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The Brazilian Agricultural Revolution in the XXI Century-Changing Paradigms in Biotechnology

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How Brazil will feed the world

The late Norman Borlaug visited Brazil some years before he passed away and said in a press release: "the next revolution in agriculture will happen in Brazil". Brazil multiplied its food production by four within fifty years. More important, this was done without substantial increase in acreage. Plant breeding, performed by a dozen of good geneticists, has continuously built cultivars to feed our seed industry. High-tech seeds and low-tech farming practices resulted in Brazil from seed laws and plant breeders. Agriculture in Brazil is competitive because it is cheap for the farmer. The country is ready to take this new revolution worldwide. This article brings to context the fact that we have to aggregate value to the products of agribusiness and that must be done with biotechnology and will require to change paradigms. Examples are expressing genes in plants and in the milk of mammalian instead in CHO cells. Biology today converged to allow that vaccine antigens be engineered in plants. I did nineteen contributions to the blog of Nature/Trade Secrets dealing with these subjects (http://www.blogs.nature.com/tradesecrets/author/lbarreto). In the nineteen eighties, the best Brazilian biotechnology scientist visited Europe. The expectation was then that we could partner with the best Biotech groups in Europe .We were so behind that we came back to Brazil frustrated. Thirty years later, we repeated the same visit only including South American scientists. We caught up to a point that now we can offer excellent opportunities to both sides. Biotech now is entangled with the agribusiness and there is competence in both areas in Brazil. We will try to demonstrate this in this paper.

A long analysis on food production, published by Nature [1], stated that to feed the world`s population in 2050 will be possible. The challenge will be the cost to be charged for high-tech seeds and low-tech farming practices. This statement does not identify who will have the task to exercise the strategy and produce enough food for the planet. A recent Science Editorial [2] quoted a statement of the Food and Agriculture Organization saying that: the challenge of "lifting a billion of people out of poverty and feeding an extra 2.3 billion by 2050 will require increasing cereal production by 70%, which is equivalent to "doubling the output of developing countries". The two statements are somewhat controversial, Brazil feels committed with what Uma Lele said in the Science Editorial. Jeff Tollefson, in one of the articles reported by Nature titled "The Global Farm" while emphasizing the growing role of Brazil in the context of food production questioned if Brazil can continue to make agricultural gains without destroying the Amazon. The

Economist [3] drew attention to "The miracle of the cerrado": the acid soil savannah conquered for agriculture in Brazil. Scientifically, there is need for consistent answers to these often-controversial questions. For Brazil to be a key actor to feed the world, it must gradually reduce the pressure over two important biomes: the Amazon and the Cerrado. During the past decades, Brazil has destroyed an area of the Amazon rainforest as big as Germany [4]. The Cerrado, we found fifty years ago when Brasilia became the capital of Brazil, has only 17% of its original vegetation left [5]. Can Brazil expand agriculture without destroying the Amazon and the Cerrado? The answer is Brazil can expand its agricultural frontier while gradually reducing deforestation in the Amazon and preserving what is left in the Cerrado. We will describe the components of this revolution in progress in Brazil to demonstrate that Brazil will be a key actor to feed the world in the coming decades, substantially supporting our arguments with the best science available. The cost of the Amazonian reforestation is extremely expensive [6]. Recovering of degraded pastures has a lower cost of approximately 800 hundred dollars per hectare. Recovering degraded pasture in the Amazon for agricultural purposes is not financially feasible or environmentally sustainable. The strategy is to increase grain production in the Cerrado, in areas, which were degraded by over grazing. Degraded pasture exists in several regions in Brazil and may reach 200 million hectares [7]. The state of Parana has more than 1.6 million hectares. The Cerrado, which totalizes 2 million Km^2 or 200 million hectares, has about 100 million hectares of degraded pasture. This huge degraded area results from a long-lasting absence of public policies for its adequate use. Recently, actions towards the sustainable use of the Cerrado have been carried out to mitigate losses of biodiversity, such as Pro Centro-Oeste and Comcerrado Networks [8]. Brazil produces almost 200 million tons of grains on 50 million hectares. It will take at least 100 million hectares to accomplish the task of doubling grain production. Doubling the area for soybean is possible. It was done in the past but will require an additional 23 million hectares. (Romeu Kihl, personal communication). Corn will require an additional 13 million hectares [9]. The area utilized for sugarcane, if tripled, will add an additional 16 million hectares for the production of bioethanol/sugar [10]. We can propose an answer to the Nature articles: the amount of area or the cost of its degraded recovery will not be a limiting factor. The limiting factors will be first the reforestation cost in the Amazon, and second a science-based effort focusing on major constraints for agricultural development in the tropics in order to provide high-tech seed and low-tech farming methods, whereas both are required. In fifty years, Brazil multiplied its food production by four. More importantly, this was done without substantial increase in acreage. This successful story started in 1965, when Brazil established the first Law to regulate the commercialization of seeds. Without this Law, the seed industry would never have prospered in Brazil. Plant breeding, performed by a dozen of good geneticists, has continuously built the cultivars to feed this seed industry. In Brazil, high-tech seeds and low-tech farming practices were offered because of seed laws and plant breeders. Agriculture in Brazil is competitive because it is cheap for the farmer. Ever since, the Brazilian Enterprise for Agricultural Research (EMBRAPA) was created in the mid-seventies, Brazil began training plant cell, molecular, and developmental biologists. They were mostly gathered at The National Research Center for Genetic Resources and Biotechnology (CENARGEN), and have helped plant geneticists and plant breeders to develop the best cultivars for the tropics. When genes were not available, partnerships were arranged with gene companies. As a proof of concept, EMBRAPA scientists will release and make available to Africa, if required, the first green beans engineered to become resistant to the Golden Mosaic Virus, using RNA interference technology [11,12] (Figure 1), that gave the Nobel Prize in medicine to Craig Mello and Andrew Fire in 2005. Few countries made use of the gene revolution, particularly when this revolution is related to developmental biology, to benefit putative advances for agriculture as we did in Brazil. Scientists at CENARGEN aware of the fact that a great number of genes, in particular constitutive genes which are common to most species, share common sequences. These common sequences may be as they call them "encrypted" in a protein that has a completely different function than the function of the "encrypted" sequence and as such the sequence is impeded to function as the common sequence. The fact however that common sequences are found in distant species phylogenetically speaking open the possibility to use these sequences (once separated from the protein where it is "encrypted") to play the same function of the sequence of the distant species I will exemplify. We find sequences in frogs which function as dermaseptin antifungic molecules and we find the same sequences in soy bean. We mobilized the "encrypted" sequence in the soy gene without transferring the gene from the frog to the soybean. We call this intrageny [13,14] and transfering the gene from the frog would be transgeny. Our definition of intrageny may differ from the definition of others [15]. Since one of the major concerns of the general public about transgenic crops relates to the mixing of genetic materials between species that cannot hybridize by natural means, two transformation concepts cisgenesis and intragenesis were developed as alternatives to transgenesis. Both concepts imply that plants must only be transformed with genetic material derived from the species itself or from closely related species capable of sexual hybridization. The goal of our concept of intrageny is to identify in genomes of the plant of interest sequences matching functionally with sequences of other plants, even if these plants cannot hybridize with each other by natural means because they are phylogenetically very distant. This fate turns transgenesis unnecessary since the function of the sequences of the two species is common. EMBRAPA is today the largest institution of its kind with offices in the five Continents, and Brazil is ranked second in the world biotech crop production. In the same vein, science has multiplied six folds in Brazil since the seventies, and the Brazilian scientific output has risen to 2.64% of the world scientific output [16]. In addition, science in Brazil is growing at a higher rate than most developed countries [17]. Funds for molecular biology, applied to plant genetic engineering and consequently to plant breeding, have substantially improved, since the Nineties. Furthermore, Sectoral Funds, promulgated by law, were made available for areas such as Biotechnology and Agribusiness [18]. Finally, Brazil has now adopted the most ambitious program to train young scientists abroad. Brazil offered, in the past 3000 to 5000 scholarships a year to train students in foreign countries. Now, we intend to fund more than 100000 scholarships over a four-year period under the "Science Without Frontiers" scheme. However, to face the challenge of Uma Lele´s Science Editorial will require the best science in the world. The gene revolution has produced plants that can defend themselves from insects and viruses but it has not advanced much with respect to resistance to bacteria and fungi. Our visit to Rainer Fischer has demonstrated that genetically modified cereal resistant to fungi is available at the Fraunhofer Institute in Germany. Plants resistant to drought are been engineered by Syngenta, Dupont, and Monsanto for corn in temperate climates. To obtain corn resistant to drought in the tropics particularly in the Semi-Arid regions of the world we need facultative CAM (Crassulacean Acid metabolism) plants. One example is *Portulaca oleraceae*. It operates as a C4 plant when water is available but switches to CAM under water stress, e.g. closes stomata during the day and opens stomata at night to fix CO_2 as malate that is stored in vacuoles. It then uses malate as a C4 plant during the day, when ATP is provided by the light mechanism of Photosynthesis, releasing CO_2, and using the C3 molecule pyruvate in the Calvin cycle. We need to know which genes *Portulaca* expresses to switch to CAM under scarcity of water, and what genes it expresses to establish the mechanism to open and close the stomata [19-20]. These genes are needed in corn. No one is dealing with this project in Brazil to date. Aluminum toxicity that affects more than half of tropical soils are also needed; as well as grasses that fix nitrogen to replace oil derived urea which pollutes the soil and underground waters [21-22]. Brazil can make the Gene Revolution to work in the same direction as the Green Revolution did decades ago, with more powerful science tools available. Poverty and hunger are world acute problems. The population of hungry people is close to a billion according to FAO. Poverty in Brazil is a small fraction of the world's poverty. Other regions in the South Sahara in Africa are much worse. Thousands die of hunger daily, mostly children. Ignoring this and not using the advances from bioscience [23-24], to attenuate the problem is morally unacceptable. For this reason, we have partnered with Elizabeth Maga and Jim Murray at UC Davis to express genes in the milk of "caprinos" that can reduce diarrhea. The genes code for Lysozime and Lactoferrin. Marcelo and Luciana Bertolini at UNIFOR in the State of Ceará in Brazil, former students of Jim Murray and Elizabeth Maga at UC Davis are expressing these genes in Brazil [25]. Diarreha causes over 100 death/100.000 infants under 5 years of age in many Countries of the South Sahara in Africa and in few Counties of the Semi-Arid in the North East of Brazil. Biotechnology must demonstrate that it can resolve crucial social problems. This is important for the public perception of the area that is understood by many as an area that will never reach the poor.

How the agricultural revolution will build a pharmaceutical industry in Brazil–Changing paradigms

Will that constitute the entire agricultural revolution in Brazil during this century? The answer is no. This component of the agricultural revolution will feed the world, but agriculture in Brazil will not survive unless it aggregates value to its products. That requires changing paradigms. I made a contribution to Trade Secrets (http://www.blogs.nature.com/tradesecrets/author/lbarreto) on this topic. I published an E-book with Bentham and Sciences last year: Opportunities and Limitations for Biotechnology Innovation in Brazil [26]. Innovation was the key word. One of the chapters was devoted to building the pharmaceutical sector in Brazil and how agriculture can contribute to this process. Why is this issue so important? A sound pharmaceutical industry is the best example of what a government can offer back to taxpayers. A sound pharmaceutical industry should mean the best drugs at affordable prices. We however do not have this in Brazil. The only way Brazil can have the best drugs today is to import them, and we import a lot of drugs. In the range of $10 billion and $20 billion worth of drugs annually to supply the SUS a Unique Health System, linked to the Ministry of Health that pays for drugs with taxes paid by the society. What about the future? How do we adjust the pharma industry to compete? The drug market in Brazil is growing continuously, but if Brazil wants to become an important player in the pharmaceutical area, I see only one way: a change of paradigms. The large pharma firms are satisfied with their paradigms and they will not adopt new ones unless they are convinced it can work and is profitable. Whatever is the goal for Brazil's pharma industry, for instance, will it be biosimilar monoclonal antibodies, a market of billions of US dollars, our products must be able to compete in Brazil and abroad. Our funds are limited, and we will not be able to compete even with South Korea, India and China, who are more experienced than we are at expressing genes in Chinese Hamster Ovary (CHO) mammalian cell lines, yeast and even bacteria. Building manufacturing units to work with CHO cells in Brazil will take too long and will cost too much. Instead of lining up against these countries to compete with Big Pharma, we need other kinds of "biofactories". We should aim to express genes in the milk of mammalians, such as REVO Biologics is doing in Boston. Ofcourse, FDA has approved only one drug from gene expression in the milk of animals: Atryn. We have to demonstrate that monoclonal antibodies produced in the milk of animals will behave as they do when produced in CHO cells and will not be rejected by the human immune system. Sialyltransferases, which transfers syalic acid to nascent carbohydrate chains in MABs, must work properly as they do in the MABs produced by CHO cells. REVO is optimistic, and they are in the process of doing these experiments with MABs coming from genetically modified animals. Luciana Bertolini and Marcelo Bertolini expressed the gene coding for Glucocerebrosidase in "caprinos" in Brazil (personal communication). Gaucher disease a genetic disorder affects 500 people in Brazil that do not have this enzyme expressed at satisfactory levels in the body, and costs hundreds of millions of dollars to Sistema Único de Saúde SUS, because the enzyme is imported from the US. Francisco Aragão expresses genes in plants, (personal communication), specifically in lettuce (*Lactuca sativa*), in the chloroplast genome where the copy number is very high. This can aggregate value to the produce of small farmers that have low returns from their crops in the conventional way. Will that become the silver bullet? The results of these initiatives will tell us. One big way to shift our paradigm is through investment. We invest modestly in science and technology. Compared to the US where R&D receives approximately $300 billion to $400 billion annually [27]. We invest $20 billion. The Gross National Products of the two countries are not 20 times apart from each other. We should at least double our investments from multiple sources. Venture capital–the US applies $30 billion to $40 billion per year–is very scanty in Brazil. Start-up companies cannot scale up their products. Where will this money be coming from? Marcia McNutt, Editor in Chief of Science, said in a recent editorial "today a growing number of billionaires invest in scientific research in the US philanthropically. They are unafraid to take risks and abhor bureaucracy." This is another paradigm that we have to establish in Brazil [28], even if we do not have a growing number of billionaires, and they are not philanthropists.

Figure 1. Transgenic geminivirus resistant common beans cultivar (a) and common beans susceptible cultivar (b) [13,14].

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