growth in *Arabidopsis* seedlings of ca. 30% but no increase in tulip, probably because of the very thick wax layer which remains thick when wax synthesis is reduced by ca. 90%. Removing wax should also increase uptake of medium components via the epidermis but this has not yet been examined.



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Soft Fruit in the European Union

Production and trade of propagating material of fruit crops is an important activity in many parts of the world. For crops such as strawberry (*Fragaria*), raspberry (*Rubus*), citrus (*Citrus*), apple (*Malus*), cherries/plums (*Prunus*) and pear (*Pyrus*), the availability of high quality and healthy plants for planting is crucial for creating orchards and soft fruit production systems.

John van Ruiten

In various countries, propagation programmes have been set up for many years for the production of 'virus-free' plants. These programmes in countries like USA, France, the Netherlands and the UK have a history going back to the early 1960s when techniques for indexing and for creating virus-free material became available. For most relevant pathogens (viruses, viroids, phytoplasmas), good techniques are available nowadays, both for rapid lab testing/indexing but also indicator plant testing (both woody indicators and herbaceous indicators) is still widely used for screening candidate material to be included in further propagation.

EPPO schemes

In the 1980s and 1990s, EPPO (the European and Mediterranean Plant Protection Organisation) has developed, in consultation with experts from many European countries, recommendations for certification schemes. In these schemes, available on the EPPO website, the requirements for various stages of propagation and also the recommended tests for relevant pathogens are presented.

These EPPO schemes have formed the basis for the creation of many national legal certification schemes in the Member States of the European Union (EU). Therefore, most of these schemes can be regarded as brothers and sisters within one family. Although they very much look alike, there are differences between these various national systems. The differences occur in test requirements, test intensity, prescribed isolation distances, numbers of allowed generations, certification norms and standards and, last but not least, labelling.

In the 1990s, in the EU, based on the introduction of legislation in 1992, an attempt was made to see if a harmonized EU certification programme could be formulated and set up. It turned out to be too difficult to do that. Obviously, differences were felt as being too great and individual Member States also wanted to continue their own system for the benefit of their own industry. But gradually the schemes in operation in Member States, step-by-step, began to show more comparable approaches.

Renewal

In 2008, the EU decided to come up with a complete

Fruit sector on the threshold of harmonized certification



The harmonized certification scheme will mean an important step forward, not only for internationally operating plant producers, but also for fruit growers.

Ir. John van Ruiten is director of Naktuinbouw, Roelofarendsveen, the Netherlands

renewal of the Directive on Marketing of fruit plants. In that directive 2 new essential changes were taken up. First of all, it was agreed that from 1 October 2012 it was necessary for every variety to be registered officially (with DUS test) before plants or seeds of that variety could be marketed. For old varieties (marketed before that time), a registration is done on the basis of a short description. All Member States of the EU have to make their list of registered varieties and these lists will be combined in an EU database by the end of 2014. The second point is to set up a harmonized certification scheme in the EU. It took somewhat longer, but it is foreseen now (although not yet finally decided) that from 1 April 2016, plant propagating material can be 'EU certified'. The exact

requirements for this certification on the basis of new directive 2008/90 are soon to be published by the EU Commission. For internationally operating producers of plants and also for fruit growers, this is an important step forward. They can use this system as the framework for production programmes.

In the Netherlands, the Test Centre of Naktuinbouw in Horst is going to offer (except for Citrus) test facilities and facilities for creation and aphid proof maintenance of pre-basic mother plants. These plants are the starting plants for big scale propagation programmes.

Vandinter Semo celebrates its first centennial

Hajo P. Strik

46

Spring 1914. Tension in Europe is rising. Nationalists and militarist parties are gathering strength, but the Great War has not yet started. The rural area around Scheemda in the northern province of Groningen, the Netherlands, is a peaceful haven in this turmoil. Two entrepreneurs, Andries Zwaan and Otto de Wiljes, start to produce and sell seeds. A wise choice as today, a century later, Scheemda is still as peaceful and the company is blooming.

The name **Zwaan & De Wiljes** has long since been removed from the front of the head office. As are Limagrain, LG Zaden and within a few months from now Vandijke Semo, the name the company has carried since 1996. The company will continue under the new name Vandinter Semo, as the former namegiver, one of the directors, will soon be retiring.

The many changes of name do not reflect a swaying strategy though. The policy line is straight, the ambitions high and every employee is still focused on delivering quality, coming up with innovative solutions, being reliable and flexible. The result: it has become one of the largest Dutch companies that produces and processes agricultural seeds. Every year, 7,000 tonnes of seed find their way to clients all over Europe. Co-owners Henk van Dijke and Bert-Jan van Dinter give a tour around the premises.

Processing plant

Vandijke Semo is a large company and that is mirrored in the size of the plant: 13,000 m². The tour starts at the entrance where trucks deliver the seeds and identification takes place. Next are several buildings where row after row of cubic metre crates are piled up, waiting for their turn. To say the number of crates is impressive is an understatement, so to avoid mix-ups they are clearly marked. "We don't use bar-coding as yet", Bert-Jan van Dinter explains. "These crates travel a lot. That is why we are testing an alternative digital system."

The central hall is filled with seed cleaning and sorting machines to ensure that every type of seed can be processed. A necessity, as Vandijke Semo handles a wide range of species, from grasses, flowers, vegetables to fodder crops, maize, fibre hemp and green fertilizers, such as yellow mustard and fodder radish, just to name a few. There are machines that sort seeds based on form and size, based on weight, based on gravity or based on colour. "Please meet our eldest employee", says Henk van Dijke, as he points at an ancient looking seed cleaning machine that stands in a corner. This so-called 'slangentrieur' was built in 1914, and in spite of its age it still works perfectly. "We keep it as a museum piece."

State-of-the-art

In another hall, the seeds are coated, pelleted,

History at a glance

- 1914 Andries Zwaan and Otto de Wiljes start the company Zwaan & De Wiljes. The company is focused on the growing and trade of vegetable, agricultural, flower and grass seeds.
- 1925 Andries Zwaan leaves the company to start a new one, together with Karel van der Molen. Zwaan & Van der Molen, a company that started with selecting spinach seeds.
- 1930 The focus of Zwaan & De Wiljes shifts from vegetable seeds to breeding, growing and trading of grass seeds and other fodder crops.
- 1960 Grain merchant E.G. Smith buys Zwaan & De Wiljes.
- 1981 The French cooperative, Limagrain, acquires Zwaan & De Wiljes.
- 1986 Limagrain splits up the commercial activities and breeding. The latter remains in Scheemda.
- 1989 Henk van Dijke is appointed Managing Director of the Limagrain subsidiary in Scheemda. The company specializes in breeding and production of grasses. The name is changed to LG Zaden.
- 1995 Limagrain sells its grass breeding activities to the Danish DLF.
- 1996 Henk van Dijke takes over the remaining activities in Scheemda. The company is renamed Vandijke Semo.
- 2007 Bert-Jan van Dinter acquires 50% of the company's shares.
- 2014 The company celebrates its centennial on July 5th, Henk van Dijke retires and the name changes to Vandinter Semo.

primed or treated in another way the client desires. "We make sure that the quality of the seeds meets the standards. We are fully accredited by the Dutch General Inspection Service", states Van Dinter. "And we also carry out research, mainly on nematodes", adds Van Dijke. "We have one of the largest research programmes focused on cruciferae in Europe. We develop our own varieties of yellow mustard and fod-



Henk van Dijke and Bert-Jan van Dinter: 'In spite of our age, we are young and dynamic'

der radish that are resistant to nematodes. Years ago, we introduced 'Emergo', the first yellow mustard that was resistant against the beet cyst nematode", he fondly recalls. "Today we have varieties that are suitable for all major production areas in Europe." Van Dinter: "We also have a programme for potato cyst nematode. Our varieties of rocket leaf diminish the amount of these nematodes by 60-90%." The state-of-the-art climate chambers where the new varieties are tested for resistances do not need cooling. "We use LED lights and these produce almost no heat."

Unique position

Vandijke Semo holds a unique position when it comes to grass seed production. As the company does not have their own varieties, they do not compete with their clients. "More and more clients seem to appreciate that fact, because it offers a unique guarantee that their property rights are respected. We also reproduce pre-basic and basic seeds of several

agricultural and horticultural crops for other companies", says Van Dinter. Van Dijke: "We co-operate with carefully selected farmers in the Netherlands. As the climate is particularly suitable for the production of grass seeds, many companies from elsewhere in Europe have discovered the route to Scheemda. Besides, the Dutch farmer is renowned for his skills and we have experienced growing advisers, so the seed production is high. And, of course, we produce certified organic seeds too."

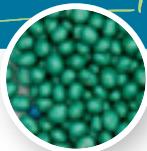
Seed production of their own varieties also takes place in Eastern European countries. "These seeds are centralised in Scheemda first, to be recleaned and repacked if needed. That way we can ensure that the quality standards meet the demands of our clients", explains Van Dijke.

Special products

A special crop that is reproduced by Vandijke Semo is spinach seed. Most of the seed production of spinach takes place in Denmark, but the Netherlands is well suited for this, according to the two directors. They have also broken into the novel market of gluten-free food. Since cardiologist William Davis published his cookery book 'Wheat Belly' in 2011, 20% of USA citizens stopped eating glutens. Davis claimed that a gluten-free diet not only prevents heart disease, but it would even reverse it in his eyes. And although science has found no grounds whatsoever for it, Europeans are following this trend. In truth, only a medical disorder, such as coeliac disease or gluten sensitivity, requires a gluten-free diet. A basic ingredient for a gluten-free diet is oats. "And we are able to deliver oat seeds that are guaranteed free of any other cereals such as wheat", Van Dijke explains. He points out one of the cleaning machines that separates the oat seeds flawlessly. "This product can now be safely used in food destined for coeliac patients."

All in all it is clear that the company is thriving. "In spite of our age, we are young and dynamic", is the conviction of Bert-Jan van Dinter and Henk van Dijke. "We are looking forward to celebrating our first centennial."

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VANDIJK SEMO
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In Vitro Grown *Arabidopsis*

Salicylic acid

Anna Czerednik, Joanna Ammerlaan, Aoife McCarthy and Anton

- In vitro conditions have a tremendous effect on the physiology of plants growing in tissue culture. One of the features that are affected is the very poor ability of tissue-cultured plants to retain water. When no special measures are taken, transfer from the humid in vitro conditions to soil leads almost instantaneously to wilting. This is a persistent problem for the application of plant tissue culture.

The failure of tissue-cultured plants to retain water leads to prompt wilting after transfer to soil. The very poor water retention is caused by the inability of stomata to close. Both stomatal structure and function are affected by the in vitro conditions. Guard cells are often raised and rounded, rather than sunken and kidney-shaped as in ex vitro plants. In many cases, in vitro plants have higher stomata densities and stomata themselves are bigger than their counterparts growing in soil. Conventional acclimatization of tissue-cultured plants involves a gradual decrease of the humidity and a slow increase of light intensity. Nevertheless, growth and survival are often still considerably impaired.

Arabidopsis seedlings grown in vitro show the

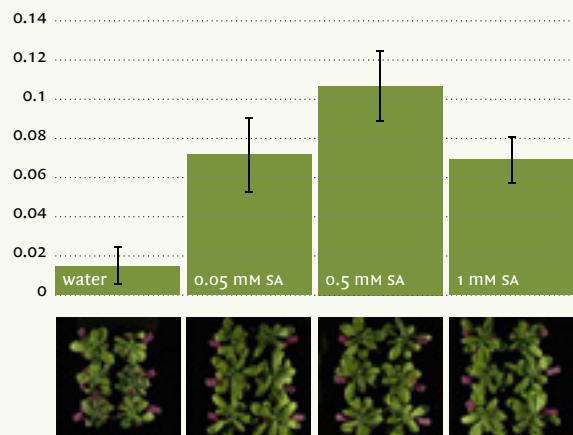
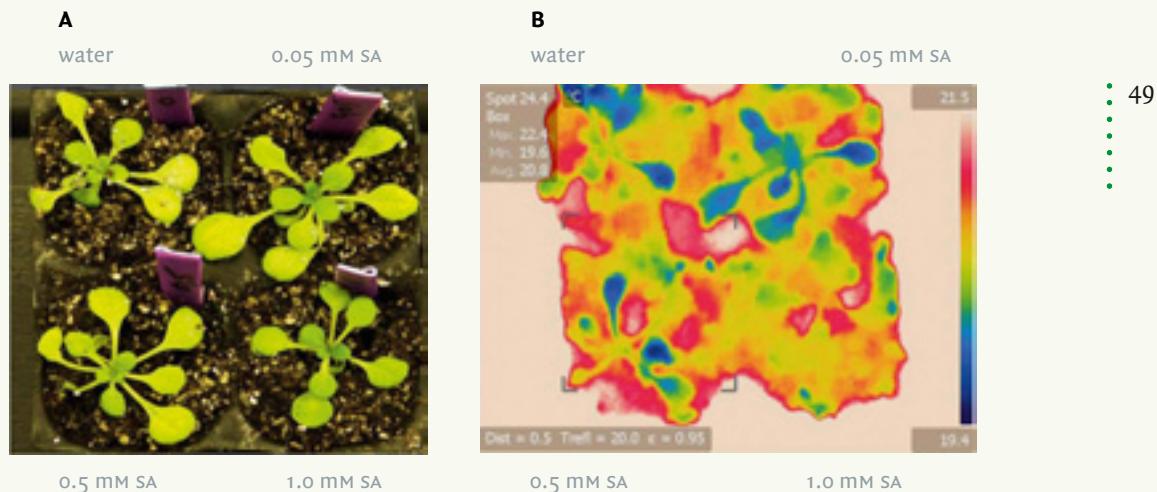


Fig. 1. *Arabidopsis thaliana* 20 days after transfer from in vitro to ex vitro after 18 days of culture in vitro. A. Plants in soil; B. Dry weight (gram). One day before transfer to soil, the seedlings were treated in vitro with different concentrations of salicylic acid (SA), or with water as a control

closes the stomata

Peeters

Fig. 2. *Arabidopsis* plantlets one hour after transfer to the soil. A. photographed using a normal digital camera and B. photographed using an infrared imager. The lower the temperature (see scale at the right, blue indicates a relatively low temperature), the higher the transpiration. Thus, transpiration was lowest at 1.0 mM SA



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same abnormalities as other tissue cultured plants, but the responses of *Arabidopsis* are amenable to genetic analysis. After several weeks of growth in vitro, we observed that the ratio of root/stem in *Arabidopsis* seedlings had changed, that the leaf area was reduced and that the cuticle had developed. However, leaf thickness increased in in vitro plants. Leaves were composed of a spongy, largely vacuolated mesophyll with large intracellular spaces and palisade tissue was often absent. Many intercellular spaces were full of water, which leads to hypoxia. Plants developed in vitro had more large stomata per area.

Application of salicylic acid (SA) improves the resistance to various abiotic and biotic stresses. SA is a monophenolic compound that plays a signaling role in many physiological processes, including flower induction, stomatal behaviour, ion uptake and membrane permeability. SA also plays a major role in disease resistance, acting as a signal transducer during biotic stress. Various physiological responses occur after application of SA. In drought-stressed chickpea, SA improves water status and dry weight significantly. Physiological parameters such as photosynthetic rate, chlorophyll concentrations and Rubisco levels are higher. SA may function in osmoregulation during drought stress and SA induced drought tolerance may be the result of accumulations of organic and inorganic solutes within roots and shoots. SA induced high-light acclimatization is related to alterations in

metabolism affecting photosynthesis and redox homeostasis.

Acclimatization

We studied the effect of SA on acclimatization using *Arabidopsis thaliana* Landsberg erecta (Ler) as a model. This ecotype had an intermediate reaction to stress, related to the in vitro conditions. After 18 days culture in vitro, the time at which they were transferred to soil, seedlings showed reduced growth. Twenty days after transfer to soil, the diameter of the rosette was smaller than in control seedlings that had been sown directly in soil. Some plantlets already developed flower buds (Fig.1). The application of SA one day before transfer ex vitro (at day 17) improved the acclimatization and did promote growth.

Observation with infrared light showed changes in transpiration after the application of SA one day before transfer to soil (Fig.2). Six hours after transfer to soil, microscopical observations showed complete closure of the stomata on both the abaxial and the adaxial side of the leaf in all SA-treated plants. In untreated plants, this ability to react to changes in humidity had recovered only after several days. Knowledge about how SA brings about stomatal closure is limited, but research has revealed the involvement of several components including ROS, NO and K⁺ channels. SA induced stomatal closure is inhibited by catalase and superoxide dismutase, indicating the role of ROS in signal transduction.

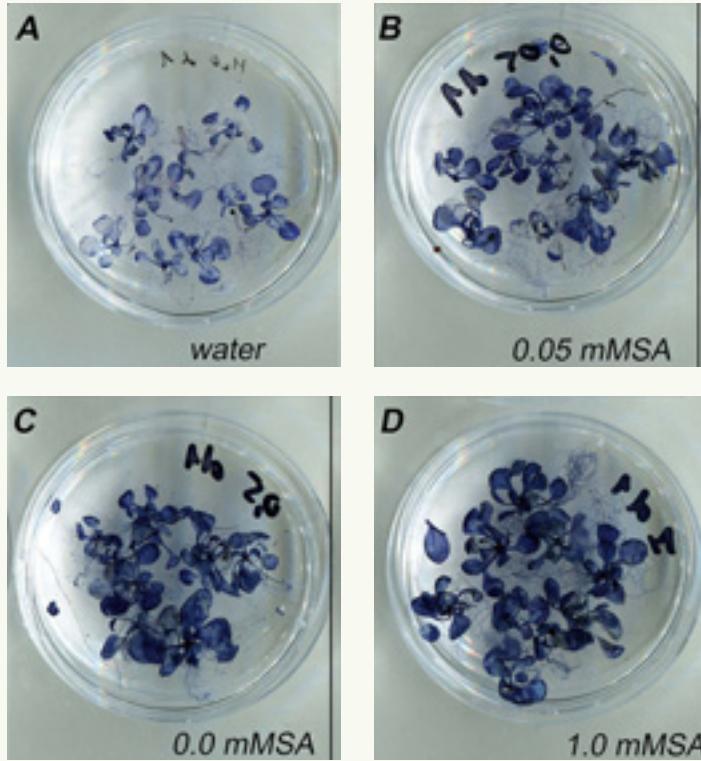


Fig.3. Visualization of superoxide radicals by nitroblue tetrazolium (NBT) staining. Visualization of Arabidopsis seedlings treated with different concentrations of SA was made one day after transfer from in vitro to soil

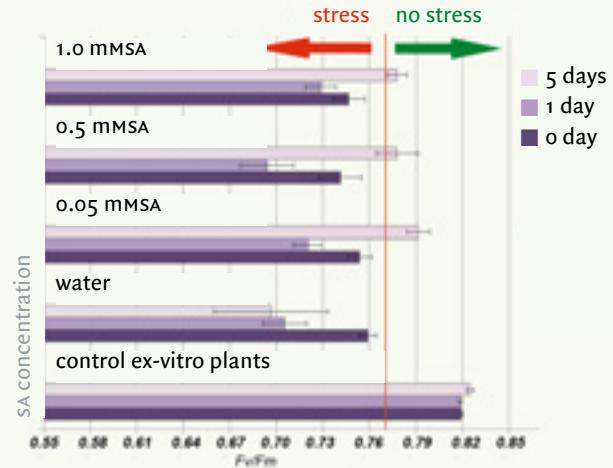


Fig.4. Chlorophyll fluorescence of control (soil-sown seedlings) and in vitro cultured Arabidopsis seedlings treated with increasing concentrations of SA and transferred to soil. The red line indicates the threshold of Fv/Fm for stressed (left side) and recovered/unstressed (right side) plants. Note that Fv/Fm ratio of SA-treated plants had normalized 5 days after transfer to soil

However, SA induced stomatal closure still occurs when NADPH oxidase is inhibited and in mutants lacking RbohD and RbohF genes. This implies that ROS involved in the SA pathway are generated in a different way from the ABA pathway.

Superoxide radicals

We analysed accumulation of superoxide radicals in treated plants using the nitroblue tetrazolium (NTB) staining. The presence of superoxide radicals was visualized by a blue precipitate (Fig.3). We suppose that an increase of superoxide dismutase could be one of the reasons of changes in stomatal aperture in SA-treated plants.

In tissue culture, high levels of sucrose inactivate Rubisco, reduce its catalytic turnover rate and reduce photosynthetic efficiency, in addition to the effects of low light intensity and low CO_2 concentrations. During successful acclimatization, plants switch from heterotrophic to photoautotrophic nutrition. New leaves generated ex vitro display higher photosynthetic activity than those generated in vitro. Leaves develop thicker epicuticular wax and mesophyll differentiates into palisade and spongy parenchyma with fewer intracellular air spaces than in vitro plants.

For an efficient estimation of the photosynthetic activity, we used chlorophyll fluorescence. This is a non-destructive method often used in ecophysiological researches. It enables the

changes in photosystem II (PSII) to be followed during acclimatization. Chlorophyll fluorescence is measured as the ratio of variable and maximum fluorescence (Fv/Fm) after dark adaptation, which shows the maximum quantum efficiency of PSII. In our experiment with different accessions of Arabidopsis, we found that the threshold of stress/unstressed plants is 0,77 Fv/Fm. Below this value, plants suffer from stress. Plants pretreated with SA recovered their photosystem ability faster. They already reached the threshold value 24 hours after transfer to soil and were completely recovered after 5 days (Fig.4).

Conclusion

Arabidopsis is an excellent model to study acclimatization of in vitro plants. It suffers from the same morphological and physiological abnormalities as micropropagated commercial plants, but offers many exciting possibilities to study the underlying mechanisms. Our results suggest that application of SA to tissue-cultured Arabidopsis seedlings improves acclimatization via stomatal closure.



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