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Señor Director
DIRECCIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 378
Actuación : 523

Referencia : Expediente No. **09.140.880**
Asunto : Solicitud de patente: **"UNA CÉLULA DE PLANTA TRANSGÉNICA QUE COMPRENDE UN POLINUCLEÓTIDO QUE CODIFICA UNA DIOXIGENASA ARILOXIALCANOATO, EL POLINUCLEÓTIDO Y EL MÉTODO DE OBTENCIÓN"**
Solicitante : DOW AGROSCIENCES LLC
Fecha de solicitud : 9 de Diciembre de 2009

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al oficio No. **9341** notificado por fijación en lista el 5 de Agosto de 2014.

2. Mediante memorial del 29 de Octubre de 2014 se solicitó oportunamente plazo para responder la acción oficial No. **9341**.

3. Mediante oficio No. **9341** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Las observaciones realizadas en el oficio se refieren a la claridad del título, a la presencia de materia que no es considerada una invención, a la claridad del pliego reivindicatorio y al nivel inventivo de las reivindicaciones 2 a 24 en vista de los documentos **D1**: WO2005107437 y **D2**: Applied and Enviromental Microbioogy.

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

BOGOTÁ - Edificio Siski - Carrera 4 No. 72 - 35 - Tel. (57-1) 347 3611 - Fax (57-1) 211 8650 - Fax in U.S.A: (1-305) 675 7743 - COLOMBIA
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www.cavelier.com
cavelier@cavelier.com

4. Respuesta a las objeciones

De este modo y con el objeto de atender las objeciones arriba mencionadas, se desea presentar las siguientes modificaciones, aclaraciones y argumentos:

4.1. Modificación de una solicitud en trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 24 reivindicaciones.

Se han introducido las siguientes modificaciones al capítulo reivindicatorio:

- Se modificaron las reivindicaciones 1, 2, 7 y 8 con el objetivo de especificar aún más la materia reclamada en la presente solicitud, aclarando las secuencias específicas del polinucleótido.
- Se eliminaron las reivindicaciones originales 5 y 6. Lo anterior no puede ser considerado como un desistimiento de la materia allí revelada y mi representada se reserva el derecho de volver sobre la misma durante el trámite de la presente solicitud de patente.

Por lo anterior, las objeciones formuladas por el Examinador en cuanto a la presencia de materia que no es considerada una invención y la claridad y concisión del pliego reivindicatorio se ven superadas. En consecuencia, se solicita atentamente tener en cuenta este nuevo pliego reivindicatorio para el nuevo análisis de patentabilidad.

4.2. Argumentos con respecto a la falta de Nivel Inventivo

El artículo 18 de la Decisión 486 señala lo siguiente:

“Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica”.

Una invención se considerará inventiva si la misma no hubiese resultado obvia a partir del arte previo para una persona del oficio normalmente versada en la materia. Esto a su vez implica que el Examinador a cargo de esta evaluación debe colocarse en el momento exacto en el que el inventor buscaba resolver el problema técnico contemplado en la solicitud y para el cual poseía la literatura y el conocimiento disponibles en dicho momento.

Siguiendo entonces los lineamientos de la Jurisprudencia aplicable al análisis del nivel inventivo de una solicitud de patente, se considera que una anterioridad o una combinación de dos anterioridades puede afectar dicho requerimiento de patentabilidad, si una persona medianamente versada en la materia encuentra enseñanzas o sugerencias claras que, a partir de un conocimiento medio en dicho campo, le permitan llegar de manera obvia a la invención reivindicada. Adicionalmente se considera que una mejora sustancial, un efecto inesperado o una ventaja con respecto al arte previo es un indicio de nivel inventivo, por cuanto aporta un salto tecnológico en la regla técnica.

A este respecto, respetuosamente solicitamos atentamente la reconsideración de las razones por las cuales se objeta el nivel inventivo, con base en la siguiente información:

Con respecto al documento D1 (caso AAD-1), fue posible notar que AAD-13 presenta actividad dual, incluso contra herbicidas oxipiridilo, mientras que AAD-1 no lo hace. En el caso del documento D2, en la presente invención se están limitando las reivindicaciones a secuencias optimizadas (y sin porcentaje de identidad), por lo que hay que tener en cuenta que las secuencias optimizadas no se dan a conocer en dicho documento.

Existen muchas maneras de optimizar una secuencia para expresión en plantas, y múltiples decisiones subjetivas para ser realizadas por PHOSITA dentro de cada proceso de optimización. D2 tampoco enseña ni sugiere los pasos exactos de optimización utilizados para producir las secuencias optimizadas reclamadas y mucho menos las secuencias como tal.

Así mismo, adjunto se presenta la declaración de Sean Rusell, una persona versada en la materia que determina que la presente invención si tiene actividad inventiva. Con esta es posible observar que la secuencia de polinucleótidos codón optimizadas de la presente invención es una secuencia única que comprende diferentes secuencias seleccionadas y que no existe ninguna sugerencia en el arte previo que permita a una persona versada en la materia hubiese llegado exactamente a la misma secuencia utilizando otro protocolos para la optimización del codón. Con base a lo anterior, la presente solicitud posee la altura inventiva necesaria para que se le conceda el privilegio solicitado.

4.3 Conclusión

Por todo lo anterior, el documento D1, el documento D2, o su combinación, no resultan anterioridades válidas capaces de afectar el nivel inventivo de la invención de la presente solicitud.

Por lo tanto, consideramos que el Examinador está pasando por alto que una sola diferencia con respecto al estado de la técnica, permite concluir que el objeto reivindicado es nuevo. Además, está excluyendo el hecho de que el inventor en el momento de solucionar el problema técnico de la solicitud tuvo que emplear habilidad inventiva, pues la presente invención no resulta de la mera extrapolación de las enseñanzas del arte previo, como se afirma. Se está desconociendo el mérito investigativo empleado y que está aportando una ventaja al arte previo.

CAVELIER

A B O G A D O S

5. Puesto que se ha dado respuesta a todas las objeciones formuladas por el Examinador, se solicita atentamente proceder al otorgamiento de la solicitud de patente. En este sentido, respetuosamente recordamos al Examinador que la patente no podrá ser negada con base en objeciones diferentes a las expuestas en la acción oficial No. 9341, toda vez que al no haber sido trasladadas a mi representada, dicha decisión violaría tanto el tenor literal del artículo 45 de la Decisión 486 como el derecho constitucional al debido proceso. En este evento, y con base en el inciso 2 del artículo 45, respetuosamente solicitamos al Examinador expedir una nueva acción oficial que permita a mi representada contra argumentar las mismas o modificar la solicitud con el fin de cumplir los requisitos de patentabilidad.

6. Anexos

- Nuevo pliego reivindicatorio conformado por 24 reivindicaciones.
- Declaración de Sean Russel.

Señor Director,


LUZ CLEMENCIA DE PÁEZ

C.C. 35.456.344 de Usaquén

T.P.A. No. 23.555

COLO-10975-0841-032-7
CPT\CPTID-275787\WPC10975
No. M-2014-275787\CPT

REIVINDICACIONES MODIFICADAS

1. Un polinucleótido que codifica una proteína que enzimáticamente degrada una estructura química de un herbicida de ariloxialcanoato, en donde dicho polinucleótido está operablemente conectado a un promotor, que es una célula de planta funcional, el polinucleótido comprende la secuencia seleccionada del grupo que consiste de SEQ ID NO: 3 y SEQ ID NO:5.
2. El polinucleótido de la reivindicación 1 en donde dicho polinucleótido es la secuencia SEQ ID NO: 3.
3. El polinucleótido de la reivindicación 1, en donde dicho polinucleótido comprende una composición codón no nativa, que tiene un sesgo hacia el empleo de codón de planta, para incrementar la expresión de dicho polinucleótido en la planta.
4. El polinucleótido de la reivindicación 3, en donde la composición codón está sesgada hacia el empleo de codón de planta dicotiledónea.
5. Un método para controlar malezas en un área, donde dicho método comprende las etapas de:
 - producir una célula vegetal transformada que comprende un polinucleótido heterólogo, plantar semillas en la tierra del área, en donde dichas semillas comprenden dicho polinucleótido que codifica una proteína que enzimáticamente degrada la estructura química de un herbicida de ariloxialcanoato, en donde dicho polinucleótido está operablemente conectado a un promotor que es una célula de planta funcional y en donde el polinucleótido comprende la secuencia seleccionada del grupo que consiste de: SEQ ID NO: 3 y SEQ ID NO: 5; produciendo plantas transformadas de dichas semillas; y
 - aplicar dicho herbicida de ariloxialcanoato a dichas plantas
8. El método de la reivindicación 7, en donde dicho polinucleótido que consiste de: SEQ ID NO: 3.

9. El método de la reivindicación 7, en donde dichas semillas comprenden un segundo polinucleótido que codifica una segunda proteína que degrada enzimáticamente un segundo herbicida, y dicho método comprende aplicar dicho segundo herbicida a dicha área.
10. El método de la reivindicación 9, en donde dichas semillas comprenden un tercer polinucleótido que codifica una tercera proteína que degrada enzimáticamente un tercer herbicida, y dicho método comprende aplicar dicho tercer herbicida a dicha área.
11. El método de la reivindicación 10, en donde dicho tercer herbicida es seleccionado del grupo que consiste de: glufosinato y dicamba.
12. El método de la reivindicación 9, en donde dicho segundo herbicida es glifosato.
13. El método de la reivindicación 9, en donde dicho herbicida de ariloxialcanoato es 2,4-D y dicho segundo herbicida es glifosato.
14. El método de la reivindicación 13, en donde dicho 4, 4-D y dicho glifosato, son aplicados desde un tanque de mezcla.
15. El método de la reivindicación 7, en donde dicho herbicida es 2,4-D.
16. El método de la reivindicación 7, en donde dichas semillas son semillas de una planta de maíz.
17. El método de la reivindicación 16, en donde dicha planta es dicotiledónea.
18. El método de la reivindicación 17, en donde dicha planta es una planta de soya.
19. El método de la reivindicación 7, en donde el herbicida ariloxialcanoato, es seleccionado del grupo que consiste de: (a) un fenoxiacetato o un herbicida ácido fenoxiacético; (b) herbicida ácido fenoxipropiónico; (c) un herbicida

ácido piridiloxialcanoico; y (d) un ácido una sal o éster de un ingrediente activo de dicho herbicida.

20. El método de la reivindicación 19, en donde dicho herbicida ácido piridiloxialcanoico es un herbicida ácido piridiloxiacético.
21. El método de la reivindicación 20, en donde dicho herbicida ácido piridiloxiacético es seleccionado del grupo que consiste de: triclopir y fluroxipir.
22. El método de la reivindicación 19, en donde (a) dicho herbicida ácido fenoxiacético es seleccionado del grupo que consiste de 2,4-D y MCPA; y (b) dicho herbicida ácido fenoxipropiónico es seleccionado del grupo que consiste de diclorprop, mecoprop, un enantiómero de diclorprop y un enantiómero de mecoprop.
23. El método de la reivindicación 7, en donde el herbicida de fenoxiacetato y el herbicida de piridiloxiacetato, son aplicados a dicha área.
24. El método de la reivindicación 7, en donde dicho método comprende adicionalmente, hacer crecer las plantas de maíz, de dichas semillas, en dicha área.

CPT\CPT\ID-275786\WPC10975

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Held et al.		Art Unit: 1638
Serial No.: 13/303,502		
Filed: August 23, 2011		Examiner: D. Kruse
For: OPTIMIZED EXPRESSION OF		
GLYPHOSATE RESISTANCE		
ENCODING NUCLEIC ACID		Conf. No. 8998
MOLECULES IN PLANT CELLS		

DECLARATION OF SEAN RUSSELL UNDER 37 C.F.R. §1.132

I, Sean Russell, declare as follows:

1. At the time of the invention, I was employed as a Research Scientist at Dow AgroSciences, LLC in Indianapolis, IN. I have fifteen years of research experience in the field of biotechnology, and received a Bachelor of Science degree (B.S.) and Master of Science (M.S.) from Kansas State University, in Biology. I am a co-inventor on fourteen U.S. patents or patent applications, and a co-author of three peer-reviewed scientific journal articles. My research has been in the areas of plant biotechnology and bacterial heterologous expression systems.
2. I have direct knowledge of the results described below, as I was involved in the design and construction of the monocotyledonous/dicotyledonous codon optimized polynucleotide sequences of the present invention on behalf of MS Technologies LLC, the assignee.
3. There are varying and different types of codon optimization strategies that can be deployed to design and produce a novel codon optimized polynucleotide sequence. The different codon optimization design strategies apply differing parameters for the design and configuration of the codon optimized polynucleotide sequence. The resulting codon optimized polynucleotide sequence is a unique sequence that is resultant upon the selected design strategy and subjective implementation of the strategy used to generate the sequence. As such, the selection of the codon optimization strategy, and its implementation, directly influences the resultant codon optimized polynucleotide sequence.
4. The codon optimization process utilized for development of the claimed invention is described in Example 1 of the application. Implementation of this process results in the incorporation of codon substitutions within a polynucleotide sequence such that the polynucleotide sequence possesses a balanced codon distribution of frequently used codons

found in monocotyledonous and dicotyledonous plant genes. An exemplary listing of codon usage for coding sequences of monocotyledonous and dicotyledonous plant genes is available at Table 1 and Table 2 of the present application. Importantly, additional refinements of the polynucleotide sequence are subsequently performed according to the codon optimization process of the present application. For example, refinements are made to eliminate undesirable and deleterious sequences. Such sequences include; restriction enzyme recognition sites, potential plant intron splice sites, long runs of A/T or C/G residues, and other motifs that might interfere with mRNA stability, transcription, or translation of the coding region in plant cells. Other changes are made to introduce desired restriction enzyme recognition sites, and to eliminate long internal Open Reading Frames (*i.e.*, frames other than +1). These potential changes must be balanced with the need to retain the monocotyledonous and dicotyledonous biased codon composition of the codon optimized sequences, which requires individualized decision-making for each codon selection based on experience and judgment.

5. The present application describes a native 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS) gene from *Zea mays*. The native EPSPS polynucleotide sequence is listed in the present application as SEQ ID NO:1. This polynucleotide sequence was modified to introduce an Isoleucine for Threonine at amino acid residue 102, and a Serine for Proline at amino acid residue 106. The modified EPSPS gene was labeled as “2mEPSPS v1” and is listed in the present application as SEQ ID NO:4. The 2mEPSPS v1 sequence was codon optimized using a balanced monocotyledonous/dicotyledonous biased codon selection protocol as described in Example 1. The resulting codon optimized polynucleotide sequence is listed in the present application as SEQ ID NO:5 and is labeled as “2mEPSPS v2”.

6. The monocotyledonous/dicotyledonous codon optimized polynucleotide sequence listed as SEQ ID NO:5 in the present application is shown in Table 1 below.

Table 1. The SEQ ID NO:5 monocotyledonous/dicotyledonous codon optimized polynucleotide sequence which is labeled as “2mEPSPS v2” in the present application.

```
GCTGGAGCTGAAGAGATTGTGCTCCAACCCATCAAGGAGATCTCTGGCACAGTCAAACCTCCCTGGCTCAAAGTCACTTTCAAACC
GTATCCTCTTGCTTGCAGCTCTTTCTGAAGGGACCACAGTGGTTGACAACCTTCTCAACTCAGAGGATGTCCACTACATGCTCGGA
GCCTTGAGGACTCTTGGCTTGTCTGTTGAAGCAGACAAAGCTGCCAAGCGTGCTGTTGTGGTTGGCTGTGGTGGAAGTTCCAG
TTGAAGATGCCAAAGAGGAAGTCCAGCTCTTCTTGGGAATGCTGGGATTGCCATGAGATCCTTGACTGCAGCTGTCACTGCAGC
TGGTGGGAATGCCACCTATGTTCTTGATGGCGTGCCACGCATGAGGGAGAGACCCATTGGCGACTTGGTGGTTGGCTTGAAGCAA
CTTGAGCTGATGTTGACTGCTTCCTTGGCACCGACTGTCCACCTGTTCTGTCAATGGGATTGGAGGTCTCCCTGGTGGCAAGGT
CAAGCTCTCTGGCTCCATCAGCTCCAGTACTTGTGAGCCTTGCTCATGGCAGCTCCCTTGGCTCTTGGTGATGTGGAGATTGAGA
TCATTGACAACTCATCTCCATTCCCTATGTGGAGATGACCTTGAGATTGATGGAAGGTTTGGTGTGAAAGCTGAGCATTCTGA
CAGCTGGGACAGATTCTACATCAAGGGAGGTCAGAAGTACAAGTACCCAAGAATGCCTATGTTGAAGGTGATGCCAGCTCTGC
CAGCTACTTCTTGGCTGGTGCTGCAATCACTGGAGGGACTGTGACAGTGGAAGGTTGTGGCACTACCAGCTTGCAAGGTGATGTG
AAGTTTGCTGAGGTGCTTGAGATGATGGGAGCAAAGGTCACCTGGACTGAAACCTCCGTCACAGTGACTGGACCTCCAAGGGAG
CCATTCGGAAGGAAACATCTCAAAGCCATTGATGTCAACATGAACAAGATGCCAGATGTTGCCATGACTCTTGCTGTGGTTGCAC
TCTTTGCCGATGGACCAACAGCCATCAGAGATGTGGCTTCCTGGAGAGTCAAGGAGACAGAGAGGATGGTTGCAATACGCACAG
AGTTGACCAAACCTTGGAGCCAGCGTTGAGGAAGGACCAGACTACTGCATCATCACACCTCCCGAGAAGCTCAACGTGACAGCCA
TAGACACCTATGATGACCACGCATGGCAATGGCTTTCTCCCTTGCAGCCTGTGCAGAAGTCCCTGTACCATAACGTGACCCTGG
GTGCACTCGCAAGACCTTCCAGACTACTTTGATGTGCTCAGCACCTTGTCAAGAACTGA
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7. The monocotyledonous/dicotyledonous codon optimized polynucleotide sequence of SEQ ID NO:5 disclosed in the present application is a novel and inventive polynucleotide sequence. The disclosed polynucleotide sequence of SEQ ID NO:5 is one of many possible polynucleotide sequences that can encode the modified EPSPS protein. A calculation of the total theoretical polynucleotide sequences that can encode the modified EPSPS protein, indicates that 1.2332×10^{230} polynucleotide sequences can encode the protein of the modified EPSPS protein of the present application. Accordingly, SEQ ID NO:5 is 1 of 1.2332×10^{230} polynucleotide sequences that can encode the modified EPSPS protein.

8. I understand that in the Office Action dated April 02, 2014 issued for the present application, the Examiner rejected claims 1-22 of the present application as allegedly being unpatentable because, according to the Examiner, they are obvious under 35 U.S.C. § 103 over LeBrun et al., (U.S. Patent No. 6,566,587; hereinafter ‘587 patent) in view of Kawabe et al., (2003, *Genes Genet. Syst.*, 78: 343-352; hereinafter Kawabe) and Green et al., (2008, *Pest Management and Science*, 64: 332-339), and in further view of Barry et al., (U.S. Patent No. 5,633,435), and Pallet et al., (U.S. Patent No. 7,250, 561).

9. The ‘587 patent describes SEQ ID NO:4 that is a polynucleotide sequence encoding a modified EPSPS protein that is 76.5% identical to SEQ ID NO:5 of the present application as indicated by the Examiner. An alignment of the protein sequence of SEQ ID NO:5 (translation of SEQ ID NO:4) from the ‘587 patent with the translated protein sequence of SEQ ID NO:5 of the present application indicates that the translated protein sequences share 100% sequence identity. The polynucleotide sequence encoding a modified EPSPS protein (i.e., SEQ ID NO:4) of the ‘587 patent was isolated from *Zea mays*.

10. The '587 patent does not describe a process for codon optimization. However, the Examiner cites Kawabe for making the claimed nucleic acid molecules (i.e., polynucleotide sequences) of the present application. Kawabe is cited as teaching that monocot coding sequences contain a higher G+C content than dicot coding sequences at page 343, and the preferred codons in monocot and dicot plants in Table 2 on page 347.

11. Kawabe teaches the analysis of hundreds of coding sequences (CDSs) from four monocot and three dicot plant species that were analyzed for codon usage bias. Common features of codon choice in the selected plant species were identified. For example, the codon bias of preferred codons was correlated with G+C content in the third codon position. As such, Table 2 at page 347 of Kawabe presents the preferred codons that were determined for each of the four monocot and three dicot plant species. Only one to three codons are identified as the preferred codon for each amino acid in the four monocot and three dicot plant species. In some instances, no preferred codons are identified for a plant species.

12. The Examiner cites the teachings of Kawabe to make the claimed nucleic acid molecules (i.e., polynucleotide sequences) of the present application. However, the application of the teachings of Kawabe to codon optimize polynucleotide sequences encoding a modified EPSPS protein is a different codon optimization process as compared to the codon optimization process of the present application. For example, the identified preferred codons described by Kawabe are different than the balanced codon distribution of frequently used codons found in monocotyledonous and dicotyledonous plant genes taught by the present application. Kawabe lists the preferred codons for each of the four monocotyledonous and three dicotyledonous plant species in Table 2 at page 347. Only one to three preferred codons are identified for each plant species. In some instances, no preferred codons are identified for a plant species. Furthermore, Kawabe does not provide a single consolidated codon usage table comprising balanced codon distribution of frequently used codons found in both monocotyledonous and dicotyledonous plant genes. Finally, Kawabe does not require complete to near-complete removal of deleterious sequences such as potential plant intron splice sites, long runs of A/T or C/G residues, and other motifs that might interfere with mRNA stability, transcription, or translation of the coding region in plant cells.

13. For example, the preferred codons of Kawabe (Table 2, page 347 of Kawabe) can be compared to the preferred codons of the present application (Table 1 or Table 2 of the present application). The comparison of the preferred codons of Kawabe in relation to the preferred codons of the present application indicates that there are significant differences in codon choice. Kawabe identifies from one to three preferred codons for each amino acid

residue. In some instances, no preferred codons are identified for a plant species. Moreover, Kawabe individually lists preferred codons for only one plant species (i.e., *T. aestivum* preferred codons are provided, *H. vulgare* preferred codons are provided, *Z. mays* preferred codons are provided, *O. sativa* preferred codons are provided, *A. thaliana* preferred codons are provided, *N. tabacum* preferred codons are provided, and *P. sativum* preferred codons are provided).

Comparatively, the present application provides a distribution of codon usage for each codon that encodes an amino acid residue. The majority of the codons¹ that encode an amino acid are incorporated into a codon optimized sequence as preferred codons. As such, the present application teaches the incorporation of a greater number of preferred codons for each amino acid and teaches the incorporation of these preferred codons into a codon optimized sequence in a distribution of a weighted average.

Table 2, listed below, provides an example of the codon distribution for the amino acids Arginine and Asparagine of the present application as compared to Kawabe. As shown in Table 2, the codon usage of the present application results in the incorporation of 4 of 6 Arginine codons at varying frequencies, such that the resulting codon optimized sequence possesses a balanced codon distribution of frequently used codons found in monocotyledonous and dicotyledonous plant genes. Conversely, the codon usage of Kawabe varies depending upon the plant species selected. Some plant species identify 2 of 6 Arginine preferred codons, other plant species list no preferred codons. Accordingly, the present application teaches the incorporation of a greater number of preferred codons for each amino acid than Kawabe.

Moreover, Kawabe fails to disclose the codon distribution frequency by which the preferred codons would be incorporated into a codon optimized polynucleotide sequence. For example, as shown in Table 2, the codon usage of the present application results in the incorporation of Asparagine codons at frequencies of 61.4% for AAC and 38.6% for AAT, such that the resulting codon optimized sequence possesses a balanced codon distribution of the Asparagine codons. Conversely, the preferred codon for Asparagine taught by Kawabe is only the AAC codon. Following the teachings of Kawabe, the AAT codon would not be incorporated into a codon optimized sequence. In direct contrast, the present application teaches the incorporation of preferred codons into a codon optimized sequence in a distribution of a weighted average.

¹ Some codons that are rarely used in monocotyledonous or dicotyledonous plants are not incorporated in the codon optimized sequence. These codons are identified in Table 1 and Table 2 of the present application as “DNU” (Do Not Use).

Table 2. Comparison of preferred codons for the amino acid Arginine (ARG) and Asparagine (ASN) for the present application as compared to the Kawabe reference.

		Present Application	Kawabe						
		Balanced Monocot-Dicot	T. aestivum	H. vulgare	Z. mays	O. sativa	A. thaliana	N. tabacum	P. sativum
ARG	AGA	27.4%	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred
	AGG	31.5%	Preferred	Not-preferred	Preferred	Preferred	Preferred	Not-preferred	Not-preferred
	CGA	DNU	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred
	CGC	21.7%	Preferred	Not-preferred	Preferred	Preferred	Not-preferred	Not-preferred	Not-preferred
	CGG	DNU	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred
	CGT	19.4%	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Preferred	Preferred	Not-preferred
ASN	AAT	38.6	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred	Not-preferred
	AAC	61.4	Preferred	Preferred	Preferred	Preferred	Preferred	Preferred	Preferred

DNU- Do Not Use

14. Furthermore, the monocotyledonous/dicotyledonous codon optimized polynucleotide sequence of the present application is relatively free of deleterious sequences. During the design of the monocotyledonous/dicotyledonous codon optimized polynucleotide sequence, the inventors, based on our experience and judgment, removed what we considered could be potentially deleterious sequences, including, for example, restriction enzyme recognition sites, potential plant intron splice sites, long runs of A/T or C/G residues, and other motifs that could potentially interfere with mRNA stability, transcription, or translation of the coding region in plant cells. In addition, other changes were made to introduce desired restriction enzyme recognition sites, and to eliminate long internal Open Reading Frames (*i.e.*, frames other than +1). Kawabe does not list any examples of identifying such sequences, nor does Kawabe teach the removal of such sequences.

15. Based on the teachings of Kawabe, a person having ordinary skill in the art would start with a native EPSPS protein sequence containing two modifications for glyphosate tolerance and utilizing the G+C content and preferred codons described for monocots and dicots in Table 2 at page 347 of Kawabe. The person having ordinary skill in the art would select the preferred codons from one plant species (either one of the three dicot plants, or one of the three monocot plants described in Kawabe) and use these preferred codons in a codon optimized sequence. Resultantly, the codon optimized sequence would comprise only one, two,

or three preferred codons for each amino acid residue. It should be further noted that there may be instances where no preferred codon has been identified for a specific plant species. In such instances, the teachings of Kawabe do not indicate which preferred codon would be incorporated into a codon optimized sequence. Furthermore, the teachings of Kawabe do not describe the frequency of codon usage for the codon optimized sequence of the present application. The person having ordinary skill in the art would select the preferred codons taught in Kawabe, but would not incorporate the codons within the codon optimized sequence of the present application at a defined codon distribution frequency.

In applying Kawabe to build a codon optimized sequence, the person having ordinary skill in the art would not produce a codon optimized sequence containing the same codon distribution and usage as the codon optimized sequence of the present application (i.e., SEQ ID NO:5). The present application incorporates codons within a codon optimized polynucleotide that are not identified as preferred codons in Kawabe. As such, the resulting codon optimized sequence of Kawabe excludes many of the non-preferred codons that are incorporated into the codon optimized sequence of the present application. The resulting codon optimized sequence of Kawabe would contain a lesser variety of codons as compared to the codon optimized sequence of the present application. Following the teaching of Kawabe to codon optimize a nucleotide sequence would not result in the production of the polynucleotide sequence of the present application (i.e., SEQ ID NO:5).

16. Accordingly, the inventive monocotyledonous/dicotyledonous codon optimized polynucleotide sequence of the present application is a unique sequence comprising different selected sequence attributes because it is produced from a different codon optimization strategy as compared to the codon optimization procedures taught by Kawabe. The codon optimization process of the present application results in the incorporation of codon substitutions within a polynucleotide sequence such that the polynucleotide sequence possesses a balanced codon distribution of frequently used codons found in monocotyledonous and dicotyledonous plant genes. In addition, the present application teaches the removal of deleterious sequences. Furthermore, the codon optimization process of the present application is subjective, in that the selection of each codon is determined based on experience and judgment of the scientist designing the codon optimized polynucleotide sequence. The choice of selecting each codon requires balancing numerous factors (e.g., presence of deleterious sequence, removal of stem loop structures, elimination of long internal Open Reading Frames, etc.) with the need to retain the monocotyledonous and dicotyledonous biased codon composition of the codon optimized sequences. Given that each amino acid comprises numerous different codons, the scientist

designing the codon optimized polynucleotide sequence is provided with many different codon choices. Accordingly, the codon optimized polynucleotide generated by the codon optimization process of the present application required individualized decision-making for each codon selection based on experience and judgment. Comparatively, the codon optimization process of Kawabe results in the incorporation of codon substitutions within a polynucleotide sequence such that the polynucleotide sequence possesses only one, two, or three preferred codons found in gene from only one plant species. Furthermore, Kawabe does not teach the removal of deleterious sequences.

17. Based on the above analysis, it is my expert opinion that different researchers faced with the problem of codon optimizing a particular 2mEPSPS polynucleotide sequence for expression in plants could not arrive at identical codon optimized polynucleotide sequences. Especially, given that there are a total of 1.2332×10^{230} different possible polynucleotide sequences that can encode the protein of the modified EPSPS protein of the present application. It is highly improbable that different researchers would arrive at an identical codon optimized polynucleotide sequences using different codon optimization protocols, and it is in my expert opinion a certainty that a researcher following the teaching of Kawabe would arrive at a different optimized sequence than the claimed inventive polynucleotide sequence.

All statements herein made of my own knowledge are true, and all statements herein made on information and belief are believed to be true; these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dated:

By:

Sean Russell