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Biologies, Agricultures, Biotechnologies

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Abstract

The aim of this paper is to discuss the objectives of 'tailored' biotechnologies within the framework of the contemporary socio-economic context, taking into account the existence of a variety of agricultural practices in different environments and societies. For this purpose, a brief account of the history of human relationships with nature and the consequent changes of the 'spirit of the times' is offered, along with a discussion of the effects of such changes on research strategies at both basic and applied levels. A distinction is made between 'modern' and 'contemporary' biologies, showing that current genetically modified plants rely mainly on obsolete technology and a strategy coherent with 'modern' science. Present-day GMPs are thus to be considered a failure in terms of the number and quality of successful products on the market, particularly when compared to previous successes of traditional breeding, notably those in the FAO CGIAR centres leading the green revolution. However, present-day GMPs are viewed as the final product of a strategy that aims at the construction of 'optimal ideotypes' for a homogeneous agriculture completely controlled by industrialized countries. The concluding section is dedicated to a preliminary discussion of future prospects for research in agriculture. Models are proposed which take into account both developments in basic biology and also the need for drastic changes in the rules of world market regulations coherently with the need to help the existence of different agricultural practises aimed at maintaining agricultural variety in diverse socio economic and environmental contexts which exploits locally the richness of human cultural traditions.

Premise

The interactions of life science with the human world, unlike such interactions in respect of other scientific disciplines, are two-fold. On the one hand, knowledge gained from biology fosters the development of biotechnologies, while on the other, biological theories and ideas directly influence our conception of ourselves as living beings and become integrated into our cultures with obvious socio-political and even ideological consequences. The interaction is, however, by no means uni-directional, as conceptual paradigms prevalent in society are shared by scientists who tend to choose the living objects to be studied and the features to be analysed accordingly, often 'suggested' by the collective request for knowledge in specific fields. As discussed at length elsewhere (Buiatti 2004a; 2004b; 2004c) living systems, being 'multiverse', show apparently contradictory aspects, which, if observed separately, may be interpreted as antinomies, used as evidence for equally contadictory conceptions of life, and waved as banners of opposing ideologies.

It is not by chance that the history of the life sciences is punctuated by the rise and fall of 'paradigms' or, better, contrasting interpretations of data and observations, often biased by the fact that different facets of living systems are being observed in different periods of our history. As is shown and discussed by many authors (see, e.g., Cini, 1995), it is the changing 'spirit of time' ("ZeitGeist")which influences the choice of the 'pair of glasses' to be worn when observing nature. The dynamics of change in the spirit of time, largely depends on modifications of human society and economic structure and, more generally, on the relationships both between humans and between our species and the rest of the planet including other living beings.

Dealing with technologies and particularly bio-technologies, the branch which directly deals with the usage for production of modified living systems, it seems to me necessary to briefly discuss the 'spirit of the present time', in order to assess the 'state of the art' of the criteria informing our present day relations system. This in turn requires a glance back at the changing history of our ways of looking at and interacting with the external world

a) A brief history of human adaptation strategies

The human general adaptation strategy has been and is unique and very innovative, although based, as those of other living beings, on an explorative model (Gerhart & Kirschner, 1995). The success of explorative strategies (Buiatti & Buiatti, 2004; in press) as adopted by all living beings depends on the presence of a sufficient amount of 'benevolent disorder of life' (Buiatti, 2004), that is, of genetic and phenotypic variability and of fast and efficient

processes of choice from the most useful variants in different environments and times.

Bacteria and in general prokariotes use mutations as the main generator of variability due to their very short individual life cycles and consequent short exposure times to environmental changes, and to the haploid nature of their genomes, allowing immediate expression of alleles with a selective advantage. Eukaryotes, on the other hand, with their far higher DNA contents, long life cycles, diploid or polyploid genomes, have developed a number of genetic and epigenetic tools and processes leading to phenotypic variation. Plants, with their limited capacity to move in the search of favorable habitats, rely essentially on their somatic plasticity and can be considered as organized, genetically heterogeneous, populations of cells. Animals, however, move from one environment to another, often changing it through the construction of more or less complex niches.

Niche construction is also the basis of human adaptive behavior but the strategy followed to adapt the environment to ourselves is qualitatively different. This is essentially due to the quantitative/qualitative change in our nervous system endowed with surprising information storage and processing capacity. Our cortex contains about 100 billion neurons, with a potential of one million billion synaptic connexions, while our genome only contains 25,000 genes. This extraordinary potential allows us not only to store 'culture' and knowledge, but to organise it into 'inventions', that is, new projects eventually to be 'imprinted' into the environment to construct it according to our needs. The pace and features of this process have changed during the last 15,000 years or so as we have learned to abstract from nature our ideas of it, use these ideas to construct projects and then impose them onto nature.

The first clear evidence of our abstraction capacity can probably be considered to be the early examples of art, paintings in a humanized form of animals, humans and objects, as art can be regarded as the projection on external objects of simplified images created in our brain. At more or less the same time we were developing fairly sophisticated objects and dwellings. Certainly, the invention of agriculture can be considered an abrupt change in our behavior, when we chose to settle down and construct human 'agro-ecosystems', that is, highly sophisticated and organized niches in selected environments, with animals, plants, and microbial flora partially adapted to our needs. We should note, of course, that this was not a single, undifferentiated process: the birth of agriculture occured at different times in different areas of the planet, and there are also documented cases of reversion from agriculture to the earlier habits of collecting, hunting and fishing, like that of the American Indians of the 'pueblo' societies probably caused by climate changes (Buiatti, 2004).

The domestication and selection of domesticated plants and animals was carried out with the aim of improving their adaptation to the local humanized ecosystems, thus increasing but also stabilizing crop and animal production through the years. This allowed the differentiation of a number of forms of agriculture in different environments, along with an extraordinary variety of cultures, food products, habits, traditions, rites, religions, etc. Many plant and animal species were used at this early stage of development and the genetic variability within species must have been very high, this being a feature necessary for crop production stability in varying environmental conditions. Present day subsistence agricultures still mantain some of the features described, but the human strategy has been changing throughout the world at an exponentially increasing pace over the last three centuries.

The success of industrial revolutions has led us to believe that the context is essentially irrelevant for our survival and progress in living conditions as we can easily change it without any side effects. Progress has therefore been identified as a linear path leading to the optimization of our human-constructed context model, to be applied easily in any part of the world. From the second half of the last century, this line of thought has been extended from industry to agriculture, its principles being used to inform the green revolution. As a plant geneticist, I remember very well that in those times we were aiming at the obtainment of the 'optimal ideotype' for each crop, to be cultivated indifferently in developed or developing countries. In our minds, this was rendered possible whatever the context by supporting life and the production of plants and animals with energy, chemical and mechanical inputs.

A very generous effort was made by the world-wide human community under the direct coordination of the UN derived worldwide Food and Agriculture Organisation (F.A.O.) and its germplasm collection and selection centres. Such centres, all coordinated by CGIAR, were built in developing countries in the area of origin of some of the major crops (CYMMIT for cereals in Mexico, CIP for potatoes in Peru, IRRI for rice in the Philippines, etc). Large amounts of money were spent, in capacity building, germplasm collection, selection and free distribution, the development of chemical and mechanical industries, etc.

This unique effort has indeed produced good results in Asia and Latin America, increasing per capita food production. Africa, however, never has received the expected benefits, and, furthermore, growth in developing countries elsewhere has slowed down and showed signs of reversion since the mid-eighties of last century: The reasons for this long term failure are manifold. Africa never really had the industrial capacity and capital to support high yielding varieties with chemistry and energy, and at the same time lost its traditional agriculture. Allover the World, production costs per unit of production kept increasing while prices steadily decreased, due especially to soil erosion, biodiversity destruction and the high impact of chemical compounds on agro-ecosystems. Finally, in many countries the beginnings of mass migrations towards booming cities led to the present day 'monsters' with their huge reservoirs of urban poverty.

To be sure, this is not all to be attributed to an incorrect research strategy aiming at a 'universal' optimal genotype. Rather, it has been the extremist industrial ideology of the last century that led scientists to believe so firmly in the existence of such things as optimal genotypes, regardless of the specifics of agriculture practices and agricultural variety.

Recently the situation has been getting worse and worse for many reasons, most of which are economic rather than scientific. One of the most important factors has been the concentration of economic power in the fields of agriculture, food production and pharmaceuticals, or, in other words, in the whole spectrum of life-science related technologies. This is the reason for the crisis of CGIAR centres, which have been increasingly forced to turn to a private-like behavior due to the lack of funds and strong pressures from the private sector and developed World Governments, which see continued research in agriculture as a tool for the control of the markets of developing countries.

An emblematic related change is the rush to patenting as *a tool for research and universitiy funding* and the creation of a single, 'open' market more and more diverted from the production of goods to the obtainment of intellectual products to be patented. Patent production is in fact becoming the alternative to public funding for many institutions and has been proposed also to CGIAR Centres at a moment when the national administrations of industrialized countries and particularly the U.S.A. have been withdrawing support.

Now, while it is understandable that the 'patent rush' may attract universities in the industrialized world it would be very negative for the improvement of access to food if adopted by centres whose mission has always been to hand over for free to developing countries cultivatars and techniques useful for the improvement of their agricultures.

The most striking example of the difficulty developing countries have developing their own cultivars comes from the biotechnological sector. In this case, as every molecular biologist knows, the development of a product through genetic engineering requires the sequential usage of a large number of techniques and molecules, from DNA extraction to the construction of vectors transformation, the analysis and selection of transgenic plants, etc. All the 'fragments' of technology can be and are patented, leading sometimes to a wholly virtual process in which entire patented inventions are developed through the usage of earlier patented steps, which results in more patents, but not necessarily in new successful products.

As the private concentration process gains pace, more and more patents are in the hands of the few who either already have or can afford to buy the tools and methods needed to proceed. It seems to me that we are, in this case as in others, rapidly proceeding towards an ever more dangerous abstraction from material reality, potentially leading to a world where the object of production is a tool to increase revenues rather than a material product to be used for the many facets of human well-being. This process, it should be noted, is by no means limited to biotechnology, being present also, for example, in informatics and nano-technology. It is not by chance that one of the first products of this latest technology to enter the market is self-cleaning clothes, a completely useless but apparently 'very fancy' object actively advertised throughout the world.

At this point of this paper many readers may be asking themselves why I am discussing at such length these problems, apparently not directly relevant to the purpose of 'tailoring biotechnologies'. The reason is, however, very simple. Present-day biotechnology applied to agriculture offers probably the best and sometimes most extreme example of abstraction from reality, a process which is the opposite of tailoring biotechnologies, this funded on the assumption of the existence in the real world of different agro-ecosystems which form the material, social and economic perspectives. The scientific background of such abstraction can be found in the very foundations of biotechnology, and

particularly in the implicit assumption that living beings, including humans, are machines which can be changed at will to follow human projects, independently from the context and without any 'unintended' effects derived from internal or external 'reactions' to such changes. The mechanical nature of living beings so conceived was in fact the prevalent position of life-sciences, fully coherent with the spirit of the times when the first biotechnological pro ducts were developed. I will now try to support this, taking examples from the history and state of the art of genetically engineered plants, and trying to find in them a few elements for a future change.

b) The conflict between modern and contemporary biology.

Genetic engineering has until now been a technology based on the following principles:

a) The phenotype of an organism is essentially based on the action of genes, meaning by this term coined by W. Bateson in 1906, the coding part of the genome.
b) Genes are not ambiguous in the sense that only one protein, with an aminoacid sequence unambiguously corresponding to the string of nucleotides in DNA, is synthesized.
c) Genes are independent from one another in the Mendelian sense, that is, their expression is not influenced by and does not influence other genes. In other words there is not such a thing as a gene network.
d) Natural and social environments do not have to be taken into account, or can anyway be considered as wholly predictable.

If these principles were true, a genetically engineered microrganism, plant, animal, could be considered as a wholly predictable machine to which a new 'piece' has been added without any unintended effect, according to a project developed by biotechnologists. Genetic engineering therefore could take genes with known and useful functions from one organism and insert them into an already existing one, possibly phylogenetically very different, to be optimized and utilized in agricultures regardless of the context. This new breeding procedure being predictable, it could introduce new functions and be much faster and safer than traditional, intra-specific and inter-specific crossing, which needs a long, tedious and expensive series of generations of selection to achieve good results. Seen this way, the basic aim was again, as in the green revolution, to obtain an optimal ideotype, now, however,using the powerful tools offered by molecular biology.

It should be recalled that genetic engineering of both plants and animals started as an entirely new branch of biotechnology in 1981, a time when very few scientists would disagree with the aforementioned principles coherent with a mechanistic conception of life. It was the time when what I call the 'industrialist utopia' of the production of a single, optimal model of life seemed to be on the verge of realization. Even in the field of life sciences, success seemed to be very near, as the 'green revolution' really had reached some very important objectives allowing for a very optimistic view of the future. It is not surprising then, that the name given to the new bio-technology was 'genetic engineering', implicitly stating the equivalence of living beings to machines, able to be planned and 'constructed' without unintended effects.

The general mechanistic feeling was not modified when the mice carrying a rat or a human growth hormone gene were found to have very short and unhappy lives, or when plants 'engineered' with a bacterial gene (Bt) did not express it properly and required a decade of further research to become productive. Even now, in the year 2005, most genetic engineers seem still to be thinking in the same way, despite the fact that our knowledge of genome functions and dynamics is wholly changed and the relevance of the socio-economic and environmental context to the success of plant and animal cultivars and breeds is widely accepted. So, while molecular biology on the one hand and social and economic sciences on the other are fast progressing, genetic engineers seem to be removed from the new knowledge, tending to limit themselves to an updated and strenuous defense of their products and the old 'modern' conception.

As often happens in science, the concepts of contemporary biology are not completely new, having been proposed several times in the past following a line of thought which assigns relevant roles to genetic interactions, the modulating effect of the environment, regulation processes, a series of influences on genotype expression and a series of uninherited changes in DNA molecules - processes now assembled under the term 'epigenetics'. Leading names in this field have been Sewall Wright, one of the founders of the 'modern synthesis' of evolution, Conrad Hal Waddington, calling in the fifties for a new 'phenotype paradigm', Barbara M Clintock, the discoverer of DNA mobile elements, and I.M. Lerner and R. Lewontin, working on the advantage of heterozygotes and gene interactions and many others.

These precursors were generally, however, a relevant but seldom considered minority, until the nineties when the first DNA sequences were deciphered and then whole genomes sequenced and their dynamics analyzed.

The results of this have been astonishing and the whole vision of biological systems changed as I have discussed at length elsewhere (Marcello and Marco Buiatti, 2001;Marco Buiatti and Marcello Buiatti 2004; M.Buiatti, 2004 b). Let us summarize some of the new concepts relevant to the subject of the present paper:

1) Eukaryotic genomes are endowed with relatively few classical genes (coding sequences) and large numbers of non-coding ones. The classical example is given by the human genome which bears only 25,000 genes covering just 1.4% of the genome. The situation is similar in plants, with the exception of *Arabidopsis* where coding sequences reach 30%.

2) Non-coding sequences are not, as previously thought, 'junk DNA', but are involved in a large number of regulatory functions. In particularly, sequences upstream of the genes, introns within them and downstream DNA, all have specific local conformations needed to form complexes, with signal proteins representing the last step of long and complex signal transduction chains. These start from the membrane where external conditions and agents are perceived. Gene activation, repression and modulation do, therefore, occur according to signals perceived and represent an 'active' homeostatic (Waddington rightly calls them 'homeorrhetic') continous resetting and repair of the system itself.

3) Genes are 'ambiguous' in the sense that the information contained in one gene may be used for the synthesis of more than one polypeptide. 'Ambiguity' derives from differential start and termination transcription sites yielding more than one RNA, and from 'alternative' splicing of the primary transcript giving rise to more than one mature RNA. Furthermore, it has recently been discovered (Fantom Consortium and Riken Genome Exploration Research Group, 2005) that in many cases not only 'sense' but also 'antisense' RNAs can be produced by the same gene, the second one inhibiting translation when needed.

These ambiguity processes are very common and allow, for instance, one gene in *Drosophila* to produce 38,000 different polypeptides and three genes in humans to give rise to more than 2,000 different forms of neurexins, a class of proteins very important for brain functioning. Ambiguity, moreover, increases through post-transcriptional 'editing' of RNAs, post-translational modifications of proteins, alternative usage of different conformations of the same protein, formation of different complexes with different functions according to the context. In all these cases the 'choice' of the part of potential variability to be used depends on recognition and complex formation events very often triggered by a wide range of signals.

4) Semi-permanent modifications of gene regulation occur frequently and are in several cases transmitted to subsequent generations. One of these processes involves differential replication of specific DNA sequences which, when incorporated into chromosomes, become inherited, resulting in copy number variation between individuals. This process was discovered by our group (Parenti, 1973) and has since been shown to be a very frequent phenomenon with phenotypic consequences (Buiatti, 1977; Cullis 2005).

DNA methylation, leading to semi-permanent gene expression inhibition, besides being extremely frequent and in many cases transmitted (genome imprinting), is one of the tools plant and animal cells use to avoid unwanted increases in gene expression also deriving from the integration of one or more copies of homologous sequences (co-suppression, Bartel and Bartel, 2002). Methylation is often mediated by a particular class of small RNAs (RNAi) and other RNAs, as antisense RNAs are powerful tools either for the suppression of transcription or in RNA degradation (Fantom Consortium and Riken GERG, 2005). Finally, DNA sequences can be spontaneously rearranged, particularly when artificially inserted into chromosomes .

5) Genes are now known to be interconnected in networks, as required in development and metabolism organisation (Papin et al., 2003). Living system network structures follow a scale-free rule and are organised into modules, for which reason they are highly resistant to random noise but easily disrupted when relevant nodes with many links are hit. Throughout networks, quantitative fluctuations are allowed but only below specific thresholds, over which the network itself reacts, either with homeostatic 'measures' or, if these are insufficient to re-establish equilibrium, collapse. Many kinds of living networks have been compared in a number of studies, on subjects ranging from proteins to ecosystems to networks of actors working in the same movies, and all have been shown to comply with these same general rules. In all cases, and this is very relevant for our subject, any 'invader' element either establishes

connections with components of the invaded network or it is 'refused' (this meaning the suppression of the invader or the collapse of the system).

One interesting example of such processes in a genetic system has been thorougly studied in our laboratory: the analysis of the evolutionary effects of a natural case of genetic engineering in the genus Nicotiana (Bogani, 2000; Intrieri, 2002). The natural 'genetic engineer' involved is *Agrobacterium rhizogenes* (a 'cousin' of *Agrobacterium tumefaciens*) which transferred its genetic complement (the 'rol' genes) in many *Nicotiana* species a long time ago. As rol genes have a relevant effect on plant hormonal equilibria, their integration presumably altered the physiological phenotype of Nicotiana spp. After a study of the evolution of physiological parameters in these species, we analysed the presence/absence of rol genes and their effect on physiology. It was thus shown that during the evolution, some Nicotiana spp. had conserved the whole rol complement, others had 'refused' it and a third group had only maintained some genes, discarding the others.

The conserved genes moreover, had been 'adapted' to the receiving genome as shown by their phylogenetic tree, coincident with Nicotiana evolution but very different from the phylogenesis of the same genes in present day Agrobacterium. A relevant side effect of this was the division of the genus into two groups, one containing rol genes which shift the hormonal equilibrium towards high cytokinin levels, the other without rol genes diverting it towards anxins. The interesting fact was that a physiological barrier to interspecific hybrid formation was thus constructed as crosses between species belonging to the two groups would form the genetically tumorous hybrids described by Kostoff in 1930 endowed with a very low fertility level. I am citing this example here because it is, to my knowledge, the only work dealing with the physiology- (phenotype-) mediated evolutionary effects induced by horizontal transfer with a mechanism identical to modern artificial genetic engineering, documenting natural (evolutionary) changes in genetic, physiological and phenotypic behavior.

Now, taken together as a whole, the concepts summarized above (points 1-5) design the main features of contemporary biology, offering us a completely different view of biological systems from that prevalent when genetic engineering began. This has led to a failure of that branch of biotechnology, derived from the somewhat surprising stubborness in sticking to the old, outdated science and its methods. Let us then rapidly describe the state of the art in this field, in order to open the way to some suggestions for the future.

c) Genetic engineering: the state of the art

I use here the phrase 'genetic engineering' to stress again that this branch of biotechnology is qualitatively different from the other methods of plant breeding, included those employing molecular biology based techniques. The rationale of genetic engineering is to improve plant genotypes through the addition of a new sequence or set of sequences bearing the information for functions entirely new for the host. Consequently, the vast majority of experimental and commercialised transgenic plants and animals derive from the insertion of bacterial or human genes. In traditional breeding, on the other hand, crosses involve the exchange of allales, not genes, and therefore the resulting genotypes are new combinations of variants (alleles) of the same genes coding for already existing functions. Even mutagenesis, carried out with chemicals, and ionizing radiations, UV, produce mutations which can also occur spontaneously at higher frequencies.

Interspecific hybridization, a technique used for the first time in the twenties, is based on crosses between interfertile, closely-related species bearing very similar genomes. It is interesting to recall that this technique was cheered by the then president of the USA, Franklin D. Roosevelt, in a famous speech in 1946 whose prophecy was that the new species derived from the crosses would solve the world famine problem. Unfortunately this prophecy has been proved wrong, because although derived from closely-related species, the new crops were not found to be productive and only a few of them gave reasonable results, even after decades of selection. To be more precise, just one species, the Triticale, a hybrid between wheat and rye, is still cultivated and used.

Similar prophecies are frequent in the mass media these days in relation to genetically engineered plants. It seems to me that, unfortunately again, they will not be fulfilled. Although 81 million of hectares (5% of total arable land) are now said by the ISAA to be cultivated with transgenic maise, soya beans, canola and cotton, the results are much below what had been expected and the plants obtained sofar certainly will not contribute in a significant way to the solution of the famine problem. In over twenty years of research carried out with considerable investment by thousands of groups in many countries, only two new characters (resistance to herbicides and to insects) have been inserted into only four species, yielding a very limited number of productive cultivars.

If we compare this branch of biotechnology with any other technology, from informatics to electronics to all energy-related technologies, etc., the failure is manifest, both in terms of number and real value of innovations. The same is true when the comparison made is between present-day PGMs, on the one hand, and the extraordinary number of useful varieties produced by CGIAR laboratories and the other participants in the green revolution, on the other. Things are no better if we consider the quality of the impact of genetically engineered products on the lives of the poor in developing countries. It is very well known that the vast cultivations of soyabeans in Argentina and Brazil are for export and that their presence, particularly in Argentina, has had devastating effects on the local agriculture - not to speak, of course, of the detrimental effects on the environment of the utilization of vast areas for the cultivation of a single crop, soyabean, which will not be utilized by Brazilians and has no relationship with local agricultures, instead dangerously accelerating the pace of deforestation and destruction of the land.

The somewhat unbelievable fact is that, far from acknowledging the present failure of genetically modified plants (not of genetic engineering per se'), producers and the mass media exert all possible pressures to convince reluctant people and states, that these products will alleviate famine and positively change the living conditions of the poor. This extraordinary effort has, in fact, a negative impact on genetic engineering itself, as it diverts public and private research from updating their knowledge-derived, technique-obtaining innovative ideas which take into account what we know now, and leaving to their fate the two extant products both based on the biology of the eighties. It would be sufficient, as a start of a re-moulding process, to understand why none of the vast number of laboratory-produced GMPs has had success in production. It would then be clear that the basic reason lies primarily in the unpredictable effects of organism reactions to transformation with genes which have not coevolved with the host network, as well as in the lack of precision of transformation procedures.

To avoid making statements without supporting data, I will here briefly summarize some recent evidence of the 'unintended effects' of genetic engineering:

a) There is as yet no way to control the place of insertion of trangenes nor their number of copies, as shown by Table 1 where ranges of copy numbers found in experiments using the biolistic or *Agrobacterium*-based transformation procedures are reported. It is worth noting the differences between the data by Shou *et al,* (2004) and the others. The reason for the high values observed in the first experiment, particularly in the case of biolistic transformation, is that in that case different detection methods were used, methods endowed with a much higher resolution power than the traditional 'Southern' analysis, which only detects whole transgene copies inserted at different sites. Unfortunately, the low resolution power method is used in the requests of permits of commercialization by the most significant companies. Very little is known about insertion sites of all these copies, a not irrelevant problem if we consider the fact that the transgene, when inserted, may inactivate the host sequence or change expression levels. Finally, changes in copy number do change expression levels in an unpredictable way, higher numbers not necessarily meaning high expression level, due to co-suppression-like effects.

b) The improvement of detection and sequencing techniques allows a much more refined analysis of the fate of transgene contructs once they have been introduced into their host plants. The results are astonishing. Practically always, transgene containing constructs are re-arranged due to large insertions and deletions, fragmentation leading to the presence of several fragments scattered throughout the host genome, recombination with the consequent formation of chimaeric stretches containing sequences of the host genome, and inclusion of vector fragments. Moreover, this newly discovered variability in construct organisation has not necessarily been selected against in commercial PGMs, as selection is generally directed to the obtainment of homogeneous plants so far as the desired character is concerned. The result of this practice is the presence of unwanted, re-arranged sequences generally not described in the original patent or in the dossiers prepared for commercialization permits (Table 2).

c) Probably the most relevant unintended effects of transgene insertion observed to date are due to the interaction with the metabolic network and, through that, with phenotype development. As shown by the few examples reported in Table 3, in most cases the change in one metabolic step induces a chain of unintended modifications difficult to control, which seem to be much more general than expected even two or three years ago, explain

whyo many transgenic plants never moved out of the laboratory or the experimental field, or, on the occasions when they did, could not meet markets demands in a reasonable way. The most recent famous episode has been that of the so-called 'golden rice', subject for several years of a worldwide promoting campaign based on its nutritional advantages, but which was withdrawn when the level of expression of the transgene was found to be too low. The lack of unintended effects, incidently, also explains why BT or herbicide resistant plants are productive and a commercial success, because in these cases the genes introduced do not interact with the host metabolism. To be more precise, this has been true only for the second character because in the case of Bt the bacterial gene sequence had to be changed to make it 'readable' at high levels by the protein synthetic apparatus of the plant.

Surprisingly, the presence of unintended effects, of sequence instability induced by transformation and the many failures of such a large number of products are generally not admitted by researchers and producers. To better control the dynamics of genetic engineering is not at all popular. Moreand more varieties are being transformed with the same old genes or variants of them and the rationale of the process has remained the same. It is to me amazing that in such a situation the principle of 'one optimal plant' for all environmental, social and economic conditions is applied to the two GMPs on the markets as if they were still the only solution to all problems. It seems to me that it is about time to start using the fast increasing biological knowledge in a different way, aiming at a "contemporary" 'tailored biotechnology' which, for the time being at least, has to be 'non invasive', i.e. not based on genetic engineering, a practice of possibly future, but still, as yet, not mature. Much better results may be obtained when the new knowledge is incorporated into the daily practice of plant transformation.

d) Future prospects

As suggested above, the GMP story is fully representative of the level of alienation of present human society. Immense efforts are devoted to the defense and diffusion of two kinds of products representing the only results of an obsolete technology never updated and modified in line with the progress of science. The existence of these products has contributed to relevant changes in the very rules of world markets, enabling the process of life patenting and becoming the banner, supported by heavy investment and dumping policies, of the movement towards a putatively homogeneous, 'optimal' project, of which the two products are supposed to be a basic component. Their failure in changing human life in a positive way is proving the failure of the general project of homogenization and the need to start again, to take into account the dynamical processes of living systems (human and non-human), the overall need of plasticity and diversity for adaptation, and the interactive nature of living networks at all levels of the hierarchical organisation of life.

The new biology, along with all other natural sciences, can play an important role in offering a better understanding of the dynamical processes needed to increase our prediction capacity and support with rightly-tailored inventions the renewal of our original adaptation strategy, thus abandoning the temptation to impose a single, man-made model of the planet. Of course science and the derived technologies can offer the tools for the needed change only if the spirit of the times and the leading socio-economic forces allow and request it. As a part of this process, scientists and bio-technologists must change their general attitudes and stop resisting, anchored as they are to the old data, and implement better channels between basic research and its applications. It is also necessary for both these categories of researchers to start taking into account the relevance of the interactions between their products, the local communities and the general rules of the market. One particularly pertinent condition for a positive change in local, diversified, often poor agriculture is a less aggressive behavior on the part of private companies still pushing hard genetically engineered crops of very little value to the poor, aided by dumping policies. Moreover, the principle of food sovereignity must be implemented, particularly if we want local populations to start mastering and using new molecular tools and techniques for the conservation and improvement of their crops. The concept of food sovereignity is at the basis of a vast movement for the right of local communities to conserve, improve, exchange and eventuall-ly sell their reserves of genetic variability, not necessarily aiming at the optimal ideotype to be spread in different contexts. Representatives of many of those communities are now connected in a network whose principles may be summarised by the following statement written at a global meeting in 2002: 'Food sovereignity is a solid alternative to the current mainstream thinking on food production.The struggle for food sovereignity incorporates such wide ranging issues as land reform, territorialcontrol, local markets, biodiversity, autonomy, cooperation, debt, health, and many other issues that are of central importance to be able to produce food locally'. In this sense, the positive part of the green revolution practice, that is, the free usage of methodological and

technical innovations by local populations, who would be thus able to adapt them to their strategies of improvement of crops and develop through them innovative agricultural practices coherent with their context, should be resumed along with a new version of participatory breeding.

Within this framework it is necessary to direct selection towards characters suggested by local farmers, but not sufficient if this means maintaining the concept of the 'optimal ideotype' putatively positive in all environments and for all societies. For this purpose, a long-term battle has to be fought to improve the autonomy of CGIAR Centres, avoiding the temptation to transform them into private companies often connected to powerful, multinational companies. At the same time, intellectual property rules have to be changed, or at least contain exploitable exceptions, allowing developing countries to build their own technologies according to the needs of the farmers and consumers. In other words, while changing the science behind it, the old model has to be changed through capacity and autonomy building aimed at a situation in which local farmers and breeders not only participate in the conservation and selection work, but also direct it, in connection with their local traditions. In all this, the careful analysis of farmers' poverty levels must always be kept in mind, thus avoiding the procurement of very productive homogeneous cultivars needing an inaffordable input of chemicals and energy. One should remember the result of a 1998 poll conducted by IPGRI on farmers living in Oaxaca Mexico. When asked what kind of variety would be better for them, the women answered, 'a cultivar which gives us some harvest every year'. The reason for this answer is very simple: in subsistence agriculture the aim is not to win competition in the market with local products, but to have enough of them for direct food consumption.

These general themes lead to some more technical considerations:

a) The response of the Oaxaca women immediately brings to my mind the following: to be productive every year, in the absence of heavy and costly-chemicals and energetic inputs, cultivars should not be homogeneous but rather more similar to genetically heterogeneous populations capable of production in an ever-changing environment. This points to the relevance-of new research at this level aiming at the maintainance of intra-cultivar diversity, especially for characters which help the plant to adapt to different environments. Particularly, resistance to different biotic and abiotic stresses has to be present in cultivars and land races, and different alleles of the required genes should be subject to introduction if not already contained, in the germplasm, and, whenever possible, maintained with stabilyzing selection. At the same time, intraspecific variability should be conserved in the case of quality characters of the plants and the derived food. This is relevant to the maintenance and continuous valorisation of local diversified traditions, and necessary to avoid migration and disruption of local communities, allowing them also to attribute to their products a possible added market and export value. Seen this way, diversity will be conserved as a valuable character per se and international public agencies canwork for its exploitation, thereby also improving qualified channels for commercialisation.

b) Biodiversity conservation should have regard for not only intraspecific butalso interspecific variability, that is crops utilized in small amounts (underutilized) but liable to be relevant for their nutritional value and/or for the different foods produced through their use. Europe already has a long experience in this field, particularly in its southern nations where old species have been reproduced, valorized and now take a significant share of the market, particularly for the good quality and taste of the derived foods.

c) The characters to be selected for as a consequence of points raised in a) and b) may be those which contribute to the improvement of a low cost agriculture based mainly on local resources carefully improved from characters also 'imported' from elsewhere, with good nutritional quality, and, whenever possible, coherent with traditions particularly regarding food products to be valorized. This approach implies strong investment in research aimed at the identification of genes for such characters as: response to stress, symbiosis with nitrogen fixing and other useful microorganisms, high absorption and utilization efficiency of organic andinorganic soil components, high photosynthetic rate, synthesis of vitaminsand other components with high nutritional value, increased amounts of compounds improving quality and taste of traditional food, etc. As stated before, final character choice is to be in the hands of local farmers once adequate general choices of methods have been made by breeders.

d) Obviously, high level molecular tools and methods are to be employed forthe study of the mechanisms of genetic control of the characters in c). Molecular and biochemical research should be integrated with the modelling of networks and their response to environmental changes. At the phenotypic level, this could be implemented by studies of biochemical correlations and their genetic control. This work in turn will allow the development of very refined, assisted breeding methods based on easy-to-handle, low cost, biochemical and molecular markers of biodiversity, particularlyfunctional markers, analyzing and monitoring variability in coding and non-coding sequences of genes relevant for production.

e) Research in d) may eventually be useful in the future to develop new metods of genetic engineering with modulated and low disturbing effects at alllevels of living networks, including the social and economic. One of the approaches to be tested may be the introduction of improved alleles fromcross-fertile, closely-related species through transformation, provided thata good control of site-insertion, copy number, re-arrangements, etc. has been achieved.

f) A favourable interaction with the social and economic environment can only be reached, however, if new, local and global, intellectual property laws are developed considering clear exceptions for the usage of up-to-date techniques and tools by public- or community-led applications, thus avoiding any increase in cost to farmers and researchers due to royalty fees. At the same time, old and new cultivars and species, methods and tools have to be preserved from patenting by third parties, through immediate publication and public description of the innovations and their putative industrial usage.

Table 1 : Some examples of inserted transgene copy numbers

Plant	**Transformation process**	**N° copies**	**References**
Oryza sativa	Agrobacterium	In 54%, more than one	Kim et al, 2003
Populus tremula	Agrobacterium	1 - 3	Kumar and Fladung, 2000
Triticum aestivum	Agrobacterium	In 87%, more than one (1 - 3)	Cheng et al, 1997
Oryza sativa	Biolistic	In 33%, 2 copies	Fu et al, 2000
Saccharum spp.	Biolistic	3 -10	Butterfield et al, 2002
Avena sativa	Biolistic	1 -10	Pawlowsky and Somers, 1998
Avena sativa	Biolistic	2	Makarevitch et al, 2003
Triticum aestivum	Biolistic	1 - 5	Srivastava et al, 1996
Zea mays	Biolistic	2 - 277	Shou et al, 2004
Zea mays	Agrobacterium	1 - 10	Shou et al, 2004

Table 2 : Some examples of unintended changes in commercial cultivars

Trangenic cultivar	Unwanted changes	Flanking sequences
Cotton LL	polylinker	n.d.
Potato NewLeaf RBITT 22-82	Plasmid sequences	n.d.
Pumpkin C20J-3	Marker gene	n.d.
Mais MON863	Marker gene and a fragment of the ble gene	n.d.
Mais-Mon 810	Truncated Cry, deletion of Nos	Re-arrangement during insertion
Papaya 63-1 and 53-1	Marker gene, plasmid sequences, re-arrangement	n.d.
Soya RR 40-3-2	Transgene fragments and unknown DNA	Deletions and re-arrangment at insertion site

Table 3 : A few examples of unexpected pleiotropic effects of transgene insertions

Transgene	Effects
Ectopic Invertase,Tobacco	Hexose increase, inhibition of photosynthesis virus resistance, salt resistance
Ectopic Invertase, Potato	Hexose increase, aromatic aminoacid increase, ornhitine production, more new metabolites
Ectopic Invertase, Vicia	Hexose increase, starch reduction, shrunken seeds, sucrose synthase inhibition, ADP-glucose pirophosphorilase inhibition
E2Fd-DPA over expression, Arabidopsis	Ectopic cell division, chromosome endoreduplication, early growth arrest, cell wall synthesis inhibition, nitrate assimilation inhibition
Antisense hexogalatturonase,Tomato	Variation in citric acid and lycopene
Asialol/nerolidolsynthase, Arabidopsis	Growth reduction
Phytoene- synthase, Arabidopsis	Increased beta-carotene, luteine, violaxanthine, chlorophyll, lycopene,ABA, germination delay
Contitutive phytoene-synthase, tobacco	Increased carotenoids, dwarf plants, chlorosis
Membrane H+ ATPase co-suppression, Tobacco	Reduced sucrose translocation, inhibited stone opening, growth, male fertility

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