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Patents and genetically modified soybean for glyphosate resistance

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ABSTRACT

The last patents protecting glyphosate herbicide tolerant soybean (Roundup Ready) expired in 2015 (US4940835, US5188642, US5804425, US5312910, US5352605, US5530196, US5627061, US5633435, US5717084, US5728925). This opens up a very big market for generic soybean. In this paper an overview of patent status will be developed which will offer records of claims, dates and owners in different world regions, including our own experience in transgenic soybean development and implementation of an open operating system will be an opportunity for applications developers from the perspective of the emerging generic transgenic crops agenda.

1. Introduction

Soybean (*Glycine max*) is a legume plant (Fabaceae) and it has been used in food and feed products [1]. Soybean is one of the most important world commodities produced and consumed around the world after rice and wheat. Most soybean production occurs in United States (~89483000 ton/y), Brazil (~81699787 ton/y) and Argentina (~49306201 ton/y) [2]. In the recent years the soybeans are being analyzed, studied, developed and enhanced by many research works, promoting an incessant increase of desirable phenotypes: higher production, stress tolerance, nitrogen fixation or seed quality. This genetic improvement is led by the increase in production, up 31.4 kg/ha on an annualized basis. This substantial progress in recent years was explained by the increase in the number and varieties of genetically modified (GM) soybean, principally glyphosate/glufosinate tolerance, oleic/stearidonic acids production, and pest insect resistance traits among others [3–6]. Herbicide tolerance is the most important trait in planted GM soybean, giving the farmers an opportunity to take part in a different approach for better, improved growing of GM soybean's performance in a certain environment with greater profits (e.g. Latin America produces 33 percent of the world total of herbicide-tolerant soybean) [6,7].

A gene codifying for a 5-enolpyruvylshikimate-3-phosphate synthase derived from CP4 strain of *Agrobacterium*, (named *cp4 epsps*) was used into soybean, so crops expressing this enzyme have glyphosate tolerance [8]. That GM soybean technology is protected by patents that include elements such as DNA sequences, vectors, bacterial strains, plant varieties, plant transformation protocols, etc. There are two types of patents applied to GM soybean production: utility (invention or novel discovery) and plant breeder rights. The GM plant patents evaluation provides a framework that generates knowledge, promotes learning and guides action as an important means of competitive advantage and sustainability of results. Fortunately regulations on GM plant patents and their requirements are very similar among countries, for example United States, Latin America or China have promoted plant patenting, plant breeder rights, assessment of possible risks to the environment and human health (laboratory and field tests, and biosafety documentation) before the marketing of the GM plant is authorised [for review see: 9,10,11].

When the GM plant patent expires, the biotechnological invention ceases to be covered by intellectual property law and then falls into the public domain and any person can use the GM

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product, without the prerequisite of obtaining authorization from its owner. In terms of lawmaking, the first step involves interpreting claims on intellectual property in a way that is favourable to the society determining whether the patent is valid or whether the patent has been infringed. A Bolar exemption will offer the opportunity to test and develop the technology and meet the regulatory requirements to avoid delaying the access of GM generics to the market after the expiration of the patent. In the field of GM plants, upon expiration of a patent, a grower can produce a generic GM crop as long as the GM product meets regulatory requirements [12]. In 2014, the first patents on GM plants were reported to have expired. The glyphosate tolerance trait (Roundup Ready soybean) patent expired in 2015 [13]. When a patent expires, the price of the GM product falls depending on the number of generics that enter the market, for example, a generic drug is normally substantially cheaper than an analog patented product. Regulatory schemes governing patent expiration are essential to generic manufacturers [14,15]. Despite what has been patented about GM soybean, there is only a small body of literature that attempts to devise means of measuring the topic and its perspectives. A paper was presented that analyzed the Canadian regulatory framework related with risk assessment for canola, corn and soybean [16], a strategic document shows the terminator technology discussion involving soybean among others GM plants [17], a paper [15] raises the uncertain prospect that any GM generic crop could be included into Generic Event Marketability and Access Agreement (GEMAA) as an agreement on access to the biosafety results for commerce concerns, a fourth article deals with the implications, from a legal perspective, of GM soybean with glyphosate tolerance in Brazil [18], finally a publication shows the Monsanto legal suit against Cargill for importation of soybean meal, derived from GM soybean containing a *epsps* gene to United Kingdom [19]. Our group seeks to contribute to such a discussion, and this paper accordingly examines the state and claims of patents protecting GM soybean for glyphosate tolerance including our technical expertise.

2. Methods

Data were recovered from The Lens suite (https://www.lens.org/lens/). The Lens is an integrated initiative comprising the European Patent Office DOCDB bibliographic data (1907–2016) and grants (1980–2016), United States Patent office database (USPTO) applications (2001–2016), grants (1976–2016) and assignments, World Intellectual Property Organization (WIPO-PCT) applications (1978–2016), and Australian patents (during the past 100 years). Indian Patent Database (IPO) was included in this project. The data framework has been decided for the 1996–2016 period covering granted patents, this point is the correct one because it includes the introduction of glyphosate tolerant soybean in the USA (at 1996) to the present day. The search was performed for combination of keywords related to GM soybean and related to the herbicide tolerance: GM, soybean, genetically engineered, genetically modified, transgenic, glyphosate tolerance, glyphosate resistance, Roundup, Monsanto. The search was completed in March 2016.

3. Results and discussion

The granted patent data was checked with respect to (a) claims (b) year of application, and (c) owners. A manual analysis of preliminary results revealed that some of them are not related to this research and were not considered. Patents related to Roundup Ready soybean (US4940835, US5188642, US5804425, US5312910, US5352605, US5530196, US5627061, US5633435, US5717084, US5728925) are not covered by this framework because they have been already explained with success [13]. 63 patents were selected which met the requirements. The results are presented as tables: Table 1 to the report presents key outcome claims, Table 2 shows the progress over the years, and Table 3 gives owners for the patents.

A freedom-to-operate strategy involves complete deconstruction of GM product development, with techniques and process, including transfer material and confidentiality agreements (Fig. 1). In our experience, we have succeeded in carving out a new set of strategies to attain a possible generic GM soybean for glyphosate tolerance on intellectual property and freedom-to-operate concerns. First of all, tissue culture procedures are an important part for a successful GM plant development. Soybean is a dicot plant considered as reproducible for being propagated in tissue culture. Patents in soybean focused on protecting appropriate methods for transformation and regeneration like *A. tumefaciens* inoculation of the cot node (or *A. rhizogenes* on stem node), and bombardment of the shoot tip to allow integration of the microcontructs into the soybean cells. Our GM soybean methodology is focused on the selection of locally important cultivars for tissue culture and transformation experiments (based on the cot node and somatic embryogenesis, which are used in the state sector), these varieties are more often adapted to specific regions of a country, are passed down through generations of growers and often have a plant breeder's right. Simultaneously, a specific synthetic expression cassette design (promoter, transit peptide, gene, and terminator) adapted to the needs of each evaluation of whether there is infringement of a patent, design or trademark rights of another entity in a definite country is developed. Genes, promoters and terminators are identified and characterized (using public databases: National Center for Biotechnology Information, European Molecular Biology Laboratory or DNA databank of Japan) by bioinformatics software such as Visual Gene Developer [20], Gene Design [21], GeMS [22], or Optimizer [23] for further analyses including codon optimization, GC content adjustment, adequate use of restriction sites, or avoidance of mRNA secondary structures, potential polyadenylation sites, cryptic splice sites and repetitive elements among others. A *cp4 epsps* gene is a good option because it is biologically characterized, and has long studies about allergenicity and environmental impacts [24] if a commercial transgenic line is planned. Several patents obtained separately provided protection to *cp4 epsps* gene such as US5627061, US5633435, US5804425, USRE39247 (E1), and US6248876. Another group of patents are protecting the soybean events that were transformed with *cp4 epsps* gene: US7632985, US7608761, or US8053184 (B2). The recombinant vectors containing *cp4 epsps* gene employ a cauliflower mosaic virus (CaMV) 35S promoter because it is constitutive and is not influenced by environment or tissues. The 35S promoter is protected by numerous patents: US5352605, US5530196, US5858742, or US6255560. We used the N-terminal petunia chloroplast transit peptide (e.g. patents US5633435 and USRE39247 (E1)) and Nos terminator (e.g. patents US5362865 and US5659122) as elements of synthetic expression cassettes. When contracting with companies for the provision of cassette synthesis service, we shall demand that they safeguard the confidentiality of the information and the opportunity to negotiate an agreement for commercial use.

We use pCambia vectors to synthetic expression cassette insertion and plant modification. pCambia vectors are improved version of pPZP vectors for GM crops, they are improved to have high copy number in *Escherichia coli* and high stability in *Agrobacterium*. This vector family are small size, with adequate cloning site for inserting a *cp4 epsps* gene, has the 35S promoter and different markers (kanamycin, hygromycin and *gusA* or GFP reporters). Importantly, pCambia vectors are an open source, build on free-to-operate, available without fees for academic or non-profit research organizations. In our laboratory pCambia vectors were useful with *A. tumefasciens* strains such as LBA4404 (Ach5 pTiAch5),