

GENETICALLY MODIFIED PAPAYAS

*A Scientific Paper by Sidar Manis and Sudhan Thiyagarajah
Gymnasium Kirschgarten 5A
Basel, Switzerland
24th April 2020*



(Handrea, Unknown)

Table of Contents

| | |
|---|-----------|
| 1. PREFACE | 3 |
| 2. INTRODUCTION | 3 |
| 3. DESCRIPTION OF ENGINEERING TECHNIQUE | 4 |
| 3.1 TRADITIONAL BREEDING AND MODERN GENETIC ENGINEERING | 4 |
| 3.2 INSIGHTS INTO GENOME ENGINEERING | 5 |
| 4. CASE STUDY: HAWAIIAN PAPAYA | 6 |
| 4.1 INFECTION OF HAWAIIAN PAPAYA WITH PRSV | 7 |
| 4.2 MANAGEMENT OF PRSV | 7 |
| 5. DISCUSSION OF ETHICAL ASPECTS | 8 |
| 5.1 ADVANTAGES: | 8 |
| 5.2 DISADVANTAGES: | 9 |
| 6. FUTURE PROSPECTS | 9 |
| 7. CONCLUSION | 10 |
| 8. BIBLIOGRAPHY | 11 |
| 9. APPENDIX | 14 |
| 9.1 INTERVIEW WITH DR. PAULSEN | 14 |

spacing irregular

1. Preface

While searching for a suitable topic, we looked at videos dealing with genome modification. As we came across the Hawaiian papaya, we thought that this is the perfect topic because we can buy this fruit in Switzerland and we have already eaten it. But the most interesting thing behind this topic is the fact, that the papaya would not exist today if it wasn't for genome modification. So why does this fruit exist today? Let us find out why.

For the Hawaiian papaya GMO was the only option. In the 1990's the Hawaiian papaya industry was under attack of the papaya ringspot virus (PRSV). In simple terms, the papaya had to be vaccinated against the virus. Without this rescuing genetic modification, the papaya would have been eradicated and disappeared from our plates. We wondered how the genome of the papaya can specifically be modified to become resistant against the ringspot virus.

(Answers, 2020)

gene modification ist not vaccination

2. Introduction

Papaya (*Carica papaya* L) has a place with the Caricaceae family and is one of the financially most significant organic product crops in numerous tropical nations. Papaya is a polygamous dicotyledonous and diploid species. The papaya initially originates from southern Mexico and Costa Rica. Papaya has been cultivated in the USA, Brazil, Mexico, Nigeria, India, Jamaica, Indonesia, Thailand, China, Taiwan, Peru and the Philippines (Jayavalli, 2011). The papaya's organic product is exceptionally nutritious and known for its medicinal worth (M. A. K. Azad, 2012). Papaya is a rich wellspring of nutrients A, B and C just as peptidases, for example, papain and chymopapain. It is a magnificent wellspring of beta-carotene, which can forestall malignant growth, diabetes and coronary illness (G. Aravind, 2013).

Papaya crops are as of now tormented by malady issues, particularly those brought about by papaya ringspot infection (PRSV) (Gonsalves, 1984), (Gonsalves, 1998). PRSV is the most genuine risk to papaya creation on the planet (S. Tripathi, 2008). PRSV is perceived in numerous tropical and subtropical regions, for example, the USA, South America, Africa (D. E. Purcifull, 1984), India (Khurana, 1974), Thailand, Taiwan, China and the Philippines (Gonsalves, 1994), Mexico (H. F. Alvizo and C. Rojkind), Australia (Dodman, 1993), Japan (T. Maoka, 1995), French Polynesia and the Cook Islands (R. I. Davis, 2005) as a dangerous ailment that prompts a decrease in organic product creation. This sickness can cause up to 100% of harvest misfortunes in certain areas (P. F. Tennant, 2007). PRSV is transmitted in a non-persistent way by a few aphid animal types in a procedure including the coat protein (CP) and the helper component proteinase (HC-Pro) (Y.-H. Peng, 1998).

aphid species

At present, transgenic papayas are developed in Hawaii, which represents over 70% of Hawaii's papaya development region. SunUp and Rainbow have been developed in the USA with to a great extent no antagonistic impacts on human wellbeing (D. Gonsalves, 2010). In nations, for example, Australia, Jamaica, Venezuela, Vietnam, Thailand, Taiwan and the Philippines, the CP quality from their topographical area has been utilized to create area-specific transgenic papayas for the control of PRSV (. A. Fermin, 2010). There have been a few investigations on the advancement of PRSV-safe assortments of *C. papaya* by gene technology yet no audit article is accessible on the administration of PRSV. Tecson Mendoza et al. (E. M. Tecson Mendoza, 2008) abridged the improvement of transgenic Papaya innovation and the exploration exercises of different nations, however, didn't cover all zones of PRSV the executives.

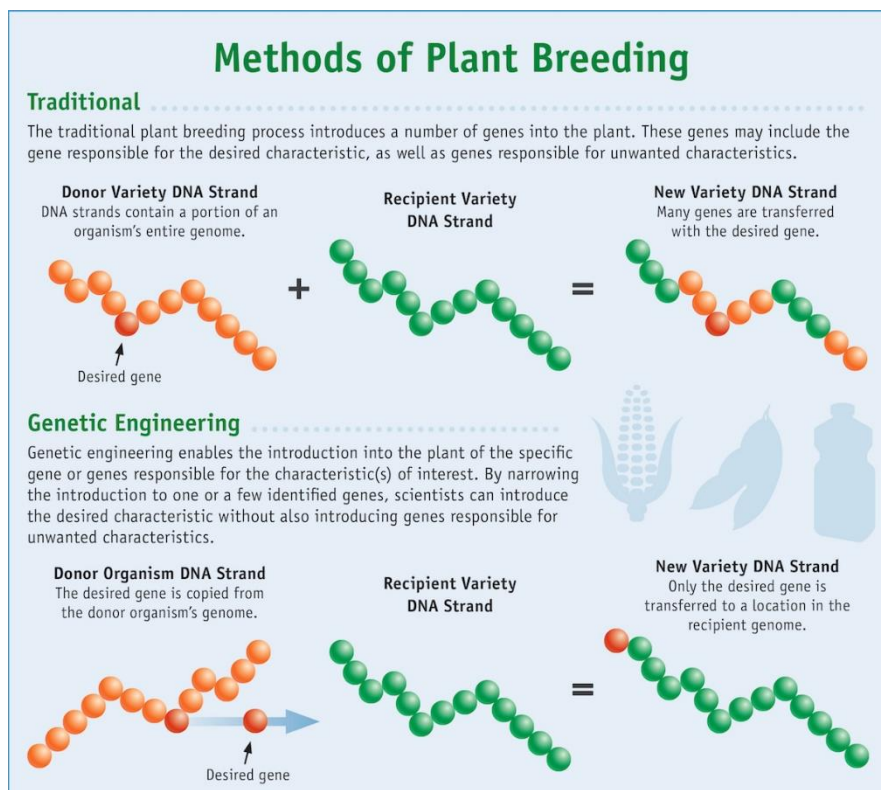
In general, genetically modifying a natural organism, such as the papaya, is a controversial topic. In the following paper, we would like to discuss the ethical aspects of the genetically engineered papaya from various perspectives. We aim to shine light on the question how the consumption of the papaya may influence people's health. Further, we are going to look into the dangers of introducing a genetically modified organism back into the natural environment and finally, we are interested in the societal opportunities the virus-resistant papaya offers for farmers and consumers.

3. Description of engineering technique

3.1 Traditional breeding and modern genetic engineering

Since traditional breeding results in permanent changes in the genome, it is a type of genetic modification. Traditional breeding and modern genetic engineering both result in a change in the genetic information of an organism. The difference between the two approaches is the technique.

In the traditional breeding method two organisms are being bred. During the process of chromosomal recombination during meiosis, the genes between the chromosomes are shuffled. The result can be unpredictable, because a large number of genes is mixed. When looking at the modern technique of genetic engineering, the work is more precise. In this context, precise means that biologists can nowadays modify, insert or delete a single gene.



bad illustration, in plant breeding strands do not get interspersed, the chromosomes get new recombination!

Figure 1: The figure highlights the difference between traditional breeding and genetic engineering. The traditional method transfers many genes including the desired gene, while genetic engineering introduces specifically only the desired gene into the recipient DNA strand.

(Fisher, 2019)

In both methods we need two DNA strands to ultimately get one resistant DNA strand. In the traditional breeding method, many genes of the donor DNA strand, including desired gene, transfer to the recipient DNA strand. Whereas in the genetic engineering method, only the desired gene transfers to the recipient DNA strand. Simplified you could say the transfer of many versus one or the only needed gene.

„genetic modification“ ≠ „gene editing“

3.2 Insights into genome engineering

During our literature research, we found three applied techniques to render the Hawaiian papaya resistant to the PRSV. But we also heard that there are a lot of ways to make a plant resistant, so probably there are even more options. At first, we didn't understand the three molecular techniques, which were called coat protein mediated resistance, RNA-interference mediated resistance and replicase gene-mediated resistance. Therefore, we asked our interview partner to explain genetic engineering of a plant in an easier way. Our interview partner was Dr. Jens Paulsen from the Department of Botany. His research interests and responsibilities are anchored in the field of botany, climate and vegetation modelling. The interview was done on the 19th of February 2020. Now, this is what we learned:

In order to save an organism from a virus, we have to transfer a small piece of DNA, the so-called resistance gene, into the nucleus of a cell of that organism. Once it is there, the resistance gene should integrate into the cell's own DNA. In case, the integration was successful, the resistance gene is transcribed, and the newly created mRNA is then translated into a protein. Finally, the protein defends the organism, in our case the Hawaiian papaya, from the virus and helps it to survive.

Dr. Paulsen said that if you want to change a plant or an animal genetically, then you have to take a specific DNA sequence (mostly a gene) which you want to transfer. This process is referred to as gene selection and isolation process cannot be done with microsurgery, since the DNA is way too small to be seen. Therefore, genetic engineering of an organism is generally performed with the help of a vehicle.

This brings us to the next step, which is inserting the selected resistance gene into the host cell. This process is called transformation, because now we are transforming the plant's DNA by integrating a foreign piece of DNA and make the plant recombinant. There are many vehicles, but often a vehicle is a virus itself, a good one though. **better: „vector“**

A virus' protein shell has a surface which is capable to hang on to a certain target cell and to transfer its content, namely the DNA, into a cell of the organism. This happens because a virus is a pathogen and it naturally infects host cells with its DNA and uses them to replicate. We can now use this fact to our advantage by putting a so-called plasmid into the virus. A plasmid is a circular piece of DNA, which is found in bacteria. The plasmid is modified in advance and must contain the resistance gene. We then let the good virus infect the plant cell and thereby the plasmid containing the resistance gene automatically gets transferred into the plant cell, where it is then able to integrate into the organism's endogenous DNA. (Unknown, 2019)

In plants specifically, a method called Agrobacterium-mediated recombination is used. The difference to the above described method with a viral vector is that, the modified plasmid containing the resistance gene is put into the bacterium *Agrobacterium tumefaciens*. This species of bacteria is very good at infecting plant cells and frequently used to genetically modify plant cells. (Unknown, 2019)

An important point to remember is that the foreign piece of DNA has to be transferred to the organism at the right time. In animals, this is done shortly after fertilization. Since all body cells

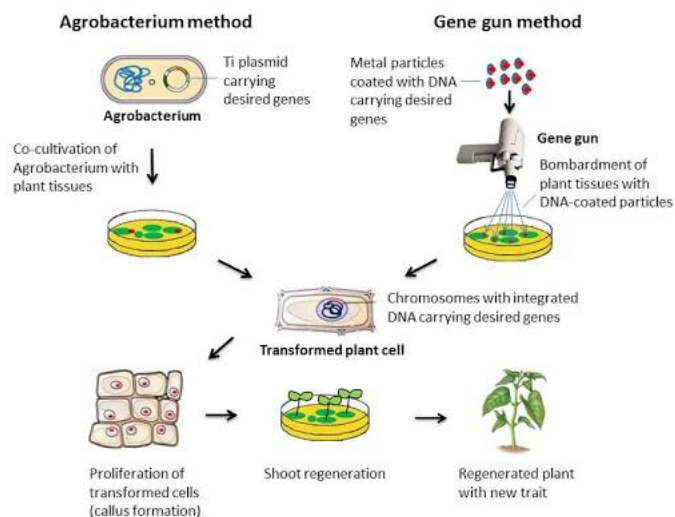
develop from a single fertilized cell, all newly formed cells will also contain the transferred piece of DNA. (Unknown, 2019)

During the interview we have not learnt something special about the gene gun respectively the Hawaiian Papaya. The interview partner gave us more general information about genetically modified organisms.

In summary, genetic engineering is a domain of biotechnology that deals with a way to integrate a sequence of DNA into an organism. The whole interview can be found in the appendix.

4. Case Study: Hawaiian Papaya

In the specific case of the Hawaiian papaya, researchers have relied on the established pathogen-derived resistance method by using a coat protein-mediated transformation in order to manage the PRSV disease. Researchers used recombinant DNA techniques to clone and isolate a very specific papaya ringspot virus (PRSV) gene. The isolated gene encodes the viral coat protein. In order to transform the Hawaiian papaya with the viral coat gene, at the experiment station special gene guns were produced. With these special guns, the gene is literally shot into the cells of the papaya plant, where it integrates into its genome. Consequently, the plant becomes resistant as the gene gets expressed (2020).



do not show the bacterial transfection, if you do not deal with it here

Figure 2 Illustration of Gene Gun

(Shengwu Ma1, 2015)

The creator of this virus-resistant papaya is Dennis Gonsalves and his team. Gonsalves says this about the project: "The only way we have affected papaya quality is to make it resistant to PSRV, which improves its survivability." (McCandless, 1996)

4.1 Infection of Hawaiian Papaya with PRSV

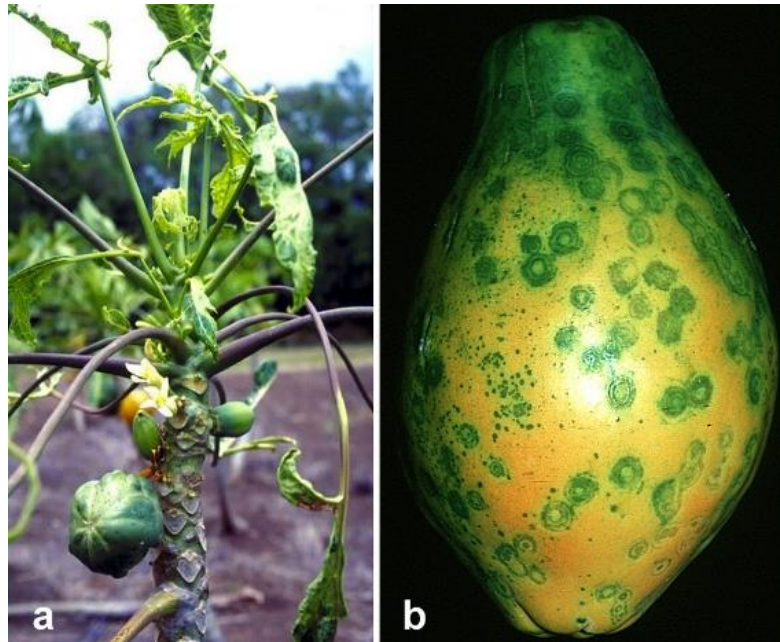


Figure 3: Here, it is shown how the papaya plant got affected by a PRSV infection. The symptoms on the tree (A) and especially on the fruit (B) are clearly visible by the typical ring and spot structure.

(Fisher, 2019)

The PRSV attacks some parts of the tree. Because of this attack no saleable fruits are produced. Symptoms are easily seen. On the leaf lamina a prominent mosaic pattern appears, wet-oily streaks on the petioles and upper part of the trunk and the distortion of young leaves occurs.

(Dennis Gonsalves, 2010)

Without the genome editing all the planted papayas become useless. In other words, without biotechnological help, there would have been a papaya crisis. Because there are a lot of plantations, a great number of people are depending on the production of the papaya for their income. If the cultivated papayas are infected, there is simply no money. For this reason, a solution had to be found to stop a disaster. Gladly, a research team found a way to genetically engineer the Hawaiian papaya and save it from PRSV infection.

(Dennis Gonsalves, 2010)

4.2 Management of PRSV

PRSV is the most damaging infectious virus of papaya. Management of PRSV includes rouging infected plants and spraying them with aphicides. However, rouging cannot stop the unfold of the malady once it's established. Similarly, spraying with aphicides is usually ineffective since the virus is transmitted to the plants before the aphids area unit killed (Litz, 1999). The PRSV virus management has been centered on developing tolerant or resistant types of papaya, however these varieties area unit seldom planted because of poor fruit quality and vigour [(S. Dillon). PRSV-resistant sequence is accessible in some wild varieties associated with the dilleniid dicot genus species. However the event of PRSV-resistant varieties through typical breeding strategies has been sophisticated because of the sexual incompatibility of untamed species and cultivated papaya (D. Gonsalves, 2006). Virus tolerance in back crosses with industrial papaya additionally limits this approach for PRSV

virus management. Cross protection was used to manage PRSV that concerned the employment of a light virus strain against economic harm caused by severe strains of an equivalent virus (Garnsey, 1989). The cross protection strategy of vaccinating papaya with a light strain of PRSV provides resistance against severe PRSV strain infection in Taiwan (S. D. Yeh, 1988). Cross protection depends on the provision of gentle strains that may be used for effective protection against the target virus. Cross protection wants further agricultural follow and care. However, strain specificity and therefore the technical difficulties related to propagating pure strains of "soft" kinds of the virus and therefore the inaccessibility of such strains limit the advantages of this approach (Cheng, 1989). Field analysis disclosed that cross protection was marginally effective for PRSV management evaluation within the field (S. Tripathi, 2008). Researchers from Cornell University and therefore the University of Hawaii initiated the event of PRSV-resistant papaya by sequence technology. The conception of infectious agent derived resistance was projected by Sanford and Johnston (Johnston, 1985) for developing resistance against pathogens. This analysis cluster has applied the conception of infectious agent derived resistance that has excited analysis into getting virus resistance through sequence technology. Infectious-agent -derived -resistance is ruled either by protein-mediated or RNA-mediated strategies. Another strategy using RNA-mediated sequence silencing with transgenic plants expressing infectious agent genes has been developed . (C.-H. Chiang, 2001)

Resistance levels of PRSV disagree with environmental factors and plant development stages despite of the success with this approach. Broad spectrum resistance against totally different PRSV isolates depends on the similarity of transgenes with infectious agent target genes and therefore the genetic divergence of various PRSV strains that area unit related to with their geographical distribution (H.-J. Bau, 2003). The transgenic papaya varieties proof against PRSV against totally different infectious agent strains should be developed separately for varied papaya growing regions. the event of PRSV-resistant lines is mostly thought-about the most effective strategy for economical PRSV malady management in papaya for semipermanent protection (G. A. Fermin, 2010).

5. Discussion of ethical aspects

In this section, we would like to discuss the ethical aspects of genetic engineering and to identify the advantages and disadvantages of the modified Hawaiian papaya in a social and economic context.

If we look at the view of the biological evolution, then this, what we call genetic engineering, is interfering with the natural course of nature. Regarding the natural selection theory of Charles Darwin, the papaya would have died-out. In a nutshell, we could say both parties, the papaya and the virus do have equal rights. For this reason, the question arises, whether humans should intervene to save the papaya? Well, if we are answering regarding the economy or better said the money, then it is clear. Genome editing is a key to earn money. A clear answer to the question is difficult to give since there are pros and cons. Let us look at some of the advantages.

What about resistance through classical breeding?

5.1 Advantages:

There are certainly clear advantages of interfering with nature and rendering the papaya resistant to the PRSV. Among them is the fact, that we can plant the Hawaiian papaya with the necessary characteristics, at least from the human perspective. We can cultivate Hawaiian papaya further. In addition, these modified plants grow faster than the ones that are grown

traditionally. Farmers can therefore increase their productivity, so that they provide the population with more food and financially profit more.

(BAWA, 2012)

In a video it is told that 53 million pounds of papaya were being produced each year. This was the case in 1992 when the ringspot virus was first discovered. But in 1998 the amount was decreased to 26 million pounds. In 2001 the industries started the success with fresh papaya free of PRSV. 46 million pounds of fresh papaya is the case after genome editing.

(Answers, 2013)

The genetically modified plants can be resistant against pathogens and therefore farmer can greatly reduce the application of pesticides and insecticides, which means the fruits are environmentally friendlier and free from potentially damaging chemicals.

(BAWA, 2012)

5.2 Disadvantages:

The experiments in the laboratory and the necessary means are expensive. A great fear in the population is that genetically modified products can have harmful effects on the human body. Because the papaya is a quite new invention, nothing is really known about long term effects on human beings.

(BAWA, 2012)

Further, it is hypothesized that some manufacturers do not label their genetically manipulated foods because they think that the label would potentially harm the business by hampering the sales. Manufactures with more than \$2.5 million receipts each year are required to have a disclosure on the package. This is the case according to the law.

(Poinski, 2020)

6. Future Prospects

Transgenic papaya is the cutting edge extant for plant disease management (Tennant, 2011). Transgenic papaya has had an extraordinary socioeconomic impact on the Hawaiian papaya industry (C. Gonsalves, 2007). However, the success of transgenic papaya depends at the continued stability of transgenic resistance and the applicable horticultural traits of papaya. The breakdown of PRSV resistance is the main trouble associated with PRSV-resistant papaya. Tennant et al. (P. F. Tennant, 2007) reported that R1 transgenic papaya of line 55-1 showed slim resistance after inoculation in greenhouse. R1 transgenic papaya plant life were proof against PRSV isolates from Hawaii however remained at risk of PRSV occurring in other countries. Moreover, transgenic resistance in papaya relies upon on growth stage, doses of transgene, and transgene homology (P. F. Tennant, 2007) Resistance to PRSV is positively correlated with a degree of homology between the CP of the infecting virus and transgene. (S. Tripathi, 2008)

Transgenic resistance in Rainbow and SunUp has proven stable for almost 10 years in Hawaii but resistance may additionally breakdown in regions in which new virus traces exist. There is superb genetic diversity within PRSV isolates from different areas of the world. PRSV-resistant transgenic papaya faces principal difficulties as no contemporary strain has resistance in opposition to geographically distinct isolates. It is important that researchers monitor the PRSV population and its variety to ensure the success of disease control of papaya. On the other hand, posttranscriptional gene silencing (PTGS) era is in all likelihood a more powerful and effective approach for the improvement of PRSV-resistant transgenic papaya. Therefore, biome-specific styles of PRSV-resistant transgenic papaya need to be developed through PTGS tech. with the using of PRSV isolates.

7. Conclusion

PRSV is the no. 1 threat for papaya cultivation. Transgenic papaya by means of GMO has been utilized for PRSV management. In this audit, we find that PRSV-safe papaya assortments have been created utilizing CP genes or RNA obstruction. The hereditary assorted variety of PRSV has been recognized all through the world. The breakdown of PRSV-safety is an important test confronting transgenic papaya development. Despite the fact that, the gene flow of PRSV-transgenic papaya is low, inquire about towards limiting this issue ought to be directed. The reception of PRSV-safe transgenic papaya is still moderate, and it relies on the interest in papaya, biosafety guidelines, and social acknowledgment of the innovation. Late examinations show that PRSV-safe transgenic papaya is naturally protected and has no antagonistic consequences for human wellbeing. Posttranscriptional quality quieting (PTGS) innovation might be reasonable for the improvement of PRSV-safe transgenic papaya in future.

8. Bibliography

- . **A. Fermin, L. T. Castro, and P. F. Tennant. 2010.** *CP-transgenic and non-transgenic approaches for the control of papaya ringspot: current situation and challenges.* s.l. : Transgenic Plant Journal, 2010. pp. 1–15. Vol. 4.
- 2020.** *Hindawi.* [Online] 14 4 2020. <https://www.hindawi.com/journals/tswj/2014/768038/>.
- Answers, . 2013.** Youtube. *How are GMOs Made? The Genetically Modified Hawaiian Papaya Case Study.* [Online] 2 August 2013. <https://www.youtube.com/watch?v=2G-yUuiqIZ0>.
- Answers, . 2020 .** How are GMOs Made? The Genetically Modified Hawaiian Papaya Case Study. [Online] 15 Februar 2020 . <https://www.youtube.com/watch?v=2G-yUuiqIZ0>.
- Baulcombe, D. C. 1996.** *RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants.* s.l. : Plant Molecular Biology, 1996. pp. 79–88. Vols. 32,.
- BAWA, . 2012.** NCBI. *Genetically modified foods: safety, risks and public concerns—a review.* [Online] 19 december 2012. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3791249/>.
- C. Gonsalves, D. R. Lee, and D. Gonsalves. 2007.** *The adoption of genetically modified papaya in Hawaii and its implications for developing countries,.* s.l. : Journal of Development Studies, 2007. pp. 177-191. Vol. 43.
- C.-H. Chiang, J.-J. Wang, F.-J. Jan. 2001.** *Comparative reactions of recombinant papaya ringspot viruses with chimeric coat protein (CP) genes and wild-type viruses on CP-transgenic papaya,.* s.l. : Journal of General Virology, 2001. pp. 2827–2836. Vol. 82.
- Cheng, S. D. Yeh and Y. H. 1989.** *Use of resistant Cucumis metuliferus for selection of nitrous-acid induced attenuated strains of papaya ringspot virus.* s.l. : Phytopathology, 1989. pp. 1257–1261. Vol. 79.
- D. E. Purcifull, J. R. Edwardson, E. Hiebert, and D. Gonsalves. 1984.** *Papaya ringspot virus.* s.l. : CMI/AAB Description of Plant Viruses, 1984. p. 8. Vol. 292.
- D. Gonsalves, A. Vegas, V. Prasartsee, R. A. Drew, J. Y. Suzuki, and S. Tripathi, . 2006.** *Developing papaya to control Papaya ringspot virus by transgenic resistance, intergeneric hybridization, and tolerance breeding.* s.l. : Plant Breeding Reviews, 2006. pp. 35-73. Vol. 26.
- D. Gonsalves, S. Tripathi. 2010.** *Papaya ringspot virus.* s.l. : The Plant Health Instructor, 2010.
- Dennis Gonsalves, Savarni Tripathi, James B. Carr, and Jon Y. Suzuki. 2010.** *APsnet. papaya ringspot virus.* [Online] 2010. <https://www.apsnet.org/edcenter/disandpath/viral/pdlessons/Pages/PapayaRingspotvirus.aspx>
- .
- Dodman, J. E. Thomas and R. L. 1993.** *The first record of papaya ringspot virus-p in Australia.* 1993.
- E. M. Tecson Mendoza, A. C. Laurena, and J. R. Botella. 2008.** *Biotechnology Annual Review.* s.l. : Biotechnology Annual Review, 2008. pp. 423-462. Vol. 14.
- Fisher, . 2019.** Libretext. *Biotechnology and Genetic Engineering.* [Online] 1 November 2019. https://bio.libretexts.org/?title=Bookshelves%2FEcology%2FBook%3A_Environmental_Biology_%28Fisher%29%2F08%3A_Food_%26_Hunger%2F8.02%3A_Biotechnology_and_Genetic_Engineering.

- G. A. Fermin, L. T. Castro, and P. F. Tennant., 2010.** *CP-transgenic and non-transgenic approaches for the control of papaya ringspot: current situation and challenges.* s.l. : Transgenic Plant Journal, 2010. pp. 1-15. Vol. 4.
- G. Aravind, D. Bhowmik, S. Duraivel, and G. Harish. 2013.** *Traditional and medicinal uses of Carica papaya.* s.l. : Journal of Medicinal Plants Studies, 2013. pp. 7–15. Vol. vol. 1.
- Garnsey, D. Gonsalves and S. M. 1989.** *Cross protection techniques for control of plant virus diseases in the tropics.* s.l. : Plant Disease, 1989. pp. 592–597. Vol. 73.
- Gonsalves, . 1994.** *Papaya ringspot virus.* s.l. : Compendium of Tropical Fruit Diseases, 1994. pp. 67–68.
- Gonsalves, . 1998.** *Control of papaya ringspot virus in papaya: a case study.,* s.l. : Annual Review of Phytopathology, 1998. pp. 415–437. Vol. 36.
- Gonsalves, M. Fuchs and D. 2008.** *Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies.* s.l. : Annual Review of Phytopathology, 2008. pp. 173–202. Vol. 45.
- Gonsalves, S. D. Yeh and D. 1984.** *Evaluation of induced mutants of papaya ringspot virus for control by cross protection.* s.l. : Phytopathology, 1984. pp. 1086–1091. Vol. 74.
- H. F. Alvizo and C. Rojkind.** *Resistencia al virus mancha anular del papayo en Carica cauliflora.*
- H.-J. Bau, Y.-H. Cheng, T.-A. Yu, J.-S. Yang, and S.-D. Yeh. 2003.** *Broad-spectrum resistance to different geographic strains of Papaya ringspot virus in coat protein gene transgenic papaya.,* s.l. : Phytopathology, 2003. pp. 112-120. Vol. 93.
- Handrea, . Unknown.** sutori. *Genetically Modified Papayas.* [Online] Unknown. <https://www.sutori.com/story/genetically-modified-papayas--CZanfGTmrdhGKMLHwcrBsH8C>.
- J. A. Teixeira da Silva, Z. Rashid, D. T. Nut, and et. al. 2007.** *Papaya (Carica papaya L.) biology and biotechnology.,* s.l. : tree and Forestry Science and Biotechnology, 2007. pp. 47–73. Vol. 1.
- Jayavalli, R. T.N. Balamohan, N. Manivannan, M. Govindaraj. 2011.** *Breaking the intergeneric hybridization barrier in carica papaya and vasconcellea cauliflora.* s.l. : scientia Horticulturae, 2011. pp. 787-794. Vol. 130.
- Jimenez, S. Horovitz and H. 1967.** *Cruzamientos interspecificos y intergenericos in Carica ceas y sus implicaciones fitotecnicas.* s.l. : Agronomia Tropical (Maracay), 1967. pp. 323-342.
- Johnston, J. C. Sanford and S. A. 1985.** *The concept of parasite-derived resistance: deriving resistance genes from the parasite's own genome.,* s.l. : Journal of Theoretical Biology, 1985. pp. 395–405., Vol. 113.
- Khurana, S. M. P. 1974.** *Studies on three virus diseases of papaya in Gorakhpur, India.,* s.l. : Proceedings 19th International Horticulture Congress, 1974. p. 260. Vol. 7.
- Litz, K. Pernezny and R. E. 1999.** *Some common diseases of papaya in Florida.,* s.l. : Florida Cooperative Extension Service Plant Pathology Fact Sheet, 1999. p. 35.
- M. A. K. Azad, M. G. Rabbani, and L. Amin., 2012.** *Plant regeneration and somatic embryogenesis from immature embryos derived through interspecific hybridization among different Carica species.* s.l. : International Journal of Molecular Science., 2012. pp. 17065–17076. Vol. vol. 13.
- McCandless, . 1996.** Cornell University. *Cornell and the University of Hawaii introduce the first genetically- engineered fruit crop cleared by the USDA.* [Online] 23 September 1996. <https://news.cornell.edu/stories/1996/09/cornell-and-university-hawaii-introduce-first-genetically-engineered-fruit-crop>.
- P. F. Tennant, G. A. Fermin, and R. E. Roye. 2007.** *Viruses infecting papaya (Carica papaya L.): etiology, pathogenesis, and molecular biology.* s.l. : Plant Viruses, 2007. Vol. 1.

- Poinski, . 2020.** Food dive. *Food manufacturers can put GMO labels on their products in 2020. Will they.* [Online] 27 January 2020. <https://www.fooddive.com/news/food-manufacturers-can-put-gmo-labels-on-their-products-in-2020-will-they/570505/>.
- R. I. Davis, L. Mu, N. Maireroa et al. 2005.** *First records of the papaya strain of Papaya ringspot virus (PRSV-P) in French Polynesia and the Cook Islands.* s.l. : Australasian Plant Pathology, 2005. pp. 125–126. Vol. 34.
- S. D. Yeh, D. Gonsalves, H. L. Wang et al. 1988.** *Control of papaya ringspot virus by cross protection.*, s.l. : Plant Disease, 1988. pp. 375–380. Vol. 22.
- S. Dillon, C. Ramage, S. Ashmore.** *Development of a codominant CAPS marker linked to PRSV-P resistance in highland papaya.*, [ed.] 2006. s.l. : Theoretical and Applied Genetics. pp. 1159–1169. Vol. 113.
- S. Tripathi, J. Y. Suzuki, S. A. Ferreira, and D. Gonsalves. 2008.** *Papaya ringspot virus-P: characteristics, pathogenicity, sequence variability and control.* s.l. : Molecular Plant Pathology, 2008. pp. 269–280. Vol. vol. 9.
- Shengwu Ma^{1, 2,*}, Yu-Cai Liao³ and Anthony M. Jevnikar¹. 2015.** World Biomedical Frontiers. *Induction of Oral Tolerance with Transgenic Plants Expressing Antigens for Prevention/Treatment of Autoimmune, Allergic and Inflammatory Diseases.* [Online] 11 November 2015. [Cited: 2 April 2020.] <http://biomedfrontiers.org/allergy-2015-21/>.
- T. Maoka, S. Kawano, and T. Usugi. 1995.** *Occurrence of the P strain of papaya ringspot virus in Japan.* s.l. : Annals of the Phytopathological Society, 1995. pp. 34–37. Vol. 61.
- Tennant, G. Fermin and P. 2011.** *Opportunities and constraints to biotechnological applications in the Caribbean: transgenic papayas in Jamaica and Venezuela.* s.l. : Plant Cell Reports, 2011. pp. 681-687. Vol. 30.
- Unknown. 2019.** Wikipedia. *Genetic engineering.* [Online] 3 april 2019. https://en.wikipedia.org/wiki/Genetic_engineering.
- Y.-H. Peng, D. Kadoury, A. Gal-On, H. Huet, Y. Wang, and B. Raccach. 1998.** *Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions.* s.l. : Journal of General Virology, 1998. pp. 897–904. Vol. 79.

9. Appendix

9.1 Interview with Dr. Paulsen

I wanted to understand the method of genetic engineering which I read about on the internet. My interview partner helped me and explained it like that:

In those methods it is going in general about that, that we have to do it with a vehicle. So we have to bring a piece of DNA to a cell. A vehicle is a genetic information, which is heritable. This is what the papaya needs in this case.

For instance, the papaya needs the skill to produce a protein. Or the skill to defend itself against a pathogen. A vehicle is a transport medium. It transports ingredients. An important point is that you have to use the vehicle in the beginning of the fertilization or production. An analogy would be a pill. A pill transports the ingredients to the organisms where it is needed. The genetic information is stored in the DNA in the nucleus and it gets read there. The RNA is transported to the cell cytoplasm and it gets translated there to the protein. There are also others examples of transferred DNA sequences, such as a gene which can turn another gene on and off, which can control the development. But if you now want to change a plant genetically or also an animal then you have to take a part of the DNA, but exactly this part which you want to function. This should be installed in the plant. This inserted part should be read exactly the same way as it happens usually in the DNA. An example in your body: Some cells of your body can produce insulin. This is a DNA sequence, this will be read and translated in a protein. When we now could install a DNA in a liver cell which codes for an insulin protein then these cells would produce another insulin protein. Most living beings have a cell walls and cell membranes and chromosomes. The genes aren't read by chance. It is quite difficult to install a sequence of DNA in a cell like that, that it also can produce the protein. There are different methods this because the whole thing is very small. We cannot do it with microsurgery, this would be still very big.

Now what exactly happens in the DNA, is there an insertion, deletion or replacement of the nucleotides?

The nucleotides code for a resistance protein. So this means a virus has got a protein shell. This protein shell has a surface which is capable to hang on certain cell and to transfer the content of the DNA in a cell. This happens because a virus is a pathogen. We have now used this, the DNA which we can put into the cell and in which we could install a genome. This way we have found to do it, because some viruses and bacteria are functioning like that. This is a method. In fact, it deals with the question how to install the DNA into the organism. How this exactly happens, I do not really know. We have here now a lot of abbreviations, these are biochemical substances. For this you really have to research with this plant (the papaya), but we here (at the institute) we are dealing with cruciferous plant. We also have our special terms for the genes, these are often abbreviations. They use the skill of pathogen microbes, to propagate with the infrastructure of the host cell. This, they use to take a part of the DNA and to place it in the cell. This is basically everything.

Is it possible that you explain to me the other two methods?

The RNA interference-mediated resistance. I only can tell so much about the RNA. It is the substance which the RNA reads out. The DNA is the hereditary material, the RNA is the

read out. It is then going to another part of the cell (the cytoplasm) and there, based on the RNA, the protein is made. Now there are so called retro-viruses which can make from RNA again DNA. Obviously, this is used.

How about this?

If I fly over this site, I can tell that RNA, which DNA happens and a certain part of the host cell can be translated or suppressed and so we can change the plant, so that it has other new features.

You said there happens a suppress or a replacement?

Yes, that is true. But this is very complicated and I do not understand it. But also here is the question how do I get the host cell to have another genome than what it normally has. And not in the way of conventional breeding, but rather in a way of a lot of combinations. I read out from all plants those and do not just take those which I like, but rather specific. I put it into the plant to get breeding more efficient. Or in other words when we do a comparison. We are breeding white rabbits, you can have a lot of rabbits and you look for those which are white. You are taking the white ones and you let them multiply. When you are lucky then you get once white rabbits which are solid heritage.

Or we can do it in another way, we can look at the genes which are responsible for producing white hairs. We can install it in the sperms of the rabbits, when we know how it works. Like so, we have much faster the white rabbits and you also know exactly why they are white. Because there can be different reasons why it is white.

Could you maybe look at the third one the Replicase Gene Mediated Resistance?

So here it is talking about the protein which is happened, has another structure, because a genome is inhibited, that this protein expresses. I understood it like that. The tertiary structure of the proteins is changed. The protein, which is produced by the RNA, is an endless chain with certain amino-sequences. But this is going to be fold, this means it comes together.

What do you mean there exactly with folded?

The tertiary structure has a certain row and length of amino-acids. Because some amino groups are doing hydrogen-bonds. If we change there something then we get different amino groups with different effect.

Another effect? What kind of effect?

As a rule for the enzyme is the tertiary structure very important to the function. And if you change there at the tertiary structure then is the effect of the enzyme maybe already gone or stronger or weaker. So this is obviously the mechanism. As I looked at it very fast. Everything is biochemically very special. A good example: when you build a house with bricks. You need someone who arranges the bricks right. But if the bricklayer knows how the bricks have to be build up to make a wall. But if he gets a wrong plan, he can do everything right but at the end it is not like the peasant the house wanted. So in this league or lever we can do something. As I said this all is very biochemical. The technique is also very important, how the company are researching on it. This is not something which can be made very fast in two or three days. There is a lot of basic thinking behind it.

Do you have maybe pictures or models to show?

I do not have them right here. As I said, we are here not working with transgenic plants, but we are here trying to understand why from one type we get two types and how they are genetically different and why they have developed like that.

I want to move now to the ethical aspects. What is your opinion/answer to this: Should the people modify plants like papayas regarding ethical aspects?

It is already happening. So the question is already answered.

Maybe you have an answer to this? As we know from the evolution theory, there is survival of the fittest. If we are looking at this theory, the Papaya would have died-out. Or there are people who would die, if there are not papayas anymore.

Both are problems in the human medicine. For instance, a kind of heritage illness, which we could heal. For example, if you miss an essential enzyme...

But if you look at it in the moral way?

I am very conservative with this question, because both aspects do have the equal right. On one hand, for this I did some notes. The advantage is that we can a cultivated plant, which has for us the right characteristics. We can cultivate them further, but this would be much more expensive, because we can not hinder the infections. When you grow it commercially it is not the same as in the laboratory. I am just saying that there are aspects for and against. For instance, we need less protecting agent and it is also a less environmental burden (=positive aspect). It is resident, we want to have bigger sweeter fruits, which are not bitter. We can do this also genetically. It is just a question of market acceptance. We can also do with some genetically modified organisms things which we could not have done before. Even things like insulin for the diabetic people. This makes also possible for the overpopulation. Now the disadvantages, a company which makes it would patent its products. This means when you want to grow this plant you have to pay licence. Like if you would pay for a software on your computer when it would not be public domain. Now it is the question when the resistance from another organism is, why can we patent it. The achievement of the company is not the resistance development, rather it is the isolated genes to put it into the plant. But the resistance could have from a wild nearby plant. This is often the case by cultured plants. Now when you want a feature to patent in a plant then it is a pirate copy. An example you find a wild plant, which has a resistance gene against a pathogen which makes the commercial extension not possible. When we the cultural plant genetically modified then is this resistance against the virus and smells the same like before. This would be the best case. This means, that the client does not know it if it would not be written on the package.

Is it true that it smells exactly the same?

Well it depends, there are some different types. It could be that something else is a bit different. In general, you do not know it when you do not know what is going on, because it really gets optimized how it should smell. The main achievement has done the wild plant, namely to be resistant against the virus and you have only found how to rig this plant. So, you cannot basically patent the characteristic itself.

What do you mean with the characteristic itself?

Let's suppose that a gene produces a protein. This all is now hypothetically. There is a step that the virus does not happen because of the protein. This protein is nontoxic and chintzy. This means you do not know that this is in the product, similar to that that does not have this protein. So, it would be great when all fruits could produce this protein. So, like this we would not have the problem anymore. But the achievement to produce such a protein to protect against the pathogen has done the wild plant. From it we have extracted the gene. So, cut out to a vector or a vehicle then let grow and then put in the host plant of the sick papaya. There it is going to express the enzymes are going to make from the DNA proteins and they do not think if it makes sense. Same if you would install a software, for the processor it does not matter what is going to be coded. He just does it. So, this is similar. It is a mechanism like in our body the cells. Let's imagine you are a programmer. You programme an algorithm like for instance the mp3 player. So, you are doing an algorithm to make music. You make this in a software. Now another is going to compress your mp3 and he combines yours with another software. So, it is counterfeit and this is forbidden. In the genetic field it is allowed, this is like that, because the wild plants are not substances with rights. But it gets complicated if you use in this way the old cultivated plants. Because there are some people

who are not enough informed about the growing of the plant. So it is possible that you could use others cultural good and when those people have it long time in their culture.

Is it not possible to reproduce this?

Yeah, this is the question now. If you want to do it because of the economic success, they just do it. Now the other question is, when someone comes to the same resistance just in another way, what happens then? Because the way is patent and not the way through it. What I just want to say is that there are different ways to be resistant. What when you now create another method to also get virus resistant papayas Does the protect patent rule still count? So, when we breed now a type of papaya, which is genetically modified. Do we now have the right to sell it? And everyone who is growing it has to pay a licence tax? To you? Because you are the software owner. This is also the reason why such a breeding happens. Because the company which are making it earn money. It is the same as Roche would make a new drug. You cannot then just copy it and sell it with your price. The problem is that every peasant can propagate the genetically modified plant. With a medicine or a drug is this not possible. For this reason, everyone, who is making or propagating this patent types has to pay patent taxes. This is the working system of them. The same is like if you would copy the excel program and the calculator, so therefore you have to pay two-times the licence. You have to do it because you are using two programs of excel. Another problem you know that the most cultured plants, also other plants are going over is fertilization pollination and junction. We know from cultured plants, that this plant is giving information through pollination.

How do they give information exactly?

So, when you have the papaya who is resistant against ringspot virus. If the dust from one papaya gets to the other papaya which is not resistant. Because there are papayas which survive the ringspot virus. So, it could be that the offspring afterwards have the resistance. I mean the genetically manipulated resistance. If you have children you give a part of your gene endowment to them. The ploy of the gene manipulation is that we make strange genes suddenly familiar. Now is the question when such plants have those resistance, do you have to pay then also licence taxes? Or not? It is a question of right. There was once a case in America with Raps. It is not that easy, because it also has to do with the busyness-model.

But what if you could prove it that they are resistant through nature?

This is not possible to show or prove. We only see if the plant is resistant or not. The licence owner has the ability to say no one else can have such plant features like mine. He does not have to prove it. So, it is quite difficult. So, another question is, what is when this feature of the plant has disadvantages which we cannot see yet. Who has then the responsibility? The thing is that this disadvantage is maybe bot remarkable in the taste of the plant. I make another example, you know what raps is? This is a useful plant, which is growing here. The wild form of the plant has a substance, which makes that the taste is very bitter, so that it does not taste very well. Because it cannot be eaten from the wild one. Now we have rapeseed soil, this does not contain this substance. If you eat the bitter one you get collateral damage. Another example, when such a plant is planted in a huge area with other plants. Then this huge area gets pollinated. The other plants, they will be crossing away. This is the reason why we don't know or do not see the wild form of our cultivated plants. Let us imagine we have the ancestor of our plant. Now you are making breed types. You are planting them in a huge area, then is the chance great that the wild plant is going to be pollinates with the fertile plant. At the end is the wild plant lost. That is something that let us think, because we are destroying or losing the culture. The other thing is if you are collecting licence pay (money) for the planting, then those people who cannot pay the tax cannot just plant the plant. Until now it was always like that, that the peasant could propagate his seed. This is with such plants not possible anymore. The last thing is maybe

not a big problem, but by animals it is the case. We call it the dignity of the creature. If you have for instants type of chicken which are going to be a food in half year time. Those are going to have joint damage, because they are growing too fast.

Is this because of the food?

Yes, it is because of the food. The type is genetically so modified or breed that it grows so fast. Such animals would never happen in pure nature. But there are some and they are going to suffer, because they are not adapted to this environmental nature. We are going to breed them because of commercial reasons. So they do not become extinct. This is a problem for us vertebrates maybe plants. If now a mildew makes a citric acid is this not a problem. It is a gradual transition. This should be thought before they start. Mostly it is done because of money. I do not say it is bad to earn money, but the question is just how. Again, I do not say we should make it and I do not say we should just make it like that. I read quickly this part. (He reads it after he says). It is basically already this what I have told you. When we grow it, it suddenly appears everywhere. We cannot rollback such things.

Why is the Hawaiian plant the first plant which is genetically modified? Why exactly this plant?

There I have to guess. I think it is relatively easy because you just have to add one characteristic. It was also a familiar activator. And probably also because of the commercial interest. A similar case would be the bananas. There were also pathogens which are making the growing difficult. The bananas which we have here are a type of banana. There are thousands of types which do not come to the market.

Is it in a nutshell said basically because of the easy features done?

No, the reasons are it should be easily modified, it should have a commercial interest. The thing is also that you would not invest millions of dollars for a research like for instants a tomato, which no one would buy. So, it does not make really sense to modify it.

Do you maybe know how this ringspot virus happened or had been created?

No, this I do not know. But I think this is to look up on the internet. The thing with the viruses is mostly like that that they are presented a long time on the cultivated plants. So, they create them big damage. Because I do not know how much you know of pathogens and host plants. But imagine now you are a pathogen. You need people which you could make ill, so that you can expand it. If you now kill everybody who comes close to you, then is the thing that the virus cannot expand itself that good. Because it is not in the interest to kill the host cell. The same is for the owner who owns a slave. It does not pay to kill his slave. The slave should be alive so that they can work. This is a problem between parasite and host or between disease and host.

So one needs the other one right?

Yeah, the host can do or live better than the parasite. But when the host kills the parasite then is he just damaging itself. So those pathogens are there but they do not create a big damage. What often happens when we seed fruits which do have for us nice characteristics is that they disturb the balance. And a Virus will be by hazard very virulent. It is a part of the research to find this out. Is this mutation or sometimes we find out that the culture plant has lost a resistance. The pant was originally in a place where the virus wasn't. And when they are not resistant against a virus like that then it is going to have a big problem. Nowadays is this happening a lot, we are dragging parasites from other places. Or the other example, maybe you know it. By the discovery of north America through the Europeans. We have brought them diseases which were harmful in Europe but which were very strong there. A lot of people died there. Meanwhile it goes better.

Can a papaya which is sick reproduce itself?

This depends on the special case which happened. There are diseases which stop the plant to reproduce itself. I mean a lot of use plants somehow or other they are going to be expanded by vegetation. For instance, bananas cannot reproduce itself because they do not have semen in the fruit.

Hearing from you that, would tell me that it is not possible to reproduce a sick papaya.

Yeah, so would the papaya be died out and the virus. Because the virus needs the papaya. Which we know from the virus is, is that the vitality is very reduced. This means that the plants do not grow so as they should. This means they brings small or crippled ones which we cannot sell anymore. This can be a consequence of the virus. It could be also that the plants have nothing but the semen are not germinating. There are a lot of possibilities. It could also be that the virus affects the plant so that it is going to susceptible to infections of bacteria or fungus. I have learned from the article, that the disease is a big problem and that it has to be resistant because of commercial reasons. But for this you have to know what a papaya really is and what its symptoms are. I know for instance from potatoes. Potatoes which are affected by the virus do not have crop.

If we look now at Switzerland, do we see gene-modified fruits in the markets?

As much I know it is still inadmissible.

Does this mean the papayas which we eat are not genetically modified?

I do not know if it is forbidden to import them, but what I know is, that it is forbidden to have or sell them agricultural. I do not really know, but what I know is that it is forbidden to let genetically modified plants grow. So papaya is not a fruit which we can let grow in Switzerland. It is also like that that the countries who are producing genetically modified plants have interests that they do not do it remarkable (=unlabelled).

So that the clients have no clue about the product?

Yeah, the producer just tells that they are good as the others. Because a lot of people do not know about it, they just do not eat it. It is like, if you would sell a sausage with cooking salt and a sausage with sodium chloride. Most people would not buy the sodium chloride one because it sounds too chemically. But in fact, it is the same. This is the view of the sellers and the view of the consumer is that I can decide what I want to buy. So, I mean that it really matters for us clients how or where the products are produced.

Are those genetic modified products damaging us eaters or our environment?

I think not directly. Because this is in the interest of the breeder. When you find a plant, which is resistant, this you can find out with laboratory experiments, then you make taste proofs. You have to do a lot of research so that you can use it for selling. Now with the technics, how I bring in a genome a piece of the DNA is extremely biotechnical. This is relative unimportant when we look at the consequences. It is similar to how are we producing the most efficient cars combustion motor. And other people are thinking how to live in cities where we have a lot of traffic. These are two topics. The one topic does not really deal with the other. Now the question is how does the plant take a foreign or an external gene. So, this question is for the society or for the nature not that important. Another thing that I want to add, we know always less what we eat. So, it would be good to know how does really is functioning. Because then we can really decide what to buy. The thing is when you are uninformed then are others going to decide for you. This is the reason why we are researching.

This Interview was translated by us from German to English.