MARC VAN MONTAGU - HISTORICAL ACCOUNT OF ACHIEVEMENTS

Marc Van Montagu is widely recognized as a world's leading plant biotechnologist. His international intellectual leadership and field-defining discoveries have steered the development of plant biotechnology.

DISCOVERY OF TI PLASMID

The celebrated discovery of the Ti-plasmid of *Agrobacterium tumefaciens* by Jeff Shell, Marc Van Montagu and colleagues, and their research on the natural mechanism of gene transfer from Agrobacterium to plants, resulted in ground-breaking plant molecular genetics tools that rocketed fundamental and applied knowledge in plant sciences

Marc Van Montagu and Jeff Shell became interested in crown-gall-inducing strains of the bacterium *Agrobacterium tumefaciens* as a research model to their laboratory at Ghent University, Belgium in the late sixties. It was because the interaction between the bacteria and their hosts displayed many unusual features. Unlike other pathogenic bacteria that are known to cause plant tissues to die, wilt, or become discoloured, *A. tumefaciens* have the ability to cause infected plant cells to proliferate and form a tumour. Most unusually, crown gall tissues could be maintained in definitively in vitro in the absence of hormones and of Agrobacterium. This observation led, in 1947, to the concept of a tumour-inducing principle (TIP), a postulation that implied that the bacteria transferred this principle to the plant cell to induce transformation (1). Much work has ensued to identify the TIP. The preferred hypothesis in the late 60's was that a bacteriophage would be the causing agent.

Marc Van Montagu and Jeff Schell explored the possibility that bacterial DNA could be involved in *Agrobacterium* tumourgenesis. They conducted a systematic search for the presence of supercoiled DNA in a number of pathogenic and non-pathogenic strains of agrobacteria. This work culminated in the landmark discovery of a large (200,000 base pairs, the first of its kind) supercoiled plasmid in all virulent strains, but interestingly, this plasmid was not detected in any of eight avirulent strains tested (2). Marc and Jeff designated these plasmids tumour-inducing (Ti) plasmids. Direct proof that the Ti-plasmids were responsible for the tumour-inducing capacity of their host strains came through isolation of plasmid-free derivatives from tumour-inducing strains – the virulent strain C58 lose both virulence and Ti-plasmid when grown at 37 °C. Shortly thereafter it was demonstrated that *A. tumefaciens* strains acquire virulence as a result of plasmid transfer (3). The finding that oncogenicity and virulence were determined by a mobile, extrachromosomal element was revolutionary. The discovery of TIP opened up hitherto unforeseen scenarios for initiating the molecular genetics of plant-microbe interaction. With increased focus on the Ti-plasmid, a worldwide effort was launched to unravel the molecular basis for tumorigenicity, and the group of Jeff Schell and Marc Van Montagu continued to dominate the field.

FUNCTIONAL MAPPING OF THE TI PLASMID AND THE MECHANISM OF GENE TRANSFER

In the late 1960s it was demonstrated that crown galls generate copious amounts of novel metabolites, octopine and nopaline, and that crown-gall cells that were free from bacteria were still able to produce them (5). The *Agrobacterium* strain, not the plant, determines the type of opine made by the tumour and each *Agrobacterium* strain can catabolize solely its own particular type of opine. It was proposed that TIP must be the cause, or it must include a gene responsible for opine synthesis in the plant. At that time, it was difficult to accept the notion that a bacterial gene could enter a plant cell and function there. With the Ti plasmid in hand, the group of Van Montagu and Schell were in a position to test the idea. The novel technique of Southern-blot showed that a DNA segment highly conserved in all Ti-plasmids was present in tobacco crown-gall cells, firmly establishing that DNA transfer from bacterium to plants does occur. The transfer involves specific segment of the Ti-plasmid, which was called T-DNA (6). Adding quickly to this discovery, at a time when little was known about DNA sequence determinants for heterologous integration, a 25-basepair direct repeat on the Ti plasmid was identified at the borders of what is incorporated into the plant genome (7, 8). It was also shown that these sequences define T-DNA on the plasmid. At the same time, elegant cell fractionation analyses revealed that the T-DNA integrated into the nuclear, and not the chloroplastic or mitochondrial, genome of plants (9).

A year after the initial discovery of the Ti-plasmid, the first genetic marker, Agrocin 84 resistance, was shown to be encoded on the Ti-plasmid, thereby opening the way for more refined genetic analysis (10). Building on expertise in bacterial genetics and the use of phage mutagenesis in functional mapping (11, 12), the Ghent's

team set about dissecting the genetic structure of the Ti plasmid, and the molecular basis of the metabolic interaction between pathogen and host.

The mapping of transposon insertion and deletion mutations in the Ti-plasmid revealed the functional organization of the nopaline pTiC58 and octopine pTiB6S3 Ti-plasmids (13, 14, 15, 16), thereby confirming the observations that the Agrobacterium strain determines the type of opine synthetized in the crown gall. Transposon hits in T-DNA were found to eliminate opine production, to alter tumour morphology, or to have no phenotypic effect at all. The morphological mutations were used to demonstrate that genes encoding a pathway for the synthesis of the plant-growth regulators auxin and cytokinin mapped to the T-DNA (17,18). These studies of transposon mutagenesis also showed that loci with opine catabolic functions and another region affecting tumour induction (*vir* region) are mapped outside the T-DNA.

As soon as the structure and function of the Ti plasmid was understood, the concept that the Ti plasmid could be used to deliver novel genes into plants was born. The molecular dissection of plant physiology through target manipulation of gene expression was in sight. Furthermore, the possibility of introducing at will genes that could confer new desirable properties to a plant and the potential for revolutionizing plant breeding and crop production was obvious. Galvanized by the realization of this outstanding opportunity in plant science, research progressed rapidly, both in analysis of the Ti plasmid and in understanding the process by which the plasmid DNA is integrated into the plant genome.

THE IMPACT OF THE DISCOVERY

MOLECULAR GENETICS OF PLANT-MICROBE INTERACTION

Agrobacterium is an extremely interesting biological system and its study has led to many fundamental insights on plant-microbe interactions.

For example, the recognition that bacterial genes could integrate into the plant genome and direct the synthesis of plant growth regulators, thereby creating the microenvironment (the tumour) for their growth, was revolutionary and formed the basis for much of what we know about horizontal gene transfer. The discovery that the T-DNA intermediate is a single-stranded molecule elicited the hypothesis that T-strand transfer occurs by a conjugative mechanism, where the recipient is a eukaryotic plant cell (19, 20). This interkingdom DNA conjugation stimulated basic research in bacterial conjugation. In the postgenomic era, we now know that horizontal DNA transfer is responsible for a great deal of adaptive responses and invasive behaviour, and that the genomes of living organisms are much more fluid than previously imagined. (c) The induction of *vir* genes by plant phenolics was one of the first examples of communication between microbes and plants in the soil environment (21).

PLANT GENE VECTORS

Conversion of the Ti plasmid of *Agrobacterium* into a gene vector progressed in multiple stages. The transposon mutagenesis approach showed that no mutation in the T-DNA could block T-DNA transfer, and that all the genes affecting the process of T-DNA export to the plant cell mapped in the so-called *vir* region, outside the T-DNA borders (15). Methods were then developed for site direct mutagenesis and for insertion of DNA into any part of the Ti plasmid by homologous recombination by means of a co-integrate plasmid, which is the product of homologous recombination through a single crossover between a small plasmid of bacterial origin and an *Agrobacterium* Ti plasmid. Integration of the two plasmids requires a region of homology present in both plasmids introduced into *A. tumefaciens* (22). Co-integration was used to manipulate and recombine genes of interest into the T-DNA of the large Ti plasmid. Such co-integrated vector system was also used to develop the first transgenic plants (23).

Screening plant transformants for their ability to synthesize nopaline, or their inability to grow in the absence of exogenous cytokinin was cumbersome, and was incompatible with the main goal of selecting few transformants from a large background of false positives. Sequencing the nopaline synthase (NOS) and the octopine (OCS) genes (24, 25) and fusing their promoter and terminator sequences to a kanamycin-resistance encoding gene enabled the creation of a selectable marker for plant cells. The bacterial regulatory sequences that were known to function *in planta* would drive the expression of a gene that would allow survival of transformants under antibiotic-selection conditions.

THE FIRST GENETICALLY MODIFIED PLANTS

The way to obtain the first transgenic plants had been paved, and in 1983 Schell and Van Montagu's, Fraley's, and Chilton's groups simultaneously announced, at the 15th Miami Winter Symposium, the success in the use of Ti-derived plant gene vectors, antibiotic selection of transformants, and regeneration of fertile plants that passed on the chimeric gene in a stable and Mendelian manner to their progeny. Only nine years after the discovery of the Ti plasmid, the "Golden Era" of plant molecular genetics had begun and the development of plant transgenic technologies would expand dramatically in the nineties.

PLANT PHYSIOLOGY AND DEVELOPMENT

The demonstration that *Agrobacterium* could be used as a vehicle to transfer and integrate any foreign gene into the plant genome (26) undoubtedly opened the door to a completely new area of plant sciences and plant engineering. For the academic world, this technology allowed researchers to study plant processes through gain of function and enabled a systematic and refined analysis of the impact of single genes on all aspects of plant biology. In the early years, the technology was applied in model plants, such as *Nicotiana tabacum* (23) and *Arabidopsis thaliana* (27). Transgenic model plants were the basis to identify and study fundamental principles, such as transcriptional regulation in plants (e.g., the signals needed to induce expression of genes by light) (28), or the translocation of proteins in the plant cell (e.g., the signal peptides to transport a protein to the chloroplast) (29). These initial successes convinced an ever-increasing number of labs throughout the world to rapidly adopt the novel genetic engineering technology. Much of today's detailed knowledge of how plants grow, develop and respond to pathogens or abiotic stresses, as well as gene regulatory mechanisms such as RNA gene silencing have been unravelled by creating transgenic plants through *Agrobacterium*-mediated transformation.

Some of the first efforts to understand the function of all genes in a genome have been launched in plants, to a large extent thanks to the availability of the efficient gene-transfer technology offered by *Agrobacterium*. The possibility of using T-DNA as an intentional mutagen had been realized early on, and the publicly available populations of T-DNA-tagged mutants in Arabidopsis are now so large that nearly every gene in the genome is tagged. In addition, reverse-genetic strategies on DNA from populations of T-DNA mutants allowed researchers to start with one gene sequence and to identify a mutant line. The use of these mutant lines in labs throughout the world has led to a thorough understanding of the physiology, biochemistry, and developmental programs of plants.

The laboratory of Marc Van Montagu has given pioneering contributions on plant gene discovery and regulation (e.g. 30, 31), plant molecular mechanisms of environmental sensing and response to abiotic stresses (e.g. 32, 33, 34), plant development (e.g. 35, 36) and gene silencing (e.g. 37).

TI-PLASMID AS AN AGENT OF PLANT EVOLUTION

Recent works have shown that gene transfer from agrobacteria to various plant species had occurred under natural circumstances. Several *Nicotiana, Linaria* and *Ipomoea* species carry T-DNAs (called cellular T-DNA or cT-DNA) from Agrobacterium spp. into their genomes. cT-DNA carrying plants are considered as natural transformants and the transferred genes do not produce the usual disease phenotype. cT-DNA inserts have been used as markers to reconstruct plant evolution. Studies suggest that cT-DNAs were introduced in an ancestor species and transmitted across speciation events (38 and 39). Also, the distribution of cT-DNAs within *I. batatas* (both cultivated and wild forms) and *I. trifida* is shedding new light on much debated question regarding the origin of the cultivated hexaploid species *I. batatas*.

It has also been proposed that cT-DNA insertions may have led to plant speciation. The pattern of acquisition of cT-DNAs may indicate the role that these sequences have played in the plant evolution. In the case of *Nicotiana Tomentosae* section, different cT-DNA combinations were found in different species and the order of cT-DNA entry corresponds to the proposed branching order of the species. This pattern is consistent with the idea of speciation by transformation (39).

INNOVATIONS IN PLANT BIOTECH

With the first gene-cloning experiments, it became evident that this technology had enormous potential to agricultural development.

Marc Van Montagu contributed to innovations in plant biotechnology by cloning of a *Bacillus thuringiensis* insecticidal protein and the engineering of tobacco plants that expressed this gene at a level to convey resistance to *Manduca sexta* (tobacco hornworm) (40). The other breakthrough was the expression in plants of a bacterial gene that detoxified an herbicide (41), produced by the companies Hoechst and Meiji, termed Basta in cooperation with Biogen.

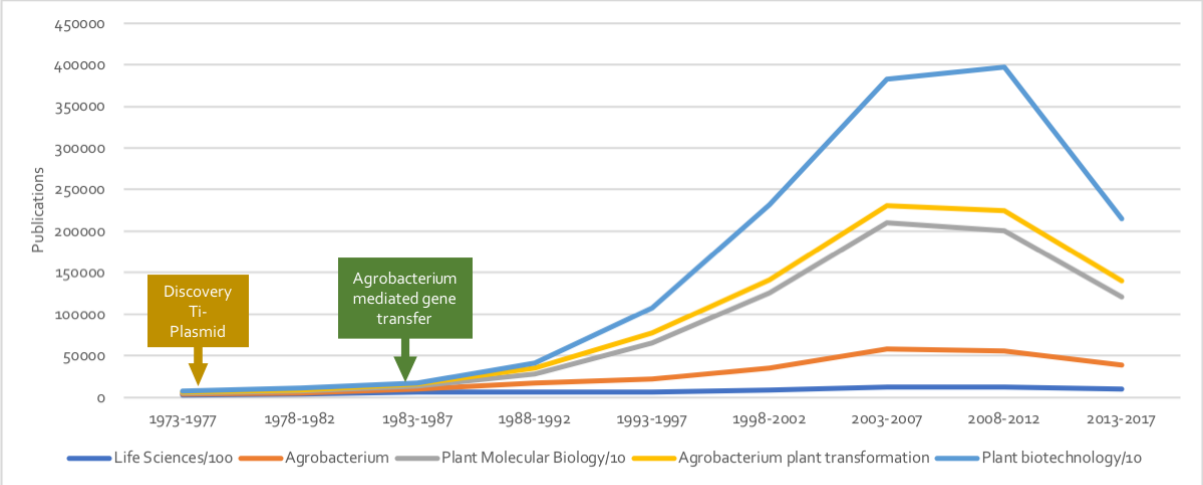
Other contributions of Marc Van Montagu to applications in plant biotechnology include the development of poplar trees with higher lignin extractability to pulp and paper industry (42)

Two companies have been established based on the work of Marc Van Montagu at Ghent University: Plant Genetic Systems in 1982 (since 2002 Bayer CropScience) and CropDesign in 1998 (since 2006 acquired by BASF).

IMPACT OF MARC VAN MONTAGU'S RESEARCH ON THE SCIENTIFIC WORLD

The discovery of the Ti plasmid is a classic case of curiosity-driven research that ultimately led to major scientific breakthroughs both in the proliferation of new research tools and quantum leaps in fundamental and applied knowledge in the agro-biotechnology sector. Direct evidence of the intellectual impact of the discovery is reflected in more than 60,000 publications on "Agrobacterium plant transformation". As shown in the figure bellow, the discovery of Ti plasmid and the development of Agrobacterium-mediated gene transfer technology were at the basis of the rapid advance of basic and applied modern plant sciences. The discoveries were also followed by a worldwide boom of academic and industrial laboratories focusing on plant biotechnology. The fact that Ti-plasmid derivatives were adopted worldwide within the year of the announcement of Agrobacterium-mediated gene transfer is evidence of the real impact of the discovery.

Molecular technologies have evolved and new genome editing methods offer accuracy not possible with earlier methods. Yet, these new approaches still involve gene transfer technology and, three decades after the firsts transgenic plants were produced, Agrobacterium-mediated plant transformation continues to be an essential tool in the palette of techniques which are enabling further cutting-edge research and development in plant sciences and biotechnology.



Source: Google Scholar

Marc Van Montagu outstanding scientific career has left an enduring footprint in plant sciences. It is evidenced by 1199 publications that have been cited more than 92,000 times (Google Scholar). For 10 years (1995-2004) Van Montagu was ranked number 1 on the ISI citation index for plant and animal scientists and even eighteen years after his retirement as director of the Laboratory of Genetics at the Ghent University he upholds an average of more than 3400 citations/year (Google Scholar).

Prof. Van Montagu's lab have been an incubator for many outstanding scientists. Hundreds of scientists were trained and they have gone to create further collaborations supporting the diffusion of modern biotechnology in a variety of labs and companies. Prof. Van Montagu has been especially active on capacity building in low- and middle-income countries. With the strong conviction that the promotion of knowledge and technology are vital for addressing the grand societal challenges, he has helped to train and empower a large number of scientists from the developing countries who went back to tackle specific challenges of low-income economies. Marc Van Montagu created, in 2000, the International Biotechnology Outreach (IPBO), a platform at VIB-

UGent, Belgium, with the mission to empower biotech capacities in less developed regions with the latest technological developments and effective science-based biosafety regulatory mechanisms.

Prof. Van Montagu has received many awards and numerous honours for his individual breakthrough achievement in founding, developing and applying modern agricultural biotechnology. These include the prestigious Japan Prize for Biotechnology and Agriculture Sciences in 1998, the Genome Valley Award from India in 2009, and to the World Food Prize in 2013. He is member of 11 academies of sciences/agriculture worldwide and recipient of 9 honorary doctorate degrees. Marc Van Montagu was the President of the European Federation of Biotechnologists from 2005-2013.

IMPACT OF THE DISCOVERIES ON AGRICULTURE

Agriculture is one of the most essential human activities with consequences for many human outcomes. The current agricultural systems heavily impact the environment and the transition toward sustainable agriculture remains a most pressing challenge. As the world grapples with how to feed the estimated 9 billion people who will inhabit the planet by the year 2050, it will be critical to continue building upon on advancements and revolutionary agricultural discoveries.

Marc Van Montagu work has triggered many biotechnology innovations that clearly have the potential to lead to a major new agricultural revolution based on the harnessing modern scientific techniques. The genetically modified (GM) crops on the market today have radically transformed agriculture. In a span of two decades GM crops have been adopted by 28 countries and planted on 13 percent of the world's arable land, producing 441 million tons of food, feed and fibre worldwide (43).

GM technology is the fasted adopted crop technology of recent times because it generates significant economic benefits to farmers. Notably, the highest gains are obtained by farmers in developing countries, many of which are resource-poor and farm small plots of land. This has a direct impact on poverty alleviation since resource-poor farmers and the rural landless dependent on agriculture represent the majority of the world's poorest people.

The farm profits are derived from yield and production gains and from cost savings, such as less ploughing, fewer pesticide sprays, and less labour (44). The transgenic traits have made possible the use of such environmentally friendly farming practices, contributing to reducing the environmental footprint of agriculture. Significantly, the adoption of GM crops has conserved biodiversity by removing 174 million hectares from agricultures. These socio-economic and environmental benefits of GM crops have arisen even though only a limited range of GM agronomic traits have so far been commercialised, in a small range of crops. Much more will be realized with the next generation GM crops. A wide variety of useful genes have been transformed into a large number of economically important plants, including most of the food crops, scores of varieties of fruits and vegetables, and many tree species. Output traits with improved quality and composition geared towards nutrition are of special interest to human health. A lot more can and should be done, in particular to achieve an agricultural revolution in low-income countries.

The innovations triggered by Marc Van Montagu ground-breaking discoveries have been applied in practice and are delivering extensive change in agriculture that can alleviate the needs of the poor and the hungry now and in the future. For this reason, the United Nations Industrial Development Organisation, in 2014, appointed Prof. Em. Marc Van Montagu UNIDO Goodwill Ambassador for the development of agriculture for food and agribusiness in low-income countries.

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