

# Functional expression of recombinant proteins in soybean seeds

**Elibio Rech** 



1996 Goal

## To generate soybean elite cultivars tolerant to imidazolinones herbicides as an additional option for the world market and balance the rotational managment system

**Approach** 

**Transgenics** 

# (12) United States Patent



### The Chemical Company

US 6,444,875 BI

\*Sep. 3, 2002

(54) IMIDAZOLINONE RESISTANT AHAS

Inventor: Gabriele Elfriede Dietrich, Rocky Hill, (75)

(73) Assignee: BASF Aktiengesellschaft, Ludwigshafen (DE) (\*) Notice:

This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C.

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

### (21) Appl. No.: 09/096,562

(22) Filed: Jun. 12, 1998

### Related U.S. Application Data

- (63) Continuation of application No. 07/894,062, filed on Jun. 8, 1992, now Pat. No. 5,767,361, which is a continuation-inpart of application No. 07/737,851, filed on Jul. 31, 1991, now Pat. No. 5,731,180.
- (51) Int. Cl.<sup>7</sup> ... A01H 5/00; C12N 5/04
- (52) U.S. Cl. 800/279; 536/23.6
- (58) Field of Search ... 800/320.1, 320. 800/260, 275; 536/23.6; 435/419, 430

#### (56)References Cited

#### U.S. PATENT DOCUMENTS

4,761,373 A \* 8/1988 Anderson et al. ...... 435/172.3 5,731,180 A \* 3/1998 Dietrich et al. .......... 435/172.3 5,767,361 A \* 6/1998 Dietrich et al. ..... 800/205

#### FOREIGN PATENT DOCUMENTS

5/1992 92/08794 WO

OTHER PUBLICATIONS Anderson et al., Genome 31:994-999, 1989. Jen et al., J. Cellular Biochem. 14E(Supp.):302, Abstract, Wiersma et al., Mol. Gen. Genet. 219:413-420, 1989.

(10) Patent No.;

(45) Date of Patent:

Lesser, W.H., ed., Animal Patents, N.Y., Stockton Press, Haughn, G. et al., Plant Physiology 92:1081-1085, Apr.

Sathasivan et al., Nucleic Acids Research 18:2188, Apr. Haughn, G. et al., Mol. Gen. Genet. 211:266-271, 1988. Old, R.W. et al., Principles of Genetic Manipulation, 4th Ed., Oxford U.K. Blackwell Scientific Publ., 1989, pp. 87-98. Lawther, R.P. et al., Proc. Natl. Acad. Sci. USA 78(2):922-925, Feb. 1981. Burr, B. et al., TIG 7(2):55-61, 1991. Lee, K.Y. et al., The EMBO Journal 7(5):1241-1248, 1988. Falco, S.C. et al., Genetics 109:21-35, 1985. Mazur, B.J. et al., Plant Physiol. 85:1110-1117, 1987. Falco, S.C. et al., Nucleic Acids Research 13(11):4011-4027, 1985.

11(15):5299-5313, 1983. \* cited by examiner

Primary Examiner-David T. Fox Assistant Examiner-David H Kruse (74) Attorney, Agent, or Firm-Anne Rosenblum

Klein, T.M. et al., Nature 327:70-73, May 1987.

Squires, C.H. et al., Nucleic Acids Research

ABSTRACT

The present invention relates to mutant AHAS enzymes that are specifically resistant to imidazolinone herbicides. Exemplary of these is a corn sequence, which encodes an amino acid substitution at position 621 of the wild-type AHAS enzyme. Polynucleotides encoding the mutant enzyme can be used in screening methods for identifying plant cells that

3 Claims, 29 Drawing Sheets

### 19) United States

12) Patent Application Publication (10) Pub. No.: US 2004/0268444 A1 Filho et al. (43) Pub. Date: Dec. 30, 2004

(54) PROCESS FOR OBTAINING TRANSGENIC LEGUMINOUS PLANTS (LEGUMINOSAE) CONTAINING EXOGENOUS DNA

nnorcod

(76) Inventors: Elibio Leopoldo Rech Filho, Brasilia (BR); Francisco Jose Lima Apacao, Brasilia (BR)

> Correspondence Address: SMITH, GAMBRELL & RUSSELL, LLP 1850 M STREET, N.W., SUITE 800 WASHINGTON, DC 20036 (US)

(21) Appl. No.: 10/861,456

(22) Filed:

Jun. 7, 2004

- Related U.S. Application Data
- (63) Continuation of application No. 09/509,982, filed on Jun. 7, 2000, now Pat. No. 6,753,458, filed as 371 of international application No. PCT/BR97/00053, filed

Publication Classification

(51)	Int. Cl.7	C12N 15/82; C12N 15/87
(52)	U.S. Cl.	A01H 5/00 800/293; 800/278; 800/300; 800/312: 800/300;
		1012, 000/298

ABSTRACT

(57)

The present invention refers to a plant produced by a process of selecting germ line-transformed leguminous plants. The process includes introducing exogenous genes into apical meristematic cells of the embryonic axis of leguminous plants by bombarding the plant cells with a DNA construct which includes a sequence which encodes a protein capable of conferring tolerance to a herbicide selected from the group consisting of an imidazolinone and a glyphosate, inducing multiple shoot formation from the transformed cells by culturing the embryonic axis in a medium containing a cytokinin, and selecting the meristematic cell-derived shoots obtained by culturing the embryonic axis on a medium containing imidazolinone or glyphosate.

3 Claims, 29 Drawing Sheets

have been transformed.

(57)



## **Partial metabolic pathway**



## genetic material





Process for obtaining transgenic leguminous plants (Leguminosae) containing exogenous DNA







- Rech et al., NATURE Protocols, 2008, 3. 410-418
- EMBRAPA; WO99/18223, US patent 6,753,458, Rech & Aragão





## Deregulation

## **Molecular characterization**

## **Protein characterization, Food / Feed safety**

Composition equivalence, nutritional equivalence

Agronomic equivalence, environmental safety







## December 2010: Brazilian authorities have approved the commercial use of <u>a new</u> <u>genetically-modified soy seed</u> which was developed jointly by BASF and EMBRAPA - a private and public partnership -



The potential environmental impact of introducing "Cultivance<sup>™</sup>" (genetically modified herbicide tolerant) soybeans into the Brazilian soybean sector

Source: Rech E.; EMBRAPA/BASF/PG Economics



## Potential <u>fuel savings</u> from adoption of "Cultivance<sup>™</sup>" soybeans



baseline is 2009-10 planting area of approximately 23.1 million hectares





baseline is 2009-10 planting area of approximately 23.1 million hectares

# Production of recombinant molecules in genetically modified plants?



## soybean seeds

## ≈ 20% oil ≈ 40% protein



Soybean protein storage vacuoles (PSVs) are temporal extension organelles of the endoplasmic reticulum specialized in accumulating and compartmentalizing proteins

PSV = protein storage vacuoles OB = oil bodies

soybean section (100 X) light microscopy

OB

**PSV** 





## Human Growth Hormone (rhGH)

Cunha et al., Transgenic Research, 2011, 20, 811-826

28 22 19 accumulation of recombinant human growth bormone (hGH) in transgenic soybean seeds

A kDa



3

Δ

5

2



Protein targeting to protein storage vacuoles (PSV) was evaluated by ultrastructural immunocytochemistry of rhGH presence in ultra-thin sections of soybean cotyledons





Full hGH recombinant protein sequence expressed in soybeans. Black bars correspond to the digestion sites of trypsin. Coloured boxes indicate sequence peptides confirmed by MS/MS spectra. Blue boxes indicate hGH peptide sequences with expected masses. The green box indicates sequences with carbamidomethyl+C modification caused by the protein digestion protocol. Numbers above the coloured boxes indicate the monoisotopic masses of the corresponding peptide.



## proteinID X MW X PI





- Biological activity stable cells express the wild-type hGH receptor (WT hGHR), and their proliferation is normally dependent on the presence of growth factors, such as hGH





## Human coagulation factor IX

Cunha et al., Transgenic Research, 2011, 20, 841-855



## recombinant human coagulation Factor IX



Ultrastructural immunocytochemistry evaluation of recombinant hFIX in ultra-thin sections of soybean cotyledon. (a) Subcellular accumulation of recombinant hFIX in protein storage vacuoles (PSV) of line 1 transgenic seeds; (b) non-transgenic seeds. OB = oil bodies. MORVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRY NSGKLEEFVOGNLERECMEERCSFEEAREVFENTERITEFWROYVDG DOCESNFCLNGGSCKDDINSYECWCPFGFEGKNCELDVTCNIKNGRC EQFCKNSADNKVVCSCTEGYRLAENOKSCEPAVPFPCGRVSVSOTSK LTRAETVFPDVDYVNSTEAETILDNITOSTOSFNDFTRVVGGEDAKP GQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEH NIEETEHTEOKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNS YVTPICIADKEYTNIFLKFGSGYVSGWGRVFHKGRSALVLOYLRVPL VDRATCLRSTKFTIYNNMFCAGEHEGGRDSCOGDSGGPHVTEVEGTS FLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKTKLT



Full hFIX recombinant protein sequence expressed in soybean. Black bars correspond to digestion sites of trypsin. Coloured boxes indicate peptide sequences confirmed by MS/MS spectra. Blue boxes indicate hFIX peptide sequences with expected masses. The green box indicates sequences with carbamidomethyl+C modification caused by protein digestion protocol. Numbers above coloured boxes indicate the monoisotopic mass of the corresponding peptide.

lotting activity of recombinant hFIX from transgenic soybean se								
Sample	Clotting	Relative hFIX activity of						
	time (s)	normal human blood (%)						
Standard hFIX in normal	human							
plasma								
1 (1:80)	40.5	75.5						
2 (1:160)	45.0	28.7						
3 (1:320)	50.1	10.7						
4 (1:640)	54.8	4.7						
5 (1:1280)	60.3	1.9						
6 (1:2560)	63.1	1.3						
7 (1:5120)	64.9	1.0						
Soybean seeds								
Transgenic Line 1	119.5	1.0						
Transgenic Line 7	117.5	1.4						
Non-transgenic	254	0						



# **CANCER ANTIGENS**



## The promise of CTAG, GAGE and PLAC1 as a candidate for specific immune recognition of cancer comes from its restricted expression in normal tissues but frequent occurrence in cancer



ARASGPGGGA	PRGPHGGAAS	GLNGUURUGA	RGPESRLLEF	A PEAULAU AUDITALIA
EAELARRSLA	QDAPPLPVPG	VLLKEFTVSG	NILTIR <mark>LTAA</mark>	DHRQLQLSIS
SCLQQLSLLM	WITQCFLPVF	LAQPPSGQRR		

Murad et al., J. Sep. Sci., 2011, 34, 2618-2630

Entry	Description	mW	pl (pH)	PLGS	Amount	% of TSP
		(Da)		Score	(ng)	
P78358	Cancer testis antigen 1	17981	8.4739	2886.386	0.2635	0.11445
O22120	$\alpha$ -subunit of $\beta$ -conglycinin Fragment OS Glycine max PE 2 SV 2	63126	4.7254	51090.21	65.47	28.43671
C6T488	Putative uncharacterised protein OS Glycine max PE 2 SV 1	24103	5.1341	49332.05	0	0
P04776	Glycinin G1 OS Glycine max GN GY1 PE 1 SV 2	55671	5.8257	34638.61	28.6832	12.45847
P19594	2S albumin OS Glycine max PE 1 SV 2	18447	5.0153	26866.75	6.9832	3.033133
Q549Z4	Proglycinin A2B1 OS Glycine max PE 2 SV 1	54356	5.2983	26163.09	9.1288	3.965068
P04405	Glycinin G2 OS Glycine max GN Gy2 PE 1 SV 2	54356	5.2983	26155.1	16.8381	7.31358
C6TKH0	Putative uncharacterised protein OS Glycine max PE 2 SV 1	31640	6.4124	25943.07	4.4002	1.911214
B3TDK4	Lipoxygenase OS Glycine max PE 3 SV 1	94352	5.8755	23234.38	11.2248	4.87546
P08170	Seed lipoxygenase 1 OS Glycine max GN LOX1 1 PE 1 SV 2	94310	5.9301	22866.77	0	0
P01063	Bowman Birk type proteinase inhibitor C II OS Glycine max PE 1 SV 2	9194	4.3797	19673.12	2.7779	1.206573
P01064	Bowman Birk type proteinase inhibitor D II OS Glycine max PE 1 SV 2	9460	4.6657	18789.71	1.434	0.622854
P24337	Hydrophobic seed protein OS Glycine max PE 1 SV 1	8353	6.0467	17254.79	0.5404	0.234721
Q39805	Dehydrin-like protein OS Glycine max PE 2 SV 1	23703	6.084	16428.98	5.4437	2.364455
Q7GC77	Glycinin A3B4 subunit OS Glycine max PE 1 SV 1	58151	5.4199	14016.68	0.3179	0.138079
Q852U4	Glycinin A1bB2 784 OS Glycine max PE 2 SV 1	54264	5.9489	12395.54	0.6519	0.283151
Q852U5	Glycinin A1bB2 445 OS Glycine max PE 2 SV 1	54183	5.7768	12393.59	0.4914	0.213438
P05046	Lectin OS Glycine max GN LE1 PE 1 SV 1	30908	5.5955	11981.52	8.6499	3.757059
C6T9Z5	Putative uncharacterised protein OS Glycine max PE 2 SV 1	42796	6.2935	11699.31	1.0956	0.475871
C6TDF5	Putative uncharacterised protein OS Glycine max PE 2 SV 1	41854	6.9985	11311.33	0.5088	0.220996
Q9SEK9	Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1	25713	4.7899	9964.625	1.0382	0.450939
Q9SEK8	Seed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV	26087	4.63	9770.589	0.9765	0.42414
Q9XET1	Seed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 1	17735	6.104	9168.433	1.5019	0.652346
Q9SEL0	Seed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1	26824	4.9752	8024.353	0.8781	0.3814



# **MICROBICIDES AGAINST HIV**





molecular and biochemical analysis of soybean transgenic plants, CV-N transcripts and mature protein in the transgenic seeds. (A) PCR; (B) RT-PCR; (C) WB



kinetics of CV-N expression at different phenological stages (*A*); (*B*) Northern blot; (*C*) Hybridization of the soy elongation factor probe/respective mRNAs; (*D*) Western blot; (*E*) SDS-PAGE loading controls TSP (~100 µg)





organ-specific Western blot analysis. Immunoassays of TSP extracts (100 µg) from roots, stems, leaves, flowers and seeds of a transgenic plant



ultrastructural immunocytochemistry of recombinant CV-N presence in ultra-thin sections of soybean cotyledons





Western blot of soybean seeds expressing cyanovirin. 1) Purified CV-N (*E.coli*); 2) Soy non-transgenic; 3) Soy line 10/11; 4) Soy line 10/13; 5) Soy line 10/17; 6) Soy line 10/22 and 7) Soy line 10/25.







Accession	Entry	Description	mW (Da)	pl (pH)	PLGS Score	Coverage	Amount	% TSP
				4 = 4 = 1		(%)	(ngrams)	
CVN1_SOYBN	E00004	Cyanovirin-N	11119	4,7401	14082,18	70,5882	0,4576	0,445096
GLYG2_SOYBN	P04405	Glycinin G2 OS Glycine max GN Gy2 PE 1 SV 2	54356	5,2983	32228,25	63,299	30,9577	30,11177
GLYG1_SOYBN	P04776	Glycinin G1 OS Glycine max GN GY1 PE 1 SV 2	55671	5,8257	52555,09	52,5253	29,2242	28,42564
Q4LER5_SOYBN	Q4LER5	Beta conglycinin alpha subunit Fragment OS Glycine max PE 2 SV 1	70021	4,8483	74914,2	53,9735	14,1308	13,74467
SBP_SOYBN	Q04672	Sucrose binding protein OS Glycine max GN SBP PE 1 SV 1	60484	6,4228	5371,13	46,5649	3,8262	3,721648
B3TDK4_SOYBN	B3TDK4	Lipoxygenase OS Glycine max PE 3 SV 1	94352	5,8755	6860,105	53,5161	3,4109	3,317696
C6SWW4_SOYBN	IC6SWW4	Putative uncharacterized protein OS Glycine max PE 2 SV 1	22432	4,6553	20889,78	42,3645	2,6463	2,573989
LEC_SOYBN	P05046	Lectin OS Glycine max GN LE1 PE 1 SV 1	30908	5,5955	5512,499	28,7719	2,552	2,482266
O64458_SOYBN	O64458	Gly m Bd 30K allergen OS Glycine max GN P34 PE 2 SV 1	42730	5,5616	912,6769	18,9974	1,5229	1,481286
7SB1_SOYBN	P13917	Basic 7S globulin OS Glycine max GN BG PE 1 SV 2	46363	8,2441	1257,887	31,3817	1,0365	1,008177
Q42795_SOYBN	Q42795	Beta amylase OS Glycine max PE 1 SV 1	56036	5,1887	1702,356	47,5806	1,0075	0,97997
ADH1_YEAST	P00330	ALCOHOL DEHYDROGENASE I EC 1 1 1 1	36668	6,2734	3226,613	36,8876	0,9173	0,892234
C6TKH0_SOYBN	C6TKH0	Putative uncharacterized protein OS Glycine max PE 2 SV 1	31640	6,4124	1984,977	48,9796	0,8329	0,810141
C6EVF9_SOYBN	C6EVF9	Elongation factor 1 alpha OS Glycine max GN EF 1A PE 2 SV 1	49365	9,2369	1960,385	29,3065	0,7952	0,773471
C6T9Z5_SOYBN	C6T9Z5	Putative uncharacterized protein OS Glycine max PE 2 SV 1	42796	6,2935	3241,802	55,6701	0,7491	0,728631
Q38JB2_SOYBN	Q38JB2	Chloroplast lipocalin OS Glycine max GN CHL PE 2 SV 1	36630	6,1227	245,1823	25,228	0,7333	0,713262
C6T7M2_SOYBN	C6T7M2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	35828	7,9616	1706,998	46,1538	0,587	0,57096
C6T8W9_SOYBN	C6T8W9	Putative uncharacterized protein OS Glycine max PE 2 SV 1	31705	7,6479	1468,348	55,9727	0,5355	0,520867
C6TDF5 SOYBN	C6TDF5	Putative uncharacterized protein OS	41854	6,9985	3264,335	53,8058	0,5302	0,515712











Accession	Entry	Description	mW (Da)	pI (pH)	PLGS Score	Coverage (%)	Amount (ngrams)	Sample %
CVN1_SOYBN	E000	Cyanovirin-N	11119	4.7401	14888.99	76.4706	34.7071	13.18248
Q9S9D0_SOYBN	Q9S9D	Glycinin G4 subunit OS Glycine max	63640	5.2386	14317.44	86.4286	44.529	16.91304
Q9SB11_SOYBN	Q9SB1	Glycinin OS Glycine max GN A5A4B3	63758	5.0043	14314.52	83.659	128.9708	48.9858
Q43452_SOYBN	Q4345	Glycinin OS Glycine max GN Gy4 PE 3	63837	5.0479	14060.63	76.1566	0	0
GLYG1_SOYBN	P04776	Glycinin G1 OS Glycine max GN GY1	55671	5.8257	7773.647	62.2222	22.5529	8.566062
C6T034_SOYBN	C6T034	Putative uncharacterized protein OS Glycine max PE 2	24261	4.3334	4555.267	49.3274	4.0634	1.543364
Q9XET1_SOYBN	Q9XET	Seed maturation protein PM31 OS Glycine max GN PM31 PE	17735	6.104	3269.925	69.281	1.1212	0.425855
2SS_SOYBN	P19	2S albumin OS Glycine max PE	18447	5.0153	3129.056	46.8354	1.3706	0.520582
C6TLF0_SOYBN	C6TLF0	Putative uncharacterized protein OS Glycine max PE 2 S	24289	4.3802	3126.956	56.9507	1.0715	0.406978
C6TN36_SOYBN	C6TN3	Putative uncharacterized protein OS Glycine max PE 2 S	36973	7.3506	2236.746	47.9412	3.6537	1.387752
C6TD56_SOYBN	C6TD5	Putative uncharacterized protein OS Glycine max PE 2 S	36991	7.3511	2225.717	47.9412	3.148	1.195676
EF1A_SOYBN	P25698	Elongation factor 1 alpha OS Glycine max GN TEFS1 PE 3 S	49346	9.3931	1948.932	47.4273	1.6341	0.620665
C6EVF9_SOYBN	C6EVF9	Elongation factor 1 alpha OS Glycine max GN EF 1A PE 2	49365	9.2369	1928.087	45.6376	0.0246	0.009344
C6TB67_SOYBN	C6TB67	Putative uncharacterized protein OS Glycine max PE 2	22971	7.7157	1468.592	42.3645	0.399	0.151549
ADH1_YEAST	P00330	ALCOHOL DEHYDROGENASE I EC 1 1	36668	6.2734	1087.198	68.5879	0.9173	0.34841
C6SWC7_SOYBN	C6SWC	Putative uncharacterized protein OS Glycine max PE 2 SV 1	25829	10.5602	871.4886	40.9483	0.2815	0.10692
C6T5M6_SOYBN	C6T5M	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17425	5.3696	837.6975	43.7909	0	0
C6SXV0_SOYBN	C6SXV0	Putative uncharacterized protein OS Glycine max PE 2 SV 1	15403	10.8699	813.1491	39.3939	0.2317	0.088004
C6TLD2_SOYBN	C6TLD2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	31374	9.9534	783.3578	23.8908	0.7223	0.274345
SBP_SOYBN	Q04672	Sucrose binding protein OS Glycine max GN SBP PE 1 SV 1	60484	6.4228	762.5027	61.0687	8.0291	3.04962
C6SV69_SOYBN	C6SV69	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17775	7.1816	414.1665	36.7089	0.1871	0.071064
Q9SEK9_SOYBN	Q9SEK9	Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1	25713	4.7899	402.9525	57.7075	0.4915	0.186682
Q39871_SOYBN	Q39871	Late embryongenesis abundant protein OS Glycine max GN MP2 PE 2 SV 1	50613	6.2924	398.0186	48.3801	2.9882	1.134981
C6T1Q7_SOYBN	C6T1Q7	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17812	5.9577	350.2724	70.3226	0.3007	0.114212
C6T2F2_SOYBN	C6T2F2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17983	5.6922	339.2488	25.1572	0.6253	0.237502
C6T9Z5_SOYBN	C6T9Z5	Putative uncharacterized protein OS Glycine max PE 2 SV 1	42796	6.2935	299.0843	41.2371	0	0
C6T5N1_SOYBN	C6T5N1	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17777	9.4995	280.1813	50	0.2588	0.098298
Q9AVK8_SOYBN	Q9AVK 8	Allergen Gly m Bd 28K Fragment OS Glycine max PE 2 SV 1	52608	5.6576	210.1718	34.0381	0.5597	0.212586
Q9SEK8_SOYBN	Q9SEK8	Seed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV 1	26087	4.63	208.6765	44.358	0.0829	0.031487
C6TJC4_SOYBN	C6TJC4	Putative uncharacterized protein OS Glycine max PE 2 SV 1	34811	6.6876	189.7686	13.5048	0.36	0.136736

Accession	Entr	Description	mW	pl (pH)	PLGS	Coverage	Amount	Sample
	У		(Da)		Score	(%)	(ngrams)	%
CVN1_SOYBN	E00 0	Cyanovirin-N	11119	4.7401	14888.99	76.4706	34.7071	13.18248
Q9S9D0_SOYBN	Q9S 9D	Glycinin G4 subunit OS Glycine max	63640	5.2386	14317.44	86.4286	44.529	16.91304
Q9SB11_SOYBN	Q9S B1	Glycinin OS Glycine max GN A5A4B3	63758	5.0043	14314.52	83.659	128.9708	48.9858
Q43452_SOYBN	Q43 45	Glycinin OS Glycine max GN Gy4 PE 3	63837	5.0479	14060.63	76.1566	0	0
GLYG1_SOYBN	P04 776	Glycinin G1 OS Glycine max GN GY1	55671	5.8257	7773.647	62.2222	22.5529	8.566062
C6T034_SOYBN	C6T 034	Putative uncharacterized protein OS Glycine max PE 2	24261	4.3334	4555.267	49.3274	4.0634	1.543364
Q9XET1_SOYBN	Q9X ET	Seed maturation protein PM31 OS Glycine max GN PM31 PE	17735	6.104	3269.925	69.281	1.1212	0.425855
2SS_SOYBN	P19	2S albumin OS Glycine max PE	18447	5.0153	3129.056	46.8354	1.3706	0.520582
C6TLF0_SOYBN	C6T LF0	Putative uncharacterized protein OS Glycine max PE 2 S	24289	4.3802	3126.956	56.9507	1.0715	0.406978
C6TN36_SOYBN	C6T N3	Putative uncharacterized protein OS Glycine max PE 2 S	36973	7.3506	2236.746	47.9412	3.6537	1.387752
C6TD56_SOYBN	C6T D5	Putative uncharacterized protein OS Glycine max PE 2 S	36991	7.3511	2225.717	47.9412	3.148	1.195676
EF1A_SOYBN	P25 698	Elongation factor 1 alpha OS Glycine max GN TEFS1 PE 3 S	49346	9.3931	1948.932	47.4273	1.6341	0.620665
C6EVF9_SOYBN	C6E VF9	Elongation factor 1 alpha OS Glycine max GN EF 1A PE 2	49365	9.2369	1928.087	45.6376	0.0246	0.009344
C6TB67_SOYBN	C6T B67	Putative uncharacterized protein OS Glycine max PE 2	22971	7.7157	1468.592	42.3645	0.399	0.151549
ADH1_YEAST	P00 330	ALCOHOL DEHYDROGENASE	36668	6.2734	1087.198	68.5879	0.9173	0.34841







## Main steps of the chemical industrial process for extraction of soy oil and protein







Expression of the Cyanovirin in non-transgenic (NT) and transgenic (T) soybean seed oil and protein samples (TSP) (Prot-dot-blot)





# synthetic domestication of biodiversity organisms





## Spider anatomy





## Parawixia bistriatra minor ampullate spidroin (GenBank: GQ275358)

## 







Teule *et al.*, NATURE Protocols, 2009, 3, 341-355







## Acknowledgements

Rech Laboratory A. Melro - Embrapa C. Lacorte - Embrapa D. Bittenccourt - Embrapa F. da Silva - Embrapa G. Vianna - Embrapa M. Coelho – Embrapa

E. Contini, F. Marques,
G. Matsunaga, K. Pereira
N. Cunha, P. Oliveira,
P. Sousa, T. Souza ,T. Lima
V. Lacerda,

Collaborators O'Keefe - National Inst. Health, USA C. Bloch - Embrapa, Brazil F. Prosdoscimi - UFRJ, Brazil G. Ritter - Ludwig Cancer Res. Inst., USA J. Ma - Univ. London, England L. Paulino - EMBRAPA, Brazil M. Waters - Univ. Queensland, Australia P. Ismael - Inst. Butantan, Brazil P. Motta - Univ. Brasilia, Brazil R. Lewis - Univ. Utah, USA

Funding: EMBRAPA; CNPQ; CAPES; FAP-DF/Pronex; AFORS; BASF;