PHARMING IN PLANTS – Anniek Bodewes and Michele Di Palma

These days lots of people are against GMO (Genetically Modified Organisms) but in some ways, GMO have always existed, and farmers have often relied on them: in fact, plants which were more suitable for cultivation (because they produced more and better fruits or maybe were less infected by pathogens) were selected and then cloned with a horticultural technique named as grafting. This technique consists in the positioning of a part of the plant, usually a branch, in a second plant, compatible with the first one. In this way, it is possible to clone plants that have a genetic modification which has some benefits for us. For example, the bananas used for consumption do not have seeds because their set of chromosomes is 3n and this makes them sterile (they are a cross between 2n and 4n bananas, which are not sterile): so, all the bananas that are for sale in supermarkets could be the fruit of clones of the same banana tree! Furthermore, fruits like lemons or grapefruits are hybrids, they are a cross between other different fruit. Gregor Mendel, who discovered the basis of genetics by crossing different type of peas, was the first one to show that lots of things can be done with plants!

A non-sterile banana
Free picture on https://en.wikipedia.org/wiki/Banana

Plants have been the basis for medical treatments through much of human history. For example, it is known from "Ebers Papyrus", a medical papyrus of herbal knowledge, that Egyptians knew a lot about the use of plants in medicine; the Greeks and the Romans too used plants for medical purposes. Such traditional medicine is still widely practised today: in China, 50% of the total use of medicines is from herbal preparations. Nowadays, everyone knows that plants are effective because of their active ingredients: lots of them have been identified, and about a quarter of prescription drugs are still of plant origin[1].

But if plants already have their own active substances, why should they not be modified to increase the amount of these substances in them, or maybe to create new ones that we need?
Thanks to genetic engeneering, this has become possible. It has been discovered that plants have a very high potential: they can protect themselves from pathogens, they can be made richer in active ingredients or in nutritional values, vitamins for example, but they can also obtain characteristics (and applications) they did not have before. They can be used for the production of biofuels and for bioremediation, a technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site, and they can be used as bioreactors for the production of substances which can treat diseases.

Pharming is a combination of the words "pharmaceutical" and "farming": it refers to the insertion of genes that code for a pharmaceutical into an organism that does not have them in its genome. Pharming in plants is the procedure carried out in these organisms.
Genetic engeneering in fact allows us also to use plants to produce drugs, vaccines, hormones, cytokines, antibodies and other specific molecules. These pharmaceuticals are produced in specific parts of the plant, which can be seeds or chloroplasts mainly. But why should plants be modified in order to create these molecules and then harvest them, rather than make them in a lab, using bacteria or mammalian cells, as usually done before? Well, the use of genetically modified plants to produce these molecules is cheaper, and it produces better molecules than the ones made by bacteria: in fact, proteins that are usually glycosylated in humans (glycosylation is a process that gives the protein its correct shape) are not glycosylated by bacteria, but they are in plants.

Factor	Transgenic plants	Plant cell cultures	Bacteria	Yeast	Mammalian cell culture	Transgenic animals
Production costs	Low	Medium	Low	Medium	High	High
Product quality	High	High	Low	Medium	High	High
Time effort	High	Medium	Low	Medium	High	High
Productivity	High	Medium	Medium	Medium	Medium	High
Contamination risk	No	No	Yes	No	Yes	Yes
Storage	RT	-20°C	-20°C	-20°C	N_2	N_2

RT = Room temperature N_2 = In liquid nitrogen

Pharming in plants compared to other types of pharming
Adapted from Fischer et al. 2000; created by Michele Di Palma

Moreover, the cost of recombinant proteins made by plants is very low compared to other ways to obtain them (about 2-10% of the cost of recombinant proteins made in E. coli, 0.1% of the ones made in mammalian cells[2]).

But how can a plant be genetically modified? There are three main ways to do that. The first one, called agroinfiltration, uses a common bacteria which usually infects plants and makes them sick (called Agrobacterium Tumefaciens). This bacteria has a plasmid, called Ti (Tumor inducing), which is responsible for the transformation of normal cells into tumoral cells. A part of the DNA in the plasmid (called T-DNA) becomes part of the DNA of the cell and modifies it. The extremities of T-DNA are essential parts for its insertion into the plant cell, so we can remove what is in the middle of T-DNA, replace it with the DNA we want to replicate and connect it to the extremities with ligase enzyme. Now the bacterium is not dangerous anymore and it modifies the plant cell so that it has the desired gene. In this plasmid there is also a gene marker which makes plants resistant to an antibiotic: in this way plant cells which have been successfully modified can be identified by putting them in a Petri dish with the chosen antibiotic. The ones which have not been modified will then be discarded.
The second way to modify a plant is called biolistic particle delivery system (or gene gun): this method consists in shooting dense particles of tungsten or gold coated with DNA into a plant tissue, but this way is less effective than agroinfiltration. The last way, called electroporation, is a technique by which an electrical field is applied to cells in order to increase the permeability of their cell membrane, allowing the DNA to be introduced into the cell.

Agroinfiltration technique
Free picture on https://en.wikipedia.org/wiki/Agroinfiltration

The first antibiotic resistant plant was produced in 1982. Since then there have been lots of trials for the production of molecules in plants, for example both the cytochine Interleukin-10 (I-10, an immunosuppressor which "breaks" your immune system) and ZMapp, a pharmaceutical which can treat the Ebola virus disease, have been produced in a tobacco plant. Also, there have been trials for glucocerebrosidase, an enzyme that, if lacking, is responsible for Gaucher's disease, and many other trials for the production of hormones and proteins in plants.
But the most interesting topic regards edible vaccines. If a plant can be modified in order to produce a molecule in any part of the plant (in fact it is possible to choose the place where this molecule should be produced: in the leaves, in the seeds, in the roots etc.), a vaccine could be produced in a fruit or a vegetable and people could then just eat it. This would be very fascinating, but there are some problems connected to it. First of all, there needs to be enough vaccine into this fruit or vegetable, and this is not as simple as it seems. It is difficult to reach amounts of the vaccine higher than 1% of total soluble proteins[3]. Furthermore, the vaccine has to pass through the digestive system without being degraded, and this one too is not an easy task.
Anyway, do not think this is impossible: the vaccine for hepatitis B has been produced in a tuber, and it is resistant to digestion!

"An apple a day keeps the doctor away!"
Created by Anniek Bodewes and Michele Di Palma

Pharming in plants can save our lives, but it can also be very dangerous. In fact, the molecules produced, stored in the plant, can pollute the air, the soil and water, and they can also accumulate in non-target organisms: that is why genetically modified plants must be cultivated in greenhouses. If I-10, for example, got into a virus which can incorporate it in its DNA, this virus could become a "doomsday pathogen", killing all of us through the inhibition of our immune system. We must be careful.

The greater use of transgenic plants for the production of biopharmaceuticals would lead to a greater availability of these molecules all around the world where required, this is the reason why this technique should be studied in depth, in order to improve it.

Maybe one day a pharmaceutical that can prevent cancer will be found. And it would be great if we could just simply find it in our food, eat it and stay healthy.

[1] Rainer Fischer & Neil Emans / Molecular farming of farmaceutical proteins, Transgenic Research 2000; 279-299
[2] J.W. Larrick et al. / Biomolecular Engeneering 2000; 18: 87-94
[3] Henry Daniell et al. / Trends in Plant Science 2001; 222

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