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(54) METHODS AND COMPOSITIONS FOR RESISTANCE TO CYST NEMATODE IN PLANTS

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(21) Appl. No.: **16/102,475**

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Publication Classification

(51) Int. Cl.
C12N 15/82 (2006.01)

(52) U.S. Cl.
CPC *C12N 15/8285* (2013.01)

(57) ABSTRACT

The disclosure relates to methods and compositions for producing plants or plant cells that exhibit improved cyst nematode resistance.

Specification includes a Sequence Listing.

1 NSF$_{Ch07}$: 14 LC

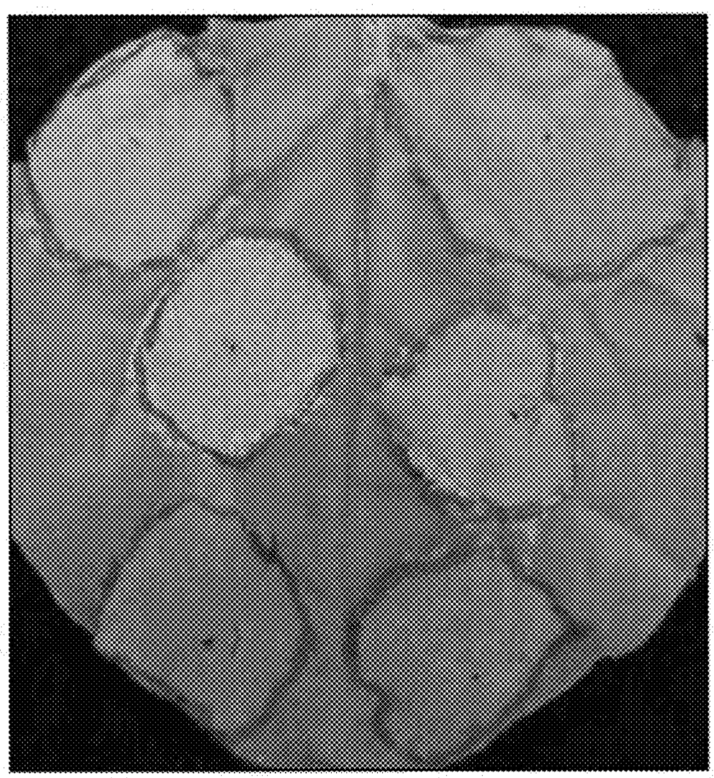
1 RAN07 : 14 LC

1 EV : 09 LC

1 NSF$_{Ch13}$: 09 LC

1 RAN07 : 09 LC

1 NSF$_{Ch07}$: 09 LC



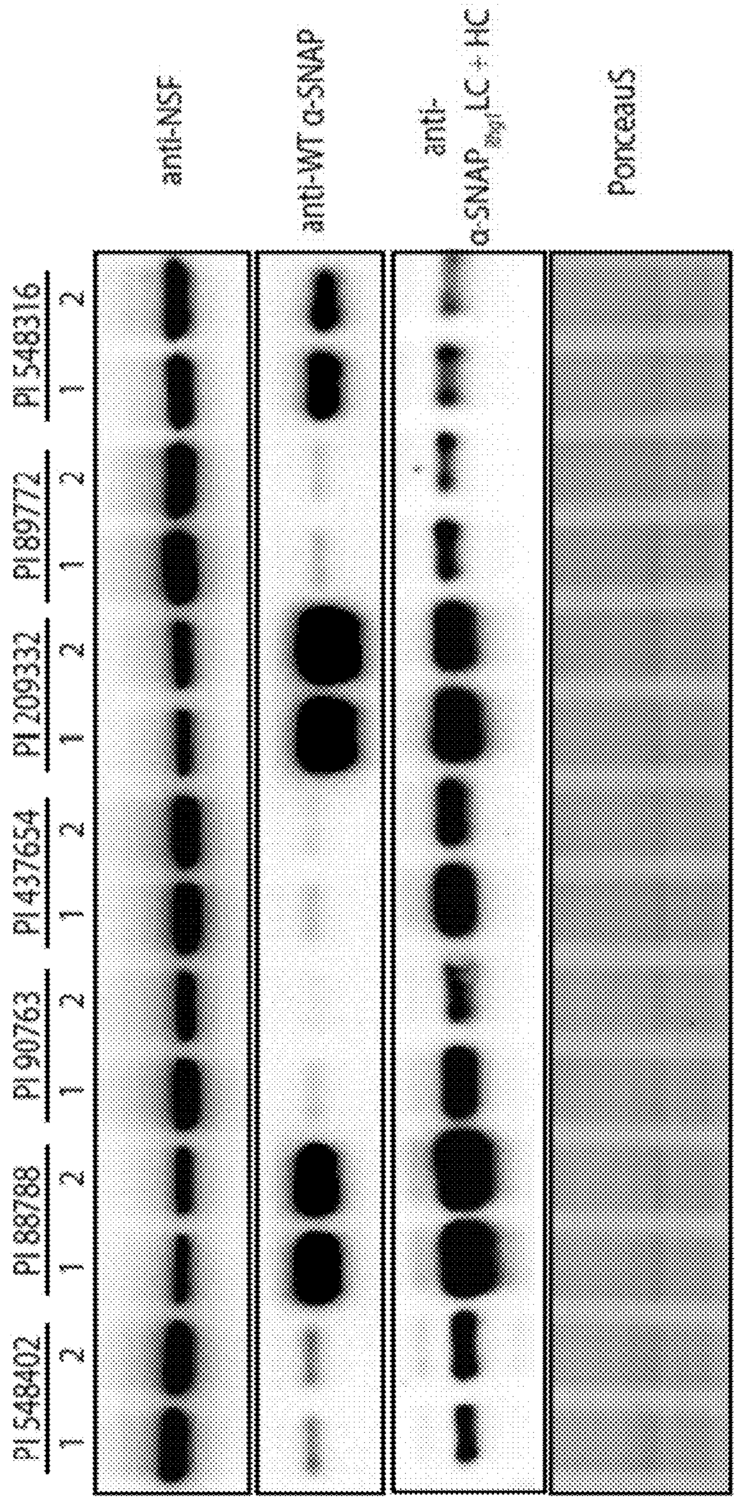

PI 548402
1 2
PI 88788
1 2
PI 90763
1 2
PI 437654
1 2
PI 209332
1 2
PI 89772
1 2
PI 548316
1 2
anti-NSF
anti-WT α-SNAP
anti-α-SNAP$_{Rhg1}$LC + HC
PonceauS

Figure 1A

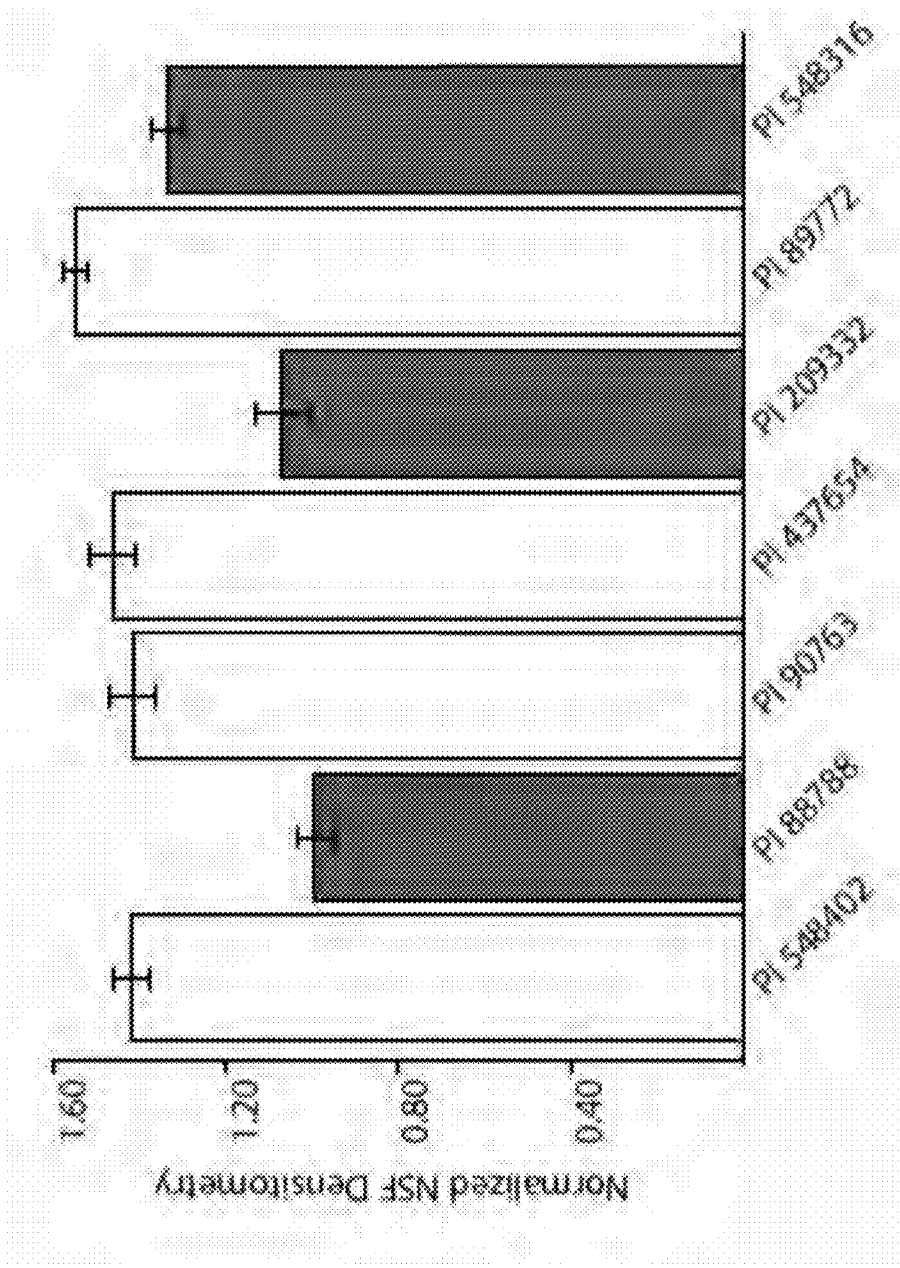

Normalized NSF Densitometry
1.60
1.20
0.80
0.40
PI 548402
PI 88788
PI 90763
PI 437654
PI 209332
PI 89772
PI 548316

Figure 1B

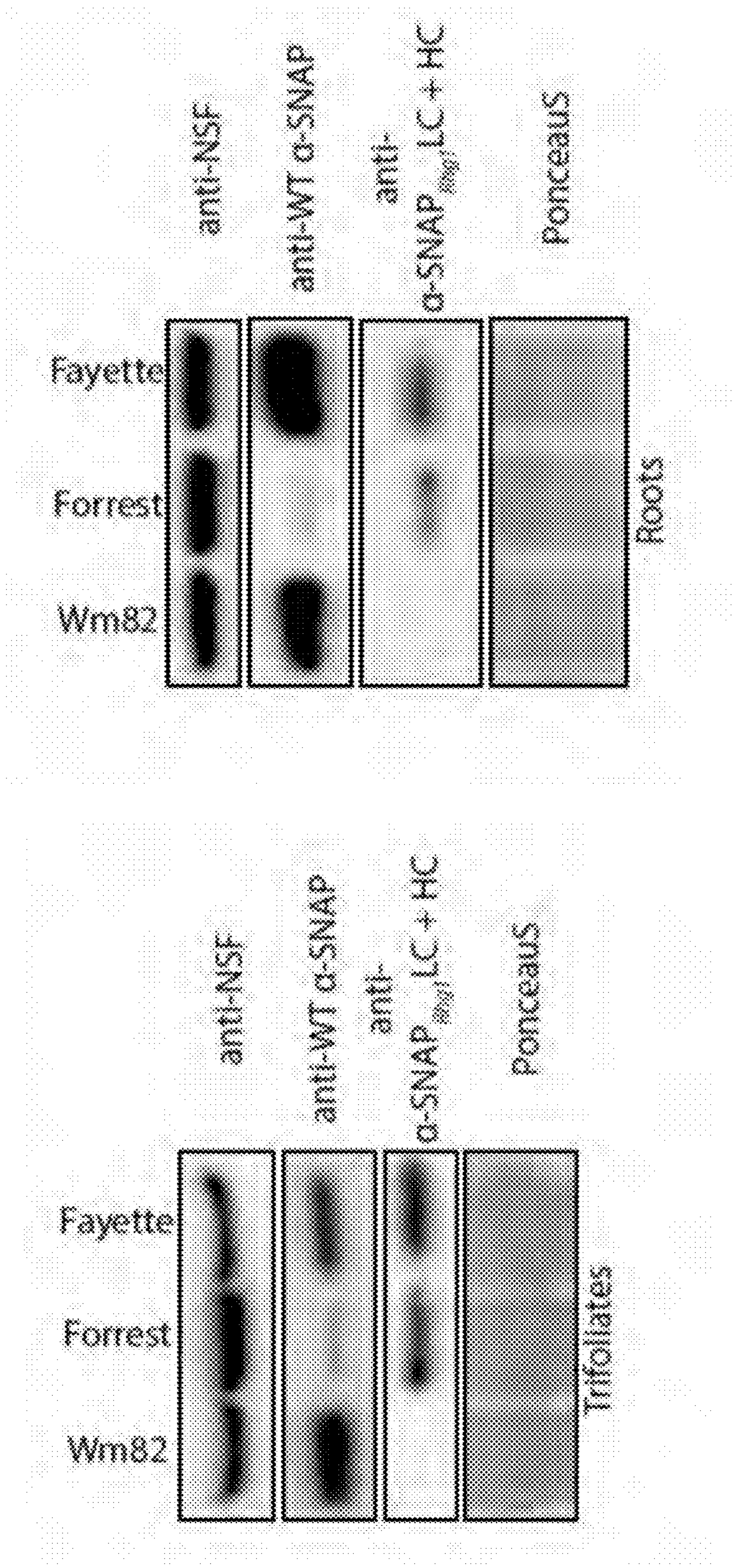

anti-NSF
anti-WT α-SNAP
anti-
α-SNAP$_{Rhg1}$LC + HC
PonceauS
Fayette
Forrest
Wm82
Roots
anti-NSF
anti-WT α-SNAP
anti-
α-SNAP$_{Rhg1}$LC + HC
PonceauS
Fayette
Forrest
Wm82
Trifoliates

Figure 1C

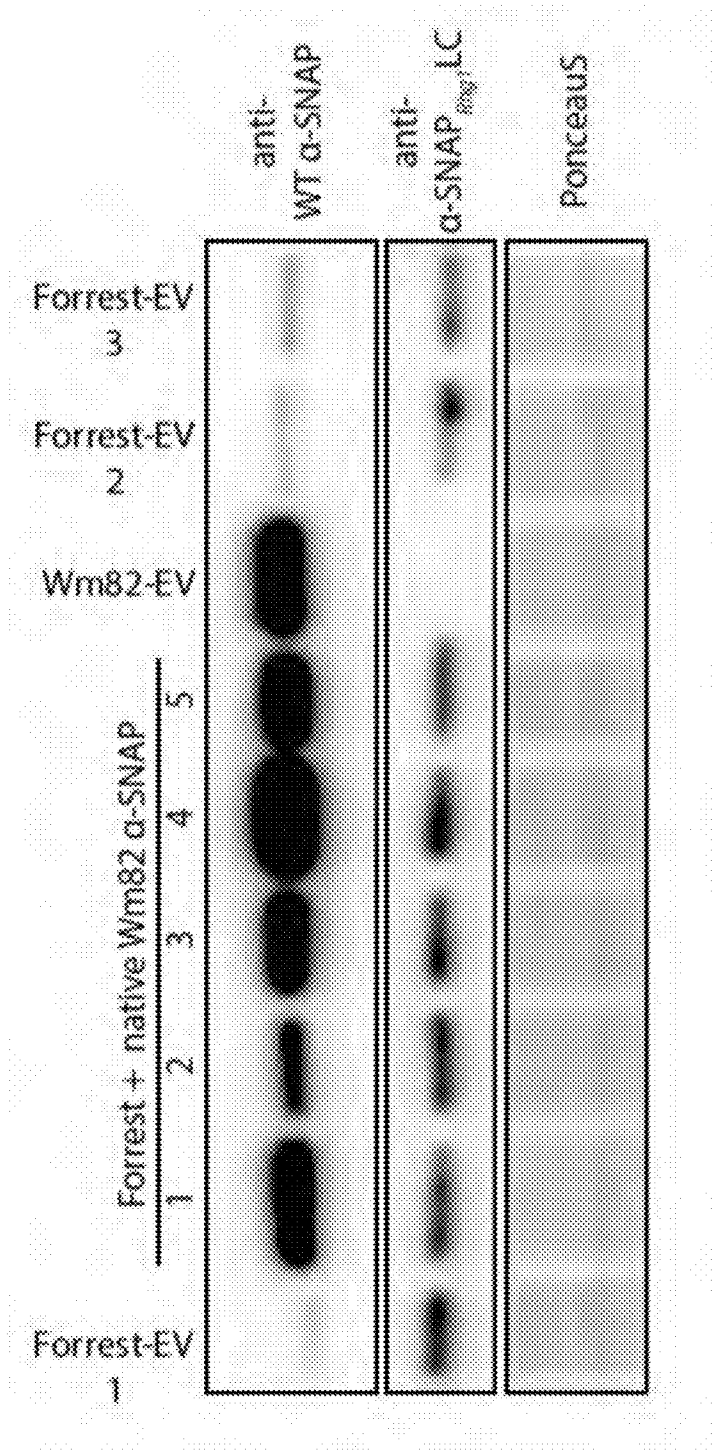

anti-
WT α-SNAP
anti-
α-SNAP$_{Rhg1}$LC
PonceauS
Forrest-EV
3
Forrest-EV
2
Wm82-EV
Forrest + native Wm82 α-SNAP
5
4
3
2
1
Forrest-EV
1

Figure 1D

```
                               1                      I          68                       II    114     III   179
SEQ ID NO: 20  NSF_Ch07        MASRFGLSSSSSSASSMRVTNTPASD....DTIGSGQIALNAVQRRCAK....LELEF-VKKGSK....RGM
SEQ ID NO: 21  NSF_BAN07       MASQFGLSSSSSSASSMRVTYTPAND....DTIGSGQIALNAVQRRCAK....LELEFFVKKGSK....RGI
SEQ ID NO: 22  NSF_Ch13        M---FGLSSSSSSASNMRVTNTPASD....DNIGSGQIALNVVQRRCVK....LDLEF-VKKGSK....RGM
```

Figure 2A

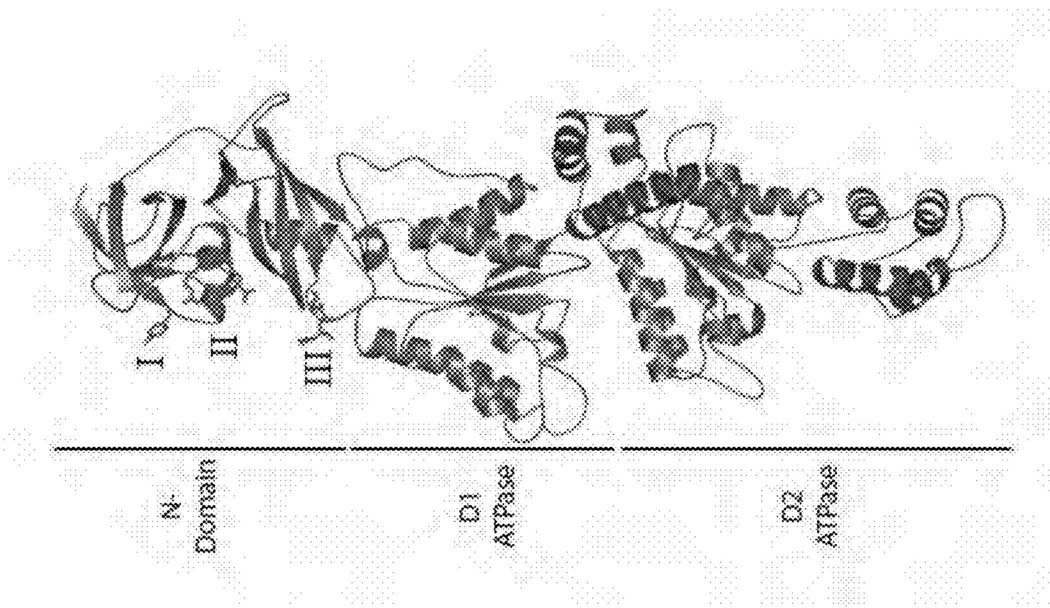


Figure 2B

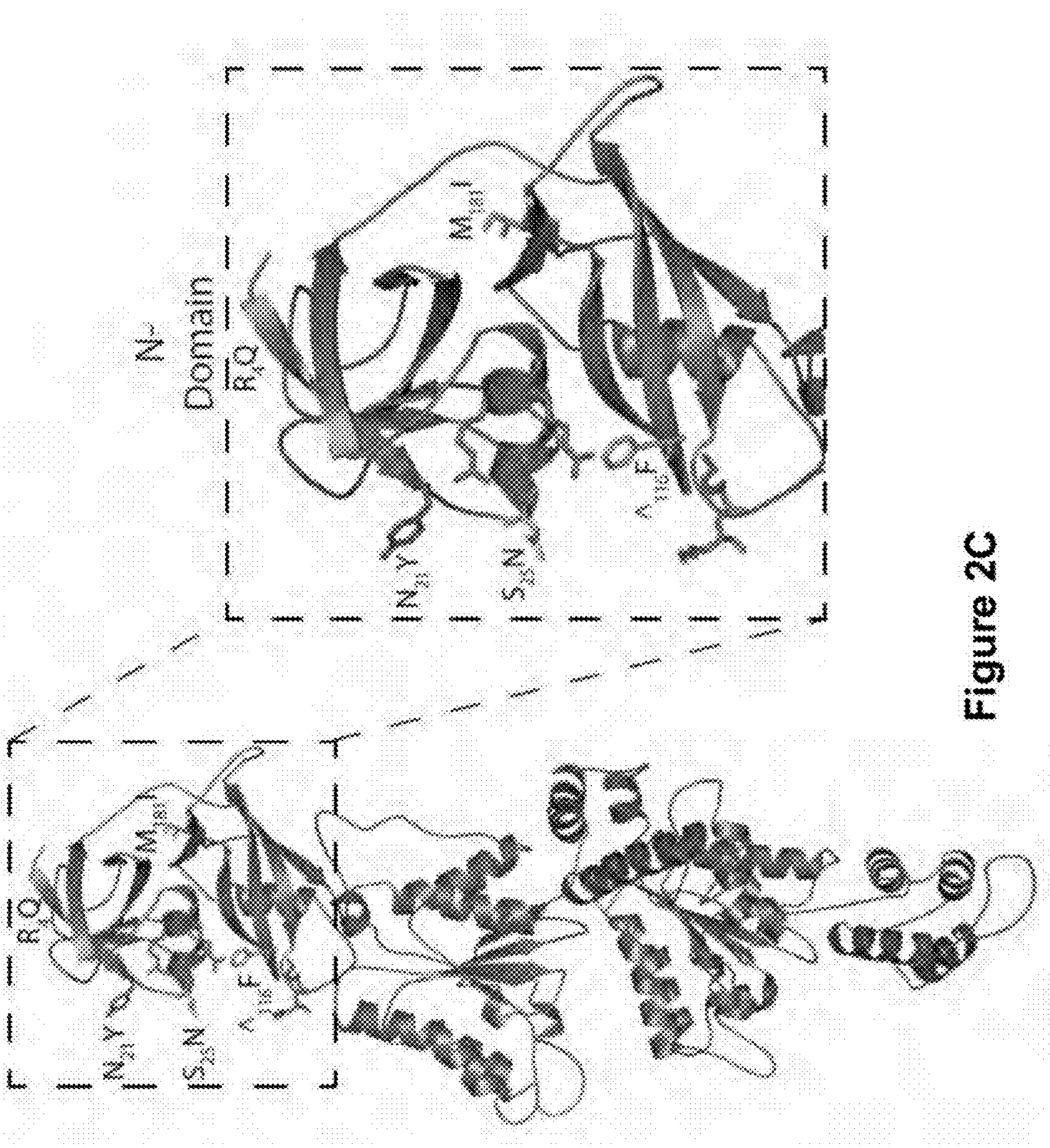


Figure 2C

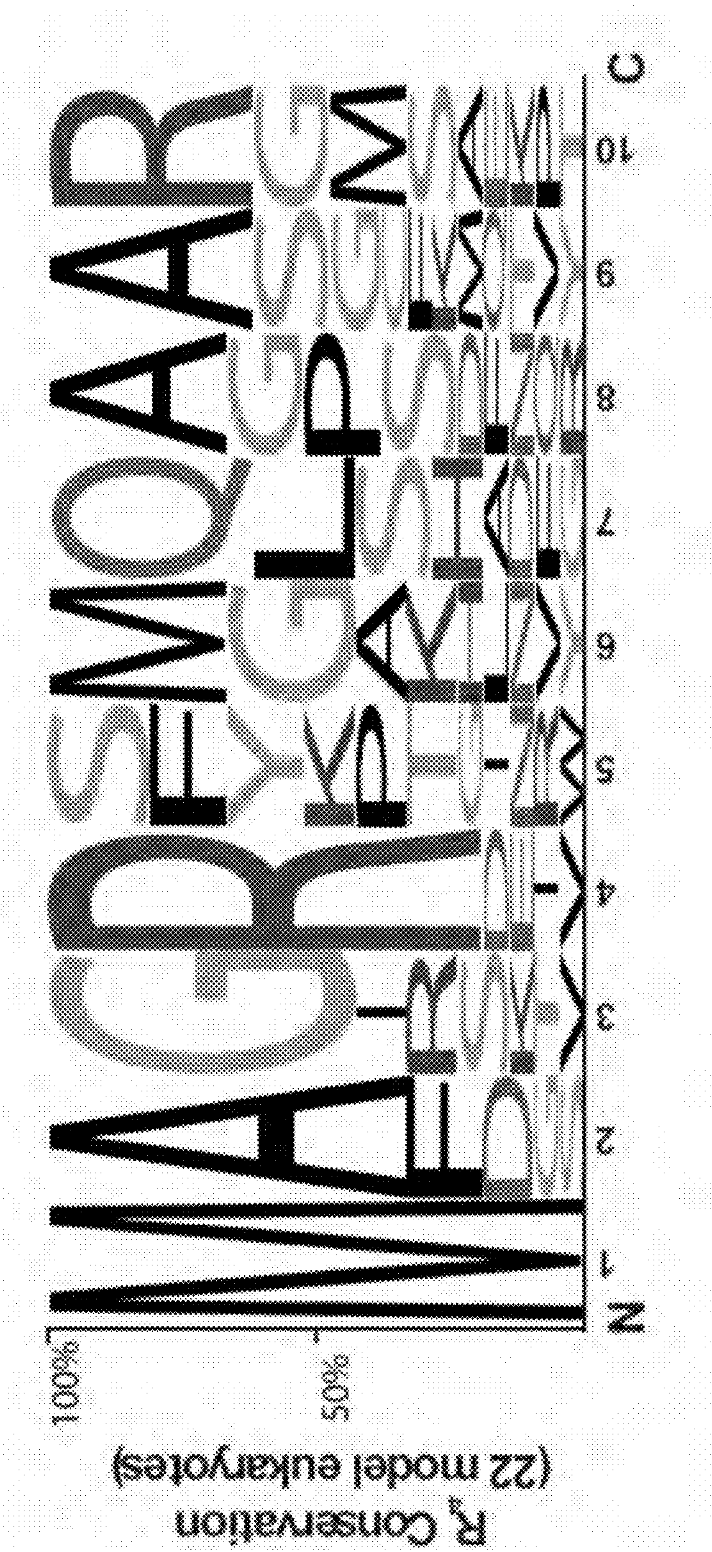


Figure 2D

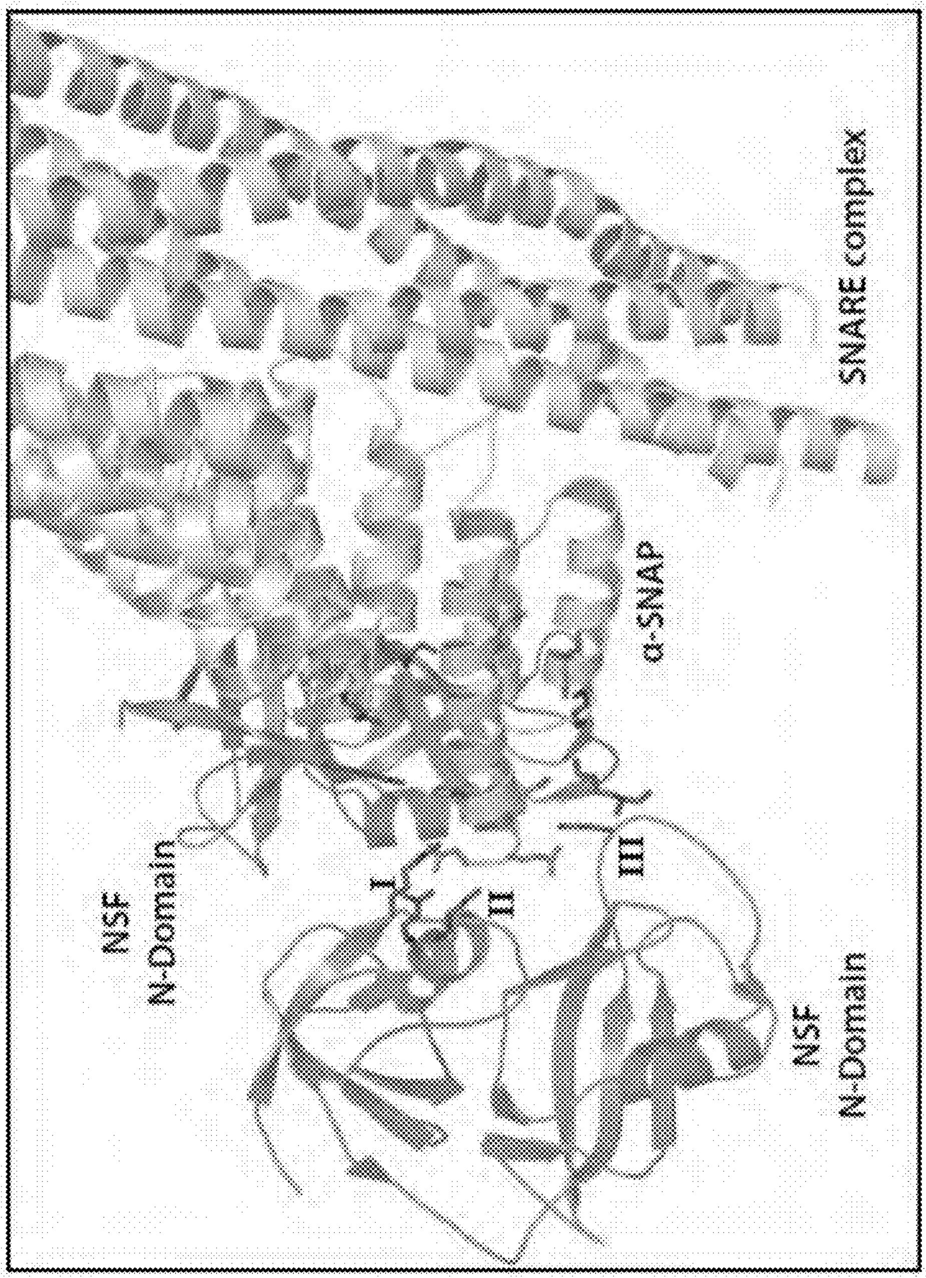


Figure 3A

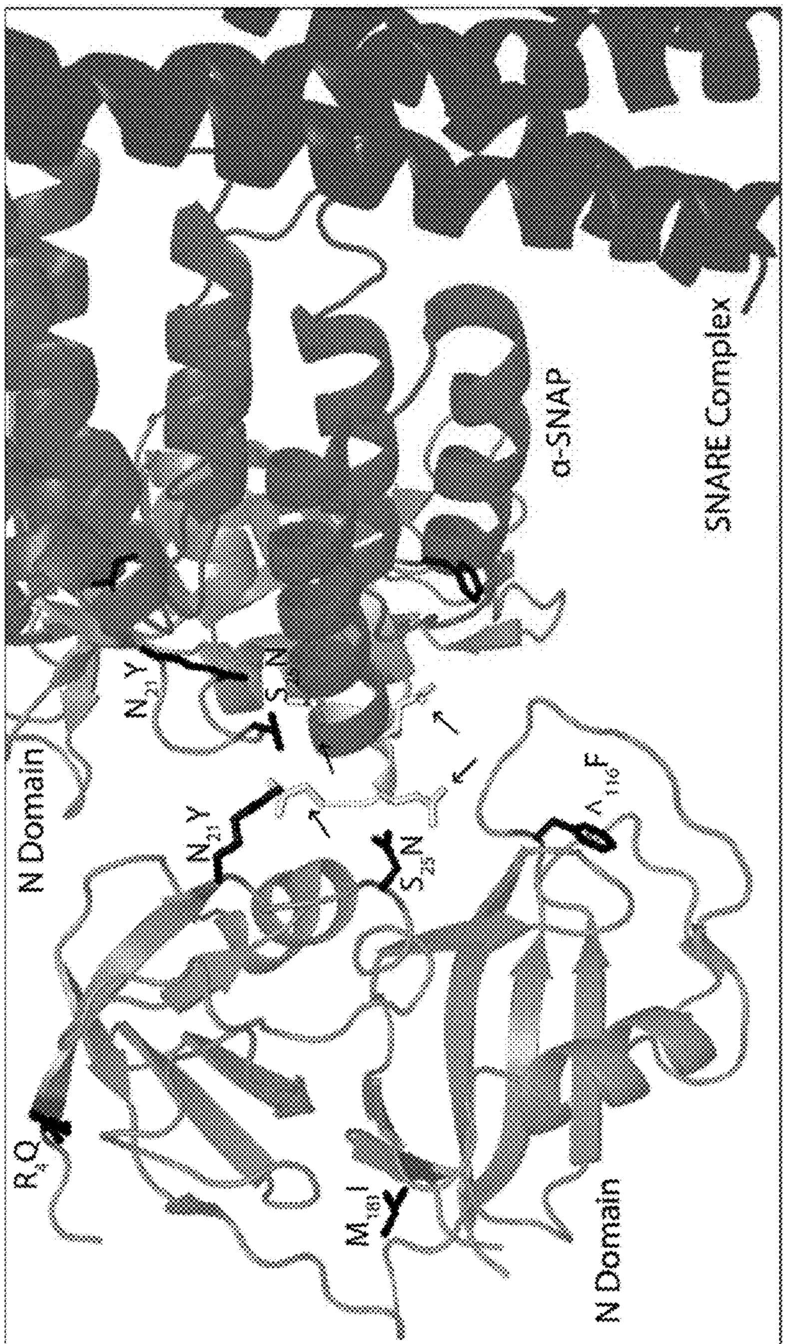


Figure 3B

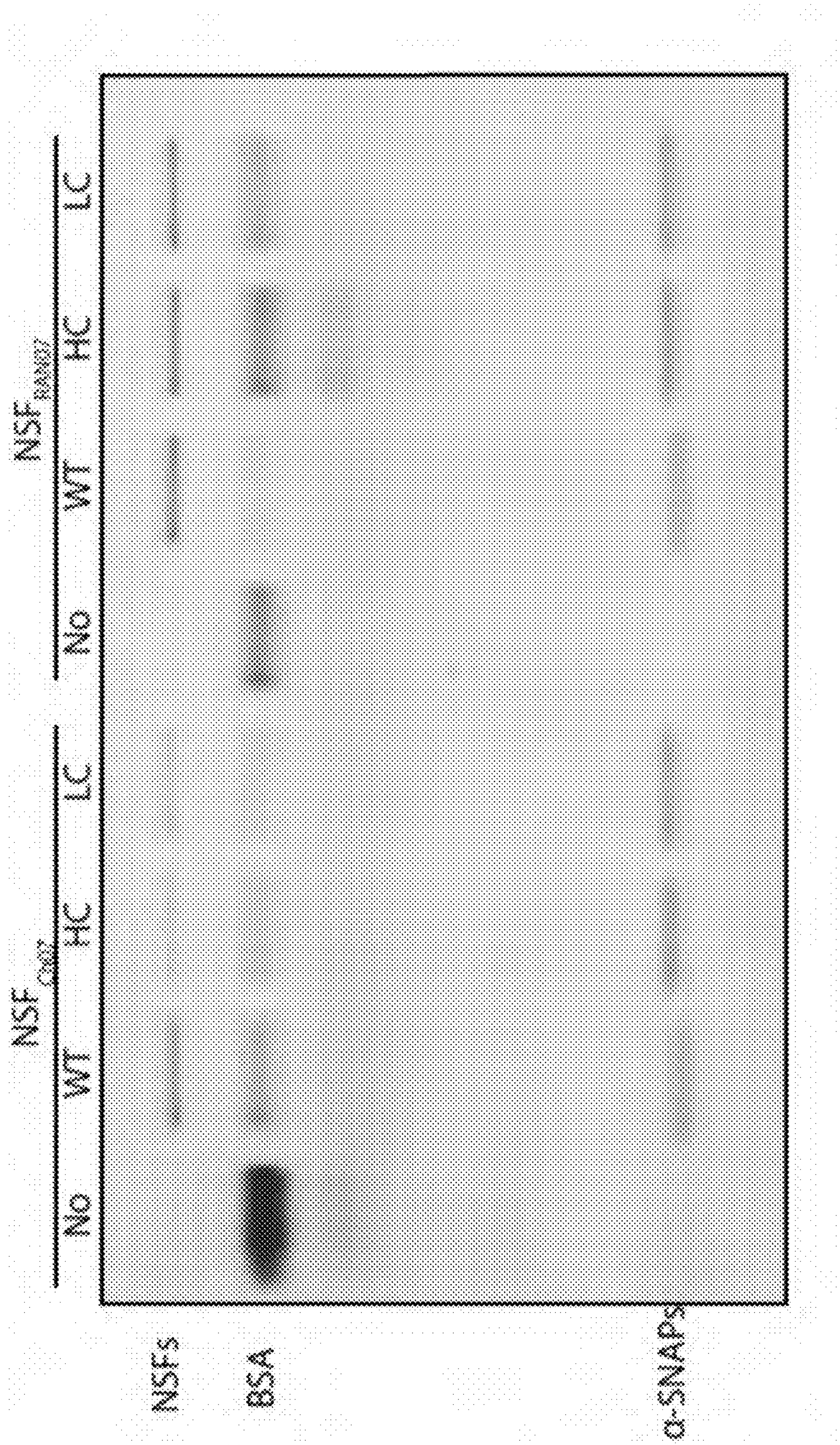


Figure 3C

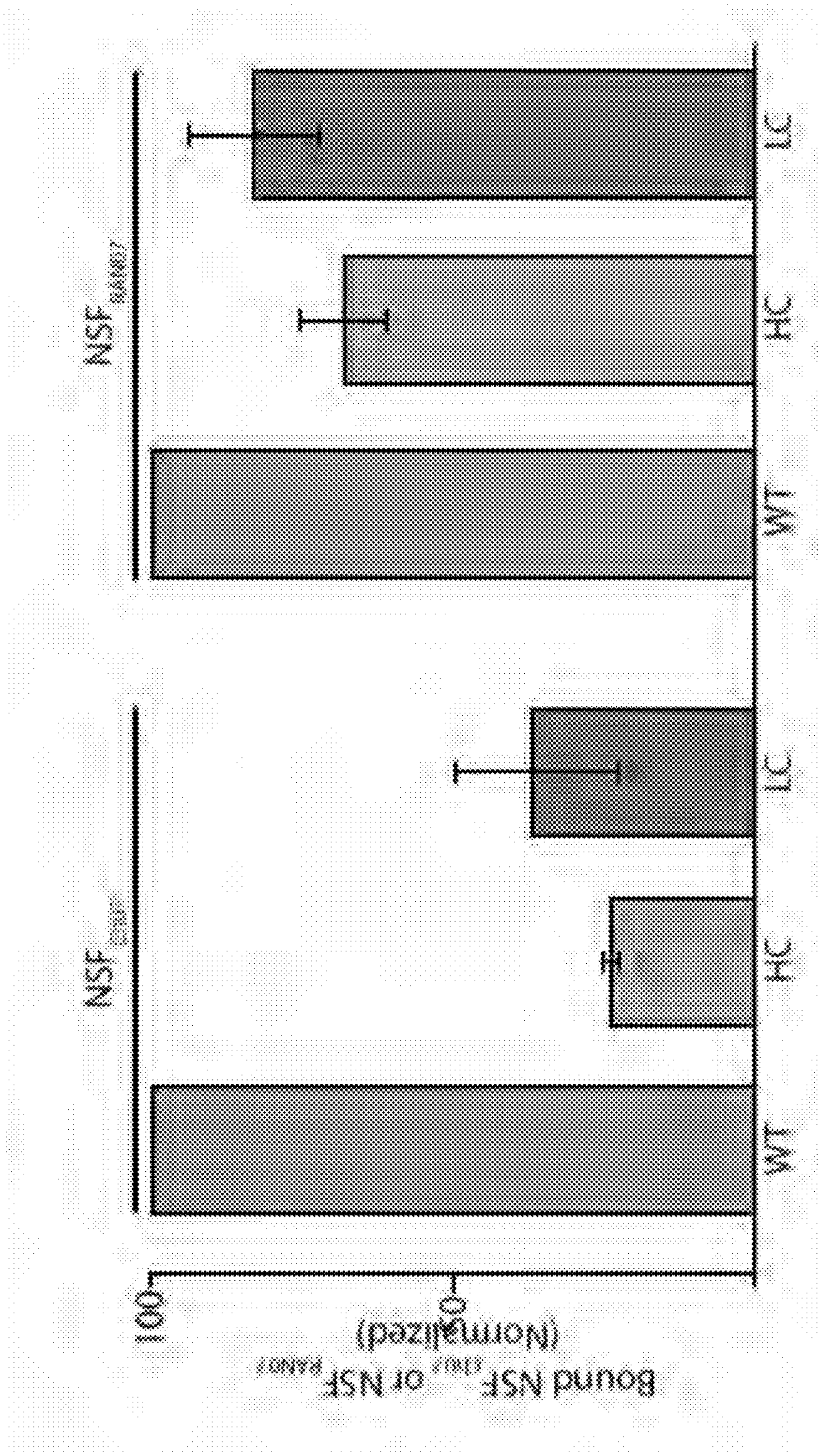


Figure 3D

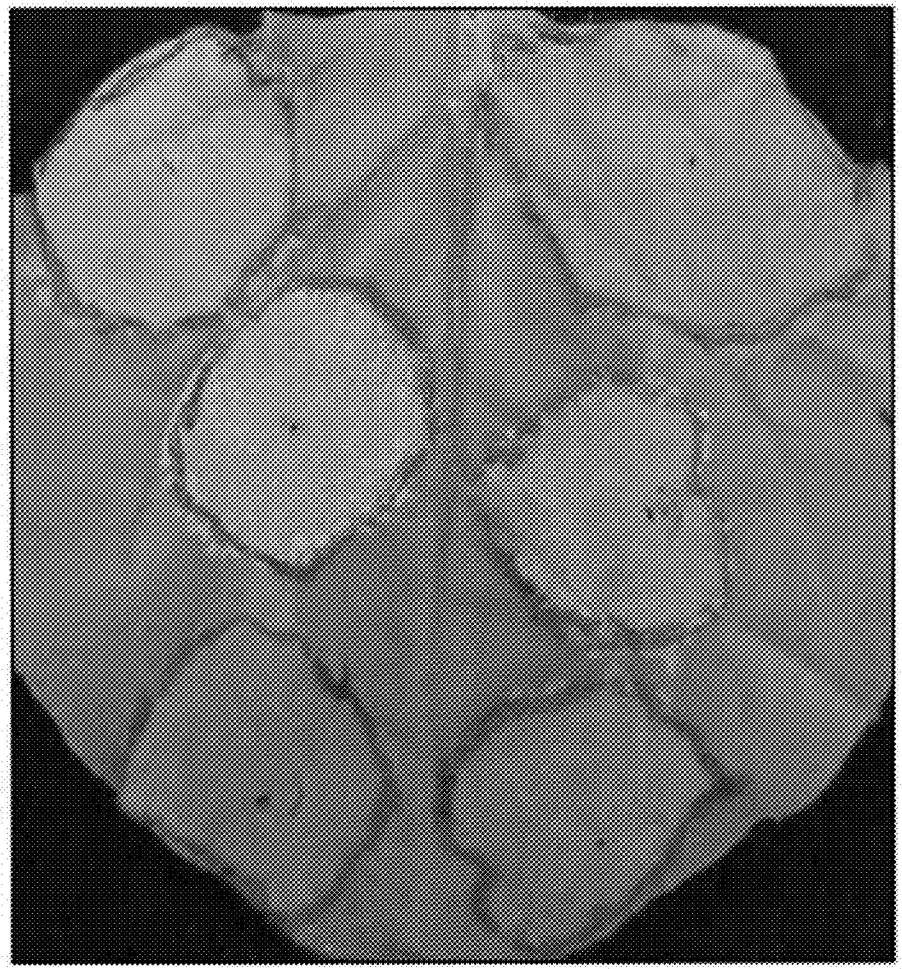

1 NSF$_{Ch07}$: 14 LC
1 RAN07 : 14 LC
1 EV : 09 LC
1 NSF$_{Ch13}$: 09 LC
1 RAN07 : 09 LC
1 NSF$_{Ch07}$: 09 LC

Figure 4A

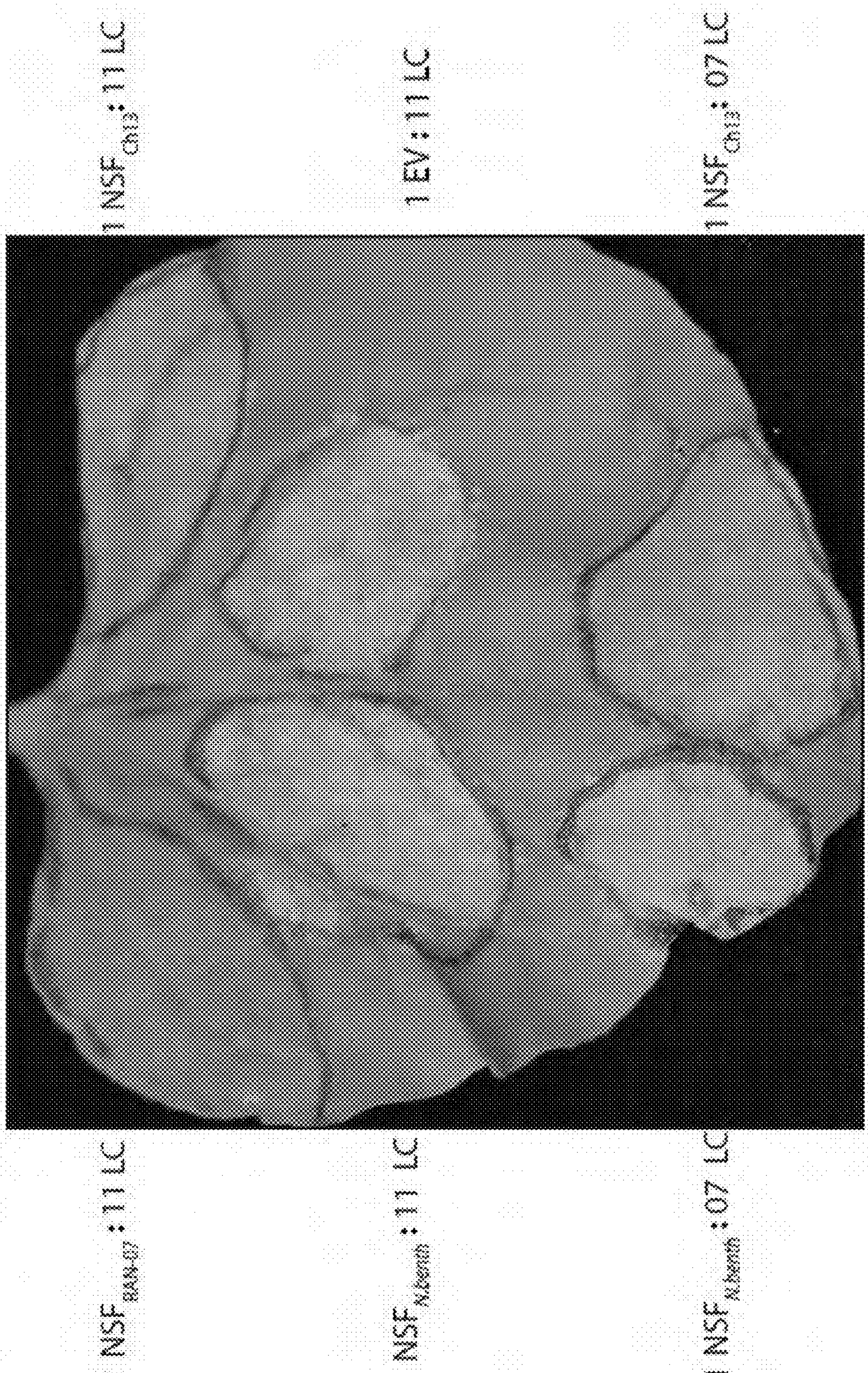

1 NSF$_{Ch13}$: 11 LC
1 EV : 11 LC
1 NSF$_{Ch13}$: 07 LC
1 NSF$_{RAN-07}$: 11 LC
1 NSF$_{N.benth}$: 11 LC
1 NSF$_{N.benth}$: 07 LC

Figure 4B

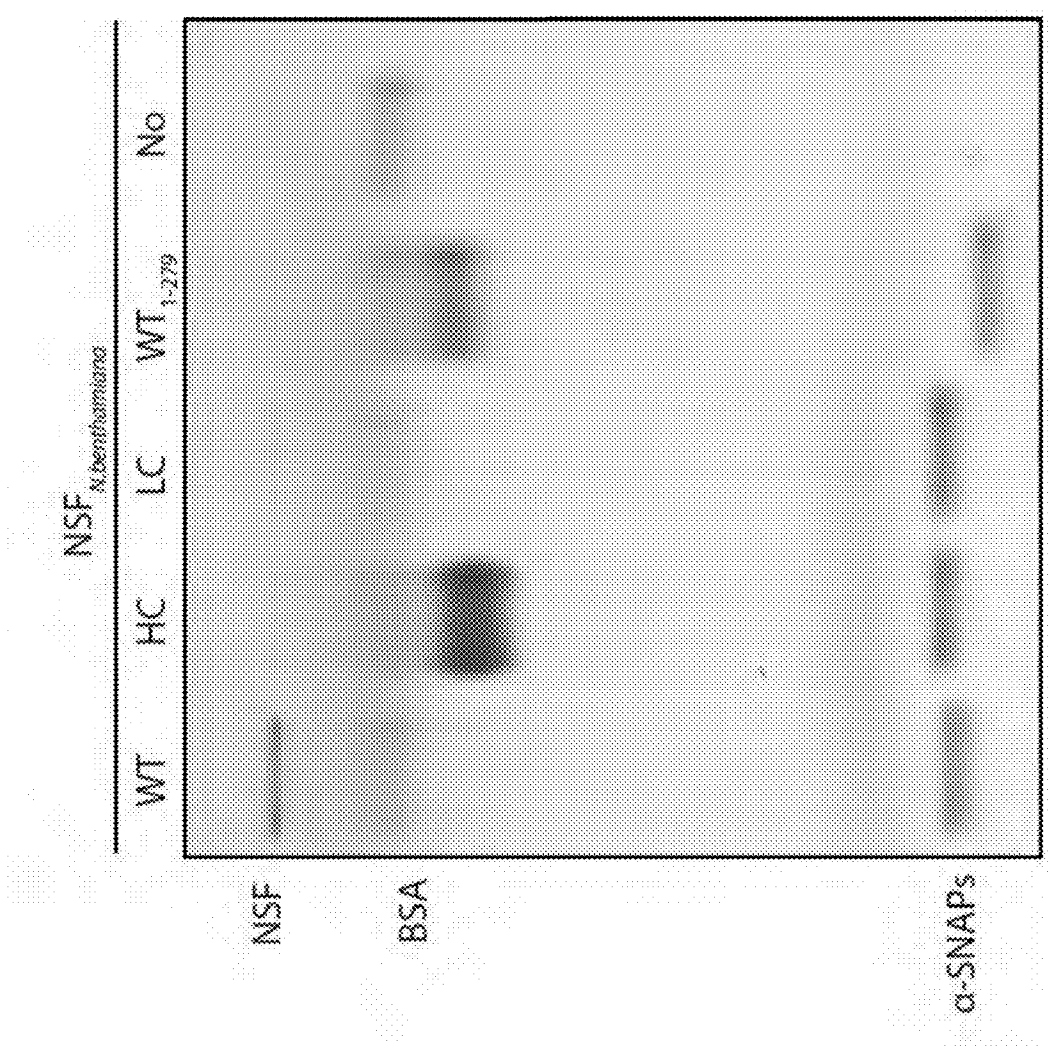


Figure 4C

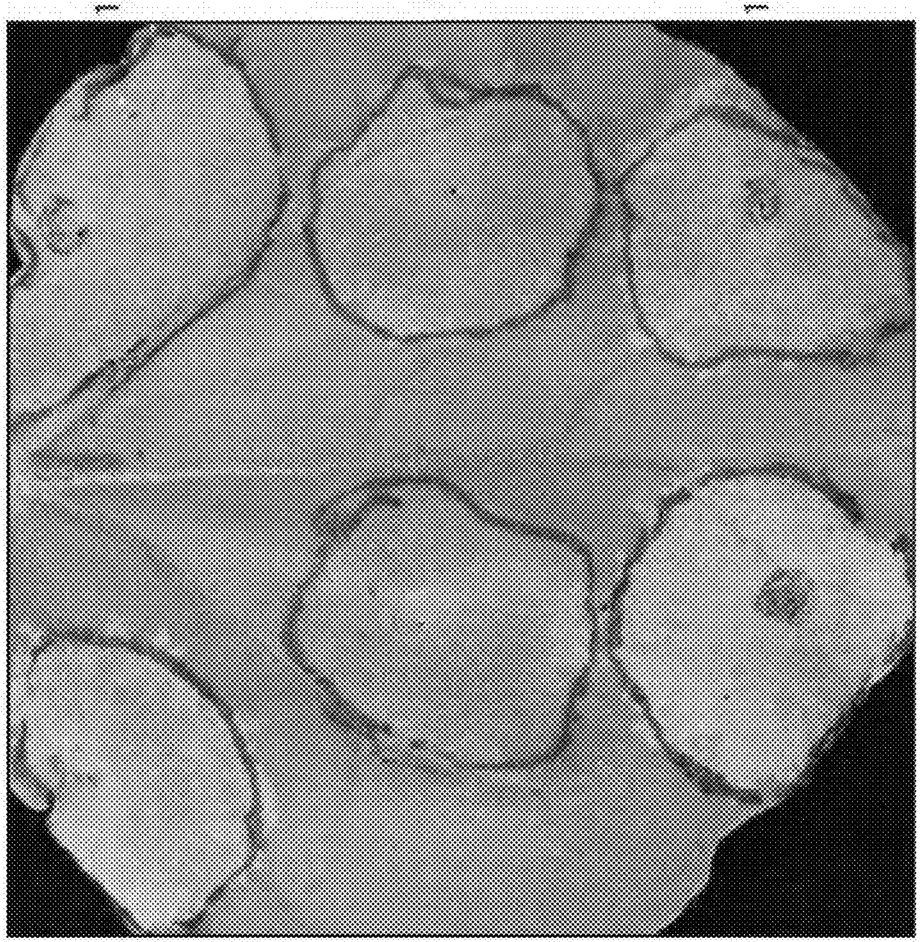

4
1 NSF_{Ch07} : 04 LC $I_{289}A$
1 RAN07 : 04 LC $I_{289}A$
1 RAN07 : 09 LC
1 NSF_{Ch07} : 09 LC
1 NSF_{Ch07} : 09 LC $I_{289}A$
1 RAN07 : 09 LC $I_{289}A$

Figure 4D

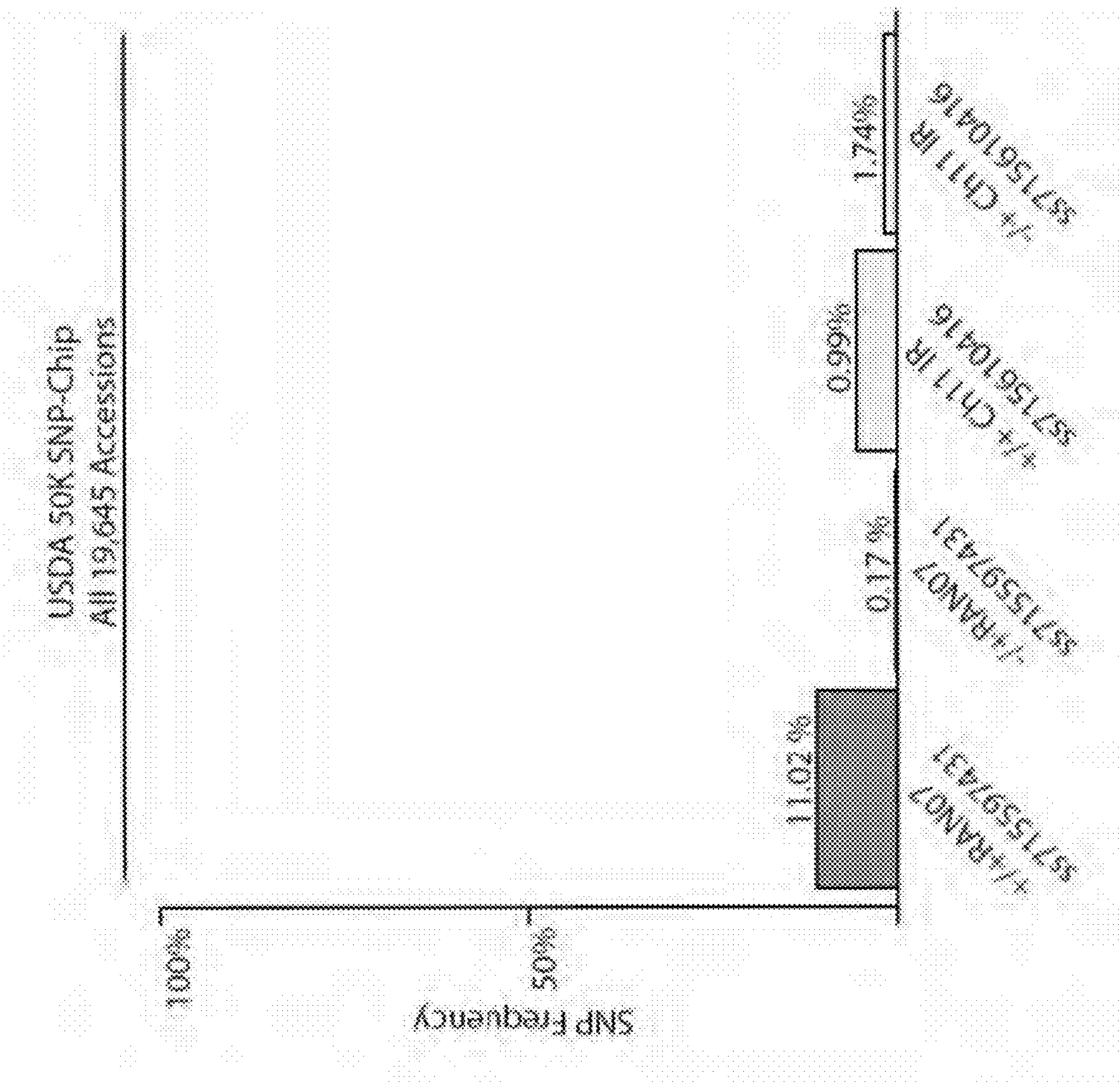

USDA 50K SNP-Chip
All 19,645 Accessions
SNP Frequency
100%
50%
11.02 %
0.17 %
0.99%
1.74%
+/+RAN07 ss715597431
+/-RAN07 ss715597431
+/+ Ch11 IR ss715610416
+/- Ch11 IR ss715610416

Figure 5A

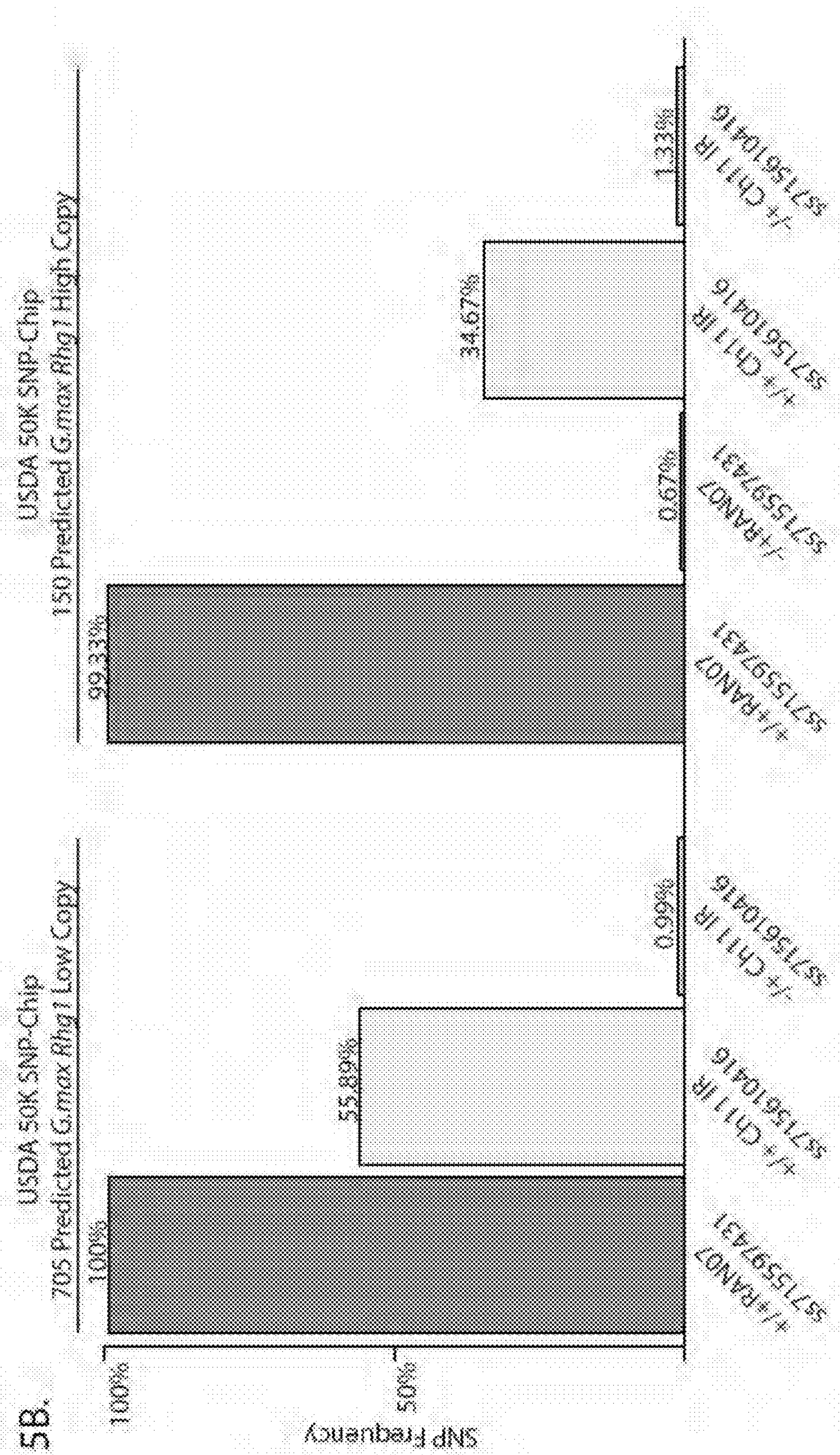
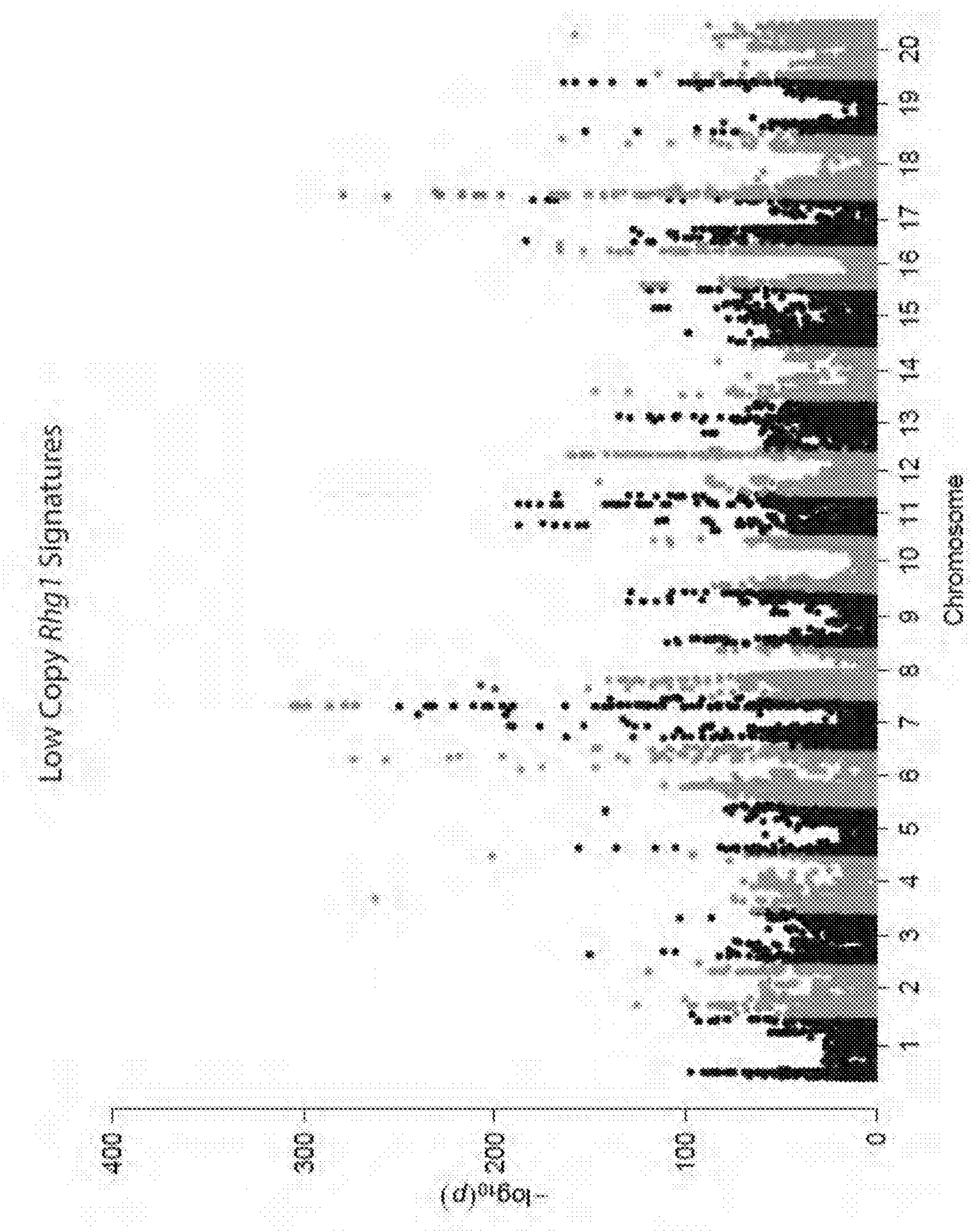

5B.
USDA 50K SNP-Chip
705 Predicted G.max Rhg1 Low Copy
USDA 50K SNP-Chip
150 Predicted G.max Rhg1 High Copy
SNP Frequency
100%
50%
100%
55.89%
0.99%
99.33%
0.67%
34.67%
1.33%
+/+RAN07 ss715597431
+/+ Chr11 IR ss715610416
-/+ Chr11 IR ss715610416
+/+RAN07 ss715597431
-/+RAN07 ss715597431
+/+ Chr11 IR ss715610416
-/+ Chr11 IR ss715610416

Figure 5B



Low Copy *Rhg1* Signatures
400
300
200
100
0
$-\log_{10}(p)$
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Chromosome

Figure 5C

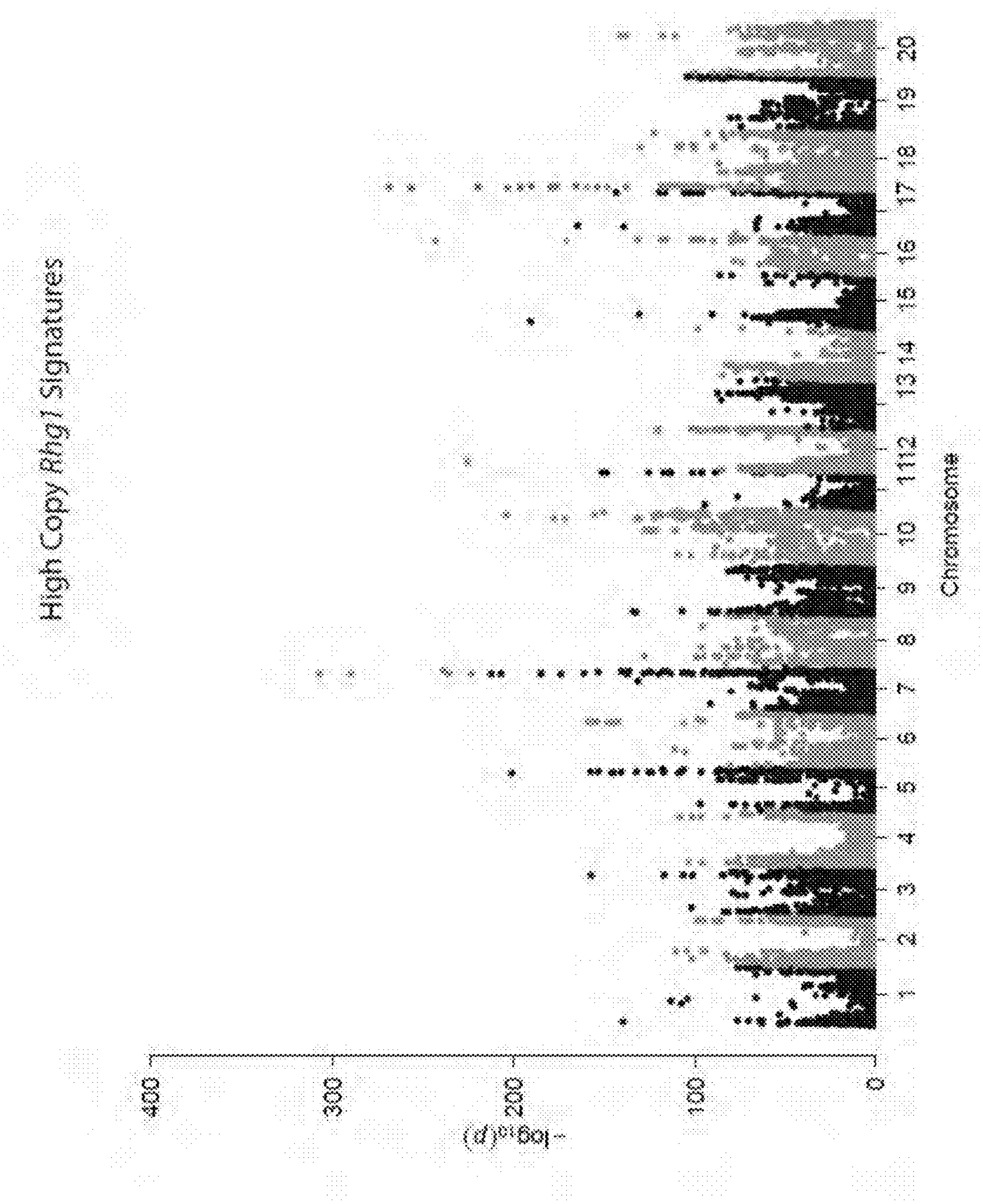

High Copy *Rhg1* Signatures
$-\log_{10}(p)$
0
100
200
300
400
1
2
3
4
5
6
7
8
9
10
1112
1314
15
16
17
18
19
20
Chromosome

Figure 5D

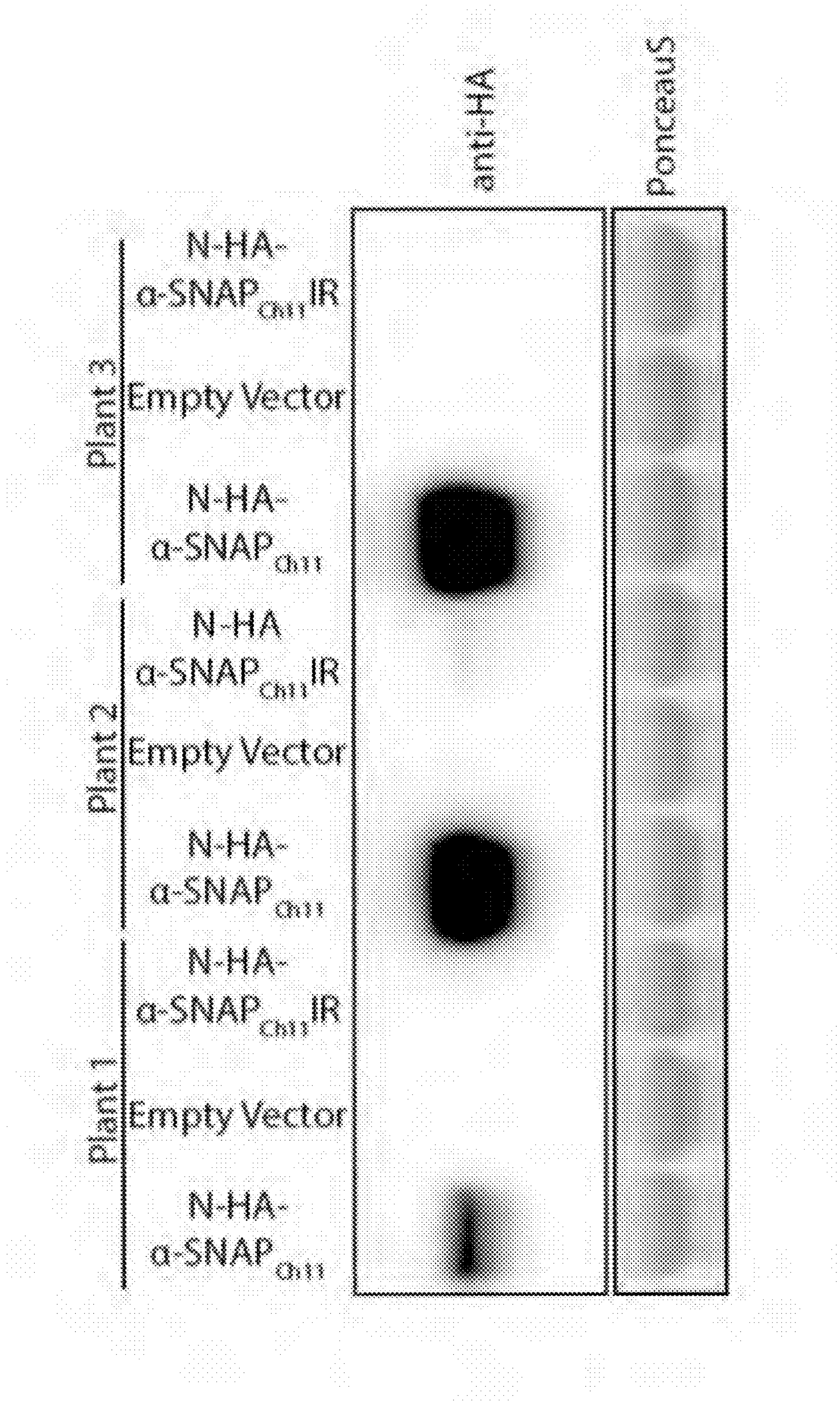

anti-HA
PonceauS
Plant 1
N-HA-α-SNAP$_{Ch11}$
Empty Vector
N-HA-α-SNAP$_{Ch11}$IR
Plant 2
N-HA-α-SNAP$_{Ch11}$
Empty Vector
N-HA α-SNAP$_{Ch11}$IR
Plant 3
N-HA-α-SNAP$_{Ch11}$
Empty Vector
N-HA-α-SNAP$_{Ch11}$IR

Figure 6A

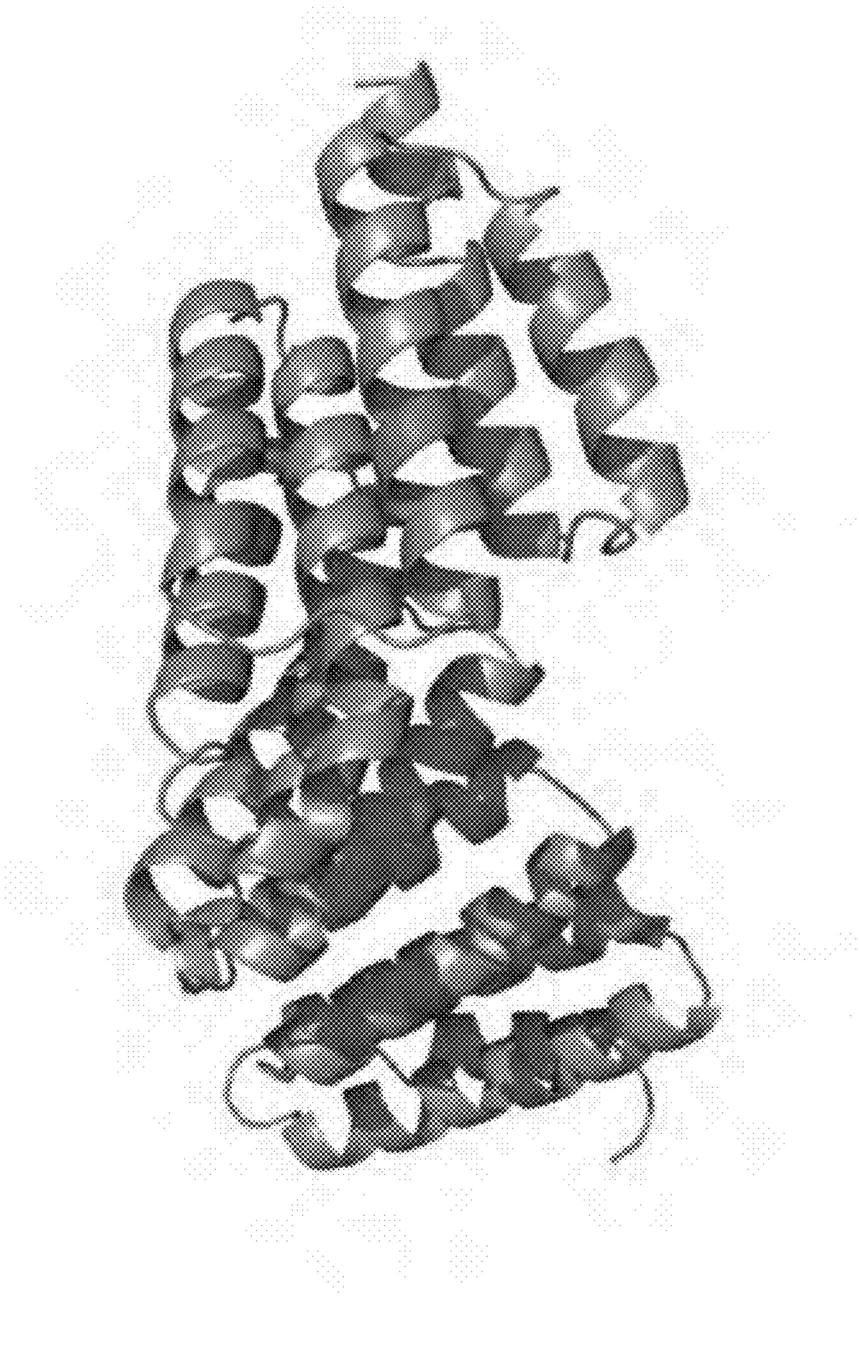


Figure 6B

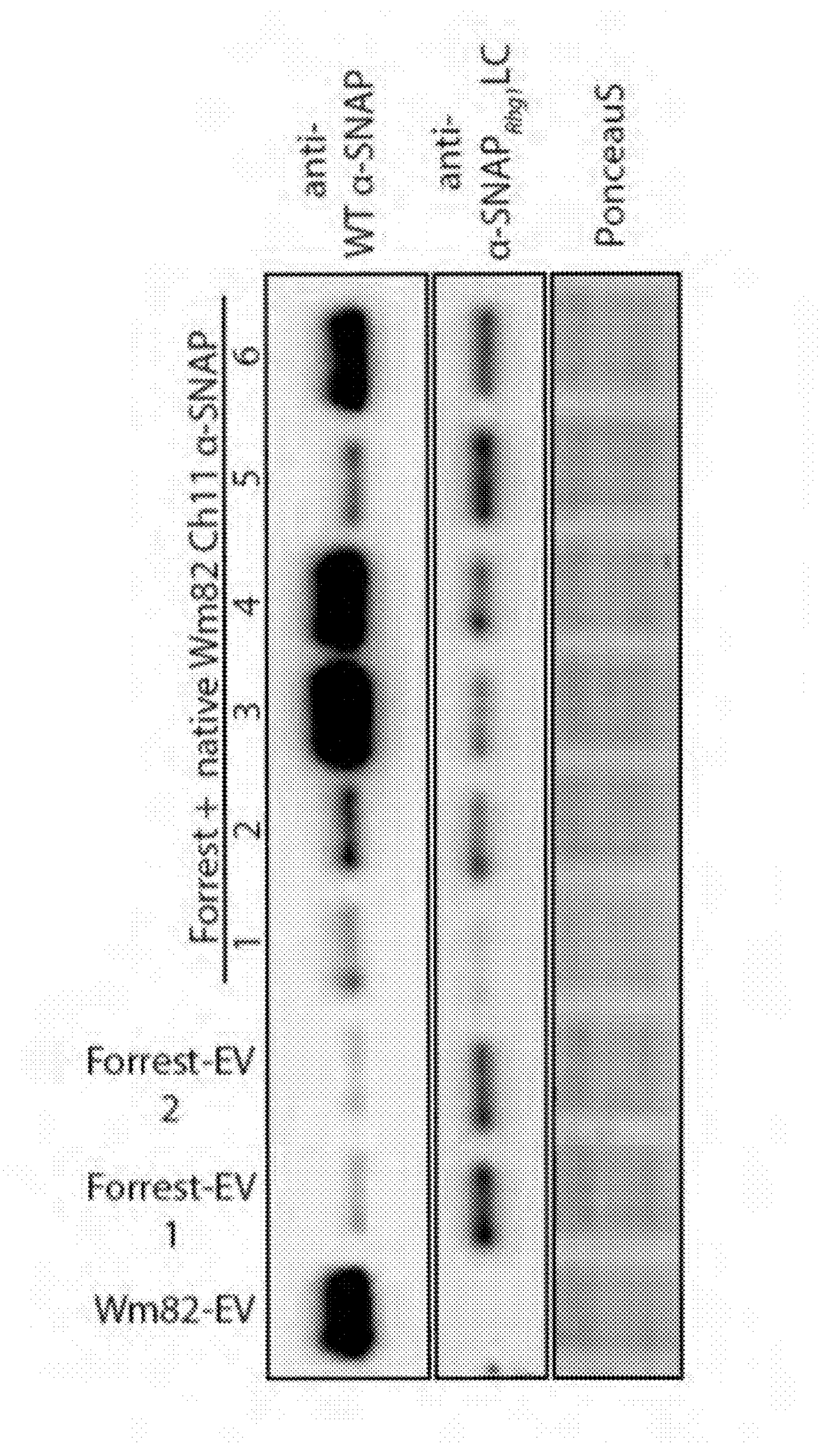

anti-
WT α-SNAP
anti-
α-SNAP$_{Rhg1}$LC
PonceauS
Forrest + native Wm82 Ch11 α-SNAP
1
2
3
4
5
6
Forrest-EV
2
Forrest-EV
1
Wm82-EV

Figure 6C

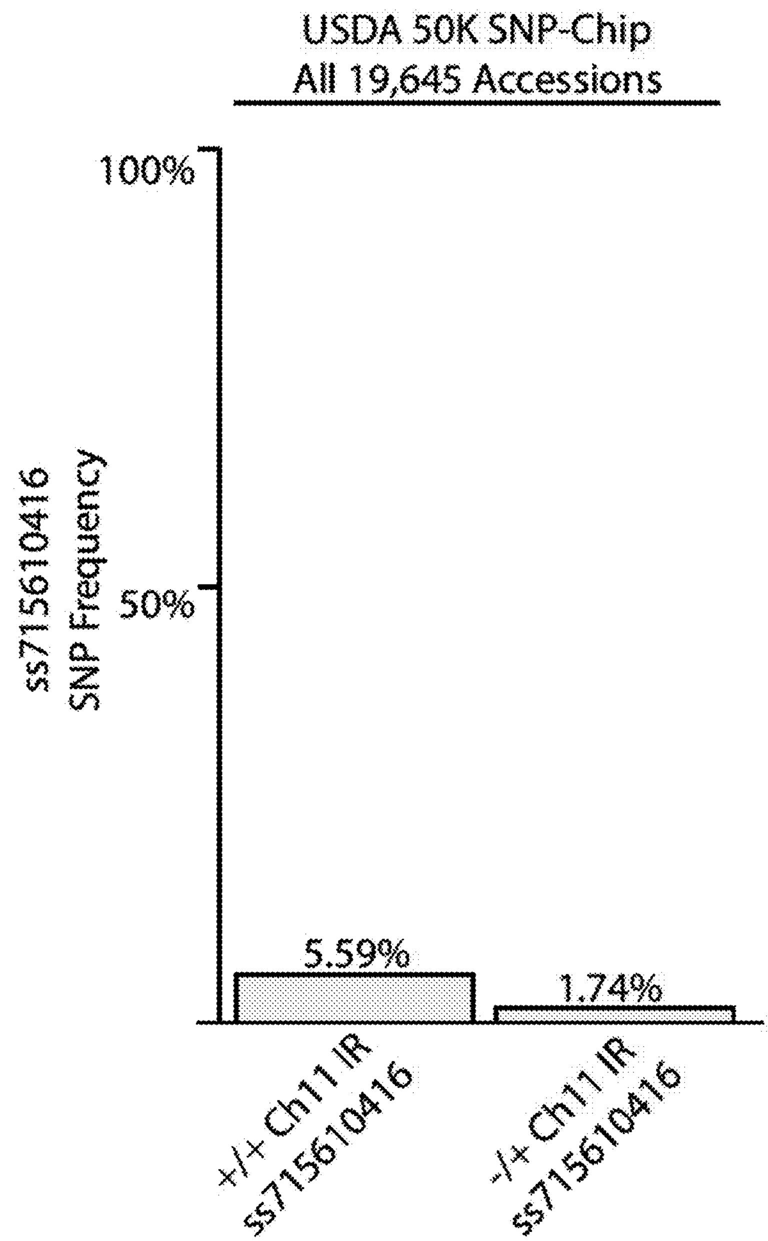

USDA 50K SNP-Chip
All 19,645 Accessions
100%
50%
ss715610416
SNP Frequency
5.59%
1.74%
+/+ Ch11 IR
ss715610416
-/+ Ch11 IR
ss715610416

Figure 6D

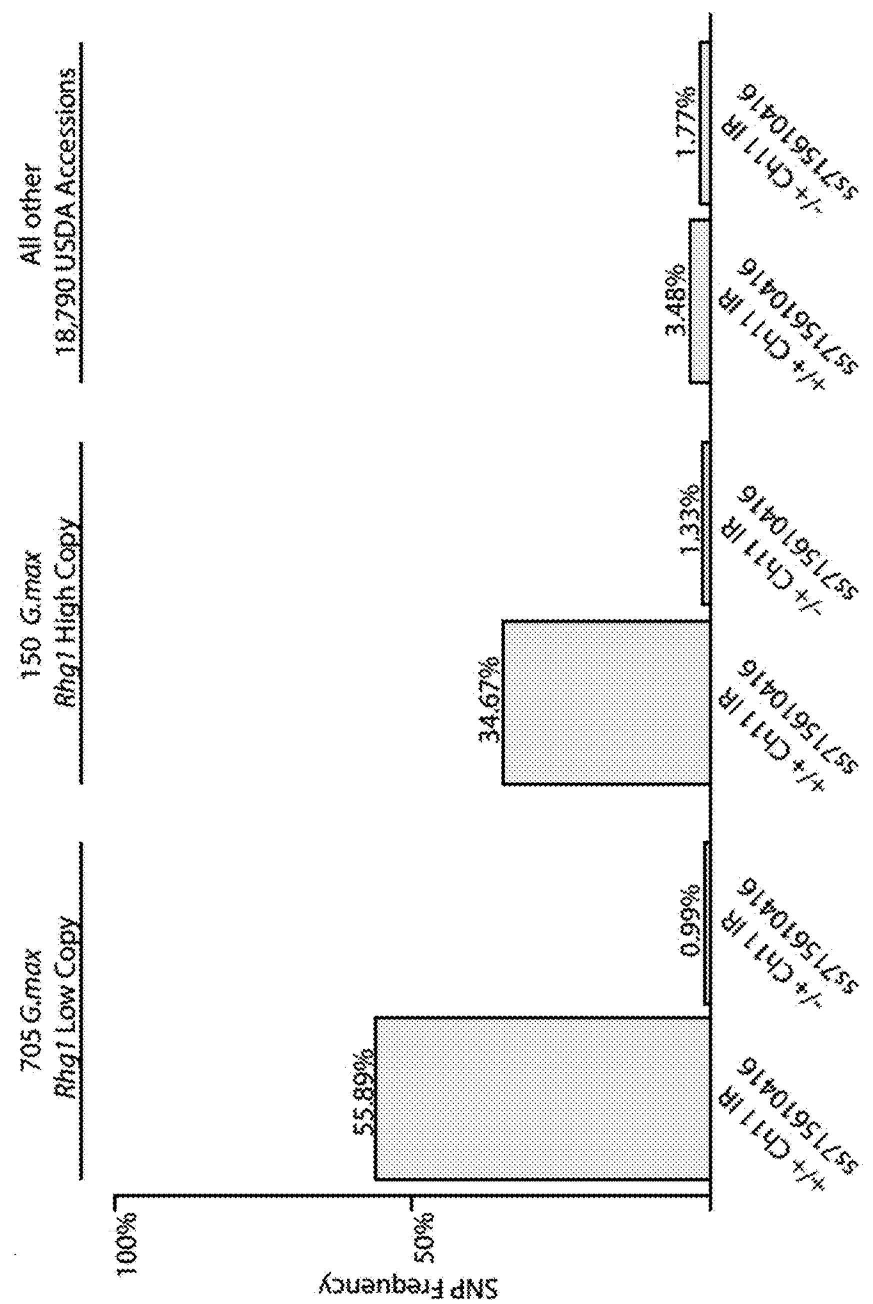

705 G.max
Rhg1 Low Copy
150 G.max
Rhg1 High Copy
All other
18,790 USDA Accessions
55.89%
0.99%
34.67%
1.33%
3.48%
1.77%
+/+ Ch11 IR
ss715610416
-/+ Ch11 IR
ss715610416
SNP Frequency
100%
50%

Figure 6E

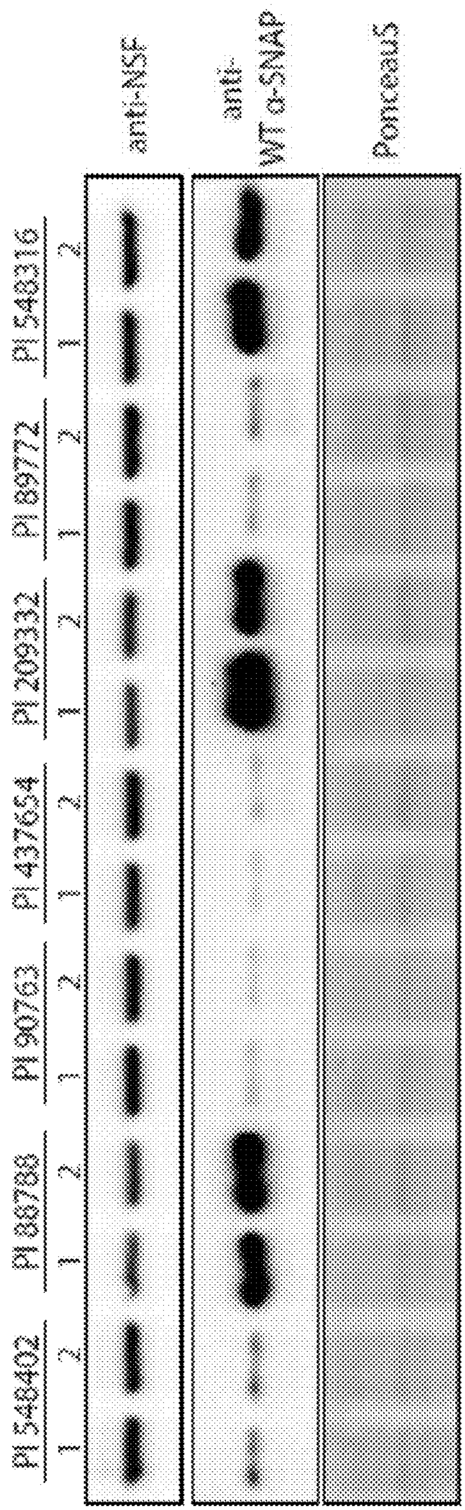

anti-NSF
anti-
WT α-SNAP
PonceauS
PI 548402
1
2
PI 88788
1
2
PI 90763
1
2
PI 437654
1
2
PI 209332
1
2
PI 89772
1
2
PI 548316
1
2

Figure 7A

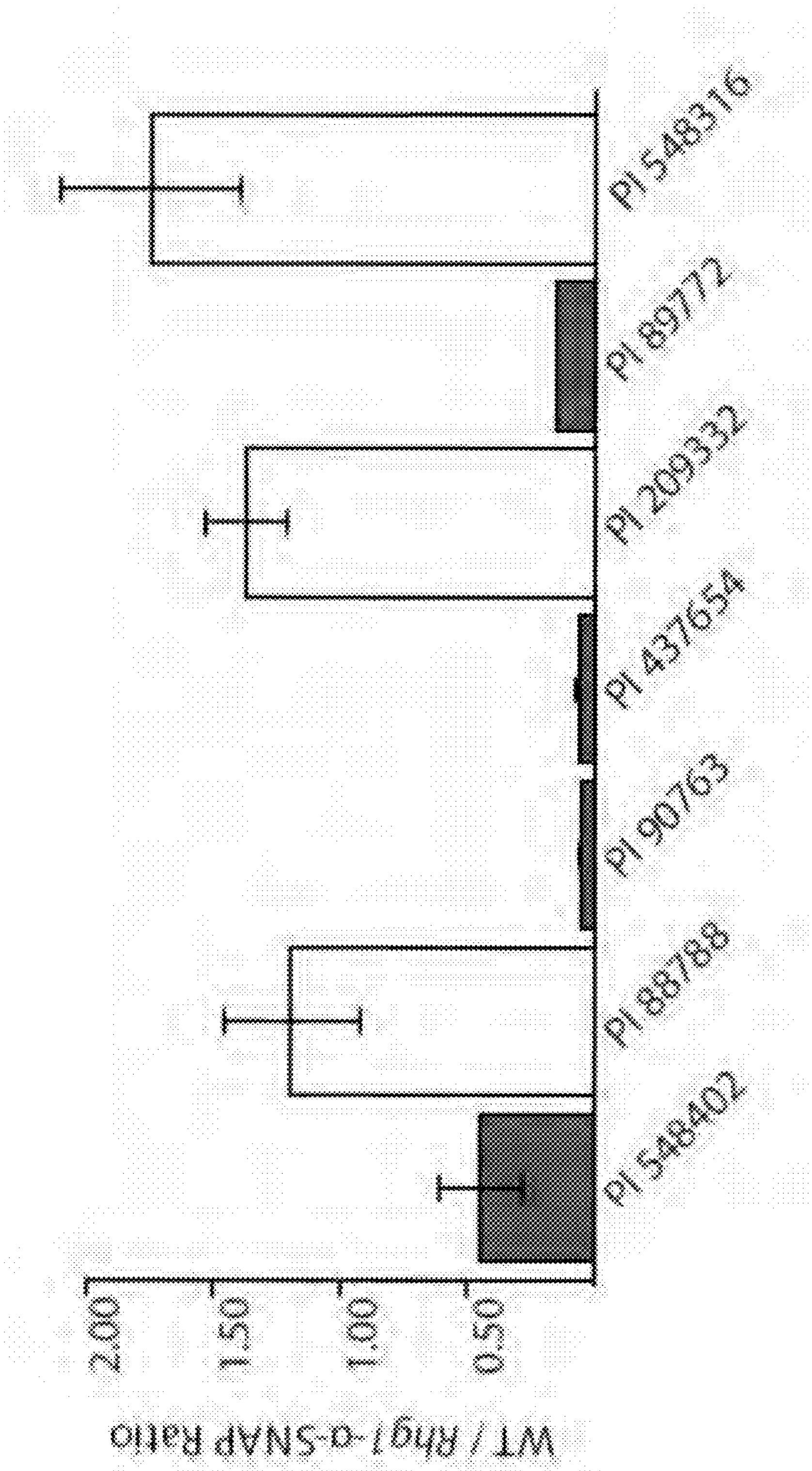

PI 548316
PI 89772
PI 209332
PI 437654
PI 90763
PI 88788
PI 548402
2.00
1.50
1.00
0.50
WT / Rhg1-α-SNAP Ratio

Figure 7B

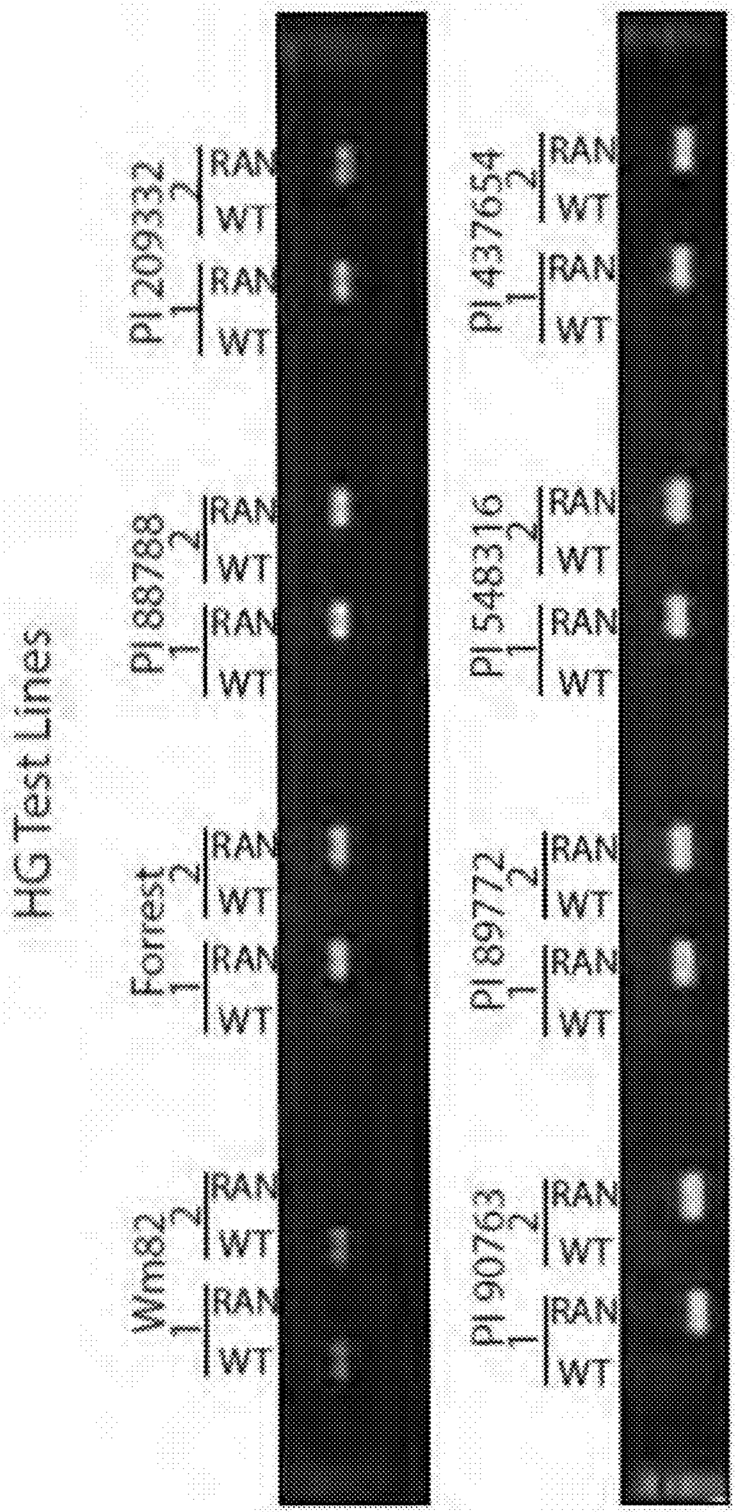

HG Test Lines
Wm82
Forrest
PI 88788
PI 209332
PI 90763
PI 89772
PI 548316
PI 437654
1
2
WT
RAN

Figure 7C

NSF RAN07 alignment to Wild-Type NSF_{Ch07} (Wm82)

```
Wms82 SEQ ID NO: 17   MASRFGLSSSSSSASSMRVTNTPASDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
RAN07 SEQ ID NO: 18   MASQFGLSSSSSSASSMRVTYTPANDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
                      ***:**************** ***.***********************************

Wms82 SEQ ID NO: 17   VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEF VKKGS
RAN07 SEQ ID NO: 18   VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEFFVKKGS
                      ******************************************************* *****

Wms82 SEQ ID NO: 17   KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGM
RAN07                 KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGI
                      ***********************************************************:

Wms82 SEQ ID NO: 17   ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
RAN07 SEQ ID NO: 18   ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
                      ************************************************************

Wms82 SEQ ID NO: 17   RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
RAN07 SEQ ID NO: 18   RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
                      ************************************************************

Wms82 SEQ ID NO: 17   TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
RAN07 SEQ ID NO: 18   TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
                      ************************************************************
```

Figure 8A

```
Wms82 SEQ ID NO: 17  IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
RAN07 SEQ ID NO: 18  IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
                     ************************************************************

Wms82 SEQ ID NO: 17  SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
RAN07 SEQ ID NO: 18  SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
                     ************************************************************

Wms82 SEQ ID NO: 17  FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
RAN07 SEQ ID NO: 18  FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
                     ************************************************************

Wms82 SEQ ID NO: 17  LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
RAN07 SEQ ID NO: 18  LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
                     ************************************************************

Wms82 SEQ ID NO: 17  ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
RAN07 SEQ ID NO: 18  ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
                     ************************************************************

Wms82 SEQ ID NO: 17  FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
RAN07 SEQ ID NO: 18  FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
                     ************************************************************

Wms82 SEQ ID NO: 17  AEAIFSGKEKISIAHFYDCLQDVVRL
RAN07 SEQ ID NO: 18  AEAIFSGKEKISIAHFYDCLQDVVRL
                     **************************
```

Figure 8B

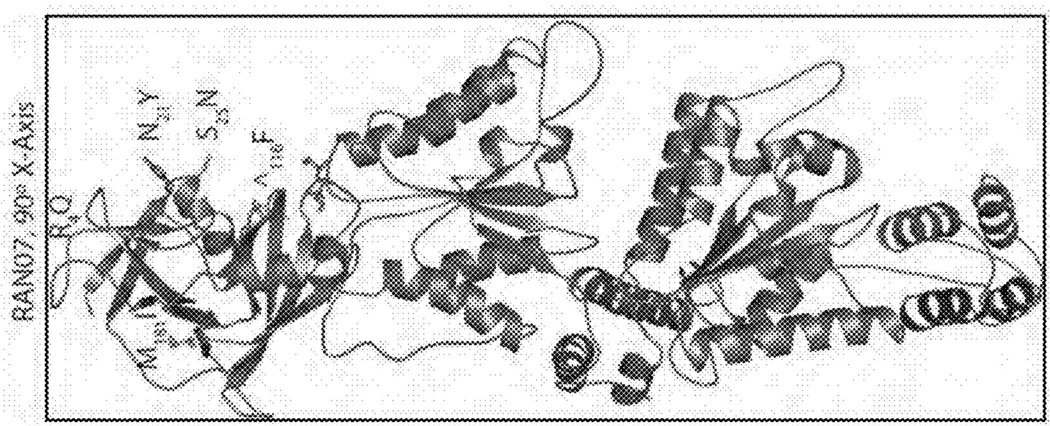


Figure 9A

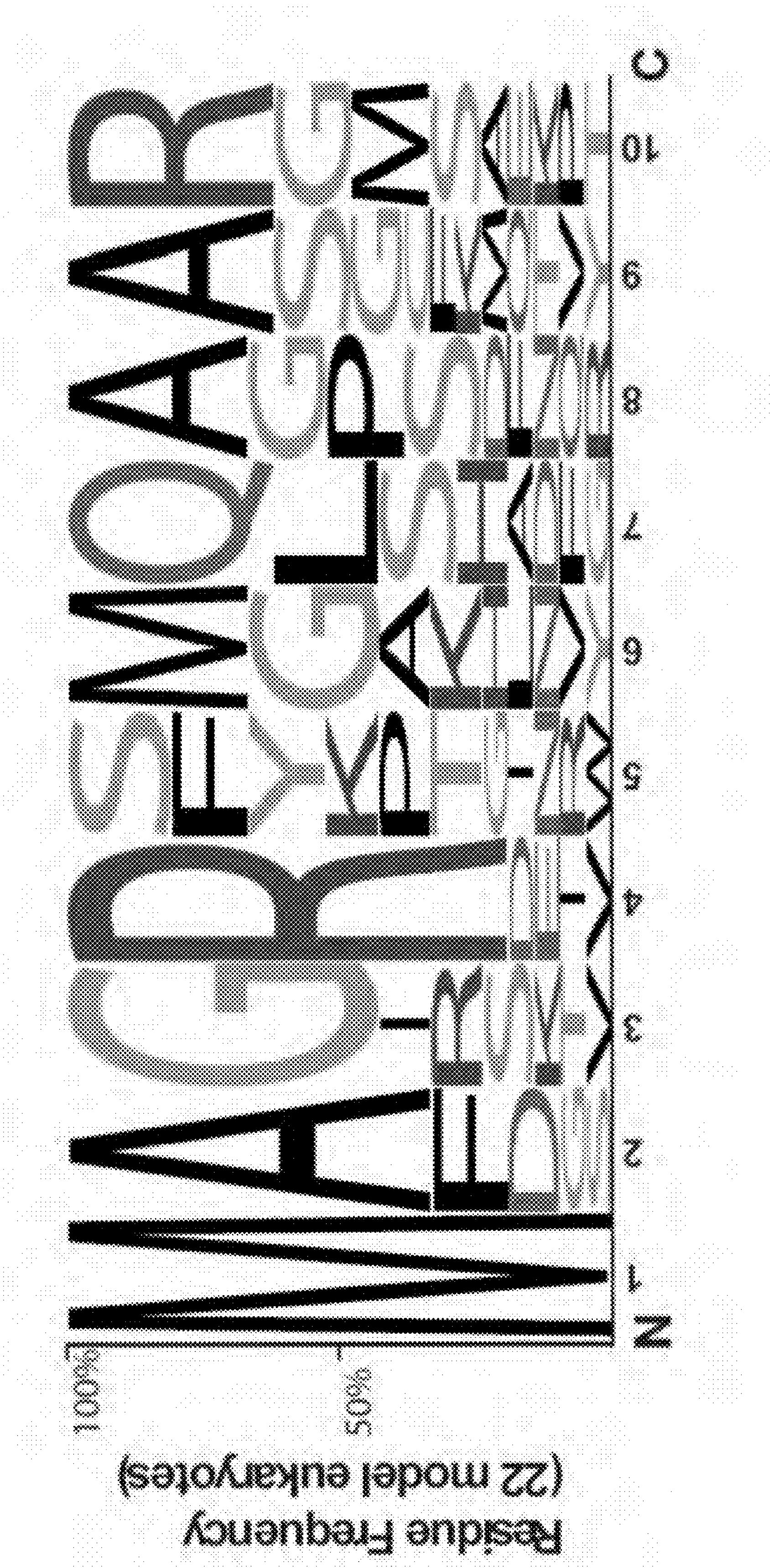

Residue Frequency
(22 model eukaryotes)
100%
50%
N
1
2
3
4
5
6
7
8
9
10
C

Figure 9B

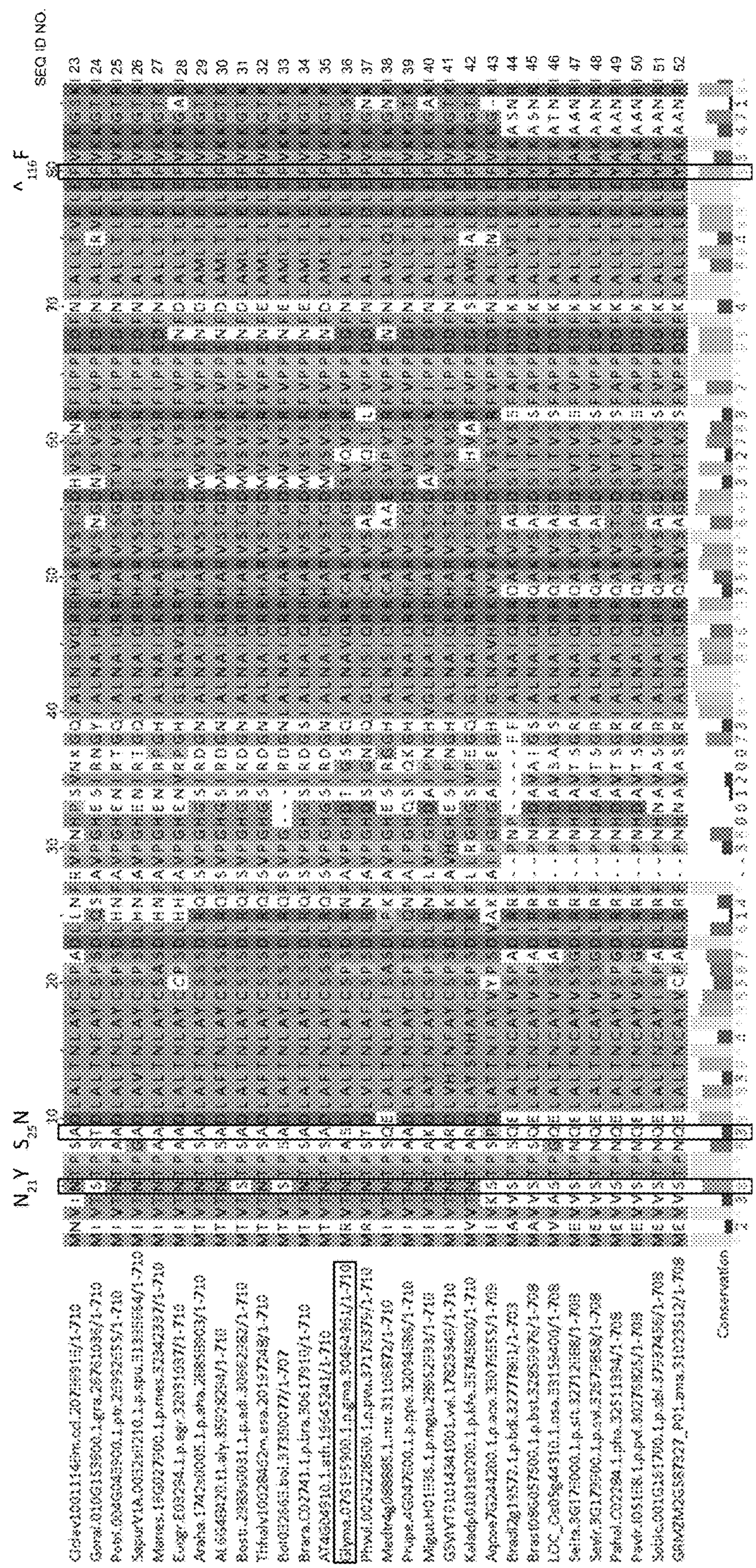


Figure 9C

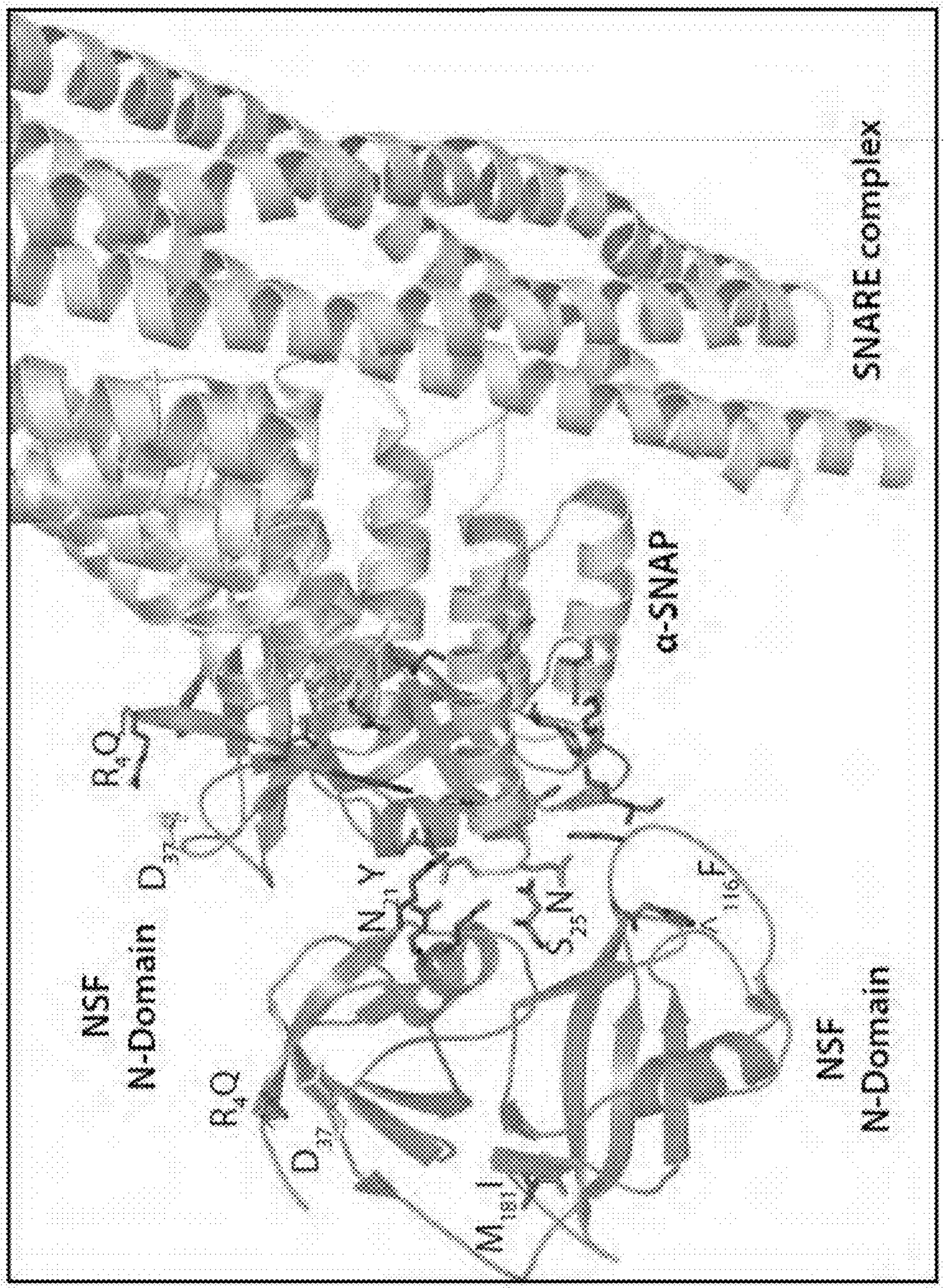


Figure 10A

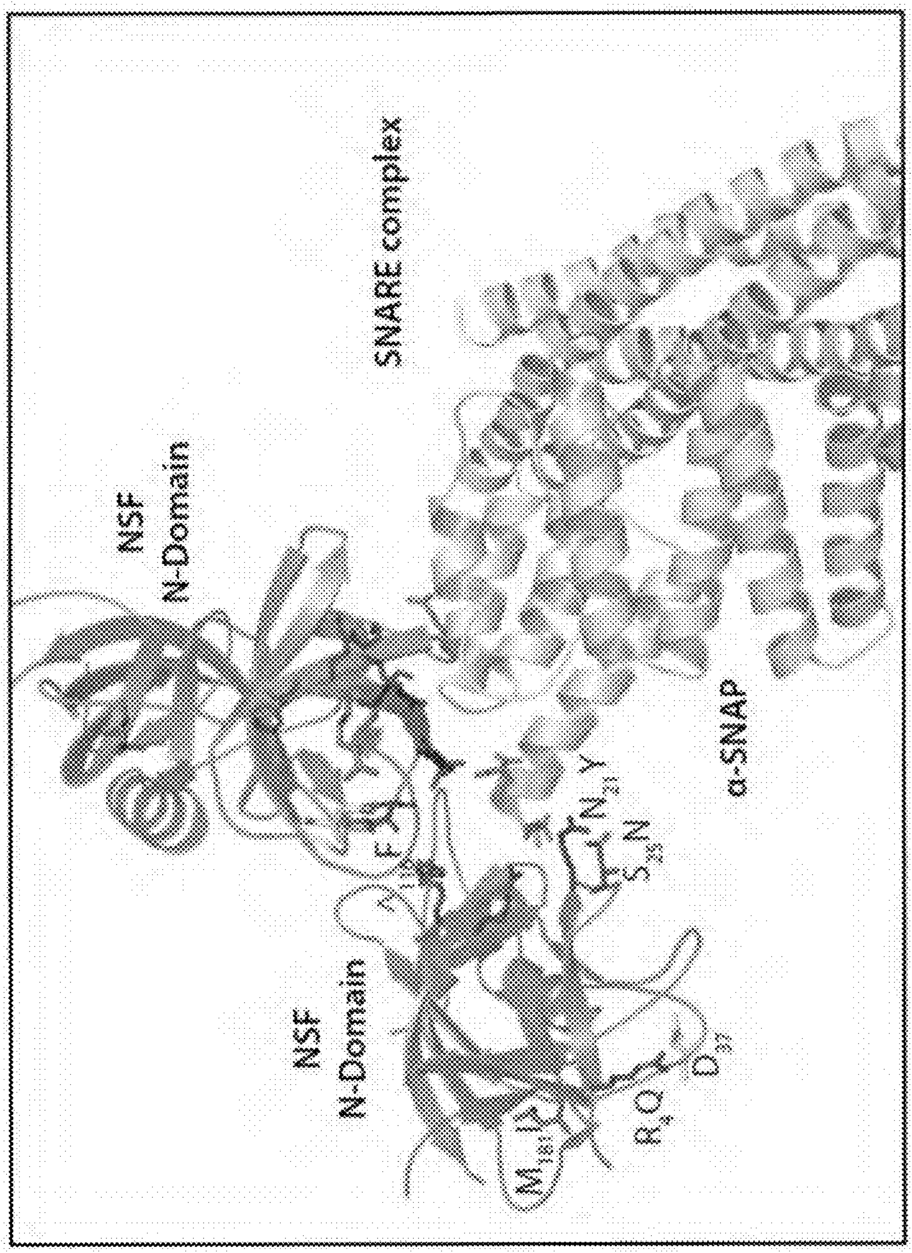


Figure 10B

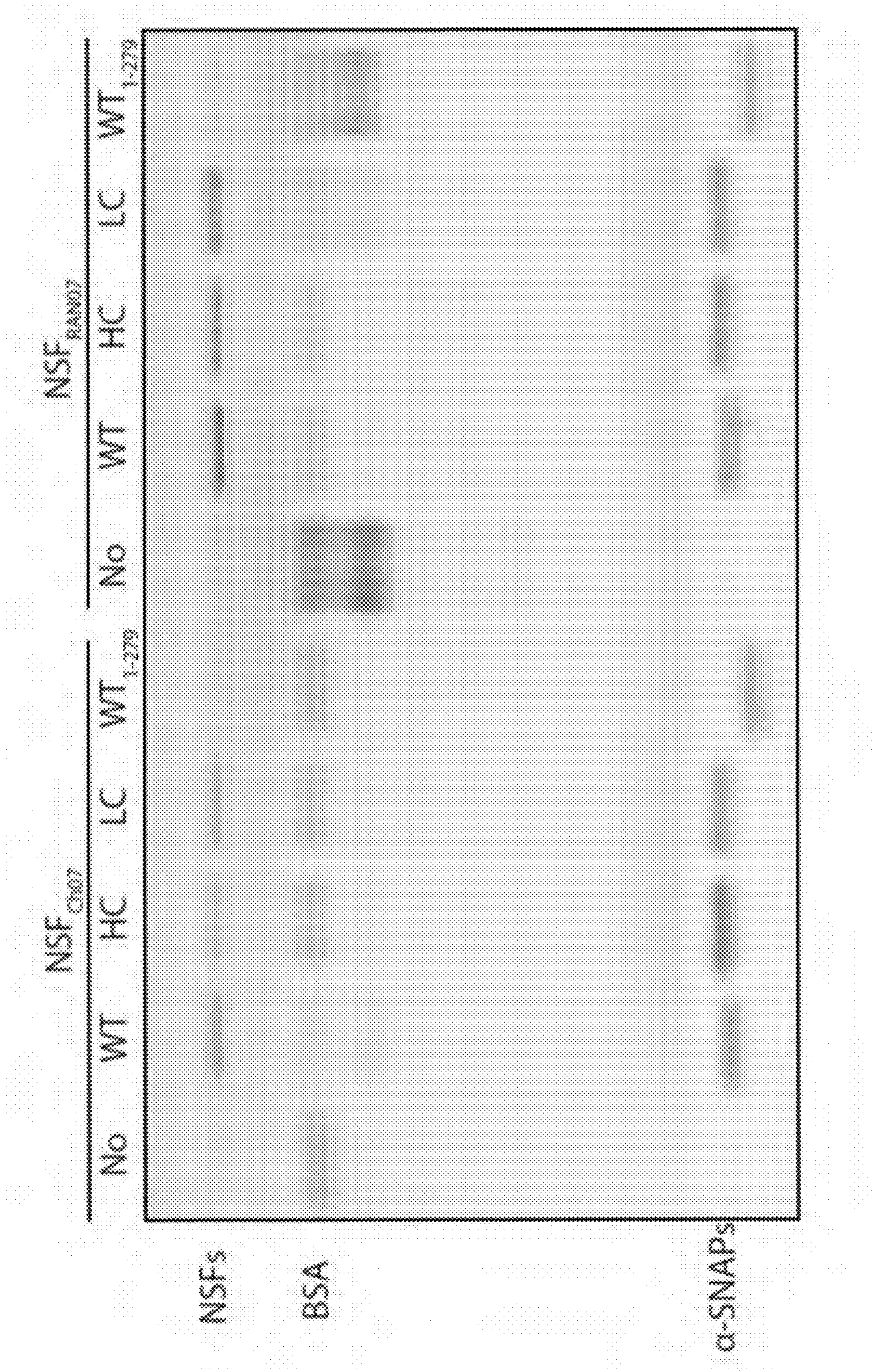


Figure 10C

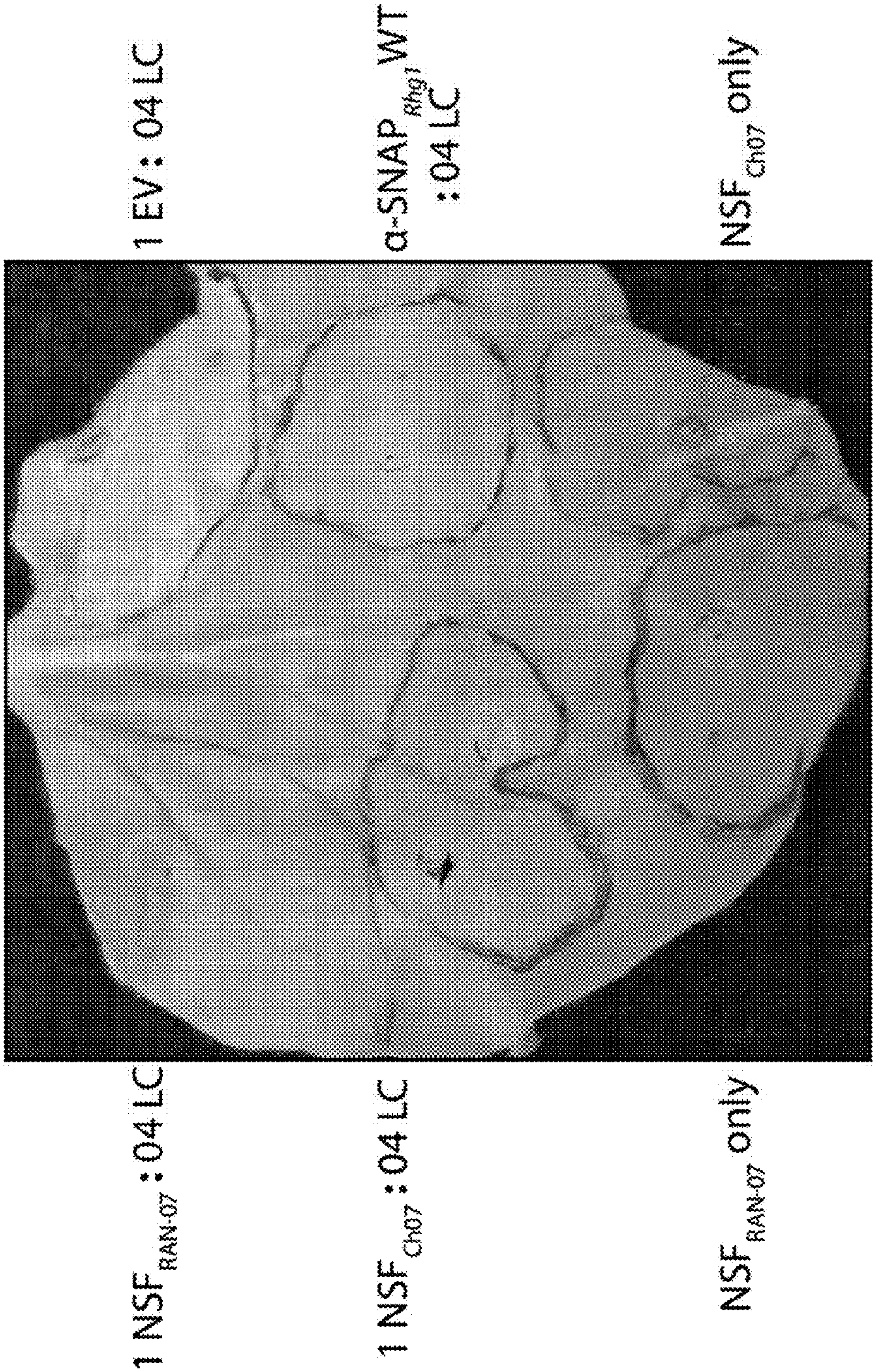

1 EV : 04 LC
α-SNAP$_{Rhg1}$ WT : 04 LC
NSF$_{Ch07}$ only
1 NSF$_{RAN-07}$: 04 LC
1 NSF$_{Ch07}$: 04 LC
NSF$_{RAN-07}$ only

Figure 11A

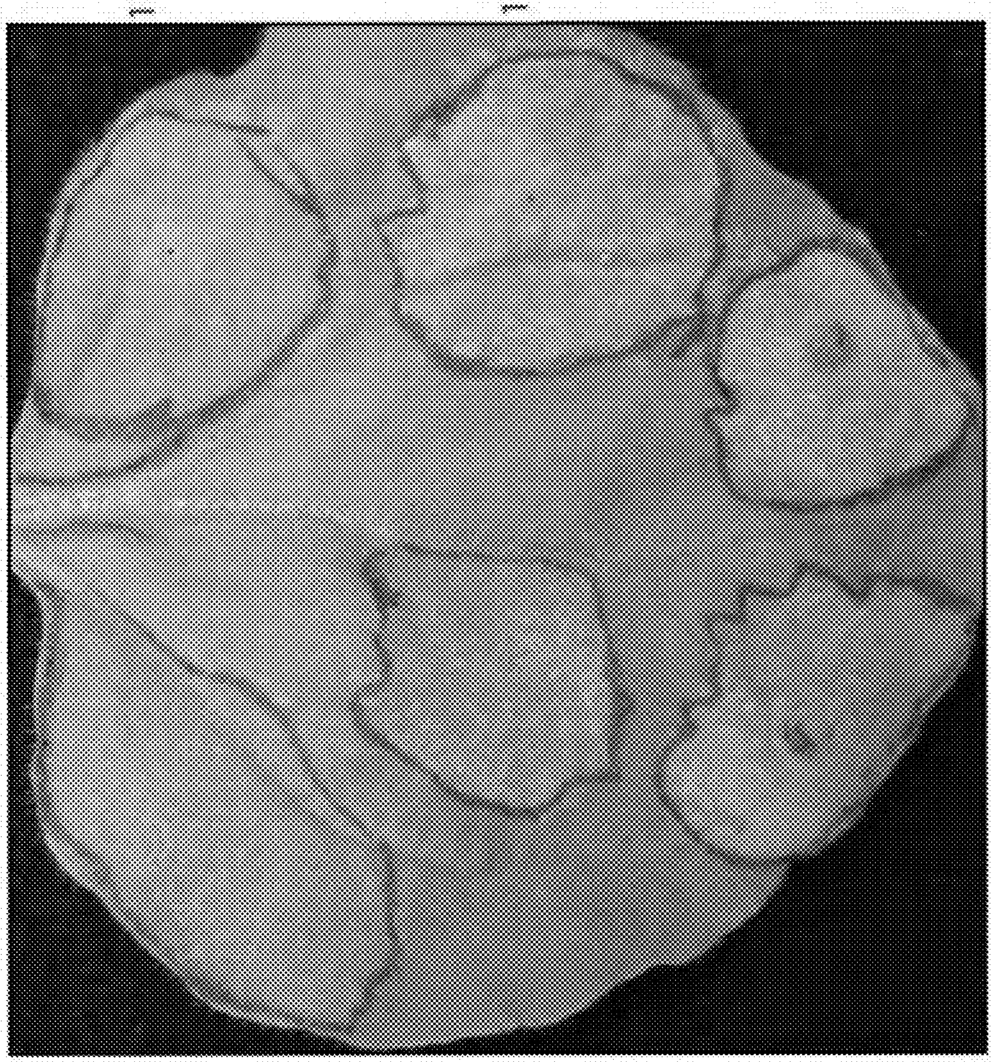

1 NSF_{Ch07} : 09 LC
1 NSF_{Ch07} : 19 LC
1 EV : 09 LC
1 RAN07 : 09 LC
1 RAN07 : 19 LC
1 NSF_{Ch13} : 09 LC

Figure 11B

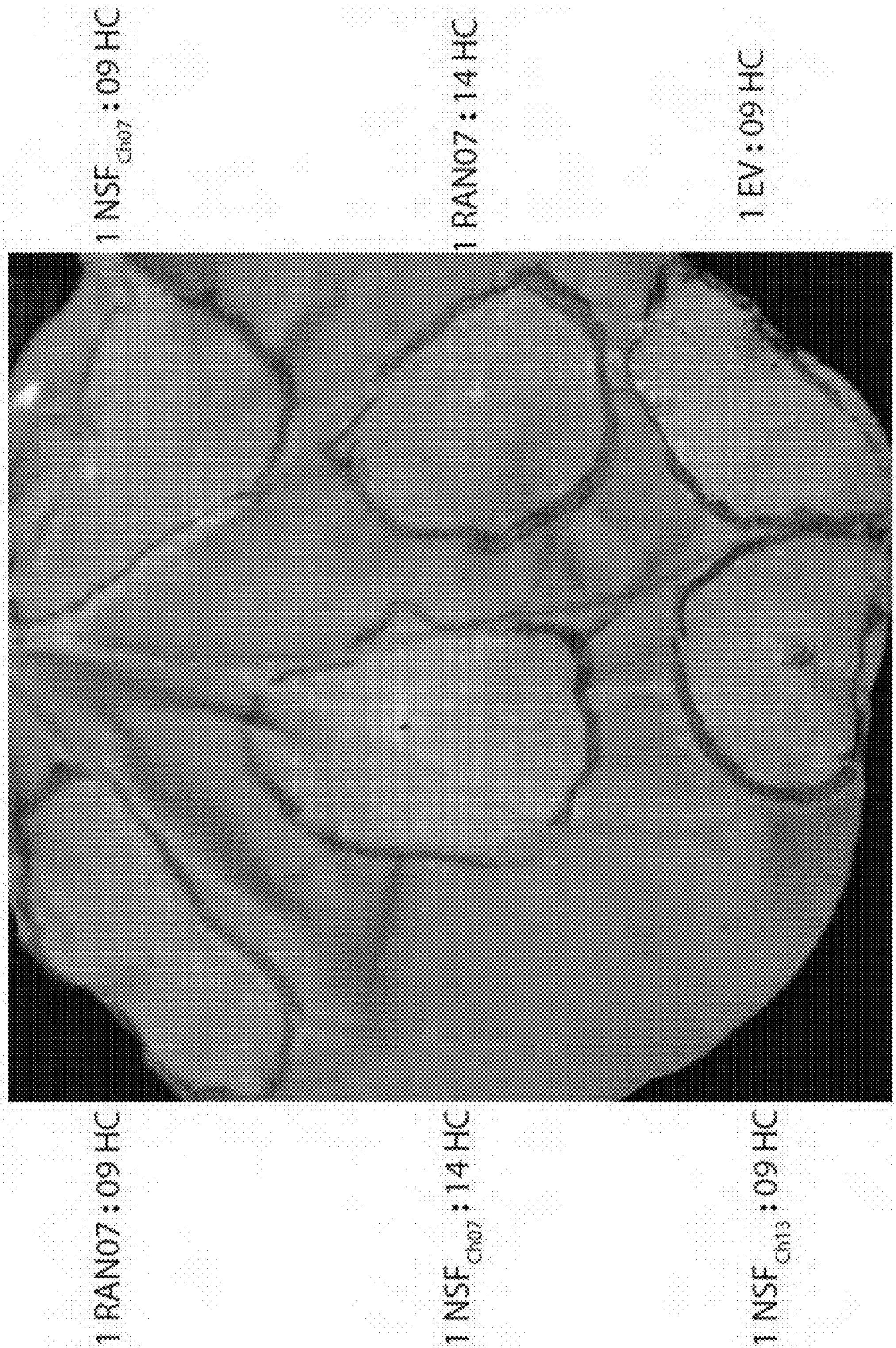

1 NSF_{Ch07} : 09 HC
1 RAN07 : 14 HC
1 EV : 09 HC
1 RAN07 : 09 HC
1 NSF_{Ch07} : 14 HC
1 NSF_{Ch13} : 09 HC

Figure 11C

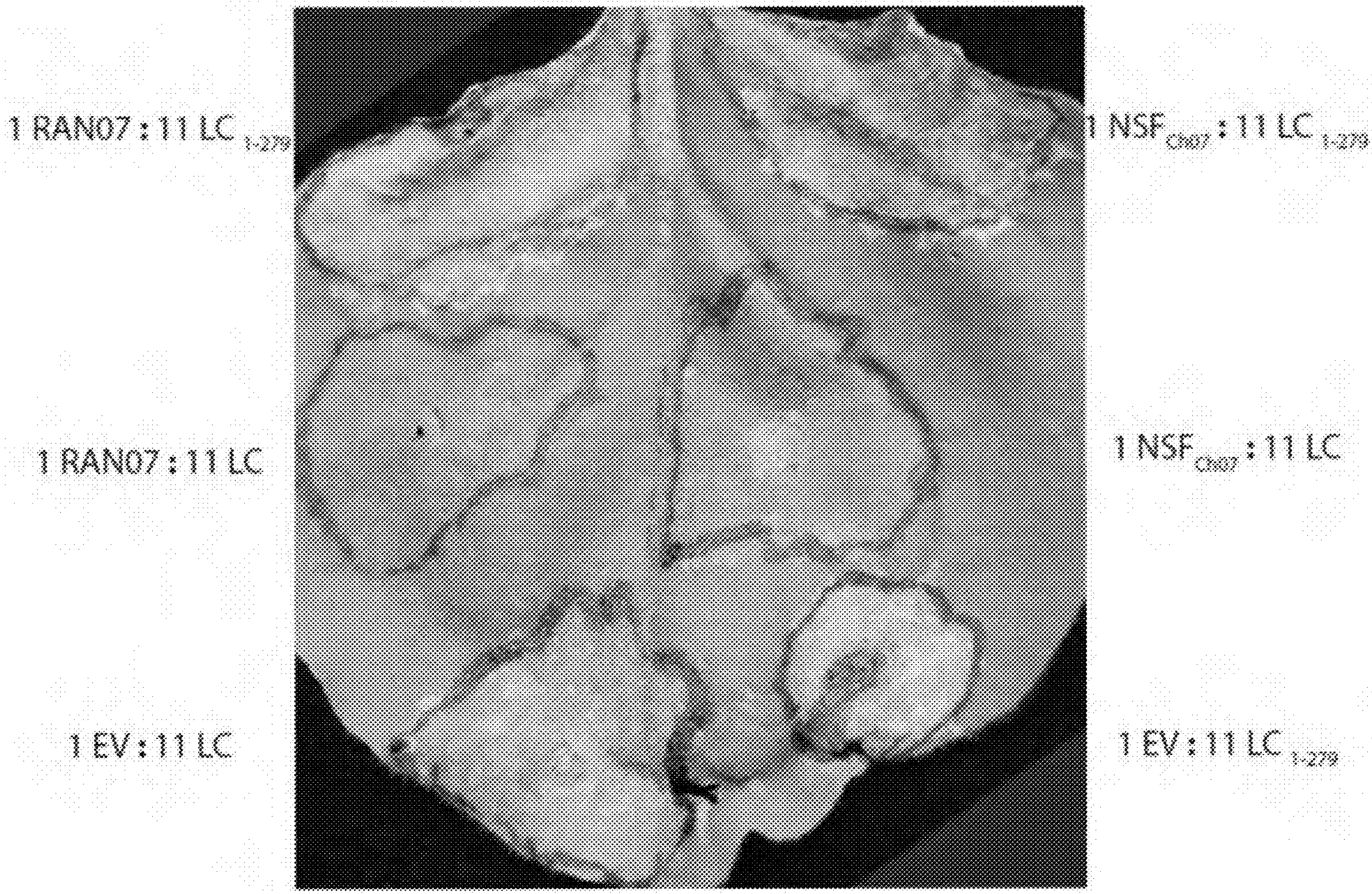

1 RAN07 : 11 LC $_{1\text{-}279}$
1 NSF$_{Ch07}$: 11 LC $_{1\text{-}279}$
1 RAN07 : 11 LC
1 NSF$_{Ch07}$: 11 LC
1 EV : 11 LC
1 EV : 11 LC $_{1\text{-}279}$

Figure 11D

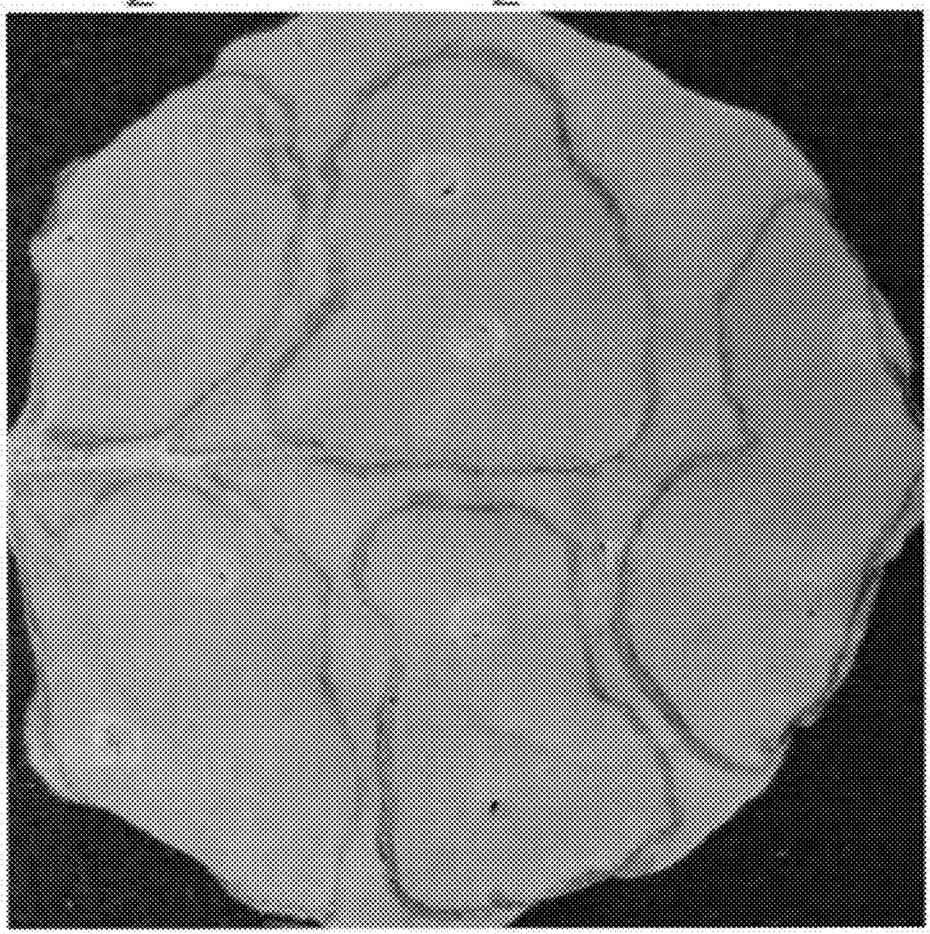


Figure 11E

```
Ch07 SEQ ID NO: 18   MASRFGLSSSSSSASSMRVTNTPASDLALTNLAFCSPSDLRNFAVPGHNNLYLAAVADSF
Nben SEQ ID NO: 53   MAGRFG-----SGASTMIVTNTPAKDLAYTNCAYCSPADLRNFLVPGSK-LAYGLIADAF
                            .  .*.**:* ******.*** ** *:***:***** *** : *   . :**:*

Ch07 SEQ ID NO: 18   VLSLSAHDTIGSGQIALNAVQRRCAKVSSGDSVQVSRFVPPEDFNLALLTLELEFVKKGS
Nben SEQ ID NO: 53   VLTLAAHDGIPNGHLGLNAIQRRYAKVSTGDTISVNRFVPPDDFNLALLTIDLEFVKKGT
                     **:*:*** * .*::.***:*** ****:**::.*.*****:********::*******:

Ch07 SEQ ID NO: 18   KSEQIDAVLLAKQLRKRFMNQVMTVGQKVLFEYHGNNYSFTVSNAAVEGQEKSNSLERGM
Nben SEQ ID NO: 53   RDEQVDAVSLANQVRKKFANQIMSTGQKVTFEYHGNSYIFTVNQATVEGQEKSN-IERGM
                     :.**:*** **:*:**:* **:*:.**** ******.* ***.:*:******** :****

Ch07 SEQ ID NO: 18   ISDDTYIVFETSRDSGIKIVNQREGATSNIFKQKEFNLQSLGIGGLSAEFADIFRRAFAS
Nben SEQ ID NO: 53   ISADTYIIFEAANSSGIKIVNQREAASSSIFRQKEFNLQSLGIGGLSAEFADIFRRAFAS
                     ** ****:**::..**********.*:*.**:*****************************

Ch07 SEQ ID NO: 18   RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKILNGKEPKIVNGPEVLSKFVGE
Nben SEQ ID NO: 53   RVFPPHVTSKLGIKHVKGMLLYGPPGTGKTLMARQIGKMLNGKEPKIVNGPEVLSKFVGE
                     **************************************:*********************

Ch07 SEQ ID NO: 18   TEKNVRDLFADAEQDQRTRGDESDLHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
Nben SEQ ID NO: 53   TEKNVRDLFADAEQDQRTKGDQSELHVIIFDEIDAICKSRGSTRDGTGVHDSIVNQLLTK
                     ******************:**:*:************************************

Ch07 SEQ ID NO: 18   IDGVESLNNVLLIGMTNRKDMLDEALLRPGRLEVQVEISLPDENGRLQILQIHTNKMKEN
Nben SEQ ID NO: 53   IDGVESLNNVLLIGMTNRKDLLDEALMRPGRLEVQVEISLPDENGRLQILQIHTNQMKEN
                     ********************:*****:****************************:****
```

Figure 12A

```
Ch07  SEQ ID NO: 18   SFLAADVNLQELAARTKNYSGAELEGVVKSAVSYALNRQLSLEDLTKPVEEENIKVTMDD
Nben  SEQ ID NO: 53   SFLSPDVNLQELAARTKNYSGAELEGVVKSAVSFALNRQLSMDDLTKPVDEESIKVTMDD
                      ***:.****************************:*******::******:**.*******

Ch07  SEQ ID NO: 18   FLNALHEVTSAFGASTDDLERCRLHGMVECGDRHKHIYQRAMLLVEQVKVSKGSPLVTCL
Nben  SEQ ID NO: 53   FLHALGEVRPAFGASTDDLERCRLNGIVDCGERHQHIYRRIMLLAEQVKVSRGSPLITCL
                      **:** ** .**************:*:*:**:**:***:*:***.******:****:***

Ch07  SEQ ID NO: 18   LEGSRGSGKTALSATVGIDSDFPYVKIVSAESMIGLHESTKCAQIIKVFEDAYKSPLSVI
Nben  SEQ ID NO: 53   LEGPSGSGKTAMAATVGIESDFPYVKIISAETMIGLSESSKCAQIVKVFEDAYKSPLSIV
                      ***. ******::*****:********:***:**** **:*****:************::

Ch07  SEQ ID NO: 18   ILDDIERLLEYVPIGPRFSNLISQTLLVLLKRLPPKGKKLMVIGTTSELDFLESIGFCDT
Nben  SEQ ID NO: 53   VLDGIERLLEYVAIGPRFSNLISQTLLVLLKRLPPKGKKILVIGTTSEAGFLDSVGLCDA
                      :**.********.**************************::******* .**:*:*:**:

Ch07  SEQ ID NO: 18   FSVTYHIPTLNTTDAKKVLEQLNVFTDEDIDSAAEALNDMPIRKLYMLIEMAAQGEHGGS
Nben  SEQ ID NO: 53   FSVTYHVPTLKTEDAKKVLQQLNVFSNDDVDSAAEALNDMPIKKLYMVVEMAAQGEHGGT
                      ******:***:* ******:*****:::*:************:****::**********:

Ch07  SEQ ID NO: 18   AEAIFSGKEKISIAHFYDCLQDVVRL
Nben  SEQ ID NO: 53   AEAIYSGKEKIQISHFYDCLQDIARY
                      ****:******.*:********:.* 
```

Figure 12B

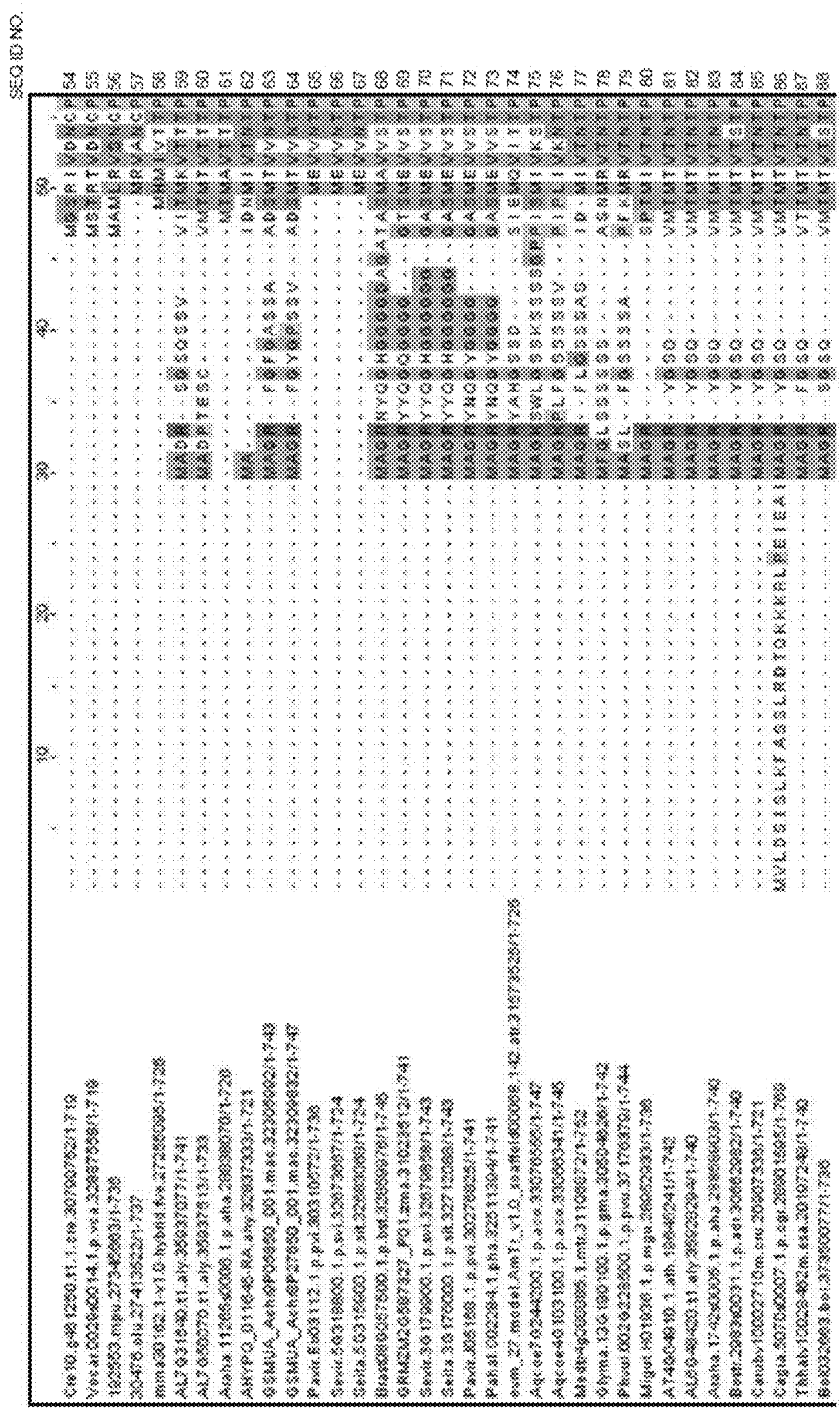


Figure 13A

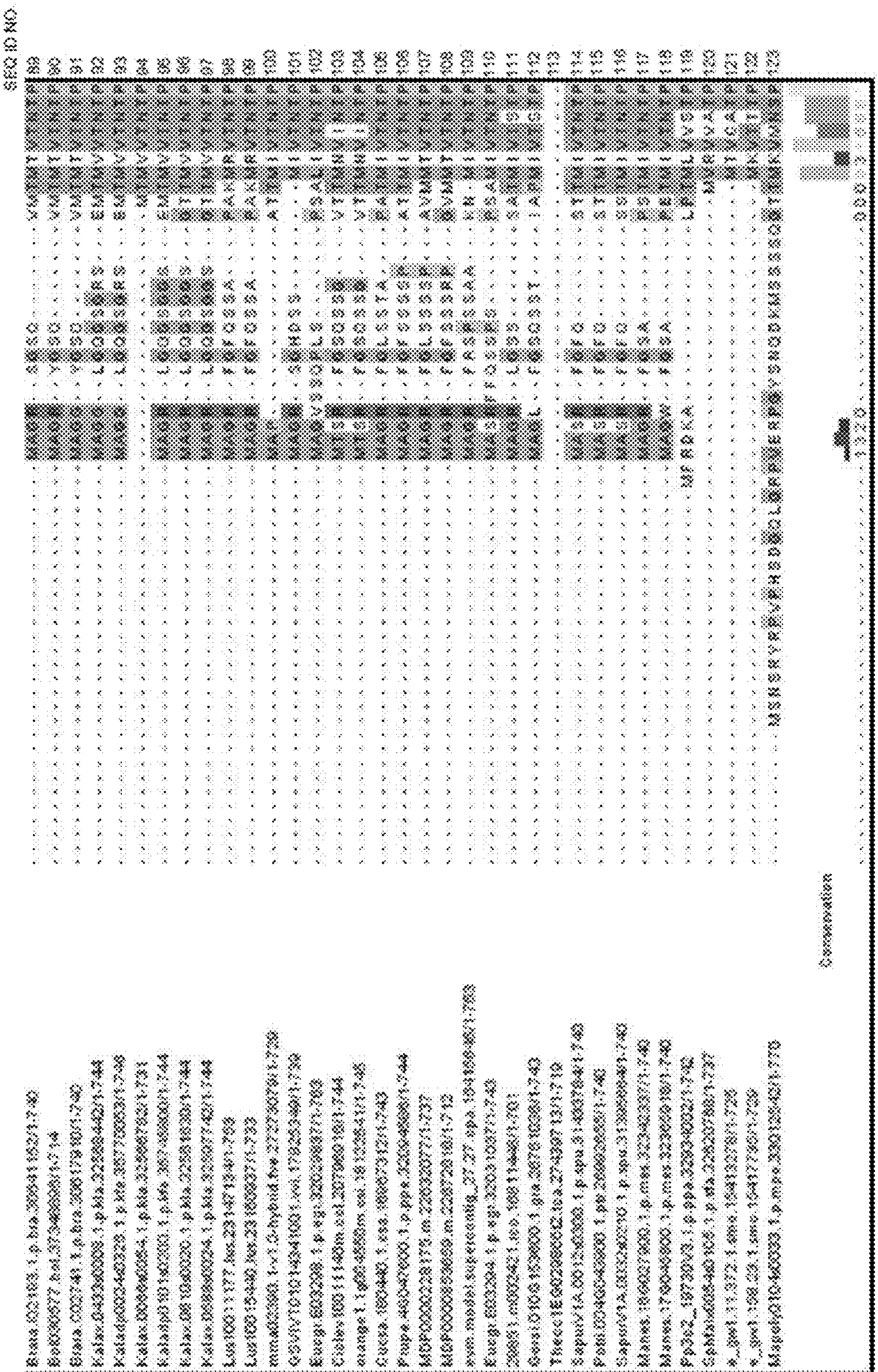


Figure 13B

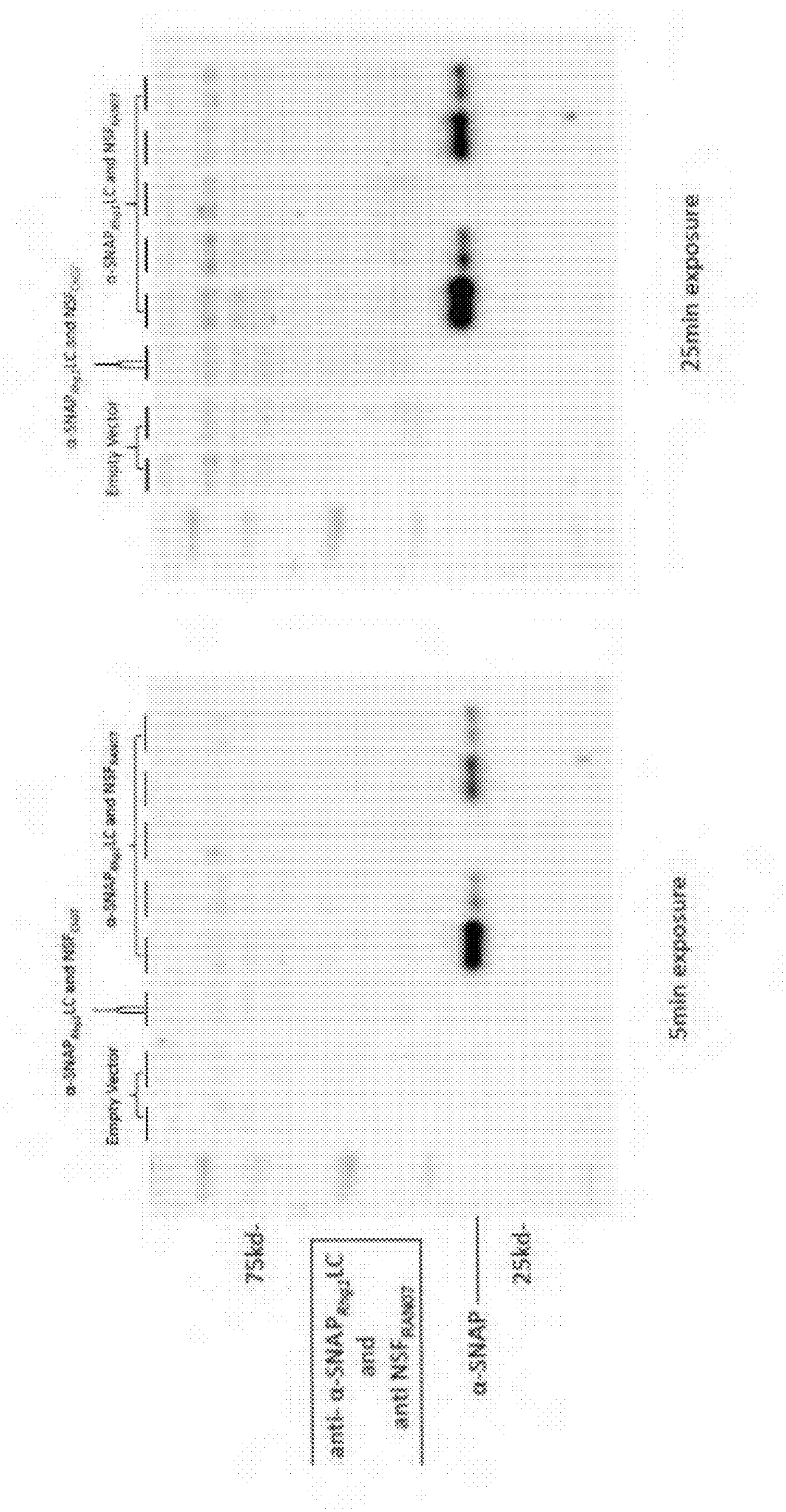

Empty Vector
25min exposure
5min exposure
75kd
25kd
α-SNAP
and

Figure 14

METHODS AND COMPOSITIONS FOR RESISTANCE TO CYST NEMATODE IN PLANTS

[0001] This application claims priority to U.S. Provisional Application Nos. 62/544,856 and 62/544,824, the disclosures of which are explicitly incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under 17-CRHF-0-6055 awarded by the USDA/NIFA. The government has certain rights in the invention.

BACKGROUND

Field of the Invention

[0003] The present disclosure provides methods and compositions for conferring or producing nematode resistance in a plant or plant cells, and nematode resistant plants or plant cells. The disclosure further provides methods for improving growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance.

Description of Related Art

[0004] Soybean cyst nematode (*Heterodera glycines*; SCN) is consistently the most damaging disease or pest of U.S. soybeans, one of the world's most important crops (Niblack et al., 2006, Annu Rev Phytopathol 44, 283-303; Jones et al., 2013, Mol Plant Pathol 14, 946-961; Mitchum, 2016, Mol Plant Pathol 5, 175-181; T. W. Allen, 2017, Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Research. doi:10.1094/PHP-RS-16-0066). Plant parasitic nematodes, including cyst nematodes, infest the roots of many valuable crops and establish elaborate feeding structures (Kyndt et al., 2013, Planta 238, 807-818). Cyst nematodes secrete a complex arsenal of effector molecules that modulate the host's physiology and promote fusion of neighboring host cells into a large unicellular feeding site, termed a syncytium (Gheysen and Mitchum, 2011, Curr Opin Plant Biol 14, 415-421; Hewezi and Baum, 2013, Mol Plant Microbe Interact 26, 9-16; Mitchum et al., 2013, New Phytologist 199, 879-894), with negative effects on the health and propagation of the involved plants.

[0005] A soybean locus, Rhg1 (Resistance to *Heterodera glycines*), has been widely used by soybean breeders and growers as the best available disease resistance locus to reduce damage caused by SCN (Concibido et al., 2004, Crop Science 44, 1121-1131; Mitchum, 2016, Id.). The complex Rhg1 locus on soybean chromosome 18 is a tandemly repeated block of four genes: Glyma.18G022400 (formerly Glyma18g02580), Glyma.18G022500 (formerly Glyma18g02590), Glyma.18G022600 (formerly Glyma18g02600) and Glyma.18G022700 (formerly Glyma18g02610), as well as the adjacent nucleotides that comprise the chromosomal segment containing the above genes, which is tandemly repeated in haplotypes that confer increased SCN resistance (Cook et al., 2012, Science 338, 1206-1209; U.S. Patent Application Publ. No. 2013-0305410 A1). (The 13-character gene names are from the Wm82.a1 genome assembly and Glyma 1.0 gene models (Schmutz et al., 2010, Nature 463, 178-183) and the more recent 15-character gene names are from the U.S. Department of Energy Joint Genome Institute Wm82.a2 soybean genome assembly and Glyma 2.0 gene model naming revision.) The relevant genes at the Rhg1 locus do not encode proteins widely associated with plant disease resistance. Instead, resistance is mediated by copy number variation of three disparate genes at the Rhg1 locus, one of which (Glyma.18G022500) encodes proteins with high similarity to known α-SNAP proteins (U.S. Patent Application Publ. No. 2013-0305410 A1; Mitchum et al., 2004, Mol Plant Pathol 5, 175-181; Jones and Dangl, 2006, Nature 444, 323-329; Dodds and Rathjen, 2010, Nat Rev Genet 11, 539-548; Cook et al., 2012, Science 338, 1206-1209; Cook et al., 2014, Plant Physiol 165, 630-647; Lee et al., 2015, Mol Ecol 24, 1774-1791).

[0006] Alpha-Soluble NSF Attachment Protein (α-SNAP or α-SNAP herein) is a ubiquitous housekeeping protein in plants and animals that facilitates cellular vesicular trafficking by mediating the disassembly and reuse of the four-protein bundles of SNARE proteins (soluble NSF attachment protein receptor proteins) that form when t-SNARE and v-SNARE proteins anneal during vesicle docking to target membranes (Jahn and Scheller, 2006, Nat Rev Mol Cell Biol 7, 631-643; Baker and Hughson, 2016, Nat Rev Mol Cell Biol 17, 465-479; Zhao and Brunger, 2016, J Mol Biol 428, 1912-1926). α-SNAP functions together with the ATPase N-ethylmaleimide Sensitive Factor (NSF) to carry out this SNARE bundle disassembly (Zhao and Brunger, 2015, J Mol Biol 428: 1912-1926).

[0007] NSF is an ATPases Associated with various cellular Activities (AAA) family protein containing three well defined domains: the N-domain, which mediates interactions with one or more α-SNAP polypeptides, the D1 ATPase domains, which couple ATP hydrolysis to force-generating conformational changes that remodel SNARE complexes, and the D2 ATPase domain, which mediates NSF hexamerization (Whiteheart et al., 2001, Int Rev Cytol 207, 71-112; Hanson and Whiteheart, 2005, Nat Rev Mol Cell Biol 6, 519-529; Zhao et al., 2010, J. Biol. Chem. 285, 761-772).

[0008] The soybean resistance-associated Rhg1 α-SNAPs comprise polymorphic variant sequences of Glyma.18G022500 that encode variant α-SNAP proteins (U.S. patent application Ser. No. 13/843,447). Rhg1 resistance-associated α-SNAPs have lower binding affinity for NSF and SNARE/NSF complexes, and disrupt vesicle trafficking in planta (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). The relative abundance of Rhg1-encoded defective α-SNAP variants increases substantially within host syncytium cells at the nematode feeding site (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA Proc. Natl. Acad. Sci. USA 113, E7375-E7382, Proc. Natl. Acad. Sci. USA 113, E7375-E7382).

[0009] Resistance-associated Rhg1 haplotypes group into structural classes based on the type of α-SNAP polymorphisms that they encode, which also correlates with the copy-number of Rhg1 repeats that are present across hundreds of soybean accessions (Cook et al., 2014, Plant Physiol 165, 630-647; Lee et al., 2015).). $Rhg1_{HC}$ (high copy) loci carry four or more and frequently nine or ten Rhg1 repeats, and $Rhg1_{LC}$ (low-copy) loci carry three or fewer Rhg1 repeats. $Rhg1_{LC}$ is also known as rhg1-a and Rhg_{HC} is also known as rhg1-b (Mitchum 2016 and Liu 2017 Nat. Commun. 8, 14822). $Rhg1_{HC}$ and $Rhg1_{LC}$ encode similar yet distinct α-SNAP variants that are impaired in normal α-SNAP/NSF interactions (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). All $Rhg1_{HC}$ loci examined to date also have one Rhg1 repeat that encodes a wildtype (WT) α-SNAP along with multiple repeats encoding a resistance-type α-SNAP, while $Rhg1_{LC}$ loci encode only resistance-type α-SNAPs and no WT α-SNAP (Cook et al., 2012, Science 338, 1206-1209; Cook et al., 2014, Plant Physiol 165, 630-647; Lee et al., 2015). Plants carrying $Rhg1_{HC}$ or $Rhg1_{LC}$ loci exhibit elevated transcript abundance that correlates approximately with copy number for the repeat genes, including the Rhg1 α-SNAP gene, and variants thereof (U.S. Patent Application Publ. No. 2013-0305410 A1; Cook et al., 2012, Science 338, 1206-1209; Cook et al., 2014, Plant Physiol 165, 630-647).

[0010] In experiments performed in *N. benthamiana* leaves, high expression of these resistance-conferring α-SNAPs hindered vesicular trafficking and eventually elicited cell death, but co-expression of wild type soybean α-SNAPs diminished this cytotoxicity (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382).

[0011] Therefore, there is a need in the art for methods and compositions that enable the generation and propagation of SCN-resistant plant cells that harbor Rhg1 resistance-associated genes, including Rhg1 resistance-associated α-SNAPs.

SUMMARY OF THE INVENTION

[0012] The present disclosure provides methods for producing plant cells resistant to nematodes. The disclosure further provides methods for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance. The present disclosure also provides compositions for producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes conferring nematode resistance. In further aspects, the disclosure provides plant cells and plants with increased resistance to nematodes, without or preferably with improved growth or survival.

[0013] In some embodiments, the disclosure provides methods and compositions for producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance, comprising increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of, one or more polynucleotides encoding α-SNAP proteins, or homologs or variants thereof, and/or one or more polynucleotides encoding NSF proteins, or homologs or variants thereof, wherein said plant cells are resistant to nematodes relative to native plant cells.

[0014] In certain embodiments, the disclosure provides methods of producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance, comprising increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of a polynucleotide encoding one or more α-SNAP proteins with at least 95% identity to a polynucleotide identified by SEQ ID NOs: 5 or 6, or an encoded polypeptide with at least 95% identity to a polypeptide identified by SEQ ID NOs: 14 or 15, or homologs or variants thereof.

[0015] In further embodiments, the disclosure provides methods of producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance, comprising increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of a polynucleotide encoding and a polynucleotide encoding one or more NSF proteins with at least 95% identity to a polynucleotide identified by SEQ ID NOS: 8 or 9, or an encoded polypeptide with at least 95% identity to a polypeptide identified by SEQ ID NOs 17 or 18, or homologs or variants thereof.

[0016] In still further embodiments, the disclosure provides methods of producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance, comprising increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of both (a) a polynucleotide encoding one or more α-SNAP proteins encoded by a polynucleotide with at least 95% identity to SEQ ID NO: 5 or SEQ ID NO: 6, and (b) a polynucleotide encoding one or more NSF proteins encoded by a polynucleotide with at least 95% identity to SEQ ID NO: 9, or homologs or functionally conserved variants of any of the aforementioned SEQ ID NOs.

[0017] In embodiments, the methods of the disclosure produce plant cells or plants resistant to nematodes. In certain embodiments, the plant cells or plants provided herein are soybean, sugar beets, potatoes, corn, wheat, pea or beans or those plants listed in Tables 6 and 7.

[0018] In embodiments, the methods of the disclosure comprise increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of a polynucleotide cells in the root of the plant. In some embodiments, the one or more polynucleotides encoding α-SNAP proteins or NSF proteins, or homologs or variants thereof, is increased by incorporation of a construct comprising a promoter operably linked to one or more of said polynucleotides in the plant cells. In embodiments, the disclosure provides a method of increasing nematode resistance in a plant, wherein at least two of the polynucleotides recited herein have increased expression, an altered expression pattern, or increased copy number.

[0019] In one aspect, the disclosure provides a method of altering the abundance of one or more α-SNAP proteins in a plant cell. In certain embodiments of the disclosed methods, an amount of an α-SNAP encoded by the sequence identified in SEQ ID NO: 2, or a polynucleotide with at least 95% identity thereof, is reduced relative to an amount of an α-SNAP encoded by either of the sequences identified in SEQ ID NO: 5 and SEQ ID NO: 6, or polynucleotides with at least 95% 75% identity, or homologs or functionally conserved variants of the SEQ ID NO: 2, SEQ ID NO: 5, or SEQ ID NO: 6.

[0020] In a further aspect, this disclosure provides compositions for producing plant cells resistant to nematodes, or for improving the growth or survival of a plant cell containing one or more Rhg1 genes capable of conferring nematode resistance. In some embodiments, the disclosure provides constructs comprising a promoter operably linked to one or more polynucleotides encoding α-SNAP proteins, one or more polynucleotides encoding NSF proteins, or homologs or variants thereof. In further embodiments, the disclosure provides a construct comprising a polynucleotide with at least 95% identity to SEQ ID NO: 5 or SEQ ID NO: 6, and/or a polynucleotide with at least 95% identity to SEQ ID NO: 9, or homologs or functionally conserved variants of the SEQ ID NOs identified herein. In certain embodiments, a construct of the disclosure comprises a plant promoter.

[0021] In still another aspect, the disclosure provides a nematode resistant transgenic plant cell, or a transgenic plant cell containing one or more Rhg1 genes capable of conferring nematode resistance comprising with improved growth or survival. In embodiments, a transgenic plant cell of the disclosure comprises one or more polynucleotides encoding α-SNAP proteins, or one or more polynucleotides encoding NSF proteins, or homologs or variants thereof. In certain embodiments, a transgenic plant or plant cells of the disclosure comprises one or more α-SNAP proteins encoded by polynucleotides with at least 95% identity to the polynucleotides identified by SEQ ID NOS: 1-7, or polypeptides with at least 95% identity to polypeptides identified by SEQ ID NOs 10-16, or homologs or variants thereof. In further embodiments, a transgenic plant cell of the disclosure comprises one or more NSF proteins encoded by polynucleotides with at least 95% identity to the polynucleotides identified by SEQ ID NOS: 8 and 9, or comprise polypeptides with at least 95% identity to polypeptides identified by SEQ ID NOs 17 and 18, or homologs or variants thereof.

[0022] Embodiments of the disclosure also provide seeds comprising the transgenic plant cells described herein, plants grown from the seeds described herein, parts, progeny or asexual propagates of the transgenic plant cells disclosed herein. In some embodiments, the transgenic plant, plant cell or seed, or part, progeny or asexual propagate thereof of the disclosure are soybeans, sugar beets, potatoes, corn, wheat, peas or beans, or a wide variety of plant species as listed in Tables 6 and 7.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The following detailed description can be best understood when read in conjunction with the following drawings in which:

[0024] FIG. **1**A shows an immunoblot of wild-type α-SNAPs, Rhg1 resistance-type α-SNAPs and NSF in HG type test soybean roots. $Rhg1_{LC}$ varieties: PI 548402 (Peking), PI 89772, PI 437654, PI 90763; $Rhg1_{HC}$ varieties: PI 88788, PI 209332, PI 548316 (7 copy). PonceauS staining shows total protein loaded per lane. FIG. **1**B illustrates densitometry indicating total NSF expression in HG type test lines. FIG. **1**C, shows immunoblots from trifoliate leaves or roots of Williams 82 (Wm82) and modern $Rhg1_{LC}$ and $Rhg1_{HC}$ varieties Forrest and Fayette (labeling as described for FIG. **1**A). FIG. **1**D shows immunoblots for total WT α-SNAPs and α-$SNAP_{Rhg1}$LC in "Forrest" ($Rhg1_{LC}$) transgenic roots transformed with an empty vector (EV) or the native Williams 82 α-$SNAP_{Rhg1}$WT locus, or in Williams 82 roots transformed with empty vector.

[0025] FIG. **2**A is an alignment of soybean NSF_{Ch07}, NSF_{Ch13}, and NSF_{RAN07} N-terminal domains (SEQ ID NOs: 20, 22, and 21, respectively). Large identical regions are omitted. N-domain residues that bind α-SNAP are shaded dark grey (N_{21}, RR_{82-83}, $KK_{117-118}$). NSF_{RAN07} polymorphisms R_4Q, $N_{21}Y$, $S_{25}N$, $_{116}F$, $M_{181}I$ are shaded light grey. FIG. **2**B shows NSF_{RAN07} modeled to NSF_{CHO} cryo-EM structure (3J97A, State II). NSF residue patches implicated in α-SNAP binding are labeled I, II or III, respectively. FIG. **2**C shows NSF_{RAN07} polymorphisms ($N_{21}Y$), with zoomed in view of polymorphic N-domain region. FIG. **2**D shows that NSF N-domain R_4 is conserved in most model eukaryotes. Frequency logo of first 10 NSF N-domain residues of the following organisms: *Homo sapiens, Bos taurus, Mus musculus, Cricetulus griseus* (Chinese hamster), *Caenorhabditis elegans, Drosophila melanogaster, Danio rerio, Xenopus laevis, Gallus gallus, Neurospora crassa, Saccharomyces cerevisiae, Schizosaccharyomyces pombe, Chlamydomonas reinhardtii, Physcomitrella patens, Zea mays, Oryza sativa, Solanum tuberosum, Cucumis sativa, Arabidopsis thaliana, Medicago truncatula, Nicotiana benthamiana*, and *Glycine max*.

[0026] FIG. **3**A is a ribbon diagram showing cryo-EM structure of mammalian 20S supercomplex, masked to show only SNARE bundle (right, "SNARE complex"), one α-SNAP (middle, "α-SNAP") and two NSF N-domains (left and middle behind, "NSF N-Domain"). Conserved NSF N-domain patches (I, R10; II, RK67-68; III, KK104-105) and α-SNAP C-terminal contacts (D217DEED290-293) are shown extending from the ribbon depiction (see also, FIG. **3**B). FIG. **3**B is a ribbon diagram showing NSF_{RAN07} polymorphisms; RAN07 residues are labeled (shown black), and arrows point out the α-SNAP interacting residues (light grey). FIG. **3**C is a photograph of silver-stained SDS/PAGE of recombinant NSF_{Ch07} or NSF_{RAN07} bound in vitro by the recombinant proteins indicated on second line: no-α-SNAP control (No) or wild-type (WT), low-copy (LC), or high copy (HC) Rhg1 α-SNAP. BSA: bovine serum albumin. FIG. **3**D shows densitometric quantification of NSF_{Ch07} or NSF_{RAN07} bound by Rhg1 α-SNAPs in FIG. **3**C; data are from three independent experiments and error bars show SEM.

[0027] FIG. **4**A is a photograph of *N. benthamiana* leaves ~6 days post agro-infiltration with 9:1 or 14:1 mixed cultures of α-$SNAP_{Rhg1}$ LC and NSF_{Ch07} or NSF_{Ch13} or NSF_{RAN07} or empty vector (nine or fourteen parts *Agrobacterium tumefaciens* that delivers α-$SNAP_{Rhg1}$LC to one part *Agrobacterium* that delivers soybean NSF or empty vector control). FIG. **4**B, same as in FIG. **4**A, but 7:1 or 11:1 mixed cultures of α-$SNAP_{Rhg1}$ LC co-expressed with $NSF_{N.benth}$ or NSF_{Ch13} or NSF_{RAN07} or empty vector. FIG. **4**C is a photograph of silver-stained SDS/PAGE of recombinant $NSF_{N.benth}$ bound in vitro by recombinant wild-type, low-copy (LC), or high copy (HC) Rhg1 α-SNAP proteins or WT α-SNAP lacking the final 10 C-terminal residues (α-SNAP1-279). BSA, bovine serum albumin. FIG. **4**D, same as in FIG. **4**A and FIG. **4**B, but 4:1 or 9:1 mixed cultures of α-$SNAP_{Rhg1}$LC or α-$SNAP_{Rhg1}$LC-1289A co-expressed with NSF_{Ch07} or NSF_{RAN07}.

[0028] FIG. **5**A shows frequency of SoySNP50K SNP ss715597431 (corresponding to NSF_{RAN07} R_4Q) in all 19,645 SoySNP50K-genotyped *Glycine max* accessions. FIG. **5**B shows frequency of ss715597431 in all USDA *G. max* with $Rhg1_{LC}$ or $Rhg1_{HC}$ haplotype signatures or in remainder of SoySNP50K-genotyped *G. max* from USDA collection. FIG. **5**C and FIG. **5**D show SNP mapping of the

NSF_{RAN07} candidate gene interval for low copy Rhg1 and high copy Rhg1 respectively, indicating relative SNP frequencies. HG type and SoyNAM populations used for SNP mapping.

[0029] FIG. **6**A is an anti-HA immunoblot of *N. benthamiana* leaves agroinfiltrated to express empty vector, N-HA-α-$SNAP_{Ch11}$ or N-HA-α-$SNAP_{Ch11}$-IR (intron-retention). PonceauS staining indicates relative total protein levels. FIG. **6**B illustrates modeling of α-$SNAP_{Ch11}$-IR to sec17 crystal structure (yeast α-SNAP, PDB ID 1QQE) suggests early termination of alpha-helix 12. FIG. **6**C shows immunoblots for total WT α-SNAP and α-$SNAP_{Rhg1}$LC levels in Forrest ($Rhg1_{LC}$) transgenic roots transformed with an empty vector (EV) or the native WT α-$SNAP_{Ch11}$ locus from Williams 82. FIG. **6**D, as described in FIG. **5**A, except frequency of SoySNP50K SNP ss715610416 allele that is closest marker for α-$SNAP_{Ch11}$-IR, in all 19,645 USDA accessions. FIG. **6**E illustrates the frequency of ss715610416 in all USDA *Glycine max* with $Rhg1_{LC}$ or $Rhg1_{HC}$ haplotype signatures vs. remainder of SoySNP50K-genotyped USDA collection.

[0030] FIG. **7**A shows immunoblot of wild-type α-SNAPs and NSF expression in HG type test soybean roots. $Rhg1_{LC}$ varieties: PI 548402 (Peking), PI 89772, PI 437654, PI 90763; $Rhg1_{HC}$ varieties: PI 88788, PI 209332, PI 548316 (7 copy). PonceauS staining shows total protein loaded per lane. FIG. **7**B shows densitometry data on the ratio of WT α-SNAPs to Rhg1 resistance type α-SNAPs. Ratios calculated using Image J densitometry as in FIG. **1**B. FIG. **7**C is an agarose gel showing PCR amplicons generated with RAN07 or NSF Ch_{07}WT specific primers on HG type soybeans and soybean genome reference variety Williams82 (Wm82). $Rhg1_{LC}$ varieties: "Forrest" (PI 548402-derived), PI 89772, PI 437654, PI 90763; $Rhg1_{HC}$ varieties: PI 88788, PI 209332, PI 548316 (7 copy).

[0031] FIG. **8**A and FIG. **8**B show NSF_{RAN07} (SEQ ID NO:18) amino acid alignment with NSF_{Ch07} of soybean reference genome Williams82 (SEQ ID NO:17). N-domain amino acid polymorphisms unique to RAN07 are indicated by boldface in the corresponding residues in Wm82 NSFCh07.

[0032] FIG. **9**A shows NSF_{RAN07} modeled to an NSF_{CHO} cryo-EM structure (as described in FIG. **2**A), but rotated 90° on the X-axis. NSF residue patches implicated in α-SNAP binding are indicated. FIG. **9**B shows that NSF N-domain R_4 is conserved in most model eukaryotes. Frequency logo of first 10 NSF N-domain residues of the following organisms: *Homo sapiens, Bos taurus, Mus musculus, Cricetulus griseus* (Chinese hamster), *Caenorhabditis elegans, Drosophila melanogaster, Danio rerio, Xenopus laevis, Gallus gallus, Neurospora crassa, Saccharomyces cerevisiae, Schizosaccharyomyces pombe, Chlamydomonas reinhardtii, Physcomitrella patens, Zea mays, Oryza sativa, Solanum tuberosum, Cucumis sativa, Arabidopsis thaliana, Medicago truncatula, Nicotiana benthamiana*, and *Glycine max*. FIG. **9**C is an alignment of NSF N-domain using available plant NSF amino acid sequences from Phytozome.org (SEQ ID NOs:23-52). The alignment was generated with Jalview starting at a conserved methionine residue corresponding to RAN07 met 17. Residues polymorphic in RAN07 are outlined with a box with the corresponding position labeled above.

[0033] FIG. **10**A shows cryo-EM structure of mammalian 20S supercomplex showing SNARE bundle similar to that of FIG. **4**A. FIG. **10**B depicts that same as FIG. **10**A but rotated 90° on Y-axis. FIG. **10**C is the same as FIG. **3**C, except the recombinant NSF_{Ch07} or NSF_{RAN07} is bound in vitro by no-α-SNAP control (No) or wild-type (WT), low-copy (LC), or high copy (HC) Rhg1 α-SNAP, or WT α-SNAP truncated at final 10 residues (WT1-279). BSA: bovine serum albumin.

[0034] FIG. **11**A shows *N. benthamiana* leaves −6 days post agro-infiltration with 1:4 or 4:1 mixed cultures of α-$SNAP_{Rhg1}$LC and NSF_{Ch07} or NSF_{RAN07} or α-$SNAP_{Rhg1}$WT or empty vector (one or three parts *Agrobacterium* that delivers α-$SNAP_{Rhg1}$LC to one part *Agrobacterium* that delivers soybean NSF, or α-$SNAP_{Rhg1WT}$ or empty vector control) as in FIG. **4**A. FIG. **11**B shows *N. benthamiana* leaves like those shown in FIG. **4**A, but with a 9:1 or 19:1 mixed culture of α-$SNAP_{Rhg1}$LC co-expressed with NSF_{Ch07} or NSF_{RAN07} or empty vector. FIG. **11**C shows *N. benthamiana* leaves as shown in FIG. **4**A, but using α-$SNAP_{Rhg1HC}$ instead of α-$SNAP_{Rhg1}$LC in the corresponding mixture cultures of NSF_{Ch07} or NSF_{RAN07} or empty vector.

[0035] FIG. **11**D depicts *N. benthamiana* leaves −6 days post agro-infiltration with 1:9 mixed cultures of NSF_{Ch07} or NSF_{RAN07} or NSF_{Ch13} or NSF_{Nbenth} to empty vector (9 parts empty vector cultures to 1part NSF expressing *Agrobacterium* culture). FIG. **11**E shows *N. benthamiana* leaves similar to those shown in FIG. **4**A, but with a 11:1 mixed culture of α-$SNAP_{Rhg1LC}$ or α-SNAPRhg1Lc1-2soα-$SNAP_{Rhg1LC1-280}$ (lacks the final 10 C-terminal residues) co-expressed with NSF_{Ch07} or NSF_{RAN07} or empty vector.

[0036] FIG. **12**A and FIG. **12**B show an amino acid alignment with NSF *N. benthamiana* (SEQ ID NO:53) and NSF_{Ch07} (SEQ ID NO:18) of soybean reference genome Williams82. NSF N-domain residues are conserved in α-SNAP binding and are shown in boldface.

[0037] FIG. **13**A (SEQ ID NOs:54-88) and FIG. **13**B (SEQ ID NOs:89-123) show an alignment of NSF N-domain starting from position 1 and depicts general conservation of R4. The alignment was generated with Jalview and includes all reliable Angiosperm NSF sequences available from Phytozome.org.

[0038] FIG. **14** is an immunoblot showing expression results for α-$SNAP_{Rhg1}$LC in independent soybean lines transformed with genes encoding α-$SNAP_{Rhg1}$LC and either wild-type NSF_{Ch07} or NSF_{RAN07}. Only one transformed plant was obtained for the α-$SNAP_{Rhg1}$LC+wild-type NSF_{Ch07} DNA construct and that plant did not actually express α-$SNAP_{Rhg1}$LC protein.

DETAILED DESCRIPTION

[0039] All publications, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes.

[0040] Before describing the disclosed methods and compositions in detail, a number of terms will be defined. As used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0041] It is noted that terms like "preferably," "commonly," and "typically" are not utilized herein to limit the scope of the claimed invention or to imply that certain features are critical, essential, or even important to the structure or function of the claimed invention. Rather, these terms are merely intended to highlight alternative or additional features that can or cannot be utilized in a particular embodiment of this invention.

[0042] For the purposes of describing and defining this invention it is noted that the term "substantially" is utilized herein to represent the inherent degree of uncertainty that can be attributed to any quantitative comparison, value, measurement, or other representation. The term "substantially" is also utilized herein to represent the degree by which a quantitative representation can vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

[0043] In addition to the methods that are more specifically described herein and/or described by reference to literature citations, methods well known to those skilled in the art (e.g., Ausubel, F., et al. (Eds.), *Current Protocols in Molecular Biology,* 2017; Acquaah, G. (Ed.), *Principles of Plant Genetics and Breeding,* 2^{nd} Edition 2012) can be used to carry out many of the manipulations disclosed herein.

[0044] As used herein, a "plant" includes any portion of the plant, including but not limited to, a whole plant, a portion of a plant such as a part of a root, leaf, stem, seed, pod, flower, cell, tissue or plant germplasm or any progeny thereof.

[0045] As used herein, soybean refers to whole soybean plant or portions thereof including, but not limited to, soybean plant cells, soybean plant protoplasts, soybean plant tissue culture cells or calli.

[0046] As used herein, a plant cell refers to cells harvested or derived from any portion of the plant or plant tissue, germplasm, cultured cells or calli.

[0047] As used herein "substantially equivalent" in terms of amino acid modification is intended to mean an amino acid that imparts, confers, or results in the substantially same function as the substituted amino acid.

[0048] As used herein, "germplasm" refers to genetic material from an individual or group of individuals or a clone derived from a line, cultivar, variety or culture, and the cells or tissues containing said genetic material. In the plural sense, "germ plasm" refers to collections of multiple lines, cultivars, varieties or cultures.

[0049] As used herein, "native polynucleotide" or "native polypeptide" refer to an endogenous polynucleotide or polypeptide in a naturally occurring chromosomal context. In contrast, an "exogenous" or "ectopic" polynucleotide or polypeptide refers to expression of a transgenic gene, or expression controlled by a non-native chromosomal context (e.g., by introduction of non-native promoters or enhancer elements).

[0050] As used herein, "nematode" is intended to mean any roundworm or unsegmented worm belonging to the phylum Nematoda

[0051] As used herein, "enhanced resistance" is intended to mean increased resistance to nematodes compared to native plants of the same species.

[0052] As used herein, "altering the expression pattern of" a gene or polypeptide comprises increasing its expression, decreasing its expression, or altering the location of its expression. As used herein, increasing, decreasing, or altering expression of a gene or polypeptide can be at the nucleotide or polypeptide level, and can comprise alterations in native or exogenous polynucleotide or polypeptide. Altering the location of expression of a gene product or polypeptide means altering the location or relative abundance in different parts of a plant. Alternatively, in some embodiments described herein, altering the location of expression means altering the sub-cellular localization of expression in a cell.

[0053] As used herein, "modification" as it refers to an amino acid, polypeptide and/or nucleotide is intended mean for example missense mutation, nonsense mutation, insertion, deletion, duplication, frameshift mutation and repeat expansion.

[0054] The Rhg1 locus is a chromosomal region identified as a region important for resistance to SCN. When used in reference to a protein, the term Rhg1 typically is not italicized, and refers to the protein products of one or more genes that are located at the Rhg1 locus. As used herein, a locus is a chromosomal region where one or more trait determinants, genes, polymorphic nucleic acids, or markers are located. A quantitative trait locus (QTL) refers to a polymorphic genetic locus where one or more underlying genes controls a trait that is quantitatively measured and contains at least two alleles that differentially affect expression of a phenotype or genotype in at least one genetic background, with said locus accounting for part but not all the observed variation in the overall phenotypic trait that is being assessed. A genetic marker is a nucleotide sequence or amino acid sequence that can be used to identify a genetically linked locus, such as a QTL. Examples of genetic markers include, but are not limited to, single nucleotide polymorphisms (SNP), simple sequence repeats (SSR; or microsatellite), a restriction enzyme recognition site change, genomic copy number of specific genes or target sequences or other sequence-based differences between a susceptible and resistant plant.

[0055] A "linked" genetic locus describes a situation in which a genetic marker and a trait are closely linked chromosomally such that the genetic marker and the trait do not independently segregate and recombination between the genetic marker and the trait does not occur during meiosis with a readily detectable frequency. The genetic marker and the trait can segregate independently, but generally do not. For example, a genetic marker for a trait can only segregate independently from the trait 5% of the time; suitably only 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25%, or less of the time. Genetic markers with closer linkage to the trait-producing locus will serve as better markers because they segregate independently from the trait less often because the genetic marker is more closely linked to the trait. Genetic markers that directly detect polymorphic nucleotide sites that cause variation in the trait of interest are particularly useful for their accuracy in marker-assisted plant breeding. Thus, the methods of screening provided herein can be used in traditional breeding, recombinant biology or transgenic breeding programs or any hybrid thereof to select or screen for resistant varieties.

[0056] A linked locus can also describe two loci that do not reside close to each other on a chromosome, and therefore are not physically linked, but exhibit lack of independent segregation (i.e. they co-segregate). In the formal genetic sense, such a pair of co-segregating loci exhibit genetic linkage. As used herein, the terms "linked locus" and "co-segregating locus" are used interchangeably, and thus refer to physical linkage (on the same chromosome) or genetic linkage (either on the same chromosome or co-segregating on different chromosomes). A gene or locus is "associated" with another gene or locus when they are linked or co-segregate with one another. For example, a gene, allele, or locus is "associated" with Rhg1 if it co-segregates or is physically linked to the Rhg1 locus.

[0057] As used herein, Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 refer to the soybean genomic nomenclature describing those genes, the proteins or polypeptides they encode, and include any polynucleotide or polypeptide variants, naturally occurring or otherwise, and any homologues or conserved portions in other plant species. In some embodiments, Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 refer to the genes or polypeptides, and any polynucleotide or polypeptide variants, naturally occurring or otherwise, in plants of the genus Glycine, and encompass any homologues or conserved portions in other plant species. The 13-character gene names are from the Wm82.a1 genome assembly and Glyma 1.0 gene models (Schmutz et al., 2010) and the more recent 15-character gene names are from the U.S. Department of Energy Joint Genome Institute Wm82.a2 soybean genome assembly and Glyma 2.0 gene model naming revision.

[0058] The present disclosure provides methods and compositions for increasing resistance of a plant or plant cells to cyst nematodes. In some embodiments, the disclosure provides methods and compositions for generating transgenic plant materials, including transgenic cells and plants. In additional embodiments, the disclosure provides compositions comprising nucleotide constructs useful for generating transgenic cells and plants resistant to nematodes. In still further embodiments, the disclosure provides nucleotide constructs encoding Rhg1 resistance-type polypeptides, or homologs or variants thereof. In certain embodiments, Rhg1 resistance-type α-SNAPs are provided. In further embodiments, the disclosure provides Rhg1 resistance-type α-SNAPs encoded by SEQ ID NO: 5 or SEQ ID NO: 6, or homologs or variants thereof.

[0059] In some embodiments, the disclosure provides alleles associated with the Rhg1 locus due to lack of independent segregation from the locus. In certain embodiments, the disclosure provides alleles that co-segregate with Rhg1 genes despite residing on a different chromosome (i.e., despite lack of physical linkage on the same chromosome). In one aspect, alleles associated with the Rhg1 locus comprise genes that improve the growth, reproduction and/or SCN resistance of plant cells, plants, or germplasm, that carry Rhg1 SCN resistance-conferring alleles. In certain embodiments, the disclosure provides alleles of an NSF gene, wherein the alleles of an NSF gene are associated with Rhg1. In some embodiments, the disclosure provides alleles of an NSF gene, wherein the alleles of an NSF gene are associated with improved growth, or completion of the life cycle, of plants that carry SCN resistance-conferring alleles of the Rhg1 locus. In particular embodiments, the NSF gene of the disclosure is Glyma.07G195900, or variants thereof. In an exemplary embodiment, the disclosure provides alleles of NSF associated with Rhg1 encoded by SEQ ID NO: 8, a protein corresponding to SEQ ID NO: 17, or homologs or variants thereof. In other exemplary embodiments, the disclosure provides alleles of NSF encoded by SEQ ID NO: 9, a protein corresponding to SEQ ID NO: 18, or homologs or variants thereof.

[0060] Also provided are Rhg1 genes that contribute to SCN resistance (SEQ ID NOS: 1-7) and the proteins they encode (SEQ ID NOs 10-16) located within a tandem repeat present in the genomes of soybeans exhibiting resistance to cyst nematodes, including, but not limited to, P188788, Peking, Hartwig, Fayette, and Forrest. Embodiments of the Rhg1 genes that contribute to SCN resistance of the present disclosure are as described in U.S. patent application Ser. No. 13/843,447, and also as described in Cook, D. E., et al. 2012, Science 338:1206-1209, and the associated Supporting Online Material, which are incorporated herein by reference in their entirety.

[0061] In certain embodiments, the Rhg1 genes that contribute to SCN are located on a tandemly repeated segment of chromosome 18 in resistant soybeans, and silencing of one or more of three genes in the segment leads to increased susceptibility to SCN in an otherwise resistant variety. In certain embodiments, the tandemly repeated segment comprises four genes, along with part of a fifth gene, and other DNA sequences in a chromosome segment that in some described soybean accessions (Cook et al., 2012, Science 338, 1206-1209) is approximately 31 kb in length. The tandemly repeated Rhg1 chromosome segment is found in at least two copies in the SCN-resistant varieties that have been characterized to have SCN resistance due in part to the Rhg1 locus. Various resistant varieties carry three, seven or ten copies, or other numbers of copies. In the published examples the higher copy number versions of Rhg1 express higher levels of transcripts for the three genes. Higher copy number versions of Rhg1 also confer more resistance to SCN on their own (exhibit less reliance on the simultaneous presence of desirable alleles of other SCN resistance QTL such as Rhg4 in order to effectively confer resistance to HG Type 0 SCN populations), relative to Rhg1 haplotypes with lower Rhg1 repeat copy numbers.

[0062] In certain aspects, the disclosure provides transgenic plants or transgenic plant cells with increased resistance to cyst nematodes, particularly SCN, carrying one or a plurality of transgenes encoding a non-native or exogenous Rhg1 derived, or Rhg1 associated, polynucleotide encoding one or more of the polynucleotides of SEQ ID NOs:1-9 or the polypeptides of SEQ ID NOs:10-18. Non-transgenic plants carrying these polypeptides, or bred or otherwise engineered to express increased levels of these polypeptides or the polynucleotides encoding these polypeptides, are also provided.

[0063] In some aspects, the disclosure provides methods and compositions for increasing resistance of a plant or plant cell to cyst nematodes, including but not limited to SCN, by increasing expression of, or altering an expression pattern of, or increasing copy number of one or more Rhg1 genes corresponding to the *Glycine max* genes designated Glyma.18G022700 (SEQ ID NO:3), Glyma.18G022500 (SEQ ID NO: 2), variants of Glyma.18G022500 (SEQ ID NO:5 or SEQ ID NO:6), and/or Glyma.18G022400 (SEQ ID NO: 1), polypeptides or functional fragments or variants thereof in cells of the plant are also provided. In another aspect, the disclosure provides methods and compositions for producing a plant or plant cell with increased resistance to cyst nematodes, including but not limited to SCN, by increasing expression of, or altering an expression pattern of, or increasing copy number of one or more Rhg1 associated genes corresponding to Glyma.07G195900 (SEQ ID NO: 8 or SEQ ID NO: 9). In embodiments, the methods and compositions of the disclosure further comprise increasing the expression of, or altering the expression pattern of, or increasing the copy number of, a polynucleotide encoding an NSF allele or a polypeptide product of said allele, in combination with one or more of the Rhg1, or Rhg1 associated, genes above. The polynucleotides of the disclosure can be 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequences provided.

[0064] In another aspect, the disclosure provides methods and compositions for increasing plant growth, seed production, or completion of the life cycle of plants in which resistance to SCN has been manipulated by increasing expression of, or altering an expression pattern of, or increasing copy number of Rhg1 genes. In certain embodiments, methods for increasing plant growth, seed production or completion of the life cycle of plants in which resistance to SCN has been manipulated comprise increasing expression of, altering expression pattern of, or increasing copy number of one or more polynucleotides encoding an NSF protein. In some embodiments, methods for increasing plant growth, seed production or completion of the life cycle of plants in which resistance to SCN has been manipulated comprise increasing expression of, altering an expression pattern of, or increasing copy number of a polynucleotide corresponding to Glyma.07G195900. In particular embodiments of the disclosure, a polynucleotide corresponding to Glyma.07G195900 comprises a polynucleotide identified in SEQ ID NO: 8 or SEQ ID NO: 9, polypeptides or functional fragments or variants thereof. The polynucleotide can be 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequences provided. In embodiments, the methods and compositions of the disclosure further comprise increasing the expression of, or altering the expression pattern of, or increasing the copy number of, a polynucleotide encoding an NSF allele or a polypeptide product of said allele, in combination with one or more of the Rhg1, or Rhg1 associated, genes above.

[0065] In still another aspect, the disclosure provides methods and compositions for increasing plant growth, seed production or completion of the life cycle of plants that contain Rhg1 alleles that contribute to SCN resistance by increasing expression of, or altering an expression pattern of, or increasing copy number of genes associated with, or linked with, Rhg1 genes that contribute to SCN resistance. In certain embodiments, the disclosure provides methods of increasing expression of, or altering an expression pattern of, or increasing copy number of a gene or protein corresponding to the *Glycine max* gene designated Glyma. 07G195900. In still further embodiments, the disclosure provides methods and compositions for increasing plant growth, seed production, or completion of the life cycle of plants that contain Rhg1 alleles that contribute to SCN resistance, by increasing expression of, or altering an expression pattern of, or increasing copy number of one or more polynucleotides identified by SEQ ID NO: 8 or SEQ ID NO:9, a polypeptide sequence identified by SEQ ID NO: 17 or SEQ ID NO:18, or homologues, or variants thereof.

[0066] In certain embodiments, the disclosure provides transgenic plants or transgenic plant cells comprising one or more polynucleotides encoding an α-SNAP protein variant. In particular embodiments, the α-SNAP protein variant or variants confer reduced or substantially disrupted cellular vesicular trafficking in cells. In some embodiments, the α-SNAP protein variant or variants exhibit disrupted disassembly and reuse of the four-protein bundles of SNARE proteins that form when t-SNARE and v-SNARE proteins anneal during vesicle docking to target membranes.

[0067] Certain embodiments of the disclosure provide an α-SNAP protein variant corresponding to the gene designated Glyma.18G022500. In some embodiments, an α-SNAP protein variant of the disclosure corresponds to the Glyma.18G022500 from Fayette or Peking soybean lines. In particular embodiments, the α-SNAP protein variant (or variants) of the disclosure are encoded by polynucleotides identified by SEQ ID NO:5 or SEQ ID NO:6, polypeptides identified by SEQ ID NO: 14 or SEQ ID NO: 15, or functional fragments or variants thereof.

[0068] In some embodiments, the α-SNAPs of the disclosure exhibit reduced or substantially disrupted binding to wild-type NSF and to SNARE/NSF complexes. For example, in certain embodiments, the α-SNAPs of the present disclosure harbor point mutations, substitutions, deletions, or other mutagenic sequence variants. In particular embodiments, the point mutations, substitutions, deletions, or other mutagenic sequence variants of the α-SNAPs disclosed herein are localized to the C-terminus of the protein. In specific particular embodiments, the α-SNAPs of the present disclosure comprise a soybean α-SNAP sequence with one or more variant C-terminal residues in the polypeptide sequence at conserved residues Q_{203}, D_{208}, $DEED_{243-246}$ (SEQ ID NO:124), or $EEDD_{284-287}$ (SEQ ID NO:125). In other embodiments, the α-SNAPs of the present disclosure comprises one or more variant c-terminal residues in the polypeptide sequence at conserved residues in rat α-SNAP at D_{217}, E_{249}, EE_{252}-253, or $DEED_{290-293}$ (SEQ ID NO:126).

[0069] In some embodiments, the α-SNAP proteins are modified by amino acids modification at positions corresponding to positions 203, 208, 284, 285, 286, and 287 by α-SNAP numbering as set forth in SEQ ID NOS: 11, 14, or 15. Positions 203 208, 284, 285, 286, and 287 correspond to the C-terminal of the Rhg1 haplotype. In one aspect modifications present in the low copy (LC) of Glyma.18G022500 is critical to nematode resistance. The modifications D208E and expression of $EEDD_{284-287}$ (SEQ ID NO:125), confer enhanced resistance of the soybean against the nematode.

[0070] In another embodiment, the modified polynucleotides encode a modified α-SNAP polypeptide, wherein the modified α-SNAP polypeptide comprises: a replacement at position D286 that is D286F, or D286W, or D286Y; and a replacement at position D287 that is D287E or remains D287; and an insertion after position 287 that is (ins)288A, (ins)288G, (ins)2881, (ins)288L, (ins)288M, or (ins)288V; and a replacement at position L288 that is L288A, L288G, L2881, L2881, L288M, or L288V, or a functional equivalent amino acid to the WT amino acid expressed at position 285, 286, 287, or 288, each by α-SNAP numbering relative to the positions set for in SEQ ID NO: 11.

[0071] In yet other embodiments the encoded modified α-SNAP has one or more polynucleotides that encode a modified an α-SNAP polypeptide wherein the modified polypeptide comprises other amino acids in the same family. In one aspect D208E can be modified to any functional equivalent amino acid. In another aspect, any or both E284 and E285 can also be modified to E284D or E285D or any functionally equivalent amino acid. In yet another aspect, any or both of D286 and D287 can be also be modified to D286E or D287E or any functional equivalent amino acid. The numbering presented herein is relative to the positions in SEQ ID NO: 11. In some embodiments the encoded modified α-SNAP polypeptides comprises amino acid modifications selected from a combination of wild type amino acids or functional equivalent amino acid substitutions at positions 208, 284, 285, 286, and 287 or adjacent residues. The number presented herein is relative to the positions in SEQ ID. NO: 11.

[0072] In some embodiments, the NSF variants of the disclosure exhibit reduced or substantially disrupted binding to α-SNAP proteins. In certain embodiments, the NSF variants of the disclosure exhibit reduced or substantially disrupted binding to "wild-type" α-SNAP proteins, such as an α-SNAP protein encoded by Glyma.18G022500 haplotype of soybean accession Williams 82 (SEQ ID NO: 2), homologues, or functionally conserved variants thereof. For example, in certain embodiments, the NSF variants of the present disclosure harbor point mutations, substitutions, deletions, or other mutagenic sequence variants. In embodiments, the point mutations, substitutions, deletions, or other mutagenic sequence variants of NSF are localized to regions near the N-terminus of the protein. In particular embodiments, the NSF variants of the present disclosure comprise an NSF protein with one or more variant N-terminal residues at conserved residues corresponding to R_{10} or $RK_{114-115}$ in the Chinese hamster NSF protein sequence. In some embodiments, the NSF of the present disclosure comprises a soybean NSF protein with one or both of an $N_{21}Y$ mutation or a A_{116F} mutation in the soybean NSF protein sequence. The A_{116F} notation refers to an insertion of an additional amino acid, in this case "F" or phenylalanine, as the one hundred sixteenth amino acid of the protein.

[0073] In some embodiments, the NSF variants of the disclosure exhibit enhanced or substantially improved binding to α-SNAP proteins associated with improved plant resistance to cyst nematodes. For example, in certain embodiments, the NSF variants of the present disclosure harbor point mutations, substitutions, deletions, or other mutagenic sequence variants that facilitate binding to, or functionally interacting with, a variant α-SNAP protein that is less capable of binding to a "wild-type" NSF protein. In embodiments, the point mutations, substitutions, deletions, or other mutagenic sequence variants of NSF that facilitate binding to, or functionally interacting with, a variant α-SNAP protein that is less capable of binding to a "wild-type" NSF protein, are localized to the regions near the N-terminus of the protein. In particular embodiments, the NSF variants of the present disclosure that facilitate binding to, or functionally interacting with, a variant α-SNAP protein that is less capable of binding to a "wild-type" NSF protein comprise an NSF protein with one or more variant N-terminal residues at conserved residues corresponding to R_{10} or $RK_{114-115}$ in the Chinese hamster NSF protein sequence. In some embodiments, the NSF variants of the disclosure that facilitate binding to, or functionally interacting with, a variant α-SNAP protein that is less capable of binding to a "wild-type" NSF protein comprises a soybean NSF protein with one or both of an $N_{21}Y$ mutation or a [<]BEGINITALm$_{116}$F mutation in the soybean NSF protein sequence.

[0074] In some embodiments, the NSF proteins are modified by amino acid mutations at positions 4, 21, 25, 116, and 181 by NSF numbering as set for in SEQ ID NOS:17 or 18. The mutations enhance growth and viability of the plant versus plants that express the wild type NSF sequence as provided in SEQ ID NO: 17. The amino acid mutations at positions 4 and 21 enhance growth and viability of the plant. In some embodiments the encoded modified polypeptides comprises amino acid modifications selected from the modifications: R4N/N21F; R4N/N21W; R4N/N21Y; R4C/N21F; R4C/N21W; R4C/N21Y; R4Q/N21F; R4Q/N21W; R4Q/N21Y; R4S/N21F; R4S/N21W; R4S/N21Y; R4T/N21F; R4T/N21W; and R4T/N21Y, each with number relative to positions set forth in SEQ ID NOS: 17 or 18.

[0075] In yet another embodiment the encoded modified NSF has one or more polynucleotides alterations that encode a modified NSF protein wherein the modified polypeptide comprises other amino acids in the same family. In one aspect, R4 can be modified to amino acids N, C, Q, S or T or any functionally equivalent amino acid. In yet another aspect the amino acid at position 21 can be modified to F, W, or any functionally equivalent amino acid. In another, aspect S25 can be optionally modified to N or a functionally equivalent amino acid. In still another embodiment the optional gap at position 116 can be optionally modified to an F or functionally equivalent amino acid. In still another aspect, the M at 181 can be optional modified to an I or functionally equivalent amino acid. The numbering herein is relative to the positions in SEQ ID NO: 17.

[0076] In certain embodiments, expression of α-SNAP variants disclosed herein is substantially toxic, or lethal, or otherwise intolerable, to a plant or transgenic plant, or plant cell in which it is expressed, unless a complementary NSF protein is co-expressed. In certain embodiments, an α-SNAP protein with point mutations, substitutions, deletions, or other mutagenic sequence variants that are toxic to a transgenic plant or plant cell, is co-expressed with one or more NSF variants with point mutations, substitutions, deletions, or other mutagenic sequence variants. In particular embodiments, one or more α-SNAP proteins with C-terminal point mutations, substitutions, deletions, or other mutagenic sequence is co-expressed with one or more NSF proteins with point mutations, substitutions, deletions, or other mutagenic sequence. In embodiments, α-SNAP proteins with C-terminal point mutations, substitutions, deletions, or other mutagenic sequence is co-expressed with one or more NSF proteins with mutations localized to the regions near the N-terminus of the protein. In particular embodiments, the NSF variants of the present disclosure comprise an NSF protein with one or more variant N-terminal residues at conserved residues corresponding to R_{10} or $RK_{114-115}$ in the Chinese hamster NSF protein sequence. In some embodiments, the NSF of the present disclosure comprises a soybean NSF protein with one or both of an $N_{21}Y$ mutation or a [<]BEGINITALm$_{116}$F mutation in the soybean NSF protein sequence. In other particular embodiments, the NSF of the present disclosure comprises a soybean NSF protein as identified in SEQ ID NO: 18 or encoded by a polynucleotide as identified in SEQ ID NO: 9, or homologues or functionally conserved variants thereof.

[0077] In certain embodiments, an NSF protein is expressed in a plant or plant cell containing the Rhg1 tandem repeat segment. In exemplary embodiments, NSF protein variants are expressed in a plant or plant cell containing the Rhg1 tandem repeat segment. In certain embodiments, the NSF variants expressed in a plant or plant cell containing the Rhg1 tandem repeat segment comprise an NSF protein with one or more variant N-terminal residues at conserved residues corresponding to R_{10} or $RK_{114-115}$ in the Chinese hamster NSF protein sequence. In some embodiments, the NSF variant expressed in a plant or plant cell containing the

Rhg1 tandem repeat segment comprises a soybean NSF protein with one or both of an R_4Q mutation, an $N_{21}Y$ mutation, or a [<]BEGINITALm$_{116}$F mutation in the soybean NSF protein sequence.

[0078] In various embodiments disclosed herein, an NSF protein is expressed in plants or plant cells that also carry Rhg1He (high copy) loci carrying four or more, and frequently nine or ten, Rhg1 repeats. In other embodiments, an NSF protein is expressed in plants or plant cells that also carry Rhg1$_{LC}$ (low-copy) loci carrying three or fewer Rhg1 repeats. (Rhg1Lc is also known as rhg1-a and Rhg1He is also known as rhg1-b.) Rhg1$_{HC}$ and Rhg1$_{Lc}$ encode similar yet distinct α-SNAP variants that are impaired in normal α-SNAP-NSF interactions (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382).

[0079] In further embodiments, the disclosure provides methods and compositions for producing plant cells with increased resistance to nematodes comprising reducing a level of a "wild-type" α-SNAP allele relative to a variant α-SNAP allele. In some embodiments, the level of an α-SNAP encoded by the sequence identified in SEQ ID NO: 2 is reduced relative to a variant α-SNAP encoded by either of the sequences identified in SEQ ID NO: 5 and SEQ ID NO: 6.

[0080] In alternative embodiments, a variant NSF protein capable of functionally complementing one or more variant α-SNAP genes is expressed in a plant cell that contains the one or more variant α-SNAP genes. In embodiments, the variant NSF protein capable of functionally complementing one or more variant α-SNAP genes improves the growth of a cell expressing the variant α-SNAP genes. In further embodiments, a variant NSF protein capable of functionally complementing one or more variant α-SNAP genes confers cyst nematode resistance on a cell expressing the variant α-SNAP genes. In certain embodiments, the one or more variant α-SNAP genes disclosed herein function analogously to α-SNAP alleles encoded by Rhg1$_{HC}$ or Rhg1$_{LC}$, and/or α-SNAP alleles similar to Rhg1$_{HC}$ or Rhg1$_{LC}$ that have been generated or introduced at other loci in the soybean genome. In still further embodiments, the one or more variant α-SNAP genes disclosed herein impact α-SNAP function in a manner similar to the αSNAPs encoded by Rhg1$_{HC}$ or Rhg1$_{LC}$ α-SNAP alleles. In yet further embodiments, the variant α-SNAP genes disclosed herein alter expression patterns relative to the wild-type α-SNAP protein encoded at the single-copy Rhg1 locus of soybean accession Williams 82.

[0081] In a certain aspect, the methods of the disclosure provide a breeding stock of a Rhg1 plant expressing an NSF variant. Also provided are methods of breeding a Rhg1 plant expressing one or more NSF variants. In addition, methods of growing or improving the lifecycle of a Rhg1 plant expressing one or more NSF variants are provided.

[0082] In other embodiments, the amino acids at the NSF and α-SNAP binding interface can be manipulated to enhance nematode resistance of plant species. In one aspect NSF amino acid residues 4, 21, 25, 116, 181 or adjacent residues with numbering relative to the NSF polypeptide set forth in SEQ ID NOS: 17 or 18 are mutated.

[0083] In another aspect residues 208, 284, 285, 286, 287, or adjacent residues of α-SNAP are mutated to impact the NSF/α-SNAP interface. The amino acid mutations at the binding interface of NSF/α-SNAP can enhance nematode resistance versus the wild type plant.

[0084] In another aspect, amino acids residing at the NSF/α-SNAP protein interaction interface can be mutated to achieve enhanced nematode resistance and plant viability and growth. For instance, NSF amino acid residues 4, 21, 25, 116, 181 or adjacent residues with numbering relative to the NSF polypeptide set forth in SEQ ID NOS: 17 or 18 interact with α-SNAP as designated in the NSF/α-SNAP/SNARE protein structure PDB ID code 3j97. Residues 208, 284, 285, 286, and 287 of α-SNAP or other α-SNAP residues that are at, or adjacent to residue at the NSF/α-SNAP 1 protein interaction interface with numbering relative to the NSF polypeptide set forth in SEQ ID NO: 11 can also be mutated to confer nematode resistance and plant cell growth viability.

[0085] In certain embodiments, the methods of the disclosure confer resistance to cyst nematode. Resistance (or susceptibility) to cyst nematode, including but not limited to SCN, can be measured in a variety of ways, several of which are known to those of skill in the art. In some embodiments of the disclosure, soybean roots are experimentally inoculated with SCN and the ability of the nematodes to mature (molt and proceed to developmental stages beyond the J2) on the roots is evaluated as compared to a susceptible and/or resistant control plant. A SCN greenhouse test is also described in U.S. Patent Application Publ. No. 2013-0305410 A1, which is incorporated herein in its entirety, and provides an indication of the number of cysts on a plant and is reported as the female index. Increased resistance to nematodes can also be manifested as a shift in the efficacy of resistance with respect to particular nematode populations or genotypes. Additionally, but not exclusively, SCN-susceptible soybeans grown on SCN-infested fields will have significantly decreased crop yield as compared to a comparable SCN-resistant soybean. Improvement of any of these metrics has utility even if all of the above metrics are not altered.

[0086] In certain embodiments, expression of one or more of the polynucleotides and polypeptides described in SEQ ID NOS: 1-18 is increased in a root of the plant. Suitably, expression of these polynucleotides and polypeptides is increased in root cells of the plant. The plant is suitably a soybean plant or portions thereof. In particular embodiments, these polynucleotides can also be transferred into other non-soybean plants, or homologs of these polypeptides or polynucleotides encoding these polypeptides from other plants, or synthetic genes encoding products similar to the polypeptides encoded or identified by SEQ ID NOS: 1-18 can be overexpressed in those plants. Example of such other plants include but are not limited to sugar beets, potatoes, corn, wheat, peas, and beans. Overexpression of these genes can increase resistance of plants from these other species to nematodes and in particular cyst nematodes, such as the soybean cyst nematode *Heterodera glycines*, the sugar beet cyst nematode *Heterodera schacthii*, the potato cyst nematodes *Globodera paflida* and related nematodes that cause similar disease on potato such as *Globodera rostochiensis*, the cereal cyst nematode *Heterodera avenae*, the corn cyst nematode *Heterodera zeae*, and the pea cyst nematode *Heterodera goettingiana*.

[0087] Expression of these polynucleotides in the various embodiments disclosed herein can be increased by increasing the copy number of these polynucleotide in the plant, in cells of the plant, suitably root cells, or by identifying plants in which this has already occurred. In some embodiments, the expression of these polynucleotides in the various embodiments can be increased using recombinant DNA technology, e.g., by using strong promoters to drive increased expression of one or more polynucleotides.

[0088] In some embodiments, expression of polynucleotides or polypeptides of the disclosure is reduced relative to the native amount. Reduction of a polynucleotide amount can be accomplished according to methods known in the art, such as reducing the mRNA level of a polynucleotide by interfering with promoter or enhancer function or modifying a promotor or enhancer. Alternatively, a polynucleotide amount can be reduced post-transcriptionally, such as by using antisense, morpholino, or small-interfering RNA, or by modifying the gene encoding the polynucleotide to reduce the stability of the mRNA or reduce or eliminate its translation. In embodiments, the amount of a protein is reduced, such as by peptide directed protein knockdown (e.g., as described in US Patent App. Publ. No. US 2015-0266935 A1), or other protein knock-down techniques known to the art (see, e.g., Bonger, K. M., et al. (2001) Nature Chemical Biology 7, 531-537; Banaszynski, L. A., et. al. (2006), Cell 126, 995-1004; Neklesa, T. K. et al. (2011) Nature Chemical Biology 7, 538-543.)

[0089] Expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can be increased in a variety of ways including several apparent to those of skill in the art and can include transgenic, non-transgenic and traditional breeding methodologies. For example, expression of the polypeptide encoded by Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 cancan be increased by introducing a construct including a promoter operational in the plant operably linked to a polynucleotide encoding the polypeptide into cells of the plant. Suitably, the cells are root cells. Alternatively, the expression of the polypeptide encoded by Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 cancan be increased by introducing a transgene including a promoter operational in the plant operably linked to a polynucleotide encoding the polypeptide into cells of the plant. The promoter can be a constitutive or inducible promoter capable of inducing expression of a polynucleotide in all or part of the plant, plant roots or plant root cells. In another embodiment, expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can be increased by increasing expression of the native polypeptide in a plant or in cells of the plant, such as the plant root cells. In another embodiment, expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can be increased by increasing expression of the native polypeptide in a plant or in cells of the plant such as the nematode feeding site, the syncytium, or cells adjacent to the syncytium. In another embodiment, expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can be increased by increasing expression of the native polypeptide in a plant or in cells of the plant such as sites of nematode contact with plant cells. In another embodiment, expression can be increased by increasing the copy number of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900. Other mechanisms for increasing expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can include, but are not limited to, increasing expression of a transcriptional activator, reducing expression of a transcriptional repressor, addition of an enhancer region capable of increasing expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900, increasing mRNA stability, altering DNA methylation, histone acetylation or other epigenetic or chromatin modifications in the vicinity of the relevant genes, altering protein or polypeptide subcellular localization, or increasing protein or polypeptide stability.

[0090] In addition, methods of increasing resistance of a plant to cyst nematodes can be achieved by cloning sequences upstream from Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 from resistant lines into susceptible lines. For these methods, nucleotide sequences having at least 60%, 70% or 80% identity to nucleotide sequences that flank the protein-coding regions of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 (or sequences having at least 75%, 80%, 85%, or 90% identity to those protein-coding regions), said flanking regions including 5' and 3' untranslated regions of the mRNA for these genes, and also including any other genomic DNA sequences that extend from the protein coding region of these genes to the protein coding regions of immediately adjacent genes can be used.

[0091] In addition to the traditional use of transgenic technology to introduce additional copies or increase expression of the genes and mediate the increased expression of the polypeptides of the disclosure in plants, transgenic or non-transgenic technology can be used in other ways to increase expression of the polypeptides. For example, plant tissue culture and regeneration, mutations or altered expression of plant genes other than those expressly recited herein, or transgenic technologies, can be used to create instability in the Rhg1 locus or the plant genome more generally that create changes in Rhg1 locus, or Rgh1 associated gene, copy number or gene expression behavior. The new copy number or gene expression behavior can then be stabilized by removal of the variation-inducing mutations or treatments, for example by further plant propagation or a conventional cross. Examples of transgenic technologies that might be used in this way include targeted zinc fingers, ribozymes or other sequence-targeted enzymes that create double stranded DNA breaks at or close to the Rhg1 locus or Rgh1 associated gene, the cre/loxP system from bacteriophage lambda, Transcription Activator-Like Effector Nucleases (TALENs), Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) systems using CRISPR-associated proteins such as Cas9 or other nucleases, artificial DNA or RNA sequences designed to recombine with Rhg1 that can be introduced transiently, or enzymes that "shuffle" DNA such as the mammalian Rag1 enzyme or DNA transposases. Mutations or altered expression of endogenous plant genes involved in DNA recombination, DNA rearrangement and/or DNA repair pathways are additional examples.

[0092] Non-transgenic means of generating soybean varieties carrying traits of interest such as increased resistance to SCN are available to those of skill in the art and include traditional breeding, chemical or other means of generating chromosome abnormalities, such as chemically induced chromosome doubling and artificial rescue of polyploids followed by chromosome loss, knocking-out DNA repair mechanisms or increasing the likelihood of recombination or gene duplication by generation of chromosomal breaks. Other means of non-transgenically increasing the expression or copy number include the following: screening for mutations in plant DNA encoding miRNAs or other small RNAs, plant transcription factors, or other genetic elements that impact Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 expression; screening large field or breeding populations for spontaneous variation in copy number or sequence at Rhg1 or Glyma.07G195900 by screening of plants for nematode resistance, Rhg1 copy number or other Rhg1 or Glyma.07G195900 gene or protein expression traits as described in preceding paragraphs; crossing of lines that contain different or the same copy number at Rhg1 or Glyma.07G195900 but have distinct polymorphisms on either side, followed by selection of recombinants at Rhg1 or Glyma.07G195900 using molecular markers from two distinct genotypes flanking the Rhg1 or Glyma.07G195900 locus; chemical or radiation mutagenesis or plant tissue culture/regeneration that creates chromosome instability or gene expression changes, followed by screening of plants for nematode resistance, Rhg1 or Glyma.07G195900 copy number or other Rhg1 or Glyma.07G195900 gene or protein expression traits as described in preceding paragraphs; or introduction by conventional genetic crossing of non-transgenic loci that create or increase genome instability into Rhg1- or Glyma.07G195900-containing lines, followed by screening of plants for either nematode resistance or Rhg1 copy number. Examples of loci that could be used to create genomic instability include active transposons (natural or artificially introduced from other species), loci that activate endogenous transposons (for example mutations affecting DNA methylation or small RNA processing such as equivalent mutations to met1 in *Arabidopsis* or mop1 in maize), mutation of plant genes that impact DNA repair or suppress illegitimate recombination such as those orthologous or similar in function to the Sgs1 helicase of yeast or RecQ of *E. coli*, or overexpression of genes such as RAD50 or RAD52 of yeast that mediate illegitimate recombination. Those of skill in the art can find other transgenic and non-transgenic methods of increasing expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900.

[0093] Polynucleotides and/or polypeptides described and used herein can encode the full-length or a functional fragment of Glyma.18G022700, Glyma.18G022500, and/or Glyma.18G022400, from the Rhg1 locus, or Glyma.07G195900, or a naturally occurring or engineered variant of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900, or a derived polynucleotide or polypeptide all or part of which is based upon nucleotide or amino acid combinations similar to all or portions of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 or their encoded products. Additional polynucleotides encoding polypeptides can also be included in the construct such as Glyma18g02600 (which encodes the polypeptide of SEQ ID NO:4). The polypeptide can be at least 75% 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequences provided herein. The polynucleotides encoding the polypeptides can be at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% identical to the sequences available in the public soybean genetic sequence database.

[0094] Expression of the polypeptide encoded by Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can be increased, suitably the level of polypeptide is increased at least 1.2, 1.5, 1.7, 2, 3, 4, 5, 7, 10, 15, 20 or 25-fold in comparison to the untreated, susceptible or other control plants or plant cells. Control cells or control plants are comparable plants or cells in which Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 expression has not been increased, such as a plant of the same genotype transfected with empty vector or transgenic for a distinct polynucleotide.

[0095] The increase in expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 in the plant can be measured at the level of expression of the mRNA or at the level of expression of the polypeptide encoded by Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900. The level of expression can be increased relative to the level of expression in a control plant as shown in the Examples. The control plant can be an SCN-susceptible plant or an SCN-resistant plant. For example, a susceptible plant such as 'Williams 82' can be transformed with an expression vector such that the roots of the transformed plants express increased levels of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 as compared to an untransformed plant or a plant transformed with a construct that does not change expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900, resulting in increased resistance to nematodes. Alternatively, the control can be a plant partially resistant to nematodes and increased expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can result in increased resistance to nematodes. Alternatively, the plant can be resistant to nematodes and increasing expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can result in further increased resistance to nematodes. Alternatively, the plant can be more resistant to certain nematode populations, races, Hg types or strains and less resistant to other nematode populations, races, Hg types or strains, and increasing expression of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 can result in increased resistance to certain of these nematode populations, races, Hg types or strains.

[0096] Increased resistance to nematodes can be measured as described above. Increased resistance in a transgenic cell of the disclosure can be measured relative to a "native" cell not having any introduced polynucleotide sequences, or exogenous polynucleotide or polypeptide control elements. Increased resistance can be measured by the plant having a lower percentage of invading nematodes that develop past the J2 stage, a lower rate of cyst formation on the roots, reduced SCN egg production within cysts, reduced overall SCN egg production per plant, and/or greater grain yield of SCN-infested soybeans on a per-plant basis or a per-growing-area basis as compared to a control plant grown in a similar growth environment. Other methods of measuring SCN resistance also will be known to those with skill in the art. In methods of increasing resistance to nematodes described herein, the resulting plant can have at least 10% increased resistance as compared to the untreated or control plant or plant cells. Suitably the increase in resistance is at least 15%, 20%, 30%, 50%, 100%, 200%, 500% as compared to a control. Suitably, the female index of the plant with increased resistance to nematodes is about 80% or less of the female index of an untreated or control plant derived from the same or a similar plant genotype, infested with a similar nematode population within the same experiment. More suitably, the female index after experimental infection is no more than 60%, 40%, or 20% of that of the control plant derived from the same or a similar plant genotype, infested with a similar nematode population within the same experiment. Suitably, when grown in fields heavily infested with SCN (for example, more than 2500 SCN eggs per 100 cubic centimeters of soil), soybean grain yields of field-grown plants are 2% greater than isogenic control plants. More suitably, the grain yield increase is at least 3%, 4%, or 5% over that of isogenic control plants grown in similar environments.

[0097] Also provided herein are constructs including a promoter operably linked to one or more of a Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 polynucleotide encoding a polypeptide comprising SEQ ID NO: 12, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 10, SEQ ID NO: 18, or a fragment or variant thereof. Also included are homologs or variants of these sequences from other soybean varieties. The constructs can further include other genes. The constructs can be introduced into plants to make transgenic plants or can be introduced into plants, or portions of plants, such as plant tissue, plant calli, plant roots or plant cells. Suitably the promoter is a plant promoter, suitably the promoter is operational in root cells of the plant. The promoter can be tissue specific, inducible, constitutive, or developmentally regulated. The constructs can be an expression vector. Constructs can be used to generate transgenic plants or transgenic cells. The polypeptide can be at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequences of SEQ ID NO: 12, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 10, or SEQ ID NO: 18. The constructs can comprise all three polynucleotides and can mediate expression of all three polypeptides.

[0098] Transgenic plants including a non-native or exogenous polynucleotide encoding the rhg1-b polypeptides identified and described herein are also provided. Suitably these transgenic plants are soybeans. The transgenic plants express increased levels of Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900 polypeptide as compared to a control non-transgenic plant from the same line, variety or cultivar or a transgenic control expressing a polypeptide other than Glyma.18G022700, Glyma.18G022500, Glyma.18G022400, and/or Glyma.07G195900. These transgenic plants also have increased resistance to nematodes, in particular SCN, as compared to a control plant. Portions or parts of these transgenic plants are also provided. Portions and parts of plants includes, but is not limited to, plant cells, plant tissue, plant progeny, plant asexual propagates, plant seeds.

[0099] Transgenic plant cells comprising a polynucleotide encoding a polypeptide capable of increasing resistance to nematodes such as SCN are also provided. Suitably the plant cells are soybean plant cells. Suitably these cells are capable of regenerating a plant. The polypeptide comprises the sequences of SEQ ID NOs:10-18, or fragments, variants or combinations thereof. The polypeptide can be 70%, 75%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequences provided. The transgenic cells can be found in a seed. A plant, such as a soybean plant, can include the transgenic cells. The plant can be grown from a seed comprising transgenic cells or can be grown by any other means available to those of skill in the art. Chimeric plants comprising transgenic cells are also provided.

[0100] Expression of polypeptides and polynucleotides encoding the polypeptides in the transgenic plant is altered relative to the level of expression of the native polypeptides in a control soybean plant. In particular the expression of the polypeptides in the root of the plant is increased. The transgenic plant has increased resistance to nematodes as compared to the control plant. The transgenic plant can be generated from a transgenic cell or callus using methods available to those skilled in the art.

EXAMPLES

[0101] The Examples that follow are illustrative of specific embodiments disclosed herein and various uses thereof. They are set forth for explanatory purposes only and are not to be taken as limiting.

Example 1: Abundance of WT and Resistance-Associated α-SNAP Proteins in $Rhg1_{HC}$ and $Rhg1_{LC}$ Soybean Varieties

[0102] To investigate the relative abundances of wildtype (WT) and resistance-associated α-SNAPs, immunoblots were performed using standard HG type test $Rhg1_{HC}$ and $Rhg1_{LC}$ soybean varieties and previously described anti-α-SNAP antibodies (Niblack et al., 2002, J Nematol 34, 279-288; Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). NSF abundance was also studied in these samples using an antibody raised to a conserved NSF domain. As shown in FIG. **1**A, immunoblots from root tissue indicated that WT α-SNAP abundance in all tested $Rhg1_{LC}$ lines (PI 548402/Peking, PI 90763, PI 437654, PI 89772) was dramatically reduced compared with the $Rhg1_{HC}$ lines (PI 88788, PI 209332, PI 548316). Probing of the same samples with antibodies that recognize α-$SNAP_{Rhg1}$LC or α-$SNAP_{Rhg1}$HC but not WT α-SNAP confirmed that, between the $Rhg1_{HC}$ and $Rhg1_{LC}$ soybean varieties, there was a pronounced difference in the abundance of WT α-SNAP relative to the abundance of Rhg1 α-SNAP (FIG. **1**A).

[0103] WT α-SNAP expression was similarly reduced in a more recent agriculturally utilized $Rhg1_{LC}$ soybean variety, "Forrest." Immunoblots on both total leaf or root proteins from Williams82 (Rhg1 single copy), Forrest ($Rhg1_{LC}$) and Fayette ($Rhg1_{HC}$), again revealed sharp decreases in total WT α-SNAP abundance in the $Rhg1_{LC}$ source Forrest (FIG. **1**C). Altogether, a sharply reduced total abundance of WT α-SNAPs was observed to be a shared trait of $Rhg1_{LC}$ soybean varieties but not $Rhg1_{HC}$ varieties. This strikingly low abundance of WT α-SNAPs is likely due to the absence of a WT-α-SNAP-encoding allele at $Rhg1_{Lc}$, low or no product from the Glyma.11G234500 (α-$SNAP_{Ch11}$) allele containing an intronic splice site mutation, and a relatively low contribution of protein from the other three putative α-SNAP-encoding loci (Table 1.)

Table 1: Normalized RNA seq reads for soybean α-SNAP transcripts from Williams82

TABLE 1

Normalized RNA seq reads for soybean α-SNAP transcripts from Willams82

Normalized RNA seq reads for soybean α-SNAP transcripts from Willams82

alpha-SNAP gene	young_ leaf	flower	one cm pod	pod shell 10DAF	pod shell 14DAF	seed 10DAF	seed 14DAF	seed 21DAF	seed 25DAF	seed 28DAF	seed 35DAF	seed 42DAF	root	module
Glyma02g42820	0	0	0	0	0	0	1	2	1	0	1	0	0	0
Glyma09g41590	4	4	3	2	2	1	1	2	2	1	1	1	10	11
Glyma11g35820	16	17	20	23	26	13	17	11	14	6	15	10	22	12
Glyma14g05920	0	5	3	2	1	10	6	2	1	1	1	2	1	9
Glyma18g02590	26	28	32	44	24	21	27	9	13	7	12	7	28	10

[0104] NSF protein abundance in the Rhg1$_{LC}$ lines was increased compared with the Rhg1$_{HC}$ lines PI 88788 and PI 209332 (FIG. **1**A, FIG. **7**A). In PI 548316, which carries only 7 copies of Rhg1$_{HC}$ and encodes an interrupted Chromosome 11 α-SNAP, total NSF expression was more similar to the Rhg1$_{LC}$ lines (FIG. **1**A, **7**A). These differences in NSF expression, across two independent experiments, were quantified using densitometry with ImageJ (FIG. **1**B)

[0105] Whether native α-SNAP$_{Rhg1}$WT locus, if expressed, could contribute to total WT α-SNAP protein abundance in Rhg1$_{LC}$ soybean lines was also investigated. Cloning native Glyma.18G022500 α-SNAP$_{Rhg1}$WT locus from Williams 82 (Wm82), transgenic Forrest (Rhg1$_{Lc}$) roots expressing native α-SNAP$_{Rhg1}$WT were generated and total WT α-SNAP abundance was assessed with immunoblots. Compared to empty vector controls, transgenic addition of the native Williams 82 α-SNAP$_{Rhg1}$WT locus increased wild type α-SNAP abundance in Forrest to levels similar to Williams 82 controls (FIG. **1**D).

Example 2: A Unique NSF$_{Ch07}$ Allele (RAN07) is Present in Rhg1-Containing NAM Parents and HG Type Test Type Varieties

[0106] Rhg1-resistance type α-SNAPs (α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$HC) exhibited compromised binding to wild-type NSFs and were toxic at high doses in *N. benthamiana* (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). (NSF and α-SNAP are essential housekeeping proteins in all eukaryotes and null mutations in either partner are lethal in animals, which typically encode only single copies of NSF or α-SNAP (Littleton et al., 2001, 98, 12233-12238; Sanyal and Krishnan, 2001, Neuroreport 12, 1363-1366; Horsnell et al., 2002, Biochemistry 41, 5230-5235; Chae et al., 2004, Nat Genet 36, 264-270).

[0107] Viability of plants harboring Rhg1-resistance type α-SNAP$_{Rhg1}$LC was investigated by examining alternative sources of α-SNAP or NSF activity. Soybean is a polyploid organism encoding multiple α-SNAP and NSF loci. Alterations in other α-SNAP (Glyma.11G234500, Glyma. 14G054900, Glyma.02G260400, Glyma.09G279400) or NSF loci (Glyma.13G180100) were examined using whole genome sequence (WGS) data from multiple Rhg1-containing varieties. Briefly, reads were assembled for all α-SNAP and NSF loci, and aligned against the Williams 82 reference genome. In all α-SNAP loci from Rhg1$_{LC}$ varieties, no obvious polymorphisms were detected other than the previously reported Glyma.11G234500 (a-SNAPch 11) allele containing an intronic splice site mutation. (Cook, 2014, Plant Physiol 165, 630-647) Among all examined Rhg1Lc and Rhg1Hc lines, a novel NSFcho1 allele was present containing five N-Domain amino acid polymorphisms (R4Q, N21Y, S25 N, A 116F, M1811) (FIG. **2**A).

[0108] Using cDNA from Forrest (Rhg1$_{LC}$), this unique NSF$_{Ch07}$ transcript was cloned and sequenced, and all 5 N-domain polymorphisms were confirmed. Additionally, two different PCR primer pairs were designed at the N_{21}Y and S_{25}N polymorphisms and this unique NSF$_{Ch07}$ allele (and absence of the wild-type NSF$_{Ch07}$allele) was verified in all HG type test lines using agarose gel electrophoresis (FIG. **7**C).

[0109] Whole genome sequencing (WGS) data from the SoyNAM (Nested Association Mapping) project (Song et al., 2017b, Plant Genome 10(2)) was used to determine that this unique NSF$_{Ch07}$ allele was in every Rhg1-containing NAM parent, while SCN-susceptible NAM parents carried the WT NSF$_{Ch07}$ allele (Table 1). The protein from this Rhg1-associated allele of Glyma.07G195900 was designated "NSF$_{RAN07}$" for "Rhg1-associated NSF from chromosome 07." In addition to NSF$_{RAN07}$, an allele of the chromosome 13 Glyma.13g180100 gene encoding an NSF$_{Ch13}$ V_{555}I protein was found in some varieties, including SCN-susceptible soybeans, but it was not present in all Rhg1$_{LC}$ or Rhg1$_{HC}$ lines (Table 2). FIG. **8**A and FIG. **8**B shows the complete NSF$_{RAN07}$ amino acid alignment to NSF$_{Ch07}$ from the Williams 82 reference genome.

TABLE 2

HG Type Test lines and Rhg1-containing NAM Parents Contain a Unique NSF$_{Ch07}$ Allele

Line	Rhg1 Haplotype	NSF$_{Ch07}$	NSF$_{Ch13}$
Peking	Rhg1$_{LC}$	Rhg1 Assoc. Allele	WT (Wm82-type)
90763	Rhg1$_{LC}$	Rhg1 Assoc. Allele	V555I
437654	Rhg1$_{LC}$	Rhg1 Assoc. Allele	WT (Wm82-type)
209332	Rhg1$_{HC}$	Rhg1 Assoc. Allele	V555I
89772	Rhg1$_{LC}$	Rhg1 Assoc. Allele	V555I
548316	Rhg1$_{HC}$	Rhg1 Assoc. Allele	V555I
Prohio	Susceptile	WT (Wm82-type)	V555I
NE3001	Susceptile	WT (Wm82-type)	Y260F
4J105-34	Rhg1$_{HC}$	Rhg1 Assoc. Allele	V555I, L738F
CL0J095-46	Rhg1$_{HC}$	Rhg1 Assoc. Allele	V555I
IA3023	Susceptible	WT (Wm82-type)	V555I
LD00-3309	Rhg1$_{HC}$	Rhg1 Assoc. Allele	WT (Wm82-type)
LD02-4485	Rhg1$_{HC}$	Rhg1 Assoc. Allele	WT (Wm82-type)
LG05-4292	Rhg1$_{HC}$	Rhg1 Assoc. Allele	WT (Wm82-type)
LD01-5907	Rhg1$_{LC}$	Rhg1 Assoc. Allele	V555I

TABLE 2-continued

HG Type Test lines and Rhg1-containing NAM Parents Contain a Unique NSF_{Ch07} Allele

Line	Rhg1 Haplotype	NSF_{Ch07}	NSF_{Ch13}
LD02-9050	$Rhg1_{HC}$	Rhg1 Assoc. Allele	V555I
Magellan	Susceptible	WT (Wm82-type)	WT (Wm82-type)
Maverick	$Rhg1_{HC}$	Rhg1 Assoc. Allele	V555I

Example 3: NSF_{RAN07} and Rhg1 α-SNAP Polymorphisms are Both at the NSF/α-SNAP Binding Interface

[0110] The NSF/α-SNAP interface consists of complementary electrostatic patches at the NSF N-domain and α-SNAP C-terminus (Zhao and Brunger, 2016, J Mol Biol 428, 1912-1926). These binding patches are conserved in yeast, animals and plants, with the soybean NSF N-domain (N_{21}, RR_{82-83}, $KK_{117-118}$) and α-SNAP C-terminus ($D_{208}DEED_{243-246}$, $EEDD_{284-287}$) corresponding to NSF_{CHO} (R_{10}, RK_{67-68}, $KK_{104-105}$) and rat α-SNAP ($D_{217}E_{249}EE_{252-253}$, $DEED_{290-293}$) respectively. Accordingly, inter-kingdom interactions between α-SNAP and NSF have been reported both in vitro and for heterologous expression systems in vivo, including between soybean WT α-SNAP and Chinese Hamster NSF (NSF_{CHO}) (Griff et al., 1992, *J. Biol. Chem.* 267, 12106-12115; Bassham and Raikhel, 1999, Plant J 19, 599-603; Rancour et al., 2002, Plant Physiol 130, 1241-1253; Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382).

[0111] To assess where the NSF_{RAN07} polymorphisms are positioned in the N-domain, NSF_{RAN07} was modeled to the NSF_{CHO} cryo-EM structure from Zhao and colleagues (Zhao, 2015, Nature 518, 61-67) (FIG. **2**B). NSFs in many plants, including soybean, encode a variable length polyserine/glycine patch, starting at ~residue 6. Therefore, modeling to NSF_{CHO} began at residue 14. The NSF_{RAN07} homology model to NSF_{CHO} placed two of the NSF_{RAN07} polymorphisms at two NSF_{CHO} regions that bind α-SNAP: $N_{21}Y$ and $S_{25}N$ at and near R_{10}, and [<]BEGINITALm$_{116}$F at $RK_{114-115}$, respectively (FIG. **2**B, FIG. **2**C, FIG. **9**A). While R_4Q was omitted from the model (because of the omission of the variable length polyserine/glycine patch), we examined R_4 frequency across 22 diverse eukaryotes (9 animals, 3 fungi, 10 plants) (FIG. **2**D). In all but four model organisms, R_4 was present in the NSF of 18 of the 22 species, while *S. cerevisiae, Drosophila, C. elegans* and *Physcomitrella* carry an R and/or K at the adjacent residue #3 and/or #5. The final NSF_{RAN07} polymorphism, $M_{181}I$, was not located near the α-SNAP binding patches and was not highly conserved among model organism NSFs. Examination of N-domain conservation in plant NSFs revealed that residues corresponding to N_{21} and F_{115} are present in a majority of plants and do not carry $N_{21}Y$ or the [<]BEGINITALm$_{118}$F insertion (FIG. **9**B). These results modeling to NSF demonstrate that three of the five NSF_{RAN07} N-domain polymorphisms are located in or adjacent to the NSF binding patches that interact with α-SNAP.

[0112] Polymorphisms of both α-$SNAP_{Rhg1}$HC and α-$SNAP_{Rhg1}$LC, are located at conserved C-terminal residues that bind and stimulate NSF (Cook et al., 2014, Plant Physiol 165, 630-647; Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). Multiple α-SNAP proteins bound to a SNARE bundle recruit six NSF proteins to form a "20S supercomplex" (4× α-SNAPs, 6×NSF, 3-4× SNAREs) and stimulate SNARE complex disassembly (Zhao et al., 2015). The proximity of the NSF_{RAN07} N-domain polymorphisms to α-SNAP C-terminal contacts was assessed by identifying and coloring the complementary NSF and α-SNAP binding residues, and then the NSF_{RAN07} and Rhg1 α-SNAP polymorphisms, on the mammalian 20S cryo-EM structure (FIG. **3**A, FIG. **3**B, FIG. **10**A, FIG. **10**B). This confirmed that NSF_{RAN07} $N_{21}Y$, $S_{25}N$, [<]BEGINITALm$_{116}$F are predicted to locate adjacent to NSF residues that bind α-SNAP residues, including residues that contact the WT α-SNAP amino acid residues that are altered in α-$SNAP_{Rhg1}$HC and α-$SNAP_{Rhg1}$LC. R_4 on the NSF_{CHO} structure was closely positioned to a D_{28} side chain, present in soybean as D_{39} (FIG. **10**B). Altogether, the location and structural modeling of the NSF_{RAN07} polymorphisms suggest that NSF_{RAN07} modifies the normal NSF binding interface that maintains complementary binding contacts with α-SNAP sites that are altered in Rhg1 α-SNAPs.

Example 4: NSF_{RAN07} Polymorphisms Promote Binding with Rhg1 Resistance-Type α-SNAPs

[0113] All Rhg1-containing HG type test and NAM lines contained NSF_{RAN07}, and α-$SNAP_{Rhg1}$HC and α-$SNAP_{Rhg1}$LC are polymorphic at C-terminal residues that bind and stimulate NSF. Therefore, the impact of NSF_{RAN07} polymorphisms on binding to both Rhg1 resistance-type α-SNAPs and α-$SNAP_{Rhg1}$WT was investigated. Recombinant NSF_{RAN07}, NSF_{Ch07} and Rhg1 α-SNAP proteins were produced for in vitro binding studies as previously described in (Barnard et al., 1997, J Cell Biol 139, 875-883; (Bayless et al. 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). NSF_{RAN07} and NSF_{Ch07} binding was quantified using ImageJ densitometry across three independent experiments (FIG. **3**D). NSF_{Ch07} binding to α-$SNAP_{Rhg1}$HC and α-$SNAP_{Rhg1}$LC was reduced compared to α-$SNAP_{Rhg1}$WT (FIG. **3**C). In contrast, NSF_{RAN07} binding to α-$SNAP_{Rhg1}$HC or α-$SNAP_{Rhg1}$LC was similar to α-$SNAP_{Rhg1}$WT binding, and was increased ~30% relative to NSF_{Ch07}.

[0114] To verify that NSF_{RAN07}/α-SNAP binding is dependent upon NSF-binding patches at the α-SNAP C-terminus, NSF_{RAN07} binding to an otherwise WT α-SNAP lacking the final 10 C-terminal residues (α-$SNAP_{Rhg1}WT_{1-279}$) was determined. Binding of NSF_{Ch07}WT or NSF_{RAN07} binding with α-$SNAP_{Rhg1}WT_{1-279}$ was disrupted, similar to the no α-SNAP binding controls (FIG. **10**C). Hence NSF_{RAN07}/α-SNAP binding requires the conserved NSF-binding contacts located at the α-SNAP C-terminus. Combined, these binding assays suggested that NSF_{RAN07} not only maintains normal binding to WT α-SNAPs, but also at least partially accommodates the unusual C-terminal NSF-binding interface of Rhg1 resistance-type α-SNAPs.

Example 5: NSF_{RAN07} Polymorphisms Guard Against Cell Death Induced by Rhg1-Resistance-Type α-SNAP

[0115] Transient expression of either α-$SNAP_{Rhg1}$HC or α-$SNAP_{Rhg1}$LC in *N. benthamiana* leaves, via *Agrobacterium* infiltration, was cytotoxic and elicited hyperaccumulation of the endogenous NSF protein (Bayless et al., 2016 Proc. Natl. Acad. Sci. USA 113, E7375-E7382). Co-expression of WT-α-SNAP with the Rhg1 α-SNAP diminished this toxicity (Bayless et al., 2016 Proc. Natl. Acad. Sci. USA 113, E7375-E7382). The penultimate leucine/isoleucine of α-SNAP, which has been implicated in stimulation of NSF ATPase, was needed for this *N. benthamiana* cytotoxicity (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382).

[0116] The ability of soybean NSF co-expression to alleviate the toxicity of Rhg1 resistance-type α-SNAPs in *N. benthamiana* was determined. Mixed *Agrobacterium* cultures containing 1 part WT α-SNAP to 3 parts α-$SNAP_{Rhg1}$LC were used for cytotoxicity complementation assays as previously described Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). NSF_{RAN07} and NSF_{Ch07} were more effective than WT α-SNAP at reducing Rhg1 α-SNAP cytotoxicity (FIG. **11**A). The proportion of NSF-delivering bacteria in the mixed *Agrobacterium* cultures was then decreased down to 1 part to 9 or 14 parts α-$SNAP_{Rhg1}$LC-delivering bacteria. Co-expressing soybean NSF_{Ch07}, NSF_{Ch13} or NSF_{RAN07} reduced cell death caused by α-$SNAP_{Rhg1}$LC compared to empty vector controls (FIG. **4**A), and NSF_{RAN07} co-expression consistently conferred greater protection than either NSF_{Ch07} or NSF_{Ch13} (FIG. **4**A). Infiltrated leaf patches had less death and/or slower death with NSF_{RAN07}. Both NSF_{RAN07} and NSF_{Ch07} were more effective than NSF_{Ch13} at complementing cell death (FIG. **4**A). NSF_{RAN07} was observed to confer at least partial protection out to a 1:19 mixture, again outperforming complementation by NSF_{Ch07} (FIG. **11**B). Complementation of α-$SNAP_{Rhg1}$HC-induced cell death with NSF_{RAN07} vs. NSF_{Ch07} produced similar results (FIG. **11**C).

[0117] Mixed cultures of *N. benthamiana* NSF ($NSF_{N. benth}$, 81% identity to NSF_{Ch07}, see FIG. **12** for alignment) and α-$SNAP_{Rhg1}$LC, were agroinfiltrated as in FIG. **4**A. EV, NSF_{Ch13} and NSF_{RAN07} were agroinfiltrated as controls. NSF_{Ch13} gave visible protection relative to an empty vector, while NSF_{RAN07} co-expression gave strong protection (FIG. **4**B). In contrast, $NSF_{N.benth}$ co-expression was similar to empty vector controls (FIG. **4**B). Expressing soybean NSFs or $NSF_{N.benth}$ with an empty vector at the same ratios used for complementation did not cause macroscopic phenotypes suggestive of stress (FIG. **11**D).

[0118] Physical binding with Rhg1 resistance-type α-SNAPs using recombinant $NSF_{N.benth}$ protein was determined. Whereas $NSF_{N.benth}$ readily bound α-$SNAP_{Rhg1}$WT, $NSF_{N.benth}$ binding to Rhg1 resistance-type α-SNAPs was much lower, only slightly over controls (α-SNAP lacking the C-terminus or no-α-SNAP) (FIG. **4**C). This suggested a biochemical explanation for why Rhg1 resistance type α-SNAPs—but not WT α-SNAPs—provoke strong cell death responses in *N. benthamiana*: the endogenous *N. benthamiana* NSF binds WT α-SNAPs but not Rhg1 resistance type α-SNAPs.

[0119] Complementation assays using NSF_{RAN07} or NSF_{Ch07} were performed to determine if either could prevent cell-death caused by α-$SNAP_{Rhg1}LC_{1-279}$, which lacks the final 10 C-terminal residues and does not bind NSF_{RAN07} or NSF_{Ch07} in vitro. Neither NSF_{RAN07} nor NSF_{Ch07} prevented the cell death caused by α-$SNAP_{Rhg1}LC_{1-279}$ whereas either complemented the cell death induced by full length α-$SNAP_{Rhg1}$LC (FIG. **11**E).

[0120] The impact of the penultimate α-SNAP residue implicated in NSF-ATPase stimulation was determined using complementation assays with NSF_{RAN07} or NSF_{Ch07}. Complementation of α-$SNAP_{Rhg1}$LC I_{289}A was evident, but was less than that observed for α-$SNAP_{Rhg1}$LC (FIG. **4**D).

Example 6: 100% of the Predicted $Rhg1^+$ Soybean Accessions in the USDA Soybean Collection, and 7% of the $Rhg1^-$ Soybean Accessions, Contain the SoySNP50K NSF_{RAN07} R_4Q Amino Acid Polymorphism

[0121] NSF_{RAN07} was present in all Rhg1-containing HG type and NAM lines, but whether this Rhg1/NSF_{RAN07} association was universal rather than "frequent" was further investigated. First, the approximate NSF_{RAN07} allele frequency was determined. In 2015, Song et al. reported genotyping the USDA soybean germplasm collection of ~20,000 accessions—collected from over 80 countries—using a 50,000 SNP DNA microarray chip (SoySNP50K iSelect BeadChip). These data were available in a searchable SNP database at Soybase (Soybase.org/snps/) (Grant et al., 2010, Nucleic Acids Res 38, D843-846; Song et al., 2013, PLoS One 8, e54985; Song et al., 2015, PLoS Genet 11, e1005200). Using the Soybase genome browser, a C/T SNP was found to be involved using the SoySNP50K (ss715597431, Gm07:36,449,014) that causes the NSF_{RAN07} R_4Q polymorphism. Analyzing all 19,645 USDA soybean accessions for ss715597431, the NSF_{RAN07} allele frequency in the USDA collection was estimated at 11.0% (2,165+/+, 33+/−) (FIG. **5**A). While NSF in most model eukaryotes contains R_4, it remained unclear whether Q_4 occurs in other plant NSFs. To determine if the NSF_{RAN07} R_4Q is unusual among plants, R_4 conservation across plant NSF sequences available on Phytozome (Goodstein et al., 2012, Nucleic Acids Res 40, D1178-D1186) was examined. Notably, Q_4 was not in the queried NSF predicted protein sequences for any other plant species (FIG. **13**).

[0122] Rhg1-mediated SCN resistance is uncommon among soybean accessions and less than 5% of the USDA soybean collection carries a multi-copy Rhg1 haplotype. Previously, Lee et al. identified SoySNP50K signatures for $Rhg1_{HC}$, $Rhg1_{LC}$ and single copy (SCN-susceptible) haplotypes, and estimated that 705 $Rhg1_{LC}$ and 150 $Rhg1_{HC}$ accessions were in the USDA *Glycine max* collection (Lee et al., 2015, Mol Ecol 24, 1774-1791). Using these 855 Rhg1-signature accessions, a 100% incidence of the ss715597431 NSF_{RAN07} signature was determined for multi-copy Rhg1-signature *Glycine max* (FIG. **5**B).

[0123] If NSF_{RAN07} is needed for the survival of Rhg1-containing soybean plants, then, all Rhg1 accessions should carry NSF_{RAN07}. As such, SNPs within the locus underlying Rhg1 co-segregation should be maintained, while SNPs at neighboring loci, though tightly linked, would not be under stringent selection and hence should be less conserved. To narrow in on the Rhg1 co-segregating locus within the interval, amino acid changes within candidate loci adjacent to RAN07 from Rhg1-carrying HG and NAM lines, between markers ss715597415 and ss715597431, were examined. NSF_{RAN07} SNPs, especially those causing the 5 N-domain polymorphisms, were 100% maintained across all Rhg1-containing varieties. On the other hand, SNPs causing amino acid changes within candidate loci adjacent to NSF_{RAN07}, were not 100% conserved across all Rhg1-containing varieties, unlike NSF_{RAN07} (Table 3). The predicted amino acid sequence of most candidate loci matches Wm82 (SCN-susceptible) sequence, and among candidate loci with amino acid substitutions, only NSF_{RAN07} has the same consistent amino acid changes across all examined Rhg1-containing germplasm (Table 3). In addition to the observed biochemical and genetic complementation of Rhg1 α-SNAPs by NSF_{RAN07}, candidate gene allele frequency further implicates NSF_{RAN07} as the gene responsible for co-segregation with Rhg1.

hub-parent to eight different soybean accessions carrying either $Rhg1_{HC}$ (seven accessions) or $Rhg1_{LC}$ (one accession) were examined. There were 122 to 139 RILs in each population and the segregation for NSF_{RAN07}:NSF_{Ch07}WT in soybean lines lacking Rhg1 did not deviate from the null hypothesis of 1:1 segregation in six of the eight populations. Across populations, there was a significant (α=0.05) deviation from a 1:1 segregation with a significantly greater number of RILs with NSF_{RAN07} than NSF_{Ch07}WT. The

TABLE 3

Amino acid polymorphisms of genes within the chromosome 07 interval co-segregating with Rhg1.

	ss715597431				ss715597413			ss715597410	
	Soybean Line								
	Glyma 07g195900 NSF	Glyma 07g195800 Rubber Elongation Factor	Glyma 07g195700 DNA Mismatch Repair MutS2	Glyma 07g195600 No annotated domains	Glyma 07g195500 TFII H Polypeptide 4	Glyma 07g195400 E3 Ubiquitin Ligase	Glyma 07g195300 Asparagine Synthase	Glyma 07g195200 Conserved Protein	Glyma 07g195100 LRR Containing Protein
PI89772	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{161}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
PI90763	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
PI209332	R_4Q, $N_{21}Y$, $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $V_{345}I$, $D_{364}N$, $M_{408}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
CLOJO95-4-6	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{408}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
IA3023	WT	$L_{42}R$, $F_{437}S$	$D_{364}N$, $M_{406}T$, $Y_{576}F$	WT	WT	WT	$E_{46}G$	$D_{60}A$, $S_{64}P$	WT
LD00-3309	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $L_{42}R$, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $G_{518}C$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
PI 437654	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
PI548402	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
Magellan	WT	$L_{42}R$, $F_{137}S$	$D_{364}N$, $M_{406}T$	WT	WT	WT	$E_{46}G$	$D_{60}A$, $S_{64}P$	WT
Maverick	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT
PI548316	R_4Q, $N_{21}Y$ $S_{25}N$, $\wedge_{116}F$, $M_{181}I$	K_3N, $F_{137}S$	$T_{21}A$, $K_{23}R$, $G_{109}C$, $H_{115}Q$, $V_{345}I$, $D_{364}N$, $M_{406}T$, $Q_{818}K$	WT	WT	WT	WT	WT	WT

Example 7: All $Rhg1^+$F5-Derived Recombinant Inbred Lines (RILs) from NAM Population Crosses Also Carry NSF_{RAN07}

[0124] The NSF_{RAN07} data from the USDA soybean germplasm collection are an indication of strong segregation distortion. However, Webb et al. (1995) reported that only 91 of 96 lines with a resistant parent marker type linked to Rhg1 also had a resistant parent marker type near the NSF_{RAN07} QTL (Webb et al., 1995, Theor Appl Genet 91, 574-581). Therefore, lines with Rhg1 were investigated for inheritance of NSF_{RAN07} in the progeny of more recent biparental crosses. From the Soybean Nested Associated Mapping (SoyNAM) project (Song et al., 2017,Plant Genome 10(2)), genotypic data for populations of RILs developed from crosses of the IA3023 (SCN-susceptible) segregation distortion for NSF_{RAN07} was obvious among RILs that carried a resistance-associated Rhg1 allele but, out of a total of 309 $Rhg1^+$RILs, 8 appeared to have possibly inherited $Rhg1_{HC}$ or $Rhg1_{LC}$ but not NSF_{RAN07} while the remainder had NSF_{RAN07}. This was based upon the lower-density SoySNP6K mapping data that that did not include perfect genetic markers for Rhg1 and NSF. Polymorphisms within Rhg1 and NSF_{RAN07} genes were genotyped using primers that detect the Rhg1 repeat junction and a WT NSF_{Ch07} vs. NSF_{RAN07} allele. All 8 re-examined RILs that inherited $Rhg1_{HC}$ or $Rhg1_{LC}$ also inherited the NSF_{RAN07} $\wedge$ 116 F and $M_{181}I$ mutations meaning that all 309 RILs that carried the resistance associated Rhg1 also carried NSF_{RAN07} (Table 4).

TABLE 4

NSF$_{RAN07}$ co-segregates with Rhg1 in all Rhg1-containing $F_{2\cdot5}$ offspring from Rhg1$^+$ × rhg1$^-$ crosses

Diverse Parent	RR (Ch07, Ch18)	RS(Ch07, Ch18)	SR(Ch07, Ch18)	SS(Ch07, Ch18)	HR(Ch07, Ch18)	HS(Ch07, Ch18)	HH(Ch07, Ch18)	RH(Ch07, Ch18)	SH(Ch07, Ch18)
4J105-3-4	41	41	2	31	9	3	1	9	0
CL0J095-4-6	35	45	0	37	6	7	0	7	1
LD00-3309	38	45	1	27	8	10	3	7	0
LD01-5907	32	32	1	42	0	6	1	6	2
LD02-4485	37	50	1	28	10	7	1	5	0
LD02-9050	43	31	2	34	10	10	1	4	0
Maverick	31	34	0	41	8	8	3	8	1
LG05-4292	44	41	1	30	1	3	0	7	0
Totals	301	319	8*	270	52	54	10	53	4

R refers to allele from Rhg1 resistant parent.

S refers to allele from SCN-susceptible parent

Genotype order: first allele is chr 7 (RAN07 interval) and second is chr 18 (Rhg1 interval)

*All 8 re-examined RILs that inherited Rhg1 $_{HC}$ or Rhg1 $_{LC}$ also inherited the NSF $_{RAN07}$ ^116 F and MI mutations meaning that all 309 RILs that carried the resistance associated Rhg1 also carried NSF $_{RAN07}$

Example 8: NSF-RAN07 Aids in the Production of Transgenic Soybean Lines that Express an SCN-Resistance-Associated Rhg1 α-SNAP

[0125] In previous work, attempts to generate transgenic soybean lines with DNA constructs derived in part from the Rhg1 locus had failed to generate lines that express α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$HC protein variants. This was despite successes within the same project in generating stably transformed transgenic soybean lines that express other genes or gene silencing constructs. That work was done using soybean variety Thorne, which does not carry an NSF$_{RAN07}$-encoding allele of Glyma.07G195900. In subsequent collaborative work with the University of Wisconsin—Madison Wis. Crop Innovation Center (Middleton, Wis.), an experiment was initiated in which soybean variety Williams 82 was transformed with DNA constructs designed to express α-SNAP$_{Rhg1}$LC or α-SNAP$_{Rhg1}$WT protein, together with either NSF$_{RAN07}$ or NSF$_{Ch07}$WT protein, or no added NSF protein. Williams 82 lacks NSF$_{RAN07}$ and lacks resistance-associated Rhg1. The respective DNA constructs, which used a *Glycine max* ubiquitin promoter sequence to drive expression of Glyma.18G022500 protein coding sequences, or Glyma.07G195900 and Glyma.18G022500 protein coding sequences on the same plasmid, were built into plasmid pC23S, a binary plasmid conferring spectinomycin resistance. Similar numbers of Williams 82 embryos were treated with the respective *Agrobacterium tumefaciens* strain for each DNA construct (approximately 300 embryos per *Agrobacterium* strain). After co-culture of the embryos with the designated *Agrobacterium* strain, counter-selection against the *Agrobacterium* was applied, and embryos were then grown on growth media containing spectinomycin. Embryos that were able to grow successfully on spectinomycin were transferred to new spectinomycin selection media, and plantlets producing new leaves and roots were then transferred to the greenhouse and grown for seed production. If the DNA used for plant transformation was phenotypically neutral, similar numbers of Williams 82 transformants would be expected for each DNA construct if using the same plasmid vector and processing all of the transformants similarly within the same experiment. However, there was a notable lack of recovery of spectinomycin-resistant transformants for soybean lines that received a DNA construct encoding α-SNAP$_{Rhg1}$LC expression. Zero lines were recovered for expression of only α-SNAP$_{Rhg1}$LC, and only one line was recovered for expression of α-SNAP$_{Rhg1}$LC+NSF$_{Ch07}$WT (Table 5). Immunoblot testing for presence of α-SNAP$_{Rhg1}$LC protein revealed that the one transgenic line for the α-SNAP$_{Rhg1}$LC+NSF$_{Ch07}$WT DNA construct failed to express α-SNAP$_{Rhg1}$LC protein (FIG. **14**). In contrast, four of the five lines that received the α-SNAP$_{Rhg1}$LC+NSF$_{RAN07}$WT DNA construct did express α-SNAP$_{Rhg1}$LC protein (FIG. **14**). These findings provide further evidence that presence of a nematode resistance-associated Rhg1 α-SNAP protein is poorly tolerated in soybean lines that express only wild-type NSF proteins, and that NSF$_{RAN07}$WT or a similarly suitable NSF partner protein is necessary to recover viable soybean lines that express a nematode resistance-associated Rhg1 α-SNAP.

TABLE 5

Recovery rate of transgenic soybean lines expressing SCN-resistance-associated Rhg1 α-SNAP

DNA construct used to transform soybean variety Williams 82	Number of Williams 82 transformants recovered
pC23S (empty vector)	11
α-SNAP-WT (no added NSF)	5
α-SNAP-Rhg1-LC (no added NSF)	0
NSF-WT + α-SNAP-WT	3
NSF-RAN07 + α-SNAP-WT	2
NSF-WT + α-SNAP-Rhg1-LC	1
NSF-RAN07 + α-SNAP-Rhg1-LC	5

Example 9: Modified NSF BLASTp Alignment in Plant Species

[0126] The WT NSF sequence for wild type *Glycine max* (accession number AWH66430.1 was entered into BLASTp and modified at R4Q, N21Y, S25N, (del)116F, and M1811. The modified sequence was then entered into BLASTp to determine the occurrence, in the NSF proteins of 100 other plant species, of amino acids at the protein residue positions of the above key NSF$_{RAN07}$ amino acids. The amino acid expressed at positions 4, 21, 25, 116 and 181 in the BLASTp results were compared against the *Glycine max* NSF$_{RAN07}$ and the data entered into Table 6. In sequences for which

BLASTp protein alignment started after the designated amino acid position, that position is marked N/A. Naturally occurring proteins encoding the R4Q or N21Y residues found in *Glycine max* NSF_{RAN07} were not present in the sequences for any of the other plant species compared via BLASTp.

TABLE 6

Modified NSF BLASTp Alignment in Plant Species

Genus Species	Plant	NSF Accession Number	% Identity	Identities	R4Q	N21Y	S25N	— 116F	M181I (Subst)	% Query Cover
Glycine Max Predicted	Soybean	XP_003529321.1	99.33	742/747	R	N	S	—	M	99.73
Glycine Max Predicted	Soybean	XP_003541535.1	97.57	724/742	N/A	N	S	—	M	98.65
Glycine Soja	Wild Soybean	KHN10009.1	95.98	717/747	N/A	N	S	—	M	97.19
Phaseolus Vulgaris	Common/ Green Bean	XP_007159324.1	92.50	691/747	L	N	T	—	L	96.79
Glycine Max	Hypo *Glycine Max*	KRH50034.1	99.00	696/703	R	N	S	—	M	99.57
Vigna Radiata var. *radiata*	Mung Bean	XP_014510227.1	92.60	688/743	N/A	N	S	—	M	96.77
Vigna angularis	Adzuki Bean	XP_017411260.1	92.60	688/743	N/A	N	S	—	M	96.64
Arachis ipaensis	Peanut	XP_016190089.1	89.83	671/747	L	N	Q	—	M	94.78
Arachis duranensis	Wild Peanut	XP_015956468.1	89.83	671/747	L	N	Q	—	M	94.91
Lupinus angustifolius	Lupin	XP_019445668.1	88.89	664/747	W	N	Q	—	M	94.78
Lupinus angustifolius	Narrow leaf Lupiin (Herb)	XP_019421896.1	90.29	660/731	N/A	N	Q	—	M	95.21
Cajanus cajan	Pigeon Pea (Legume)	XP_020225776.1	90.85	665/732	N/A	N	Q	—	M	95.36
Cajanus cajan	Pigeon Pea (Legume)	KYP76270.1	90.45	663/733	N/A	N	Q	—	M	95.23
Vigna angularis	Adzuki Bean	KOM31050.1	88.69	659/743	N/A	N	S	—	M	92.6
Medicago truncatula	BarrelClover (small Mediterranean Legume)	XP_024637282.1	85.41	638/747	R	N	Q	I	M	93.04
cephalotus follicularis	Australian Pitcher Plant	GAV67671.1	84.74	633/747	R	N	A	—	I	92.37
Quercus suber	Cork Oak	XP_023924241.1	86.44	631/730	N/A	N	A	—	M	95.07
Citrus clementina	Clementine	XP_006428558.1	84.739	633/747	R	N	A	—	I	93.57
Medicago truncatula]	BarrelClover (small Mediterrian Legume)	KEH31080.1	84.707	637/752	R	N	Q	I	M	92.29
Cicer arietinum	ChickPea	XP_004505051.1	88.615	646/729	N/A	N	T	I	M	95.2
Citrus sinensis	Sweet Oranges (blood, navel)	KDO54905.1	84.626	633/748	R	N	A	—	I	93.45
Populus trichocarpa	Black cottonwood	XP_002305796.2	84.605	632/747	R	N	A	—	M	91.97
Herrania umbratica	Colombian Cocoa	XP_021294427.1	84.584	631/746	R	S	T	—	M	92.63
Populus euphratica	Desert Poplar	XP_011027829.1	84.605	632/747	R	N	A	—	M	91.83
Jatropha curcas	Jatropha curcas	XP_012091606.1	85.346	629/737	N/A	N	P	—	M	93.22
Ziziphus jujuba	Jujube red date	XP_015890094.1	85.121	635/746	R	N	P	—	M	92.63
Durio zibethinus	*Durio zibethinus*	XP_022720468.1	84.471	631/747	R	G	T	—	M	92.5
Manihot esculenta	Yuca	XP_021597323.1	84.987	634/746	R	N	A	—	M	91.69
Pyrus × *bretschneideri*	Chinese white pear	XP_009339728.1	85.007	635/747	R	N	P	—	M	93.17
Morus notabilis	Black Mulberry	XP_024029108.1	84.718	632/746	R	S	T	—	M	93.16
Gossypium raimondii	Cotton Plant	XP_012450449.1	84.07	628/747	L	S	T	—	M	92.37
Citrus unshiu	Mandarin Orange	GAY44590.1	84.337	630/747	R	N	A	—	I	92.9
Quercus suber	Cork Oak	XP_023927780.1	85.753	626/730	T	N	A	—	M	94.38
Malus domestica	Apple Tree	XP_008364158.1	84.873	634/747	R	N	P	—	M	93.04
Gossypium arboreum	Cotton Tree	XP_017642474.1	84.853	633/7465	W	S	P	—	M	91.82

TABLE 6-continued

Modified NSF BLASTp Alignment in Plant Species										
Genus Species	Plant	NSF Accession Number	% Identity	Identities	R4Q	N21Y	S25N	— 116F	M181I (Subst)	% Query Cover
Gossypium arboreum	Cotton Tree	XP_017646058.1	83.668	625/747	L	S	T	—	M	92.1
Gossypium hirsutum	Mexican Cotton Tree	XP_016676150.1	83.534	624/747	L	S	T	—	M	92.1
Hevea brasiliensis	Rubberwood	XP_021641739.1	84.584	631/746	R	N	S	—	M	91.42
Durio zibethinus	Durian Tree	XP_022724072.1	84.048	686/746	R	S	T	—	M	91.96
Lupinus angustifolius	Lupin	OIV94352.1	91.215	623/683	N/A	N/A	N/A	—	M	96.34
Gossypium hirsutum	Mexican Cotton Tree	XP_016683459.1	83.802	626/747	L	S	T	—	M	91.97
Gossypium raimondii	Cotton	XP_012450761.1	84.048	627/746	W	S	P	—	M	91.96
Gossypium raimondii	Cotton	KJB68632.1	83.936	627/747	W	S	P	—	M	91.83
Prunus avium	Sweet/wild Cherry tree	XP_021825850.1	83.78	625/746	R	N	A	—	M	92.76
Hevea brasiliensis	Rubberwood	XP_021640046.1	83.512	623/746	R	N	L	—	M	91.69
Lupinus angustifolius	Blue Lupine	OIW10410.1	85.007	635/747	W	N	Q	—	M	90.36
Gossypium raimondii	Cotton Plant	XP_012450763.1	84.048	686/746	W	S	P	—	M	91.96
Theobroma cacao	Cacao Tree	XP_007025619.2	83.78	625/746	R	S	A	—	M	92.23
Populus trichocarpa	Black cottonwood	XP_006377363.1	83.936	627/747	R	N	A	—	M	91.43
Gossypim raimondii	Cotton Plant	XP_012450762.1	84.048	627/746	W	S	P	—	M	91.96
Hevea brasiliensis	Rubber Tree	XP_021657769.1	84.316	629/746	R	N	D	—	M	91.15
Eucalyptus grandis	Eucalyptus or Rose Gum	XP_010057417.1	83.914	626/746	R	N	A	—	K	92.36
Populus trichocarpa	Black Cottonwood	PNT11917.1	83.936	627/747	R	N	A	—	M	91.43
Prunus persica	Peach	XP_007214647.1	83.78	691/746	R	N	A	—	M	92.63
Prunus mume	Japanese Apricot	XP_008225100.1	83.646	624/746	R	N	A	—	M	92.76
Pyrus × *bretschneideri*	Chinese white pear	XP_009352914.1	83.802	626/747	R	N	A	—	M	92.77
Hevea brasiliensis	Rubber Tree	XP_021640045.1	83.378	622/746	R	N	L	—	M	91.55
Gossypium hirsutum	Mexican Cotton	XP_016751989.1	83.668	625/747	W	S	P	—	M	91.7
Gossypium barbadense	Extra long staple cotton (Sea Island Cotton)	PPS13789.1	83.202	634/762	W	S	P	—	M	89.76
Gossypium hirsutum	Upland Cotton	XP_016751992.1	83.78	625/746	W	S	P	—	M	91.82
Theobroma cacao	Cacao tree	XP_017978707.1	83.556	625/746	R	S	A	—	M	91.98
Gossypium hirsutum	Upland Cotton	XP_016751991.1	83.78	625/746	W	S	P	—	M	91.82
Gossypium hirsutum	Upland Cotton	XP_016751990.1	83.78	625/746	W	S	P	—	M	91.82
Tarenaya hassleriana	Spider Flower	XP_010529424.1	83.133	621/747	R	N	A	—	M	92.1
Juglans regia	Walnut Tree	XP_018860049.1	84.146	621/738	N/A	S	P	—	M	92.95
Populus euphratica	Desert Poplar	XP_011043386.1	83.534	624/747	R	N	A	—	M	90.9
Prunus yedoensis var. *nudiflora*	King Cherry (Korean Cherry)	PQM34143.1	83.512	623/746	R	N	A	—	M	92.09
Carica papaya	Papaya	XP_021902227.1	84.182	628/746	R	N	S	—	M	92.36
Cucumis melo	Muskmelon	XP_008463616.1	82.038	612/746	R	N	Q	—	M	92.23
Manihot esculenta	Yuca	XP_021598339.1	83.244	680/746	W	N	A	—	M	91.15
Populus trichocarpa	Black cottonwood	PNT11918.1	82.827	627/757	R	N	A	—	M	90.22
Gossypium barbadense	Extra long staple cotton (Sea Island Cotton)	PPD95675.1	82.26	626/761	W	S	P	—	M	90.01
Cucurbita pepo subsp. *Pepo*	Winter Squash	XP_023519438.1	81.66	610/747	L	S	A	—	M	91.7
Tarenaya hassleriana	Spider Flower	XP_010538665.1	82.597	617/747	R	N	A	—	M	91.43

TABLE 6-continued

Modified NSF BLASTp Alignment in Plant Species										
Genus Species	Plant	NSF Accession Number	% Identity	Identities	R4Q	N21Y	S25N	— 116F	M181I (Subst)	% Query Cover
Cucurbita moschata	Pumpkin	XP_022927355.1	81.769	610/746	L	S	A	—	M	91.69
Cucumis sativus	Cucumber	XP_004139535.1	81.769	610/746	R	N	Q	—	M	92.09
Cucurbita maxima	Squash	XP_023001327.1	81.769	610/746	L	S	A	—	M	91.69
Trifolium subterraneum	Clover	GAU38492.1	82.097	642/782	R	N	Q	I	M	88.75
Nicotiana tabacum Tobacco	Cultivated	BAA13101.1	81.233	606/746	R	Y	K	—	M	91.96
Vitis vinifera	Grape Vine	XP_002284987.1	82.568	611/740	R	N	R	—	I	92.03
Nicotiana tomentosiformis	Tobacco Plant	XP_009626763.1	81.233	606/746	R	N	K	—	M	91.96
Theobroma cacao	Cacao Tree	EOY28241.1	85.278	614/720	N/A	N/A	N/A	—	M	93.06
Sesamum indicum	Seasame	XP_011098317.1	82.763	605/731	N/A	N	K	—	I	91.93
Malus domestica	Apple	XP_008383736.1	83.802	626/747	R	N	A	—	M	92.64
Nicotiana attenuata	Coyote Tobacco	XP_019251692.1	80.965	604/746	R	N	K	—	M	91.69
Actinidia chinensis var. *chinensis*	Kiwifruit	PSR95688.1	81.511	604/741	N/A	N	K	—	I	91.36
Punica granatum	Pomegranate	PKI69442.1	83.469	616/738	N/A	N	A	—	M	92.14
Capsicum annuum	Chili Peppers	XP_016574871.1	80.697	602/746	R	N	K	—	M	91.82
Ipomoea nil	Morning Glory	XP_019187191.1	81.905	602/735	N/A	N	K	—	L	91.97
Handroanthus impetiginosus	Pink Trumpet Tree	PIN22741.1	82.538	605/733	N/A	N	K	—	M	92.22
Vitis vinifera	Grape Vine	CBI20305.3	82.027	607/740	N/A	N	R	—	I	91.62
Daucus carota subsp. Sativus	Carrot	XP_017252931.1	83.083	609/733	N/A	S	K	—	M	91.68
Solanum Pennellii	Tomato	XP_015062393.1	83.083	599/746	R	N	K	—	M	91.82
Solanum tuberosum	Potato	XP_006351809.1	80.295	599/746	R	N	K	—	M	91.69
Solanum lycopersicum	Tomato	XP_004230528.1	80.295	598/746	R	N	K	—	M	91.96
Helianthus annuus	Sunflower	XP_022013369.1	81.351	607/740	N/A	N	K	—	M	91.35
Gossypium raimondii (Hypo)	Cotton Plant	KJB66715.1	81.928	612/747	L	S	T	—	M	89.69
Macleaya cordata	Plume Poppy	OVA14922.1	81.325	614/755		N	S	—	M	89.4

Example 10: Modified α-SNAP BLASTp Alignment in Plant Species

[0127] The Rhg1 LC haplotype Glyma.18G022500 encoded protein sequence was entered into BLASTp and the results for 100 plant species were further examined. The BLASTp results at the α-SNAP C-terminus amino acid residues of interest (amino acid positions 208, 284, 285, 286, and 287, in the soybean Glyma.18G022500 product) were compared against the Rhg1 LC haplotype and entered into Table 7. The majority of plant species alignments terminated prior to the sequences of interest and are represented in the table as N/A.

TABLE 7

Modified α-SNAP BLASTp Alignment in Plant Species										
Genus Species	Plant	α-SNAP Accession Number	% Identity	Identities	D208E	E284	E285	D286	D287	% Query Cover
Glycine Max Predicted	Soybean	NP_001242059.2	100	289/289	D	E	E	D	D	100
Glycine Max Predicted	Soybean	ACU19524.1	99.308	287/289	D	E	E	D	D	99.65
Glycine Max Predicted	Soybean	ARD05064.1	99.649	284/285	E	E	E	N/A	N/A	100
Glycine Max Predicted	Soybean	ACU18668.1	99.298	283/285	D	E	Q	N/A	N/A	100
Glycine Max Predicted	Soybean	NP_001344346.1	97.578	282/289	D	E	E	D	D	98.96
Cajanus cajan	Pigeon Pea (Legume)	XP_020237258.1	95.848	277/289	D	E	E	D	D	97.92
Trifolium subterraneum	Clover	GAU29434.1	91.003	263/289	D	E	E	D	D	96.89

TABLE 7-continued

Modified α-SNAP BLASTp Alignment in Plant Species										
Genus Species	Plant	α-SNAP Accession Number	% Identity	Identities	D208E	E284	E285	D286	D287	% Query Cover
Medicago truncatula	Barrel Clover (small Mediterranean Legume)	XP_003601014.1	89.619	259/289	D	E	E	D	D	96.19
Quercus suber	Cork Oak	XP_023896842.1	89.273	258/289	D	E	E	D	D	96.89
Durio zibethinus	*Durio zibethinus*	XP_022774310.1	88.581	256/289	D	E	E	D	D	95.16
Lupinus angustifolius	Lupin	XP_019456553.1	88.235	255/289	D	E	E	D	D	96.54
Phaseolus vulgaris	Common/ Green Bean	XP_007163598.1	94.464	273/289	D	E	E	D	D	97.58
Glycine Max Predicted	Soybean	KRH14886.1	87.889	254/289	D	E	E	D	D	96.89
Vigna angularis	Adzuki Bean	XP_017407790.1	93.772	271/289	D	E	E	D	D	97.23
Cajanus cajan	Pigeon Pea (Legume)	XP_020237651.1	87.543	253/289	D	E	E	D	D	96.54
Juglans regia	Walnut Tree	XP_018821859.1	88.235	255/289	D	E	E	D	D	95.85
Vigna radiata var. *radiata*	Mung Bean	XP_014490390.1	94.118	272/289	D	E	E	D	D	96.89
Medicago truncatula	BarrelClover (small Mediterranean Legume)	XP_003616738.1	86.505	250/289	D	E	E	D	D	96.19
Theobroma cacao	Cacao Tree	EOY02634.1	88.235	255/289	D	E	E	D	D	95.5
Herrania umbratica	Colombian Cocoa	XP_021299224.1	87.889	254/289	D	E	E	D	D	95.5
Theobroma cacao	Cacao Tree	XP_007031708.2	87.889	254/289	D	E	E	D	D	95.5
Cicer arietinum	ChickPea	XP_004500538.1	92.042	266/289	D	E	E	D	D	96.89
Phaseolus vulgaris	Common/ Green Bean	XP _007141718.1	86.159	249/289	D	E	E	D	D	96.19
Phaseolus vulgaris	Common/ Green Bean	AHA84269.1	93.38	268/287	D	E	E	D	E	96.86
Vigna angularis	Adzuki Bean	XP_017429402.1	84.775	277/289	D	E	E	D	D	95.85
Lotus japonicus	Trefoil/Wild Legume	AFK46359.1	91.696	265/289	D	E	E	D	D	97.23
Juglans regia	Walnut Tree	XP_018838975.1	90.311	261/289	D	E	E	D	D	97.58
Vigna radiata var. *radiata*	Mung Bean	XP_014504530.1	84.775	245/289	D	E	E	D	D	95.85
Gossypium raimondii	Cotton Plant	XP_012453802.1	85.467	247/289	D	E	E	D	D	95.85
Gossypium hirsutum	Mexican Cotton Tree	NP_001314193.1	86.851	251/289	D	E	E	D	D	95.16
Glycine Max Predicted	Soybean	XP_003519412.1	85.813	248/289	D	E	E	D	D	94.81
Arachis ipaensis	Peanut	XP_016180830.1	91.003	263/289	D	E	E	D	D	95.85
Gossypium raimondii	Cotton Plant	XP_012445339.1	86.505	250/289	D	E	E	D	D	94.81
Glycine Max Predicted	Soybean	NP_001242555.1	85.813	248/289	G	E	G	D	D	94.81
Lupinus angustifolius	Lupin	XP_019437582.1	90.311	261/289	D	E	E	D	D	95.85
Lupinus angustifolius	Lupin	XP_019415244.1	90.657	262/289	D	E	E	D	D	95.16
Gossypium hirsutum	Mexican Cotton Tree	XP_016677490.1	84.775	245/289	D	E	E	D	D	95.5
Manihot esculenta	Yuca	XP_021617295.1	85.121	246/289	D	E	E	D	D	95.5
Malus domestica	Apple Tree	XP_008369314.1	83.737	242/289	D	E	E	D	D	94.81

TABLE 7-continued

Modified α-SNAP BLASTp Alignment in Plant Species

Genus Species	Plant	α-SNAP Accession Number	% Identity	Identities	D208E	E284	E285	D286	D287	% Query Cover
Cicer arietinum	ChickPea	XP_004491041.1	83.737	242/289	D	E	E	D	D	95.85
Cucumis melo	Muskmelon	XP_008456753.1	85.813	248/289	D	E	E	D	D	93.43
Pyrus × *bretschneideri*	Chinese white pear	XP_009369241.1	83.045	240/289	D	E	E	D	D	94.81
Corchorus capsularis	White Jute	OMO73552.1	84.88	247/291	D	E	E	D	D	92.44
Gossypium raimondii	Cotton Plant	XP_012489506.1	84.775	245/289	D	E	E	D	D	93.08
Prunus avium	Sweet/wild Cherry tree	XP_021824795.1	83.391	241/289	D	E	E	D	D	94.12
Lupinus angustifolius	Lupin	OIW15090.1	88.176	261/296	D	E	E	D	D	93.58
Gossypium barbadense	Extra long staple cotton (Sea Island Cotton)	PPR99271.1	79.677	247/310	D	E	E	D	D	89.35
Glycine soja	Wild Soybean	KHN38559.1	86.17	243/282	D	E	E	D	D	95.39
Rosa chinensis	China rose/Chinese rose	XP_024170812.1	83.737	242/289	D	E	E	D	D	93.43
Gossypium barbadense	Extra long staple cotton (Sea Island Cotton)	PPS02529.1	84.083	243/289	D	E	E	D	D	93.08
Parasponia andersonii	*Parasponia andersonii*	PON79077.1	87.889	254/289	D	E	E	D	D	96.89
Morus notabilis	Black Mulberry	XP_024018217.1	87.889	254/289	D	E	E	D	D	96.19
Jatropha curcas	Jatropha curcas	XP_012091205.1	84.083	243/289	D	E	E	D	D	94.12
Citrus clementina	Clementine	XP_006435852.1	86.851	251/289	D	E	E	D	D	95.85
Cephalotus follicularis	Australian Pitcher Plant	GAV62462.1	87.197	252/289	D	E	E	D	D	95.85
Durio zibethinus	*Durio zibethinus*	XP_022741218.1	87.543	253/289	D	E	E	D	D	95.16
Populus euphratica	Desert Poplar	XP_011005868.1	84.321	242/287	D	E	E	D	D	93.73
Populus *trichocarpa*	Black cottonwood	XP_002312193.1	83.972	241/287	D	E	E	D	D	93.73
Populus trichocarpa	Black cottonwood	XP_006378643.2	83.624	240/287	D	E	E	D	D	94.43
Gossypium arboreum	Cotton Tree	XP_017628059.1	83.391	241/289	D	E	E	D	D	92.73
Trema orientalis	Charcoal-tree/Indian charcoal-tree/pigeon wood/Oriental trema	PON83245.1	87.543	253/289	D	E	E	D	D	96.54
Cucumis sativus	Cucumber	XP_004138403.1	84.429	244/289	D	E	E	D	D	92.73
Gossypium hirsutum	Mexican Cotton Tree	XP_016708559.1	83.391	241/289	D	E	E	D	D	92.73
Cucurbita pepo subsp. *Pepo*	Winter Squash	XP_023515361.1	84.775	245/289	D	E	E	D	D	93.08
Manihot esculenta	Yuca	XP_021626521.1	86.851	251/289	D	E	E	D	D	96.19
Durio zibethinus	*Durio zibethinus*	XP_022750516.1	87.591	240/274	D	N/A	N/A	N/A	N/A	94.16
Arachis duranensis	Wild Peanut	XP_015973743.1	82.007	237/289	D	E	E	D	D	94.46
Carica papaya	Papaya	XP_021904215.1	87.889	254/289	D	E	E	D	D	94.46
Arachis ipaensis	Peanut	XP_016165661.1	81.661	236/289	D	E	E	D	D	94.12
Cucurbita maxima	Squash	XP_022991069.1	84.083	243/289	D	E	E	D	D	92.73

TABLE 7-continued

Modified α-SNAP BLASTp Alignment in Plant Species

Genus Species	Plant	α-SNAP Accession Number	% Identity	Identities	D208E	E284	E285	D286	D287	% Query Cover
Corchorus olitorius	Jute Mallow	OMO69109.1	83.162	242/291	D	E	E	D	D	91.41
Hevea brasiliensis	Rubberwood	XP_021668979.1	87.197	252/289	D	E	E	D	D	94.81
Populus euphratica	Desert Poplar	XP_011015133.1	82.578	237/287	D	E	E	D	D	94.08
Cucurbita moschata	Pumpkin	XP_022964687.1	84.429	244/289	D	E	E	D	D	92.73
Hevea brasiliensis	Rubberwood	XP_021688775.1	86.159	249/289	D	E	E	D	D	95.16
Erythranthe guttata	Seep monkeyflower/ yellow monkeyflower	XP_012840021.1	80.969	234/289	D	E	E	D	D	93.77
Sesamum indicum	Sesame	XP_011084853.1	84.083	243/289	D	E	E	D	D	94.46
Medicago truncatula	BarrelClover (small Mediterranean Legume)	XP_024639705.1	87.97	234/266	D	N/A	N/A	N/A	N/A	97.37
Ricinus communis	Castor bean or castor oil	XP_002520820.1	85.813	248/289	D	E	E	D	D	95.5
Ziziphus jujuba	Jujube red date	XP_015877477.1	80.969	234/289	D	E	D	D	D	93.77
Eucalyptus grandis	Eucalyptus or Rose Gum	XP_010067574.1	81.661	236/289	D	E	E	D	D	93.43
Cucurbita moschata	Pumpkin	XP_022956354.1	80.969	234/289	D	E	E	D	D	93.77
Cucurbita maxima	Squash	XP_022991930.1	80.969	234/289	D	E	E	D	D	93.77
Momordica charantia	Bitter Melon	XP_022146873.1	80.969	234/289	D	E	E	D	D	93.43
Morus notabilis	Black Mulberry	EXB25858.1	81.613	253/310	D	E	E	D	D	89.68
Malus domestica	Apple Tree	XP_008374460.1	84.083	243/289	D	E	E	D	D	94.81
Prunus persica	Peach	XP_007218769.1	83.391	241/289	D	E	E	D	D	93.77
Prunus mume	Japanese Apricot	XP_008233838.1	83.045	240/289	D	E	E	D	D	93.77
Sesamum indicum	Sesame	XP_011076626.1	82.699	239/289	D	E	E	D	D	92.04
Cucurbita maxima	Squash	XP_022992586.1	85.467	247/289	D	E	E	D	D	94.46
Momordica charantia	Bitter Melon	XP_022134286.1	85.813	248/289	D	E	E	D	D	94.12
Olea europaea var. *sylvestris*	Wild-olive	XP _022880461.1	81.661	236/289	D	E	E	D	D	92.73
Cucurbita moschata	Pumpkin	XP_022939232.1	85.121	246/289	D	E	E	D	D	94.46
Handroanthus impetiginosus	Pink Trumpet Tree	PIN13349.1	82.007	237/289	D	E	E	D	D	91.7
Nicotiana attenuata	Coyote Tobacco	XP_019225807.1	79.585	230/289	D	E	E	D	D	92.39
Punica granatum	Pomegranate	PKI40618.1	78.547	227/289	D	E	E	D	D	91.35
Nicotiana sylvestris	Woodland tobacco/ Flowering tobacco	XP_009798526.1	79.585	230/289	D	E	E	D	D	92.73
Nicotiana tomentosiformis	Tobacco Plant	XP_009614295.1	79.585	230/289	D	E	E	D	D	92.73
Erythranthe guttata	Seep monkeyflower/ yellow monkeyflower	XP_012858890.1	79.239	229/289	D	E	D	D	D	92.39
Solanum lycopersicum	Tomato	XP_004240900.1	79.585	230/289	D	E	E	D	D	92.04

Materials & Methods

Recombinant Protein Production

[0128] Vectors encoding recombinant α-SNAP$_{Rhg1}$HC, α-SNAP$_{Rhg1}$LC, α-SNAP$_{Rhg1}$WT, α-SNAP$_{Rhg1}$WT$_{1-285}$ and the WT alleles of NSF Glyma.07G195900 (NSF$_{Ch07}$) and Glyma.13G180100 (NSF$_{Ch13}$) were generated in Bayless et al., 2016. The open reading frames (ORFs) encoding the soybean NSF$_{RAN07}$ allele of Glyma.07G195900 or *N. benthamiana* NSF were cloned into the expression vector pRham N-His-SUMO Kan according to manufacturer instructions (Lucigen). Recombinant α-SNAP and NSF proteins were also produced and purified as in Bayless et al. 2016. All expression constructs were chemically transformed into the expression strain "E. cloni 10G" (Lucigen), grown to OD_{600}~0.60-0.70, and induced with 0.2% L-Rhamnose (Sigma) for either 8 hr at 37° C. or overnight at 28° C. Soluble, native recombinant His-SUMO-α-SNAPs or His-SUMO-NSF proteins were purified with PerfectPro Ni-NTA resin (5 PRIME), and eluted with imidazole, though no subsequent gel filtration steps were performed. Following the elution of the His-SUMO-fusion proteins, overnight dialysis was performed at 4° C. in 20 mM Tris (pH 8.0), 150 mM NaCl, 10% (vol/vol) glycerol, and 1.5 mM Tris (2-carboxyethyl)-phosphine. The His-SUMO affinity/solubility tags were cleaved from α-SNAP or NSF using 1 or 2 units of SUMO Express protease (Lucigen) and separated by rebinding of the tag with Ni-NTA resin and collecting the recombinant protein from the flowthrough. Recombinant protein purity was assessed by Coomassie blue staining and quantified via a spectrophotometer.

In Vitro NSF-α-SNAP Binding Assays

[0129] In vitro NSF binding assays were performed essentially as described in Barnard et. al. (1997) J Cell Biol 139(4): 875-883; and Bayless et al. (2016), Proc Natl Acad Sci USA 113(47): E7375-E7382; Briefly, 20 μg of each respective recombinant α-SNAP protein was added to the bottom of a 1.5-mL polypropylene tube and incubated at 25° C. for 20 min. Unbound α-SNAP proteins were then washed by adding α-SNAP wash buffer [25 mM Tris, pH 7.4, 50 mM KCl, 1 mM DTT, 0.4 mg/mL bovine serum albumin (BSA)]. After removal of wash buffer, 20 μg of recombinant NSF (1 μg/μL in NSF binding buffer), was then immediately added and incubated on ice for 10 min. The solution was then removed, and samples were immediately washed 2× with NBB to remove any unbound NSF. Samples were then boiled in 1×SDS loading buffer and separated on a 10% Bis-Tris SDS-PAGE, and silver-stained using the ProteoSilver Kit (Sigma-Aldrich), according to the manufacturer directions. The percentage of NSF bound by α-SNAP was then calculated using densitometric analysis with ImageJ.

Antibody Production and Validation

[0130] Affinity-purified polyclonal rabbit antibodies raised against α-SNAP$_{Rhg1}$HC, α-SNAP$_{Rhg1}$LC and wild-type α-SNAPs were previously generated and validated using recombinant proteins in Bayless 2016. The epitopes for these custom antibodies are the final six or seven C-terminal α-SNAP residues: "EEDDLT" (SEQ ID NO: 127), "EQHEAIT" (SEQ ID NO: 128), or "EEYEVIT" (SEQ ID NO: 129) for wild-type, high-, or low-copy α-SNAPs, respectively. For NSF, a synthetic peptide, "ETEKNVRDLFADAEQDQRTRGDESD" (SEQ ID NO: 130), corresponding to residues 300 to 324 of Glyma.07G195900 was used. This NSF antibody was previously shown to be cross-reactive with the *N. benthamiana*-encoded NSF.

Immunoblotting

[0131] Tissue preparation and immunoblots were performed essentially as in (Song et al., 2015a; Bayless et al., 2016). Soybean roots or *N. benthamiana* leaf tissues were flash-frozen in N_2(L), massed, and homogenized in a PowerLyzer 24 (MO BIO) for three cycles of 15 seconds, with flash-freezing in-between each cycle. Protein extraction buffer [50 mM Tris.HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.2% Triton X-100, 10% (vol/vol) glycerol, 1/100 Sigma protease inhibitor cocktail] was then added at a 3:1 volume to mass ratio and samples were centrifuged and stored on ice. In noted experiments, Bradford assays were performed on each sample, and equal OD amounts of total protein were loaded in each sample lane for SDS/PAGE. Immunoblots for either Rhg1 α-SNAP were incubated overnight at 4° C. in 5% (wt/vol) nonfat dry milk TBS-T (50 mM Tris, 150 mM NaCl, 0.05% Tween 20) at 1:1,000. NSF immunoblots were performed similarly, except incubations were for 1 h at room temperature. Secondary horseradish peroxidase-conjugated goat anti-rabbit IgG was added at 1:10,000 and incubated for 1 h at room temperature on a platform shaker, followed by four washes with TBS-T. Chemiluminescence detection was performed with SuperSignal West Pico or Dura chemiluminescent substrate (Thermo Scientific) and developed using a ChemiDoc MP chemiluminescent imager (Bio-Rad).

Transgenic Soybean Root Generation

[0132] Binary expression constructs were transformed into *Agrobacterium rhizogenes* strain, "Arqua1". Transgenic soybean roots were produced as described in (Cook et al., 2012, Science 338, 1206-1209).

[0133] Transient *Agrobacterium* Expression in *Nicotiana benthamiana. Agrobacterium tumefaciens* strain GV3101 was used for transient protein expression of all constructs via syringe-infiltration at OD_{600} 0.60 for NSF constructs or OD_{600} 0.80 for α-SNAP constructs into young leaves of ~4-wk-old *N. benthamiana* plants. GV3101 cultures were grown overnight at 28° C. in 25 μg/mL kanamycin and rifampicin and induced for ~3.5 h in 10 mM Mes (pH 5.60), 10 mM MgCl2, and 100 μM acetosyringone prior to leaf infiltration. *N. benthamiana* plants were grown in a Percival set at 25° C. with a photoperiod of 16 h light at 100 μE·m-2·s-1 and 8 h dark. For α-SNAP complementation assays, GV3101 cultures were well-mixed with one volume of an empty vector control, or of the respective NSF construct immediately before co-infiltration. NSF$_{RAN07}$ or the *N. benthamiana* NSF were PCR amplified from a root cDNA library of Rhg1$_{LC}$ variety, "Forrest" or a *N. benthamiana* leaf cDNA library using KAPA HiFi polymerase, respectively. Expression cassettes for NSF$_{N.benthamiana}$, NSF$_{Ch13}$, NSF$_{Ch07}$ and NSF$_{RAN07}$ ORFs were directly assembled into a pBluescript vector containing the soybean ubiquitin (GmUbi) promoter and NOS terminator using Gibson assembly. The NSF expression cassettes were then digested with the restriction enzymes NotI-SalI and ligated with T4 DNA ligase into the previously described binary vector, pSM101-linker, which was cut with PspOMI-SalI restriction sites. The ORF encoding the α-SNAP$_{Ch11}$ Intron-Retention (IR) allele was amplified with Kapa HiFi from a root cDNA library of Rhg1$_{LC}$ variety "Forrest" while the ORF encoding WT α-SNAP$_{Ch11}$ was previously generated in (Bayless et al., 2016, Proc. Natl. Acad. Sci. USA 113, E7375-E7382). Both α-SNAP$_{Ch11}$ and α-SNAP$_{Ch11}$ IR were Gibson assembled into a pBluescript vector containing a GmUbi-N-HA tag and NOS terminator, cut with PstI-XbaI and ligated into the binary vector, pSM101, cut with the same restriction pair. An 11.14 kb native genomic region encoding α-SNAP$_{Rhg1}$WT was amplified with Kapa HiFi from a previously described fosmid subclone (Fosmid 19) with AvrII-SbfI restriction ends, and then digested and ligated into the binary vector, pSM101, cut with XbaI-PstI. A 6.85 kb native locus encoding α-SNAP$_{Chi\ I}$ was amplified from gDNA of Williams82 into two fragments (3.25 kb and 3.60 kb fragments) and Gibson assembled into pSM101 vector cut with BamHI-PstI.

Protein Structure Modeling and Sequence Logo

[0134] NSFRAN07, α-SNAPCh11 and α-SNAPCh11IR structural homology models were generated using SWISS-MODEL and output PDB files viewed and labeled using PyMol. NSFRAN07 was modeled to NSFCHO (Chinese hamster ovary) (PDB 3j97.1) cryo-EM structure from Zhao et al (Brunger group). 20S supercomplex modeling also generated using PDB 3j97, with α-SNAPs and SNAREs of *Rattus norvegicus* origin (Zhao et al., 2015, Nature 518: 61-67). α-SNAPCh11 and α-SNAPCh11IR were modeled to sec17 (yeast α-SNAP) crystal structure 1QQE donated courtesy of Rice et al (Rice and Brunger, 1999, Mol Cell 4: 85-95).

[0135] The R4Q NSF amino acid consensus logo was generated using WebLogo. (Crooks G E, et al. (2004), Genome Res 14: 1188-1190).

Whole-Genome Sequencing Data Analysis

[0136] Whole-genome sequencing data of 12 soybean varieties was obtained from previously published studies (Song et al., 2017, The Plant Genome 10); Cook et al., 2014 Plant Physiol 165, 630-647)). Illumina sequencing reads were aligned to the Williams 82 reference genome (Wm82.a2.v1; www.phytozome.org/) using BWA (version 0.7.12) (Li and Durbin, 2009, Bioinformatics, 25:1754-60). Reads were initially mapped using the default settings of the aln command with the subsequent pairings performed with the sampe command. Alignments were next processed using the program Picard (version 2.9.0) to add read group information (AddOrReplaceReadGroups), mark PCR duplicates (MarkDuplicates, and merge alignments from separate sequencing runs (MergeSamFiles). The processed .bam files were then converted to vcf format using a combination of samtools (version 0.1.19) and bcftools (version 0.1.19). Finally, consensus sequences were generated from these .vcf files using the FastaAlternateReferenceMaker tool within GATK (version 3.7.0; DePristo et al., 2011, Nat Genet 43: 491-498).

[0137] Having described the invention in detail and by reference to specific embodiments thereof, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims. More specifically, although some aspects of the present invention are identified herein as particularly advantageous, it is contemplated that the present invention is not necessarily limited to these particular aspects of the invention.

SEQ ID NO	Gene Designator	Nucleotide Sequence
1	Glyma.18G022 400	ATGTCTCCGGCCGCCGGAGTCAGCGTCCCCCTCCTGGGG GATTCCAAAGGAACGCCGCCGCCGGCTTCCGTCCCCGGC GCGGTGTTCAACGTGGCCACCAGCATAGTCGGCGCCGGA ATCATGTCGATTCCGGCGATCATGAAGGTTCTCGGCGTAG TTCCCGCTTTCGCGATGATTCTCGTGGTGGCCGTGCTGGC GGAACTGTCCGTGGACTTCCTGATGCGGTTCACGCACTCC GGCGAAACGACGACGTACGCTGGCGTCATGAGGGAGGC GTTCGGATCGGGTGGAGCATTgGCCGCGCAAGTTTGCGT CATCATCACCAACGTTGGGGGTTTAATTCTCTACCTTATCA TCATCGGAGATGTGCTATCTGGAAAGCAAAATGGAGGGGA AGTGCATTTGGGCATTTTGCAACAGTGGTTTGGAATTCACT GGTGGAATTCCCGGGAATTTGCTTTGCTTTTCACCTTGGT CTTTGTTATGCTTCCATTGGTATTGTACAAACGTGTAGAGT CCTTGAAGTACAGCTCTGCAGTGTCAACTCTTCTTGCAGT GGCATTTGTTGGCATATGTTGTGGGTTGGCTATCACAGCT CTGGTGCAAGGAAAAACACAAACTCCTAGATTGTTTCCTC GGCTAGACTACCAAACCTCATTCTTTGATCTGTTCACTGCA GTTCCTGTTGTTGTCACAGCCTTCACATTTCACTTTAATGT GCACCCCATTGGGTTTGAGCTTGCCAAGGCATCCCAAATG ACAACAGCAGTTCGATTAGCATTATTGCTTTGTGCTGTGAT CTACCTTGCAATAGGCTTATTTGGGTACATGTTATTTGGGG ATTCAACCCAGTCAGACATTCTCATCAATTTTGACCAGAAT GCTGGTTCAGCAGTTGGTTCCTTGCTCAATAGTTTGGTCC GTGTAAGCTATGCCCTCCACATCATGCTGGTGTTTCCTCT CTTGAACTTCTCTTTGAGAACCAACATAGATGAAGTTCTCT TCCCTAAGAAGCCTATGCTAGCCACAGACAACAAAAGATT TATGATCCTCACTCTGGTGCTGCTTGTATTCTCCTACCTTG CAGCTATAGCAATCCCAGATATTTGGTACTTCTTTCAGTTC CTGGGATCCTCATCCGCAGTGTGCCTTGCCTTCATTTTCC

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SEQ ID NO	Gene Designator	Nucleotide Sequence
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2	Glyma.18G022 500	ATGGCCGATCAGTTATCGAAGGGAGAGGAATTCGAGAAAA AGGCTGAGAAGAAGCTCAGCGGTTGGGGCTTGTTTGGCT CCAAGTATGAAGATGCCGCCGATCTCTTCGATAAAGCCGC CAATTGCTTCAAGCTCGCCAAATCATGGGACAAGGCTGGA GCGACATACCTGAAGTTGGCAAGTTGTCATTTGAAGTTGG AAAGCAAGCATGAAGCTGCACAGGCCCATGTCGATGCTG CACATTGCTACAAAAAGACTAATATAAACGAGTCTGTATCT TGCTTAGACCGAGCTGTAAATCTTTTCTGTGACATTGGAAG ACTCTCTATGGCTGCTAGATATTTAAAGGAAATTGCTGAAT TGTACGAGGGTGAACAGAATATTGAGCAGGCTCTTGTTTA CTATGAAAAATCAGCTGATTTTTTTCAAAATGAAGAAGTGA CAACTTCTGCGAACCAATGCAAACAAAAAGTTGCCCAGTT TGCTGCTCAGCTAGAACAATATCAGAAGTCGATTGACATTT ATGAAGAGATAGCTCGCCAATCCCTCAACAATAATTTGCT GAAGTATGGAGTTAAAGGACACCTTCTTAATGCTGGCATC TGCCAACTCTGTAAAGAGGACGTTGTTGCTATAACCAATG CATTAGAACGATATCAGGAACTGGATCCAACATTTTCAGG AACACGTGAATATAGATTGTTGGCGGACATTGCTGCTGCA ATTGATGAAGAAGATGTTGCAAAGTTTACTGATGTTGTCAA GGAATTTGATAGTATGACCCCTCTGGATTCTTGGAAGACC ACACTTCTCTTAAGGGTGAAGGAAAAGCTGAAAGCCAAAG AACTTGAGGAGGATGATCTTACTTGA
3	Glyma.18G022 700	ATGCGCATGCTCACCGGCGACTCCGCCGCCGACAACTCC TTCCGATTCGTTCCGCAGTCCATCGCCGCCTTCGGCTCCA CCGTCATCGTCGAGGGCTGCGACTCCGCCCGCAACATTG CCTGGGTCCACGCCTGGACCGTCACTGATGGGATGATCA CTCAAATCAGAGAGTACTTCAACACCGCCCTCACCGTCAC TCGCATCCACGATTCCGGCGAGATTGTTCCGGCCAGATCC GGCGCCGGCCGTTTGCCCTGCGTCTGGGAGAGCAGCGT CTCCGGTCGGGTCGGGAAATCCGTCCCCGGTTTGGTTCT CGCAATATAA
4	Glyma.18G022 600	ATGGTTTCGGTTGATGATGGGATTGTGAATCCCAATGATG AAATTGAGAAATCTAACGGGAGTAAAGTGAATGAGTTTGC ATCTATGGATATTTCAGCAACTCAAAAATCATATCTGAACA GTGAAGATCCTCAGAGAAGGCTTCAGGGAACCTTAATAAG TTCTTCTGTTACTAATAGGATAAACTTTCTTAAATTTGGTTC TGCATCTGCCAAATTCAAAAGGCTTGCTACTGAGAGAGAC CAGGTTTCTATATCTGTGCCTTCTCCTCGTTCAAAGAGCCT AAGATCACGTTTCAGTGGCATGTTTGCTCAGAAACTTGACT GGGCTTCAGTCAAGAAAATGTGCATGGAATGGATTAGAAA TCCAGTGAACATGGCCCTTTTTGTGTGGATCATTTGTGTC GCGGTTTCGGGTGCTATTCTGTTCCTTGTCATGACAGGCA TGTTGAATGGTGTGCTACCAAGAAAGTCTAAGAGAAATGC ATGGTTTGAAGTAAACAACCAAATACTCAATGCAGTGTTTA CACTCATGTGTTTGTACCAACACCCTAAGAGATTCTACCAC CTTGTTCTTCTGACCAGATGAAGACCAAATGACATCTCTAG CCTTAGGAAGGTATATTGCAAGAATGTCACTTACAAGCCC CATGAGTGGACACATATGATGGTAGTTGTCATTCTCCTTCA TGTTAACTGTTTTGCTCAATATGCACTTTGTGGTCTAAACT TAGGGTATAAAAGGTCCGAGAGACCTGCCATTGGAGTTGG AATATGCATATCTTTTGCAATTGCTGGTTTGTACACCATTC TTAGCCCACTTGGGAAGGACTATGATTGTGAGATGGATGA AGAAGCACAGGTTCAAATTACAGCTTCTCAAGGGAAAGAG CAGCTGAGAGAGAAACCAACTGAGAAGAAATATTCATTTG CATCCAAAGATCAACAAAGGGTTGTTGAAAATAGACCAAA GTGGAGTGGAGGAATACTTGACATTTGGAACGATATTTCC TTAGCATATCTCTCACTTTTCTGCACCTTTTGTGTGCTTGG GTGGAATATGAAGAGGCTTGGCTTTGGAAACATGTATGTT CACATTGCCATTTTTATGCTGTTCTGTATGGCTCCTTTCTG GATTTTTCTTTTGGCTTCCGTTAACATAGATGATGACAATG TTAGGCAGGCTCTAGCAGCTGTTGGAATCATTCTTTGTTTT CTTGGTTTATTGTATGGTGGATTTTGGAGGATCCAAATGAG AAAGAGGTTCAATTTACCAGCCTATGACTTCTGTTTTGGCA AACCTTCAGCTTCTGATTGCACACTTTGGCTACCCTGTTGC TGGTGCTCTCTCGCTCAAGAAGCGCGTACCAGGAATAACT ATGATCTTGTAGAAGATAAATTCTCAAGGAAAGAAACTGAT

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SEQ ID NO	Gene Designator	Nucleotide Sequence
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5	Glyma.18G022 500. Fayette	ATGGCCGATCAGTTATCGAAGGGAGAGGAATTCGAGAAAA AGGCTGAGAAGAAGCTCAGCGGTTGGGGCTTGTTTGGCT CCAAGTATGAAGATGCCGCCGATCTCTTCGATAAAGCCGC CAATTGCTTCAAGCTCGCCAAATCATGGGACAAGGCTGGA GCGACATACCTGAAGTTGGCAAGTTGTCATTTGAAGTTGG AAAGCAAGCATGAAGCTGCACAGGCCCATGTCGATGCTG CACATTGCTACAAAAAGACTAATATAAACGAGTCTGTATCT TGCTTAGACCGAGCTGTAAATCTTTTCTGTGACATTGGAAG ACTCTCTATGGCTGCTAGATATTTAAAGGAAATTGCTGAAT TGTACGAGGGTGAACAGAATATTGAGCAGGCTCTTGTTTA CTATGAAAAATCAGCTGATTTTTTTCAAAATGAAGAAGTGA CAACTTCTGCGAACCAATGCAAACAAAAAGTTGCCCAGTT TGCTGCTCAGCTAGAACAATATCAGAAGTCGATTGACATTT ATGAAGAGATAGCTCGCCAATCCCTCAACAATAATTTGCT GAAGTATGGAGTTAAAGGACACCTTCTTAATGCTGGCATC TGCAAACTCTGTAAAGAGGACGTTGTTGCTATAACCAATG CATTAGAACGATATCAGGAACTGGATCCAACATTTTCAGG AACACGTGAATATAGATTGTTGGCGGACATTGCTGCTGCA ATTGATGAAGAAGATGTTGCAAAGTTTACTGATGTTGTCAA GGAATTTGATAGTATGACCCCTCTGGATTCTTGGAAGACC ACACTTCTCTTAAGGGTGAAGGAAAAGCTGAAAGCCAAAG AACTTGAGCAGCATGAGGCTATTACTTGA
6	Glyma.18G022 500 Peking	ATGGCCGATCAGTTATCGAAGGGAGAGGAATTCGAGAAAA AGGCTGAGAAGAAGCTCAGCGGTTGGGGCTTGTTTGGCT CCAAGTATGAAGATGCCGCCGATCTCTTCGATAAAGCCGC CAATTGCTTCAAGCTCGCCAAATCATGGGACAAGGCTGGA GCGACATACCTGAAGTTGGCAAGTTGTCATTTGAAGTTGG AAAGCAAGCATGAAGCTGCACAGGCCCATGTCGATGCTG CACATTGCTACAAAAAGACTAATATAAACGAGTCTGTATCT TGCTTAGACCGAGCTGTAAATCTTTTCTGTGACATTGGAAG ACTCTCTATGGCTGCTAGATATTTAAAGGAAATTGCTGAAT TGTACGAGGGTGAACAGAATATTGAGCAGGCTCTTGTTTA CTATGAAAAATCAGCTGATTTTTTTCAAAATGAAGAAGTGA CAACTTCTGCGAACCAATGCAAACAAAAAGTTGCCCAGTT TGCTGCTCAGCTAGAACAATATCAGAAGTCGATTGACATTT ATGAAGAGATAGCTCGCCAATCCCTCAACAATAATTTGCT GAAGTATGGAGTTAAAGGACACCTTCTTAATGCTGGCATC TGCCAACTCTGTAAAGAGGAGGTTGTTGCTATAACCAATG CATTAGAACGATATCAGGAACTGGATCCAACATTTTCAGG AACACGTGAATATAGATTGTTGGCGGACATTGCTGCTGCA ATTGATGAAGAAGATGTTGCAAAGTTTACTGATGTTGTCAA GGAATTTGATAGTATGACCCCTCTGGATTCTTGGAAGACC ACACTTCTCTTAAGGGTGAAGGAAAAGCTGAAAGCCAAAG AACTTGAGGAGTATGAGGTTATTACTTGA
7	Glyma.18G022 500 Peking Iso	ATGGCCGATCAGTTATCGAAGGGAGAGGAATTCGAGAAAA AGGCTGAGAAGAAGCTCAGCGGTTGGGGCTTGTTTGGCT CCAAGTATGAAGATGCCGCCGATCTCTTCGATAAAGCCGC CAATTGCTTCAAGCTCGCCAAATCATGGGACAAGGCTGGA GCGACATACCTGAAGTTGGCAAGTTGTCATTTGAAGTTGG AAAGCAAGCATGAAGCTGCACAGGCCCATGTCGATGCTG CACATTGCTACAAAAAGACTAATATAAACGAGTCTGTATCT TGCTTAGACCGAGCTGTAAATCTTTTCTGTGACATTGGAAG ACTCTCTATGGCTGCTAGATATTTAAAGGAAATTGCTGAAT TGTACGAGGGTGAACAGAATATTGAGCAGGCTCTTGTTTA CTATGAAAAATCAGCTGATTTTTTTCAAAATGAAGAAGTGA CAACTTCTGCGAACCAATGCAAACAAAAAGTTGCCCAGTT TGCTGCTCAGCTAGAACAATATCAGAAGTCGATTGACATTT ATGAAGAGATAGCTCGCCAATCCCTCAACAATAATTTGCT GAAGTATGGAGTTAAAGGACACCTTCTTAATGCTGGCATC TGCCAACTCTGTAAAGAGGAGGAACTGGATCCAACATTTT CAGGAACACGTGAATATAGATTGTTGGCGGACATTGCTGC

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SEQ ID NO	Gene Designator	Nucleotide Sequence
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atctgcaaac tctgtaaaga ggacgttgtt gctataacca atgcattaga acgatatcag    660
gaactggatc caacattttc aggaacacgt gaatatagat tgttggcgga cattgctgct    720
gcaattgatg aagaagatgt tgcaaagttt actgatgttg tcaaggaatt tgatagtatg    780
acccctctgg attcttggaa gaccacactt ctcttaaggg tgaaggaaaa gctgaaagcc    840
aaagaacttg agcagcatga ggctattact tga                                 873
```

<210> SEQ ID NO 6
<211> LENGTH: 873
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 6

```
atggccgatc agttatcgaa gggagaggaa ttcgagaaaa aggctgagaa gaagctcagc     60
ggttggggct tgtttggctc caagtatgaa gatgccgccg atctcttcga taaagccgcc    120
aattgcttca agctcgccaa atcatgggac aaggctggag cgacatacct gaagttggca    180
agttgtcatt tgaagttgga aagcaagcat gaagctgcac aggcccatgt cgatgctgca    240
cattgctaca aaaagactaa tataaacgag tctgtatctt gcttagaccg agctgtaaat    300
cttttctgtg acattggaag actctctatg gctgctagat atttaaagga aattgctgaa    360
ttgtacgagg gtgaacagaa tattgagcag gctcttgttt actatgaaaa atcagctgat    420
ttttttcaaa atgaagaagt gacaacttct gcgaaccaat gcaaacaaaa agttgcccag    480
tttgctgctc agctagaaca atatcagaag tcgattgaca tttatgaaga gatagctcgc    540
caatccctca acaataattt gctgaagtat ggagttaaag gacaccttct taatgctggc    600
atctgccaac tctgtaaaga ggaggttgtt gctataacca atgcattaga acgatatcag    660
gaactggatc caacattttc aggaacacgt gaatatagat tgttggcgga cattgctgct    720
gcaattgatg aagaagatgt tgcaaagttt actgatgttg tcaaggaatt tgatagtatg    780
acccctctgg attcttggaa gaccacactt ctcttaaggg tgaaggaaaa gctgaaagcc    840
aaagaacttg aggagtatga ggttattact tga                                 873
```

<210> SEQ ID NO 7
<211> LENGTH: 837
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 7

```
atggccgatc agttatcgaa gggagaggaa ttcgagaaaa aggctgagaa gaagctcagc     60
ggttggggct tgtttggctc caagtatgaa gatgccgccg atctcttcga taaagccgcc    120
aattgcttca agctcgccaa atcatgggac aaggctggag cgacatacct gaagttggca    180
agttgtcatt tgaagttgga aagcaagcat gaagctgcac aggcccatgt cgatgctgca    240
cattgctaca aaaagactaa tataaacgag tctgtatctt gcttagaccg agctgtaaat    300
cttttctgtg acattggaag actctctatg gctgctagat atttaaagga aattgctgaa    360
ttgtacgagg gtgaacagaa tattgagcag gctcttgttt actatgaaaa atcagctgat    420
ttttttcaaa atgaagaagt gacaacttct gcgaaccaat gcaaacaaaa agttgcccag    480
```

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```
tttgctgctc agctagaaca atatcagaag tcgattgaca tttatgaaga gatagctcgc    540

caatccctca acaataattt gctgaagtat ggagttaaag gacaccttct taatgctggc    600

atctgccaac tctgtaaaga ggaggaactg gatccaacat tttcaggaac acgtgaatat    660

agattgttgg cggacattgc tgctgcaatt gatgaagaag atgttgcaaa gtttactgat    720

gttgtcaagg aatttgatag tatgacccct ctggattctt ggaagaccac acttctctta    780

agggtgaagg aaaagctgaa agccaaagaa cttgaggagt atgaggttat tacttga       837
```

<210> SEQ ID NO 8
<211> LENGTH: 2241
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 8

```
atggcgagtc ggttcgggtt atcgtcttcg tcttcctctg cgtccagcat gagagttacc     60

aacacgcccg cgagcgacct cgccctcacc aacctcgcct tctgttcccc ctccgatctc    120

cgcaatttcg ccgtccctgg ccacaataac ctctacctcg ccgccgtcgc cgattccttc    180

gtcttatctc tctctgctca tgacaccata ggcagcggtc agattgcgtt gaatgccgtt    240

caacgccggt gtgccaaagt ttcttccggt gattccgtac aagtgagccg atttgtgccg    300

cctgaagatt tcaacctcgc actgctaact cttgaattgg aatttgttaa aaaggggagt    360

aagagtgagc agattgatgc tgttctactg gctaagcaac ttcgtaagag atttatgaac    420

caggttatga ctgtggggca gaaagtatta tttgagtatc acggaaataa ttatagcttt    480

actgtcagta atgctgctgt tgagggccaa gaaaagtcta attctcttga aagagggatg    540

atttcagatg acacatacat tgtttttgaa acatcacgtg atagtggaat taagattgtc    600

aatcaacgag agggtgccac tagcaacatt ttcaagcaga aagaatttaa ccttcagtca    660

ctgggtattg gtggcctgag tgcagaattt gcagatatat ttcgaagagc ttttgcctct    720

cgtgttttcc caccccatgt gacatctaaa ttaggaatca agcatgtgaa gggcatgctt    780

ctttatgggc ctcctggaac tggaaagaca cttatggcac gccaaattgg aaaaattttg    840

aatgggaagg aacccaagat tgtaaatggc cctgaagttt tgagcaaatt tgttggtgaa    900

actgaaaaga atgtgagaga cctttttgct gatgctgaac aggatcagag gacccgaggg    960

gatgaaagtg atttgcatgt tataatcttt gatgaaattg atgctatttg caagtcaaga   1020

ggttcaactc gagatggtac tggagttcat gatagtattg taaatcagct tcttactaag   1080

atagatggtg tggagtcact aaataatgtt ttacttattg gaatgactaa cagaaaggac   1140

atgcttgatg aagctctctt aagaccaggg aggttggaag tccaggttga gataagcctt   1200

cctgatgaaa atggtcgatt gcaaattctt caaatccata ctaacaaaat gaaagagaat   1260

tcttttctag ctgctgatgt gaaccttcaa gagcttgctg ctcgaacgaa aaactacagt   1320

ggtgcagaac ttgaaggtgt tgtgaaaagt gctgtctcat atgctttaaa tagacaattg   1380

agtctagagg atctcactaa gccagtggag gaagagaaca ttaaggttac aatggatgac   1440

tttttgaatg cactccacga agttacttcc gcatttggag cttcaactga tgatcttgaa   1500

agatgcagac tccatggcat ggttgagtgt ggtgatcgac ataagcacat ttatcaaaga   1560

gcaatgctac ttgtggagca agttaaagtg agtaaaggaa gcccacttgt cacttgtctc   1620

ctggaaggtt cccgtggcag tggtaaaact gcactttcag ctactgttgg tatcgacagc   1680
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```
gacttcccat acgtcaagat agtttcagct gaatcaatga ttggtctaca tgagagcacc   1740

aaatgtgcac agattattaa ggtttttgag gatgcataca agtcaccatt gagtgtcatc   1800

attcttgatg acattgagag attattggag tatgtgccca ttggtcctcg attttcaaac   1860

ttgatttctc agacactgct ggttctgctc aaacggcttc ctccaaaggg gaaaaaacta   1920

atggttattg gcacaacaag tgaactagat ttcttggaat caattggatt ttgtgatacc   1980

ttctctgtta cttaccatat tcctaccttg aacacaacgg atgcaaagaa ggtcctagaa   2040

cagttgaatg tgtttactga tgaagatatt gattctgctg cagaggcgtt gaatgatatg   2100

cctatcagga aactatacat gttgatcgag atggcagcgc aaggggagca tggtggatct   2160

gcagaagcca tcttttctgg caaagagaag attagtatcg ctcatttcta tgattgcctc   2220

caggatgttg ttaggttata a                                             2241
```

<210> SEQ ID NO 9
<211> LENGTH: 2244
<212> TYPE: DNA
<213> ORGANISM: Glycine max

<400> SEQUENCE: 9

```
atggcgagtc agttcgggtt atcgtcttcg tcttcctctg cgtccagcat gagagttacc     60

tacacgcccg cgaacgacct cgccctcacc aacctcgcct tctgttcccc ctccgatctc    120

cgcaatttcg ccgtccctgg ccacaataac ctctacctcg ccgccgtcgc cgattccttc    180

gtcttatctc tctctgctca tgacaccata ggcagcggtc agattgcgtt gaatgccgtt    240

caacgccggt gtgccaaagt ttcttccggt gattccgtac aagtgagccg atttgtgccg    300

cctgaagatt tcaacctcgc actgctaact cttgaattgg aattttttgt taaaaagggg    360

agtaagagtg agcagattga tgctgttcta ctggctaagc aacttcgtaa gagatttatg    420

aaccaggtta tgactgtggg gcagaaagta ttatttgagt atcacggaaa taattatagc    480

tttactgtca gtaatgctgc tgttgagggc caagaaaagt ctaattctct tgaaagaggg    540

attatttcag atgacacata cattgttttt gaaacatcac gtgatagtgg aattaagatt    600

gtcaatcaac gagagggtgc cactagcaac attttcaagc agaaagaatt taaccttcag    660

tcactgggta ttggtggcct gagtgcagaa tttgcagata tatttcgaag agcttttgcc    720

tctcgtgttt tcccacccca tgtgacatct aaattaggga tcaagcatgt gaagggcatg    780

cttctttatg ggcctcctgg aactggaaag acacttatgg cacgccaaat tggaaaaatt    840

ttgaatggga aggaacccaa gattgtaaat ggccctgaag ttttgagcaa atttgttggt    900

gaaactgaaa agaatgtgag agaccttttt gctgatgctg aacaggatca gaggacccga    960

ggggatgaaa gtgatttgca tgttataatc tttgatgaaa ttgatgctat ttgcaagtca   1020

agaggttcaa ctcgagatgg tactggagtt catgatagta ttgtaaatca gcttcttact   1080

aagatagatg gtgtggagtc actaaataat gttttactta ttggaatgac taacagaaag   1140

gacatgcttg atgaagctct cttaagacca gggaggttgg aagtccaggt tgagataagc   1200

cttcctgatg aaaatggtcg attgcaaatt cttcaaattc atactaacaa aatgaaagag   1260

aattcttttc tagctgctga tgtgaacctt caagagcttg ctgctcgaac gaaaaactac   1320

agtggtgcag aacttgaagg tgttgtgaaa agtgctgtct catatgcttt aaatagacaa   1380

ttgagtctag aggatctcac taagccagtg gaggaagaga acattaaggt tacaatggat   1440
```

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```
gactttttga atgcactcca cgaagttact tccgcatttg gagcttcaac tgatgatctt   1500

gaaagatgca gactccatgg catggttgag tgtggtgatc gacataagca catttatcaa   1560

agagcaatgc tacttgtgga gcaagttaaa gtgagtaaag gaagcccact tgtcacttgt   1620

ctcctggaag gttcccgtgg cagtggtaaa actgcacttt cagctactgt tggtatcgac   1680

agcgacttcc catacgtcaa gatagtttca gctgaatcaa tgattggtct acatgagagc   1740

accaaatgtg cacagattat taaggttttt gaggatgcat acaagtcacc attgagtgtc   1800

atcattcttg atgacattga gagattattg gagtatgtgc ccattggtcc tcgattttca   1860

aacttgattt ctcagacact gctggttctg ctcaaacggc ttcctccaaa ggggaaaaaa   1920

ctcatggtta ttggcacaac aagtgaacta gatttcttgg aatcaattgg attttgtgat   1980

accttctctg ttacttacca tattcctacc ttgaacacaa cggatgcaaa gaaggtccta   2040

gaacagttga atgtgtttac tgatgaagat attgattctg ctgcagaggc gttgaatgat   2100

atgcctatca ggaaactata catgttgatc gagatggcag cgcaagggga gcatggtgga   2160

tctgcagaag ccatcttttc tggcaaagag aagattagta tcgctcactt ctatgattgc   2220

ctccaggatg ttgttaggtt atga                                          2244
```

```
<210> SEQ ID NO 10
<211> LENGTH: 436
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 10

Met Ser Pro Ala Ala Gly Val Ser Val Pro Leu Leu Gly Asp Ser Lys
1               5                   10                  15

Gly Thr Pro Pro Pro Ala Ser Val Pro Gly Ala Val Phe Asn Val Ala
            20                  25                  30

Thr Ser Ile Val Gly Ala Gly Ile Met Ser Ile Pro Ala Ile Met Lys
        35                  40                  45

Val Leu Gly Val Val Pro Ala Phe Ala Met Ile Leu Val Val Ala Val
    50                  55                  60

Leu Ala Glu Leu Ser Val Asp Phe Leu Met Arg Phe Thr His Ser Gly
65                  70                  75                  80

Glu Thr Thr Thr Tyr Ala Gly Val Met Arg Glu Ala Phe Gly Ser Gly
                85                  90                  95

Gly Ala Leu Ala Ala Gln Val Cys Val Ile Ile Thr Asn Val Gly Gly
            100                 105                 110

Leu Ile Leu Tyr Leu Ile Ile Ile Gly Asp Val Leu Ser Gly Lys Gln
        115                 120                 125

Asn Gly Gly Glu Val His Leu Gly Ile Leu Gln Gln Trp Phe Gly Ile
    130                 135                 140

His Trp Trp Asn Ser Arg Glu Phe Ala Leu Leu Phe Thr Leu Val Phe
145                 150                 155                 160

Val Met Leu Pro Leu Val Leu Tyr Lys Arg Val Glu Ser Leu Lys Tyr
                165                 170                 175

Ser Ser Ala Val Ser Thr Leu Leu Ala Val Ala Phe Val Gly Ile Cys
            180                 185                 190

Cys Gly Leu Ala Ile Thr Ala Leu Val Gln Gly Lys Thr Gln Thr Pro
        195                 200                 205
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Arg Leu Phe Pro Arg Leu Asp Tyr Gln Thr Ser Phe Phe Asp Leu Phe
    210                 215                 220

Thr Ala Val Pro Val Val Val Thr Ala Phe Thr Phe His Phe Asn Val
225                 230                 235                 240

His Pro Ile Gly Phe Glu Leu Ala Lys Ala Ser Gln Met Thr Thr Ala
                245                 250                 255

Val Arg Leu Ala Leu Leu Leu Cys Ala Val Ile Tyr Leu Ala Ile Gly
            260                 265                 270

Leu Phe Gly Tyr Met Leu Phe Gly Asp Ser Thr Gln Ser Asp Ile Leu
        275                 280                 285

Ile Asn Phe Asp Gln Asn Ala Gly Ser Ala Val Gly Ser Leu Leu Asn
    290                 295                 300

Ser Leu Val Arg Val Ser Tyr Ala Leu His Ile Met Leu Val Phe Pro
305                 310                 315                 320

Leu Leu Asn Phe Ser Leu Arg Thr Asn Ile Asp Glu Val Leu Phe Pro
                325                 330                 335

Lys Lys Pro Met Leu Ala Thr Asp Asn Lys Arg Phe Met Ile Leu Thr
            340                 345                 350

Leu Val Leu Leu Val Phe Ser Tyr Leu Ala Ala Ile Ala Ile Pro Asp
        355                 360                 365

Ile Trp Tyr Phe Phe Gln Phe Leu Gly Ser Ser Ser Ala Val Cys Leu
    370                 375                 380

Ala Phe Ile Phe Pro Gly Ser Ile Val Leu Arg Asp Val Lys Gly Ile
385                 390                 395                 400

Ser Thr Arg Arg Asp Lys Ile Ile Ala Leu Ile Met Ile Ile Leu Ala
                405                 410                 415

Val Val Thr Ser Val Leu Ala Ile Ser Thr Asn Ile Tyr Asn Ala Phe
            420                 425                 430

Ser Ser Lys Ser
        435


<210> SEQ ID NO 11
<211> LENGTH: 289
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 11

Met Ala Asp Gln Leu Ser Lys Gly Glu Glu Phe Glu Lys Lys Ala Glu
1               5                   10                  15

Lys Lys Leu Ser Gly Trp Gly Leu Phe Gly Ser Lys Tyr Glu Asp Ala
            20                  25                  30

Ala Asp Leu Phe Asp Lys Ala Ala Asn Cys Phe Lys Leu Ala Lys Ser
        35                  40                  45

Trp Asp Lys Ala Gly Ala Thr Tyr Leu Lys Leu Ala Ser Cys His Leu
    50                  55                  60

Lys Leu Glu Ser Lys His Glu Ala Ala Gln Ala His Val Asp Ala Ala
65                  70                  75                  80

His Cys Tyr Lys Lys Thr Asn Ile Asn Glu Ser Val Ser Cys Leu Asp
                85                  90                  95

Arg Ala Val Asn Leu Phe Cys Asp Ile Gly Arg Leu Ser Met Ala Ala
            100                 105                 110

Arg Tyr Leu Lys Glu Ile Ala Glu Leu Tyr Glu Gly Glu Gln Asn Ile
        115                 120                 125
```

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Glu Gln Ala Leu Val Tyr Tyr Glu Lys Ser Ala Asp Phe Phe Gln Asn
    130                 135                 140

Glu Glu Val Thr Thr Ser Ala Asn Gln Cys Lys Gln Lys Val Ala Gln
145                 150                 155                 160

Phe Ala Ala Gln Leu Glu Gln Tyr Gln Lys Ser Ile Asp Ile Tyr Glu
                165                 170                 175

Glu Ile Ala Arg Gln Ser Leu Asn Asn Asn Leu Leu Lys Tyr Gly Val
            180                 185                 190

Lys Gly His Leu Leu Asn Ala Gly Ile Cys Gln Leu Cys Lys Glu Asp
        195                 200                 205

Val Val Ala Ile Thr Asn Ala Leu Glu Arg Tyr Gln Glu Leu Asp Pro
    210                 215                 220

Thr Phe Ser Gly Thr Arg Glu Tyr Arg Leu Leu Ala Asp Ile Ala Ala
225                 230                 235                 240

Ala Ile Asp Glu Glu Asp Val Ala Lys Phe Thr Asp Val Val Lys Glu
                245                 250                 255

Phe Asp Ser Met Thr Pro Leu Asp Ser Trp Lys Thr Thr Leu Leu Leu
            260                 265                 270

Arg Val Lys Glu Lys Leu Lys Ala Lys Glu Leu Glu Glu Asp Asp Leu
        275                 280                 285

Thr


<210> SEQ ID NO 12
<211> LENGTH: 80
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 12

Met Arg Met Leu Thr Gly Asp Ser Ala Ala Asp Asn Ser Phe Arg Phe
1               5                   10                  15

Val Pro Gln Ser Ile Ala Ala Phe Gly Ser Thr Val Ile Val Glu Gly
            20                  25                  30

Cys Asp Ser Ala Arg Asn Ile Ala Trp Val His Ala Trp Thr Val Thr
        35                  40                  45

Asp Gly Met Ile Thr Gln Ile Arg Glu Tyr Phe Asn Thr Ala Leu Thr
    50                  55                  60

Val Thr Arg Ile His Asp Ser Gly Glu Ile Val Pro Ala Arg Ser Gly
65                  70                  75                  80


<210> SEQ ID NO 13
<211> LENGTH: 536
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 13

Met Val Ser Val Asp Asp Gly Ile Val Asn Pro Asn Asp Glu Ile Glu
1               5                   10                  15

Lys Ser Asn Gly Ser Lys Val Asn Glu Phe Ala Ser Met Asp Ile Ser
            20                  25                  30

Ala Thr Gln Lys Ser Tyr Leu Asn Ser Glu Asp Pro Gln Arg Arg Leu
        35                  40                  45

Gln Gly Thr Leu Ile Ser Ser Ser Val Thr Asn Arg Ile Asn Phe Leu
    50                  55                  60
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```
Lys Phe Gly Ser Ala Ser Ala Lys Phe Lys Arg Leu Ala Thr Glu Arg
65                  70                  75                  80

Asp Gln Val Ser Ile Ser Val Pro Ser Pro Arg Ser Lys Ser Leu Arg
                85                  90                  95

Ser Arg Phe Ser Gly Met Phe Ala Gln Lys Leu Asp Trp Ala Ser Val
            100                 105                 110

Lys Lys Met Cys Met Glu Trp Ile Arg Asn Pro Val Asn Met Ala Leu
        115                 120                 125

Phe Val Trp Ile Ile Cys Val Ala Val Ser Gly Ala Ile Leu Phe Leu
    130                 135                 140

Val Met Thr Gly Met Leu Asn Gly Val Leu Pro Arg Lys Ser Lys Arg
145                 150                 155                 160

Asn Ala Trp Phe Glu Val Asn Asn Gln Ile Leu Asn Ala Val Phe Thr
                165                 170                 175

Leu Ile Pro Asn Asp Ile Ser Ser Leu Arg Lys Val Tyr Cys Lys Asn
            180                 185                 190

Val Thr Tyr Lys Pro His Glu Trp Thr His Met Met Val Val Val Ile
        195                 200                 205

Leu Leu His Val Asn Cys Phe Ala Gln Tyr Ala Leu Cys Gly Leu Asn
    210                 215                 220

Leu Gly Tyr Lys Arg Ser Glu Arg Pro Ala Ile Gly Val Gly Ile Cys
225                 230                 235                 240

Ile Ser Phe Ala Ile Ala Gly Leu Tyr Thr Ile Leu Ser Pro Leu Gly
                245                 250                 255

Lys Asp Tyr Asp Cys Glu Met Asp Glu Glu Ala Gln Val Gln Ile Thr
            260                 265                 270

Ala Ser Gln Gly Lys Glu Gln Leu Arg Glu Lys Pro Thr Glu Lys Lys
        275                 280                 285

Tyr Ser Phe Ala Ser Lys Asp Gln Gln Arg Val Val Glu Asn Arg Pro
    290                 295                 300

Lys Trp Ser Gly Gly Ile Leu Asp Ile Trp Asn Asp Ile Ser Leu Ala
305                 310                 315                 320

Tyr Leu Ser Leu Phe Cys Thr Phe Cys Val Leu Gly Trp Asn Met Lys
                325                 330                 335

Arg Leu Gly Phe Gly Asn Met Tyr Val His Ile Ala Ile Phe Met Leu
            340                 345                 350

Phe Cys Met Ala Pro Phe Trp Ile Phe Leu Leu Ala Ser Val Asn Ile
        355                 360                 365

Asp Asp Asp Asn Val Arg Gln Ala Leu Ala Ala Val Gly Ile Ile Leu
    370                 375                 380

Cys Phe Leu Gly Leu Leu Tyr Gly Gly Phe Trp Arg Ile Gln Met Arg
385                 390                 395                 400

Lys Arg Phe Asn Leu Pro Ala Tyr Asp Phe Cys Phe Gly Lys Pro Ser
                405                 410                 415

Ala Ser Asp Cys Thr Leu Trp Leu Pro Cys Cys Trp Cys Ser Leu Ala
            420                 425                 430

Gln Glu Ala Arg Thr Arg Asn Asn Tyr Asp Leu Val Glu Asp Lys Phe
        435                 440                 445

Ser Arg Lys Glu Thr Asp Thr Ser Asp Gln Pro Ser Ile Ser Pro Leu
    450                 455                 460

Ala Arg Glu Asp Val Val Ser Thr Arg Ser Gly Thr Ser Ser Pro Met
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465                 470                 475                 480

Gly Ser Thr Ser Asn Ser Ser Pro Tyr Met Met Lys Thr Ser Ser Ser
                485                 490                 495

Pro Asn Ser Ser Asn Val Leu Lys Gly Tyr Tyr Ser Pro Asp Lys Met
            500                 505                 510

Leu Ser Thr Leu Asn Glu Asp Asn Cys Glu Arg Gly Gln Asp Gly Thr
        515                 520                 525

Met Asn Pro Leu Tyr Ala Gln Lys
    530                 535


<210> SEQ ID NO 14
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 14

Met Ala Asp Gln Leu Ser Lys Gly Glu Glu Phe Glu Lys Lys Ala Glu
1               5                   10                  15

Lys Lys Leu Ser Gly Trp Gly Leu Phe Gly Ser Lys Tyr Glu Asp Ala
            20                  25                  30

Ala Asp Leu Phe Asp Lys Ala Ala Asn Cys Phe Lys Leu Ala Lys Ser
        35                  40                  45

Trp Asp Lys Ala Gly Ala Thr Tyr Leu Lys Leu Ala Ser Cys His Leu
    50                  55                  60

Lys Leu Glu Ser Lys His Glu Ala Ala Gln Ala His Val Asp Ala Ala
65                  70                  75                  80

His Cys Tyr Lys Lys Thr Asn Ile Asn Glu Ser Val Ser Cys Leu Asp
                85                  90                  95

Arg Ala Val Asn Leu Phe Cys Asp Ile Gly Arg Leu Ser Met Ala Ala
            100                 105                 110

Arg Tyr Leu Lys Glu Ile Ala Glu Leu Tyr Glu Gly Glu Gln Asn Ile
        115                 120                 125

Glu Gln Ala Leu Val Tyr Tyr Glu Lys Ser Ala Asp Phe Phe Gln Asn
    130                 135                 140

Glu Glu Val Thr Thr Ser Ala Asn Gln Cys Lys Gln Lys Val Ala Gln
145                 150                 155                 160

Phe Ala Ala Gln Leu Glu Gln Tyr Gln Lys Ser Ile Asp Ile Tyr Glu
                165                 170                 175

Glu Ile Ala Arg Gln Ser Leu Asn Asn Asn Leu Leu Lys Tyr Gly Val
            180                 185                 190

Lys Gly His Leu Leu Asn Ala Gly Ile Cys Lys Leu Cys Lys Glu Asp
        195                 200                 205

Val Val Ala Ile Thr Asn Ala Leu Glu Arg Tyr Gln Glu Leu Asp Pro
    210                 215                 220

Thr Phe Ser Gly Thr Arg Glu Tyr Arg Leu Leu Ala Asp Ile Ala Ala
225                 230                 235                 240

Ala Ile Asp Glu Glu Asp Val Ala Lys Phe Thr Asp Val Val Lys Glu
                245                 250                 255

Phe Asp Ser Met Thr Pro Leu Asp Ser Trp Lys Thr Thr Leu Leu Leu
            260                 265                 270

Arg Val Lys Glu Lys Leu Lys Ala Lys Glu Leu Glu Gln His Glu Ala
        275                 280                 285
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Ile Thr
    290


<210> SEQ ID NO 15
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 15

Met Ala Asp Gln Leu Ser Lys Gly Glu Glu Phe Glu Lys Lys Ala Glu
1               5                   10                  15

Lys Lys Leu Ser Gly Trp Gly Leu Phe Gly Ser Lys Tyr Glu Asp Ala
            20                  25                  30

Ala Asp Leu Phe Asp Lys Ala Ala Asn Cys Phe Lys Leu Ala Lys Ser
        35                  40                  45

Trp Asp Lys Ala Gly Ala Thr Tyr Leu Lys Leu Ala Ser Cys His Leu
    50                  55                  60

Lys Leu Glu Ser Lys His Glu Ala Ala Gln Ala His Val Asp Ala Ala
65                  70                  75                  80

His Cys Tyr Lys Lys Thr Asn Ile Asn Glu Ser Val Ser Cys Leu Asp
                85                  90                  95

Arg Ala Val Asn Leu Phe Cys Asp Ile Gly Arg Leu Ser Met Ala Ala
            100                 105                 110

Arg Tyr Leu Lys Glu Ile Ala Glu Leu Tyr Glu Gly Glu Gln Asn Ile
        115                 120                 125

Glu Gln Ala Leu Val Tyr Tyr Glu Lys Ser Ala Asp Phe Phe Gln Asn
    130                 135                 140

Glu Glu Val Thr Thr Ser Ala Asn Gln Cys Lys Gln Lys Val Ala Gln
145                 150                 155                 160

Phe Ala Ala Gln Leu Glu Gln Tyr Gln Lys Ser Ile Asp Ile Tyr Glu
                165                 170                 175

Glu Ile Ala Arg Gln Ser Leu Asn Asn Asn Leu Leu Lys Tyr Gly Val
            180                 185                 190

Lys Gly His Leu Leu Asn Ala Gly Ile Cys Gln Leu Cys Lys Glu Glu
        195                 200                 205

Val Val Ala Ile Thr Asn Ala Leu Glu Arg Tyr Gln Glu Leu Asp Pro
    210                 215                 220

Thr Phe Ser Gly Thr Arg Glu Tyr Arg Leu Leu Ala Asp Ile Ala Ala
225                 230                 235                 240

Ala Ile Asp Glu Glu Asp Val Ala Lys Phe Thr Asp Val Val Lys Glu
                245                 250                 255

Phe Asp Ser Met Thr Pro Leu Asp Ser Trp Lys Thr Thr Leu Leu Leu
            260                 265                 270

Arg Val Lys Glu Lys Leu Lys Ala Lys Glu Leu Glu Glu Tyr Glu Val
        275                 280                 285

Ile Thr
    290


<210> SEQ ID NO 16
<211> LENGTH: 278
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 16
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Met Ala Asp Gln Leu Ser Lys Gly Glu Glu Phe Glu Lys Lys Ala Glu
1               5                   10                  15

Lys Lys Leu Ser Gly Trp Gly Leu Phe Gly Ser Lys Tyr Glu Asp Ala
            20                  25                  30

Ala Asp Leu Phe Asp Lys Ala Ala Asn Cys Phe Lys Leu Ala Lys Ser
        35                  40                  45

Trp Asp Lys Ala Gly Ala Thr Tyr Leu Lys Leu Ala Ser Cys His Leu
    50                  55                  60

Lys Leu Glu Ser Lys His Glu Ala Ala Gln Ala His Val Asp Ala Ala
65                  70                  75                  80

His Cys Tyr Lys Lys Thr Asn Ile Asn Glu Ser Val Ser Cys Leu Asp
                85                  90                  95

Arg Ala Val Asn Leu Phe Cys Asp Ile Gly Arg Leu Ser Met Ala Ala
            100                 105                 110

Arg Tyr Leu Lys Glu Ile Ala Glu Leu Tyr Glu Gly Glu Gln Asn Ile
        115                 120                 125

Glu Gln Ala Leu Val Tyr Tyr Glu Lys Ser Ala Asp Phe Phe Gln Asn
    130                 135                 140

Glu Glu Val Thr Thr Ser Ala Asn Gln Cys Lys Gln Lys Val Ala Gln
145                 150                 155                 160

Phe Ala Ala Gln Leu Glu Gln Tyr Gln Lys Ser Ile Asp Ile Tyr Glu
                165                 170                 175

Glu Ile Ala Arg Gln Ser Leu Asn Asn Asn Leu Leu Lys Tyr Gly Val
            180                 185                 190

Lys Gly His Leu Leu Asn Ala Gly Ile Cys Gln Leu Cys Lys Glu Glu
        195                 200                 205

Glu Leu Asp Pro Thr Phe Ser Gly Thr Arg Glu Tyr Arg Leu Leu Ala
    210                 215                 220

Asp Ile Ala Ala Ala Ile Asp Glu Glu Asp Val Ala Lys Phe Thr Asp
225                 230                 235                 240

Val Val Lys Glu Phe Asp Ser Met Thr Pro Leu Asp Ser Trp Lys Thr
                245                 250                 255

Thr Leu Leu Leu Arg Val Lys Glu Lys Leu Lys Ala Lys Glu Leu Glu
            260                 265                 270

Glu Tyr Glu Val Ile Thr
        275


<210> SEQ ID NO 17
<211> LENGTH: 746
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 17

Met Ala Ser Arg Phe Gly Leu Ser Ser Ser Ser Ser Ser Ala Ser Ser
1               5                   10                  15

Met Arg Val Thr Asn Thr Pro Ala Ser Asp Leu Ala Leu Thr Asn Leu
            20                  25                  30

Ala Phe Cys Ser Pro Ser Asp Leu Arg Asn Phe Ala Val Pro Gly His
        35                  40                  45

Asn Asn Leu Tyr Leu Ala Ala Val Ala Asp Ser Phe Val Leu Ser Leu
    50                  55                  60

Ser Ala His Asp Thr Ile Gly Ser Gly Gln Ile Ala Leu Asn Ala Val
65                  70                  75                  80
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Gln Arg Arg Cys Ala Lys Val Ser Ser Gly Asp Ser Val Gln Val Ser
                85                  90                  95

Arg Phe Val Pro Pro Glu Asp Phe Asn Leu Ala Leu Leu Thr Leu Glu
            100                 105                 110

Leu Glu Phe Val Lys Lys Gly Ser Lys Ser Glu Gln Ile Asp Ala Val
        115                 120                 125

Leu Leu Ala Lys Gln Leu Arg Lys Arg Phe Met Asn Gln Val Met Thr
    130                 135                 140

Val Gly Gln Lys Val Leu Phe Glu Tyr His Gly Asn Asn Tyr Ser Phe
145                 150                 155                 160

Thr Val Ser Asn Ala Ala Val Glu Gly Gln Glu Lys Ser Asn Ser Leu
                165                 170                 175

Glu Arg Gly Met Ile Ser Asp Asp Thr Tyr Ile Val Phe Glu Thr Ser
            180                 185                 190

Arg Asp Ser Gly Ile Lys Ile Val Asn Gln Arg Glu Gly Ala Thr Ser
        195                 200                 205

Asn Ile Phe Lys Gln Lys Glu Phe Asn Leu Gln Ser Leu Gly Ile Gly
    210                 215                 220

Gly Leu Ser Ala Glu Phe Ala Asp Ile Phe Arg Arg Ala Phe Ala Ser
225                 230                 235                 240

Arg Val Phe Pro Pro His Val Thr Ser Lys Leu Gly Ile Lys His Val
                245                 250                 255

Lys Gly Met Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu Met
            260                 265                 270

Ala Arg Gln Ile Gly Lys Ile Leu Asn Gly Lys Glu Pro Lys Ile Val
        275                 280                 285

Asn Gly Pro Glu Val Leu Ser Lys Phe Val Gly Glu Thr Glu Lys Asn
    290                 295                 300

Val Arg Asp Leu Phe Ala Asp Ala Glu Gln Asp Gln Arg Thr Arg Gly
305                 310                 315                 320

Asp Glu Ser Asp Leu His Val Ile Ile Phe Asp Glu Ile Asp Ala Ile
                325                 330                 335

Cys Lys Ser Arg Gly Ser Thr Arg Asp Gly Thr Gly Val His Asp Ser
            340                 345                 350

Ile Val Asn Gln Leu Leu Thr Lys Ile Asp Gly Val Glu Ser Leu Asn
        355                 360                 365

Asn Val Leu Leu Ile Gly Met Thr Asn Arg Lys Asp Met Leu Asp Glu
    370                 375                 380

Ala Leu Leu Arg Pro Gly Arg Leu Glu Val Gln Val Glu Ile Ser Leu
385                 390                 395                 400

Pro Asp Glu Asn Gly Arg Leu Gln Ile Leu Gln Ile His Thr Asn Lys
                405                 410                 415

Met Lys Glu Asn Ser Phe Leu Ala Ala Asp Val Asn Leu Gln Glu Leu
            420                 425                 430

Ala Ala Arg Thr Lys Asn Tyr Ser Gly Ala Glu Leu Glu Gly Val Val
        435                 440                 445

Lys Ser Ala Val Ser Tyr Ala Leu Asn Arg Gln Leu Ser Leu Glu Asp
    450                 455                 460

Leu Thr Lys Pro Val Glu Glu Glu Asn Ile Lys Val Thr Met Asp Asp
465                 470                 475                 480
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Phe Leu Asn Ala Leu His Glu Val Thr Ser Ala Phe Gly Ala Ser Thr
485 490 495

Asp Asp Leu Glu Arg Cys Arg Leu His Gly Met Val Glu Cys Gly Asp
500 505 510

Arg His Lys His Ile Tyr Gln Arg Ala Met Leu Leu Val Glu Gln Val
515 520 525

Lys Val Ser Lys Gly Ser Pro Leu Val Thr Cys Leu Leu Glu Gly Ser
530 535 540

Arg Gly Ser Gly Lys Thr Ala Leu Ser Ala Thr Val Gly Ile Asp Ser
545 550 555 560

Asp Phe Pro Tyr Val Lys Ile Val Ser Ala Glu Ser Met Ile Gly Leu
565 570 575

His Glu Ser Thr Lys Cys Ala Gln Ile Ile Lys Val Phe Glu Asp Ala
580 585 590

Tyr Lys Ser Pro Leu Ser Val Ile Ile Leu Asp Asp Ile Glu Arg Leu
595 600 605

Leu Glu Tyr Val Pro Ile Gly Pro Arg Phe Ser Asn Leu Ile Ser Gln
610 615 620

Thr Leu Leu Val Leu Leu Lys Arg Leu Pro Pro Lys Gly Lys Lys Leu
625 630 635 640

Met Val Ile Gly Thr Thr Ser Glu Leu Asp Phe Leu Glu Ser Ile Gly
645 650 655

Phe Cys Asp Thr Phe Ser Val Thr Tyr His Ile Pro Thr Leu Asn Thr
660 665 670

Thr Asp Ala Lys Lys Val Leu Glu Gln Leu Asn Val Phe Thr Asp Glu
675 680 685

Asp Ile Asp Ser Ala Ala Glu Ala Leu Asn Asp Met Pro Ile Arg Lys
690 695 700

Leu Tyr Met Leu Ile Glu Met Ala Ala Gln Gly Glu His Gly Gly Ser
705 710 715 720

Ala Glu Ala Ile Phe Ser Gly Lys Glu Lys Ile Ser Ile Ala His Phe
725 730 735

Tyr Asp Cys Leu Gln Asp Val Val Arg Leu
740 745

<210> SEQ ID NO 18
<211> LENGTH: 747
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 18

Met Ala Ser Gln Phe Gly Leu Ser Ser Ser Ser Ser Ser Ala Ser Ser
1 5 10 15

Met Arg Val Thr Tyr Thr Pro Ala Asn Asp Leu Ala Leu Thr Asn Leu
20 25 30

Ala Phe Cys Ser Pro Ser Asp Leu Arg Asn Phe Ala Val Pro Gly His
35 40 45

Asn Asn Leu Tyr Leu Ala Ala Val Ala Asp Ser Phe Val Leu Ser Leu
50 55 60

Ser Ala His Asp Thr Ile Gly Ser Gly Gln Ile Ala Leu Asn Ala Val
65 70 75 80

Gln Arg Arg Cys Ala Lys Val Ser Ser Gly Asp Ser Val Gln Val Ser
85 90 95

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Arg Phe Val Pro Pro Glu Asp Phe Asn Leu Ala Leu Leu Thr Leu Glu
            100                 105                 110

Leu Glu Phe Phe Val Lys Lys Gly Ser Lys Ser Glu Gln Ile Asp Ala
        115                 120                 125

Val Leu Leu Ala Lys Gln Leu Arg Lys Arg Phe Met Asn Gln Val Met
    130                 135                 140

Thr Val Gly Gln Lys Val Leu Phe Glu Tyr His Gly Asn Asn Tyr Ser
145                 150                 155                 160

Phe Thr Val Ser Asn Ala Ala Val Glu Gly Gln Glu Lys Ser Asn Ser
                165                 170                 175

Leu Glu Arg Gly Ile Ile Ser Asp Asp Thr Tyr Ile Val Phe Glu Thr
            180                 185                 190

Ser Arg Asp Ser Gly Ile Lys Ile Val Asn Gln Arg Glu Gly Ala Thr
        195                 200                 205

Ser Asn Ile Phe Lys Gln Lys Glu Phe Asn Leu Gln Ser Leu Gly Ile
    210                 215                 220

Gly Gly Leu Ser Ala Glu Phe Ala Asp Ile Phe Arg Arg Ala Phe Ala
225                 230                 235                 240

Ser Arg Val Phe Pro Pro His Val Thr Ser Lys Leu Gly Ile Lys His
                245                 250                 255

Val Lys Gly Met Leu Leu Tyr Gly Pro Pro Gly Thr Gly Lys Thr Leu
            260                 265                 270

Met Ala Arg Gln Ile Gly Lys Ile Leu Asn Gly Lys Glu Pro Lys Ile
        275                 280                 285

Val Asn Gly Pro Glu Val Leu Ser Lys Phe Val Gly Glu Thr Glu Lys
    290                 295                 300

Asn Val Arg Asp Leu Phe Ala Asp Ala Glu Gln Asp Gln Arg Thr Arg
305                 310                 315                 320

Gly Asp Glu Ser Asp Leu His Val Ile Ile Phe Asp Glu Ile Asp Ala
                325                 330                 335

Ile Cys Lys Ser Arg Gly Ser Thr Arg Asp Gly Thr Gly Val His Asp
            340                 345                 350

Ser Ile Val Asn Gln Leu Leu Thr Lys Ile Asp Gly Val Glu Ser Leu
        355                 360                 365

Asn Asn Val Leu Leu Ile Gly Met Thr Asn Arg Lys Asp Met Leu Asp
    370                 375                 380

Glu Ala Leu Leu Arg Pro Gly Arg Leu Glu Val Gln Val Glu Ile Ser
385                 390                 395                 400

Leu Pro Asp Glu Asn Gly Arg Leu Gln Ile Leu Gln Ile His Thr Asn
                405                 410                 415

Lys Met Lys Glu Asn Ser Phe Leu Ala Ala Asp Val Asn Leu Gln Glu
            420                 425                 430

Leu Ala Ala Arg Thr Lys Asn Tyr Ser Gly Ala Glu Leu Glu Gly Val
        435                 440                 445

Val Lys Ser Ala Val Ser Tyr Ala Leu Asn Arg Gln Leu Ser Leu Glu
    450                 455                 460

Asp Leu Thr Lys Pro Val Glu Glu Glu Asn Ile Lys Val Thr Met Asp
465                 470                 475                 480

Asp Phe Leu Asn Ala Leu His Glu Val Thr Ser Ala Phe Gly Ala Ser
                485                 490                 495
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Thr Asp Asp Leu Glu Arg Cys Arg Leu His Gly Met Val Glu Cys Gly
            500                 505                 510

Asp Arg His Lys His Ile Tyr Gln Arg Ala Met Leu Leu Val Glu Gln
        515                 520                 525

Val Lys Val Ser Lys Gly Ser Pro Leu Val Thr Cys Leu Leu Glu Gly
    530                 535                 540

Ser Arg Gly Ser Gly Lys Thr Ala Leu Ser Ala Thr Val Gly Ile Asp
545                 550                 555                 560

Ser Asp Phe Pro Tyr Val Lys Ile Val Ser Ala Glu Ser Met Ile Gly
                565                 570                 575

Leu His Glu Ser Thr Lys Cys Ala Gln Ile Ile Lys Val Phe Glu Asp
            580                 585                 590

Ala Tyr Lys Ser Pro Leu Ser Val Ile Ile Leu Asp Asp Ile Glu Arg
        595                 600                 605

Leu Leu Glu Tyr Val Pro Ile Gly Pro Arg Phe Ser Asn Leu Ile Ser
    610                 615                 620

Gln Thr Leu Leu Val Leu Leu Lys Arg Leu Pro Pro Lys Gly Lys Lys
625                 630                 635                 640

Leu Met Val Ile Gly Thr Thr Ser Glu Leu Asp Phe Leu Glu Ser Ile
                645                 650                 655

Gly Phe Cys Asp Thr Phe Ser Val Thr Tyr His Ile Pro Thr Leu Asn
            660                 665                 670

Thr Thr Asp Ala Lys Lys Val Leu Glu Gln Leu Asn Val Phe Thr Asp
        675                 680                 685

Glu Asp Ile Asp Ser Ala Ala Glu Ala Leu Asn Asp Met Pro Ile Arg
    690                 695                 700

Lys Leu Tyr Met Leu Ile Glu Met Ala Ala Gln Gly Glu His Gly Gly
705                 710                 715                 720

Ser Ala Glu Ala Ile Phe Ser Gly Lys Glu Lys Ile Ser Ile Ala His
                725                 730                 735

Phe Tyr Asp Cys Leu Gln Asp Val Val Arg Leu
            740                 745


<210> SEQ ID NO 19
<211> LENGTH: 740
<212> TYPE: PRT
<213> ORGANISM: Cricetulus griseus

<400> SEQUENCE: 19

Met Ala Gly Arg Ser Met Gln Ala Ala Arg Cys Pro Thr Asp Glu Leu
1               5                   10                  15

Ser Leu Ser Asn Cys Ala Val Val Ser Glu Lys Asp Tyr Gln Ser Gly
            20                  25                  30

Gln His Val Ile Val Arg Thr Ser Pro Asn His Lys Tyr Ile Phe Thr
        35                  40                  45

Leu Arg Thr His Pro Ser Val Val Pro Gly Ser Val Ala Phe Ser Leu
    50                  55                  60

Pro Gln Arg Lys Trp Ala Gly Leu Ser Ile Gly Gln Glu Ile Glu Val
65                  70                  75                  80

Ala Leu Tyr Ser Phe Asp Lys Ala Lys Gln Cys Ile Gly Thr Met Thr
                85                  90                  95

Ile Glu Ile Asp Phe Leu Gln Lys Lys Asn Ile Asp Ser Asn Pro Tyr
            100                 105                 110
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Asp Thr Asp Lys Met Ala Ala Glu Phe Ile Gln Gln Phe Asn Asn Gln
        115                 120                 125

Ala Phe Ser Val Gly Gln Gln Leu Val Phe Ser Phe Asn Asp Lys Leu
    130                 135                 140

Phe Gly Leu Leu Val Lys Asp Ile Glu Ala Met Asp Pro Ser Ile Leu
145                 150                 155                 160

Lys Gly Glu Pro Ala Ser Gly Lys Arg Gln Lys Ile Glu Val Gly Leu
                165                 170                 175

Val Val Gly Asn Ser Gln Val Ala Phe Glu Lys Ala Glu Asn Ser Ser
            180                 185                 190

Leu Asn Leu Ile Gly Lys Ala Lys Thr Lys Glu Asn Arg Gln Ser Ile
        195                 200                 205

Ile Asn Pro Asp Trp Asn Phe Glu Lys Met Gly Ile Gly Gly Leu Asp
    210                 215                 220

Lys Glu Phe Ser Asp Ile Phe Arg Arg Ala Phe Ala Ser Arg Val Phe
225                 230                 235                 240

Pro Pro Glu Ile Val Glu Gln Met Gly Cys Lys His Val Lys Gly Ile
                245                 250                 255

Leu Leu Tyr Gly Pro Pro Gly Cys Gly Lys Thr Leu Leu Ala Arg Gln
            260                 265                 270

Ile Gly Lys Met Leu Asn Ala Arg Glu Pro Lys Val Val Asn Gly Pro
        275                 280                 285

Glu Ile Leu Asn Lys Tyr Val Gly Glu Ser Glu Ala Asn Ile Arg Lys
    290                 295                 300

Leu Phe Ala Asp Ala Glu Glu Glu Gln Arg Arg Leu Gly Ala Asn Ser
305                 310                 315                 320

Gly Leu His Ile Ile Ile Phe Asp Glu Ile Asp Ala Ile Cys Lys Gln
                325                 330                 335

Arg Gly Ser Met Ala Gly Ser Thr Gly Val His Asp Thr Val Val Asn
            340                 345                 350

Gln Leu Leu Ser Lys Ile Asp Gly Val Glu Gln Leu Asn Asn Ile Leu
        355                 360                 365

Val Ile Gly Met Thr Asn Arg Pro Asp Leu Ile Asp Glu Ala Leu Leu
    370                 375                 380

Arg Pro Gly Arg Leu Glu Val Lys Met Glu Ile Gly Leu Pro Asp Glu
385                 390                 395                 400

Lys Gly Arg Leu Gln Ile Leu His Ile His Thr Ala Arg Met Arg Gly
                405                 410                 415

His Gln Leu Leu Ser Ala Asp Val Asp Ile Lys Glu Leu Ala Val Glu
            420                 425                 430

Thr Lys Asn Phe Ser Gly Ala Glu Leu Glu Gly Leu Val Arg Ala Ala
        435                 440                 445

Gln Ser Thr Ala Met Asn Arg His Ile Lys Ala Ser Thr Lys Val Glu
    450                 455                 460

Val Asp Met Glu Lys Ala Glu Ser Leu Gln Val Thr Arg Gly Asp Phe
465                 470                 475                 480

Leu Ala Ser Leu Glu Asn Asp Ile Lys Pro Ala Phe Gly Thr Asn Gln
                485                 490                 495

Glu Asp Tyr Ala Ser Tyr Ile Met Asn Gly Ile Ile Lys Trp Gly Asp
            500                 505                 510
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Pro Val Thr Arg Val Leu Asp Asp Gly Glu Leu Leu Val Gln Gln Thr
        515                 520                 525

Lys Asn Ser Asp Arg Thr Pro Leu Val Ser Val Leu Leu Glu Gly Pro
    530                 535                 540

Pro His Ser Gly Lys Thr Ala Leu Ala Ala Lys Ile Ala Glu Glu Ser
545                 550                 555                 560

Asn Phe Pro Phe Ile Lys Ile Cys Ser Pro Asp Lys Met Ile Gly Phe
                565                 570                 575

Ser Glu Thr Ala Lys Cys Gln Ala Met Lys Lys Ile Phe Asp Asp Ala
            580                 585                 590

Tyr Lys Ser Gln Leu Ser Cys Val Val Val Asp Asp Ile Glu Arg Leu
        595                 600                 605

Leu Asp Tyr Val Pro Ile Gly Pro Arg Phe Ser Asn Leu Val Leu Gln
    610                 615                 620

Ala Leu Leu Val Leu Leu Lys Lys Ala Pro Pro Gln Gly Arg Lys Leu
625                 630                 635                 640

Leu Ile Ile Gly Thr Thr Ser Arg Lys Asp Val Leu Gln Glu Met Glu
                645                 650                 655

Met Leu Asn Ala Phe Ser Thr Thr Ile His Val Pro Asn Ile Ala Thr
            660                 665                 670

Gly Glu Gln Leu Leu Glu Ala Leu Glu Leu Leu Gly Asn Phe Lys Asp
        675                 680                 685

Lys Glu Arg Thr Thr Ile Ala Gln Gln Val Lys Gly Lys Lys Val Trp
    690                 695                 700

Ile Gly Ile Lys Lys Leu Leu Met Leu Ile Glu Met Ser Leu Gln Met
705                 710                 715                 720

Asp Pro Glu Tyr Arg Val Arg Lys Phe Leu Ala Leu Leu Arg Glu Glu
                725                 730                 735

Gly Ala Ser Pro
            740
```

What is claimed is:

1. A method of producing plant cells with enhanced nematode resistance, comprising:

a) increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of,

(i) one or more polynucleotides encoding alpha-soluble N-ethylmaleimide-sensitive factor Attachment Protein (α-SNAP), or resistance-promoting variants thereof, or

(ii) one or more polynucleotides encoding soluble N-ethylmaleimide-sensitive factor (NSF) proteins, or homologs or variants thereof,

wherein the plant cells exhibit increased resistance to nematodes.

2. The method of claim **1**, wherein,

a polynucleotide encoding one or more α-SNAP proteins has at least 75% identity to a polynucleotide identified by SEQ ID NOs: 2, 5 or 6, or

an encoded polypeptide has at least 75% identity to a polypeptide identified by SEQ ID NOs: 11, 14 or 15, or homologs or variants thereof, and

a polynucleotide encoding one or more NSF proteins has at least 75% identity to a polynucleotide identified by SEQ ID NOS: 8 or 9, or

an encoded polypeptide has at least 75% identity to a polypeptide identified by SEQ ID NOs 17 or 18, or homologs or variants thereof.

3. The method of claim **1**, wherein the one or more polynucleotides encodes a modified α-SNAP polypeptide, wherein:

the modified α-SNAP polypeptide comprises one or a plurality of amino acid modifications at positions corresponding to positions 203, 208, 285, 286, 287, and 288 with numbering relative to the α-SNAP polypeptide set forth in SEQ ID NO: 11 or to positions 203, 208, 285, 286, 287, 288, or 289 with numbering relative to the α-SNAP set forth in SEQ ID NOS: 14 or 15;

the modified α-SNAP polypeptide comprises the amino acid modification or amino acid modifications compared to the α-SNAP set forth in SEQ ID NOS 11, 14, or 15; whereby the modified α-SNAP polypeptide comprises a sequence of amino acids that has less than 100% identity or has 100% identity to the modified and more than 75% identity to the α-SNAP polypeptide as set forth in SEQ ID NO 11; and the modified α-SNAP polypeptide comprises a sequence of amino acids that has greater than 75% sequence identity to the α-SNAP set forth in SEQ ID NOS: 11; and the modified α-SNAP confers enhanced nematode resistance in the plant cell that is greater than the nematode resistance in the plant cell without the α-SNAP amino acid modification or amino acid modifications.

4. The method of claim **3**, wherein the encoded modified α-SNAP comprises amino acid modifications at positions corresponding to positions 208, 285, 286, 287, and 288 by α-SNAP numbering relative to position in the α-SNAP polypeptide set forth in SEQ ID NO: 11.

5. The method of claim **3**, wherein the modified polynucleotides encode a modified α-SNAP polypeptide, wherein the modified α-SNAP polypeptide comprises:

a replacement at position D286 that is D286F, or D286W, or D286Y; and

a replacement at position D287 that is D287E or remains D287; and

an insertion after position 287 that is (ins)288A, (ins) 288G, (ins)2881, (ins)288L, (ins)288M, or (ins)288V; and

a replacement at position L288 that is L288A, L288G, L2881, L2881, L288M, or L288V, or

a functional equivalent amino acid to the WT amino acid expressed at position 285, 286, 287, or 288, each by α-SNAP numbering relative to the positions set for in SEQ ID NO: 11.

6. The method of claim **5**, wherein the encoded modified NSF polypeptide comprises same family amino acid modifications selected from among modifications corresponding to:

D286F/D287E/(del)288A/L289A;
D286F/D287E/(del)288A/L289G;
D286F/D287E/(del)288A/L2891;
D286F/D287E/(del)288A/L289L;
D286F/D287E/(del)288A/L289M;
D286F/D287E/(del)288A/L289V;
D286F/D287E/(del)288G/L289A;
D286F/D287E/(del)288G/L289G;
D286F/D287E/(del)288G/L2891;
D286F/D287E/(del)288G/L289L;
D286F/D287E/(del)288G/L289M;
D286F/D287E/(del)288G/L289V
D286F/D287E/(del)2881/L289A;
D286F/D287E/(del)2881/L289G;
D286F/D287E/(del)2881/L2891;
D286F/D287E/(del)2881/L289L;
D286F/D287E/(del)2881/L289M;
D286F/D287E/(del)2881/L289V;
D286F/D287E/(del)288L/L289A;
D286F/D287E/(del)288L/L289G;
D286F/D287E/(del)288L/L2891;
D286F/D287E/(del)288L/L289L;
D286F/D287E/(del)288L/L289M;
D286F/D287E/(del)288L/L289V;
D286F/D287E/(del)288M/L289A;
D286F/D287E/(del)288M/L289G;
D286F/D287E/(del)288M/L281;
D286F/D287E/(del)288M/L289L;
D286F/D287E/(del)288M/L289M;
D286F/D287E/(del)288M/L289V;
D286F/D287E/(del)288V/L289A;
D286F/D287E/(del)288V/L289G;
D286F/D287E/(del)288V/L281;
D286F/D287E/(del)288V/L289L;
D286F/D287E/(del)288V/L289M;
D286F/D287E/(del)288V/L289V;
D286F/D287/(del)288A/L289A;
D286F/D287/(del)288A/L289G;
D286F/D287/(del)288A/L2891;
D286F/D287/(del)288A/L289L;
D286F/D287/(del)288A/L289M;
D286F/D287/(del)288A/L289V;
D286F/D287/(del)288G/L289A;
D286F/D287/(del)288G/L289G;
D286F/D287/(del)288G/L2891;
D286F/D287/(del)288G/L289L;
D286F/D287/(del)288G/L289M;
D286F/D287/(del)288G/L289V;
D286F/D287/(del)2881/L289A;
D286F/D287/(del)2881/L289G;
D286F/D287/(del)2881/L2891;
D286F/D287/(del)2881/L289L;
D286F/D287/(del)2881/L289M;
D286F/D287/(del)2881/L289V;
D286F/D287/(del)288L/L289A;
D286F/D287/(del)288L/L289G;
D286F/D287/(del)288L/L2891;
D286F/D287/(del)288L/L289L;
D286F/D287/(del)288L/L289M;
D286F/D287/(del)288L/L289V;
D286F/D287/(del)288M/L289A;
D286F/D287/(del)288M/L289G;
D286F/D287/(del)288M/L281;
D286F/D287/(del)288M/L289L;
D286F/D287/(del)288M/L289M;
D286F/D287/(del)288M/L289V;
D286F/D287/(del)288V/L289A;
D286F/D287/(del)288V/L289G;
D286F/D287/(del)288V/L281;
D286F/D287/(del)288V/L289L;
D286F/D287/(del)288V/L289M;
D286F/D287/(del)288V/L289V;
D286W/D287E/(del)288A/L289A;
D286W/D287E/(del)288A/L289G;
D286W/D287E/(del)288A/L2891;
D286W/D287E/(del)288A/L289L;
D286W/D287E/(del)288A/L289M;
D286W/D287E/(del)288A/L289V;
D286W/D287E/(del)288G/L289A;
D286W/D287E/(del)288G/L289G;
D286W/D287E/(del)288G/L2891;
D286W/D287E/(del)288G/L289L;
D286W/D287E/(del)288G/L289M;
D286W/D287E/(del)288G/L289V;
D286W/D287E/(del)2881/L289A;
D286W/D287E/(del)2881/L289G;
D286W/D287E/(del)2881/L2891;
D286W/D287E/(del)2881/L289L;
D286W/D287E/(del)2881/L289M;
D286W/D287E/(del)2881/L289V;
D286W/D287E/(del)288L/L289A;
D286W/D287E/(del)288L/L289G;
D286W/D287E/(del)288L/L2891;
D286W/D287E/(del)288L/L289L;
D286W/D287E/(del)288L/L289M;
D286W/D287E/(del)288L/L289V;
D286W/D287E/(del)288M/L289A;
D286W/D287E/(del)288M/L289G;
D286W/D287E/(del)288M/L281;

D286W/D287E/(del)288M/L289L;
D286W/D287E/(del)288M/L289M;
D286W/D287E/(del)288M/L289V;
D286W/D287E/(del)288V/L289A;
D286W/D287E/(del)288V/L289G;
D286W/D287E/(del)288V/L281;
D286W/D287E/(del)288V/L289L;
D286W/D287E/(del)288V/L289M;
D286W/D287E/(del)288V/L289V;
D286W/D287/(del)288A/L289A;
D286W/D287/(del)288A/L289G;
D286W/D287/(del)288A/L2891;
D286W/D287/(del)288A/L289L;
D286W/D287/(del)288A/L289M;
D286W/D287/(del)288A/L289V;
D286W/D287/(del)288G/L289A;
D286W/D287/(del)288G/L289G;
D286W/D287/(del)288G/L2891;
D286W/D287/(del)288G/L289L;
D286W/D287/(del)288G/L289M;
D286W/D287/(del)288G/L289V;
D286W/D287/(del)2881/L289A;
D286W/D287/(del)2881/L289G;
D286W/D287/(del)2881/L2891;
D286W/D287/(del)2881/L289L;
D286W/D287/(del)2881/L289M;
D286W/D287/(del)2881/L289V;
D286W/D287/(del)288L/L289A;
D286W/D287/(del)288L/L289G;
D286W/D287/(del)288L/L2891;
D286W/D287/(del)288L/L289L;
D286W/D287/(del)288L/L289M;
D286W/D287/(del)288L/L289V;
D286W/D287/(del)288M/L289A;
D286W/D287/(del)288M/L289G;
D286W/D287/(del)288M/L281;
D286W/D287/(del)288M/L289L;
D286W/D287/(del)288M/L289M;
D286W/D287/(del)288M/L289V;
D286W/D287/(del)288V/L289A;
D286W/D287/(del)288V/L289G;
D286W/D287/(del)288V/L281;
D286W/D287/(del)288V/L289L;
D286W/D287/(del)288V/L289M;
D286W/D287/(del)288V/L289V;
D286Y/D287E/(del)288A/L289A;
D286Y/D287E/(del)288A/L289G;
D286Y/D287E/(del)288A/L2891;
D286Y/D287E/(del)288A/L289L;
D286Y/D287E/(del)288A/L289M;
D286Y/D287E/(del)288A/L289V;
D286Y/D287E/(del)288G/L289A;
D286Y/D287E/(del)288G/L289G;
D286Y/D287E/(del)288G/L2891;
D286Y/D287E/(del)288G/L289L;
D286Y/D287E/(del)288G/L289M;
D286Y/D287E/(del)288G/L289V;
D286Y/D287E/(del)2881/L289A;
D286Y/D287E/(del)2881/L289G;
D286Y/D287E/(del)2881/L2891;
D286Y/D287E/(del)2881/L289L;
D286Y/D287E/(del)2881/L289M;
D286Y/D287E/(del)2881/L289V;
D286Y/D287E/(del)288L/L289A;
D286Y/D287E/(del)288L/L289G;
D286Y/D287E/(del)288L/L2891;
D286Y/D287E/(del)288L/L289L;
D286Y/D287E/(del)288L/L289M;
D286Y/D287E/(del)288L/L289V;
D286Y/D287E/(del)288M/L289A;
D286Y/D287E/(del)288M/L289G;
D286Y/D287E/(del)288M/L281;
D286Y/D287E/(del)288M/L289L;
D286Y/D287E/(del)288M/L289M;
D286Y/D287E/(del)288M/L289V;
D286Y/D287E/(del)288V/L289A;
D286Y/D287E/(del)288V/L289G;
D286Y/D287E/(del)288V/L281;
D286Y/D287E/(del)288V/L289L;
D286Y/D287E/(del)288V/L289M;
D286Y/D287E/(del)288V/L289V;
D286Y/D287/(del)288A/L289A;
D286Y/D287/(del)288A/L289G;
D286Y/D287/(del)288A/L2891;
D286Y/D287/(del)288A/L289L;
D286Y/D287/(del)288A/L289M;
D286Y/D287/(del)288A/L289V;
D286Y/D287/(del)288G/L289A;
D286Y/D287/(del)288G/L289G;
D286Y/D287/(del)288G/L2891;
D286Y/D287/(del)288G/L289L;
D286Y/D287/(del)288G/L289M;
D286Y/D287/(del)288G/L289V;
D286Y/D287/(del)2881/L289A;
D286Y/D287/(del)2881/L289G;
D286Y/D287/(del)2881/L2891;
D286Y/D287/(del)2881/L289L;
D286Y/D287/(del)2881/L289M;
D286Y/D287/(del)2881/L289V;
D286Y/D287/(del)288L/L289A;
D286Y/D287/(del)288L/L289G;
D286Y/D287/(del)288L/L2891;
D286Y/D287/(del)288L/L289L;
D286Y/D287/(del)288L/L289M;
D286Y/D287/(del)288L/L289V;
D286Y/D287/(del)288M/L289A;
D286Y/D287/(del)288M/L289G;
D286Y/D287/(del)288M/L281;
D286Y/D287/(del)288M/L289L;
D286Y/D287/(del)288M/L289M;
D286Y/D287/(del)288M/L289V;
D286Y/D287/(del)288V/L289A;
D286Y/D287/(del)288V/L289G;
D286Y/D287/(del)288V/L281;
D286Y/D287/(del)288V/L289L;
D286Y/D287/(del)288V/L289M; and
D286Y/D287/(del)288V/L289V, each with number relative to positions set forth in SEQ ID NOS: 11, 14, or 15.

7. The method of claim **3**, wherein the one or more polynucleotides encode a modified α-SNAP polypeptide, wherein:

the encoded α-SNAP polypeptide comprises at least one modification corresponding to D208E, numbering corresponding by alignment with the polypeptide of SEQ ID NO: 14, or Q203K, numbering corresponding by alignment with the polypeptide of SEQ ID NO:15.

8. The method of claim **3**, wherein the encoded modified α-SNAP further comprises optional amino acid replacements, including amino acid insertions or deletions, at positions 285, 286, 287, and 288, that alter α-SNAP protein interactions with NSF proteins, with numbering relative to the α-SNAP polypeptide set forth in SEQ ID NOS: 11.

9. The method of claim **1** wherein the plant cells with enhanced resistance to nematodes are produced in plants that also express wild type α-SNAP polypeptide sequences.

10. The method of claim **1**, wherein the one or more polynucleotides encodes a modified NSF polypeptide, wherein:

the modified NSF polypeptide comprises one or a plurality of amino acid modifications at positions corresponding to 4 and 21 and optionally positions 25, 116, and 181, with numbering relative to the NSF polypeptide set forth in SEQ ID NOS: 17 or 18;

the modified NSF polypeptide comprises one or a plurality of amino acid modifications compared to the NSF polypeptide set forth in SEQ ID NO 17; whereby the modified NSF polypeptide comprises a sequence of amino acids that has less than 100% identity and more than 75% identity to the NSF polypeptide as set forth in SEQ ID NO 17; and

the modified NSF is a growth promoting and survival variant of the plant cell that is greater than the growth or survival of the plant cell without the NSF amino acid modification or amino acid modifications.

11. The method of claim **10**, wherein the encoded modified NSF comprises amino acid modifications at positions corresponding to positions 4 and 21 by NSF numbering relative to position in the NSF polypeptide set forth in SEQ ID NOS: 17 or 18.

12. The method of claim **10**, wherein the encoded modified NSF one or more polynucleotides encode a modified NSF polypeptide, wherein the modified NSF polypeptide comprises:

a modification at position R4 that is R4N, R4C, R4Q, R4S, or R4T; and

a modification at position N21 that is N21F, N21W, or N21Y, or

or a functional equivalent amino acid to the WT amino acid expressed at position 4 and 21 each by NSF numbering relative to the positions set for in SEQ ID NO: 17.

13. The method of claim **12**, wherein the encoded modified NSF polypeptide comprises amino acid modifications selected from among modifications corresponding to:

R4N/N21F;
R4N/N21W;
R4N/N21Y;
R4C/N21F;
R4C/N21W;
R4C/N21Y;
R4Q/N21F;
R4Q/N21W;
R4Q/N21Y;
R4S/N21F;
R4S/N21W;
R4S/N21Y;
R4T/N21F;
R4T/N21W; and
R4T/N21Y, each with number relative to positions set forth in SEQ ID NOS: 17 or 18.

14. The method of claim **10**, wherein the one or more polynucleotides encode a modified NSF polypeptide, wherein:

the encoded NSF polypeptide comprises at least one modification corresponding to R4Q and N21Y numbering with reference to the positions set forth in SEQ ID NOS: 8 or 9, and corresponding amino acids are identified by alignment with the polypeptide of SEQ ID NOS: 17 or 18.

15. The method of claim **10**, wherein the encoded modified NSF further comprises optional amino acid modifications at positions 25, 116, and 181 corresponding to:

S25N;
(del)116F; and
M1811,

with numbering relative to the NSF polypeptide set forth in SEQ ID NOS: 17 or 18.

16. The method of claim **1** wherein the plant cells with enhanced resistance to nematodes are produced in the plants comprising NSF polypeptides having amino acid sequence modifications identified in Table 5.

17. The method of claim **1**, wherein expression of one or more polynucleotides is increased in plant cells in the root of the plant.

18. The method of claim **1** wherein expression of one or more native polynucleotides is increased.

19. The method of claim **1**, wherein an amount of an α-SNAP is decreased.

20. The method of claim **19**, wherein an amount of an α-SNAP encoded by the sequence identified in SEQ ID NO: 2 or a polynucleotide with at least 75% identity thereof, or homologs or functionally conserved variants thereof, is reduced relative to an amount of an α-SNAP encoded by either of the sequences identified in SEQ ID NO: 5 and SEQ ID NO: 6 or a polynucleotide with at least 75% identity thereof, or homologs or functionally conserved variants thereof.

21. The method of claim **1**, wherein expression of one or more polynucleotides encoding α-SNAP proteins, or homologs or variants thereof, or one or more polynucleotides encoding NSF proteins, or homologs or variants thereof, is increased by incorporation of a construct comprising a promoter operably linked to one or more of the polynucleotides in the plant cells.

22. The method of claim **1** wherein at least two of the recited polynucleotides have increased expression, an altered expression pattern, an altered abundance or localization of a polypeptide product of, or increased copy number.

23. The method of claim **1**, wherein the plant cells comprise a nematode-resistant plant.

24. A recombinant expression construct comprising a promoter operably linked to one or more of:

(i) one or more polynucleotides encoding α-SNAP proteins, or homologs or variants thereof, or

(ii) one or more polynucleotides encoding NSF proteins, or homologs or variants thereof.

25. The construct of claim **24**, comprising a polynucleotide according to SEQ ID NO: 5 or SEQ ID NO: 6, or a polynucleotide with at least 75% identity to SEQ ID NO: 5 or SEQ ID NO: 6, or a polynucleotide according to SEQ ID NO: 9, or with at least 75% identity to SEQ ID NO: 9, or homo logs or functionally conserved variants thereof.

26. The construct of claim **24**, wherein the promoter is a plant promoter.

27. A nematode-resistant transgenic plant cell comprising:

(i) one or more polynucleotides encoding α-SNAP proteins, or homologs or variants thereof, or

(ii) one or more polynucleotides encoding NSF proteins, or homologs or variants thereof.

28. The transgenic plant cell of claim **27**, wherein the one or more α-SNAP proteins are encoded by polynucleotides with at least 75% identity to the polynucleotides identified by SEQ ID NOS: 1-7, or comprise polypeptides with at least 75% identity to polypeptides identified by SEQ ID NOS 10-16, or homologs or variants thereof, and the one or more NSF proteins are encoded by polynucleotides with at least 75% identity to the polynucleotides identified by SEQ ID NOs: 8 and 9, or comprise polypeptides with at least 75% identity to polypeptides identified by SEQ ID Nos: 17 and 18, or homologs or variants thereof.

29. A seed comprising the transgenic plant cells of claim **27**.

30. A plant grown from the seed of claim **22**.

31. A transgenic plant comprising the cell of claim **27**.

32. A part, progeny or asexual propagate of the transgenic plant of claim **25**.

33. The transgenic plant, plant cell or seed, or part, progeny or asexual propagate thereof of claim **27**, comprising NSF polypeptides having amino acid sequence modifications set forth in Table 6.

34. A method of improving growth or survival of a plant cell containing one or more Rhg1 genes conferring nematode resistance, comprising:

a) increasing expression of, altering an expression pattern of, altering a polynucleotide sequence of, altering abundance or localization of a polypeptide product of, or increasing copy number of,

(i) one or more polynucleotides encoding α-SNAP proteins, or homologs or variants thereof, or

(ii) one or more polynucleotides encoding NSF proteins, or homologs or variants thereof.

35. The method of claim **27**, wherein said one or more Rhg1 genes conferring nematode resistance are identified by SEQ ID NOs: 1-7.

36. The method of claim **1**, wherein the encoded NSF protein carries changes at amino acid residues 4, 21, 25, 116, with numbering relative to the NSF polypeptide set forth in SEQ ID NOS: 17 or 18, or at adjacent residues in the folded protein that interact with α-SNAP as designated in the NSF/α-SNAP/SNARE protein structure PDB ID code 3j97, or at NSF residues that are physically adjacent to the NSF residues that directly contact α-SNAP protein as identified in the NSF/α-SNAP/SNARE protein structure PDB ID code 3j97.

37. The method of claim **36**, wherein modification of the amino acid residues 4, 21, 25, 116 or the other specified residues at the α-SNAP/NSF protein interface enhance growth and survival of plants expressing said α-SNAP proteins with improvements in plant resistance to cyst nematodes relative to the plant prior to this modification.

38. The method of claim **3**, wherein the modified polynucleotides encode a modified α-SNAP polypeptide, wherein the modified α-SNAP polypeptide comprises:

a replacement at position E285 that is E285Q, or E285N; and

a replacement at position D286 that is D286H, or D286K, or D286R; and

a replacement at position D287 that is D287E or remains D287; and

an insertion after position 287 that is (ins)288A, (ins) 288G, (ins)2881, (ins)288L, (ins)288M, or (ins)288V; and

a replacement at position L288 that is L288A, L288G, L2881, L288M, or L288V, or a

functional equivalent amino acid to the WT amino acid expressed at position 285, 286, 287, or 288, each by α-SNAP numbering relative to the positions set for in SEQ ID NO: 11.

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