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From the tumor-inducing principle to plant biotechnology and its importance for society

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ABSTRACT This dialogue was held between the Guest Editors of the Special Issue on "Plant Transgenesis" of the *Int. J. Dev. Biol.* and Marc Van Montagu. Research in the group of Marc Van Montagu and Jeff Schell in the 1970s was essential to reveal how the phytopathogenic bacterium *Agrobacterium tumefaciens* transfers DNA to host plants to cause crown gall disease. Knowledge of the molecular mechanism underlying gene transfer, subsequently led to the development of plant transgene technology, an indispensable tool in fundamental plant research and plant improvement. In the early 1980s, Marc Van Montagu founded a start-up company, *Plant Genetic Systems*, which successfully developed insect-resistant plants, herbicide-tolerant plants and a hybrid seed production system based on nuclear male sterility. Even before the first transgenic plant had been produced, Marc Van Montagu realized that the less developed countries might benefit most from plant biotechnology and throughout his subsequent career, this remained a focus of his efforts. After becoming emeritus professor, he founded the *Institute of Plant Biotechnology Outreach* (IPBO), which aims to raise awareness of the major role that plant biotechnology can play in sustainable agricultural systems, especially in less developed countries. Marc Van Montagu has been honored with many prizes and awards, the most recent being the prestigious World Food Prize 2013. In this paper, we look to the past and present of plant biotechnology and to the promises this technology holds for the future, on the basis of the personal perspective of Marc Van Montagu.

KEY WORDS: *Agrobacterium tumefaciens, crown gall, genetically modified plants, plant genetic engineering, Ti plasmid, World Food Prize*

Introduction

Marc Van Montagu was born on November 10th 1933 in Ghent, Belgium. He obtained a Master's degree in Chemistry and a PhD in Biochemistry from Ghent University and was later appointed as professor at the same university. Research in the group of Marc Van Montagu and his colleague Jeff Schell demonstrated that *Agrobacterium tumefaciens*, the causal agent of crown gall disease in plants, carries a large plasmid, the Ti plasmid, that is responsible for the tumor-inducing ability of this bacterium and that part of the Ti plasmid, the T-DNA, is transferred to plant cells. Knowledge of this gene transfer mechanism led to the development of plant transgene technology, and thus paved the way for many fundamental discoveries in plant science as well as for transgenic crop plants that are now widely used in agriculture.

Why did you decide to study sciences and how did you become interested in scientific research?

The secondary school that I attended at the end of the 1940s, the Atheneum in Ghent, actually offered a very good science education; I was especially fascinated by chemistry and, in the attic of our house, I even experimented with chemicals that I obtained from pharmacies in the neighbourhood. At that time, the Atheneum was one of the few schools where "Organic Chemistry" was taught, which stimulated my interest in the chemistry of living organisms. Incidentally, I read the novel "Arrowsmith" by Sinclair Lewis for the English class. For me, it was a captivating account of the life of a

Abbreviations used in this paper: GMO, genetically modified organism; IPBO, Institute of Plant Biotechnology Outreach; PGS, Plant Genetic Systems; T-DNA, transfer DNA.

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scientist, Dr. Martin Arrowsmith, who established his own laboratory at home and did cell biology experiments. Reading this novel, I realized there was more to biology than the descriptive lectures I got at school. In real life, this was also the period in which the later Nobel Prize winners Albert Claude and Christian De Duve developed cell fractionation methods, allowing the study of the structural and functional organization of the cell. I also had the chance to meet Professor Lucien Massart, lecturer of biochemistry in Ghent; in these days it was quite an honour for a high-school kid to talk to a university professor! He advised me to study pharmacy, which would satisfy my interests in biology and chemistry; however, as I feared to end up in a pharmacist shop, I decided to study chemistry.

In the last year of my chemistry studies, I followed the Biochemistry course of Professor Massart, which was optional, and I also chose a subject in his laboratory for my master thesis: I investigated β-amylases of barley, and the importance of the sulfhydryl groups for enzymatic activity. It was in Professor Massart's laboratory that I met Walter Fiers (Fig. 1A) and together with Walter, I attended the Youth World Congress of Biochemistry in Moscow in 1957. Many Russian scientists had visited Belgium the previous year for the International Conference on Biochemistry, organized in Brussels. As we came from Belgium and were introduced by Professor Massart, we were welcome to visit the laboratories of all the "big names" in biochemistry in Moscow. Thus, my first international scientific travel became very inspiring and also started a life-long connection with Walter Fiers.

What did you investigate during your PhD and in the subsequent years?

In 1955 I became research assistant with Professor Laurent Vandendriessche and I started a PhD, together with Walter Fiers on the nature of the phosphodiester bond in RNA, and a search for RNases with novel specificities. I left after a year to become vice director of a school for technical engineers for a few years, I did my military service, and I was also teaching in the brewery school in Ghent. Subsequently, I took up again the PhD research in nucleic acid chemistry with Vandendriessche, now on a slightly ambitious goal, i.e. the synthesis of the nucleotide triplets; this meant I was actually competing with the later Nobel Prize winner Gobind Khorana!

In the second half of the 1960s, I worked on phage genetics, mostly on RNA phages, again together with Walter Fiers and on phage λ together with René Thomas from the Université Libre de Bruxelles (Van Montagu *et al.*, 1967). Because mutations in RNA phages could not be mapped through recombination, I worked with one of my first PhD students, Joël Vandekerckhove, on sequencing proteins from phage MS2. At the beginning, we used really artisanal and time-consuming chromatography and elution methods, because we had no money to buy an amino acid analyser. What was interesting is that we could correlate the RNA sequences that had been determined in Walter Fiers's group with our protein sequences, allowing us to experimentally verify the predictions made from the phage nucleotide sequences (Contreras *et al.*, 1973; Vandekerckhove *et al.*, 1973).

In that same period, the scientific contacts with Jeff Schell (Fig. 1B) also intensified. I knew Jeff already as a student: he was the president of the humanist student organization, of which my future wife was vice-president. Jeff Schell's PhD research concerned biochemical and taxonomic studies of acetic acid bacteria. However, after several short-term stays abroad – among others in Bill Hayes's laboratory – Jeff also became fascinated by bacterial genetics, studying phage λ and DNA restriction and modification.

A remarkable aspect of phage genetics research was that it had no applications whatsoever, although it formed the basis of microbiology and molecular genetics. As a result, everyone in the field was very open and freely discussed new results. It was also in this spirit that we later started the crown gall research. We also welcomed very much researchers from countries outside Europe and the US in scientific meetings. Indeed in the 1960s the idea that

Fig. 1. Marc Van Montagu and colleagues. (A) *Marc Van Montagu (left) with Walter Fiers (Laboratory of Molecular Biology, Ghent University) (middle) in 1993.* **(B)** *Symposium in honor of Marc Van Montagu's retirement in 1999 (middle, front row), his wife, Nora Podgaetzki (on the left), and Jeff Schell (Max Planck Institute für Züchtungsforschung, Köln, Germany) (on the right).* **(C)** *James Watson (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA) (on the right) with Marc Van Montagu (on the left) and collaborators Ann Depicker (in the middle) and Geert Angenon (in the back) at the Laboratory of Genetics in 1989.* **(D)** *Marc Van Montagu together with Patricia Zambryski (University of California, Berkeley, CA, USA) at Howard Goodman's (Massachusetts General Hospital, Boston, MA, USA) birthday party in 2004.*

knowledge needs to be globalized was already present; science as a privilege of Europe and the US did not seem acceptable to us.

How did the work on *Agrobacterium* start?

At the end of the 1960s, Walter, Jeff and I started to have regular journal clubs, discussing what was new in molecular biology and related fields. From our discussions, it was clear that we wanted to start investigating eukaryotic systems. Walter was more inclined to medical research, whereas Jeff and I decided to study plant tumors, called crown galls, induced by *Agrobacterium tumefaciens*. We thought we could uncover important fundamental mechanisms by investigating these plant tumors; moreover working with plants or plant cells would require less sophisticated equipment than what would be needed for animal cell culture. Interestingly, the laboratory where Jeff had done his PhD had a large collection of *Agrobacterium* strains, including *A. tumefaciens*, *A. rhizogenes* and the non-pathogenic *A. radiobacter* (De Ley *et al*., 1966).

Did you immediately start with plant experiments?

Of course, we had no experience at all in handling plants or plant tissues and Jeff argued we should perhaps ask Rob Schilperoort from the University of Leiden, who had already several publications on *Agrobacterium*, to perform these experiments. However, when Jeff went to the US for a couple of months, I asked the advice of a colleague in plant biochemistry, Roger Van Parijs, on how to infect carrot tissue with *Agrobacterium*. He suggested to simply buy some carrots in a grocery store, and to surface sterilize and slice them before inoculation. And so began our first plant experiments. Tumors were obtained without problems on the carrot slices and we also used Kalanchoe plants for infections. When Jeff came back from the US, the experiments were well underway and he agreed that it was best to perform them in Ghent. I actually think there are still interesting discoveries to be made on tumor induction. In those first plant experiments we saw for example the phenomenon of the "teratoma" strains, *Agrobacterium* strains that induce tumors with profuse roots and thick leaf-like structures. Other strains never induced such teratomas, but smooth tumors. I still wonder whether this is just due to the auxin/cytokinin ratio in the induced tumors or whether other genes than the auxin and cytokinin biosynthetic genes are involved. Many regions in the T-DNA have never been investigated in much detail; they may encode interesting gene products, such as small RNAs.

What were the first crucial experiments to reveal the nature of the tumor-inducing principle?

The first key observation was that we could isolate, by gradient centrifugation, large plasmids from various crown gall-inducing *Agrobacterium* strains, whereas such plasmids were absent in non-pathogenic strains (Zaenen *et al*., 1974). From electron micrographs the size of the plasmids could be estimated and corresponded to 150-200 kbp. We formulated the hypothesis that these plasmids could be the tumor-inducing principle, although no direct evidence for this was available. In hindsight, we were also lucky that the technical capability to detect very large plasmids was lacking. Indeed, several *Agrobacterium* strains possess very large plasmids, such as the >500-kbp plasmid pAtC58 in the *A. tumefaciens* C58 strain, but their presence is not correlated with virulence. If we had been able to isolate these very large plasmids, perhaps we wouldn't have stated our hypothesis!

Subsequently, it was shown that loss of the 200-kbp plasmid from *A. tumefaciens* C58, by growth of the strain at 37°C, always led to a loss of tumorigenicity (Van Larebeke *et al*., 1974). Thus, the large plasmid was necessary for tumor induction, but we also had to prove that introduction of such a plasmid in a non-oncogenic strain, would make that strain oncogenic. Here the work of Alan Kerr, an Australian phytopathologist, was important; through careful observation, he had discovered that non-oncogenic *Agrobacterium* strains could acquire virulence when they were – at first accidentally, then intentionally – incubated on crown gall tumors containing oncogenic agrobacteria (Kerr, 1969; 1971). Transfer of virulence was not observed when the two *Agrobacterium* strains were mixed in liquid cultures, but only occurred in crown gall tumors and only after prolonged incubation times (up to six weeks) (Kerr, 1971). We wanted to show that the transfer of virulence as observed by Alan Kerr, was actually caused by transfer of the large plasmid. Jean-Pierre Hernalsteens, at that time one of my PhD students at the Vrije Universiteit Brussel, conducted thereto what became known as *in planta* conjugation experiments: non-oncogenic recipient *Agrobacterium* strains (made resistant to rifampicin and streptomycin) and oncogenic donor *Agrobacterium* strains were mixed and inoculated on wounded plants; after eight weeks of incubation, a large fraction of the antibiotic-resistant recipient bacteria had become oncogenic and were shown to possess the large plasmid originally present in the donor strain (Van Larebeke *et al*., 1975). At that moment we were sure that the large plasmids in virulent *Agrobacterium* strains carried the tumor-inducing principle and they were therefore named the tumor-inducing or Ti plasmids.

What gave a lot of impetus to our research in that period was the 1974 EMBO Meeting that Walter Fiers, Jeff Schell and I organised in Drongen near Ghent on cloning and DNA sequencing. All important people in the field were present: Walter Gilbert, Fred Sanger, Stanley Cohen, Herbert Boyer, Richard Roberts, and many others. For me it was a continuation of the international contacts I had established when I was working on phages. In 1966, I attended the first Spetsai Summer School where I met, for example, James Watson (Fig. 1C), scientists from Harvard and Cambridge University, and others. At the Spetsai Summer School I also came into contact with Howard Goodman, with whom we later collaborated extensively. In 1969 and 1970, I attended the Cold Spring Harbor Symposia and, at the same time, I visited James Watson's group and discussed with his collaborators such as Jeffrey Roberts, who discovered the rho factor. Many of these people became personal friends. Everything was based on personal contacts in those days and the atmosphere was really warm and cordial. Fax and e-mail did not exist, so we wrote letters or made phone calls, or talked in the bar during scientific meetings. I had especially good contacts with Richard Roberts, who, after his PhD, started isolating restriction enzymes and we collaborated on this. For example, Jef Seurinck, a technician in my group, isolated the enzyme *Msp*I from bacteria: it is a methylation-insensitive isoschizomer of *Hpa*II and is still used in DNA methylation studies today. Through Rich Roberts we had access to a range of restriction enzymes with which Ti plasmids were characterized by Ann Depicker (Fig. 1C) and others.

When did you realize that *Agrobacterium* could really be used for plant transgenesis?

As soon as it was established that the tumor-inducing ability of *Agrobacterium* depended on the presence of the Ti plasmid

(Zaenen *et al.*, 1974; Van Larebeke *et al.,* 1974; 1975), it seemed most logic that gene transfer from *Agrobacterium* to plant cells occurred and we formulated the possibility to use *Agrobacterium* as a vector for plant transformation. However in the mid-1970s, the focus of the research was on unravelling the mechanism of crown gall induction and on convincingly demonstrating that interkingdom gene transfer took place. This was certainly a controversial concept; remember that tumors would also form spontaneously in certain hybrids obtained by crossing particular *Nicotiana* species, the so-called "genetic tumors" (Smith, 1958). Therefore, quite a few plant physiologists remained skeptical of the genetic transformation hypothesis that we, molecular biologists, put forward, but for which we did not have direct evidence! Southern blots (Schell *et al.*, 1979; Thomashow *et al.*, 1980; Lemmers *et al.,* 1980) and DNA renaturation kinetics experiments (Chilton *et al.*, 1977) demonstrating the presence of T-DNA in plant cells only came a few years later. At the same time experiments were done to delimit the parts of the Ti plasmid that were transferred to the plant and thus formed the T-DNA (Depicker *et al.*, 1978; 1980; Chilton *et al.*, 1978; Zambryski *et al.*, 1980).

You have always been technology driven, haven't you?

Absolutely, techniques are essential to answer the biological questions. If you don't have access to appropriate technology, the questions remain out in the sky without a stairway to reach them. Already in the 1960s, I visited Edouard Kellenberger in Switzerland to learn electron microscopy techniques for visualisation of nucleic acids. Later we made extensive use of electron microscopy to study heteroduplexes. As you know, seeing is believing…. It was a technique for finding homologous regions in Ti plasmids of different *Agrobacterium* strains, and for mapping restriction fragments and transposon insertions on the Ti plasmid (Engler *et al.*, 1977; Hernalsteens *et al.*, 1980).

The Southern blotting technique was also crucial, and therefore our contacts with Richard Roberts, then at the Cold Spring Harbor Laboratory, were very important to get access to restriction enzymes, most of which were not yet commercially available. I spent a few months in Rich Roberts's laboratory in the winter of 1975, where I saw a first version of Southern's publication (Southern, 1975). Back in Ghent I could instruct Marc De Beuckeleer and other collaborators on the use of the DNA gel blotting technique. The restriction enzymes and the Southern blotting technique were essential to delineate and characterize the T-DNAs (Depicker *et al.*, 1978, 1980; De Vos *et al.*, 1981) and, of course, also to show that T-DNA was present in transformed plant cells (Schell *et al.*, 1979; Lemmers *et al.*, 1980).

Once the T-DNA transfer to plant cells was clearly demonstrated, how did you proceed to insert other foreign genes into plants?

At first, a transposon, Tn7, was introduced into a wild-type T-DNA and, subsequently, shown to be present in plant DNA (Hernalsteens *et al.*, 1980); it revealed that *Agrobacterium* can indeed be used as a vector to transfer foreign DNA, but of course this did not lead to a readily observable phenotype. Moreover, as the oncogenes were still present in this T-DNA, tumors were still formed. Therefore the ensuing steps, undertaken by Patricia Zambryski (Fig. 1D), Luis Herrera-Estrella and Marc De Block, were on the one hand, "disarming" the Ti plasmid by replacing the wild-type T-DNA – including the oncogenes, but not the 25-bp directly repeated T-DNA border sequence – with other sequences through homologous recombination (Zambryski *et al.*, 1983); and, on the other hand, on creating Ti plasmid-derived vectors with chimeric antibiotic resistance genes that conferred a selectable phenotype when transferred to plant

Fig. 2. Marc Van Montagu over the years during professional activities. (A) *Marc with suitcase ready to leave for his next journey.* **(B)** *As academic at the Université Catholique de Louvain (Belgium) in 1997.* **(C)** *At his 60th birthday party at the Laboratory of Genetics.* **(D)** *In his office as head of the Laboratory of Genetics at Ghent University.* **(E)** *In his office at the Department of Plant Systems Biology (VIB-Ghent University) during this interview.*

cells (Herrera-Estrella *et al.*, 1983a; 1983b). This ultimately allowed us to obtain fertile transgenic tobacco plants that transmitted the transgene to progeny (De Block *et al.*, 1984). For a more detailed account of these experiments, see Zambryski (2013) and Van Lijsebettens *et al.,* (2013) (this volume).

How did the idea arise in the early 1980s to establish the start-up company Plant Genetic Systems?

Genetic engineering started with the experiments of Herbert Boyer and Stanley Cohen in 1973. Soon thereafter, Herbert Boyer, whom I personally knew very well, founded Genentech. We noticed how medical biotechnology became successful and how pharmaceutical companies were investing in the production of peptides and proteins. Thus, there was no reason why plant biotechnology would not go the same way. In fact, Jeff Schell and I were asked at the end of the 1970s to be member of the scientific board of a start-up agrobiotech company, called AGS, in the United States. So we saw first-hand how such start-ups could attract venture capital, allowing them to build well-equipped laboratories and providing excellent opportunities for applied research. In addition, Ernest Jaworski tried to convince us to join Monsanto in St. Louis. However, neither Jeff nor I were inclined to go to the US and hence grew the idea to create Plant Genetic Systems (PGS) in Ghent, of which I was the first scientific director.

What interested you most, applications of plant transgenesis or the possibility to get insight into how plants function?

I was certainly interested in both aspects and it was generally thought that progress in basic knowledge and applications would develop hand in hand. It was a period of great optimism; for example, some people claimed that the ability to fix nitrogen could soon be engineered in all plants. However we rapidly realized that asking fundamental questions is easier than developing useful new crop varieties. Nevertheless we were immediately successful with several projects at PGS. While Monsanto was struggling to find a useful glyphosate resistance gene, we rapidly got access to the *bar* herbicide resistance gene through a collaboration with Julian Davies from the Biogen company in Switzerland (Thompson *et al.*, 1987), allowing us to develop transgenic plants with resistance to the herbicide Basta (De Block *et al.*, 1987). Simultaneously, cloning of insecticidal protein genes from *Bacillus thuringiensis* led to the development of insect-resistant plants (Vaeck *et al.*, 1987). Finally, a few years later, a tapetum-specific promoter provided by Robert Goldberg, allowed to introduce nuclear male sterility in plants, a trait that formed the basis for an efficient hybrid production system (Mariani *et al.*, 1990; 1992).

In the meantime, I remained director of the Laboratory of Genetics at Ghent University (Fig. 2). As very few processes were characterized at the molecular level in the middle of the 80s, nearly every subject was new and potentially interesting. For example, a superoxide dismutase was cloned from *N. plumbaginifolia* (Bowler *et al.*, 1989); this looked quite intriguing and worth investigating further; so our research on oxidative stress developed. In a similar way cell cycle research was initiated (Ferreira *et al.*, 1991), because such a strongly conserved mechanism certainly appeared worth exploring and new discoveries were likely with plants as study object. We could discern plenty of interesting scientific questions, and so the laboratory expanded considerably.

Indeed, you have had a very large number of collaborators. What were your criteria for recruiting students or post-docs?

Pragmatically, I tried to attract the students with the highest scores, because they can easily get scholarships. Among them, were those who lost themselves in details, which often interfered with creativity. At the other end of the spectrum, were the students with the wildest ideas. I preferred the latter, assuming that somebody would bring them back on the right track. As a student I was like that myself, being involved in all kinds of cultural and political activities, besides my studies. When Jeff Schell and I started to collaborate, one of our professors warned Jeff: "Do you know Van Montagu was president of the University Film Club as a student, this cannot possibly be a serious scientist!" Luckily, for me too, there were always sensible people around, for example Walter Fiers, who brought me back on the right track!

There always was a lot of diversity among the members of your laboratory. Was that a deliberate choice?

Yes, certainly, diversity was important. I always welcomed people from abroad, including researchers from developing countries, because I was convinced that good education is key to development. The international presence also resulted from the numerous contacts and exchanges I had in scientific meetings. Also women were well represented among the senior staff of the laboratory: approximately 50% of the group leaders were female. I could rationalize this now as a deliberate strategy, but I rather think this situation grew organically as a result of the general atmosphere in the laboratory where there was absolutely no bias regarding culture, nationality or gender. I did not believe in a strong hierarchical structure of the research group either. I rather tried to create an environment of interaction among the group members, to whom I gave sufficient freedom to pursue their own scientific interests.

In the year 2000 you established the Institute of Plant Biotechnology Outreach (IPBO). When and why did you start considering it important to focus on developing countries?

The concept gradually matured, but I started thinking about it early on. Even before the first transgenic plants were produced, I had been asked to give lectures and summer schools on plant genetic engineering at the University of São Paulo in Brazil. I did so for several years and at the same time I visited agricultural institutes in Piracicaba and Campinas. In the beginning of the 1980s, I was also invited to China as well as to Cuba, where the Center for Biotechnology and Genetic Engineering was being established. Through these visits I realized that the highest needs are exactly encountered in the less developed countries and that the promise of genetic engineering was even more important there than in the industrialised world. Moreover, many scientists from developing countries came to the laboratory from the early 1980s onward, mostly as PhD students. Luis Herrera-Estrella (Mexico) and Kan Wang (China), who both made very substantial contributions to the *Agrobacterium* field, were among the first. When we started with research with possible applications in agriculture, it was logical to strongly involve people from developing countries including Vietnam, Thailand and especially Brazil. Vice versa, I also encouraged people to go to laboratories in developing countries. One of the first was Jan Leemans, whom I advised to go for a post-doc to Cuernavaca (Mexico), where I knew high-quality research on

biological nitrogen fixation was going on. Several post-docs from the group also went to the University of Rio de Janeiro.

Later, we had collaborative projects with several countries such as Vietnam or Sénégal, and with CGIAR centres such as the International Rice Research Institute (IRRI, Philippines), the International Institute of Tropical Agriculture (IITA, Nigeria) and the International Center for Tropical Agriculture (CIAT, Colombia). In 1999, when I had become emeritus professor, I left the direction of the laboratory to Marc Zabeau and, later, to Dirk Inzé and, as an obvious continuation of my previous involvements, I founded IPBO with substantial financial support from Suri Sehgal, and also from Bayer. We started research on crops important for low-income countries and on folate metabolism and biofortification. We also provided post-doctoral scholarships to researchers from developing countries who had previously been trained in our research group.

What do you now consider as the most important mission of IPBO?

Undoubtedly, the main goal is to raise awareness for the major role that plant biotechnology can play in agricultural systems of developing countries. The IPBO staff tries to explain worldwide how genetically modified (GM) plants can contribute to increase crop yields, while minimizing the impact on the environment. We especially need to convince political authorities that GM plants are not inherently harmful, but, on the contrary, are needed for sustainable intensification, a now widely accepted concept.

Very important in this regard is our post-graduate programme "Biosafety in Plant Biotechnology". Many developing and emerging countries still lack a regulatory framework to commercialize transgenic plants. Especially in some African countries, intractable forces with good access to top politicians seem to raise new barriers preventing adoption of GM technology.

Therefore, it is important to educate professionals who understand biotechnology and its applications and who are able to set up affordable and science-based biosafety regulations. In the long run, more rational and less cumbersome biosafety laws are needed. The regulatory burden that exists at this moment prohibits, for example, small and medium-sized enterprises to commercialize GM plants. Through the United Nations Industrial Development Organization (UNIDO) and the Flemish Government, we also support Bt cotton development in Ghana and, more generally, promote plants as replacement of fossil feedstocks for industrial use. Besides food production, such non-food applications of plants are certainly important for developing countries. Take the example of Brazil, where ethanol has been produced for many years, but where, more recently, also plastic production from renewable plant resources has started. This approach sets an example for many other countries.

Finally, I see it as an important task for IPBO to stress the importance of classical breeding and to promote interactions between breeders and plant molecular biologists. This link is absolutely needed to obtain useful new crop varieties. In this respect, field trials with GM plants are very important; that is where you see the real phenotypes. Therefore it is such a pity that the costs for field trials are prohibitively high, mainly due to regulatory hurdles.

How do you think the difference in perception of GMOs in different regions of the world can be explained?

In nearly every country there are organizations or individuals that are opposed to GMOs [genetically modified organisms] for various reasons. The question is whether society at large is following them, and this depends on several factors, such as the point of view of influential politicians or other opinion leaders. A good example is Luiz Inácio Lula da Silva who protested together with José Bové against GMOs in Porto Alegre in 2002. However, when he became president of Brazil, he saw the advantages of the technology and changed his mind and, since then, the country is very supportive of plant biotechnology. What is also important in Brazil is that they have cases of GM plants developed in the country itself and of benefit to their own farmers, such as the virus-resistant bean plants (Aragão and Faria, 2009)

How do you see future developments in plant biotechnology? For example, do you think single gene overexpression will remain a worthwhile strategy?

It is difficult to generalize, but expression of single genes can certainly remain an important strategy. In this respect, I regret the recent emphasis on "cisgenesis" (transformation with sequences from the same species) because I expect the strongest effect from expression of transgenes, derived from other species. Endogenous genes are subject to many regulatory mechanisms, some of which we undoubtedly still have to discover. As foreign genes probably escape some of these regulation mechanisms, the chances are higher that they exert a major effect; see for example to the success of expression of *Bt* or herbicide resistance genes. Therefore, I predict that the fastest progress will be seen in engineering of pathogen resistance. Increasing yield, improving photosynthetic capacity and enhancing abiotic stress tolerance will be more dif-

Fig. 3. Marc Van Montagu, laureate of the World Food Prize 2013. *Marc Van Montagu (left) together with Mary-Dell Chilton (Syngenta Biotechnology Inc., Research Triangle Park, NC, USA) (middle) and Robert T. Fraley (Monsanto Company, St. Louis, MO, USA) (right), the 2013 laureates of the World Food Prize for their independent, individual breakthrough achievements in founding, developing, and applying modern agricultural biotechnology.*

ficult, but these are problems we absolutely need to tackle. The task may seem daunting, but I am convinced that progress in sequencing technology, in computational methods, and in techniques to study chromatin modifications and DNA methylation will allow us to unravel these processes. Indeed, epigenetic regulation is of enormous importance. The knowledge of epigenetic mechanisms added a whole new dimension to genetics and will continue to do so. Epigenetic modifications can be a prelude to genetic changes, in the sense that epigenetic changes in gene expression that are beneficial, may later give rise to more permanent genetic changes. This is a plausible mechanism to explain the high phenotypic variability seen in natural as well as in breeding populations.

Considering all the levels of regulation present in cells and organisms, another challenge for future research is to understand how crop plants function under real life conditions. So far, our investigations were necessarily limited to model organisms. However, studies of a mouse under sterile laboratory conditions may not be relevant to what happens in a patient and likewise crops in the field may react in a totally different manner than an *Arabidopsis thaliana* plant in a greenhouse.

It is only now we start to have the tools to address this complexity in crop plants grown under highly variable conditions and we can combine this knowledge with plant breeding efforts. This should generate the new plant varieties that are badly needed to develop a high-efficiency agriculture that is less polluting and makes minimal use of agrochemicals. Although I am sure that the chemical companies perform the necessary toxicological analyses, it is important to limit the use of synthetic molecules in agriculture, because they always bear the risk of collateral damage and harm to non-target organisms, including beneficial ones. Dealing with pests and diseases will no doubt be less detrimental when done via genetic engineering than via the use of pesticides.

Coming back to plant transformation, is further research in this area required?

This is certainly the case for many local varieties of orphan crops and it will be crucial for forestry species. We need improved trees for the production of biomass, energy and high-quality wood in plantations. This may contribute to save the natural forests that are left. However, breeding of tree species, which often have very long generation times, is extremely slow. At the moment this is overcome by clonal selection and vegetative propagation of the best clones. But reforestation with clonal forests entails the risk of spreading pests and pathogens. To keep genetic diversity and still speed up tree improvement, genetic engineering is required. Moreover, we need transformation methods for a wide range of genotypes to avoid monoculture. This is more generally the case for many crop species: the available transformation methods are mostly genotype specific and for example not applicable to many landraces. Therefore research in transformation technology and the use of *Agrobacterium* remains necessary, to be able to transform a wide range of genotypes and guarantee a maximum diversity in the varieties used in agriculture and forestry.

Epilogue

After this dialogue took place, the 2013 World Food Prize was awarded to Marc Van Montagu, Mary-Dell Chilton, and Robert Fraley (Fig. 3), in recognition of their ground-breaking work on *Agrobacterium tumefaciens*, the gene transfer mechanism and the development of the first plant transformation technology. They are honored because their work directly led to the development of a range of GM crops that, by 2012, were grown worldwide on more than 170 million hectares by 17.3 million farmers, over 90% of whom were small resource-poor farmers in developing countries. It is further recognized that the achievements of the 2013 World Food Prize laureates can play a critical role to face the global challenges of the 21st century of producing more food, in a sustainable way, under a changing climate.

The World Food prize, conceived by the 1970 Nobel Peace Prize winner Norman Borlaug, is the foremost international award recognizing the achievements of individuals who have advanced human development by improving the quality, quantity or availability of food in the world (http://www.worldfoodprize.org).

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