9th International Conference on Public Communication of Science and Technology (PCST)
Seoul, South Korea, 17-19 May 2006

"ECO-FRIENDLY" GENES: FROM SCIENTIFIC RESEARCH TO RISK MANAGEMENT, ETHICAL ISSUES AND COMMUNICATION

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Abstract

The scientific community needs to care for increasing social demand for safety in plant gene-transfer techniques by exploiting "sustainable" practices based on technological progress and risk-management expertise. The adoption of a bioethical approach is becoming more and more critical in determining laboratory practices. The need for good communication of scientific results is an essential tool for properly managing conflicts among the various actors of the debate on plant transgenic research. The acknowledgment of these issues constitutes a milestone of our project. This was conceived for developing multidisciplinary research on the question of risk management for plant exogenous gene transfer, by bringing together the expertise of genetics, social sciences, bioethics, communication and dissemination of scientific results. In the laboratory activity, we are assessing potential approaches for exogenous gene transfer into plants based on "clean" tools. We are also conducting social research to analyze the scientific community's perception towards exogenous gene transfer hazards and their management. Moreover, we analyze—from a bioethical point of view—the concept of "sustainable development". We are also testing tools to engage the public in debate on controversial scientific issues. Finally, the project is attempting to work out strategic guidelines for a good scientific communication.

Keywords: Bioethics, Communication, Gene transfer, Risk management

1. Introduction

Biotechnology offers powerful tools to study various crucial aspects of plant sciences. In model plants, the joint advance of molecular biology, *in vitro* tissue culture and gene transfer techniques is opening up new experimental opportunities for gene discovering and functional genomics analysis [1]. Besides these opportunities, genetic improvement of crops for various traits of interest is a relevant application of agrobiotechnology, and after the first introduction in the global market dated to the beginning of 1990s, cultivation of genetically modified plants is increasingly spreading around the world.

When this type of study is limited to lab research, it is much better tolerated by the public than when it aims at practical applications. In this case, in fact, it tends to raise a whole range of collective fears. This is why in the field of the biotechnologies, and specifically when it comes to the exogenous gene transfer into plants, the question of risk evaluation and management is of great importance [2, 3].

Even if through these techniques it is possible to bring out quality products, what counts is the capacity to guarantee their environmental and social compatibility. Therefore, risk management should be the top priority for the scientific and political communities if they want to have a consensus over the choices made and the decisions taken. All this requires a stronger presence of civil society thanks to processes of social empowerment involving the spheres of culture, education and research [4, 5, 6]. In this "*social empowerment*" process, culture, training / education, and research has to play a role of relevance [7, 8, 9, 10].

In this framework, the scientific community needs to take into account increasing social demand for safety in plant gene-transfer techniques by exploiting "sustainable" practices based on technological progress and risk-management expertise. In addition, the adoption of a bioethical approach is becoming more and more critical in determining laboratory practices. Finally, the need to establish a dialogue between the general public and scientists is widely acknowledged as an important element of scientific research projects that are currently in progress.

We believe that this aspect of communication is one of the most stimulating and fascinating challenges for modern society. For this reason, in the last years we have embarked in a research aiming to consider in a multidisciplinary approach the vast field of GMOs, from traceability in the feed and food chains to the risk management on transgenic plant production. Our study is a multidisciplinary approach, involving laboratory research, social sciences, bioethics, and communication.

In this paper, we describe the methodology chosen in a specific part of this broad research, i.e. the aspects related to the question of risk management for plant exogenous gene transfer techniques. Besides, we will report on the activity already developed in a first year of project.

The final goal of the overall work is to provide the scientific community and the public institutions with safety procedures, tools, and strategies for suitable risk management. Because of its peculiarity, our project brings together the expertise of genetics, social sciences, bioethics, communication and dissemination of scientific results to promote research that thoughtfully considers environmental questions, biological risk management, and the complex relationship between science and society.

In our laboratory activity (described in section 2), we are actively assessing potential approaches for exogenous gene transfer into plants based on "eco-compatible" tools, i.e. the elimination of the antibiotic resistant genes, the transfer of alternative marker genes, etc. At the same time, we are conducting social research to analyze the scientific community's perception towards exogenous gene transfer hazards and their management (as reported in section 3). Moreover, we analyze—from a bioethical point of view—the concept of "sustainable development" and the implications of scientific research on nature (as depicted section 4). We are testing tools to engage the public in the debate on modern biotechnology applications and on the risk evaluation of scientific research (as described in section 5). Finally, the project is attempting to work out strategic guidelines for a good scientific communication (detailed in section 6).

2. Research on the "eco-friendly" constructs

Due to the recent public concern for the environment and for the reduction of biological risk, a few researchers have undertaken the serious commitment to start a dialogue with the public on this matter. As a consequence, a new line of research has been launched, which represents a turning point in the field of plant biotechnologies—the employ of "eco-friendly" strategies for plant gene transfer [11]. These techniques are based on the use of constructs that are exploited to allow the transfer of low environmental impact marker genes to plant tissues [12, 13, 14], and even only of the target gene without the marker gene through co-transformation [15, 16], transposable elements [17], site-specific recombination [18, 19, 20, 21]. Marker genes —the ones that are associated to the gene that must be transferred to the plant—are a crucial aspect of the whole gene transfer strategy because they allow us to distinguish tissues that contain the exogenous gene from those that don't [22]. Normally, the marker genes employed are those that activate antibiotic resistance. This, however, is becoming one of the more controversial and less socially accepted issues, so much so that such genes are now going to be banned (regulation 2001/18/CE).

At the IASMA Cellular and Molecular Biology Unit, there is an acknowledged experience on grape molecular breeding [23, 22, 24, 25]. This plant is recently object of advanced research aimed at studding its genome, and to understanding the activity of genes involved in important biologic processes (http://www.vitaceae.org/). Moreover, the recent characterization of genes involved in basilar pathways (sugar transport, and production of polyphenol, pigments, organic acids, aminoacids and polyamines, structural and functional proteins) obtained in various laboratories [26], requires further in depth studies. In this aspect, the gene transfer technique result crucial. Also for grape, thus, the development of a gene transfer strategy based on the "eco-friendly" vectors is of relevance. Moreover, in grape, this field is almost unexplored.

2.1 Marker gene elimination

To eliminate the marker genes after the insertion of the target gene, the self-excision can be obtained with the site-specific recombination, a strategy toady suitable also for the plants with vegetative propagation, such as grape. In our activity, embryogenic calli or somatic embryos of *V. vinifera* (cvs. Chardonnay, Brachetto), 110 Richter and *V. rupestris* were co-cultured with *Agrobacterium tumefaciens* carrying the chemically-inducible site-specific *cre/loxP* pX6 vector with the gene for the Green Fluorescent Protein (GFP), and the gene for the nemicin phosfphotransferase (NPTII) as marker gene. In this construct, the expression of the *cre* recombinase is regulated by the 17-β-estradiol [27]. The construct was kindly provided by The Rockefeller University of New York (USA), prof. Nam-Hai Chua [21]. Putatively transgenic cultures were selected on kanamycin, and individual somatic embryos were isolated and converted into plantlets. Preliminary molecular assays showed the transfer of the GFP gene into the plant genome. Inductions of the cultures on different concentration and exposition time on 17-β-estradiol were performed. Observations at the fluorescence stereomicroscope gave encouraging results, showing the expected GFP gene expression: this is, in fact, the result of the successful induction of the marker gene self-excision mechanism.

2.2 Alternative marker genes

Among the possible strategies that appear promising for grape, the employ of low environment impact marker genes that make cells able to metabolize specific carbon sources -such as mannose as alternative to sucrose (Posytech, Sygenta licence)- has proved successful with various plants [14], however gave different results in grapes [28, 29]. In our activity, preliminary essays have been performed to verify the effect of the selective medium on the callus growth ability. Embryogenic calli of *V. vinifera* cv. Brachetto were grown and monthly subcultured on different formulations of the same medium containing mannose, sucrose or free of the carbohydrate source, respectively. Calli cultured on the substrate containing mannose or sucrose showed a similar growth rate. No growth was observed in calli placed on the medium without carbohydrate source, which progressively turned brown and died. A more specific assay is in progress

to test the impact of the selective medium on the morphogenic activity of the embryogenic callus.

Just as interesting is the VHb gene for *Vitreoscella* hemoglobin through which it is possible to obtain a selection based on the increase of cell metabolism in the cells that express it [30, 31, 32, 13]. In our activity, embryogenic calli of *V. vinifera* cv. Chardonnay and Brachetto were co-cultured with *A. tumefaciens* strain EHA 105 carrying the VHb gene expressed constitutively by the 35S promoter (kindly provided by Prof. C. Fogher, Piacenza University, Italy). At present, observations did not showed peculiar traits in co-cultured callus maintained on solid media, although a better reactivity was recently noticed in embryos liquid cultures. Chemical and molecular assays of crucial importance are in progress.

At this point of our work, we can consider that the removal of the marker genes seems to be, in our experience, the most promising between the strategies we are exploiting in the view to apply more sustainable gene transfer strategies for grape. Tissue culture conditions related to the marker genes at low environmental impact still require a labor-intensive optimisation. Moreover, the use of this strategy necessarily implies the holdover of the marker genes after performing the selection. In conclusion, the elimination of the marker allow us to take advantage of an effective selection method, and also to perform the actual elimination from the grape cells of all the non-desired genetic material that were inserted during the gene transfer process.

3. Study on Management and Safety Practices

Today, the detention and the manipulation of microganisms in the research labs involved in the plant gene transfer are specifically regulated (in Italy: DL n° 224, 8/7/03), while the European law concerning the in vivo release of GM plants has been recently approved in Italy (Dir. 201/18/CE). Due to the strong attention to the public information of such regulation, specific programs for the public involvement in the problems of biotechnology risk management are necessary.

In this framework, our research is concentrated on the analysis of hazard perception concerning the exogenous gene transfer to plants, and its management by the scientific community in order to define a framework of appropriate knowledge.

In the first year of activity, we concentrated on the reconstruction of the regulations framework in selected both Italian and European research laboratories, in the view of comparing the different national and international methods of analysis and risk management procedures.

Then, a group of 15 laboratories involved in the gene transfer techniques has been selected. In these labs, some fundamental themes concerning the safety management, such as the laboratory practice, the application of regulations, the behaviors towards problems and any problem of communication with the Public are going to be examined. Moreover, the opinion of the researchers with respect to the need to define “eco-friendly techniques” to transfer genes into plants shall be evaluated.

The research techniques used is as following: the ethnographic observation and the interview to the persons in charge of the laboratories and of the research guidelines, also with on-site visits. The collected material will be analyzed and worked out by the research staff in order to obtain interpretations on the themes of safety management and on the definition of innovative procedures to apply it in the laboratories.

For this part of the research, we involved the Observa Association (Vicenza, Italy), a non-profit organization and a research centre founded by sociologists, economists and political scientists, whose mission is bridging scientific research, political decisions and public opinion. The Association has main experience in analysis, development and evaluation of solutions for public participation in scientific, technological, health and environmental contexts, of innovative procedures to involve the public in complex decisions and, in general, of dialogue forms between scientists and the wider public [33, 34].

4. Analysis of the Bioethical Relevance of Scientific Conceptual Categories

This part of the project is developed by ITC-Istituto per le Scienze Religiose, and aims at clarifying the bioethical relevance of scientific categories such as the idea of nature and the naturalness of evolution processes, as well as the responsibility of the subjects operating on those processes. From a bioethical point of view, in fact, we face today a particular concern for the naturalness of the procedures adopted. The debate over man’s capacity and right to intervene and modify nature is central if we want to address issues of competence and liability. Recently, advances have been made in the ethical-teleological analysis of the foundations of the decision-making process. [35, 36, 37, 38]. These questions are generally addressed in local, national and international bioethical committees. The results of this activity will converge in scientific publications that will serve as a platform for discussion also for the other partners involved in the project. Furthermore, we would expect they will provide the starting point of the debate with other research institutions, as well as the basis for the education of the public opinion.

We are devoting particular attention to the identification and classification of the different meanings of the idea of development and of social and environmental sustainability in relation to the technological and genetic interventions on the environment and on its influence on social behavior. Our approach privileges the question of the relation between an intervention in the present and its consequences in the future on the following generations whose expectations and rights will have to be clarified. Such analysis aims at correcting a prejudicial attitude of hostility to any technological

intervention on nature, but also its opposite, that is, an excessive faith in scientific progress without any consideration for its environmental, cultural and social costs.

A first start of our work required efforts for providing the group with some definite notions in order to form a sort of common language. An interesting debate involved the group, concerning as basic question, the find out of the more appropriate denomination for describing the technology we are exploiting for the gene transfer into plants. Among various options, such as "eco-friendly", "eco-compatible", "sustainable", and "clean", two notions revealed as particularly important for the bioethical point of view, i.e. the "cleaned genetic technology" and the "eco-compatible" one. The category of "clean" seemed to motivate a particular attention because of its use both in the scientific and in the ordinary language. However, the common language could not correspond to a univocal attribution of meaning to be precisely applied into the two languages, producing a possible incomprehension that would be crucial in the moral evaluation. This aspect may represent a problem. Besides, the term "eco-compatible" and "eco-friendly"proved to be worthy of investigation because it is strongly linked with the idea of nature, responsibility, development and social and environmental sustainability with specific attention to the rights of future generations which is a specific task pf our project.

Another part of our work on the ethical problems concerning the "eco-friendly" technologies produced a definite and detailed bibliography collection. This resulted essential for focusing the *status quaestionis* regarding the moral evaluation of biotechnologies, with particular regards to the complexity of the moral value to be given to the notion of nature. For instance, the expression "natural" can be perceived as a synonym of "morally good". Besides, it can be used as an expression to appoint something as "morally neutral".

Besides, our research demonstrated that often bioethics flattened into some political and juridical aspects. There is a strict linkage between these two disciplines, however tasks and requirements are quite different. A study is in progress in the view of deeply analyzing the interaction between bioethics and bio-law.

5. Public knowledge diffusion tools

This part of our project is dedicated to the development of tools for public engagement, to test means to involve citizens in the debate on modern biotechnologies, and is carried out by Museo Tridentino di Scienze Naturali, an institution with an established experience in scientific culture diffusion in general (see the website www.mtsn.tn.it) that has in recent years committed itself in the development of tools and approaches that encourage public debate on controversial scientific issues [39].

This activity is structured in two phases. In the first, a group of teenage students (over 100) from five different schools are involved in a programme of activities spread over a period of 8 months, to come into contact with and discuss the different issues that are related to applications of modern agrobiotechnology. The students are developing their own tools to speak about this topic, and will present them in a final event that will take place in May 2006. This will also involve the families of the participants, and will be a precious opportunity to verify if and how the students vehicle the information and experience they make to their close ones. The feedback collected during this phase (questionnaires filled in by the students and by their families both at the beginning and end of the project, interviews and group discussion with the participants) will be used to define the public events for the second phase of the project (2006 – 2007).

6. Innovative strategies for the communication

This part of the activity focuses on the perception and communication of risk and innovation in the field of GMO applications and involves all the researchers of the project. The aim is twofold: firstly, to define effective methodologies that would support researchers in the dissemination of the results of their activity; in second place, to set the laboratory aspect within the specific social setting in which it is performed. This allows us to take into account at an early stage its potential applications and the associated risks, as well as the reactions of the public towards these. Our project's multidisciplinary trait provides an excellent opportunity for this.

During the first phase of the project, we concentrated on two different aspects.

An intense debate is in progress inside our group, in the view of sharing the different point of views on crucial issues and interpretations of the GM transfer techniques. This has the main purpose of creating a necessary common ground of knowledge between the molecular biologists and the researchers of the other disciplines, through continuous exchange of expertise, information, technical language, experience and competence. Our meetings would produce articles, publications and events that would support dialogue between the project group and the public. In particular, the concerns of the lay-citizens on the gene transfer techniques have been discussed, with major emphasis on the attitude of the "scientists" to the lay-citizens. As previous research suggested, in fact, "non-experts" perceive the existence of incommunicability and distance from the scientific community [3, 40].

An other relevant aspect of our activity concerns with the development of research aiming to assay the attitude and the preference construction of the consumers on GM food. We are assessing reactions, opinions, attitudes and feelings towards GM techniques on a representative sample of the population of Trentino region (Italy). Our goal is to find out which priorities and critical points are perceived by the Society when considering the risks and benefits associated to GM techniques. An *on-field* research is conducted in order to acquire more extensive knowledge of how people evaluate

the implications of genetic modification and to examine the factors that affect the final choice when GMOs are used in food processing. We adopted the Discrete Choice Methodology (DCM) approach, using hypothetical health, environment and price attributes [41, 42, 43] to evaluate the attitudes of potential GM food purchasers. This on-field research is expected to provide us with knowledge of the society's attitudes, priorities and interests with regard to agro-biotechnology applications. Finally, the results would offer insights about public understanding of risks and technologies that, in turn, will help us to define a strategic risk communication plan.

7. Conclusion

The work here presented is an original multidisciplinary research on the question of risk management for plant exogenous gene transfer techniques. The laboratory aspect is the central part of the overall project. Here, the attempt to exploit more sustainable practices for gene transfer into plants represent a conciliation effort between technology progress and attention to the society concern. In this view, the technical aspect has to be considered as a relevant communication practice. The cooperation of the other disciplines is an essential requisite. Social science would enable us to investigate the scientists' consciousness in the matter, while bioethics would focus on the responsibility of the subjects. Finally, the analysis of the perception of risk and innovation in the field of GMO applications would lead us to combine our scientific formation with an improved skill in the communication processes.

8. Acknowledgments

This research is supported by Autonomous Province of Trento, Project EcoGenEtic.Com (scientific coordinator, Lucia Martinelli).

9. References

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