Monitoring the Utilization of Glyphosate Resistance Genes Based on Patent Analysis

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Abstract. As a widely used herbicide in the world, glyphosate occupies an important position in the global agrochemical market. The first genetically modified glyphosate-resistant crop was successfully studied in the United States. Monsanto, as a giant in the agrochemical field, has extensive influence in the field of genetically modified glyphosate resistance. Phosphoenolpyruvate shikimate synthase ((EPSPS)) is a key enzyme that catalyzes the synthesis of EPSP from PEP and S3P in shikimic acid pathway. As an important prerequisite for branched acid synthesis in photosynthesis, shikimate synthase plays an important regulatory role. Glyphosate is the structural analogue of S3P, which competitively inhibits the enzyme activity of EPSPS, resulting in the interruption of shikimic acid pathway, which leads to the death of plants. According to their conserved domains, EPSPS can be divided into type I EPSPS and type II EPSPS, type I EPSPS sensitive to glyphosate, while type II EPSPS is inherently tolerant to glyphosate, and the two types of EPSPS have specific mutants, showing different degrees of sensitivity to glyphosate inhibition in the catalytic shikimic acid pathway. Based on China knowledge Network and other databases, mining the current types, sequence characteristics and transformation events of glyphosate-resistant genes will help our country to occupy a favorable position in the field of glyphosate-resistant transgenic and maintain the agricultural security of our country.

Keywords: Glyphosate Resistance Gene, Glyphosate Resistance, Patent, EPSPS

1. Introduction

1.1. Introduction to Glyphosate

Glyphosate is a highly effective spectral herbicide and has been widely used in agricultural production. It has been found that the application of glyphosate can effectively control annual weeds and perennial weeds that are propagated by roots. Glyphosate and phosphoenolpyruvate (PEP) are structural analogues and competitive inhibitors of EPSPS [1].
Glyphosate is an organophosphate herbicide, and it has the killing effect on most monocotyledons and dicotyledons. Glyphosate can be absorbed by the keratinocytes and stomata of plants. When glyphosate is absorbed into plants, it will be transported from "source" to "reservoir". In other words, glyphosate entering plants will be transported from the phloem to the site of vigorous growth along with the photosynthetic products produced by plants [2]. This means of transport from "source" to "repository" is fast, so glyphosate can be transported quickly to the roots, shoots and young leaves of plants. When plants are at different stages of growth and development, the amount of glyphosate assigned to each site varies. For young plants, after glyphosate is absorbed, it will be transmitted to the young leaves and buds of the plants along with the flow of organic matter inside the plants, causing damage to the young leaves and buds. The plants will soon die after being sprayed with glyphosate at this stage. Plant leaves are also affected by glyphosate. After spraying glyphosate on plant leaves, it will cause water loss in leaf cells, stomatal closure and reduced CO$_2$ uptake in leaves. Meanwhile, glyphosate also inhibits chlorophyll synthesis in leaves, which will eventually lead to failure of photosynthesis in leaves and lead to plant death. In mature plants, glyphosate is absorbed into the plant as organic matter flows toward the flowers and young fruits, eventually causing the plant to wilt, turn yellow, and wither to death.

Glyphosate is a non-selective herbicide that competitively inhibits the activity of 5-enol acetone shikimate 3-phosphate synthase (EPSPS) in the shikimic acid pathway. Glyphosate, EPSPS, and shikimic acid triphosphate (S3P) combine to form the EPSPS-S3P-glyphosate complex, which is very stable and not easily destroyed [3]. It can block the synthesis of branch acids in shikimic acid pathway by inhibiting the activity of EPSPS, and then block the biosynthesis of aromatic amino acids and aromatic compounds, and finally disturb the normal metabolism of organisms and cause plant death.

1.2. Mechanism of Action of Glyphosate Resistance Genes
Some genes that have been reported to have glyphosate resistance were obtained by searching the patent database. Glyphosate resistance genes can be roughly divided into three regions. EPSPS from plants belong to Type I EPSPS, EPSPS from microorganisms belong to type II EPSPS, and glyphosate degradation related genes that can be polymerized with glyphosate acetyltransferase GAT. The first type of EPSPS (Type I) is an enzyme coded for Salmonella and Escherichia coli genes. The second type of EPSPS are enzymes encoded by Pseudomonas sp. PG2982, Achromobacter sp. LBAA and Agrobacterium Tumefaciens SP. CP4. The background of EPSPS from plants does not have glyphosate resistance. Most of the EPSPS of Type I used in the current production have site-directed mutations to reduce the inhibition of glyphosate on them and improve their affinity with PEP and S3P. LsEPSPS1, LsEPSPS2 and LsEPSPS3 are EPSP synthase from ophiopogonis, ophiopogonis soil and Ophiopogonis broadleaf respectively [4]. The patent shows that the occurrence of partial amino acid mutations in conserved domain 1 (280-294) and domain 2 (415-433) of LsEPSPS will effectively improve glyphosate resistance of LsEPSPS. Specifically, amino acid mutations in conserved domain 1 are optimized to occur at 284, 285 and 289. The amino acid mutation in conserved region 2 was optimized at 416, 421, 422, 424 and 425 positions. Described the optimal amino acid mutations as the 284th amino acid mutations as arginine, 285th amino acid mutations for tyrosine, 289th amino acid mutation of histidine, 416th amino acid mutations to glutamate, 421th amino acid mutations as glycine, 422th amino acid mutations as valine, 424th for cysteine amino acid mutations, 425th amino acid mutation of tyrosine [5].

1.3. Current Utilization of Glyphosate-Resistant Transgenic Crops
Glyphosate-resistant crops play an important role in weed control. At present, our commercial cultivation of glyphosate resistant genes used in genetically modified (gm) crops mainly comes from the soil Agrobacterium (Agrobacterium tumefaciens) CP4 and E. coli (Escherichia coli) 5-enol form of acetone acyl - shikimic acid - 3 - phosphate synthetase (5-enolpyruv - shikimate - 3 - phosphate synthase, EPSPS) gene, because of the resistance gene resource is too narrow, so we it is necessary to expand the types of available resources. Glyphosate-resistant crops play an important role in weed control.
control [6]. At present, our commercial cultivation of glyphosate resistant genes used in genetically modified (gm) crops mainly comes from the soil Agro bacterium (Agrobacterium tumefaciens) CP4 and E. coli (Escherichia coli) 5 - enol form of acetone acyl - shikimic acid - 3 - phosphate synthetase (5 - enolpyruvy - shikimate - 3 - phosphate synthase, EPSPS) gene, because of the resistance gene resource is too narrow, so we it is necessary to expand the types of available resources. Since the introduction of glyphosate-resistant crops, we have found that more than a dozen species of weeds have developed resistance [7]. At present, China has not grown glyphosate-resistant crops, but there are always some reports about glyphosate-resistant crops. With further research, the chances of planting glyphosate-resistant crops may increase, along with the risk of the development of resistant weeds [8].

According to literature review, there are potential ecological problems in glyphosate-resistant transgenic crops, which are mainly manifested in three aspects: the drift of resistance genes, the emergence of resistant weeds, and the impact on biological communities [9]. We must pay full attention to the potential ecological problems of glyphosate-resistant gm crops. Historical experience tells us that we should not go down the old road of first destroying and then governing. Ecological problems are the biggest concern of Today's China, and we must strictly control them [10]. So we have to work harder to find new technologies and new ways to solve problems.

1.4. The Purpose and Significance of This Study
The target of glyphosate is EPSPS, which has attracted extensive attention in the field of genetic engineering. EPSPS play an important role in the target of glyphosate, a widely used herbicide. Plant-derived EPSPS are widely recognized for their higher security [2]. Glyphosate-resistant genetically engineered crops are mainly based on the regulation of EPSPS coding genes. The study found that the EPSPS gene was resistant to glyphosate. The increase of copy number of EPSPS gene would lead to the increase of titer of EPSPS enzyme, the target of glyphosate, thus showing resistance to glyphosate. The analysis of the sites closely related to glyphosate activity showed that the key amino acid sites affect glyphosate resistance, and the site-directed mutation is one of the ways for organisms to produce glyphosate resistance [3]. This study is helpful to understand the biological information of EPSP synthase and provide reference for further research on glyphosate resistance.

2. The Research Methods
Search "patent" and "literature" on Cn.COM to analyze glyphosate gene mining, mechanism of action, source, patent protection, patent timeliness, type, current situation and market potential of glyphosate-resistant transgenic crops.

3. Results and Analysis

3.1 The Excavation, Mechanism and Origin of Glyphosate Gene
Glyphosate is an active chemical ingredient in many herbicides developed by Monsanto in the 1970s.Glyphosate, also known as Roundup, phosphonic acid, azaldine, etc., chemical name N-(phosphonate methyl) glycine (C3H8NO5P), white powder, melting point and boiling point of 230 °C.It is one of the most widely used herbicides in the world[9].

Glyphosate causes weed death mainly by inhibiting the activity of EPSPS in plants. EPSPS is shikimic acid pathway of tyrosine, tryptophan, phenylalanine, one of key enzymes in the biosynthesis, glyphosate is the PEP (phosphoric acid dilute alcohol acetone acid) competitive inhibitor, it is competitive with EPSPS and S3P formed EPSPS S3P - to inhibit the activity of EPSPS glyphosate, hinder the aromatic amino acid compound, the plant protein cannot normal synthesis, and inhibition of cell wall polymer formed in the process of lignin in plant growth, and eventually led to the deaths of a plant[10].

By retrieving the patent database, scan obtained at home and abroad in recent years the cloning of glyphosate resistance genes, the resistance genes in glyphosate resistance to maintain and carried on
the thorough analysis of the role of mechanism, system research of glyphosate resistant access, for the deep understanding of glyphosate resistance genes in glyphosate resistance molecular mechanism of laid a foundation [11].

3.2 The Type, Current Situation and Market Potential of Glyphosate-Resistant Transgenic Crops
With the deepening of research, researchers gradually introduced herbicide resistant genes from different sources into crop cells, reintegrated the genome, obtained herbicide resistant transgenic crops, and increased the planting scale. At present, such gm crops as sulfonylurea-resistant soybean (STS) and sugar beet (CR I2B) have been popularized. Imidazolinone of maize (IT), Rice (Clearfield Rice), rape (IM I2Canola), sunflower (Cleanfield), etc. With the development of ALS inhibitor herbicide resistance genes, herbicides not only greatly reduce the harm to crops, but also play an important role in the research and control of resistant weeds.

Nowadays, making plants resistant to herbicides and producing high-quality varieties are mostly accomplished through transgenic technology. Herbicide-resistant traits have attracted much attention and played an important role in transgenic plants since the emergence of tobacco with herbicide-resistant genes in the 1980s. At present, there are more than 300 herbicide-resistant transgenic plants [12]. The author believes that with the gradual optimization of herbicide resistance genes in the future, the performance of herbicides will be given more play, and the yield and quality of crops will be improved to a greater extent.

4. Conclusion and Discussion
By searching the database and analyzing the patent and application of glyphosate resistance successfully applied in recent years, the research status of glyphosate resistance gene was more comprehensively understood, and the ideas for further development of glyphosate resistance gene were provided. Plant EPSPS of type I can obtain glyphosate resistance through natural and site-directed mutations, providing a feasible means for further improving glyphosate resistance in plants.

The issue of agriculture is a major event related to the national economy and people's livelihood of our country, and it occupies an important basic position in the development of our national economy. While planting a large number of crops, the problem of weeds has become one of the important factors restricting crop planting area and crop yield. Biotechnology accounts for an increasing proportion in modern agricultural production, transgenic herbicide-resistant crops are developing rapidly, and there is still a certain gap between China's modern bio-agriculture and foreign developed countries. Because of its many advantages, glyphosate has become the first choice for the cultivation of herbicide-resistant transgenic crops [12]. However, at present, the resources of glyphosate resistant genes are narrow, and most of the glyphosate resistant genes with excellent characters are protected by all-round patents by foreign companies, which limits the development of modern agriculture in our country. Based on this, mining some new glyphosate resistance genes is of strategic significance to the agricultural development of our country.

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References
[1] Hui Wang, XiaoHong Yan, Jie Xu, FuGui Mi, Lei Yao. Current status of glyphosate-resistant genes in China [J]. Journal of agricultural biotechnology,2014,22(1):109-118.
[2] HongWei Wang, YeHong Liang, ZhenSheng Shi, ShiHuang Zhang. Overview of transgenic crops resistant to glyphosate. Journal of Crops,2007.4.
[3] YunYan Fei, TuanJie Zhao, JiaZheng Ji. Disputes between "accompanying brothers" glyphosate and glyphosate-resistant genetically modified crops. Quality and Safety of agricultural products. 2012.4.

[4] RongRong Chen, GaoYi Cao, YunJun Liu. 2014. Site-directed mutations of the 5-enol pyruvate-shimimic acid-3-phosphate synthase gene (EPSPS) of Arabidopsis thaliana and acquisition of glyphosate-resistant transgenic Arabidopsis thaliana [J]. Acta Agri-Gazette (4):397-405.

[5] Wei Liu, JinXin Wang, GuangLing Yang. Progress in weed resistance to acetyllactic synthase inhibitors [J]. Acta Agrosciences, 2006, 6(4): 7-12.

[6] Zulet-Gonzalez A, Barco-Antonanzas M, Gil-Monreal M, Royuela M, Zabalza A. Increased Glyphosate-Induced Gene Expression in the Shikimate Pathway Is Abolished in the Presence of Aromatic Amino Acids and Mimicked by Shikimate[J]. Front Plant Sci,2020, 11:459.

[7] Beres ZT, Giese LA, Mackey DM, Owen MDK, Page ER, Snow AA. Target-site EPSPS Pro-106-Ser mutation in Conyza canadensis biotypes with extreme resistance to glyphosate in Ohio and Iowa, USA[J]. 2020,Sci Rep 10 (1):7577.

[8] Achary VMM, Sheri V, Manna M, Pandiati V, Borphukan B, Ram B, Agarwal A, Fartyal D, Teotia D, Masakapalli SK, Agrawal PK, Reddy MK. Overexpression of improved EPSPS gene results in field level glyphosate tolerance and higher grain yield in rice[J]. 2020,Plant Biotechnol J.

[9] Karthik K, Nandigant M, Thangaraj A, Singh S, Mishra P, Rathinam M, Sharma M, Singh NK, Dash PK, Sreevathsa R. Transgenic Cotton (Gossypium hirsutum L.) to Combat Weed Vagaries: Utility of an Apical Meristem-Targeted in planta Transformation Strategy to Introgress a Modified CP4-EPSPS Gene for Glyphosate Tolerance[J]. 2020, Front Plant Sci 11:768.

[10] Cechin J, Piasecki C, Benemann DP, Kremer FS, Galli V, Maia LC, Agostinetto D, Vargas AL. Transcriptome Analysis Identifies Candidate Target Genes Involved in Glyphosate-Resistance Mechanism in Lolium multiflorum[J]. 2020, Plants (Basel) 9 (6).

[11] Mollaee M, Matloob A, Mobli A, Thompson M, Chauhan BS. Response of glyphosate-resistant and susceptible biotypes of Echinochloa colona to low doses of glyphosate in different soil moisture conditions. 2020, PLoS One 15 (5):e0233428.

[12] Franci J, Lam KW, Chua T, Cha T. Genetic diversity and in silico evidence of target-site mutation in the EPSPS gene in endowing glyphosate resistance in Eleusine indica (L.) from Malaysia[J]. 2020, Pestic Biochem Physiol 165:104556.