KTH Biotechnology

The Competitive Effects of Adopting Modern Biotechnical Methods in Plant Breeding Programs

An economic and regulatory evaluation of the agrobiotech industry

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Abstract

The industry of agrobiotechnology is a relatively young industry dominated by multinational companies. The regulations surrounding the use of biotechnology to develop genetically modified crops have made it very hard for small or medium sized companies to compete in this industry due to high regulatory costs. The first part thesis describes the regulatory system for commercialization of GMOs in the EU and also presents estimations of the costs experienced by a company from this system. The second part of this thesis describes how biotechnology is used in plant breeding programs, using potato breeding as a specific example. With the help of researchers from Mistra Biotech, a new process for developing plant varieties using site-directed mutagenesis has been economically evaluated using a cost/benefit analysis. The results of this case study shows that site-directed mutagenesis using TALEN has the potential of greatly reducing the time and cost of conventional breeding programs. Benefits arise from the shortening of the breeding program which translates into higher net present values of released varieties and also on the ability of producing new varieties faster. The competitive advantage of adopting new biotechnical methods can be reduced developing cost, a more dynamic and faster developing process and a way of circumventing the GMO regulations. This could have different impacts on the industry since it could allow smaller companies to compete with multinational agrochemical companies. It could however also lead to a regained interest from the multinational companies in the European market which would force European companies to compete with much larger companies.

Keywords: Site-directed mutagenesis, TALEN, Plant-breeding, Competitive advantage

Sammanfattning

Agrobioteknik är en relativt ung industri som idag domineras of multinationella storföretag. Regleringarna kring användandet av bioteknik för att utveckla genetiskt modifierade grödor har gjort det väldigt svårt för små och medelstora företag att konkurrera i denna industri på grund av höga regulatoriska kostnader. Första delen av den här uppsatsen beskriver hur användandet av bioteknik för utveckling av GMO är reglerad i EU samt vilka kostnaderna för ett företag är av detta system. Den andra delen av uppsatsen beskriver hur bioteknik används i växtförädlingsprogram med potatisförädling som specifikt exempel. Fokus läggs vid att beskriva site-directed mutagenesis och TALEN tekniken. Med hjälp av forskare från Projektet Mistra Biotech har en ny process för förädling av potatis genom användandet av site-directed mutagenesis utvärderats. Utvärderingen har fokuserat på de ekonomiska aspekterna av metoden utifrån ett cost/benefit-perspektiv. Resultaten visar att site-directed mutagenesis med hjälp av TALEN skulle kunna reducera tiden och kostnaden för förädling jämfört med konventionell förädling. Genom att förkorta förädlingsprocessen kan nya sorters grödor utvecklas och kommersialiseras snabbare vilket ger högre inkomster för ett företag. Genom minskade utvecklingskostnader, en snabbare utvecklingsprocess och genom att undgå regleringar för GMO kan konkurrensfördelar erhållas. Detta skulle kunna göra att mindre företag kan ta sig in i och konkurera med de multinationella företagen i industrin. Det skulle också kunna innebära att företag som idag övergivit den Europeiska marknaden på grund av hårda regleringar på nytt intresserar sig för EU.

The industry of agrobiotechnology is a relatively young industry dominated by multinational companies. The regulations surrounding the use of biotechnology to develop genetically modified crops have made it very hard for small or medium sized companies to compete in this industry due to high regulatory costs. The first part thesis describes the regulatory system for commercialization of GMOs in the EU and also presents estimations of the costs experienced by a company from this system. The second part of this thesis describes how biotechnology is used in plant breeding programs, using potato breeding as a specific example. The use of site-directed mutagenesis and TALEN technology is explored in more detail. With the help of researchers from Mistra Biotech, a new process for developing plant varieties using site-directed mutagenesis has been economically evaluated using a cost/benefit analysis. The results of this case study shows that site-directed mutagenesis using TALEN has the potential of greatly reducing the time and cost of conventional breeding programs. Benefits arise from the shortening of the breeding program which translates into higher net present values of released varieties and also on the ability of producing new varieties faster. The competitive advantage of adopting new biotechnical methods can be reduced developing cost, a more dynamic and faster developing process and a way of circumventing the GMO regulations. This could have different impacts on the industry since it could allow smaller companies to compete with multinational agrochemical companies. It could however also lead to a regained interest from the multinational companies in the European market which would force European companies to compete with much larger companies.

Content

Introduction

The agrobiotech industry is a relatively young industry. It was created by the transition of agrochemical companies into the seed industry. The differences in size and business strategy between the two industries created a new industry with fierce competition where the multinational agrochemical companies have come to determine the movement of the industry. (Tait, et al., 2002) (Gravalos, et al., 2002). The adoption of technology for genetic modification of crops was what led the evolution of the agrobiotech industry. The ability to introduce new genes and traits like herbicide resistance into common field crops was complementary to the selling of herbicides by agrochemical companies. With the use of methods for genetic engineering came concerns about the safety of such methods and the impact that genetically engineered organisms could have on the environment. Regulations were initially adapted based on already existing legislations about chemicals and environmental laws. These regulations demanded that data was to be provided about new crops and traits in crops that where developed using genetic modifications. The production of data proved to be expensive and evaluations from different groups and researchers have found that the high costs involved in commercialization of genetically modified crops made it impossible for small or medium sized companies to sustain in the agrobiotech industry (Gravalos, et al., 2002), (Tait, 2004).

In 2003 the European Union introduced a new regulatory framework for the commercialization and introduction of genetically modified organisms into the environment. The new regulations have however also proven to be expensive and have been accused of being both unpredictable and of slowing down the industry (Food Chain Evaluation Consortium, 2010). With the development of biotechnology and introduction of new techniques used for plant breeding, the new regulations have today also shown to be insufficient or out of date. New technologies have emerged that challenge the definitions of genetic engineering as defined in the regulations and this makes for an interesting time in the industry where the lines between conventional breeding techniques and the creation of genetically modified organisms (GMOs) are not as clear as before. One of the methods that have been given attention is site-directed mutagenesis. This allows for alteration of plant genomes without necessarily creating a GMO. The implications of the technology are yet to be determined and this thesis aims to give a first glimpse of how modern biotechnology can change the competitiveness of the crop breeding and agrobiotech industry.

Purpose & Research questions

The purpose of this thesis is to investigate the business side of the development of biotechnology. Modern biotechnology and the agrobiotech industry are both young and subjected to regulations that influence the commercial applications. This thesis will investigate what technology development can do in terms of changing the competitive conditions of the agrobiotech industry and also how regulations have affected the structure of the industry. There are mainly two questions around which the work in this thesis has been built:

- How does the adoption of modern biotechnology affect the competitiveness of companies in the agrobiotech or plant breeding industry?
- How do regulations affect the industry and the adoption of new technology?

Delimitations

When talking about the agrobiotech industry, only companies working with plants and crops are considered. The industry surely does also incorporate animal breeders but in order to limit this thesis, this part of the industry has been left out.

Regulations are of high importance in the industry. The regulations that have been considered in this thesis are:

- Regulation EC 1829/2003 on the authorization of genetically modified food and feed
- Regulation EC 1830/2003 on the labeling and traceability of food and feed products produced from GMO.
- Directive 2001/18/EC on the deliberate release of GMO in the environment.

These regulations are the most influential when considering genetically modified plants.

A big part of the analysis of competitiveness in the industry and the competitive impact of regulations is based on costs. The regulatory costs have however not been estimated first-hand but are based on estimations from the literature. In the literature it was clearly stated that this kind of cost estimations are highly dependent on the assumptions made and also very limited by the restricted access to data from private companies. The estimations found in the literature and presented in this thesis are however considered to be good enough to give a good picture of the regulatory impacts on the industry and to give a good enough basis for the analysis and the conclusions.

Methodology & Structure of the Thesis

This thesis is based on literature research and interviews. The industry evolution, the regulatory framework and process, and the technical overview of site-directed mutagenesis are based on literature findings. The case study where one particular case of site-directed mutagenesis in potato breeding is studied and evaluated is based on interviews with the responsible scientists. For the understanding of the patents and the licensing process governing the chosen technique (the use of Transcription Activator-Like Effector Nuclease) an interview with a representative of the foundation holding the proprietary rights (the Two Blades Foundation) was conducted.

The term competitiveness is mainly based on the Theory of Competitive Strategy by Michael Porter. This theory describes the different components of competition and strategies that are used in order to create competitive advantages by companies. In order to give an understanding of competition and the ideas on which the conclusions and discussions are based, this thesis will start of by giving an introduction to the Porter theory and specifically describe the importance of costs as a competitive element. The results from the literature research will then be presented with an overview of the evolution of the agrobiotech industry and a description of the regulations governing the industry. The regulatory process will be described to give an understanding of what it is that creates the costs of the regulations.

The specific technology that has been evaluated is site-directed mutagenesis mediated by Transcription Activator-Like Effector Nuclease. This method will be thoroughly described followed by a case study of a process still in its early stage that has been evaluated using a cost/benefit analysis. In this analysis, a spread sheet approach was used to determine the costs of using the technology for

developing a new potato variety. The costs are then compared to potential benefits assumed to originate mainly from a shortening of the breeding process. The thesis will finish with a discussion of the overall results of the work.

Competition

Michael Porter's Competitive Forces

Competition is one of the key determinants of industrial landscapes all over the world. For a company to be successful on a market it has to have a strategy for interacting with other companies. Competition arises when several companies meet on a market in order to satisfy the same demand. The strive for a company to earn profits will lead the different companies to compete with each other for the market in order to secure as large a share of the potential profits as possible. The way that companies compete has been described in several ways and one of the most famous theories on competitive strategies has been developed by MIT economist Michael E. Porter. In his books on the competitive advantage of nations and on how to create and sustain competitive advantage, Porter created a general framework that defines competition in industries. The framework consists of different forces acting on industries and driving the competition between the different actors. There are five competitive forces as described by Porter, all acting together to create an environment in which companies compete with each other. The framework is often described and illustrated as shown below in Figure 1.

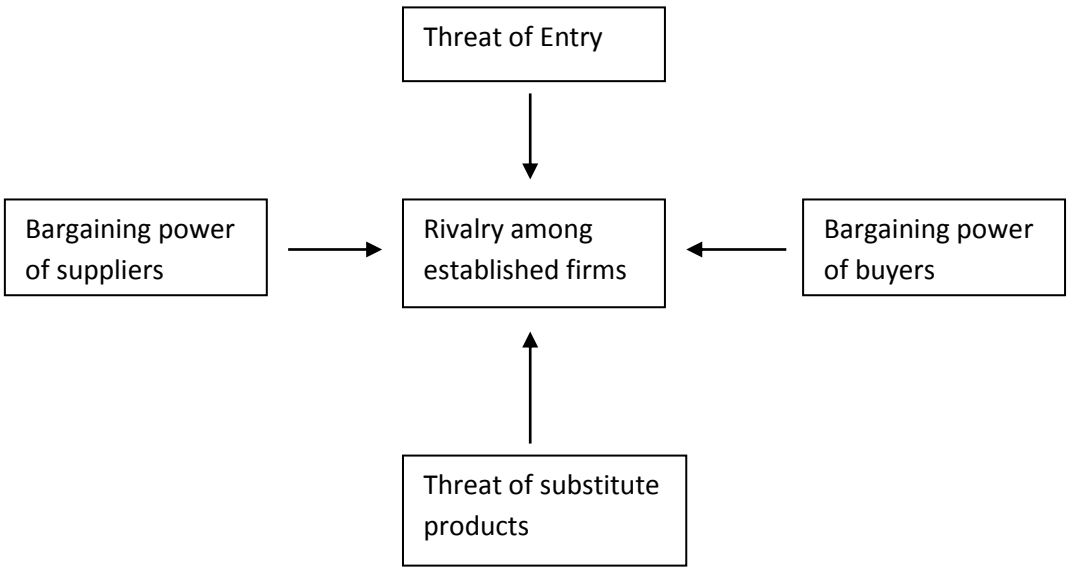

Figure 1: The competitive forces shaping an industry as described by Michael E. Porter. (Porter, 2004)

The different forces shaping an industry also describe the different actors involved in that same industry:

- The established firms already existing in the industry
- Suppliers to the existing firms
- Buyers, customers of the goods and/or services produced by the industry
- Potential new entrants that might enter the industry
- Other similar products (substitute products)from related or similar industries, threatening to replace the industry's products

A sixth force is often added in the shape of governmental regulations as the government is often involved in different industries through laws, regulations, environmental standards, etc. This force is applied outside of the five main forces as it often acts by influencing the other forces (Porter, 2004).

The relative magnitude of the forces acting in an industry is different for different industries and the competitive environment will therefore also be different. However, in an industry where companies compete with each other, the framework provides insights into how a company can create a competitive advantage for itself compared to its competitors. By studying the different forces and the causes for them, it is theoretically possible to create a competitive strategy that will give a company a sustainable advantage in the industry. Starting from the framework shown above, Porter has suggested three basic strategies used by companies to compete in any given industry. The first two strategies are:

Cost leadership: A company invests in a process that allows it to produce products or services at a cost lower than its competitors. This strategy is a broad strategy where the company cuts its cost on behalf of diversifying its products. This is often achieved through large-scale production and by creating economies of scale. This usually generates big companies with heavy investments in certain cost-lowering techniques (Porter, 2004).

Diversification: A company diversifies its brand or products from its competitors, thus convincing the buyers that its products are in some ways superior to its competitors' and worth a higher price than the one charged by e.g. a cost leader. The company using diversification has to be able to adapt to changing demands and have a more flexible organization than a cost leader (Porter, 2004).

The third strategy is a sub-strategy to the above mentioned strategies. It is called a **focus strategy:** A company finds a niche market within the industry and focuses its efforts on becoming the cost leader or achieves diversification within this smaller niche. This focused strategy can often outrival the two major strategies in those smaller niches. A focused company can never gain as large a market shares as in the first two strategy cases (Porter, 2004).

Cost as a Competitive Element

In this thesis, costs will be the main focus when evaluating competitiveness. Costs are not only important as being one of the different strategies a firm can use to compete with, costs also play an important role in shaping the competitive environment as part of the different forces in the framework.

If an industry is profitable, that is, it sells its products at a price higher than the marginal cost of producing those products; the industry will attract more competitors. These new competitors are the threat of entry, one of the forces mentioned above. The entry into a new industry is however not usually free. Depending on the industry, there might be certain requirements, costs, of entering the industry that might deter potential entrants from entering (Porter, 2004). In the case of agricultural biotechnology, some barriers to entry can be identified, for example:

- Costs of research & development
- Equipment costs
- Patents
- Regulatory costs

Certain industries are tightly regulated and in order to be active in the industry, there might be several regulatory permissions that have to be obtained. This is for example the case in agriculture where the use of biotechnology in order to produce genetically modified crops is carefully regulated. The demands in order to receive permission to sell products in Europe as well as in the US and most other markets are high and very expensive (Kalaitzandonakes, et al., 2007). Costs are incurred directly as costs for producing the data needed to obtain permission, and also indirectly due to the lengthy regulatory process of obtaining approval. The process delays product launch which incurs costs and the process can also be used by competitors to obtain information about the intentions of new entrants (Porter, 2004) (Tait, 2004). The regulatory framework and their effect on the market will be briefly discussed in a later chapter. Expenses coupled to research and development and commercialization can often be considered sunk costs: the costs cannot be reversed by for example selling a regulatory approval to a third party. For research, much of the research that is done does not lead to a product generating profits. These sunk costs often act as powerful barriers to entry since only companies that can afford to comply with the regulations or that can afford to invest in extensive research will be able to act in the industry. The case with regulations acting as barriers to entry is an example of how the government can affect the forces.

In the case of research and development in agricultural biotechnology where new crops are developed, a lot of resources might have to be invested in research that may not generate products. The research and development of new products is usually also a very time consuming process where a company has to be able to sustain itself through long periods of product development (Tait, 2004). This often incurs large costs that will affect which companies will be able to enter the market.

Another important factor to consider, especially in biotechnology, is the immaterial property rights. The use of certain key techniques, methods or material is often heavily guarded by patents preventing new companies from performing certain activities without first obtaining licenses of doing so. In agricultural biotechnology, many of the important techniques needed for the most common operations such as transformation of plants, belong to a few of the major companies already well entrenched in the industry (McElroy, 2003). They thus have the power to determine how these techniques are to be used and can therefore actively influence some of the entry barriers.

Among the existing firms (rivalry between established firms), costs are also of high importance. Regardless of if the strategy is to become a cost leader or to diversify, costs will be of significance. There are several methods of both cutting costs and diversify that involves different choices. In the case of lowering costs, focus will be on building a structure where as much output can be produced to as low a cost as possible. This will involve investment in facilities, process development and technology (Porter, 2004). When producing new crops there are many different methods for breeding new varieties and there are also many different crops that can be developed. The urge to produce products to the lowest costs will influence which technologies are the more suitable and which crops that will be the most profitable. This is also the case when the strategy is diversification: In crop breeding, diversification can be obtained either by developing varieties with traits not developed by competitors or by focusing on crops not targeted by other competitors. The traits that can be developed are restricted by the available germplasm and the techniques used in the breeding program. Some traits can be developed through traditional breeding where crossing is employed, whereas other traits such as pest resistance may need the use of biotechnology and transgenesis. Depending on which techniques are used, costs can differ substantially. The choice of what

technology to use and which traits to develop will therefore be affected by the costs of using different techniques and on what kind of products they yield. Depending on how a new crop variety is developed, if biotechnology is used, it can be classified as either a genetically modified (GM) crop or a non-GM crop. This classification will have a huge impact on the cost of product development and commercialization and has to do with the regulatory framework surrounding agricultural biotechnology.

The Agrobiotech Industry

The agro-biotechnology industry has developed mainly from the agrochemical industry that faced maturation in the middle of the 1980's (Tait, et al., 2002). With patents getting out of date, several big, multinational companies had to deal with the challenges that come in such a phase in an industry's evolution. New, smaller companies started to appear and generic products copying the best sellers of the big companies started to reduce the profits in the industry. (Tait, et al., 2002), (Gravalos, et al., 2002)

At the same time, biology made great progress. The applications of biotechnology as a tool in crop protection, the main focus of agrochemical companies, started to attract the attention of the same companies. The new applications that seemed possible; resistance to insects, fungi and herbicides, did however also pose a threat to the existing chemical alternatives in the product catalogues and the product strategies of many agrochemical companies. The development of insect resistant or disease resistant crop varieties could potentially cannibalize on the profits from chemical crop protection products. Being well established companies based in chemistry, a transition into crops and seeds would also mean big efforts in acquiring the necessary biological and biotechnological knowledge for research and product development. (Tait, et al., 2002)

For one of the major agrochemical companies at this time, Monsanto, the product portfolio did not rely very heavy on the crop protection products used for battling insects and diseases. The main portion of the revenues was instead generated by one weed control product; the herbicide RoundUp (Tait, et al., 2002), (Chataway, et al., 2004). For Monsanto, the development of insect resistant crops would not pose a threat to the current revenues and herbicide resistant crops would not be a substitute but rather a complement, to its commonly used, broad spectrum herbicide.

The biological base needed by agrochemical companies for a move into seeds and genomics was possible to obtain through acquisitions of companies in the seed industry. The seed industry, compared to the agrochemical industry, was not as profitable (Gravalos, et al., 2002). The margins were lower and there were many more and smaller companies than in the agrochemical industry. This made it possible for big agrochemical companies to buy seed companies. The agrochemical companies gained the necessary germplasm base and biological know-how to develop seeds and crops with biotechnical traits by acquiring seed companies, thus creating the industry; agro-biotechnology (Tait, et al., 2002), (Gravalos, et al., 2002). Using Monsanto as an example as it is a pioneering company in agrobiotechnology, they used an aggressive acquisition strategy in order to buy their way into the seed market and gaining the biological base needed for this industry (Tait, 2004). Their annual reports show many strategic acquisitions and collaborations with companies in order to strengthen Monsanto as an industry leader in agrobiotechnology. They explicitly stated that they had moved from being an agrochemical company dominated by the selling of RoundUp to a company instead focusing on traits and seeds in 2003 (Monsanto, 2003).

Smaller companies in the agrochemical sector were unable to follow the big ones in this transition due to a lack of sufficient resources to compete in this new industry. Instead they had the option to adapt to the new environment in the agrochemical sector. Not only was it getting increasingly hard to maintain product monopolies because of expiring patents, a tougher regulatory regime was also established which aimed at phasing out old chemicals according to new environmental guidelines (Gravalos, et al., 2002). This created the opportunity for smaller companies to adapt a focused strategy and develop new bio-products that would be less environmentally harmful, and also to create new strategies to crop protection all together. One way was the transition into biotechnology and introduction of resistance traits in crops which was also the strategy of the big companies. For the smaller companies, opportunities also included developing new products for crops that were now abandoned by larger companies or where old crop protection solutions fully disappeared with the phasing out of harmful chemicals (Gravalos, et al., 2002). In many cases, the smaller agrochemical companies adapted and started to find niche markets where they did not have to compete with the bigger companies (Gravalos, et al., 2002).

The entrance of agrochemical companies into the seed industry changed the conditions for competition in this industry. The environment changed due to both the new competitors and the new technology available, but also due to a new governmental activity regarding biotechnology. Policies to promote new companies and create public benefits from this new technology as well as regulations to govern the use of biotechnology were implemented. The biotechnology-promoting climate gave rise to new small agrobiotech companies (Gravalos, et al., 2002). When the initial regulations regarding the development of genetically modified crop varieties were formulated however, they have been accused of being targets for heavy lobbying from the agrochemical companies. They argued for onerous restrictions in order to raise barriers to entry into the developing agrobiotech industry (Miller, 1996). This was done with the belief that the need for government approval of developed products would act as a quality stamp for the products, which would be beneficial when competing with substitutes on the market (Miller, 1996). Also, the cost of producing a great volume of data to fulfill regulatory requirements would keep smaller potential competitors from entering the market.

A 32 month long project; "*PITA project-Policy influences on technology for agriculture-chemicals, biotechnology and seeds*" by the European Commission was conceived in 1997 and analyzed the industries involved in agrochemicals, seeds and agro-biotechnology (Tait, 2004). The results were presented in a report in 2004 as well as in several research articles. When looking at biotechnology it was found that in the agrobiotech industry there were very few small or medium sized companies (Tait, 2004). Instead the promotion of new biotech companies had resulted in a growing pharmaceutical sector where the use of biotechnology is differently regulated and the competitive climate is different from the agricultural industry. Small and medium sized enterprises (SMEs) in agro biotechnology did not have the resources to bring products to the market due to the high costs involved (Chataway, et al., 2006). Instead, successful companies were bought and incorporated into the big agrochemical companies. The agrobiotech SMEs still in the industry seemed to be either doing research for larger companies or trying to avoid competing with bigger companies by finding niche markets (Gravalos, et al., 2002).

The already established seed industry contained quite a big number of companies, many focused on local markets because of the very differing agricultural needs due to local differences and challenges

(Tait, 2004). The industry was also found to be much smaller than the agrochemical industry and the companies had smaller margins and were generally much smaller than the agrochemical companies. The market sizes for the different industries in the year 2000 were estimated to US$ 30 billion for agrochemicals compared to US$ 5 billion for seeds (Chataway, et al., 2004), (Tait, 2004). The introduction of biotechnology and agrochemical companies to the seed industry created new challenges. The seed companies had to adapt to the new technology, the new competitors and also to the governmental response to the applications of biotechnology.

The large companies transitioned into the seed industry by acquiring leading companies to gain access to resources needed to establish successful research and development activities. For some companies this transition was quite fast. They were able to develop seeds with biotech traits that were relatively easy to develop such as herbicide resistance and resistance to some major insect pests (Tait, et al., 2002). The industry was however very concentrated and focus was on the major field crops soybean, maize and cotton. In the year 2000, 7 companies were estimated to hold a total 80% of the market (Syngenta, 2000).The focus on only the largest crops granted possibilities to achieve economies of scale since the global feature of these commodity crops made it possible to spread costs over several different markets (Fulton & Giannakas, 2001). This can be pointed out to be perfectly in line with strategies for achieving competitive advantages as suggested by Porter (Porter, 2004). When the agrochemical companies entered the seed industry the seed companies had to decide on their strategic moves. They could either adapt by utilize biotechnology to compete with the new competitors or they could try to avoid competition. The regulations in place for the agrobiotech industry meant large costs for developing biotechnical products for the market. In the EU, the regulations were mainly based on Directive 90/220 on deliberate release of genetically modified organisms. The implementations of this regulation, together with a public that was able to heavily influence the risk assessment and release of GM products, created a market characterized by uncertainty (Tait, 2004). The risk assessment of new GM products was viewed as uncertain as it was hard to predict when or if a product would be accepted in the European Union. The process of registering a product for release to the market was coupled to large costs both through the data that needed to be produced and also due to the long lead times expected (Bijman & Tait, 2002). The seed companies were not equipped to cope with such uncertainties, and could not rely on sales from profitable agrochemicals while waiting for approvals. They also had disadvantages in size compared to the agrochemical companies. The agrochemical companies were able to spend very large amounts of money on R&D and they mainly focused on the biggest, most profitable crops where they also could reap benefits from economies of scale (Gravalos, et al., 2002). Being multinational, they also had the choice to simply choose other markets if one market was hard to penetrate or compete in. Coming from the agrochemical industry, they were also used to having long time horizons when formulating their strategies and could so cope with lengthy processes of product approval (Chataway, et al., 2006). Given these circumstances, the major agrochemical companies were able to develop whole-crop strategies where they could reap profits from both the agrochemical industry and the seed industry.

The seed companies, not able to cope with the risks of using biotechnology, could not compete with the whole-crop strategy. Many seed companies continued to use non-biotech methods of producing seeds, relying instead on traditional breeding and marker-assisted breeding and focusing on niches and markets where they would not have to compete with the big companies. This made them

targets for acquisition as the agrochemical companies saw successful seed companies as profitable prospects in their strategies (Tait, et al., 2002) , (Chataway, et al., 2004).

Seed companies that chose to incorporate biotechnology into their R&D had to deal with the higher risks and costs of doing so. This mainly led to close collaborations with multinational companies or being run as subsidiaries by the same companies as seed companies could not themselves afford to bring new products to the market (Bijman & Tait, 2002). The risks and costs were mainly based in the uncertainty of using biotechnology since the regulations were not very clear and also had a direction towards stricter regulations which would give higher costs. Another important factor, as stated earlier, was the size of the smaller seed companies which meant they could not compete with companies reaping economies of scale.

Many biotechnical products such as nutrition enhanced feed crops have substitutes which come with low switching costs. Because of this threat, it might not be possible for a small company to increase the price of its products in order to recoup the development costs since the buyers always have other alternatives that might be less efficient but cheaper (Arundel, 2002). The multinational companies can however offer whole crop strategies and also minimize their developing costs and production costs because of their size.

Small or medium sized companies working with biotechnology and agriculture have come to adopt either a strategy where they avoid competition with larger companies or they have the strategy to be acquired by large companies (McElroy, 2003). In the first case the direction of innovation has mainly been to abandon biotechnology and focus on traditional breeding techniques or by collaborating with an agrochemical company and do part of their research. The other option has been for the SMEs to make themselves more attractive for acquisition by a larger competitor by focusing their research and innovation strategy on areas that might be of interest to the potential buyers. Thus, in the agrobiotech industry, the innovation trajectories are mainly in line with the largest companies in the industry (Gravalos, et al., 2002).

For seed companies choosing not to develop GM varieties they have an advantage on the European market as long as the attitudes toward GMOs remain hostile and as long as substitute GMO products are not allowed by regulators or chosen by farmers and consumers. The lack of investment in biotechnology by these firms would however give them a great disadvantage would the attitude towards GMOs shift on the market. They would then be in a position where similar companies outside of Europe find themselves and most probably they would not be able to compete with the dominant multinational companies (Tait, 2004). The benefits of being an early adaptor and the ability to climb the experience curve earlier than the competitors was pointed out by Monsanto as two of the most important reasons to their leading position in the industry (Monsanto, 2003).

In the PITA project many of the major companies in the industry were interviewed, as were SMEs. The main problem from the industry's point of view was found to be the uncertainty of the risk assessment process in Europe. The industry was supportive of a revision of the directive 90/220 and also supportive of labeling as this was perceived to be increasing consumer choice and hopefully also allay the critique against GMO. A revision of the regulatory system in Europe would however have to result in an increased level of certainty. (Tait, 2004)

Regarding the heavy opposition against GMOs in Europe, most actors agreed that the solution probably does not lie in regulations or public policies, but rather in developing GMO products that will be attractive for end consumers. The first generation of GMO crops are exclusively focused on input traits and marketed to the farmers, giving benefits in their crop management and increasing yields (Levidow, et al., 2002). Development of genetically modified crops that have increased value to end consumers is believed to show the public the benefits of GMOs, thus changing the opinion (Levidow, et al., 2002).

A third issue that was brought up but not explored much in the PITA report was the system for intellectual property rights (IPR) which is perceived as too vague, slow and expensive. It also increases the uncertainty since it sometimes is possible to infringe on other companies patents unknowingly due to the layout of the system (Gravalos, et al., 2002). On the world-wide level there is also a lack of harmonization of IPR. Especially for SMEs lacking the oversight of the world that the multinational companies have, the IPR system was viewed as troublesome and expensive (Gravalos, et al., 2002).

The regulatory climate in Europe and the problem with releasing GM crops due to moratoriums and delays have caused many of the biggest agrobiotech companies to abandon Europe. Most recently, Monsanto declared that they would stop their development of GMOs in Europe an also withdraw all current applications that has been filed (ATL Lantbrukarnas affärstidning, 2013).BASF Plant Science announced in 2012 that they will discontinue their breeding efforts in Europe and instead focus on markets with more relaxed regulatory systems (BASF, 2012).In both cases the resistance towards GMOs and the difficulty of commercializing new products in Europe were stated as reasons to the decisions.

From a global perspective, the industry has been seen to become more and more concentrated. The consolidation pattern in the industry can partly be explained by the huge costs needed to commercialize products which promote economies of scale (Fulton & Giannakas, 2001). Another explanation that has been researched is the system of protection of intellectual properties. It has been found that the largest companies in the industry hold a significant amount of the patents. More important is that even fewer firms hold patents for key techniques for the development of genetically modified crops (Brennan, et al., 1999), (McElroy, 2003). These patents have often been acquired through the acquisition of other companies and through licenses from public sector research. Many patents are also claimed to be very broad, giving the owner-company a lot of power over the whole invention and development process of new crops in the industry (Fulton & Giannakas, 2001). With broad and ill-defined patents, it is hard for competitors to negotiate solutions where they can effectively license the use of techniques to one another or form strategic alliances. To deal with such problems, acquisitions have been a solution; IP-regulations thus influenced the consolidation of the industry (Fulton & Giannakas, 2001).

Regulation of GMOs for Food & Feed in the European Union

The commercialization of GMOs for the use in food and feed products in the EU is governed by regulations and directives in order to assure the safety of humans, animals and the environment. This legislation has been adjusted since GMOs first started to be commercialized in the early 1990's and today they consist mainly of:

- Regulation EC 1829/2003 on the authorization of genetically modified food and feed
- Regulation EC 1830/2003 on the labeling and traceability of food and feed products produced from GMO.
- Directive 2001/18/EC on the deliberate release of GMO in the environment.

The Directive 2001/18/EC relates to the cultivation of GMOs and the risk of GMOs spreading in the environment which today is also covered by regulation 1829/2003. In order to put a GM food or feed product on the market, authorization must be granted for their release into the environment and for their use as food or feed. The regulation that was introduced in 2003 is an amendment to directive 2001/18/EC and takes both these aspects into consideration by requiring a complete technical dossier on the release of the GMO into the environment with reference to annexes in Directive 2001/18/EC. In practice this has led to applicants submitting their applications according to regulation 1829/2003 rather than according to the directive 2001/18/EC which would require a separate application for the use of the GMO based products as food or feed. (Food Chain Evaluation Consortium, 2010), (EPEC, 2009)

The Regulatory Process

The risk assessment process preceding approval for commercialization of a genetically modified food or feed product is outlined below in Figure 2 as it was described in (Food Chain Evaluation Consortium, 2010).

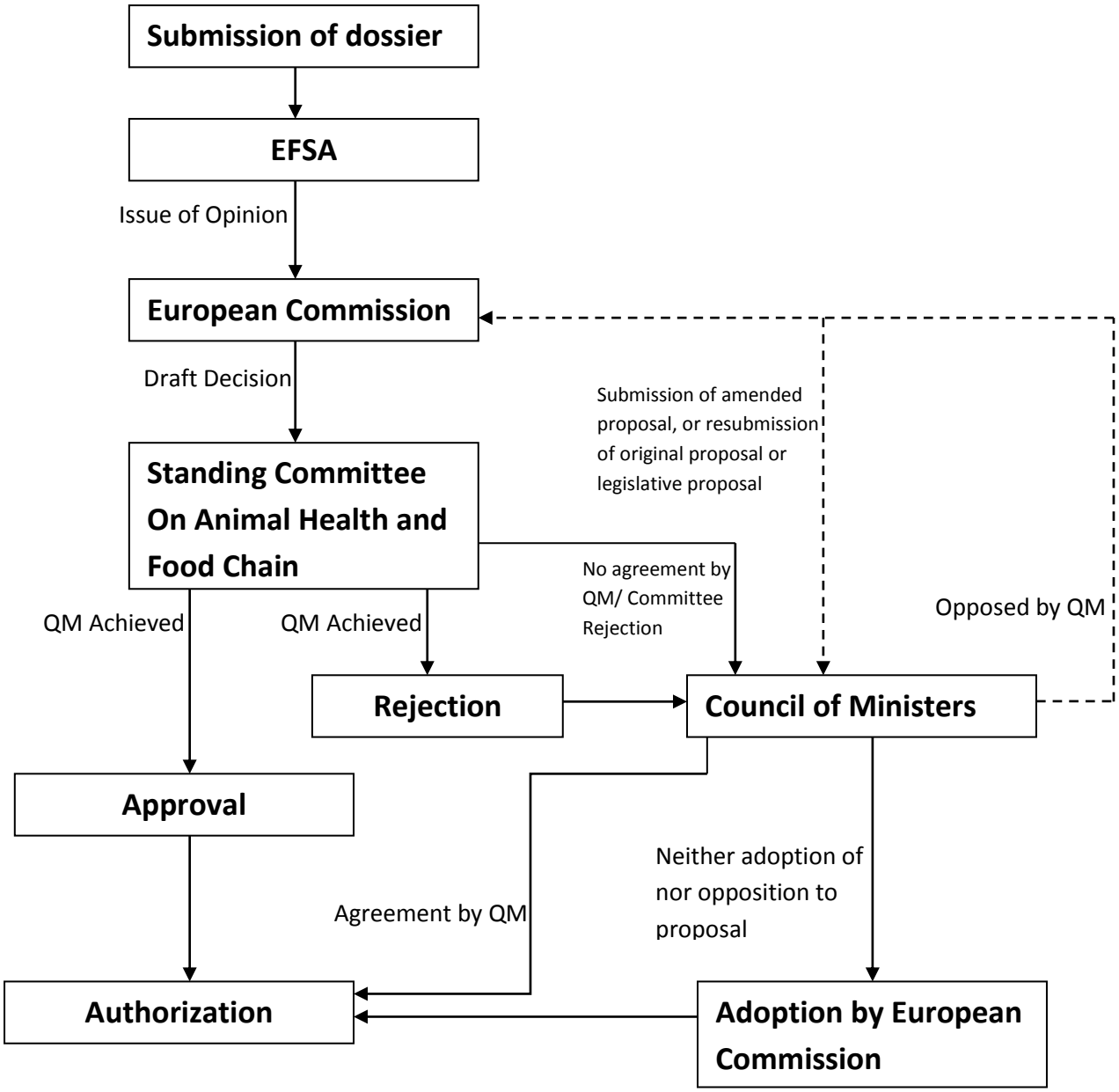

Figure 2: Approval process under Regulation 1829/2003. QM is the abbreviation for Qualified Majority, a prerequisite for decisions. Picture adapted from (Food Chain Evaluation Consortium, 2010)

The process outlined is the general path of an application for commercialization where the intended market is the European Union. An application that is submitted to a member state under regulation 1829/2003 is forwarded to the European Food Safety Agency (EFSA). The regulation contains a list of requirements for a dossier to be complete. Regulation 1829/2003 also refers to the Directive 2001/18/EC for the data needed for the environmental risk assessment. The annexes of the Directive contain lists of what data should be submitted and is also supported and clarified by an EFSA guidance document (EFSA Panel on Genetically Modified Organisms (GMO), 2010).

Beside information on the crop, the trait(s), the method of the modification and the intended use, the dossier should also include (Food Chain Evaluation Consortium, 2010), (annex IIIB of Regulation 2001/18/EC):

- Methods for detection, sampling and identification of the GM event
- Proposal for post-market monitoring regarding the use for human consumption
- Survivability of the crop & the extent of pollen dissemination
- Interactions with intended target organisms and other organisms in the ecosystem as well as the abiotic environment
- Etc…

The applicant submits the dossier to the relevant agency in a European member state. The receiving member state authority then forwards the dossier to EFSA within 2 weeks. After the dossier is submitted to EFSA, a member state is appointed do an initial appraisal of the risk assessment. The member state is appointed by EFSA from a pool of volunteers that have been deemed qualified with regard to their experience with GMOs (EPEC, 2009). The dossier is checked for its completeness. This initial process has a timeline of 6 weeks during which the time can be stopped in order to collect additional data from the applicant. When the dossier has been deemed valid and complete, EFSA will do a thorough scientific evaluation of the application and issue an opinion to the European Commission where they advise for or against the release of the GMO to the market. When EFSA starts assessing the application they have 6 months before an opinion should be issued. These six months can be prolonged if the agency finds that more data has to be provided by the applicant. In that case, the "countdown" of the 6 months is stopped and will not start until the additional data has been assessed by EFSA. On average this adds an extra 328 days to the EFSA evaluation: It takes on average 154 days for the applicants to provide the requested data and then 274 days for EFSA to evaluate that data and the time can be stopped more than once during an application process (EPEC, 2009). When the opinion has been received by the European Commission they have 3 months to formulate and submit a draft decision to the Standing Committee on the Food Chain and Animal Health. The decision should be based on the opinion provided by EFSA and has to be explained if it differs from that opinion (EPEC, 2009).

The Standing Committee has to propose rejection or authorization of the received draft. This is done by voting where a qualified majority is needed for a decision. If a majority votes for authorization the application will be approved and the GM product can be released on the European market. If a majority votes against the approval or if no qualified majority is obtained, the application proceeds to the Council who has to come to a decision by a qualified majority (EPEC, 2009). The Council has 3 months to come to a decision. If the Council votes against the draft proposal by the European Commission, the Commission has to either:

- Submit their draft decision again
- Submit a new version of the decision
- Bring forward a legislative proposal

If the Council achieves a qualified majority in favor of the draft decision it will be adopted. If the Council fails to come to a decision within three months, either due to failure to obtain a qualified majority or due to inactivity, the draft decision will be automatically adopted by the Commission (Food Chain Evaluation Consortium, 2010).

If approved, the product is authorized for commercialization in the European Union for 10 years after which a new application has to be submitted for renewed authorization.

In theory, an application could be handled in 57 weeks from the time of initial application to a member state. Due to several occasions of "clock-stopping" this has however never been the case. Instead, the average time it takes for an application to be processed is 187 weeks (Food Chain Evaluation Consortium, 2010). The process is shown graphically below in Figure 3. It should be noted that there is no time frame for the voting in the Standing Committee and that there are no system in place to assure that the time-line that is set out is kept (Food Chain Evaluation Consortium, 2010).

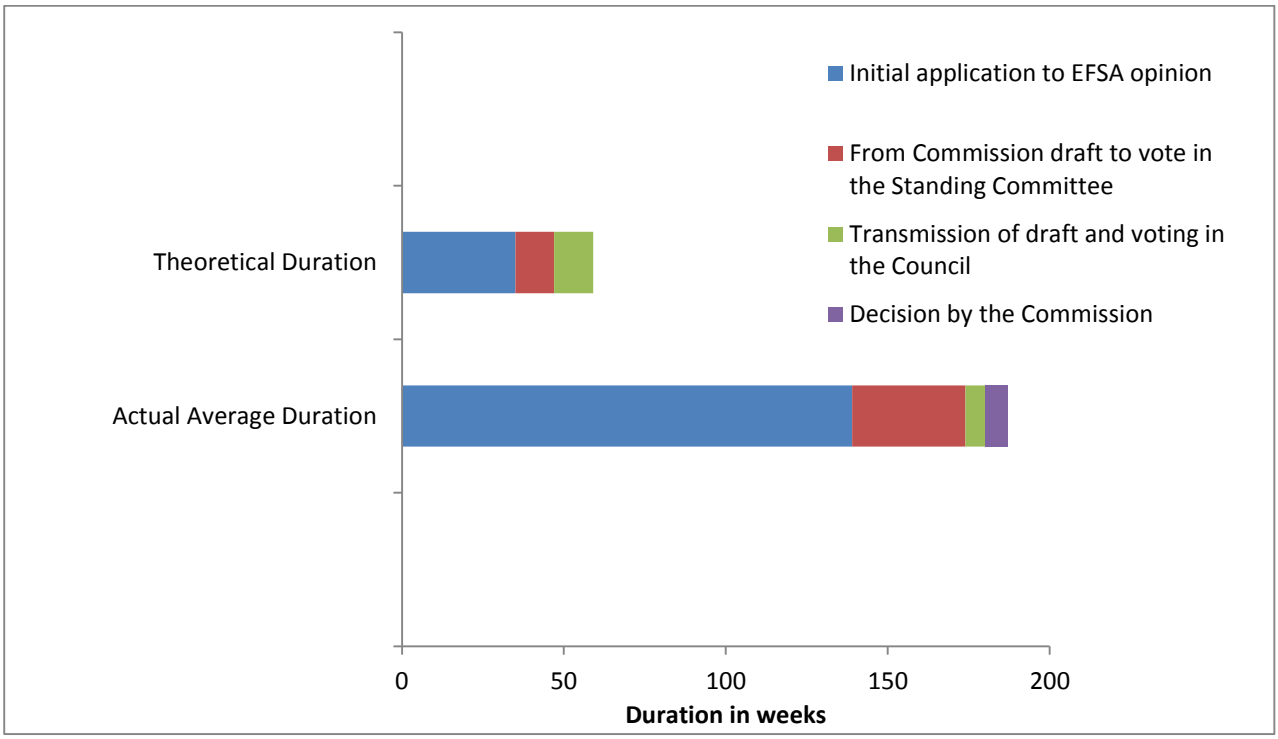

Figure 3: Graphic overview of the duration of an application under Regulation 1829/2003 in the European Union. Based on data from (Food Chain Evaluation Consortium, 2010).

Figure 3 illustrates the unpredictability of the application process. Among applicants, there is a consensus that a risk assessment process is necessary in order to validate new products, but the process takes on average too much time and is hard to predict (EPEC, 2009). In 2009, only one GM-product was allowed for cultivation in the European Union (EPEC, 2009).

Costs of the Regulations

The costs of complying with the regulations and of getting products approved for the market have not been accurately determined for private companies since the access to data about such costs is very restricted (Kalaitzandonakes, et al., 2007). There are however several different estimations based on the data that needs to be provided by an applicant. In the report by the European Commission (Food Chain Evaluation Consortium, 2010), data is presented that has been obtained by the Dutch consulting agency Schenkelaars Biotechnology Consultancy who reviewed 7 different dossiers and estimated the costs. Here will be presented the data as it was presented in (Food Chain Evaluation Consortium, 2010) since the original report was only available in Dutch.

The average cost of getting a novel GM-crop through the approval process in the EU was estimated to € 6 788 000. The cost estimation given was a range: € 3 820 000-€ 10 388 000 (Food Chain Evaluation Consortium, 2010). A big portion of the costs stem from field trials needed to assess the

environmental impact and the phenotypic differences between the GM-variety and its non-GM relatives. In the evaluation process of the dossiers, with regard to the detection method of the GM trait, there is a mandatory fee to the Joint Research Centre (JRC) for validating the proposed detection method. This fee is € 90 000 (Food Chain Evaluation Consortium, 2010).

The cost for the approval process has also been estimated in a study by (Kalaitzandonakes, et al., 2007) where the focus is more on the US approval process. The study does however also consider costs in Europe. In the report, estimations have been made for two types of maize; herbicide-resistant and insect-resistant. The data is based on cost data provided by global private companies. In Table 1 below is shown the results from this study, together with the data from the European study.

	Min. Cost (€ x 1000)	Max. Cost (€ x 1000)	Average Cost (€ x 1000)
Cost of the European approval process (EPEC, 2009)	3 820	10 388	6 788
Cost of the US approval process (EPEC, 2009)	3 335	8 257	5 474
Cost of the US approval process (Herbicide-resistant maize) (Kalaitzandonakes, et al., 2007)	4 623	10 853	10 345
Cost of the US approval process (Insect-resistant maize) (Kalaitzandonakes, et al., 2007)	7 060	15 440	11 250

Table 1: Estimated cost for the approval process in the EU and in the US. The costs for the US maize varieties has been converted to Euros using exchange data from 2007. (1 USD=0,748 EUR). The cost data has been obtained from (Food Chain Evaluation Consortium, 2010), (Kalaitzandonakes, et al., 2007).

The table above shows big differences both in the min and max values for the different costs and also between the different studies. In the report by Kalaitzandonakes, et al. it is pointed out that the costs depend on what kind of crop is being assessed, what kind of genetic modification that is the case and what country the application regards. In the European study, it is emphasized that the approval process is more expensive in the EU compared to US which is also seen in the European comparison of the two approval processes. The difference in magnitude between the studies can depend on the exact costs that are included, how costs are allocated in the budgets of different companies, how many events that are assessed in the different dossiers, etc. In the study by Kalaitzandonakes, et al. costs for overhead and management are included. These are fixed costs that may be hard to accurately distribute between different products. This is also pointed out in the report (Kalaitzandonakes, et al., 2007). Even though the deviation between all the numbers is big, they still give an intuition of the costs that are added by the regulatory process. An important aspect is that these costs only are the direct costs coupled to the approval processes. Costs related to

delayed product launch, inventory costs or other costs due to the delay in the regulatory process are not considered (EPEC, 2009) (Kalaitzandonakes, et al., 2007).

It might be interesting to put the estimated costs of regulations in relation to the research budgets of Swedish crop breeding companies. In a database search it was found that this industry is very small in Sweden with only a few actors. The database used was Affärsdata and a part of the result of a search for companies performing plant breeding in Sweden is shown below in Table 2.

Company	Year	Research & Development (Thousand SEK)	Turnover (Thousand SEK)	Research & Development (Thousand EUR)	Turnover (Thousand EUR)
Crop Tailor AB	2011	371	1 100	40	130
Lantmännen SW Seed	2012	94 000	140 000	10 900	16 300
Syngenta Seeds AB	2012	95 000	380 000	11 100	44 200

Table 2: Breeding companies active in Sweden and their budgets for R&D. Data was obtained from a search in the database Affärsdata.

The data given in Table 1 and Table 2 show the burden of a Swedish actor if they were to commercialize a genetically modified crop. Syngenta may of course be an exception as their Swedish branch belongs to the multinational Syngenta corporate group. The costs shown in Table 1 do not (as mentioned) include indirect costs or loss of profits due to regulatory delay. There are reports showing that some firms find it reasonable to budget US$ 50 million for launching a new GM-crop on the market (Redenbaugh & McHughen, 2004). Regardless of which of the numbers mentioned above for the regulatory costs, the market size of the crop has to be big enough to give enough revenues to recoup the cost. For many specialty crops, the market is too small to be able to generate sufficient revenues from new varieties and GM-technology can therefore only be applied to the large commodity crops such as maize, soybean, rice and cotton (Redenbaugh & McHughen, 2004). Given all of this, it should be clear that a small or medium sized company, such as a Swedish breeding company, cannot bear the cost of commercializing GMO products and thus have to avoid the use of such methods in their breeding programs.

An estimation of potential profits that are lost due to a delayed product launch has been provided by the Joint Research Center (JRC). When trying to account for the delayed launch of a product, the JRC estimated that if a product could be launched one year earlier this could add a net present value of € 0,7-70 million (Food Chain Evaluation Consortium, 2010). This is a highly uncertain figure, intended to somehow bring to mind the effects of delays in the approval process.

Another aspect that may influence the uncertainty in accurately estimating the costs of obtaining approval in the EU is stacked traits. Since the launch of the first of the genetically modified crops found today where the modifications were single traits such as herbicide resistance or insect tolerance, more and more of the applications submitted for authorization are for stacked traits (Food Chain Evaluation Consortium, 2010). Crops with stacked traits are crops that have been genetically modified to contain more than one trait, e.g. both insect tolerance and herbicide resistance in the same plant. In the current European legislation, GMOs with stacked traits have to be individually authorized independently of if the single events have already been authorized. Few

stakeholders and competent authorities view this as a strength with the European legislation (Food Chain Evaluation Consortium, 2010). This has especially been pointed out as a risk of heavily increasing the workload of EFSA in the future as more and more stacked events are expected to be developed. From the industry side, a fast-track process for stacked events is requested (EPEC, 2009). A suggestion from the industry is that special attention is given to stacked events only if there is reason to believe that the interaction of traits within a crop causes other effects than simply the sum of the single events. In the risk assessment of stacked events, all combinations of stacks and sub-stacks that are possible from a stacked plant need to be assessed (Food Chain Evaluation Consortium, 2010), (EPEC, 2009).

The uncertainty about the time it will take to get authorization for a product and launch it on the market is seen by the industry as the main constraint on the development of biotechnology in agriculture. Together with the costs coupled to getting authorization, this is especially damaging for small firms and also impacts the type of innovations launched on the market (EPEC, 2009) From a European perspective it has also been pointed out that the very strict regulations in the EU and the time-consuming process of getting authorization for cultivation leads to companies focusing on markets outside of the EU. Since 2006, a decline in field trials in the EU has been observed, and with less field trials being conducted, fewer products may be developed for the European market and the local variations in the EU (Food Chain Evaluation Consortium, 2010), (EPEC, 2009).

Over the last decade, a range of new biotechnical techniques that can be used in plant breeding programs have been developed and is gaining increasing attention from the industry. These new techniques have created new challenges for the regulators and also more uncertainty for the industry since they are not covered by the legal definitions governing the GMO regulations today (Lusser, et al., 2011). The new techniques for manipulation of genomes is different from the transgenic techniques used to develop the GMOs found on the market today. In many cases they do not insert any foreign genetic material into the genome of the plants. Techniques such as zinc finger nucleases, oligonucleotide directed mutagenesis, RNA-dependent DNA methylation and reverse breeding make use of the native system of a cell to alter the genetic code (Lusser, et al., 2011). Some methods induce mutations by damaging the DNA of a cell and then letting the cell repair-system repair the damage, thereby either inducing random mutations or by repairing according to a desired template creating planned mutations. At the end of this thesis is a case study describing one such technique in more detail, but a short description will be given here in order to show some of the difficulties with today's regulations. The techniques using this approach insert enzymes or genetic material encoding enzymes in a parental plant cell. When the mutation has occurred it will be part of the plant genome and passed on to the subsequent generations. The initial genetic modification responsible for producing the enzyme will however not be passed on to the progeny. In this way, the progenies will be carrying the genetic modifications but they will not be carrying any transgenic material. The modifications will be the result of the native repair-system of the cell and cannot be identified as a genetic modification different from what is achieved through random mutation or conventional breeding (Lusser, et al., 2011). RNA-dependent DNA methylation (RdDM) is an example of epigenetics where the functions of a cell are altered without altering the DNA. Instead of manipulating the DNA, the system with which the DNA is transcribed is altered. By affecting different molecules or the way transcription factors interacts with DNA it is possible to repress or enhance the expression of certain target genes without inflicting any change in the genome (Lusser, et al., 2011). With RdDM a transgenic parent plant is produced where alterations to the transcription process will

take place. In the progeny of this plant however, the transgenic material will be absent but the alterations to the transcription process will remain. These alterations cannot be identified as caused by different means than random processes or conventional breeding. There are several other examples of techniques where transgenes are not present in the final organism or where the genes inserted are no different from what could be achieved through conventional breeding. Common for all of these techniques is that they all fall outside of the current definitions of how a GMO is produced and their legal status is thus uncertain (Lusser, et al., 2011). If they will be legally defined as GMOs they will be regulated according to the same legislation as current GMOs and consensus in the industry is that this would have a negative impact on the development and use of these techniques in the EU (Lusser, et al., 2011). If however they are classified as non-GMO producing techniques, they would be much less regulated and the regulatory costs for developing plants would possibly be less than € 7000 which was the cost for getting a plant developed through marker assisted breeding in 2010 (Lusser, et al., 2011). Opinions from EFSA regarding some of these new techniques (e.g. site-directed nucleases) show that they think that these new techniques should not automatically be regulated as GMO-producing techniques. Instead attention should be given to the specific genome alterations produced in different cases (EFSA Panel on Genetically Modified Organisms (GMO), 2012).

Up until 2011, the new techniques were researched mostly by public institutes in the EU, leading over the US in publications of research related to these new techniques (Lusser, et al., 2011). Despite this, the patents that have been filed in the same time are mainly from US-based private companies who have filed patents in both the US and the EU, indicating a possible commercial interest in both regions. Among the private companies holding the majority of the patents, Dow Agrosciences LLC and Pioneer Hi Bred Int. can be recognized from the major multinational biotechnology companies active in the agricultural sector (Lusser, et al., 2011).

Site-Directed Mutagenesis

Transcription Activator-Like Effector Nucleases

Transcription Activator-Like Effectors (TALEs) are proteins used by several species of the bacteria *Xanthomonas* (Baker, 2012). TAL effectors are used by the plant pathogen to facilitate infection. The protein is injected into the plant nucleus via the type III secretion pathway of the bacteria. Well inside the nucleus, TAL effectors bind to specific sites in the genome and activates genes involved in the plant metabolism, hijacking the system in order to facilitate microbe infection and propagation (Baker, 2012), (Bogdanove, et al., 2010). The binding precision of TAL effectors was first revealed in 2009 when the structure of the protein was beginning to be understood in more detail (Boch, et al., 2009). The middle part of the protein is made up of almost identical repeats of varying length depending on the exact origin of the protein. These repeats are almost perfect repeats but have two hypervariable residues, Repetitive Variable Di-residues (RVDs) at specified residue sites 12 and 13 (Boch, et al., 2009), (Bogdanove, et al., 2010). When trying to figure out what determined the binding sites of TAL effectors, attention was given to the repeats and especially to the hypervariable regions. When investigating the binding sites of certain TAL effectors, it was found that the number of nucleotides in the binding sites seemed to correlate with the number of repeats in the corresponding TAL effectors (Boch, et al., 2009). It was hypothesized that the RVDs, since they were

the only thing differing between the repeats, were determining the binding specificity. With the use of simulations and bioinformatics, a code for which amino acids corresponded with which nucleotides was formulated and validated (Boch, et al., 2009), (Moscow & Bogdanowe, 2009). Based on this code, predictions about target sequences of different TAL effectors were made and validated. The findings suggested an almost degenerative code where mainly a few amino acid pairs are determining the binding specificity as shown below in Figure 4 (Bogdanove & Voytas, 2011) (Boch, et al., 2009). The almost degenerative character of the code refers to the most common RVDs mainly recognizing certain nucleotides as shown below. The specificity for certain nucleotides is not absolute but in most cases very low affinities for other nucleotides than the main targets has been found. Some RVDs however have the same affinity for different nucleotides as shown in Figure 4 below where RVD (NN) has the same affinity for A and G (Bogdanove & Voytas, 2011). This code has since its discovery been validated by many different research groups.

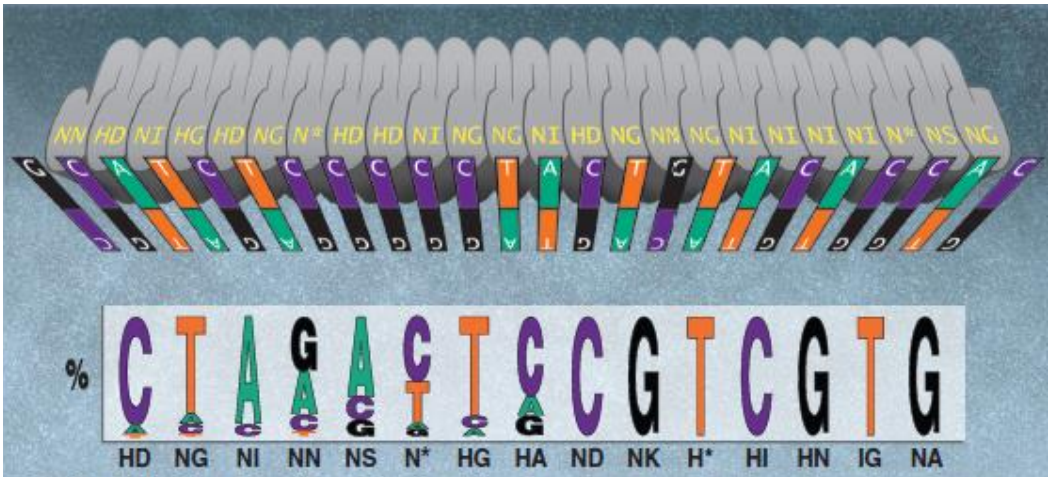

Figure 4: Example of the interaction between a TAL effector and its corresponding binding site. The figure shows the RVDs of the tandem repeat region of the protein connected to a strand of DNA. The table shows the affinity of different RVDs to the nucleotides. The figure is adapted from (Bogdanove & Voytas, 2011).

When being able to predict the binding of TAL effectors by knowing the repeat sequences, ideas emerged that it should be possible to use TAL effectors to direct other enzymes by fusing them to a TAL effector. This had already been done and experienced with Zinc Fingers which are other proteins able to bind to specific DNA sequences (Baker, 2012). Zinc Fingers are among other things used for creating genetic mutations in a genome by fusing the zinc finger to a nuclease yielding a Zinc finger nuclease (ZFN). Zinc Finger Nucleases bind to specific target sites in a genome and can with the nuclease activity create double-stranded breaks (DSBs). The binding specificity of ZFNs is determined by different finger-like domains of the protein, each recognizing and binding to specific nucleotide triplets. However, the binding affinity of ZFNs has been found to be context dependent, i.e. it depends on the order in which the fingers bind to their respective triplets (Mussolino & Cathomen, 2012). ZFNs have been found to be able to bind to targets with only 66% of the right similarity, thus tolerating a miss-match ratio of about 33% (Mussolino & Cathomen, 2012). This can potentially give rise to unwanted off-target effects when ZFNs are used for manipulating genes. Also, ZFNs are quite laborious to construct and their use is guarded by immaterial property rights which raise the cost of using ZFNs (Sanjana, et al., 2012). The possibility to instead use TAL effectors for the same applications was therefore enthusiastically welcomed. Using the same idea as for ZFNs, the restriction enzyme FokI was attached to TAL effectors, yielding TAL effector nucleases. (Baker, 2012).

The restriction enzyme FokI is the same that has been used to create zinc finger nucleases. It is an enzyme that cuts DNA randomly outside its binding site. Attached to a targeting enzyme like TALE or ZF however, the DNA will be cut at a certain distance from the specific binding site of the TALE or ZF (Baker, 2012). The enzyme is only active in its dimeric form which implies the need for two TALEN to create a DSB. The TALENs need to bind to the DNA close enough to allow the restriction enzyme to dimerize or else it will not be active (Mussolino & Cathomen, 2012).

The binding specificity of TAL effectors has been found to (apart from the RVDs) also depend on a highly conserved repeat that is initiating the repeat domain of the protein. This repeat contains an RVD that is specific for thymidine which is always found at the very beginning of a TAL effector binding-site (Mussolino & Cathomen, 2012), (Boch, et al., 2009), (Bogdanove & Voytas, 2011).

The application of TALEN in research has been to create site-directed mutations in genomes. The nuclease creates double-stranded breaks at the target site. These breaks are then sealed by the DNA-repairing machinery of the cell, either through non-homologous end joining (NHEJ) or through homologous recombination (HR) (Bogdanove & Voytas, 2011). The easiest application is NHEJ were the cell repair-system tries to rejoin the broken DNA strands, but does so in a way that creates random substitutions, insertions or deletions. These alterations can for example shift the reading frame, thus inactivating genes (Wei, et al., 2013), (Bogdanove & Voytas, 2011), (Mussolino & Cathomen, 2012).

If instead the intention is to create carefully monitored mutations, homologous recombination is utilized. With HR, the cell is trying to repair the DNA according to a template. If that template is provided in large quantities, it can be designed to give either predetermined small mutations or to insert short sequences of DNA (Bogdanove & Voytas, 2011), (Wei, et al., 2013).

Structure of TAL Effectors and TAL Effector Nucleases

TAL effectors like other proteins have an N-terminus and a C-terminus. The N-terminus of the native protein contains the localization signal required for secretion and the C-terminus contains the nuclear localization signal and an Acidic activation domain typical for transcription factors (Bogdanove, et al., 2010). The middle part of the protein contains a tandem repeat region made up of several almost identical repeats carrying the repetitive variable di-residues. The repeat units consist of 33-35 amino acids depending on the origin of the protein (Bogdanove, et al., 2010). The repeat region is preceded by a repeat often called the 0^{th} repeat that recognizes the thymidine residue found at the start of TAL effector binding sites. The last tandem repeat is a half repeat, also carrying an RVD (Baker, 2012), (Boch, et al., 2009), (Mussolino & Cathomen, 2012). In Figure 5 below is shown a schematic picture of a TAL effector and the amino acid sequence of one of the repeat units.

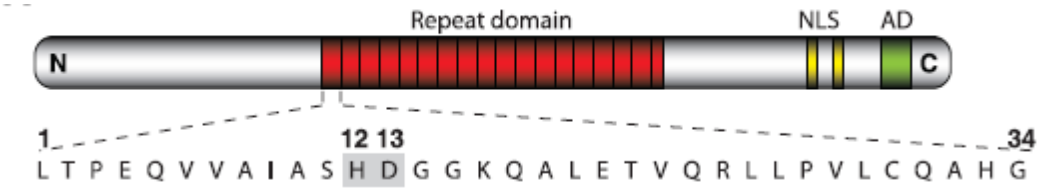

Figure 5: Schematic picture of a TAL effector showing the repeat domain with 17.5 repeat units. The amino acid sequence of the first repeat unit is shown with the RVDs at position 12 and 13 marked in grey. At the C-terminus is shown the nuclear localization signal (NLS) and the acidic activation domain (AD). The figure is found in (Boch, et al., 2009).

When constructing artificial TAL effectors different guidelines have been developed. TAL effectors have been observed with as few as 1.5 tandem repeats and up to 28.5 (Boch, et al., 2009). For successful binding however, it has been found that >10.5 repeats are necessary for strong recognition of a site and binding to that site (Boch, et al., 2009), (Mussolino & Cathomen, 2012). For TALENs, the average binding site is made up of 19 nucleotides, corresponding to 18.5 repeats, not counting the 0^{th} repeat (Boch, et al., 2009). To create a TALEN, the binding domain of the TAL effector, together with the NLS is fused to a nuclease as shown below in Figure 6 (Mussolino & Cathomen, 2012).

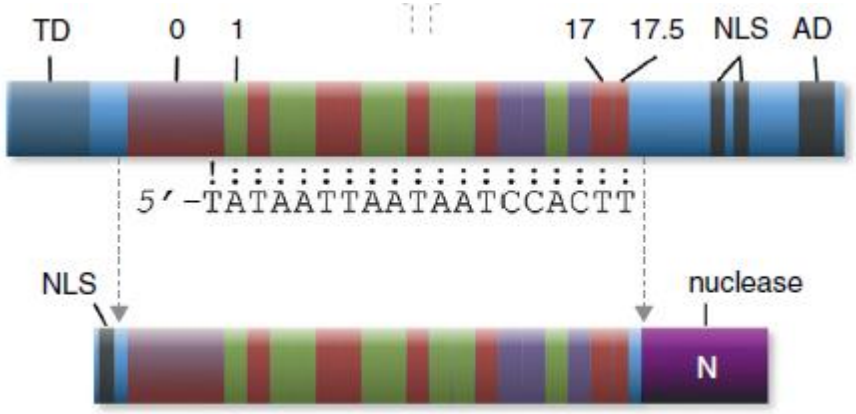

Figure 6: To create a TALEN, the repeat domain of a TAL effector is fused to a nuclease. TALEN consists of the repeat domain mediating DNA binding, the nuclear localization signal (NLS), and the nuclease. Picture from (Mussolino & Cathomen, 2012).

The nuclease monomer is separated from the binding domain with a short spacer sequence to give room for two TALEN monomers to dimerize and thus creating an active nuclease (Mussolino & Cathomen, 2012).

Assembly of TAL Effectors

The assembly of novel TAL effectors involves assembling the right sequence and the appropriate number of tandem repeats to match an intended target in the genome. Several methods for achieving this have been developed, including PCR-base methods, clonal methods and solid-phase based methods (Wei, et al., 2013). One of the most commonly mentioned methods is the Golden Gate cloning strategy. This is a method where two reaction steps are used to assemble the repeat units. The repeat units are first inserted into an assembly vector in shorter stretches and then assembled into a final vector to give the complete repeat domain (Cermak, et al., 2011). The amplification of the assembled product is achieved by clonal amplification in *E.coli* using blue/white screening. The method needs access to a library of the monomer repeats with all different RVDs. The

repeats are flanked by restriction sites that determine their position in the final repeat domain. In order to create a TAL effector, a library of all different combinations of repeats is thus necessary, 4 repeat units (one per possible nucleotide) for each position in the binding domain of the final protein (Cermak, et al., 2011).

In the reaction, Type IIS restriction endonucleases are used. These nucleases cleave outside their restriction sites, leaving 4 base-pair sticky-ends and removing the restriction sites (Weber, et al., 2011), (Cermak, et al., 2011). This allows the reaction to proceed with a ligation step in the same reaction mixture. The single repeat units that are to be used in the full length repetitive domain are mixed in the reaction mixture together with a preassembly vector, ligase and endonuclease. The repeat units are carefully chosen so that they all have different restriction sites that will allow the repeat units to be ligated together in a predetermined order. In this first step, the repeat units will be assembled into the preassembly vector. In the preassembly vector the repeat domain is flanked by other restriction sites that will allow for the whole repeat stretch to be released in a later step (Cermak, et al., 2011).

The preassembly vector contains a LacZ gene for blue/white screening and also a gene for Ampicillin resistance. A successfully assembled vector contains no Lac Z gene but has the gene for Ampicillin resistance and can thus be identified after cloning in a bacterial host (Weber, et al., 2011), (Cermak, et al., 2011). A schematic overview of this process is shown below in Figure 7 .

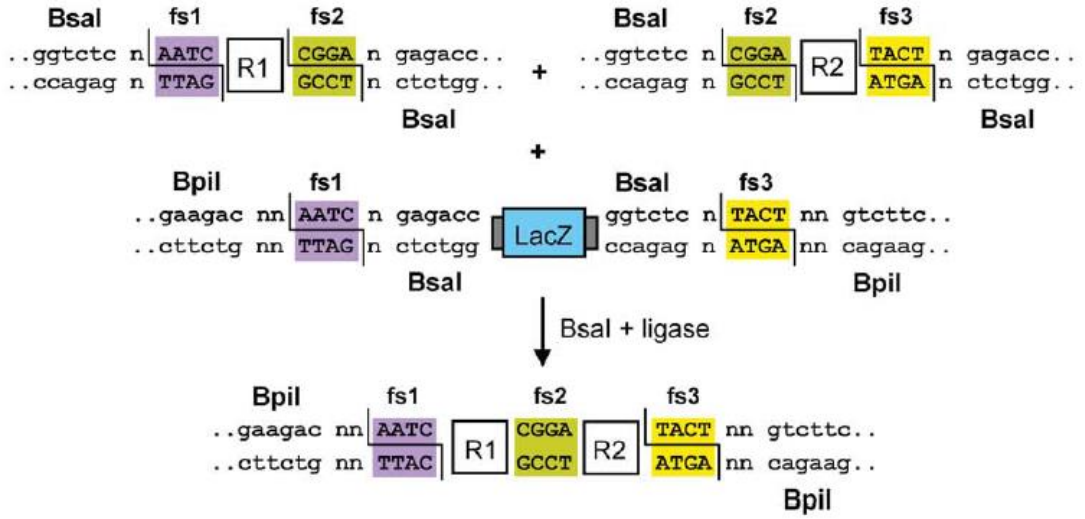

Figure 7: Assembly of single repeat units into a preassembly vector. The process is shown here for only two repeat units (R1 and R2) for simplicity. The single repeats are flanked by fusion sites (fs1, fs2, fs3) and BsaI restriction sites. Treatment with BsaI and ligase will connect the repeats to each other and assemble them into the preassembly vector. In the preassembly vector the assembled repeat domain will be flanked by other restriction sites; BpiI. The initial backbone of the preassembly vector contains a LacZ gene for blue/white selection, as well as an Ampicillin resistance gene. A successfully assembled vector contains no active LacZ gene but has the gene for Ampicillin resistance. Figure from (Weber, et al., 2011)

The successfully assembled vectors are amplified in *E.coli*. When creating an artificial TAL effector to be used as a TALEN, the repeat sequence is typically made up of about 19 repeats (Cermak, et al., 2011). The efficiency of the Golden Gate cloning procedure has been found not to be efficient when trying to create this full-length repeat domain at once. Instead the above described procedure is

used to create shorter repeat domains, containing 5-6 repeat units that can be assembled to form the final, full-length repeat stretch in the last step (Weber, et al., 2011), (Cermak, et al., 2011).

After all the preassembly vectors have been amplified they are added to a new mixture containing endonuclease, ligase and the final vector. The endonuclease will now cut out the repeat domains from each preassembly vector. Their restriction sites will have been designed in a way so that the different repeat domains will be assembled in the right order into the final vector (Cermak, et al., 2011). The final vector consists of the TAL effector backbone, including the 0th repeat and the final half repeat. It also has all the final units needed at the TAL effector N- and C-terminus. Like the preassembly vector, the final vector has a LacZ gene for blue/white screening as well as a gene for Streptomycin resistance. Correctly assembled, the final vector will not have the functional LacZ gene but has the gene for Streptomycin resistance which will allow for identification and selection of successfully assembled vectors after cloning. This second reaction step is shown below in Figure 8 (Weber, et al., 2011), (Cermak, et al., 2011).

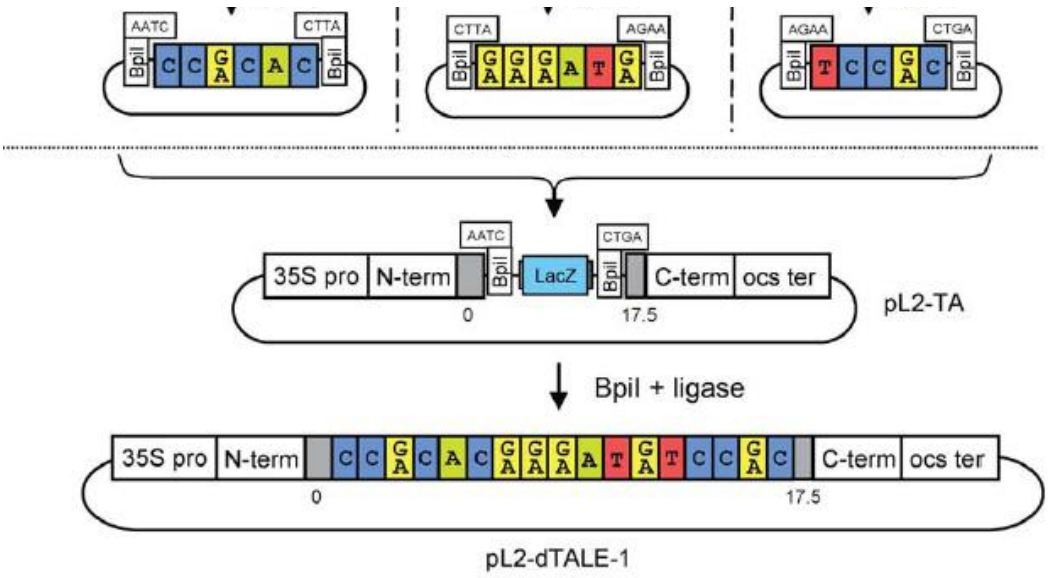

Figure 8: Second reaction step in the Golden Gate cloning procedure. Three successfully assembled preassembly vectors are mixed with the final vector (pL2-TA). The preassembly vectors (top) have restriction sites flanking the repeat domains that will mediate correct assembly into the final vector backbone (middle). The final vector has a LacZ gene, restriction sites corresponding to the preassembly vectors, the 0th repeat, the half repeat (here the 17.5th), as well as N- and C terminals and a 35S promoter. The final vector also contains a gene for Streptomycin resistance. The successfully assembled final vector (bottom) has no functional LacZ gene, has the complete repeat domain, and has the gene for Streptomycin resistance. The picture was adapted from (Weber, et al., 2011).

The final vector is amplified trough cloning in *E.coli* followed by assessment of repeat length and sequence. This can be done by cutting out the full repeat domain and running a gel electrophoresis followed by sequencing (Cermak, et al., 2011).

Variations to the Golden Gate cloning protocol exist where PCR is used to amplify the assembled repeat domains. There are different opinions regarding the accuracy of PCR amplification (Sanjana, et al., 2012). Cloning is also questioned as this is a more laborious and time consuming method compared to PCR (Sanjana, et al., 2012).The time needed to construct and verify a new TALEN has been approximated to 6-8 days using a PCR based approach whereas some more days may have to be added to a clonal procedure to allow for the clonal steps.

PCR methods and especially the Golden Gate clonal method have been accused of not being suitable for high-throughput production of TALENs. Instead, a solid-phase based method was suggested by (Reyon, et al., 2012). Fast Ligation-based Automatable Solid-phase High-throughput (FLASH) was suggested as a method that can produce repeat units of any length needed with possibility of high automatization and reduced cost. Using a library of plasmids coding for 1, 2, 3 and 4 repeats with every single combination of RVDs for each length, they showed that it is possible to create sequence validated TALE expression vectors in under 1 week (Reyon, et al., 2012). With this method, the cost of producing one pair of sequence validated TALEN plasmids could be lower than US$ 200 including the cost of labor (Reyon, et al., 2012). The method uses the library of the different repeat fragments (376 plasmids in total) that are iteratively assembled on solid-phase magnetic beads to obtain the required repeat length. With full automatization they were able to construct 96 different TALE repeat arrays in less than 1 day. However, the automatization required highly specified equipment, so a semi-automate method was also suggested where the same process would take 1-2 days (Reyon, et al., 2012).

Biotechnology in Potato Breeding Programs

Potato is one of the most important crops produced today. With about 330 million tons of produced potatoes per year it is the fourth biggest crop in the world (Gebhardt, 2013). The importance of potato as a staple-food in the world was exemplified by the famine disaster caused by Late Blight on Ireland in the early 19th century. Even today late blight caused by the oomycete *Phytophthora infestans* is perceived as the worst disease when cultivating potatoes (Oosumi, et al., 2009). Causing world-wide losses of billions of US-dollars each year it is a highly prioritized problem targeted in breeding programs (Slater, et al., 2008). Early attempts to breed away susceptibility to *P. Infestans* by introducing major disease resistance genes from potato cultivars with known resistance proved to be insufficient already in the middle of the 1900's (Oosumi, et al., 2009). Resistance based on single genes was quickly overcome by the pathogen and today *P. infestans* is mainly battled with fungicides. The efficiency of fungicides is however diminishing as fungi are developing better resistance to the fungicides (Oosumi, et al., 2009). The need to find new ways of improving crops through breeding is therefore of great importance.

Typically, breeding of potato is carried out by crossing elite potato lines and then screen thousands of seedlings for phenotypic characteristics to in the end arrive at a new variety (Lindhout, et al., 2011). Phenotypic breeding has evolved to be, in many cases, very efficient and new varieties of crops are produced every year based on traditional breeding methods. For some crops, potato being a good example, phenotypic breeding may however have some inbuilt shortcomings. Commercial potato varieties today mainly consist of tetraploid genotypes (Lindhout, et al., 2011). The tetraploid genome provides great opportunities for unwanted alleles to remain hidden during initial breeding generations when only phenotypic characterization is utilized (Lindhout, et al., 2011). After several generations the "bad" alleles may surface and can then cause delays in the breeding process. Since this has to be taken into account, the number of seedlings initially cultivated and screened can reach 100 000 and the time from initial crossing to a final new potato variety has been reported to take 10-12 years (Gebhardt, 2013), (Ortega & Lopez-Vizcon, 2012).

An analysis of utilizing marker technology in a traditional maize breeding program performed in 2003 showed that the possibility of shortening the time to develop a new maize variety by approximately 3 years translated into net gains of US$ 134 000 based on the earlier release of the

new variety (Morris, et al., 2003). Numbers showing the same result for other crops such as rice were also reported. Even though potato is a smaller crop world-wide this shows that there are possible economic gains from utilizing new techniques to further improve established breeding protocols. Also, apart from only being able to sell a new variety earlier, there may also be competitive advantages from introducing new varieties faster.

With conventional breeding, when crossing of elite lines is used to produce progeny with new combinations of traits, or where new traits are crossed into commercial varieties using wild relatives, there are limitations to what can be accomplished. The germplasm available for crossing may not contain all the material that is necessary to produce varieties of the highest value. It has also been proven to be problematic to fixate new traits in potato since it is very sensitive to inbreeding (Lindhout, et al., 2011). Also, in order to evaluate if a new trait has been successfully introduced through crossing, several years of cultivation is needed before the plants produces enough tubers to allow phenotypic analysis of certain traits (Gebhardt, 2013) (Slater, et al., 2013). Methods that allow for accurate and controllable introduction of new traits into elite potato lines could both reduce the time needed to introduce the new trait and also broaden the pool of traits possible to utilize.

Case Study: TALEN-mediated Development of a New Potato Variety

Mistra Biotech is an interdisciplinary research program that aims to use biotechnology to improve Swedish agriculture. The program involves several research areas such as biotechnology, economics and ethics. The goal is to develop new crop varieties and biotechnical tools for agriculture, both crops and livestock. Economical and ethical aspects of the use of biotechnology in agriculture are also evaluated in order to understand the economic impact of using new technologies in breeding as well as understanding consumer and public attitudes towards biotechnology. (Mistra Biotech, 2013)

One of the projects in Mistra Biotech is to use TALEN as a tool for improving disease resistance in potato. At this stage in the program, the method is being developed for application on potato and a protocol is on its way.

Results of the Interviews

For this thesis, access was given to the experimental protocols, the progress and the results that have been obtained up to this point of the project. Open interviews with scientists directly involved with the TALEN process and with good knowledge and experience with potato research and potato transformation were carried out. The interviews have been used to estimate the costs and possible benefits of using site-directed mutagenesis. The cost/benefit analysis has been done using a spreadsheet approach where costs of consumables and labor have been determined based on information from the interviews. The benefit calculation has been done using the same approach as in (Morris, et al., 2003) where the Net Present Value (NPV) was calculated. For the purpose of this thesis the NPV has been estimated using the same assumptions as in Morris, et al. The NPV has then been compared between the cases where TALEN are used and when a traditional breeding method is used.

With the use of TALEN the crop genome (here potato) can be altered by introducing site-directed mutations. As described above, these mutations can be of varying kind but most often they are so called indels (insertions or deletions). If the mutations are targeted to specific sites in the genome

affecting genes or regulatory domains, it is possible to reduce or enhance sensibility of the crop to pathogens, etc. This requires thorough knowledge of the genes and their phenotypic expression.

The process that is being developed in the Mistra Biotech program will be described followed by alternative methods that could give the same end results for comparison. The case study will be concluded by a discussion with conclusions about how the use of site-directed mutagenesis could change the competitive climate in the agrobiotech industry. This discussion will use results from the case study as well as the results obtained in the literature research which has been presented earlier.

The TALEN process used is based on transformation of protoplasts that are extracted from leaves. Much of what gives the timeline of the process has to do with the growing of plant material which starts with 4 weeks of plant propagation to obtain leaves and protoplasts. After the transformation the goal is to achieve seedlings with the desired mutation, a process that requires the development of *in vitro* plants from protoplasts. This will take another 26 weeks.

For the transformation and mutation, TALENs have to be constructed. The TALENs needed have been constructed using the Golden Gate method described earlier and according to the protocol found in (Cermak, et al., 2011). This method requires the use of plasmids, enzymes, antibiotics and other chemicals. The plasmids needed for the Golden Gate reactions (the plasmids containing intermediate vectors and the repeat monomers) can be purchased as kits. The rest of the material (ligases, restriction enzymes, antibiotics, plant expression plasmids, etc.) have to be purchased separately. The TALENs have been validated by sequencing which has been done externally. Several different samples need to be sequenced over the whole process and these are collected and sequenced on a 96-well plate. During the process PCR is carried out. This requires the use of primers which are bought externally. The expression plasmids needed to transfect protoplasts need to be obtained elsewhere since they are not included in the Gateway kit. In this process these plasmids were available in-house and thus came at no extra cost. It was however suggested that plasmids could be acquired at a cost of about 5000 SEK per 2-3 plasmids. This would add 5000 SEK to the process. The price details have been obtained from supplier websites (Fermentas, 2013), (Sigma-Aldrich, 2013) where they were given without taxes. The prices are also based on the quantities that where purchased in this particular process which usually were the smaller quantities giving a higher price/unit and the total process price is thus in the upper range of a cost estimation. The total cost including taxes is given with an added 25% in taxes. When the prices were given in USD this has been converted into SEK using the average exchange rate 6,49 SEK/USD.[1] The conversion between SEK and EUR has been done using the exchange rate 8,60 SEK/EUR.[2] Table 3 below summarizes the material costs of constructing one TALEN pair using the protocol in (Cermak, et al., 2011).

[1] Average exchange rate SEK/USD as given by Riksbanken for October 2013.
[2] Average exchange rate SEK/EUR as given by Riksbanken for October 2013.

	Cost (SEK)	Cost (EUR)
Golden Gate kit & extraction kits	4228	492
Enzymes & primers	998	116
Chemicals & antibiotics	329	38
Sequencing	4000	465
Expression plasmids	Available in-house, otherwise: 5000	Available in-house, otherwise: 581
Total cost	9555 or 14 555	1111 or 1692
Total cost inc. tax	11 944 or 18 194	1389 or 2116

Table 3: Material costs for the construction of a TALEN pair.

The transformation of the protoplasts and the growing of shoots were done according to an experimental and confidential protocol . This involved the use of several media and stock solutions in order to carry out the transformation and then mediate plant growth and propagation. The components are usually prepared in large volumes and then used for several different experiments or at different occasions. In the cost calculations the cost of using only part of the stock solutions has therefore been considered. For some of the costs however, larger volumes than what was used in the process have been used and the cost estimation may therefore be a little high. Also here the prices and exchange rates are based on the same sources as above.

	Cost (SEK)	Cost (EUR)
Chemicals	1174	137
Total cost inc. taxes	1467	171

Table 4: Transformation and growing of protoplasts.

The labor needed for the process is based on estimations of time requirements for the different steps. These estimations are based on the successful transformation of protoplasts according to the method protocol that has been established so far. The actual time that has been used in developing this protocol is however longer since much time has been spent on gaining experience with the methods and doing research before starting the lab work. This protocol is under development as it needs optimization to reach e.g. higher transformation frequency. It was also pointed out that while the process described here involves the construction of one TALEN pair, more TALENs could be produced in parallel if needed, thus reducing the average labor cost per TALEN. The labor presented in Table 5 below shows the time used for hands-on work in the lab when preparing stock solutions and running the reaction steps and analyses needed. The actual time for completing the process is however longer due to the time needed for cultivation of bacteria, cultivation of plant cells and the actual reaction times. The costs are based on the average salary for a postdoc at a Swedish university, 33 000SEK (SACO, 2013). The payroll tax 31,42% has been added (Skatteverket, 2013).

Process Step	Time (Hours)	Cost (SEK)	Cost (EUR)
TALEN construction	60	16 263	1891
Protoplast transformation & plant growth	43	11 655	1355
Research	400	108 420	12 607
Total	503	136 340	15 853

Table 5: Hands-on work needed for the development of a plant variety using TALEN and the corresponding cost.

When summing up the costs for material and labor from the tables above the cost of the total process amounts to:

	Cost (SEK)	Cost (EUR)
Total cost of the process	149 751	17 413

Table 6: Total cost for the development of a plant variety using TALEN. The cost includes taxes and the time needed to research the target gene.

Additional work was put into researching the method of using and constructing TALENs, etc. This has however been omitted as this is a cost inferred only once and then shared by all other activities afterwards. In the case of only producing one TALEN pair and use it for one protoplast transformation, this would add several months of extra labor cost to the process. In a more realistic case however, this cost would be inferred just once (sunk cost) and then be spread out on all activities giving a different cost/TALEN. Since labor cost is the single largest cost, this clearly shows how economies of scale can be reaped if the process would be used commercially.

The process described here is a pilot process carried out in order to evaluate and establish a methodology of transforming potato protoplasts using TALEN. After *in vitro* plants have been developed, these have to be screened in order to find successful mutants and these mutants have to be sequenced in order to prove the mutation. This will add additional cost to the process. This cost will however depend heavily on the mutagenesis efficiency which is currently not optimized. Accurate estimations of the cost of identifying and sequencing mutants can therefore not be made at this point in the process but it will add the cost of sequencing shown above in Table 3 times a number of plants that will be sequenced. The use of machines, glassware, pipette-tips, etc. has not been included since this could not be accurately estimated. In the interviews it was mentioned that this cost could be kept very low, referring to the possibility of leasing space in the research facility with access to all necessary machines and equipment for about 3000 SEK/month (€349/month) when doing research.

The process described has been chosen and designed in a way so that the final result should not be a GMO. The expression plasmid that is inserted into the protoplast is delivered in a way so that it is not incorporated in the plant genome which can be the case when e.g. Agrobacterium is used for the transformation. Instead, a transient expression of the TALEN plasmid is achieved which has some benefits: The transient expression means that the final crop will not carry any foreign DNA which should keep it from being classified as a GMO. Also, the transient expression assures that stable mutants will be produced. As long as the TALEN plasmid is expressed, TALENs will be produced that will create mutations. If the expression is not transient, TALENs will be produced that may reverse

already acquired mutations or keep on mutating the genome past the desired point. If instead the expression is transient, the plasmid will disappear after some days and no more TALENs will be produced. This will give stable mutants which are carrying the mutations that have been induced while the TALENs were expressed.

After mutants have been identified and sequenced, the *in vitro* plants would proceed to field trials in order to evaluate performance in the field. This part of the process is however the same as for other breeding methods where a newly developed variety has to go through field trials in order to evaluate the commercial performance of the variety. Since this will be the same process regardless of the technique used for developing the new variety (techniques resulting in GMOs are excluded) this cost is not considered as it would be the same in all cases.

In a best case scenario a new potato variety could be developed up to the point of field trials in less than 1 year taking into account the time it takes to develop plants, allowing for plant growth etc. Using potato as an example this is however probably only just a best case scenario due to the tetraploid genome. Depending on the trait that is developed, this infers the possibility of having to target up to four alleles for each new trait or mutation. The numbers of alleles that have to be targeted depend on the trait, and the number of alleles that can be targeted at once using TALEN remains to be determined. If all four alleles have to be targeted, up to 4 transformation events could be needed for one locus. This could possibly prolong the process fourfold. This is however purely speculative since this part of the process has not yet been evaluated and many more parameters may arise that has to be taken into consideration before doing these kinds of estimations.

Even though the process has been designed not to yield a GMO as the end-result, GMOs are used in the developing process, e.g. for the construction of TALENs. The use of GMOs in closed settings is also regulated and requires special permissions. These permissions regard both the facilities in which the research takes place and also the crops and traits that are being developed. The permission for the facilities covers the general use of GMOs in those facilities whereas permission for crops and traits need to be obtained for the particular cases. The permissions are (in Sweden) granted by the Swedish Board of Agriculture (Jordbruksverket) (Jordbruksverket (Swedish Board of Agricuture), 2013). The costs are stated on their website and are shown below in Table 7.

Permission	Cost (SEK)	Cost (EUR)
Facilities	3000	349
Species	5000/specie	582/specie
Traits	2000/trait	233s/trait

Table 7: Costs of permissions for the use of GMOs in closed environments (Jordbruksverket (Swedish Board of Agricuture), 2013).

The application fees are quite low and for common crops such as potato not a lot of data needs to be provided in the application since the crop is well known. Information of the trait needs to be enclosed which requires good knowledge of the planned process, this should however be covered by the time it takes to e.g. research the gene construct. The permission for facilities is valid not only for one single crop or process but is a general permission that is granted for the use of GMOs in that facility. This means a cost like this can be spread over several products or processes.

Comparison with conventional breeding programs

If site-directed mutagenesis would not be used to alter the genome, the same or similar results could also be obtained using traditional crossing methods, chemical mutagenesis or transgenesis.

The use of a GM technology where the result would be a GMO infers the addition of the regulatory process and costs of commercializing a GMO to the cost of breeding a new crop variety. As shown earlier, these costs are too onerous to bear for a Swedish company or a company other than very big multinational companies and will therefore not be further considered. If instead traditional crossing methods would be used, several aspects need to be considered. First of all; in order to introduce a change in the genome of a recipient plant, this change has to be found in a donor plant. This limits the possible results that can be achieved using crossing to the variability that is found in the compatible donor pool. In order to find the right donor, an extensive screening process is needed. After this initial identification, the donor and the recipient would be crossed. This is usually done by crossing a large number of individuals which are then screened in order to find individuals where the crossing has been successful and the desired trait has been introduced (Lindhout, et al., 2011) (Ortega & Lopez-Vizcon, 2012). The crossing does however not only introduce the specific and desired change; it will also introduce other changes throughout the genome. When the goal is to enhance an elite crop line the genome of the offspring should be as close to the recipient genome as possible except from in the gene that is being altered. This requires several rounds of backcrossing in order to restore the background genome to that of the recipient. The backcrossing progress can be assessed either by observing the phenotype or by using molecular markers. Depending on how many changes that are introduced, the time of this process will vary. It has been found that at least 6 generations of backcrossing should be done when using phenotypic assessment (Hospital, 2003), (Morris, et al., 2003). Each generation of backcrossing will add time to the process and in the case of potato, each generation corresponds to about one year. This would give a total of 6 years to introduce a modification into the genome of potato using traditional crossing methods with backcrossing and phenotypic assessment. If molecular markers are employed, the number of backcross generations can be reduced. This will however depend on how large the population is that will be genotyped and this in turn should depend on the cost of using a specific marker platform. Through simulations and cost assumptions, it has been found that at least 3-4 generations of backcrossing should be considered if genetic markers are used in order to keep the population size down to manageable proportions (Hospital, 2003). This would shorten the traditional phenotypic process with about 2 years. The cost of applying marker-assisted selection in a potato breeding program has been estimated in (Slater, et al., 2013). They evaluated a potato breeding process and compared the cost of using phenotypic screening and marker-assisted screening. The process was modeled for trials where the aim was to introduce resistance to potato cyst nematodes (PCN) or virus resistance. The model is based on a 3 generation long selection. In order to simulate common breeding programs, large numbers of seedlings were initially planted and crossed and then selected with different intensity. The results from their models are shown below in Table 8 where the conversion from AUD to EUR has been done using the exchange rate 0,669[3]. The numbers of seedlings correspond to different intensity of the selection which was 2%, 10% and 30% respectively. The seedling numbers were chosen to give the same number of genotypes in the second generation (Slater, et al., 2013).

[3] Exchange rate for AUD/EUR from NASDAQ OMX, 2013-11-29.

	Conventional screening			Marker-assisted screening		
Number of seedlings	100 000	20 000	6 667	100 000	20 000	6 667
Trial cost (AUS$)	633 040	257 248	194 618	605 556	229 764	167 133
Trial cost (€)	423 504	172 099	130 199	405 117	153 712	111 811

Table 8: Estimated trial costs in a potato breeding program using conventional or marker-assisted breeding (Slater, et al., 2013).

The trial cost for both approaches shown in Table 8 are much higher than the suggested estimation for the TALEN process shown above. The TALEN process does lack some costs as pointed out and the mutation frequency has the potential to raise the cost, as do the number of alleles that have to be targeted separately. The major cost found in all studies is however the cost of labor which increases with the length of the breeding program and the number of analyses that has to be done. In the conventional cross breeding program (both with marker-assisted screening and phenotypic screening) a much larger population of plants has to be handled. This should infer more labor and a higher cost than for the TALEN process even though the program lengths would be the same.

Chemical or physical mutagenesis could also be used. Here the target plant is treated with a chemical agent or radiation in order to introduce mutations. This will introduce random mutations in the whole genome and screening and backcrossing will be needed just like in the case of crossbreeding. The benefit with this kind of mutagenesis is that the mutation that will be introduced does not need to be found in a gene pool, but can be created by random mutations.

Benefits of Early Release: Net Present Value

The benefit of releasing a new variety on the market early can be visualized by comparing the Net Present Value (NPV) of doing so. This was done by (Morris, et al., 2003) for a maize breeding program. The same assumptions about adoption of a new crop variety and the benefits thereof are made in this thesis as this is stated to be reasonable assumptions for medium-sized breeding programs and common field crops (Morris, et al., 2003). Even though the size of a Swedish breeding program might be smaller and potato is smaller than other field crops such as maize and rice the assumptions are useful to give a ground for a discussion.

Maximum area planted	10 000ha
Time to reach maximum adoption	4 Years
Time to complete disadoption	10 Years from initial adoption
Net benefits/ha	25 USD = 162 SEK = 17 EUR

Table 9: Assumptions made when calculating the NPV. The assumptions are the same as in (Morris, et al., 2003). The same exchange rates as stated earlier have been used to convert USD to SEK and EUR.

The NPVs calculated in Table 10 below have been calculated using the above assumptions and a discount factor of 5%. This 5% has been chosen arbitrary to allow for some compensation for risk

and also not to choose the current interest rate form the national bank which is close to 0% which does not give any good calculations.

Time to initial release (years)	NPV (SEK)	NPV (EUR)
5	5 665 188	658 743
8	4 893 802	569 047
10	4 438 823	516 142

Table 10: Net present values of the release of a new variety of a common crop.

Table 10 clearly shows one important benefit of being able to release new varieties early. In the best case scenario when a new variety could be developed in about 1 year and compared to a traditional breeding process with backcrossing and phenotypic selection, the benefit of using TALEN is more than € 140 000. The underlying assumption here is that the mutation is done on an elite line where no backcrossing is needed when using site-directed mutagenesis. The additional time (4 years) to release is added to account for field trials and other activities prior to commercial release and adoption. The 10 years to release is thus the average 6 years that has been reported as a minimum value for a breeding program relying on phenotypic selection and repeated backcrossing (Hospital, 2003), (Morris, et al., 2003). The 8 year value is the value for a scenario where more alleles are targeted with site-directed mutagenesis and this prolongs the development process to 4 years. This is also a reasonable time-to-release of a variety if a breeding program with backcrossing would utilize markers for the screening process (Hospital, 2003), (Morris, et al., 2003).

Patent Situation for the Commercial Use of the TALEN technology in Plants

The patent for the use of TAL-effectors to produce commercial varieties of crops belongs to the Two Blades Foundation. This is a charitable organization with the aim “to support the use of safe, environmentally-benign and sustainable strategies for crop production so as to provide long-term protection from crop losses due to plant disease.” (Two Blades Foundation, 2013). An interview with one of the representatives of the foundation has been performed in which it was concluded that the foundation whishes to enable the use of the TALEN technology rather than hinder its adoption due to expensive patents and licenses. According to the interviewee; If TAL effectors are used to produce commercial crop varieties a license needs to be purchased. This is done as a one-time payment where the actual cost depends on the licensee. Several non-exclusive licenses have been granted to different companies, among them also big multinationals (The Two Baldes Foundation, 2013). In the case of smaller companies, it was stated that there has not been a problem in negotiating a mutually acceptable fee. It is also possible to adapt the fee to the needs of a particular applicant with regards to the number of crops or traits that are being developed or if the process is in its research stage or its commercial stage. When Zinc Fingers were developed, their use in crop biotechnology was restricted by patents that were held by a commercial company. The cost of using the ZF-technology is expensive due to the licensing costs which has led this technology to be used very restrictive in plant biotechnology. By holding the patent for the commercial use of TAL effectors in plant biotechnology, the Two Blades Foundation hopes to avoid such a development for this technology as they view it as a technique that can benefit plant breeding.

In terms of licensing costs of using TALEN for breeding purposes this has not been estimated as it will (according to the interview) differ from case to case. The spirit of the patent-holder does however

suggest that this should not stand in the way of the technique being chosen for commercial development of crop varieties.

Discussion & Conclusions

This thesis has covered the regulatory aspects of using biotechnology in plant breeding and also shown the possible economic impacts of the use of biotechnology in plant breeding. The regulatory review which also covered estimations of the costs incurred by the regulations and the regulatory process showed that the regulations have created a barrier to entry into the agrobiotech industry. Without sufficient capital to be able to handle the regulatory costs and also to sustain a lengthy approval process, a company cannot compete in this industry which is dominated by companies capable of using transgenic approaches to developing new crop varieties. The cost of commercializing GM-crops has also led to biotechnology (transgenesis) being used on a few common field crops. Also for large companies, the cost of using transgenic breeding methods is too large for this method to be applied to smaller crops, e.g. horticultural crops. From a European perspective, this has led to the European Union being a market where GMOs are not being commercialized. This is due to both a public resistance but also to the regulatory process which has discouraged global companies from performing research in or for the European market. The absence of the biggest multinational industry actors may have several effects. Since the research is not aimed at the EU, crops developed by companies such as Monsanto or BASF Plant Science will not be adapted to European agriculture. These companies invest large amounts of money in researching new crop varieties with new traits, and potential losses of not having these crops adapted to the EU remains to be evaluated. Returning to one of the core questions of this thesis, the difference in how GMOs are treated in the EU compared to e.g. North America may have competitive consequences for Europe and European companies. Since GMOs are hard and expensive to commercialize in Europe, not much research is done on this. For European breeders adapted and well experienced in conventional breeding methods, this may give a local advantage over firms focusing on global competition. It does also infer that European companies are late in accumulating knowledge in technologies involving genetic modifications and they may also be resistant to try new methods that are being developed and where the regulatory status is unclear. The late start or lack of experience give a considerable competitive disadvantage to European companies if GMO crops would be looser regulated in the future or if new technologies in the grey area between GMO-producing and non-GMO-producing would become popular. The head-start of climbing the experience curve was (as stated before) according to Monsanto, one of the reasons why they have managed to become leaders in the industry. From the perspective of the competitive theory of Porter, an experience curve can also be a powerful barrier to entry, along with high regulatory costs.

The case study where the potential of site-directed mutagenesis was explored showed that this method has a potential of greatly shortening the plant-breeding process, reduce costs and increase revenues. This is of course based on many assumptions but the potential should be clear. The implications of the result could be several. From the view of a company focusing on the European market (e.g. a Swedish breeder) the adoption of the technique could give several competitive advantages when competing with breeders employing conventional breeding and backcrossing. The ability to develop new varieties faster than the competitors gives a dynamic advantage: A company able to produce new varieties faster than the competition could always reap the benefits of being a first-mover which e.g. includes obtaining a larger market share. A faster development process also

infers that a company could produce more different varieties than the competition which can give advantages in the form of patents or protection of new varieties. If new traits are being developed and genes are successfully modified, it may be possible to obtain patents for the final product and also process patents which could prove to be valuable.

Another scenario could also be imagined if methods such as site-directed mutagenesis prove to be commercially successful. Given the history of the agrobiotech industry and the habit of multinational companies to buy their way into the industry, the possibility of successful European companies being acquired by larger companies should be high. If European companies would adopt methods such as the TALEN technology and be successful in commercializing valuable new varieties, they may catch the attention of the companies that abandoned their European research. The European companies should then make for interesting targets for acquisition if the larger companies want to make their way back to the European market.

It is of course also a possibility that the same multinational companies simply develop the techniques and skills themselves and use them to go around the GMO regulations in order to return to the European market. This would force smaller European companies to compete with the multinational companies who can reap benefits from their scales. This may lead to a similar development as in the early stage of the agrobiotech industry where smaller companies were observed to take on strategies compatible with the larger companies'.

One benefit of using site-directed mutagenesis that is indicated is the lower cost compared to conventional methods due to the possibility of running breeding programs of smaller scale. The lower cost for a company can give important competitive advantages. The price a company can charge for its products depends (among other things) on the cost of producing said product and the price charged by competitors. If the product is a commodity product or if the consumer is more interested in the price of the product than in its differences to other products, price is likely to be determined by the company with the lowest cost. A low cost competitor can set a price low enough to squeeze the margins of other competitors or use its low cost to increase its own profit margins given the present price situation in the industry. One possible competitive (and devastative according to Porter) scenario in and industry is when competition is focused on price and each actor tries to improve their market share by reducing its price. This may cause a price war which reduces profit margins of everyone in the industry and a low cost competitor will then be better equipped to sustain such a scenario. Pursuing a low cost strategy is also a good protection against new entrants that may enter the industry and reduce prices.

The conclusions seem to be that the regulatory climate with regards to the commercialization of GMOs has caused large barriers to entry in the agrobiotech industry. Only companies big enough can sustain the lengthy approval process and afford to produce the data needed in the risk assessment.

The coming of new biotechnical techniques that aim to improve agriculture and circumvent the regulations may alter the competitive conditions in the industry. Site-directed mutagenesis has the potential to reduce costs and save time which could allow more small or medium sized breeding companies to compete in the agrobiotech industry. It may however also lead to further consolidation of the industry if the same small or medium sized companies become targets for acquisition of multinational companies. The technology could also be used directly by large

companies to regain their interest in the European market which would force European breeders to compete with multinational companies.

Much of the work done in this thesis is based on one breeding process that is being developed and it would be very interesting to return and evaluate the process once it has been completed. It was also indicated in the interviews that other methods than the TALEN method are gaining interest and it would be interesting to look at these ones as well. A more thorough analysis of the economic impact of using new technologies would also be interesting. This thesis focused on the cost/benefit analysis of a company that was to adopt the technology and a study where the economic impact in other parts of the value chain would also be interesting to perform.

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References

Arundel, A., 2002. Agro-biotechnology, innovation and employment. *Science and public policy,* Volume 29(4), pp. 297-306.

Baker, M., 2012. Gene Editing Nucleases. *Nature Methods,* 9(1), pp. 23-26.

BASF, 2012. *Plant Biotechnology at BASF.* [Online]
Available at: http://www.basf.com/group/corporate/en/products-and-industries/biotechnology/plant-biotechnology/index
[Accessed 27 11 2013].

Bijman, J. & Tait, J., 2002. Public policies influencing innovation in the agrochemical, biotechnology and seed industries. *Science and Public Policy,* Volume 29(4), pp. 245-251.

Boch, J. & al, e., 2009. Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors. *Science,* Volume 326, pp. 1509-1512.

Bogdanove, A. J. & Voytas, D. F., 2011. TAL Effectors: Customizable Proteins for DNA Targeting. *Science,* Volume 333, pp. 1843-1846.

Bogdanove, A., Schormack, S. & Lahaye, T., 2010. TAL effectors: finding plant genes for disease and defense. *Current Opinion in Plant Biology,* Issue 13, pp. 394-401.

Brennan, M., Pray, C. & Courtmanche, A., 1999. *Impact of Industry Concenration on Innovation in U.S. Plant Biotech Industry.* Washington, DC, s.n.

Cermak, T. et al., 2011. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Research,* 39(12), pp. 1-11, e82.

Chataway, J., Tait, J. & Wield, D., 2004. Understanding company R&D strategies in agro-biotechnology: trajectories and blindspots. *Research Policy,* Volume 33, pp. 1041-1057.

Chataway, J., Tait, J. & Wield, D., 2006. The governance of agro- and pharmaceutical biotechnology innovation: Public policy and industrial strategy. *Technology Analysis and Strategic Management,* Volume 18(2), pp. 169-185.

EFSA Panel on Genetically Modified Organisms (GMO), 2010. Guidance on the environmental risk assessment of genetically modified plants.. *EFSA Journal,* 11(8), pp. 1-111.

EFSA Panel on Genetically Modified Organisms (GMO), 2012. Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. *EFSA Journal,* 10(2943).

EPEC, 2009. *Evaluation of the EU legislative Framework in the Field of Cultivation of GMOs under Directive 2001/18/EC and Regulation(EC) No 1829/2003 and marketing of their other uses under Directive 2001/18/EC (Interim report),* s.l.: s.n.

Fermentas, 2013. *Fermentas Molecular Biology Tools.* [Online]
Available at: http://www.thermoscientificbio.com/fermentas/
[Accessed 23 10 2013].

Food Chain Evaluation Consortium, 2010. *Evaluation of the EU legislative framework in the field of GM food and feed,* s.l.: s.n.

Fulton, M. & Giannakas, K., 2001. Agricultural Biotechnology and Industry Structure. *AgBioForum,* Volume 4(2), pp. 137-151.

Gebhardt, C., 2013. Bridging the gap between genome analysis and precision breeding in potato. *Trends in Genetics,* 29(4), pp. 248-256.

Gravalos, E., Garcia, A. & Barnes, N., 2002. Policy influences on innovation strategies of small and medium enterprises in the agrochemical, seed and plant biotechnology sectors. *Science and Public Policy,* Volume 29(4), pp. 277-285.

Hospital, F., 2003. Marker-assisted breeding. In: H. J. Newbury, ed. *Plant Molecular breeding.* London: Blackwell Scientific, pp. 30-56.

Jordbruksverket (Swedish Board of Agricuture), 2013. *Jordbruksverket.* [Online]
Available at: www.jordbruksverket.se
[Accessed 19 11 2013].

Kalaitzandonakes, N., Alston, J. & Bradford, K., 2007. Compliance costs for regulatory approval of new biotech crops. *Nature Bitechnology,* Volume 25(5), pp. 509-511.

Levidow, L., Oreszczyn, S., Assouline, G. & Joly, P.-B., 2002. Industry responses to the European controversy over agricultural biotechnology. *Science and Public Policy,* Volume 29(4), pp. 267-275.

Lindhout, P. et al., 2011. Towards F1 Hybrid Seed Potato Breeding. *Potato Research,* Volume 54, pp. 310-312.

Lusser, M., Parisi, C., Plan, D. & Rodriguez-Cereso, R., 2011. *New plant breeding techniques State-of-the-art and prospects for commercial development,* s.l.: European Union.

McElroy, D., 2003. Sustaining agrobiotechnology through lean times. *Nature Biotechnology,* Volume 21(9), pp. 996-1002.

Miller, H., 1996. Comment: Biotechnology giants lobby for overregulation. *Biotechnology Law Report,* Volume 15(6), p. 949.

Mistra Biotech, 2013. *Start SLU/Mistra Biotech.* [Online]
Available at: http://www.slu.se/en/collaborative-centres-and-projects/mistra-biotech/
[Accessed 28 11 2013].

Monsanto, 2003. *Annual Report,* Missouri, USA: s.n.

Morris, M., Dreher, K., Ribaut, J. & Khairallah, M., 2003. Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. *Molecular Breeding,* Volume 11, pp. 235-247.

Moscow, M. J. & Bogdanowe, A. J., 2009. A Simple Cipher Governs DNA Recognition by TAL Effectors. *Science,* Volume 326, p. 1501.

Mussolino, C. & Cathomen, T., 2012. TALE nucleases: tailored genome engineering made easy. *Current Opinion in Biotechnology,* Issue 23, pp. 644-650.

Oosumi, T. et al., 2009. Gene Rpi-bt1 from Solanum bulbocastanum Confers Resistance to Late Blight in Transgenic Potatoes. *American Journal of Potato Research,* Volume 86, pp. 456-465.

Ortega, F. & Lopez-Vizcon, C., 2012. Application of Molecular Marker-Assisted Selection (MAS) for Disease Resistance in a Practical Potato Breeding Programme. *Potato Research,* Volume 55, pp. 1-13.

Porter, M. E., 2004. *Competitive Strategy.* s.l.:Free Press, Simon & Schuster Inc.

Redenbaugh, K. & McHughen, A., 2004. Regulatory challenges reduce opportunities for horticultural biotechnology. *California Agriculture,* Volume 58(2), pp. 106-115.

Reyon, D. et al., 2012. FLASH assembly of TALENs for high-throughput genome editing. *Nature Biotechnology,* 30(5), pp. 460-465.

SACO, 2013. *Lärare/forskare vid universitet och högskola.* [Online]
Available at: http://www.saco.se/Yrken-A-O/Larareforskare-vid-universitet-och-hogskola/
[Accessed 24 10 2013].

Sanjana, N. E. et al., 2012. A transcription activator-like effector toolbox for genome engineering. *Nature Protocols,* 7(1), pp. 171-192.

Sigma-Aldrich, 2013. *Sigma-Aldrich Sweden.* [Online]
Available at: http://www.sigmaaldrich.com/sweden.html
[Accessed 23 10 2013].

Slater, A., Scott, N. W. & Fowler, M. R., 2008. *Plant Biotechnology.* 2nd ed. ed. s.l.:Oxford University Press.

Slater, A. T., Cogan, N. O. I. & Forster, J. W., 2013. Cost analysis of the application of marker-assisted selection in potato breeding. *Molecular Breeding,* Volume 32, pp. 299-310.

Syngenta, 2000. *Annual Review,* Basel, Switzerland: Syngenta International AG.

Tait, J., Chataway, J. & Wield, D., 2002. The life science industry sector: evolution of agro-biotechnology in Europe. *Science and Public Policy,* Volume 29(4), pp. 253-258.

Tait, J. e. a., 2004. *PITA project - Policy influences on technology for agriculture - chemicals, biotechnology and seeds,* s.l.: s.n.

Two Blades Foundation, 2013. *Two Blades Foundation.* [Online]
Available at: http://2blades.org/
[Accessed 26 11 2013].

Weber, E. et al., 2011. Assembly of Designer TAL Effectors by Golden Gate Cloning. *PLoS ONE,* 6(5), pp. 1-5.

Wei, C. et al., 2013. TALEN or Cas9 e Rapid, Efficient and Specific Choices for Genome Modifications. *Journal of Genetics and Genomics,* Volume 40, pp. 281-289.