

**The Biotechnological Promises and Bottlenecks for a Sustainable and Intensified  
Agriculture in Africa**

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## **Summary**

On a continent that has to cope with high rates of poverty, diseases, and malnutrition, the agricultural and farming practices have evolved only slightly for centuries. Of all the recently developed technologies, biotechnology represents an innovation toolbox that could significantly improve livelihoods of sub-Saharan populations. Its impact could be far beyond that reached in the industrialized world, in which agricultural biotechnology has become a multibillion industry. In sub-Saharan Africa, the absence of this foundation constitutes a constraint on the elaboration of a sustainable biotechnology-based industry. A critical element should be the development of a regionally innovative community in the field of agricultural biotechnology that focuses on crops relevant for Africa, agricultural practices, and economic needs, but that is also sensitive to public concerns about the use of genetic modifications.

**Keywords:** Biotechnology, GM crops, Africa, sustainable agriculture, agricultural research

## Les promesses et contraintes de la biotechnologie pour une agriculture durable et intensive en Afrique

### **Résumé**

Sur un continent fortement touché par la pauvreté, les maladies et la malnutrition, les pratiques agricoles n'ont que peu évolué depuis des siècles. De toutes les technologies récemment développées, la biotechnologie pourrait améliorer de manière significative les conditions de vie des populations en Afrique subsaharienne. Son impact pourrait dépasser celui atteint dans le monde industrialisé où l'industrie de la biotechnologie agricole s'est développée et vaut des milliards d'Euro. En Afrique subsaharienne, l'absence de cette base constitue un frein à l'extension d'une industrie biotechnologique durable. Un élément critique est le développement d'une communauté régionale innovatrice concernant la biotechnologie agricole qui se consacrerait à la culture de plantes africaines, aux pratiques agricoles et aux impératifs économiques tout en étant sensible aux préoccupations du consommateur quant à l'utilisation controversée des technologies de modifications génétiques.

Mots clés: Biotechnologie, Afrique, agriculture durable, OGM

De beloftes en beperkingen van de biotechnologie voor een duurzame en intensieve landbouw in Afrika

### **Samenvatting**

Tot op de dag van vandaag hebben de huidige landbouwpraktijken maar weinig impact op het Afrikaanse continent dat sinds eeuwen geteisterd wordt door honger, ziektes en ondervoeding. Van alle recent ontwikkelde technologieën is biotechnologie er één die de levensomstandigheden van de bevolking in Sub-Sahara Afrika aanzienlijk kan verbeteren. De impact van biotechnologie kan in deze regio zelfs groter zijn dan deze die bereikt werd in de geïndustrialiseerde wereld. In Sub-Sahara Afrika ontbreekt deze basis, wat een belangrijke belemmering vormt voor de ontwikkeling van een duurzame op biotechnologie-gebaseerde industrie. Voor de landbouw in Sub-Sahara Afrika is het heel belangrijk om een lokale en innovatieve gemeenschap te ontwikkelen die zich wil inzetten voor de implementatie van biotechnologie in de landbouw, gefocust op gewassen die belangrijk zijn voor de Afrikaanse bevolking, die landbouwpraktijken optimaliseert alsook de economische impact ervan evalueert. Tenslotte dient ze ook een antwoord te bieden op de lokale belangen en bezorgdheden rond het gebruik van genetische modificaties in de landbouw.

Sleutelwoorden: Biotechnologie, Afrika, duurzame landbouw, GGO

## **Main Text**

For centuries, agriculture has undergone major changes through technical and scientific innovations and by the evolution of farming practices that allowed farmers to increase yield and productivity. An important challenge of today's agriculture is the need to decrease the environmental footprint by reducing the required amount of natural resources, such as land, water, and inputs (e.g., seeds, fertilizers, and machinery), while still meeting the world's increasing needs in food, fuel, and fiber.

Whereas agriculture in the Western countries nowadays strongly focuses on optimizing production, land, and resource utilization by application of more technological tools and data (known as precision farming), developing countries, and especially those of sub-Saharan Africa, are still struggling to get access to efficient germplasms and inputs that could already strongly improve yield and production. Indeed, the last 50 years, thanks to the so-called "green revolution", many countries were able to increase their food production and reach an enhanced level of food security. This green revolution has been represented by the determinant work of Norman Borlaug, an American agronomist, who instigated wheat variety breeding programs and resulting lines. These new varieties combined with new agronomical methods were beneficial not only for American and south American countries, but also for Asia, where the productivity, not only in wheat, but also in other cereals, was highly enhanced. In 1970, Norman Borlaug was honored with the Nobel Prize for peace because of his influential work in globally reducing hunger and poverty. This so-called green revolution made the use of inorganic fertilizers and pesticides more methodical, with exponential yield increases as a consequence. However, these rationalized agricultural practices have been largely criticized as well, because in many cases they have led to an intensified, large-scale monoculture type of agriculture. Still, in that period, the cereal crop production tripled when the world population doubled, hence,

overcoming repetitive food shortage (Pingali, 2012). The green revolution had mainly been initiated to boost the yield of staple crops cultured under favorable conditions, thus neglecting areas with poor soil and marginal productivity, such as considerably the African continent. Although enormous progress has been made, Africa still has to undergo its own green revolution to be able to reduce famine and indigence.

Meanwhile, research on crop improvement has undergone radical changes with the rise of new knowledge and technologies. The increased understanding of the molecular mechanisms behind plant growth and development has opened a whole range of possibilities. Biotechnology from “bio” (life) “technos” (tool) and “ology” (study of) is defined by the use of living organisms to develop new products or processes. Its current impact on agriculture results mainly from the introduction of ameliorated crops that belong to the class of genetically modified (GM) organisms (GMOs).

Plant biotechnology is considered to have started approximately 8000 years ago, when humans begun domesticating plants and selecting the most suitable ones for cultivation, such as those with large seeds, short ripening time, etc. This tedious process changed when fundamental scientific knowledge was applied. For example, the laws of heredity discovered by Mendel have been significant to set up systematic breeding programs. From the 1930s, mutation breeding was implemented to enhance crop qualities. This technique that uses mutagenic radiation or chemicals to induce mutations in plants accelerates considerably the discovery of beneficial mutations. Mutagenesis has been applied for decades to improve the crops, legumes, and fruits that we eat today. The elaboration and application of molecular biology techniques have later generated new tools to amend breeding with, for example, the development of marker-assisted selection that has provided a genetic basis for the selection of new traits.

The discovery of the genetic transformation technology has enabled a big jump forward in the creation rate of new traits over the past three decades. Through genetic transformation specific pieces of DNA could be integrated into the genome of a plant, thus inserting desired new functions, such as insect resistance. This DNA fragment could belong to any living organism, because all share the same genetic code. Genetic modification through transformation has led to the so-called GMOs. However, noteworthy, genetic modifications are the essence of variety amelioration, for which the final aim is to provide genetic diversity with new traits of interest for the farmers and/or the consumers, either through conventional breeding techniques, genetic transformation, or any other available technology. Hereby, two notions should be distinguished, namely transgenesis and cisgenesis. Transgenesis implies the integration into a crop genome of a DNA piece from another organism that is not sexually compatible, whereas cisgenesis integrates a DNA fragment that could have been obtained through successive crosses with sexually compatible organisms, as, for instance, a resistance gene from a relative of wild potato (*Solanum tuberosum*) integrated into a commercial potato variety. Both cisgenesis and transgenesis allow the insertion of a (or multiple) new trait(s), still keeping all the genetic characteristics of the original variety.

The first genetic transformations were published in the early 1980s and the group of Marc Van Montagu and Jeff Schell at Ghent University were pioneers in the field. Indeed, the soil bacterium *Agrobacterium tumefaciens* had been found to induce tumors in plants (Smith and Townsend, 1907), of which later the tumor-inducing agent had been discovered to be a small piece of circular DNA, designated tumor-inducing (Ti) plasmid (Zaenen et al., 1974). By means of this Ti plasmid from *Agrobacterium* as a vector, new genes could be integrated into a plant by replacing the genes responsible for the tumor formation with genes of interest (Herrera-Estrella et al., 1983).

In the meantime, science has made tremendous progress. One of the most remarkable discoveries was the bacterial adaptive immunity to viruses and plasmids (Doudna and Charpentier, 2014). This research led to the creation of a new genome editing tool, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), abbreviated as CRISPR-Cas9 (Mojica et al., 2005; Pourcel et al., 2005), that is transforming the field of biology and biotechnology research (Doudna and Charpentier, 2014). This new technology together with other gene-editing techniques allows genetic modifications at a previously unknown precision level, marking the starting point of innovations that might well revolutionize the agricultural sector in the near future.

Technically, the CRISPR-Cas9 technology is based on a bacterial defense mechanism that can be adapted to target a specific DNA fragment. The technology consists of the Cas9 enzyme and a guide RNA (gRNA) responsible for directing the enzyme to the DNA. The gRNA binds to the Cas9 protein and, upon binding, induces a conformational change in the protein that converts the inactive protein into its active form. When the Cas9 protein/gRNA finds a potential target sequence, it executes a “cut and paste” that allows minor modifications into a specific site of the genome of a living organism. This technology is more precise than previous genetic transformation techniques, because the modification site can be selected. Furthermore, plants can be obtained without any foreign DNA, only the desired change in their own genome is kept. Traditionally, transgenic crop development has been reserved to a few big companies because of the tremendous costs for development and the linked regulatory expenses. These high expenditures could only be paid back for a few major crops of global importance. Plants obtained with the more recent gene-editing technologies, such as CRISPR-Cas9, if not regulated in the same manner as GMOs, could offer new opportunities for small and medium-sized enterprises and for the public sector to ameliorate crops that had been disregarded until now because of the low market value and little commercial interest. In other words, this technology

could be a major game changer in the development of efficient crops in Sub-Saharan Africa, where many crops are of regional or national interest, but represent only a small fraction of the international commodity trade.

Developing countries, especially in Africa, face a number of challenges that cannot be tackled and solved easily. In many African countries, a certain number of gaps need to be filled to reach a satisfactory level of agricultural efficiency, including, for example, development of the proper infrastructure to ensure collection, transportation, and storage of the harvest. A wide range of pests and diseases (favored by climatic conditions) and a low access to high-quality inputs (seeds, fertilizers, and pesticides) further undermine the productivity. In addition, strong policies supporting the implementation of a competitive agriculture are essential for the expansion and professionalization of the agricultural sector.

Moreover, when production and capacity can be increased, a fair access to markets, either regional, national, or international is needed. The low industrialization level does currently not offer a sufficiently developed network to process the agricultural production, so that it has to be conveyed as raw material that also depends largely on the market seasonal fluctuations. Additionally, Africa lacks the added value and job opportunities provided by the processing of the raw production. Food processing would add price stability for the producer on the local and regional markets, because processed products that can be preserved and easily stored, are less dependent on farming cycles and market prices, also allowing a superior access to international markets.

Nevertheless, science and innovation can propose solutions not to be ignored in hunger-suffering countries. Today, the African agricultural productivity for a whole range of crops is much lower than that in the European Union, North and South America, and Asia. According to the Food and Agricultural Organization, the average yield of maize (*Zea mays*) cultivation reached 10.7 tons/ha in the USA in 2014, whereas the average yield in Africa was only

2.1 tons/ha (<http://www.fao.org/faostat/en/#data>). This difference is not only true for maize, but also for crops, such as banana (*Musa* sp.), cassava (*Manihot esculenta*), cowpea (*Vigna unguiculata*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), and others. Together with an exponentially growing demography and all challenges mentioned above, this yield disparity leads to food shortage and societal crises with a worldwide impact that need to be taken care of with all the tools and support available, including biotechnology.

Although a wide variety of products obtained with the recent CRISPR-Cas9 technology is not yet available, the genetic engineering technology has already been used since the 1990s and it is still being adopted at an increasing rate. In 1996, in the initial phase for GMO commercialization, 1.7 Mha were grown with GMO crops versus 189.8 Mha worldwide in 2017 (ISAAA, 2017). Remarkably, the spectrum of developers is also enlarging and involves developing and growing economies. The cultivated surface in these emerging economies (including Brazil, Russia, India, China, South Africa) is now more important than that in the traditionally high-tech agricultural regions of the USA and Europe (ISAAA, 2017). Still, on this vast surface mainly a few crops of global economic importance are cultivated, such as soybean (*Glycine max*), maize, cotton (*Gossypium hirsutum*), and oilseed rape (*Brassica napus*). With the aim at developing a sustainable agriculture in Africa, one might wonder whether these approaches would really be game changers and whether they would be possibly applicable in the context of the African agricultural systems.

Although no GM product developed in Africa is on the market yet, the number of scientific research initiatives has augmented and the proportion of African products in the pipeline to commercialization is higher than that of other continents (Parisi et al., 2016). Currently, most, if not all, GM products are merchandized by a small number of actors that can afford the high developmental and regulatory costs and involve mainly a few major crops, such as maize, cotton, or soybean. However, an increasing number of research and field trial

initiatives are being taken on so-called “orphan crops” that are potentially of high importance at a regional or national level and less relevant for international trade (ISAAA, 2016).

Many of these orphan crops, especially on the African continent, are an essential part of local diets and economies, e.g., cassava, yam (*Dioscorea* sp.), sweet potato (*Ipomoea batatas*), cowpea, and grass pea (*Lathyrus sativus*). Moreover, these crops are often better adapted to the local environment and agricultural practices and, thus, potentially more resilient to the biotic or abiotic consequences of climatic disturbances than other crops. Thus, orphan crops or, more correctly, crops that have been neglected by the regional, national, and international research programs are slowly gaining the attention they deserve by the biotechnology field. Until recently, these geographically limited markets had raised only a poor interest by the agrobiological industry, but the public sector through public or private partnerships gradually performs research on these crops. This interest can even be amplified and extended to small and medium-sized enterprises, when cheaper and less effort-intensive technologies, such as CRISPR-Cas9, can be applied for crop improvement.

Currently, regarding the GM traits in the Research and Development pipeline, for instance provitamin A-enriched crops, private as well as public initiatives seem to carry out research to develop consumer-beneficial crops in addition to the previous more farmer-oriented traits, such as herbicide resistance. Although these products might not always be relevant for the global industrial sector, they might potentially have a higher impact in developing regions and be more beneficial to small-holder farmers and consumers than in the industrialized countries.

The most mediatized example of such a GM crop developed by the public sector has probably been the so-called golden rice. Golden rice has been improved genetically to produce provitamin A (Ye et al., 2000) and to become an additional tool in the fight against nutritional imbalances in the diet of the poorest, *i.e.*, a lack of provitamin A in a child’s diet can cause immune deficiency with total blindness as a possible consequence. Although it has taken a long

time to clear scientific and regulatory issues, golden rice is now approved in New Zealand, Australia, Canada, and lately by the Federal Drug Authority of the USA, but in the Philippines and Bangladesh, where it could have the greatest impact on the children's health the approval is still pending. Importantly, this golden rice has no commercial purpose, only a humanitarian reason of existence. In this same humanitarian spirit, a series of projects are running on previously neglected crops, such as the development of virus resistance in cassava, biofortification in sorghum and banana, aflatoxin resistance in groundnut (*Arachis hypogaea* L.), and drought tolerance in tropical maize. All these projects could bring specific solutions to challenges faced locally or regionally by farmers and consumers.

For instance, The Queensland University of Technology (Australia) and the National Agriculture Research Organization (NARO) of Uganda coordinate a project funded by the Bill and Melinda Gates foundation to create a biofortified banana enriched in provitamin A. These banana plants have been successfully tested in the field at the NARO of Uganda (Paul et al., 2018). Provitamin A-enriched bananas could have a strong impact for the Ugandan population that relies on cooking bananas as main starch source. In addition, the International Institute for Tropical Agriculture (IITA) is developing banana plants resistant to either bacterial wilt or nematodes. Diseases, such as bacterial wilt, can destroy a whole plantation and no direct alternative solutions are available besides the use of resistance genes from another plant, for instance, sweet pepper (*Capsicum annuum*) (Tripathi et al., 2017).

The impact of climate change on the African continent is predicted to be severe, especially because of the mostly rain-fed African agriculture. The climatic variability and especially droughts and extreme weather events can highly affect yield and crop quality. To address this challenge, two major initiatives have been undertaken on maize, which is a major food crop in African countries, namely the Drought-Tolerant Maize for Africa (DTMA) project implemented by the Centre for International Maize and Wheat Improvement (CIMMYT) and

IITA and the Water-Efficient Maize for Africa (WEMA) project, a public/private partnership, in which the associated company provides the technology free of royalties (De Buck, 2017). The latter project is currently testing varieties in field trials in several countries of the African continent.

Moreover, research and engineering of legume crops have been undertaken as well. As leading example, cowpea is grown on more than 8 million hectares in West and Central Africa, with Nigeria the largest producer with 4 million hectares (Gómez, 2004). Still, the demand is higher than the production. Hence, the creation of a variety resistant to the pod borer would be a real solution for the yield losses the producers are facing because of this disease. This project is a private/public partnership funded by the United States Agency for International Development (USAID).

These initiatives are all at different developmental stages, namely from research to product release. Currently, only a few products, such as GM maize and GM cotton, are cultivated in South Africa and South Sudan, but still no products made in Africa for Africa are on the market, although as mentioned above several products are in the pipeline.

Among the African countries, currently only two (South Africa and Sudan) grow GM crops for commercial purposes, but already 13 countries host research programs and/or conduct field trials with different GM crops produced by private companies or by public or public/private partnerships (ISAAA, 2017). Ethiopia and Nigeria, the two most populated countries of Africa have recently approved the commercialization of a GM cotton and, also in Nigeria, an insect-resistant (Bt) cowpea has been released in the meantime.

Not only for commercialization and cultivation of these GM crops, but also for research and their growth in field trials, an efficiently working regulatory framework is essential. Such a legal framework that exists in most countries regulates the use of GM technology-based products, *i.e.* from seed to final product. Since 2017, 14 African countries have a regulatory

framework and have implemented it, for example, to control imports, laboratory research, field trials, or even commercialization and cultivation, as in South Africa and Sudan. In addition, eight other countries have a law in place that has still never been applied for field trials or other activities. However, of the 54 (55 with Occidental Sahara) African countries, only less than half of them have a legislation that allows the regulation of GMO research, cultivation, or import. Furthermore, in many of the countries that possess a biosafety law, a capacity-building effort of the responsible governmental bodies needs to be strengthened. Indeed, many countries use the African law model for biotechnological safety that has been enacted by the African Union in 2007 as a base for the establishment of a national legislation. This step is essential to comply with the obligations under the Cartagena Protocol for Biosafety (Secretariat of the Convention of Biological Diversity, 2000) that has been ratified by most African countries. Nevertheless, as any model law, it can serve only as a base and needs to be adapted to the national regulations to allow proper endorsement. Inaction from authorities leads to an unclear situation that frightens potential stakeholders willing to invest in the country. Such a reluctance has been noticed not only in Africa, but also in Europe, where the regulatory framework and the administrative implementation have not evolved in parallel with the technologies and the knowledge acquired over the years. As a consequence, decisions are often taken without proper consideration of scientific facts.

In spite of regulatory frameworks installed or adopted after the appearance of any new major technological advance, each innovation has had its own acceptance history, some more tumultuous than others. Every day, new technical innovations are available and marketed. Some innovative technical products have a rather fast adoption rate by the public, such as, for example, the internet or the smartphone. In sharp contrast, GMOs have undergone a much more difficult and diverse acceptance rate, depending on region or country. The late Professor Calestous Juma illustrated very nicely in his book how the adoption of some products, such as

coffee, margarine, or even alternative electrical current, had faced struggles (Juma, 2016). Many parameters behind the fear for novelty are of social, cultural, and economic order and, thus, emotional parameters weigh potentially more than rational or scientific arguments (Blancke et al., 2015, 2016; Couée, 2016). Although scientific facts in the case of GMOs might be not so straightforward to be understood by the broad public, the immediate benefits for the consumer might also be an important factor that can be evident, as, for example, in the case of the smartphone technology. Concerning the first GMOs on the market, the direct advantage was for the farmer, because initially the new traits were herbicide tolerance and insect resistance. Furthermore, in a period in which Europe was the scene of food safety scandals, such as the bovine spongiform encephalopathy and the dioxin crisis, GMO acceptance became problematic, but by adopting a precautionary principle to its extreme, development and implementation of new technologies can be blocked and, hence, prevent countries or regions to enjoy the benefits of a significant agricultural development. In Africa, in contrast to Europe or the USA, a large proportion of its population is still involved in agriculture with, for instance, 69% farmers in Uganda versus only 1.3% in Belgium (<https://data.worldbank.org/indicator/SL.AGR.EMPL.ZS?view=map>). Thus, the advantages of the GM technology are more obvious and direct for a large population.

In other words, the African countries have to weigh the risks and benefits in adopting technologies, such as GM or gene editing. This evaluation to make strong political decisions can be done only, when the decision makers have access to well-balanced and science-based information.

## **References**

- Blancke, S., Van Breusegem, F., De Jaeger, G., Braeckman, J., and Van Montagu, M. (2015). Fatal attraction: the intuitive appeal of GMO opposition. *Trends Plant Sci.* 20, 414-418.
- Blancke, S., Van Breusegem, F., De Jaeger, G., Braeckman, J., and Van Montagu, M. (2016). The need to understand GMO opposition: reply to Couée. *Trends Plant Sci.* 21, 92-92.
- Couée, I. (2016). Hidden attraction: empirical rationality in GMO opposition. *Trends Plant Sci.* 21, 91-91.
- De Buck, S. (2017). *Maize in Africa*. Fact Series. Gent, VIB/International Plant Biotechnology Outreach, 56 p.
- Doudna, J.A., and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, 1258096.
- Gómez, C. (2004). *COWPEA: Post-Harvest Operations*. Rome, Food and Agriculture Organization of the United Nations (FAO), 70 p.
- Herrera-Estrella, L., Depicker, A., Van Montagu, M., and Schell, J. (1983). Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* 303, 209-213.
- ISAAA (2017). *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years* (ISAAA Brief, no. 53). Ithaca, NY, International Service for the Acquisition of Agri-biotech applications, 143 p [ISBN 9781892456672].
- Juma, C. (2016). *Innovation and Its Enemies, Why People Resist New Technologies*. Oxford, Oxford University Press, 432 p. [ISBN 9780190467036].
- Mojica, F.J.M., Díez-Villaseñor, C., García-Martínez, J., and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* 60, 174-182.

- Parisi, C., Tillie, P., and Rodriguez-Cerezo, E. (2016). The global pipeline of GM crops out to 2020. *Nat. Biotechnol.* 34, 31-36.
- Paul, J.-Y., Harding, R., Tushemereirwe, W., and Dale, J. (2018). Banana21: from gene discovery to deregulated golden bananas. *Front. Plant Sci.* 9, 558.
- Pingali, P.L. (2012). Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. USA* 109, 12302-12308.
- Pourcel, C., Salvignol, G., and Vergnaud, G. (2005). CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology* 151, 653-663.
- Secretariat of the Convention of Biological Diversity. (2000). *Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Text and Annexes*. Montreal, Canada, Secretariat of the Convention of Biological Diversity, 12 pp. [ISBN 92-807-1924-6].
- Smith, E.F., and Townsend, C.O. (1907). A plant-tumor of bacterial origin. *Science* 25, 671-673.
- Tripathi, L., Atkinson, H., Roderick, H., Kubiriba, J., and Tripathi, J.N. (2017). Genetically engineered bananas resistant to *Xanthomonas* wilt disease and nematodes. *Food Energy Secur.* 6, 37-47.
- Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., and Potrykus, I. (2000). Engineering the provitamin A ( $\beta$ -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287, 303-305.
- Zaenen, I., Van Larebeke, N., Teuchy, H., Van Montagu, M., and Schell, J. (1974). Supercoiled circular DNA in crown gall inducing *Agrobacterium* strains. *J. Mol. Biol.* 86, 109-127.