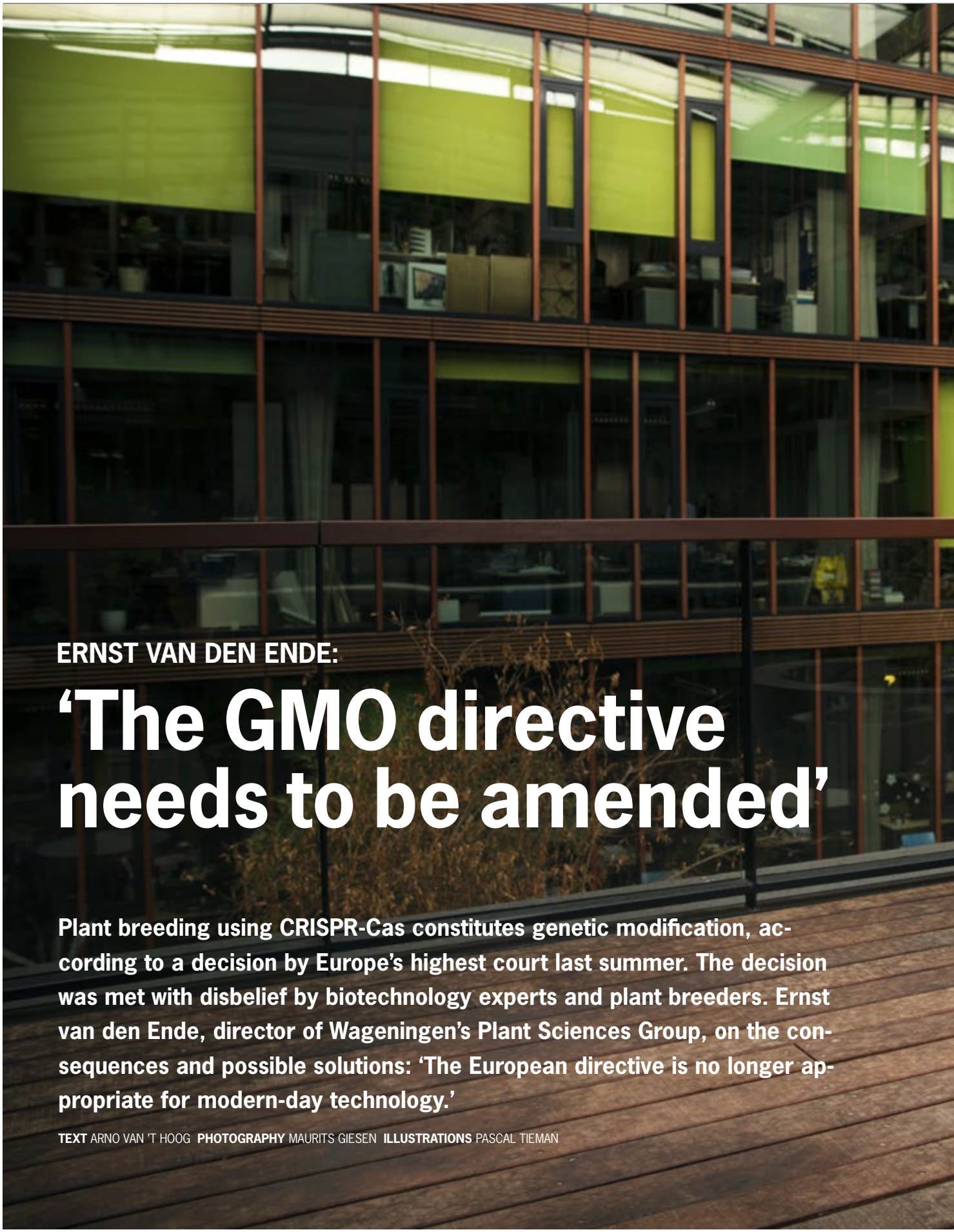

ERNST VAN DEN ENDE:

‘The GMO directive needs to be amended’

Plant breeding using CRISPR-Cas constitutes genetic modification, according to a decision by Europe’s highest court last summer. The decision was met with disbelief by biotechnology experts and plant breeders. Ernst van den Ende, director of Wageningen’s Plant Sciences Group, on the consequences and possible solutions: ‘The European directive is no longer appropriate for modern-day technology.’

TEXT ARNO VAN ’T HOOG **PHOTOGRAPHY** MAURITS GIESEN **ILLUSTRATIONS** PASCAL TIEMAN

‘We are talking about changing just a single DNA letter in a billion’

‘In lectures, I often try to clarify things by using the tomato as an example,’ says Ernst van den Ende, director of the Plant Sciences Group at Wageningen University & Research. ‘That species has one billion DNA letters. If you compare the DNA of a commercial tomato with its wild forebear, then you see that an average of about 20 million DNA letters are different. Those differences arose in the past through classic plant breeding and mutation breeding, which are not subject to controls. Now we are talking about changing one or two letters in the DNA with CRISPR-Cas and this is supposed to need legal controls.’

The ruling by Europe’s highest court, which deemed CRISPR-Cas to be genetic modification, dates from July 2018. One consequence of this ruling is that European seed companies will have to compile a large dossier if they want to apply this technology in their plant breeding programmes. Market approval for crops that are officially genetically modified costs years and tens of millions of euros in safety tests, field trials and risk assessment. That is a major barrier to breeding crops with new properties, says Van den Ende.

STRICT RULES

‘It could have been so great,’ he says. ‘This technology is used in labs all over the world. We can keep on doing this, of course, but we can’t test plants outdoors that were developed using CRISPR because you need so many different licences for that. You have to work in greenhouses under strict GMO rules.’

‘Aurélie Jouanin, who recently obtained her doctorate in Wageningen, showed that you can use CRISPR-Cas to breed wheat in which the gluten has been modified in a way that lets people suffering from coeliac disease eat it. There are other interesting applications of CRISPR-Cas, such as improving resistance to disease. The Dutch government aims to reduce the use of crop protection products to zero by 2030. But how are we going to do that? There is an urgent need to come up with better, more resistant varieties soon. CRISPR-Cas could play a major role in this.’

DEBATE

CRISPR-Cas is a technique that has attracted a great deal of interest among numerous researchers in Wageningen and far beyond, whether they are working on plants, bacteria or animals. Never before have scientists been able to make changes in genes so quickly, accurately and relatively simply. After the first publications on CRISPR-Cas appeared in 2013, it did not take long for a debate to arise about how this technology should be treated: exempt from controls, or included in the regimen of extensive tests for environmental risks that applies to genetically modified organisms such as transgenic maize?

CRISPR-Cas technology is based on elements of a bacterial immune system that recognizes viruses attacking the bacterium and renders them harmless by cutting the virus DNA. Scientists in labs have managed to reengineer CRISPR-Cas such that it can also make cuts in the genetic material of plants, for instance. A CRISPR-Cas cut results in a DNA letter being removed or added. Researchers can use these subtle changes in the DNA code – genome editing – to switch off genes or introduce different properties. This technique particularly appeals to plant breeding specialists as it lets them create new genetic variation. New mutations are the starting point in the quest for improved properties, such as firmer stalks in cereals, higher yields or resistance to infections. In the past few decades, plant breeders have created mutations artificially by irradiating plants or treating them with chemicals; these are methods that produce large-scale changes in the DNA. The Institute of Radiation Breeding in Japan, for instance, has developed various new plant varieties by growing crops in fields around a strong cobalt-60 radiation source. That used to be done in Wageningen too, at the Institute for the Application of Nuclear Energy in Agriculture, which researched the irradiation of crop plants between 1964 and 1980. The institute introduced new chrysanthemum mutations to the market with unusual colours.

The application of radiation and chemicals in plant breeding is termed ‘classical mutagenesis’. Although these techniques involve changes to the genetic material, they are not regarded as a risk to the environment in the European legislation on genetically modified organisms. They are exempt from controls: no licence is required for the cultivation, introduction and processing of crops with mutations caused by classical methods, and there are no labelling requirements either. No such exemption applies for maize, for example, where genes from a different species have been inserted to make the plant resistant to herbicides or insects. Those are GMOs, which are only allowed in Europe under strict conditions.

DIRECTIVE OUTDATED

Various new techniques have been introduced for modifying DNA since the GMO directive came into force in 2001. So there was already a debate about legislating for new technology, says Van den Ende. ‘In fact, science has rendered the directive outdated so

GMO REGULATION

European legislation does not see conventionally mutated crops as an environmental risk. Because the technique has been in use since 1930, there is a lot of knowledge about its safety. Techniques such as CRISPR-Cas do not have that kind of track record yet, and therefore come under the strict GMO regulation.

The DNA of a tomato has one billion DNA letters. If you compare a modern tomato with a wild ancestor, 20 million DNA letters differ. Those differences came about through breeding using radiation or chemical treatments: **classical mutagenesis** techniques which cause DNA changes on a large scale.

CRISPR-Cas is a new technology for creating genetic variety. This precise gene-editing technology is used to cut through a DNA letter at a particular location. The technology is developing very fast. There are now variants that not only cut through the DNA, but also change DNA letters, from a C to a T, for example. This makes the outcome more predictable.

that the text needs to be amended. It is simply no longer appropriate for modern-day technology. The European Parliament had already put off that debate because the subject matter is complex. And now another new technology has been added: CRISPR-Cas.'

In October 2016, the debate about new plant-breeding techniques moved to the courtroom after the French court of appeal asked the European Court of Justice for a ruling on the question of whether organisms with a CRISPR-Cas mutation are genetically modified. The issue had arisen in a case instigated by nine French civil society organizations. 'The strange thing was that the Advocate General at the European Court of Justice – who advises the court – had suggested quite a flexible approach to CRISPR-Cas. That is why it came as such a surprise when the Court jumped to considering CRISPR crops to be genetically modified organisms.' >

GENESPROUT INITIATIVE

Plant biotechnology student Damian Boer was disappointed when he read about the European ruling on plant breeding techniques such as CRISPR-Cas during his summer holiday in Georgia. 'I've spent five years learning about new techniques and their interesting and useful applications. You're on the verge of graduating and you hear: sorry guys, the techniques are being cancelled – we have to continue with the traditional methods, so we won't be able to use that new knowledge. I'm seriously considering a career in America or China where these technologies are subject to fewer rules.'

This was what prompted Boer to start GeneSprout, an initiative for young plant scientists at Wageningen who want to become involved in the debate about new plant-breeding techniques. 'We're focusing on providing accessible information about CRISPR-Cas. And we want to throw open the public debate about this new technique, including among students in Wageningen. There are a lot of different kinds of plant scientists, but they have little contact with one another, and each individual has their own assumptions and strong opinions. Students who focus on organic farming seldom talk to plant biotechnology specialists and vice versa. Whereas we are all pursuing the same goal – sustainable food production. We could reduce some of the polarization.'

The European Court of Justice argued that while the techniques of classical mutagenesis make comparable genetic interventions, there is much more knowledge about their safety because they have been applied since 1930. 'Techniques developed after 2001 don't have that history. And so they are covered by the strict GMO rules.'

SONNY PERDUE

This decision has created two worlds. In March 2018, the American government decided not to impose controls on plants that have been bred using CRISPR-Cas as long as the mutations that were introduced are indistinguishable from natural mutations or classical mutagenesis. According to US Secretary of Agriculture Sonny Perdue, this will let plant breeders work faster and more accurately. Canada and China have adopted a similar view.
It is a simple and obvious argument, says Van den Ende. 'In the United States they look at the DNA in the final product. If that is no different from what could occur in nature, then they don't consider it to be a GMO product. In Europe they look mainly at the technological process and what exactly is involved in the intervention. If someone in the lab does something artificial to the DNA, something unnatural is happening and so we call it a GMO.'
If CRISPR-Cas is seen in Europe as a GMO technique, that will have quite a few consequences, for example for import controls, labelling and the approval of new varieties. There will be new issues for international trade too. In Canada, for example, CRISPR-Cas is permitted. Europe has a trade agreement with Canada (CETA), which has informally already come into effect although it has not yet been ratified by

'There is an urgent need to come up with better varieties soon'

all the EU countries. In principle, Canadian exporters will be able to sell new CRISPR products to the European market, says Van den Ende. 'We will have to accept that because no technology exists that can detect whether or not CRISPR-Cas has been used.'
'If these rules are kept, only the big companies will be able to afford the European approval process. The larger companies in Europe don't see this as a positive development either but at the same time it does protect their position of power because little companies won't stand a chance any more. Whereas CRISPR-Cas is a fairly cheap and simple technology that smaller plant breeding companies and start-ups could use too. That would help do something about the concentration of power in the agro-industry that a lot of people have been concerned about in recent years. But that innovation is now being nipped in the bud.'
The Netherlands is one of the biggest players globally in the breeding and production of seed for vegetables, and the market leader in breeding and exporting seed potatoes. Van den Ende has noticed that project proposals for plant breeding with CRISPR-Cas are being withdrawn because it will not be easy to market any resulting product in Europe. 'Some breeding companies are stopping their work on CRISPR; that's simply a choice in what to spend their R&D money on. Companies breeding tomatoes and cucumbers, for instance, are however using CRISPR-Cas in the laboratory to track down genes. CRISPR lets them switch genes on and off, and thereby figure out what the promising genes are for growth, yields or disease resistance. They then look for interesting gene variants in their collection of wild relatives and cultivars. Then they use those plants in a targeted manner in cross-breeding.'

RELOCATING POTATO BREEDING

The potato breeding sector was loudest in its protests at the decision by the Court. Potato breeding company HZPC in Joure has already said that it is thinking of relocating part of its plant-breeding programme to the United States. The potato has a complex genome map with eight copies of each gene, compared with two copies in a cucumber for instance. As a result, potato cross-breeding is a particularly lengthy process and CRISPR-Cas could speed that process up. Van den Ende says that people are only now starting to realize the consequences of the

‘Smaller companies in particular could use CRISPR-Cas’

ruling for research and international trade. Because of this, attempts are being made to find new solutions. Various meetings are being planned over the next few months in Brussels with MEPs and representatives of NGOs. One solution would be to rewrite the European directive to make it fit better with the current state of science. But that process can take five to ten years in the European Parliament. A faster route would be to draw up an annex to the directive, comparable to the annex for classic mutagenesis. Van den Ende: ‘We are seeing a majority emerge – including in the Netherlands – for such an annex that exempts CRISPR-Cas technology when it introduces minor mutations.’
At the annual international conference CRISPRcon, started a few years ago, participants discuss the science, technology and public debate relating to gene editing. In June the conference will be held in Wageningen, and Van den Ende is responsible for the local organization. CRISPRcon is expected to bring 500 to 700 participants to Wageningen. ‘Proponents and opponents will have their say. Not to trade insults but to inform people comprehensively of all the arguments that the different groups have. Hopefully we will have enough understanding of one another to find a golden mean.’ ■

www.wur.eu/crispr-cas

CRISPR-CAS IN PLANT BREEDING

In the past few years, researchers have used CRISPR-Cas to edit various food crops in search of improved properties. In January, Aurélie Jouanin received her doctorate in Wageningen for genome editing using CRISPR-Cas with the aim of changing the immunological properties of gluten so that wheat flour could become suitable for people who are gluten-intolerant (who have coeliac disease). The approach works but more mutations are needed to produce wheat that does not spark off any immune response at all.
Chinese scientists have switched off a gene in tropical indica rice in order to get the plant to develop shorter stalks, a property associated with improved yields. Yield-improving mutations have also been made in soybeans. And an American laboratory has mutated a gene in tomatoes so that the plant’s growth is more compact and it flowers (and consequently bears fruit) earlier in the season. Furthermore, mushrooms have been developed with CRISPR-Cas that have less of a tendency to turn brown thanks to mutations in an enzyme. It also turns out to be possible to modify the composition of fatty acids in oil-bearing crops.