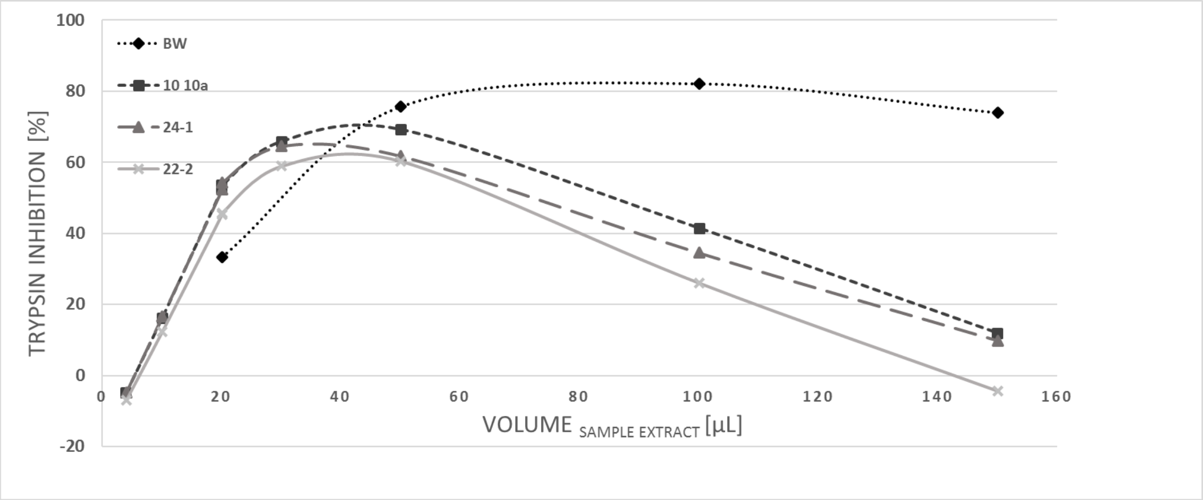

Fig. S1: Progress of trypsin inhibition with varying sample extract volumes.

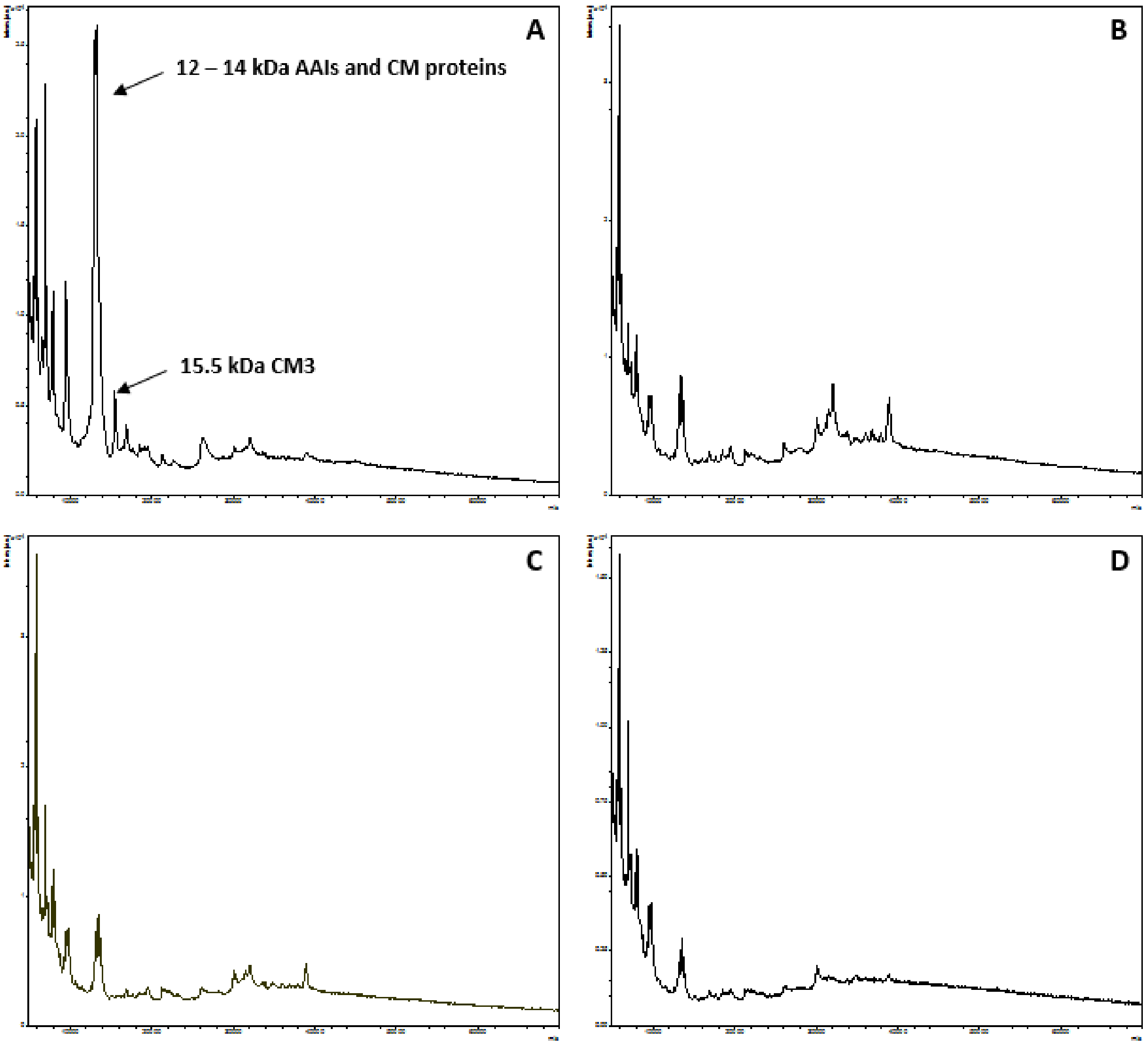

Fig. S2: MALDI-TOF spectra of salt-soluble proteins of (A) Bobwhite, (B) 10-10a, (C) 24-1 and (D) 22-2 in the range of 5 – 70 kDa.

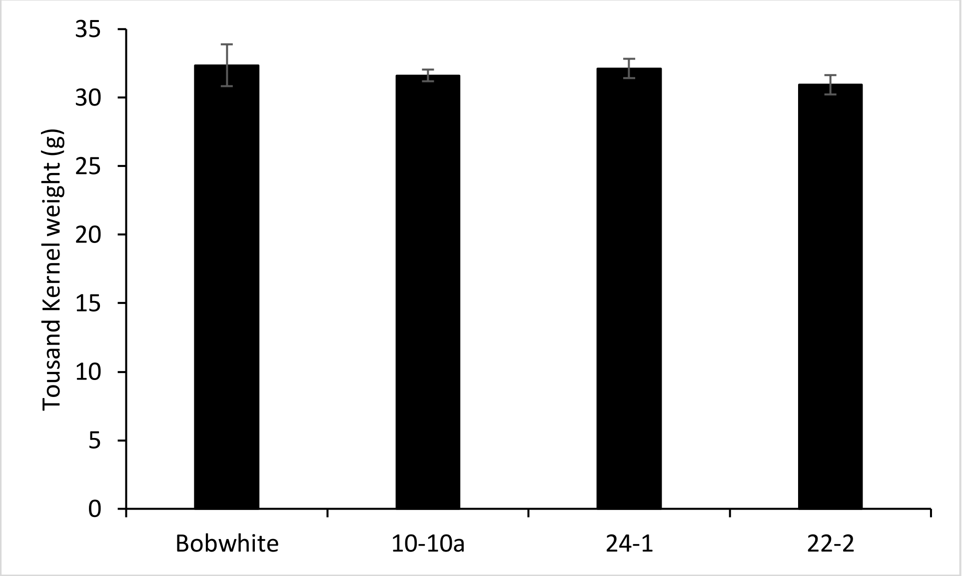

Fig. S3: Comparison of the thousand kernel weight (TKW) between the RNAi transgenic lines and Bobwhite control plants. Two independent experiments were performed. Data were subjected to ANOVA analysis, but no significant differences were detected

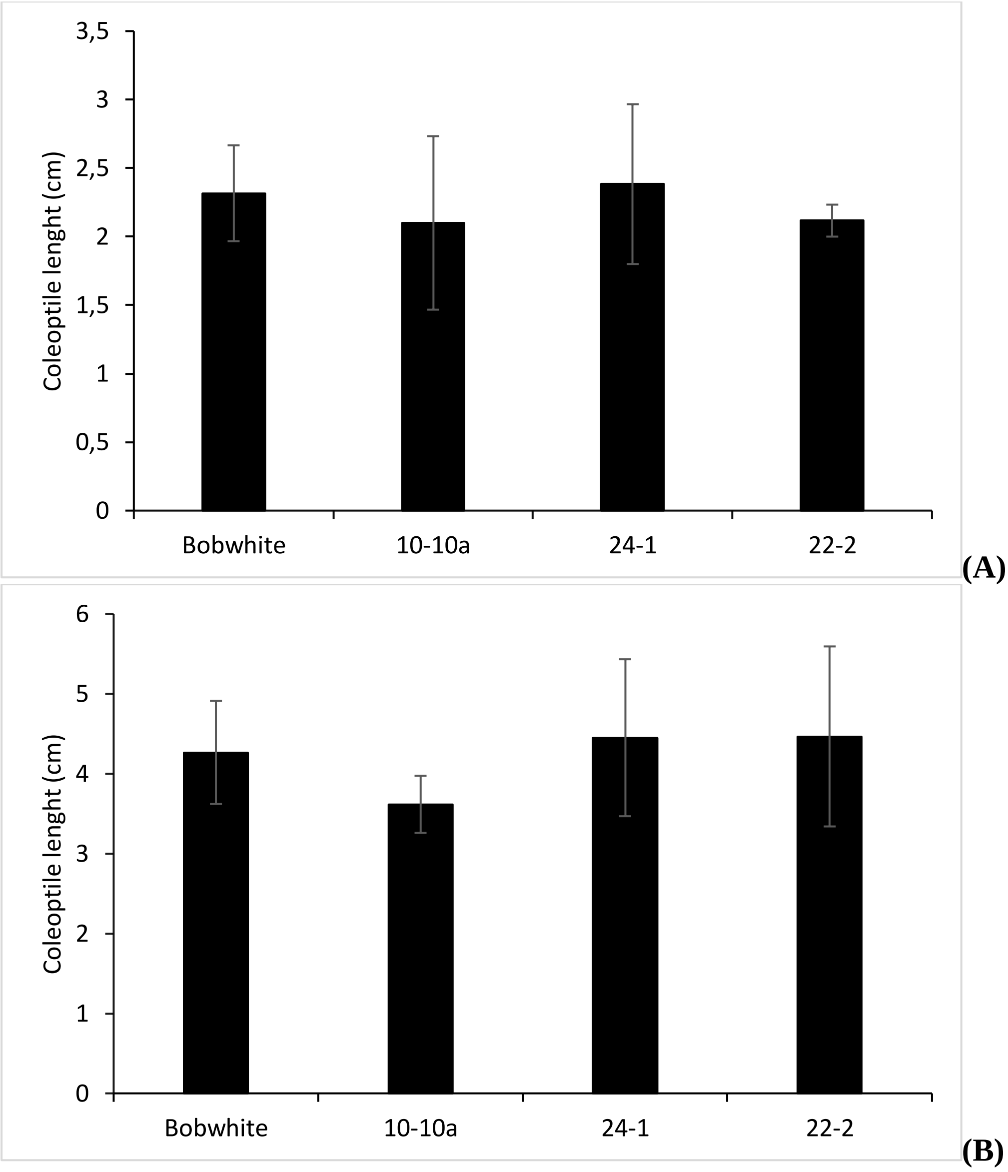

Fig. S4: Comparison of the coleoptile length (cm) at (A) 5 and (B) 7 days post imbibition (DPI) between RNAi transgenic lines and Bobwhite control plants. Two independent experiments were performed. Data were subjected to ANOVA analysis, but no significant differences were detected

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127 TACAAGGTGAGCGCACTCACGGGCTGCCGGGCA-ATGGTGAAGCTCCAGT    175
    | |.|||.||||.|.||...||..|||| |||| .|.|||.|.|..||||
104 t-ccaggagagctcgcttgaggcatgcc-ggcaggttgtggaccaacagt    151

176 GTGTGGGCAGTCAGGTGCC--------CGAGGCTGTCCTAAGAGAT----    213
       |||.|.|||.|.||||        ||.|||  |||    ||||
152 ---tggccggtcggctgccatggagcacggggc--tcc----agatgcga    192

214 TGCTGCCAGCAGCTGGCCGACAT--------CAACAACGAATGGTGCAGG    255
    ||||||||||||||  ||||.||        |||         ||||.|.
193 tgctgccagcagct--ccgagatgttagcgccaa---------gtgccgc    231

256 TGCGGGGACCTCAGCAGCATGTTGCGTAGTGTTTATCAG-GAGCTCGGCG    304
    |.||..|.|.|||||  ||.||.|| .||....|||.|| .|.||    |
232 tccgtcgccgtcagc--caagtcgc-aagacaatatgagcaaact----g    274

305 TGCGTG-------AGGGGAAGGAGGTGCT----CCCAGGTTGCCGGAAGG    343
    || |||       ||||  .|||  |.||    |||.|||
275 tg-gtgccgcccaaggg--cgga--tccttctaccctggt----------    309
```

Fig. S5. Comparison between homologous regions of *Dy10* and *0.28* genes. In uppercase the sequence corresponding to *0.28* and in lower case that of *Dy10*. In green the conserved sequence is indicated, whereas the red square includes also the further partially conserved region

Table S1: List of primers used for cloning of *CM3*, *CM16* and *0.28* genes in pRDPT vector and screening for presence of the construct in Bialaphos-resistant plants and their progeny.

Primer name	**Sequence 5´-3´**
iRNA_Sal-CM3-F	AATAGTCGACATGGCGTGCAAGTCCAGCTG
iRNA ClaI-CM3-R,	AATAATCGATGAGGCCGCTCTCACCAACATTG
iRNA XhoI-CM3-F	AATACTCGAGGAGGCCGCTCTCACCAACATTG
iRNA XbaI-CM3-R	AATATCTAGAATGGCGTGCAAGTCCAGCTG
iRNA SalI-CM16-F	AATAGTCGACATGGCGTCCAAGTCCAACTG
iRNA ClaI-CM16-R	AATAATCGATCTAGCTCCACTGAGACTCCTCC
iRNA XhoI-CM16-F	AATACTCGAGCTAGCTCCACTGAGACTCCTCC
iRNA XbaI-CM16-R	AATATCTAGAATGGCGTCCAAGTCCAACTG
: iRNA Sal-AAI0.28-F	AATAGTCGACATGTGGATGAAGACCGTGTT
iRNA ClaI-0.28 R	AATAATCGATCTAGACGTCCGGATA
iRNA XhoI-0.28_F	AATACTCGAGCTAGACGTCCGGATAC
iRNA XbaI-0.28-R	AATATCTAGAATGTGGATGAAGACCGTGTT
Intron R	ACCTCTAGACTCGAGCCACCTTCATGTTGGTCAATAG
Dx5_Promo 2F	TGGTCCTGAACCTTCACCTC
UBI-49F	TCGATGCTCACCCTGTTGTTT
BAR 2	GAAACCCACGTCATGCCAGT
Actin 77F	TCCTGTGTTGCTGACTGAGG
Actin 312R	GGTCCAAACGAAGGATAGC

Table S2: Labelling of samples for iTRAQ analysis

Sample name	Amount of protein labelled (µg)	iTRAQ reagent
Bobwhite	29	114
24-1	29	115
22-2	29	116
10-10a	29	117

Table S3: IgE reactivity of patient sera expressed in ng/mL. IgE tot: total IgE; IgE spe A/G: IgE specific to albumin/globulin fraction from bread wheat cv Récital; s: saturated

Serum N°	134	610	639	642	779	858	1026	1120
IgE tot	>2000	>2000	8151	524	815	6095	2267	468
IgE spe A/G	s	281	333	154	110	172	164	100

Serum N°	1157	1265	1266	1353	1494	1572	1638	1674
IgE tot	11113	11486	13098	363	1454	15843	6741	1468
IgE spe A/G	119	s	140	1	327	877	300	208

Serum N°	1747	1766	1826	1830	1862	1875
IgE tot	2196	339	32065	1854	4724	5609
IgE spe A/G	192	123	1181	137	216	s

Table S4: Differential expression of the ATI proteins in the three RNAi transgenic lines compared to Bobwhite control plants.

Protein Name	**Line 24-1**		**Line 22-2**		**Line 10-10a**		**No of Peptides matched (at 95% confidence level)**	**Sequence Coverage (%)**	**Accession No (Uniprot Protein ID)**
	Fold Change	**P-Val**	**Fold Change**	**P-Val**	**Fold Change**	**P-Val**			
Alpha-amylase inhibitor ATI CM16	-90.91	0.00	-24.63	0.01	-17.21	0.01	14	59	tr|B9VRI3
Monomeric alpha-amylase inhibitor ATI 0.28	-79.37	0.00	-23.98	0.00	-23.31	0.00	17	68	tr|X2KYP9
Alpha amylase inhibitor ATI CM3	-72.46	0.00	-24.88	0.01	-23.98	0.01	29	66	tr|Q6S5B1
CM 17 protein	-59.17	0.01	-27.78	0.01	-29.07	0.01	21	71	tr|Q41540
Alpha amylase-trypsin inhibitor	-50.51	0.11	-46.51	0.12	-11.38	0.15	24	55	tr|A0A4P8DL25
Alpha-amylase/trypsin inhibitor CM1	-28.33	0.09	-9.55	0.12	-11.92	0.11	16	37	tr|R7W9W1
Dimeric alpha-amylase inhibitor	-18.18	0.10	-9.37	0.13	-7.73	0.13	20	77	tr|I6PZ03
Dimeric alpha-amylase inhibitor	-10.37	0.01	-13.93	0.01	-6.08	0.01	10	55	tr|C3VW67
Thaumatin-like xylanase inhibitor	-10.09	0.00	-4.66	0.01	-5.55	0.01	2	12	tr|Q0WX48
Trypsin inhibitor CMc	-6.79	0.03	-5.20	0.02	-4.21	0.03	18	34	tr|N1QTW5
Bowman-Birk type trypsin inhibitor	-6.61	0.01	-13.05	0.01	-6.37	0.01	6	56	sp|P81713
Xylanase inhibitor protein	-4.97	0.04	-6.79	0.04	-2.13	0.09	2	7	tr|R9UNY9
Bowman-Birk type trypsin inhibitor	-2.75	0.30	-5.75	0.19	-2.63	0.34	2	31	tr|M8BFX0
Trypsin inhibitor Cme	-2.63	0.08	-1.77	0.19	-2.23	0.11	3	30	tr|M7ZXQ3
Xylanase inhibitor	-2.33	0.25	1.79	0.91	1.19	0.40	16	29	tr|Q8H0K8
Xylanase inhibitor 725ACCN	-1.04	0.84	1.82	0.66	2.07	0.60	21	43	tr|A7UME2
Xylanase inhibitor TAXI-IV	1.14	0.00	1.28	0.00	1.28	0.00	15	25	tr|Q5TMB2
Subtilisin-chymotrypsin inhibitor-2A	1.24	0.14	1.31	0.08	1.19	0.17	2	28	tr|M8BXE2

Notes:
All the fold change values of silenced lines are compared to the parental cultivar Bobwhite.
Negative fold change means lesser amount in folds and vies versa.
Minimum 2 peptides matching have been used as cut off value.

Table S5: Differential expression of the glutenin and gliadin proteins in the three RNAi transgenic lines compared to Bobwhite control plants.

Protein Name	Line 24-1		Line 22-2		Line 10-10a		No of Peptides matched (at 95% confidence level)	Sequence Coverage (%)	Accession No (Uniprot Protein ID)
	Fold Change	**P-Val**	**Fold Change**	**P-Val**	**Fold Change**	**P-Val**			
Low-molecular-weight glutenin subunit	-3.63	0.00	0.14	0.00	-4.29	0.00	22	31	tr|F8SGN6
Low-molecular-weight glutenin subunit	-4.79	0.07	-6.73	0.04	-7.31	0.05	32	39	tr|R4JBK0
Low molecular weight glutenin subunit P-13	-12.59	0.18	-9.81	0.18	-10.47	0.18	13	17	tr|A0A2R3C1P7
High-molecular-weight glutenin subunit Bx7.1	-26.81	0.40	-15.55	0.82	-6.14	0.89	130	35	tr|G4Y3Y0
High-molecular-weight glutenin subunit 1By15	-3.37	0.20	-2.91	0.23	-2.70	0.24	27	24	tr|W0C8U3
High-molecular-weight glutenin subunit	-3.63	0.17	-2.00	0.39	-1.79	0.41	47	71	tr|Q7XAH1
High-molecular-weight glutenin subunit	-4.74	0.07	-2.21	0.15	-3.47	0.14	9	38	tr|Q7XZB4
High molecular weight glutenin subunit	-42.02	0.09	-32.47	0.09	-19.23	0.00	72	36	tr|A9YSK3
High molecular weight glutenin subunit	-1.71	0.43	-1.94	0.35	-1.89	0.36	85	33	tr|A0A0X9BSF8
High molecular weight glutenin subunit	-13.05	0.25	-9.55	0.32	-4.41	0.30	63	22	tr|A0MZ38
High molecular weight glutenin subunit	-1.63	0.20	-1.58	0.21	-1.57	0.22	63	38	tr|W6AX70
High molecular weight glutenin subunit 1Dy12	-11.59	0.17	-6.31	0.22	-4.13	0.24	14	15	tr|A0A3G2C3E3
High molecular weight glutenin subunit	-14.73	0.14	-23.75	0.09	-15.41	0.16	100	37	tr|A0A060MZP1
High molecular weight glutenin 1Dx1.6t	-64.10	0.04	-21.69	0.01	-16.89	0.00	24	12	tr|Q0GLF2
Gamma-gliadin	-1.13	0.40	-5.75	0.08	-3.19	0.14	191	45	tr|B6UKS0
Gamma-gliadin	-1.14	0.48	-1.36	0.31	-1.33	0.31	42	49	tr|A0A4D6QBN8
Gamma-gliadin	1.04	0.29	-3.94	0.16	-1.89	0.22	33	26	tr|L7R664
Gamma-gliadin	1.04	0.98	1.09	0.79	1.08	0.72	104	57	tr|R9XV71
Gamma-gliadin	2.05	0.78	-1.07	0.84	1.27	0.89	95	51	tr|B6UKM6
Gamma-gliadin	1.20	0.71	-1.14	0.78	1.03	0.91	59	37	tr|W8E1A4
Alpha-gliadin	3.47	0.19	3.05	0.21	2.75	0.23	98	53	tr|A0A1K0IT04

Notes:
All the fold change values of silenced lines are compared to the parental cultivar Bobwhite.
Negative fold change means lesser amount in folds and vies versa.
Minimum 2 peptides matching have been used as cut off value.

Table S6: Differential expression of the non-target proteins in the three RNAi transgenic lines compared to Bobwhite control plants.

Protein Name	**Line 24-1**		**Line 22-2**		**Line 10-10a**		**No of Peptides matched (at 95% confidence level)**	**Sequence Coverage (%)**	**Accession No (Uniprot Protein ID)**
	Fold Change	**P-Val**	**Fold Change**	**P-Val**	**Fold Change**	**P-Val**			
Uncharacterized protein	-44.05	0.00	-16.75	0.00	-15.27	0.00	48	53	tr|A0A3B6RB62
Avenin-like b2	-36.36	0.00	-21.10	0.00	-20.88	0.00	10	28	sp|P0CZ05
Puroindoline b	-32.26	0.00	-10.09	0.00	-10.76	0.00	10	37	tr|G8CNL6
AAI domain-containing protein	-22.27	0.00	-12.24	0.00	-18.02	0.00	32	70	tr|A0A3B6JR20
AAI domain-containing protein	-14.33	0.03	-16.29	0.02	-8.47	0.03	23	65	tr|A0A446WXS8
Grain softness protein-1A	-12.36	0.08	-8.87	0.02	-4.70	0.03	27	45	tr|Q5BLR0
Grain softness protein	-11.59	0.00	-6.20	0.04	-9.29	0.00	25	42	tr|A8QRP5
Farinin protein	-11.06	0.03	-7.94	0.03	-6.08	0.10	87	60	tr|W8Q2K7
Grain softness protein-1D	-10.18	0.00	-11.17	0.09	-8.87	0.00	25	30	tr|Q5BLQ4
Superoxide dismutase [Cu-Zn]	-10.00	0.05	-14.45	0.02	-7.11	0.05	3	27	tr|C3VQ50
Alpha purothionin	-10.00	0.03	-7.38	0.02	-10.00	0.01	7	28	tr|Q9T0P1
rRNA N-glycosidase	-8.79	0.01	-5.30	0.01	-5.39	0.01	2	10	tr|Q7M1Z3
AAI domain-containing protein	-8.32	0.05	-8.55	0.04	-7.73	0.05	32	56	tr|A0A3B6I2R2
Eukaryotic translation initiation factor 3 subunit J	-8.32	0.01	-3.77	0.02	-3.10	0.03	2	10	tr|M8C1L9
Prefoldin subunit 4	5.20	0.00	4.74	0.00	4.29	0.00	3	21	tr|R7W9V1
Tubulin beta chain	5.30	0.03	4.49	0.05	5.06	0.04	4	8	tr|I1HXL8
EF1_GNE domain-containing protein	5.40	0.01	5.75	0.01	5.60	0.01	6	29	tr|A0A3B6ATD7
Protein disulfide-isomerase	6.08	0.00	7.52	0.00	7.24	0.00	30	34	tr|Q9FE55
Eukaryotic translation initiation factor 5A	9.55	0.03	13.18	0.02	19.05	0.02	2	12	tr|Q3S4I1
WHy domain-containing protein	16.60	0.00	15.42	0.00	16.44	0.00	4	27	tr|W5CUW2
60S acidic ribosomal protein P2B	17.54	0.00	20.14	0.00	18.54	0.00	12	78	tr|M8A407
Uncharacterized protein	21.88	0.01	16.90	0.08	16.29	0.11	20	38	tr|A0A453HBR5
DUF89 domain-containing protein	31.62	0.02	22.28	0.02	28.84	0.02	2	6	tr|M8BFM0
Uncharacterized protein	99.08	0.01	99.08	0.01	99.08	0.01	3	3	tr|W5GLX4

Notes:
All the fold change values of silenced lines are compared to the parental cultivar Bobwhite. Negative fold change means lesser amount in folds and vies versa.
Minimum 2 peptides matching have been used as cut off value.