development of resistance to pesticides, impact on crop genetic diversity, identity preservation issues, etc and inadequate current frameworks for environmental risk assessment and monitoring.

Delay in sector development: Twenty years have passed since the concept of biopharming was born, but little has happened in terms of the development of products. A key reason is thought to be the reluctance of the large pharmaceutical companies to get involved. For them the value of new or replacement vaccines produced in plants is balanced against the potential risks in their production and use, and the cost of not deploying the technology versus the risk of continuing with the status quo (Kirk et al, 2005). Significant investment is needed by the pharmaceutical industry to complete the development of human vaccines from plant based technology (Kirk & Webb, 2005) and this financial support is currently not being provided to the research sector. This perceived risk is largely due to the complexity of issues involved, and the fact that the cheap, edible vaccines that were dreamed of now require processing, packaging and distributing, and are hence not as cost saving as originally thought (Rybicki, 2009a). In addition, few facilities exist around the world that are able to process bulk plant material to an acceptable degree of purity for human vaccine use (Rybicki, 2009a).

Targeting of "orphan" vaccines – Rather than using this technology to produce products that compete with existing therapeutics produced by other means, providing a negligible cost advantage, this technology should be applied where it can have the greatest impact due to the rapid and scale of response possible (Rybicki, 2009c).

Processing and quality control: The dream of producing edible vaccines does not look like it will become a reality. Although it could reduce costs and enable vaccines to be taken orally, which is easier and safer than injection, the practical and potentially ethical problems prevent this from being implemented (Rybicki, 2009c). Quality control would be required to ensure plants actually contain vaccine and of appropriate quality and quantity. Plus, the dosage, i.e. the amount taken, would have to be regulated to prevent over or under dosing. It is now acknowledged that a level of processing is required and administered with supervision to ensure reproducibility (Rybicki, 2009c).

Confinement and containment: Mitigation of biopharming risk could be enabled through confinement and containment. Confinement is the use of the biology of the plant to confine it to a specific area i.e. through buffer zones, cytoplasmic male sterility etc. Containment is the physical isolation of the plant i.e. in a greenhouse. Since relatively small amounts of specific proteins would be required i.e. a few kilos could provide the annual global supply, greenhouse containment could be the route to take to avoid environmental impact.

Public engagement and awareness: As with other technologies of this nature, it is essential that the public are engaged and are invited to participate in debating the issues around biopharming. Rather than providing one way "education", the public needs to be actively engaged in a two-way dialogue based on factually based and credible information, to enable them to reach their own, informed decision.

Ultimately, the original vision of biopharming has been revised, and a significant amount of both research and investment is still required. However, it is still thought that biopharming can make a valuable contribution to supplying high quality pharmaceutical products at a very low cost in poor areas where there is a high burden of disease, expensive vaccines or no vaccines (i.e. for "orphan" diseases).

This fact sheet has been reviewed by independent experts and has followed SAASTA's Scientific Editorial Process.
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PUBLIC UNDERSTANDING OF BIOTECHNOLOGY

BioPharming for Africa?

What is biopharming?

Biopharming is the production of pharmaceutical proteins in genetically modified (GM) plants and animals (Byrne, 2008). When involving plants only, it is known as plant-based pharmaceuticals (PBP). Genes that code for useful proteins or components are inserted into plants or animals that would otherwise not express those genes. The hosts then produce the pharmaceutical product in large quantity, which can be purified and used as a drug product, such as a vaccine (Wikipedia, 2009). Diseases that can potentially benefit from these plant produced products include cancer, HIV/AIDS, heart disease, Alzheimers disease, cystic fibrosis, multiple sclerosis, hepatitis C, arthritis and human papillomavirus (HPV).

Biopharming is one of several recent biotechnology applications that go beyond the traditional agricultural products of feed and fibre, bringing further complexity to the only partially understood fields of biosafety and environmental assessments of GM plants (Chakauya et al, 2006). The original vision of biopharming included the use of edible plants or fruit to deliver vaccines directly to those that needed them the most, which could reduce transport and cold storage requirements and costs as well as increase safety through oral administration rather than injection for vaccine delivery (Rybicki, 2009c). However, during the past two decades, many practical and ethical problems with this concept have emerged (Rybicki, 2009c).

Why is it needed?

The current supply of drugs and treatments (therapeutics) that is produced using conventional manufacturing techniques, such as using mammalian cells, does not meet the demand (Elbehri, 2005). This makes them expensive and unaffordable to most people. Production capacity cannot be increased any further without heavy capital investment or use of new technologies and this has implications for human health around the globe.

Partly due to this perceived need, biotechnology has, undoubtedly, impacted the pharmaceutical industry significantly in the past 20 years In the past, the emphasis has been upon manufacture of small molecule drugs which are produced by chemical synthesis. Over a certain size, chemical synthesis becomes inefficient and economically unviable. Biotechnology has enabled the expansion of large-molecule drugs or "biologics" including proteins, viruses, therapeutic serum, vaccines, and blood components for drugs and treatments, which are usually found in living things (plants, animals and micro-organisms) (Elbehri, 2005).

Drugs made from these biologics or "recombinant" proteins potentially have greater efficacy and fewer side effects than small organic molecules because their action can be more precisely targeted toward the cause of a disease rather than the treatment of symptoms (Wikipedia, 2009). Recombinant proteins are usually produced using bacteria or yeast in a bioreactor, but pharming (i.e. using plants) offers the advantage that it does not require expensive infrastructure, and production capacity can be quickly scaled to meet demand. It is estimated that the expense of producing a recombinant protein drug via pharming will be less than 70% of the current cost (Wikipedia, 2009).

How is it done?

Following much research, a small number of proven systems have been identified and developed for industrial scale production of vaccines in plants. Although various other techniques are being developed, the better known include:

- **Transgenic plants** - Genetically modified plants are potentially a stable and cheap propagation source, but can take a long time to select a suitable line. There are also challenges in producing a high enough and stable yield (Rybicki, 2009a). Further work is needed to investigate the stability of vaccine expression in transgenic plants.
- **Transgenic single-cell cultures** - i.e. a few cells of a plant or algae grown in reactors. Using reactors, the protein production is contained in a reactor, but with low yields (Fischer, 2004). Chloroplasts (involved in photosynthesis) have also been transformed to produce proteins often resulting in high yields, but are unsuitable for a large number of proteins that need further adaptation for use as a vaccine.
- **Seed specific expression** – where the medicinal proteins are produced and found in the seeds. This is preferred since they are

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easier to purify because the protein is stored in the seed, making it stable for long-term storage. The disadvantage is that the presence and level of the medicinal protein can only be assessed when the plant goes to seed, which takes many months (Rybicki, 2009a). Moreover, separating the transgenic seed from the conventional seed is usually an issue with the regulatory authorities.

- **Transient expression systems** - the plant is temporarily modified via an inserted virus or bacteria at a certain developmental stage of a plant for a specific period of time. Some of these are based on *Agrobacterium tumefaciens* which "transports" the new genes into the plant where it integrates and is expressed (produces protein). This provides a high level of expression and the timing of the protein production can be controlled, without some of the problems associated with transgenic plants. The use of *A. tumefaciens* rather than virus "transport" systems means there is the potential to simultaneously express a large number of proteins (Rybicki, 2009a).

Numerous plants have been used in research, including tobacco, *Arabidopsis thaliana*, alfalfa, spinach, potatoes, duckweed, strawberries, carrots, tomatoes, aloe, single-celled algae, maize, rice and beans (Rybicki, 2009c). Different parts of the plants including the leaves, seeds etc are used to produce the protein depending on the species and the system used.

Benefits

Some of the benefits of biopharming include:

- Faster and easier production of large quantities of vaccine proteins (Rybicki 2009b).
- Significantly lower production costs than current practices.
- Safer vaccine antigen production.
- Production (in plants) of proteins of greater complexity than is possible with microorganisms (Collins, 2003), and to produce proteins that cannot be produced in mammalian cell cultures (Anonymous, 2002).

Risks & concerns

The risks of biopharming vary depending upon the nature of the pharmaceutical product, the crop and tissues in which the PBP is produced, and the environment in which the crop is grown (Byrne, 2008). Many of the concerns related to biopharming or PBPs are the same concerns as those related to GMOs. These include: detrimental effects on non-target organisms, gene flow to wild relatives/non-transgenic varieties, the inadvertent creation of weeds, development of resistance to pesticides and tolerance to traditional pathogens and impacts upon crop biodiversity etc (McGeoch and Pringle, 2005).

There are also some concerns that are specific to biopharming, which generally include:

- **Contamination of food chain:** If a food crop is used, the biopharm crop may inter-breed through pollen drift with the non-GM food crop and "contaminate" the food chain. This is a greater risk with outbreeding/cross pollinated crops like maize. Food crops could also become mixed or mingled with biopharm crops through improper labelling, or mixing of seed in planting, harvesting, transportation, processing or through "volunteer" plants (i.e. remnants of previous harvests) not being eliminated (Byrne,2008) . Both these routes are a cause for concern due to the potential harm to human health by contamination of food chain (Biology on-line, 2006). Two incidents involving the same company, ProdiGene, in the US have shown these concerns to be a reality. In 2001, ProdiGene failed to eliminate volunteer biopharm plants from a soybean crop planted later in the same field (Fox, 2003). The company was fined by the US Department of Agriculture (USDA) and was required to reimburse the government for expenses related to the destruction of the potentially contaminated soybean harvest (Byrne, 2008). ProdiGene was involved in a similar event in 2004 where volunteer transgenic maize contaminated an oat crop which was harvested and baled for use as on-farm animal feed, and was also found growing and flowering in a nearby sorghum field (Rybicki, 2009c). As a result of these two incidents, there is an effective moratorium on vaccine pharming in edible crops worldwide.
- **Allergenicity:** Plants process proteins differently from animals or humans - Thus, some experts are concerned that a plant-produced "human" protein could be perceived as foreign by the body and elicit an allergic reaction, including life-threatening anaphylactic shock (Biology on-line, 2006).
- **Impact upon non-target organisms:** this is a known concern related to GM crops, but is a more significant risk for biopharming since the introduced gene or its product may have negative effects on the natural environment. For example, wildlife feeding on the crop may ingest harmful levels of the PBP, or soil micro-organisms may be inhibited by decomposing crop residue or substances exuded from roots of PBP plants (Byrne, 2008).
- **Exposure:** Farm workers may be exposed to unhealthy levels of a biopharmaceutical by absorbing products from leaves through their skin, inhaling pollen, or breathing in dust at harvest (Kirk et al, 2005).
- **Inconsistent dosage:** Variations between the levels of proteins and other products expressed mean that the dosage has to be quality controlled and administration supervised.
- U**nexpected effects on drug:** Unexpected toxins or residues of pesticides used on the crop may contaminate the final drug product (Kirk et al, 2005).

What is happening in South Africa?

Within South Africa there are two main groups focusing on PBP research. One group is based at the University of Cape Town, headed by Prof Ed Rybicki. This work is focusing on developing human and animal vaccines using plants. These include vaccines for mucosal human papillomaviruses (HPV) and HIV type 1 subtype C. The other group is led by Prof Rachel Chikwamba based at the CSIR in Pretoria. This group is part of the Pharma-Planta research consortium representing 39 academic and industrial institutions in Europe and South Africa, funded by the European Union under the Framework 6 programme for Research and Development. This has evolved into the Combined Highly Active Antiretroviral Microbicide (CHAARM) consortium under European Union FP7.

Biopharming is now considered a focal area of national importance in-line with the strengthening of the "Farmer to Pharma" value chain to intensify the bioeconomy. This is one of the "grand challenges" of the Department of Science and Technology's (DST) ten year Innovation Plan. This will involve South Africa becoming a leader in pharmaceuticals, based on the national indigenous resources and expanding knowledge base. In May 2009, a workshop involving various biopharming stakeholders was held to identify all ongoing biopharming research activity in SA and to determine the optimal way that this industry could be supported and promoted by the government. Priority research areas for the future were also identified to accelerate biopharming research and plans are underway for a biopharming centre of competence (DST, 2009).

What is happening elsewhere?

The first vaccine-relevant protein produced in plants was reported in 1989 (Hiatt, A. et al. (1989). After many years of hype around biopharming, the Prodigene incidents (see "Risks") as well as the realisation of the complex regulation of these crops, a more realistic vision and role for biopharming has become clear. Although much research is focusing on the use of plants (non-food crops) to produce human targeted therapeutics, some of the most advanced research has been undertaken for those working on producing medicinal proteins for animals (Rybicki, 2009c). The leading research group in Buenos Aires have developed vaccines for cattle against diarrhoea (Rybicki, 2009c).

Worldwide to-date, only two products have made it through the regulatory processes to be licensed. The first was the use of transgenic tobacco in Cuba to produce an antibody for use in a recombinant HBV vaccine (Rybicki, 2009a). The second was a vaccine for poultry, produced in a suspension-cultured tobacco cell line in the USA, but this is not for sale.

Regulation & Legislation

Regulation of biopharming is complex and the development and future use of such crops will require tight regulation. For Africa to benefit from this technology, proper foundations in the form of policies for R&D, regulation and the relevant infrastructures for growing PBPs need to be put into place (Chakauya, 2006). In South Africa, there are a number of existing Acts implemented by different government departments that relate to PBPs already, including the Genetically Modified Organisms Act (No. 15 of 1997), the Medicines and Related Substances Control Act, (Act 101 of 1965), the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972) and the National Biodiversity Act (including the National Environmental Management Act, 1998 [Act No. 107 of 1998] and the National Environmental Management: Biodiversity Act, 2004 [Act No. 10 of 2004]) due to the potential impact on the environment (Groenewald, 2009). The overall regulation of PBPs would have to be managed by one of the departments involved, and specific consideration and resulting guidelines developed. The practicalities of this are unknown until a precedent has been set.

The first European guidelines for growing biopharm crops were published in August 2009 by the European Food Safety Authority (EFSA), and are consistent with the guidelines previously published in the US by the US Food and Drug Administration (FDA) and USDA.

Key issues

Choice of plant host: Some plants are better suited to the task of biopharming than others. Although a range of plants have been tried and tested, the tobacco plant is thought to be the ideal vehicle (USDA, 2003) and probably the most suitable African candidate for biopharmaceutical production (Chakuaya, 2006). It is not a food crop and so reduces the risk of contamination of the food chain, it is easy to transform and its genetics are well known, and is well suited to the agronomic conditions found in Africa. It can also produce large amounts of plant material (and by default, the medicinal proteins), quickly (USDA, 2003).

Food crops versus non-food crops: Although there is effectively a moratorium imposed on the use of edible crops worldwide, there are pros and cons associated with the use of food crops for biopharming:

- **Advantages:** Protocols for genetically modifying food crops are well developed and proven, they can be produced and processed at low cost, and contain minimal toxicants and so remain a favourite choice for biopharming (Chakuaya, 2006).
- **Disadvantages:** Aside from the normal risks associated with GM crops (as outlined above), the key disadvantage is the risk of contaminating food crops. The inserted gene can "escape" into non-modified counterparts, creation of weed,