

Title

Expression analysis of HPPD W336 and 2mEPSPS in transgenic soybean event FG72

Authors

**Veerle Habex
Jurgen Debaveye**

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Testing Facility

**Bayer BioScience N.V.
BioAnalytics - Molecular Characterization
Technologiepark 38
B-9052 Gent
Belgium**



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APPROVALS PAGE

Study manager

Veerle Habex

29/10/09

Date

Study director

Jurgen Debaveye

29/10/09

Date

Molecular Characterization Manager
Gent

Dirk Nennstiel

29/10/09

Date

Sponsor
Global Regulatory Affairs Manager

Russ Essner

29/10/2009

Date

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**ABBREVIATIONS**

µg	microgram
CV	Coefficient of Variance
DNA	DeoxyriboNucleic Acid
DW	Dry Weight
ELISA	Enzyme Linked ImmunoSorbent Assay
FW	Fresh Weight
g	unit for relative centrifugal force
g	gram
KCl	Potassium chloride
kDa	kiloDalton
KH ₂ PO ₄	Potassium dihydrogen phosphate
LOD	Limit of Detection
LOQ	Limit of Quantification
mg	milligram
mL	milliLiter
mM	milliMolar
NA	Not Applicable
Na ₂ HPO ₄ ·H ₂ O	Disodium hydrogen phosphate hydrated
NaCl	Sodium chloride
ND	Not Determined
ng	nanogram
nm	nanometer
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
rpm	Revolutions per minute
SD	Standard Deviation
TEP	Total Extractable Protein
USA	United States of America
V	Vegetative growth stage
WT	Wild Type

SUMMARY

Soybean plants were transformed by means of direct gene transfer with plasmid pSF10, containing a *2mepsps* gene construct, conferring tolerance to glyphosate and a *hppdPFW336* gene construct, conferring tolerance to isoxaflutole. The explants were grown to complete plants, and an elite event (FG72) was selected.

In this study, the HPPD W336 and 2mEPSPS protein expression levels were determined in different tissues at different growth stages of FG72.

FG72 transgenic and Jack wild type plants were grown under greenhouse conditions. leaf samples taken at three different growth stages (V4, V6 and V8), stem and root samples taken at two different growth stages (V4 and V8) and seeds used to grow the plants were analyzed for the HPPD W336 and 2mEPSPS protein content.

The range of the expressions levels, expressed per gram dry weight are summarized in the table below.

Matrix	Growth stage	Range of HPPD W336 expression levels (µg/g dry weight)	Range of 2mEPSPS expression levels (µg/g dry weight)
Leaf	V4	16.7 – 65.7	283 – 958
	V6	12.8 – 96.0	216 – 753
	V8	11.6 – 51.8	351 – 1180
Stem	V4	8.29 – 24.6	68.0 – 350
	V8	2.49 – 13.0	76.1 – 151
Root	V4	2.98 – 11.0	10.8 – 54.5
	V8	1.51 – 12.4	19.9 – 81.2
Seed	NA	0.79 – 2.96	1.48 – 4.13

The average expressions levels and the ranges of the expression levels both expressed per gram fresh weight and per gram dry weight are summarized in Table 5.

1. Introduction

Soybean plants were transformed by means of particle bombardment with plasmid pSF10, containing a *2mepsps* gene construct, conferring tolerance to glyphosate and an *hppdPfw336* gene construct, conferring tolerance to isoxaflutole. The explants were grown to complete plants, and an elite event (FG72) was selected.

2. Objective

The goal of this study was to analyze the protein expression levels of HPPD W336 and 2mEPSPS in leaf, root, stem and seed of FG72 plants..

Different growth stages were analyzed to obtain expression data covering the life cycle of the transgenic soybean event FG72. Information on the HPPD W336 and 2mEPSPS protein contents in these tissues will be used to support the safety assessment of transgenic soybean event FG72.

3. Test and reference items, control items

Plants of the transgenic soybean event FG72 and plants of the non-transgenic soybean (variety Jack) line were grown under conventional conditions described in protocol number 09BAGM0004 in the greenhouses of Bayer BioScience N.V. (Astene, Belgium).

The transgenic plants were selected by spraying with glyphosate before harvesting.

The test items used in this study are different tissues from FG72 plants, the reference items are different tissues from WT soybean plants, variety Jack.

All available leaf, root and stem tissue of FG72 and WT soybean was harvested from individual plants. Leaf samples were taken at 3 different growth stages (V4, V6 and V8, see Table 1). Root and stem samples were taken at 2 different growth stages (V4 and V8, see Table 1). The samples were directly frozen in liquid nitrogen and shipped on dry ice. Samples were stored at -70°C.

Seeds were collected from the same seedlots and were stored at room temperature until crushing.

Seedlot FG72: 32RRMM0497

Seedlot Jack: 32CON0521

Table 1: Tissue samples harvested at different growth stages

Growth Stage	Tissue	Harvest date
V4	leaf, stem, root	26/03/2009
V6	leaf	31/03/2009
V8	leaf, stem, root	06/04/2009

For all FG72 plants, the FG72 identity and zygosity (homozygous) was confirmed by means of PCR.

All non-transgenic plants were analyzed using a discriminating PCR to test for adventitious presence of P35S, 3'nos and cp4-epsps.

For non-transgenic seed, 25 pools of each 10 crushed seeds were analyzed using a discriminating PCR to test for adventitious presence of P35S and 3'nos.

As control items, the HPPD W336 protein produced in bacteria (Batch no. LB020309) and the 2mEPSPS protein produced in bacteria (Batch no. LEJ5838) were used to generate a standard curve for the ELISAs.

4. Methods

In order to analyze the presence of HPPD W336 and 2mEPSPS protein in these tissues, samples were crushed, extracted and the total amount of extractable protein (TEP) was determined using the Bradford method. The amount of HPPD W336 in the protein extracts was measured using a quantitative ELISA developed by Agdia (Elkhart, IN 46514, USA). The amount of 2mEPSPS in the protein extracts was measured using an ELISA developed by Strategic Diagnostics Inc. (SDI, Newark, DE, USA).

4.1 Extraction

For the expression analysis of HPPD W336 and 2mEPSPS protein, ten separate samples per tissue and per growth stage were chosen randomly and the complete samples were crushed with dry ice in a blender, resulting in homogeneous tissue samples. The powders were collected in pre-cooled 50 mL Falcon tubes, stored at -20 °C overnight to let the dry ice evaporate, and subsequently stored at -70 °C.

The amount of approximately 100 mg crushed material was weighed in pre-cooled 15 mL tubes. A volume of 4 mL PBST extraction buffer, prepared using the PBST powder supplied in the Agdia kit, was added. The composition of the PBST extraction buffer is shown in Table 2.

Samples were vortexed for 15 seconds or until all powder was in suspension, incubated on a rotary mixer at 30 rpm for 10 minutes at 4 °C and centrifuged at 4000 g for 10 minutes at 4°C. Supernatants were partly transferred to a 1.5 mL eppendorf tube and centrifuged at 20000 g for 5 minutes at 4°C. Clear supernatants were used as such or in dilutions to load on the ELISA plate.

Table 2 : Composition of the extraction buffer PBST

Extraction buffer PBST	
8 g/l	NaCl
1.15 g/l	Na ₂ HPO ₄
0.2 g/l	KH ₂ PO ₄
0.2 g/l	KCl
0.5 g/l	Tween-20
pH	7.4

4.2 Determination of Total Extractable Protein

After extraction, the total extractable protein (TEP) was measured using the Bradford protein assay with bovine serum albumin (BSA) as reference protein and by measuring the optical density (OD) at 595 nm (Bradford, 1976). Clear supernatants of the extracts were diluted in MQ water so that the total protein concentration of all samples could be fit to the standard curve. The BSA concentrations used to generate the standard curve (36-25-20-15-12-10-5 µg/mL) were prepared using PBST diluted with MQ water to the same extend as the samples, in order to compensate background signal from the PBST buffer.

The value of the TEP measurement was used as an internal control to quality check the protein extraction process. The value was not used in any calculation of ELISA results.

4.3 Determination of Dry Weight

Per soybean tissue and per growth stage, 2 pooled samples were used to determine dry weight. The analysis was performed by SGS Belgium, Agro Food Services, CTS, Antwerpen.

Crushed samples were weighed, dried overnight in an oven at 103 ± 2 °C and weighed again. This weight data was used to calculate the % moisture and the % dry weight of the samples.

The conversion of the expression data from fresh weight to dry weight was done using these values.

4.4 HPPD and 2mEPSPS ELISA

The HPPD ELISA kit from Agdia and the EPSPS ELISA kit commercialized by Strategic Diagnostics Inc. were used to determine respectively the HPPD W336 and the 2mEPSPS content in the protein extracts of FG72 tissues.

The procedures and the validation of these procedures can be found in the ELISA validation report (Habex and Debaveye, 2009).

Per tissue and growth stage, each of the 10 samples was assayed 3 times on the ELISA plate.

In order to fit the concentrations of HPPD W336 and 2mEPSPS to the respective standard curves, the protein extracts had to be diluted as described in Table 3.

Table 3: Final dilutions for protein extracts of the soybean FG72 tissues used for the HPPD and the EPSPS ELISA

Matrix	Final dilutions used for (fold dilution)	
	HPPD ELISA	EPSPS ELISA
Leaf	32	128
Stem	8	16
Root	4 or 8	16
Seed	8	4

5. Results and discussion

5.1 Determination of Total Extractable Protein

The TEP data, determined to assess the quality of the extractions, was per tissue for all samples always in the same range. Therefore, no ELISA results coming from samples having a poor TEP content were excluded.

5.2 Determination of Dry Weight

You can find an overview of the obtained results in Table 4.

Table 4 : Results of dry weight determination on FG72 samples

Matrix	Dry weight, as % of Fresh weight		
	Growth stage 1 (V4)	Growth stage 2 (V6)	Growth stage 3 (V8)
Leaf	15.88	18.11	17.23
Stem	8.94	NA	11.44
Root	15.05	NA	13.15
Seed	90.54		

5.3 Expression analysis of HPPD W336 and 2mEPSPS in FG72 tissue samples

The averages and the range of the HPPD W336 and the 2mEPSPS protein contents per gram fresh weight and per gram dry weight in the different transgenic tissues are given per growth stage in Table 5.

The ranges as well as the averages and standard deviations were derived and calculated from all 30 data points. All values were calculated with full precision and then rounded to 2 or 3 significant figures.

The values of the HPPD W336 protein contents of the ten individual plants and the calculated averages are given in Appendix 1.

For the HPPD W336 protein content in the root samples of V8 stage, one sample got lost during handling it. The average and SD were calculated on triplicate measurements of nine individual samples.

The values of the 2mEPSPS protein contents of the ten individual plants and the calculated averages are given in **Appendix 2**.

6. Conclusions

In all transgenic samples covering all analyzed growth stages and analyzed tissues, HPPD W336 and 2mEPSPS protein was detected.

Expression levels (average \pm SD and range), expressed per gram fresh weight and per gram dry weight can be found in Table 5.

Table 5 : HPPD W336 and 2mEPSPS contents per gram fresh and dry weight of the different FG72 soybean tissues and growth stages

Matrix	Growth stage	HPPD W336 protein content				2mEPSPS protein content			
		µg/g fresh weight		µg/g dry weight		µg/g fresh weight		µg/g dry weight	
		Average ± SD	Range	Average ± SD	Range	Average ± SD	Range	Average ± SD	Range
Leaf	V4	6.10 ± 2.78	2.65 – 10.4	38.4 ± 17.5	16.7 – 65.7	90.4 ± 26.1	44.9 – 152	569 ± 164	283 – 958
	V6	6.48 ± 4.08	2.31 – 17.4	35.8 ± 22.5	12.8 – 96.0	79.1 ± 29.6	39.2 – 136	437 ± 163	216 – 753
	V8	4.69 ± 1.87	2.00 – 8.91	27.2 ± 10.9	11.6 – 51.8	115 ± 38.2	60.5 – 203	668 ± 222	351 – 1180
Stem	V4	1.48 ± 0.42	0.74 – 2.20	16.6 ± 4.65	8.29 – 24.6	18.8 ± 6.16	6.08 – 31.3	211 ± 68.9	68.0 – 350
	V8	0.69 ± 0.35	0.29 – 1.49	6.04 ± 3.10	2.49 – 13.0	13.4 ± 2.62	8.71 – 17.3	117 ± 22.9	76.1 – 151
Root	V4	0.87 ± 0.35	0.45 – 1.66	5.81 ± 2.30	2.98 – 11.0	4.89 ± 1.99	1.63 – 8.21	32.5 ± 13.2	10.8 – 54.5
	V8	0.84 ± 0.50	0.20 – 1.64	6.42 ± 3.82	1.51 – 12.4	5.75 ± 2.31	2.62 – 10.7	43.7 ± 17.6	19.9 – 81.2
Seed	NA	1.27 ± 0.42	0.71 – 2.68	1.41 ± 0.47	0.79 – 2.96	2.37 ± 0.75	1.34 – 3.74	2.62 ± 0.83	1.48 – 4.13



REFERENCES

N°	Dart N°	Report N°	Author(s), year, title, source, edition, pages
1.	M-268549-01-1		Bradford M.M.; 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.
2.		BIO2-026_Express.prot_311	Habex and Debaveye, 2009, Validation of the quantitative HPPD and EPSPS ELISAs in soybean leaf, stem, root and seed tissue

Appendix 1: HPPD protein content in transgenic soybean FG72 tissues at different growth stages

FG72 LEAF tissue

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 leaf	V4	1	205.2	170.0	142.5	172.6	31.5	18.2	105	7.82	6.48	5.43	6.58	1.20	18.2
FG72 leaf	V4	2	207.4	172.6	146.6	175.5	30.5	17.4	104	7.98	6.64	5.64	6.75	1.17	17.4
FG72 leaf	V4	3	261.8	220.8	219.6	234.1	24.0	10.3	104	10.07	8.49	8.45	9.00	0.92	10.3
FG72 leaf	V4	5	252.6	273.7	209.0	245.1	33.0	13.5	105	9.62	10.43	7.96	9.34	1.26	13.5
FG72 leaf	V4	7	263.6	269.2	265.4	266.1	2.9	1.1	105	10.04	10.26	10.11	10.14	0.11	1.1
FG72 leaf	V4	8	93.3	90.8	72.9	85.7	11.1	13.0	110	3.39	3.30	2.65	3.12	0.41	13.0
FG72 leaf	V4	11	105.6	93.9	93.0	97.5	7.0	7.2	100	4.22	3.76	3.72	3.90	0.28	7.2
FG72 leaf	V4	12	184.6	163.1	144.7	164.2	20.0	12.2	102	7.24	6.40	5.68	6.44	0.78	12.2
FG72 leaf	V4	15	79.8	82.7	77.8	80.1	2.5	3.1	110	2.90	3.01	2.83	2.91	0.09	3.1
FG72 leaf	V4	17	74.5	72.4	71.9	73.0	1.4	1.9	103	2.89	2.81	2.79	2.83	0.05	1.9

Dilution: 1/32

Average \pm SD : 6.10 \pm 2.78 µg/g fresh weight, CV=45.5%

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 leaf	V6	30	102.2	107.4	119.1	109.6	8.7	7.9	102	2.31	2.87	2.87	2.68	0.32	12.0
FG72 leaf	V6	32	58.3	72.4	72.4	67.7	8.1	12.0	101	4.01	4.21	4.67	4.30	0.34	7.9
FG72 leaf	V6	35	207.8	182.4	218.9	203.0	18.7	9.2	101	3.75	3.76	4.41	3.98	0.37	9.4
FG72 leaf	V6	36	247.0	250.0	260.2	252.4	6.9	2.7	107	5.09	5.69	6.44	5.74	0.68	11.8
FG72 leaf	V6	37	460.6	401.5	417.5	426.5	30.6	7.2	106	2.73	2.62	2.95	2.77	0.17	6.2
FG72 leaf	V6	43	212.6	219.2	237.6	223.2	13.0	5.8	104	8.18	8.43	9.14	8.58	0.50	5.8
FG72 leaf	V6	48	78.8	78.4	94.6	83.9	9.2	11.0	107	2.95	2.93	3.54	3.14	0.35	11.0
FG72 leaf	V6	49	96.7	96.9	113.5	102.4	9.6	9.4	103	17.38	15.15	15.75	16.09	1.15	7.2
FG72 leaf	V6	50	131.0	146.6	166.0	147.9	17.5	11.8	103	8.23	7.22	8.67	8.04	0.74	9.2
FG72 leaf	V6	51	68.2	65.4	73.8	69.1	4.3	6.2	100	9.24	9.35	9.73	9.44	0.26	2.7

Dilution: 1/32

Average \pm SD : 6.48 \pm 4.08 µg/g fresh weight, CV=63.1%

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 leaf	V8	58	78.3	82.6	78.5	79.8	2.4	3.0	108	2.90	3.06	2.91	2.96	0.09	3.0
FG72 leaf	V8	59	169.2	173.0	167.5	169.9	2.8	1.7	103	6.57	6.72	6.51	6.60	0.11	1.7
FG72 leaf	V8	60	118.3	110.8	123.6	117.6	6.4	5.5	100	4.73	4.43	4.95	4.70	0.26	5.5
FG72 leaf	V8	61	131.1	116.6	145.8	131.2	14.6	11.1	109	4.81	4.28	5.35	4.81	0.54	11.1
FG72 leaf	V8	62	114.9	117.0	123.8	118.6	4.7	3.9	110	4.18	4.25	4.50	4.31	0.17	3.9
FG72 leaf	V8	63	110.6	102.4	113.3	108.8	5.6	5.2	102	4.34	4.02	4.44	4.27	0.22	5.2
FG72 leaf	V8	66	64.4	71.3	85.3	73.7	10.7	14.5	105	2.45	2.72	3.25	2.81	0.41	14.5
FG72 leaf	V8	67	234.0	202.2	232.3	222.8	17.9	8.0	105	8.91	7.70	8.85	8.49	0.68	8.0
FG72 leaf	V8	68	134.0	124.4	182.6	147.0	31.2	21.2	102	5.26	4.88	7.16	5.77	1.22	21.2
FG72 leaf	V8	81	58.7	54.9	70.5	61.4	8.1	13.2	110	2.13	2.00	2.56	2.23	0.29	13.2

Dilution: 1/32

Average \pm SD : 4.69 \pm 1.87 µg/g fresh weight, CV=39.9%

FG72 SEED tissue

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 seed	na	1	21.8	22.4	24.1	22.8	1.2	5.2	103	0.85	0.87	0.94	0.88	0.05	5.2
FG72 seed	na	3	23.8	21.3	23.9	23.0	1.5	6.4	100	0.95	0.85	0.96	0.92	0.06	6.4
FG72 seed	na	4	18.9	17.3	18.4	18.2	0.8	4.6	97	0.78	0.71	0.76	0.75	0.03	4.6
FG72 seed	na	2	42.4	46.2	43.6	44.0	1.9	4.4	106	1.60	1.74	1.64	1.66	0.07	4.4
FG72 seed	na	5	38.8	37.5	40.5	38.9	1.5	3.8	110	1.41	1.36	1.47	1.42	0.05	3.8
FG72 seed	na	5	35.4	32.4	37.7	35.2	2.6	7.5	102	1.39	1.27	1.48	1.38	0.10	7.5
FG72 seed	na	6	35.2	33.6	29.2	32.7	3.1	9.5	103	1.37	1.31	1.13	1.27	0.12	9.5
FG72 seed	na	6	34.0	37.0	32.6	34.5	2.2	6.4	106	1.28	1.40	1.23	1.30	0.08	6.4
FG72 seed	na	7	27.8	28.3	26.1	27.4	1.1	4.2	108	1.03	1.05	0.97	1.01	0.04	4.2
FG72 seed	na	8	71.0	51.1	47.2	56.5	12.8	22.6	106	2.68	1.93	1.78	2.13	0.48	22.6

Dilution: 1/8

Average \pm SD : 1.27 \pm 0.42 µg/g fresh weight, CV=33.2%

FG72 STEM tissue

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 stem	V4	4	43.1	40.0	39.3	40.8	2.0	5.0	105	1.64	1.52	1.50	1.56	0.08	5.0
FG72 stem	V4	5	42.0	41.4	39.7	41.0	1.2	2.9	108	1.56	1.53	1.47	1.52	0.04	2.9
FG72 stem	V4	6	30.7	29.1	30.2	30.0	0.8	2.6	109	1.12	1.07	1.11	1.10	0.03	2.6
FG72 stem	V4	18	56.0	55.6	55.3	55.6	0.4	0.7	102	2.20	2.18	2.17	2.18	0.01	0.7
FG72 stem	V4	19	26.1	24.7	24.8	25.2	0.8	3.1	109	0.96	0.91	0.91	0.93	0.03	3.1
FG72 stem	V4	20	20.1	18.9	18.5	19.2	0.8	4.3	100	0.80	0.76	0.74	0.77	0.03	4.3
FG72 stem	V4	21	42.6	45.6	43.1	43.7	1.6	3.6	109	1.56	1.67	1.58	1.60	0.06	3.6
FG72 stem	V4	22	46.2	45.7	46.9	46.3	0.6	1.3	104	1.78	1.76	1.80	1.78	0.02	1.3
FG72 stem	V4	23	43.2	47.5	45.9	45.5	2.2	4.8	104	1.66	1.83	1.76	1.75	0.08	4.8
FG72 stem	V4	24	39.8	40.4	43.0	41.1	1.7	4.1	102	1.56	1.58	1.69	1.61	0.07	4.1

Dilution: 1/8
Average ± SD : 1.48 ± 0.42 µg/g fresh weight, CV=28.1%

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 stem	V8	58	15.1	13.8	15.5	14.8	0.9	5.9	106	0.57	0.52	0.58	0.56	0.03	5.9
FG72 stem	V8	59	31.1	20.2	28.9	26.7	5.8	21.5	104	1.19	0.78	1.11	1.03	0.22	21.5
FG72 stem	V8	60	26.1	24.3	20.9	23.8	2.6	11.1	105	0.99	0.93	0.80	0.91	0.10	11.1
FG72 stem	V8	61	38.8	37.7	35.2	37.3	1.8	4.9	104	1.49	1.45	1.36	1.43	0.07	4.9
FG72 stem	V8	62	11.9	10.2	11.2	11.1	0.9	7.7	104	0.46	0.39	0.43	0.43	0.03	7.7
FG72 stem	V8	63	10.4	8.0	8.7	9.0	1.2	13.2	103	0.40	0.31	0.34	0.35	0.05	13.2
FG72 stem	V8	65	10.8	14.2	8.2	11.0	3.0	27.4	106	0.41	0.54	0.31	0.42	0.11	27.4
FG72 stem	V8	77	14.0	13.3	17.0	14.8	2.0	13.3	105	0.53	0.51	0.65	0.56	0.07	13.3
FG72 stem	V8	78	9.8	7.5	10.6	9.3	1.6	17.5	105	0.37	0.29	0.41	0.35	0.06	17.5
FG72 stem	V8	52	22.7	22.4	20.3	21.8	1.3	6.0	100	0.91	0.89	0.81	0.87	0.05	6.0

Dilution: 1/8
Average ± SD : 0.69 ± 0.35 µg/g fresh weight, CV=51.3%

FG72 ROOT tissue

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (%)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 root	V4	1 ²	37.7	33.9	31.4	34.3	3.2	9.2	106	1.42	1.28	1.18	1.30	0.12	9.2
FG72 root	V4	7 ²	21.4	24.0	21.3	22.3	1.6	7.0	103	0.83	0.93	0.83	0.86	0.06	7.0
FG72 root	V4	9 ²	39.0	41.5	39.0	39.9	1.4	3.6	100	1.56	1.66	1.56	1.59	0.06	3.6
FG72 root	V4	10 ²	13.2	11.6	13.5	12.8	1.0	8.0	100	0.53	0.46	0.54	0.51	0.04	8.0
FG72 root	V4	12 ²	24.1	24.0	22.9	23.7	0.7	2.8	101	0.96	0.95	0.91	0.94	0.03	2.8
FG72 root	V4	15 ²	14.9	13.2	12.6	13.6	1.2	8.6	107	0.56	0.49	0.47	0.51	0.04	8.6
FG72 root	V4	17 ²	22.5	25.0	23.6	23.7	1.2	5.1	108	0.83	0.92	0.87	0.88	0.05	5.1
FG72 root	V4	19 ²	16.0	17.7	18.1	17.3	1.1	6.4	103	0.62	0.69	0.70	0.67	0.04	6.4
FG72 root	V4	20 ¹	17.6	12.0	11.3	13.7	3.4	25.2	101	0.70	0.48	0.45	0.54	0.14	25.2
FG72 root	V4	24 ²	21.8	24.8	24.7	23.8	1.7	7.2	101	0.86	0.98	0.98	0.94	0.07	7.2

Average ± SD : 0.87 ± 0.35 µg/g fresh weight, CV=39.6%

Genotype	Sample growth stage	Plant n°	HPPD expression #1 (ng/ml)	HPPD expression #2 (ng/ml)	HPPD expression #3 (ng/ml)	Average HPPD expression (ng/ml)	SD HPPD expression (ng/ml)	CV HPPD expression (µg/g fresh weight)	Sample weight (mg)	HPPD expression #1 (µg/g fresh weight)	HPPD expression #2 (µg/g fresh weight)	HPPD expression #3 (µg/g fresh weight)	Average HPPD expression (µg/g fresh weight)	SD HPPD expression (µg/g fresh weight)	CV HPPD expression (%)
FG72 root	V8	58 ¹	30.7	29.4	26.6	28.9	2.1	7.2	110	1.12	1.07	0.97	1.05	0.08	7.2
FG72 root	V8	59 ²	40.8	38.9	39.8	39.9	1.0	2.4	100	1.63	1.56	1.59	1.59	0.04	2.4
FG72 root	V8	60 ¹	5.0	5.4	5.4	5.3	0.2	4.1	101	0.20	0.21	0.21	0.21	0.01	4.1
FG72 root	V8	61 ²	43.7	43.9	44.6	44.1	0.5	1.1	109	1.60	1.61	1.64	1.62	0.02	1.1
FG72 root	V8	62 ¹	22.5	21.8	22.8	22.4	0.5	2.4	102	0.88	0.85	0.90	0.88	0.02	2.4
FG72 root	V8	63 ¹	24.1	24.3	23.3	23.9	0.5	2.2	102	0.94	0.95	0.91	0.94	0.02	2.2
FG72 root	V8	65 ¹	17.3	18.0	16.4	17.2	0.8	4.5	108	0.64	0.67	0.61	0.64	0.03	4.5
FG72 root	V8	77 ¹	7.1	7.4	9.1	7.8	1.1	13.6	103	0.27	0.29	0.35	0.30	0.04	13.6
FG72 root	V8	78 ¹	8.6	11.8	9.3	9.9	1.7	17.2	108	0.32	0.44	0.35	0.37	0.06	17.2

Average ± SD : 0.84 ± 0.50 µg/g fresh weight, CV=59.5%

¹ = dilution 1/4 to measure between 1 - 8 ng/ml

² = dilution 1/8 to measure between 1 - 8 ng/ml

Appendix 2: 2mEPSPS protein content in transgenic soybean FG72 tissues at different growth stages
FG72 LEAF tissue

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 leaf	V4	1	3693	4043	4145	3960	237	6.0	109	135.5	148.4	152.1	145.3	8.7	6.0
FG72 leaf	V4	2	2437	2682	2643	2587	132	5.1	103	94.6	104.2	102.6	100.5	5.1	5.1
FG72 leaf	V4	3	2484	2824	2447	2585	208	8.0	106	93.7	106.6	92.3	97.5	7.8	8.0
FG72 leaf	V4	5	2303	2517	2493	2438	117	4.8	104	88.6	96.8	95.9	93.8	4.5	4.8
FG72 leaf	V4	7	1813	1833	1795	1814	19	1.0	100	72.5	73.3	71.8	72.6	0.8	1.0
FG72 leaf	V4	8	2590	2998	2750	2780	206	7.4	106	97.7	113.1	103.8	104.9	7.8	7.4
FG72 leaf	V4	11	2362	2471	2182	2338	146	6.2	103	91.7	96.0	84.7	90.8	5.7	6.2
FG72 leaf	V4	12	2309	2511	2496	2439	113	4.6	109	84.7	92.2	91.6	89.5	4.1	4.6
FG72 leaf	V4	15	1494	1730	1696	1640	128	7.8	109	54.8	63.5	62.2	60.2	4.7	7.8
FG72 leaf	V4	17	1223	1473	1298	1332	128	9.6	109	44.9	54.1	47.6	48.9	4.7	9.6

Dilution: 1/128
Average ± SD : 90.4 ± 26.1 µg/g fresh weight, CV=28.9%

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 leaf	V6	30	1010	1009	1202	1073	111	10.3	103	39.2	39.2	46.7	41.7	4.3	10.3
FG72 leaf	V6	32	1116	1188	1174	1159	39	3.3	99	45.2	48.1	47.5	46.9	1.6	3.3
FG72 leaf	V6	35	1340	1580	1493	1471	122	8.3	108	49.6	58.5	55.3	54.5	4.5	8.3
FG72 leaf	V6	36	2748	2948	2970	2889	122	4.2	100	109.9	117.9	118.8	115.6	4.9	4.2
FG72 leaf	V6	37	2235	2838	2691	2588	314	12.1	108	82.8	105.1	99.7	95.9	11.6	12.1
FG72 leaf	V6	43	3210	3714	3705	3543	289	8.1	109	117.8	136.3	136.0	130.0	10.6	8.1
FG72 leaf	V6	48	1896	2112	1957	1988	111	5.6	104	72.9	81.2	75.3	76.5	4.3	5.6
FG72 leaf	V6	49	1424	1574	1527	1508	76	5.1	104	54.8	60.5	58.7	58.0	2.9	5.1
FG72 leaf	V6	50	2541	2659	2380	2527	140	5.6	101	100.6	105.3	94.3	100.1	5.6	5.6
FG72 leaf	V6	51	1842	1862	1673	1792	104	5.8	100	73.7	74.5	66.9	71.7	4.2	5.8

Dilution: 1/128
Average ± SD : 79.1 ± 29.6 µg/g fresh weight, CV=37.4%

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 leaf	V8	58	2518	2640	2860	2673	173	6.5	106	95.0	99.6	107.9	100.9	6.5	6.5
FG72 leaf	V8	59	3224	3265	3306	3265	41	1.3	109	118.3	119.8	121.3	119.8	1.5	1.3
FG72 leaf	V8	60	2180	2223	2244	2216	33	1.5	105	83.0	84.7	85.5	84.4	1.2	1.5
FG72 leaf	V8	61	2446	2403	2467	2439	33	1.3	105	93.2	91.5	94.0	92.9	1.2	1.3
FG72 leaf	V8	62	2276	2400	2808	2494	278	11.2	103	88.4	93.2	109.0	96.9	10.8	11.2
FG72 leaf	V8	63	3501	3845	3901	3749	217	5.8	100	140.0	153.8	156.0	150.0	8.7	5.8
FG72 leaf	V8	66	3485	3570	3441	3498	66	1.9	104	134.0	137.3	132.3	134.6	2.5	1.9
FG72 leaf	V8	67	5453	5498	5430	5460	35	0.6	108	202.0	203.6	201.1	202.2	1.3	0.6
FG72 leaf	V8	68	2312	2741	2962	2671	330	12.4	102	90.7	107.5	116.1	104.8	13.0	12.4
FG72 leaf	V8	81	1573	1793	1686	1684	110	6.5	104	60.5	69.0	64.8	64.8	4.2	6.5

Dilution: 1/128

Average \pm SD : 115 \pm 38.2 µg/g fresh weight, CV=33.2%

FG72 SEED tissue

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 seed	na	1	90.4	87.1	90.0	89.1	1.8	2.0	99	3.66	3.52	3.64	3.61	0.07	2.0
FG72 seed	na	1	58.8	59.2	55.3	57.7	2.1	3.7	104	2.26	2.27	2.12	2.22	0.08	3.7
FG72 seed	na	2	38.9	44.6	39.0	40.9	3.3	8.0	108	1.45	1.66	1.45	1.52	0.12	8.0
FG72 seed	na	2	60.4	59.2	53.1	57.6	3.9	6.8	107	2.26	2.21	1.98	2.15	0.15	6.8
FG72 seed	na	3	71.0	75.1	75.3	73.8	2.5	3.3	105	2.70	2.86	2.87	2.81	0.09	3.3
FG72 seed	na	4	62.2	60.3	50.3	57.6	6.4	11.1	108	2.30	2.23	1.86	2.13	0.24	11.1
FG72 seed	na	5	65.9	68.5	61.3	65.3	3.6	5.6	107	2.47	2.56	2.29	2.44	0.14	5.6
FG72 seed	na	6	38.6	37.0	34.8	36.8	1.9	5.1	104	1.48	1.42	1.34	1.42	0.07	5.1
FG72 seed	na	7	44.7	45.5	46.0	45.4	0.6	1.4	100	1.79	1.82	1.84	1.82	0.03	1.4
FG72 seed	na	8	93.5	90.1	89.0	90.9	2.3	2.6	100	3.74	3.60	3.56	3.63	0.09	2.6

Dilution: 1/4

Average \pm SD : 2.37 \pm 0.75 µg/g fresh weight, CV=31.8%

FG72 STEM tissue

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 stem	V4	4	605	353	447	469	127	27.2	105	23.1	13.5	17.0	17.8	4.9	27.2
FG72 stem	V4	5	466	420	507	465	44	9.4	108	17.3	15.6	18.8	17.2	1.6	9.4
FG72 stem	V4	6	377	312	372	354	36	10.1	103	14.6	12.1	14.5	13.7	1.4	10.1
FG72 stem	V4	18	470	402	436	436	34	7.7	60	31.3	26.8	29.1	29.1	2.2	7.7
FG72 stem	V4	19	470	468	445	461	14	3.0	100	18.8	18.7	17.8	18.4	0.5	3.0
FG72 stem	V4	20	198	165	160	174	21	12.0	105	7.5	6.3	6.1	6.6	0.8	12.0
FG72 stem	V4	21	474	507	525	502	26	5.1	108	17.6	18.8	19.5	18.6	1.0	5.1
FG72 stem	V4	22	656	665	677	666	10	1.6	104	25.2	25.6	26.0	25.6	0.4	1.6
FG72 stem	V4	23	563	548	579	563	16	2.8	100	22.5	21.9	23.2	22.5	0.6	2.8
FG72 stem	V4	24	471	493	457	474	18	3.8	102	18.5	19.3	17.9	18.6	0.7	3.8

Dilution: 1/16
Average ± SD : 18.8 ± 6.16 µg/g fresh weight, CV=32.7%

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 stem	V8	52	416	436	427	426	10	2.4	109	15.3	16.0	15.7	15.7	0.4	2.4
FG72 stem	V8	58	278	276	268	274	6	2.0	103	10.8	10.7	10.4	10.6	0.2	2.0
FG72 stem	V8	59	352	363	362	359	6	1.7	105	13.4	13.8	13.8	13.7	0.2	1.7
FG72 stem	V8	60	396	467	415	426	36	8.5	109	14.5	17.1	15.2	15.6	1.3	8.5
FG72 stem	V8	61	403	464	436	434	31	7.0	107	15.0	17.3	16.3	16.2	1.1	7.0
FG72 stem	V8	62	343	360	316	340	22	6.5	103	13.3	14.0	12.3	13.2	0.9	6.5
FG72 stem	V8	63	287	299	263	283	18	6.4	109	10.5	11.0	9.7	10.4	0.7	6.4
FG72 stem	V8	65	454	460	465	460	6	1.2	109	16.6	16.9	17.1	16.9	0.2	1.2
FG72 stem	V8	77	261	323	328	304	37	12.3	101	10.3	12.8	13.0	12.0	1.5	12.3
FG72 stem	V8	78	222	260	275	253	28	10.9	102	8.7	10.2	10.8	9.9	1.1	10.9

Dilution: 1/16
Average ± SD : 13.4 ± 2.62 µg/g fresh weight, CV=19.5%

FG72 ROOT tissue

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 root	V4	1	201.4	209.2	223.7	211.4	11.3	5.4	109	7.39	7.68	8.21	7.76	0.42	5.4
FG72 root	V4	7	128.0	124.5	133.7	128.7	4.6	3.6	101	5.07	4.93	5.30	5.10	0.18	3.6
FG72 root	V4	9	93.7	100.6	101.4	98.6	4.2	4.3	105	3.57	3.83	3.86	3.76	0.16	4.3
FG72 root	V4	10	47.7	52.0	43.2	47.7	4.4	9.2	106	1.80	1.96	1.63	1.80	0.17	9.2
FG72 root	V4	12	173.8	180.4	180.5	178.2	3.8	2.1	109	6.38	6.62	6.62	6.54	0.14	2.1
FG72 root	V4	15	43.0	49.8	45.8	46.2	3.4	7.5	100	1.72	1.99	1.83	1.85	0.14	7.5
FG72 root	V4	19	164.1	194.4	181.6	180.0	15.2	8.4	105	6.25	7.41	6.92	6.86	0.58	8.4
FG72 root	V4	20	105.8	118.5	119.2	114.5	7.6	6.6	102	4.15	4.65	4.67	4.49	0.30	6.6
FG72 root	V4	21	164.8	181.4	175.9	174.0	8.4	4.9	109	6.05	6.66	6.45	6.39	0.31	4.9
FG72 root	V4	24	100.6	116.8	111.4	109.6	8.2	7.5	101	3.99	4.63	4.41	4.34	0.33	7.5

Dilution: 1/16
Average ± SD : 4.89 ± 1.99 µg/g fresh weight, CV=40.7%

Genotype	Sample growth stage	Plant n°	2mEPSPS expression #1 (ng/ml)	2mEPSPS expression #2 (ng/ml)	2mEPSPS expression #3 (ng/ml)	Average 2mEPSPS expression (ng/ml)	SD 2mEPSPS expression (ng/ml)	CV 2mEPSPS expression (%)	Sample weight (mg)	2mEPSPS expression #1 (µg/g fresh weight)	2mEPSPS expression #2 (µg/g fresh weight)	2mEPSPS expression #3 (µg/g fresh weight)	Average 2mEPSPS expression (µg/g fresh weight)	SD 2mEPSPS expression (µg/g fresh weight)	CV 2mEPSPS expression (%)
FG72 root	V8	58	213.2	175.8	185.8	191.6	19.4	10.1	101	8.44	6.96	7.36	7.59	0.77	10.1
FG72 root	V8	59	207.4	173.8	183.5	188.2	17.3	9.2	101	8.18	6.86	7.24	7.43	0.68	9.2
FG72 root	V8	60	116.9	104.0	104.6	108.5	7.3	6.7	109	4.30	3.83	3.85	3.99	0.27	6.7
FG72 root	V8	61	290.8	250.9	239.2	260.3	27.1	10.4	109	10.67	9.21	8.78	9.55	0.99	10.4
FG72 root	V8	62	161.4	146.8	162.3	156.8	8.7	5.6	100	6.47	5.88	6.51	6.29	0.35	5.6
FG72 root	V8	63	243.4	183.0	182.1	202.8	35.2	17.3	101	9.63	7.24	7.20	8.03	1.39	17.3
FG72 root	V8	65	115.5	105.6	127.5	116.2	10.9	9.4	108	4.28	3.91	4.72	4.30	0.41	9.4
FG72 root	V8	77	82.1	85.1	79.8	82.3	2.6	3.2	103	3.19	3.31	3.10	3.20	0.10	3.2
FG72 root	V8	78	73.4	66.8	66.2	68.8	4.0	5.8	101	2.91	2.65	2.62	2.72	0.16	5.8
FG72 root	V8	75	114.4	113.6	103.8	110.6	5.9	5.3	101	4.53	4.50	4.11	4.38	0.23	5.3

Dilution: 1/16
Average ± SD : 5.57 ± 2.31 µg/g fresh weight, CV=40.3%