Request for EPA Confirmation of Exemption Eligibility per 40 CFR 174.90

Submitting Company:
Company X
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Research Triangle Park, NC 27709

Contact information:
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(XXX) XXX-XXXX

Company X requests EPA confirmation that its PIP meets the exemption criteria under 40 CFR 174.26(a)(1) and 174.541(a)(1). Company X has genetically engineered *Zea mays* corn line Y to contain the corn-derived *hm1* gene, which confers specific resistance against a leaf blight and ear mold disease of corn, caused by *C. carbonum* race 1 (CCR1). This request does not contain CBI.

Information related to each of the required documentation as outlined in 40 CFR 174.95 is below:

(a) Biology of the plant.

(1) The identity of the recipient plant, including genus and species.

Zea mays

(2) If the plant-incorporated protectant was derived from a plant species other than the recipient plant species, provide the identity of the source plant including genus and species and information to support the determination that the recipient plant and the source plant are sexually compatible (*e.g.*, through peer-reviewed literature rationale).

N/A

(b) Description of the pesticidal trait and how the trait was engineered into the plant.
Include a description of the measures that were taken to ensure that no engineering components (*e.g.*, Cas proteins) are present in the final plant product and the measures taken to maximize the likelihood that the modification to the recipient plant is limited to the intended modification.

Hm1 gene confers specific resistance against a leaf blight and ear mold disease of corn, caused by the fungus *C. carbonum* race 1 (CCR1). The *hm1* gene controls both race-specific resistance to the fungus and expression of the NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)-dependent HC toxin reductase (HCTR), which inactivates HC toxin, a cyclic tetrapeptide produced by the fungus to permit infection.

The *hm1* gene was inserted into corn line Y protoplasts using PEG-mediated Cas9 protein-gRNA ribonucleoproteins and native gene template transfections as described in Woo et al., 2015. Integration of the native gene into the *Zea mays* genome was confirmed (see sequence under part c), but these plants showed no detectable integration of the Cas9 and guide RNA genes, indicating the transient expression of CRISPR/Cas9. To maximize the likelihood that the modification is limited to the intended modification, the CRISPR Genome Analysis Tool (Brazelton et al., 2015) was used during guide RNA design to identify potential target sites for CRISPR gene editing. Identified target sequences were then used to search the *Zea mays* genome for potential off-target matches; target sequences with the highest specificity were then used during genetic engineering.

(c) Molecular characterization of the plant-incorporated protectant. A nucleic acid sequence comparison of the plant-incorporated protectant between the recipient plant and the comparator(s). A deduced amino acid sequence comparison is additionally required when the pesticidal substance is proteinaceous. The relevant comparator(s) for the sequence comparison(s) are determined by the type of modification:

(1) For 174.26(a)(1), sequences in the source plant and in the recipient plant.
(2) For 174.26(a)(2), sequences in the recipient plant before the modification, after the modification, and the sequence in the source plant. The polymorphic site(s) must be indicated.

Insertion of the *hm1* gene [174.26(a)(1)] was verified by PCR and sequencing. Nucleic acid sequence comparison indicates 100% identity of the insert with the source gene (see nucleic acid alignment below). Because the nucleic acid sequence is 100% identical, the deduced amino acid sequence will also be 100% identical and is therefore not provided.

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Alignment details
Score =  2093 bits (3866.16),  Expect = 0
Identities = 2093/2093 (1.0000%), Gaps = 0 (0.0000%)
Strand = Plus / Plus

Query = Native gene sequence from source plant
Sbjct = Inserted native gene sequence in recipient plant

Query  1          AACCACAGGCCGACAACCGAGTAAGCCGGTCAATTTTGGTATCCTGCTCATGACTCATAT  60
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103566  AACCACAGGCCGACAACCGAGTAAGCCGGTCAATTTTGGTATCCTGCTCATGACTCATAT  204103625

Query  61         CAGGCGGTAGCCGAGCAGCCGCCCAGCTTTCTCATGCCAGAGCAAACCCATAGGTCCAGT  120
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103626  CAGGCGGTAGCCGAGCAGCCGCCCAGCTTTCTCATGCCAGAGCAAACCCATAGGTCCAGT  204103685

Query  121        CCAAATCCAATCCCTGTTGCCATCAGAATTTCAGGGGCAGCCATGGCCGAAAAGGAGAGC  180
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103686  CCAAATCCAATCCCTGTTGCCATCAGAATTTCAGGGGCAGCCATGGCCGAAAAGGAGAGC  204103745

Query  181        AACGGAGTGCGGGTGTGCGTCACCGGAGGAGCCGGGTTCATCGGCTCCTGGCTCGTCAGG  240
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103746  AACGGAGTGCGGGTGTGCGTCACCGGAGGAGCCGGGTTCATCGGCTCCTGGCTCGTCAGG  204103805

Query  241        AAGCTCCTCGAGAAAGGCTACACCGTCCACGCCACCCTGCGGAACACCGGTGCGTCTGAT  300
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103806  AAGCTCCTCGAGAAAGGCTACACCGTCCACGCCACCCTGCGGAACACCGGTGCGTCTGAT  204103865

Query  301        GGCGGCTCCTCAGCTCGATCCGCGCGTCGCGAAAGGCGAAACACGCCAAAGGCGAAAGGA  360
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  204103866  GGCGGCTCCTCAGCTCGATCCGCGCGTCGCGAAAGGCGAAACACGCCAAAGGCGAAAGGA  204103925

Query  361        GTGGGTCGGGTCTCGTGTGTGGCTGCCTGATGATTCGGGAATCTTAGCCGGATTCGTGTG  420
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103926  GTGGGTCGGGTCTCGTGTGTGGCTGCCTGATGATTCGGGAATCTTAGCCGGATTCGTGTG  204103985

Query  421        CTGGTGGGTGTGCAGGGGACGAGGCGAAGGCGGGGCTGCTGCGTCGGCTGGTCCCCGGCG  480
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204103986  CTGGTGGGTGTGCAGGGGACGAGGCGAAGGCGGGGCTGCTGCGTCGGCTGGTCCCCGGCG  204104045

Query  481        CGGCGGAGCGGCTGCGGTTGTTCCAGGCCGACCTCTTCGACGCCGCCACCTTCGCGCCGG  540
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104046  CGGCGGAGCGGCTGCGGTTGTTCCAGGCCGACCTCTTCGACGCCGCCACCTTCGCGCCGG  204104105

Query  541        CGATCGCTGGGTGCCAGTTCGTCTTCCTCGTCGCCACGCCATTCGGGCTCGATAGCGCCG  600
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104106  CGATCGCTGGGTGCCAGTTCGTCTTCCTCGTCGCCACGCCATTCGGGCTCGATAGCGCCG  204104165

Query  601        GCTCCCAGGTGAAGCTTGCCGTCGCGTTCGCTCCCTTCACTGTTTTACTAGTCAACGAGT  660
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104166  GCTCCCAGGTGAAGCTTGCCGTCGCGTTCGCTCCCTTCACTGTTTTACTAGTCAACGAGT  204104225

Query  661        GGTCAGGCGGCGACGCGCCGTGCTGTCTCTTCTTAATTTTAAGTTGTGGAAAATTACTGT  720
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104226  GGTCAGGCGGCGACGCGCCGTGCTGTCTCTTCTTAATTTTAAGTTGTGGAAAATTACTGT  204104285

Query  721        CCTTGCAAAGGAAAAATTTGATCAGACTGAGTATGAGTAAAGACCAGTAAGACAGCACAA  780
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104286  CCTTGCAAAGGAAAAATTTGATCAGACTGAGTATGAGTAAAGACCAGTAAGACAGCACAA  204104345

Query  781        GGATTGCGAGCTGCGACTGCGAGCAGAGGAAACGTCTTCACAGTTATTCTTGTCTGCCTT  840
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104346  GGATTGCGAGCTGCGACTGCGAGCAGAGGAAACGTCTTCACAGTTATTCTTGTCTGCCTT  204104405

Query  841        GTTTTTCTCTATCTGAAGCAAAATCTTGTGGTGCCCATCGACCGCGTATAGCAGTATAAG  900
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104406  GTTTTTCTCTATCTGAAGCAAAATCTTGTGGTGCCCATCGACCGCGTATAGCAGTATAAG  204104465

Query  901        AGCACGGCGGAAGCTGTGGTGGACGCGGTGCGCGCGATCCTCCGGCAATGCGAGGAGTCC  960
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104466  AGCACGGCGGAAGCTGTGGTGGACGCGGTGCGCGCGATCCTCCGGCAATGCGAGGAGTCC  204104525

Query  961        CGGACGGTGAAGCGAGTGATCCACACAGCCTCCGTAGCGGCCGCCTCGCCGTTGCTGGAG  1020
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104526  CGGACGGTGAAGCGAGTGATCCACACAGCCTCCGTAGCGGCCGCCTCGCCGTTGCTGGAG  204104585

Query  1021       GAGGAGGTCTCCGCCTCCGGCGTCGGGTACAGAGACTTCATCGACGAATCTTGTTGGACT  1080
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104586  GAGGAGGTCTCCGCCTCCGGCGTCGGGTACAGAGACTTCATCGACGAATCTTGTTGGACT  204104645

Query  1081       TCGCTCAACGTTGACTATCCTCTCCGAAGCGCACACTTCGACGTAAGTAGTATACAAGCG  1140
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104646  TCGCTCAACGTTGACTATCCTCTCCGAAGCGCACACTTCGACGTAAGTAGTATACAAGCG  204104705

Query  1141       AAGCTTCTTCTGATTTCTGAACTGGAACGCCTGATCACATTAATATTTTTAGCTGACGG  1200
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104706  AAGCTTCTTCTGATTTCTGAACTGGAACGCCTGATCACATTAATATTTTTAGCTGACGG  204104765

Query  1201       CCATTTGATTTGCAGAAGTACATACTGTCGAAGCTGCGGTCAGAGCAGGAGCTCCTGAGC  1260
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104766  CCATTTGATTTGCAGAAGTACATACTGTCGAAGCTGCGGTCAGAGCAGGAGCTCCTGAGC  204104825

Query  1261       TACAACGGCGGCGAGAGCCCGGCGTTCGAGGTGGTGACCCTGCCGCTGGGGCTCGTGGCG  1320
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104826  TACAACGGCGGCGAGAGCCCGGCGTTCGAGGTGGTGACCCTGCCGCTGGGGCTCGTGGCG  204104885

Query  1321       GGCGACACGGTCCTCGGCCGCGCCCCGGAGACGGTGGAGAGCGCCGTGGCGCCCGTGTCC  1380
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                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104886  GGCGACACGGTCCTCGGCCGCGCCCCGGAGACGGTGGAGAGCGCCGTGGCGCCCGTGTCC  204104945

Query  1381       CGCAGCGAGCCCTGCTTCGGCCTCCTGCGCATACTGCAGCAGCTCCTGGGGTCGCTGCCG  1440
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204104946  CGCAGCGAGCCCTGCTTCGGCCTCCTGCGCATACTGCAGCAGCTCCTGGGGTCGCTGCCG  204105005

Query  1441       CTGGTGCACGTGGACGACGTCTGCGACGCGCTCGTCTTCTGCATGGAGCGGCGCCCCTCC  1500
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105006  CTGGTGCACGTGGACGACGTCTGCGACGCGCTCGTCTTCTGCATGGAGCGGCGCCCCTCC  204105065

Query  1501       GTCGCCGGCCGCTTCCTCTGCGCCGCCGCGTACCCGACGATCCACGACGTGGTCGCCCAC  1560
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105066  GTCGCCGGCCGCTTCCTCTGCGCCGCCGCGTACCCGACGATCCACGACGTGGTCGCCCAC  204105125

Query  1561       TACGCCAGCAAGTTCCCTCACCTCGACATCTTGAAAGAGTAAGATCAAAAGCGTCCACAG  1620
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105126  TACGCCAGCAAGTTCCCTCACCTCGACATCTTGAAAGAGTAAGATCAAAAGCGTCCACAG  204105185

Query  1621       CGACAGCATCACCCTGCACACAAGAACTGACTGCCGATTTACGTTTCTGTTGCGATTGGT  1680
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105186  CGACAGCATCACCCTGCACACAAGAACTGACTGCCGATTTACGTTTCTGTTGCGATTGGT  204105245

Query  1681       TGGATTGATCTGCGTCAGGACGGAGGCGGTGGCGACGGTGCGGCCTGCCCGGGACAGGTT  1740
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105246  TGGATTGATCTGCGTCAGGACGGAGGCGGTGGCGACGGTGCGGCCTGCCCGGGACAGGTT  204105305

Query  1741       GGGCGAGCTGGGCTTCAAGTACAAGTACGGCATGGAAGAGATTCTGGATAGCAGCGTTGC  1800
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105306  GGGCGAGCTGGGCTTCAAGTACAAGTACGGCATGGAAGAGATTCTGGATAGCAGCGTTGC  204105365

Query  1801       CTGTGCGGCGAGATTAGGTTCCCTTGACGCATCCAAGCTCGGCCTACAGAAAGGATAAAA  1860
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105366  CTGTGCGGCGAGATTAGGTTCCCTTGACGCATCCAAGCTCGGCCTACAGAAAGGATAAAA  204105425

Query  1861       GCTCGAAGCTTACTCATAAGCACCATGGGGAACTTGGATTGTTCGCTGTCCACTATACGC  1920
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105426  GCTCGAAGCTTACTCATAAGCACCATGGGGAACTTGGATTGTTCGCTGTCCACTATACGC  204105485

Query  1921       GTTCGAAATTTGGAAACTAGACATACTCCAATAAAACAAGAGGTAAAGAAACGTGGGCTA  1980
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105486  GTTCGAAATTTGGAAACTAGACATACTCCAATAAAACAAGAGGTAAAGAAACGTGGGCTA  204105545

Query  1981       ACTGATACGCGTTGAGCAGTTGAGCTAGCCTAGTTTAGTCCACCTGTGTGCAGGGTTTAA  2040
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105546  ACTGATACGCGTTGAGCAGTTGAGCTAGCCTAGTTTAGTCCACCTGTGTGCAGGGTTTAA  204105605

Query  2041       AACTTCGACGAAATTTTATGACTTGCGATAATTTTAGGCCTCTAAATATCAAC  2093
                  |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204105606  AACTTCGACGAAATTTTATGACTTGCGATAATTTTAGGCCTCTAAATATCAAC  204105658
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(d) Information on the history of safe use of the plant-incorporated protectant.

(1) If the pesticidal substance is a known allergen or mammalian toxin/toxicant (*e.g.*, solanine), describe how conventional breeding practices are being used to ensure that it does not exceed human dietary safety levels in the recipient food plant (*i.e.*, ensure residues of pesticidal substance are not present in food at levels that are injurious or deleterious and are within the ranges of levels generally seen in plant varieties currently on the market and/or known to produce food safe for consumption).

N/A. The HM1 protein is not a known mammalian toxin or toxicant. Per AllergenOnline (Goodman et al., 2016), the HM1 protein does not have significant sequence identity to known allergens.

> (2) If the source plant is a wild relative of the recipient plant, describe why the plant-incorporated protectant is not anticipated to pose a hazard to humans or the environment (*e.g.*, Are levels of the pesticidal substance produced in the recipient plant within the ranges of levels generally seen in plant varieties currently on the market and/or known to produce food safe for consumption? Is the pesticidal mode of action non-toxic? Does the plant-incorporated protectant lack sequence similarity to known mammalian toxins, toxicants, or allergens? Is the plant-incorporated protectant a commonly screened substance and therefore familiar to plant breeders?).

N/A. The *hm1* gene is found in domesticated corn, not a wild relative.

References

Goodman, R.E., Ebisawa, M., Ferreira, F., Sampson, H.A., van Ree, R., Vieths, S., Baumert, J.L., Bohle, B., Lalithambika, S., Wise, J. and Taylor, S.L., 2016. AllergenOnline: a peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. *Molecular nutrition & food research*, *60*(5), pp.1183-1198.

Brazelton Jr, V.A., Zarecor, S., Wright, D.A., Wang, Y., Liu, J., Chen, K., Yang, B. and Lawrence-Dill, C.J., 2015. A quick guide to CRISPR sgRNA design tools. *GM crops & food*, *6*(4), pp.266-276.

Woo, J.W., Kim, J., Kwon, S.I., Corvalán, C., Cho, S.W., Kim, H., Kim, S.G., Kim, S.T., Choe, S. and Kim, J.S., 2015. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nature biotechnology*, *33*(11), pp.1162-1164.

Request for EPA Confirmation of Exemption Eligibility per 40 CFR 174.90

Submitting Company:
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456 Innovation Ln
Research Triangle Park, NC 27709

Contact information:
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(XXX) XXX-XXXX

Company A requests EPA confirmation that its PIP meets the exemption criteria under 40 CFR 174.26(a)(2) and 174.541(a)(2). Company A has modified the native *pto* gene in tomato line B (*Lycopersicum esculentum*) to contain polymorphisms found in the native *pto* allele of the wild relative tomato *Solanum pennellii.* This request does not contain CBI.

Information related to each of the required documentation as outlined in 40 CFR 174.95 is below:

> (a) Biology of the plant.
>
> (1) The identity of the recipient plant, including genus and species.

Lycopersicum esculentum

> (2) If the plant-incorporated protectant was derived from a plant species other than the recipient plant species, provide the identity of the source plant including genus and species and information to support the determination that the recipient plant and the source plant are sexually compatible (*e.g.*, through peer-reviewed literature rationale).

The source plant is a species of wild tomato, *Solanum pennellii*. Sexual compatibility is demonstrated by the successful crossing of *S. pennellii* and *L. esculentum* (Rick, 1960).

> (b) Description of the pesticidal trait and how the trait was engineered into the plant.
> Include a description of the measures that were taken to ensure that no engineering components (*e.g.*, Cas proteins) are present in the final plant product and the measures taken to maximize the likelihood that the modification to the recipient plant is limited to the intended modification.

The *pto* gene encodes a serine-threonine kinase that confers resistance in tomato to *Pseudomonas syringae pv tomato* strains expressing the avirulence gene *avrPto*, thereby conferring resistance to bacterial speck disease. The *pto* encoded serine-threonine kinase acts with Prf, a NOD-like receptor (NLR) protein, to recognize the pathogen effector AvrPto, which leads to the activation of NLR-triggered immunity. Polymorphisms identified in *S. pennellii* allow for increased resistance to *P. syringae*.

The tomato line B protoplasts were transfected using PEG-mediated Cas9 protein-gRNA ribonucleoproteins as described in Woo et al., 2015 targeting three locations in the *pto* gene. Modification of the native gene in the recipient plant was confirmed (see sequence under part c), but these plants showed no detectable integration of the Cas9 and guide RNA genes, indicating the transient expression of CRISPR/Cas9. To maximize the likelihood that the modification is limited to the intended modification, CRISPR-PLANT v2 (Minkenberg et al., 2019) was used during guide RNA design to predict specificity and activity; sequences with the highest specificity were then used during genetic engineering.

(c) Molecular characterization of the plant-incorporated protectant. A nucleic acid sequence comparison of the plant-incorporated protectant between the recipient plant and the comparator(s). A deduced amino acid sequence comparison is additionally required when the pesticidal substance is proteinaceous. The relevant comparator(s) for the sequence comparison(s) are determined by the type of modification:
(1) For 174.26(a)(1), sequences in the source plant and in the recipient plant.
(2) For 174.26(a)(2), sequences in the recipient plant before the modification, after the modification, and the sequence in the source plant. The polymorphic site(s) must be indicated.

Modification of the *pto* gene [174.26(a)(2)] was verified by PCR and sequencing. Nucleic acid sequence alignment with polymorphic sites indicated:

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CLUSTAL O(1.2.4) multiple sequence alignment


sourceplant                 ------TATTTTATAATATATTTTTTTATTATATTAACATGA-AAAAAGTTGCAATAATT     53
recipientplant              AATAAATATTTTATAATATATTTATTTATTATATTAACAGTGAAAAAAGTTG------CA     54
modified_recipientplant     AATAAATATTTTATAATATATTTATTTATTATATTAACAGTGAAAAAAGTTG------CA     54
                                  ***************** ***************    *********

sourceplant                 ATCCTAAGGCAAAGGCAAAGGCAGCTGCACAGTAAAACTTGTCAGTCTCTCCTCCAAATC     113
recipientplant              ATAATTATCCAAAGGCAAAGGCAGCTGCACAGTAAAACTTGTCAGTCTCTCCTCCAAATC     114
modified_recipientplant     ATAATTATCCAAAGGCAAAGGCAGCTGCACAGTAAAACTTGTCAGTCTCTCCTCCAAATC     114
                            **  * *  ***************************************************

sourceplant                 TCCATTGCTCTGAAATTTGCGCTGTCAAATACTAGTAGATTTATTTATGTATTAATGGGA     173
recipientplant              TCCATTGCTCTGAAATTTGCGCTGTCAAATACTAGTAGATTTATTTATGTATTAATGGGA     174
modified_recipientplant     TCCATTGCTCTGAAATTTGCGCTGTCAAATACTAGTAGATTTATTTATGTATTAATGGGA     174
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sourceplant                 AGCAAGTATTCCAAGGCAACAAATTCCATAAGTGATGCTTCAAACTCTTTTGAAAGTTAT     233
recipientplant              AGCAAGTATTCCAAGGCAACAAATTCCATAAGTGATGCTTCAAACTCTTTTGAAAGTTAT     234
modified_recipientplant     AGCAAGTATTCCAAGGCAACAAATTCCATAAGTGATGCTTCAAACTCTTTTGAAAGTTAT     234
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sourceplant                 CGATTTCCTTTAGAAGATTTGGAGGAAGCAACCAACAATTTTGATGACAAGTTTTTCATT     293
recipientplant              CGATTTCCTTTAGAAGATTTGGAGGAAGCAACCAACAATTTTGATGACAAGTTTTTCATT     294
modified_recipientplant     CGATTTCCTTTAGAAGATTTGGAGGAAGCAACCAACAATTTTGATGACAAGTTTTTCATT     294
                            ************************************************************

sourceplant                 GGAGAGGGTGCATTTGGGAAGGTTTACAAGGGTGTTTTGCGTGATGGAACAAAGGTCGCC     353
recipientplant              GGAGAGGGTGCATTTGGGAAGGTTTACAAGGGTGTTTTGCGTGATGGAACAAAGGTCGCC     354
modified_recipientplant     GGAGAGGGTGCATTTGGGAAGGTTTACAAGGGTGTTTTGCGTGATGGAACAAAGGTCGCC     354
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sourceplant                  CTGAAAAGGCAAAATCGTGACTCCCGACAAAGTATTGAAGAGTTCGGAACAGAAATTGAG    413
recipientplant               CTGAAAAGGCAAAATCGTGACTCCCGGCAAGGTATTGAAGAGTTCGGAACAGAAATTGGG    414
modified_recipientplant      CTGAAAAGGCAAAATCGTGACTCCCGGCAAGGTATTGAAGAGTTCGGAACAGAAATTGGG    414
                             ************************** *** *************************** *

sourceplant                  ATACTCTCACGCCGTAGCCATCCGCATCTGGTTTCATTGATAGGATACTGTGATGAAAGA    473
recipientplant               ATACTCTCACGCCGTAGCCATCCGCATCTGGTTTCATTGATAGGATACTGTGATGAAAGA    474
modified_recipientplant      ATACTCTCACGCCGTAGCCATCCGCATCTGGTTTCATTGATAGGATACTGTGATGAAAGA    474
                             ************************************************************

sourceplant                  AATGAGATGGTTCTAATTTATGACTACATGGAGAATGGGAACCTCAAGAGCCATTTGACT    533
recipientplant               AATGAGATGGTTCTAATTTATGACTACATGGAGAATGGGAACCTCAAGAGCCATTTGACT    534
modified_recipientplant      AATGAGATGGTTCTAATTTATGACTACATGGAGAATGGGAACCTCAAGAGCCATTTGACT    534
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sourceplant                  GGCTCAGATCTACCCTCCATGAGCTGGGAGCAGAGGCTGGAGATATGCATAGGGGCAGCC    593
recipientplant               GGCTCAGATCTACCCTCCATGAGCTGGGAGCAGAGGCTGGAGATATGCATAGGGGCAGCC    594
modified_recipientplant      GGCTCAGATCTACCCTCCATGAGCTGGGAGCAGAGGCTGGAGATATGCATAGGGGCAGCC    594
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sourceplant                  AGAGGTCTACACTACCTTCATACTAACGGAGTTATACATCGTGATGTCAAATCTTCAAAC    653
recipientplant               AGAGGTCTACACTACCTTCATACTAACGGAGTTATGCATCGTGATGTCAAATCTTCAAAC    654
modified_recipientplant      AGAGGTCTACACTACCTTCATACTAACGGAGTTATGCATCGTGATGTCAAATCTTCAAAC    654
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sourceplant                  ATATTGCTTGATGGGAATTTTGTGCCAAAAATTACTGATTTTGGACTATCCAAGACATGG    713
recipientplant               ATATTGCTTGATGAGAATTTTGTGCCAAAAATTACTGACTTTGGACTATCCAAGACAAGG    714
modified_recipientplant      ATATTGCTTGATGAGAATTTTGTGCCAAAAATTACTGACTTTGGACTATCCAAGACATGG    714
                             ************* ************************ ****************** **

sourceplant                  CATCAGCTTTATCAAACCCATGTAAGCACAAACGTGAAAGGAACTTACGGCTACATTGAC    773
recipientplant               CCTCAGCTTTATCAAAC---------CACAGACGTGAAAGGAACTTTCGGCTACATTGAC    765
modified_recipientplant      CATCAGCTTTATCAAAC---------CACAGACGTGAAAGGAACTTTCGGCTACATTGAC    765
                             * ***************         **** *************** *************

sourceplant                  CCTGAATATGTTATACGGCAAAAGCTGACAGAAAAATCTGATGTTTATTCTTTCGGAGTT    833
recipientplant               CCTGAATATTTTATAAAGGGACGACTTACAGAAAAATCTGATGTTTATTCTTTCGGTGTT    825
modified_recipientplant      CCTGAATATTTTATAAAGGGACGACTTACAGAAAAATCTGATGTTTATTCTTTCGGTGTT    825
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sourceplant                  GTTTTATTTGAAGCTCTTTGTGGTAGGTCTACCATAGAACCATCTCTTCCAAGGGATATG    893
recipientplant               GTTTTATTTGAAGTTCTTTGTGCTAGGTCTGCCATGGTTCAATCTCTTCCAAGGGAGATG    885
modified_recipientplant      GTTTTATTTGAAGTTCTTTGTGCTAGGTCTGCCATGGTTCAATCTCTTCCAAGGGATATG    885
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sourceplant                   GTTGCTTTAGCTGATTGGGCAGTGAAGTCGCATAATAATGGACAGTTGGAACAAATCGTA    953
recipientplant                GTTAATTTAGCTGAATGGGCAGTGGAGTCGCATAATAATGGACAGTTGGAACAAATCGTA    945
modified_recipientplant       GTTAATTTAGCTGAATGGGCAGTGGAGTCGCATAATAATGGACAGTTGGAACAAATCGTA    945
                              ***  ********* ********* ***********************************

sourceplant                   GATCCCAATCTTGCAGCTAAAATAAGACCAGAGTCCCTCAGGAAGTTTGGAGAAATAGGG    1013
recipientplant                GATCCCAATCTTGCAGATAAAATAAGACCAGAGTCCCTCAGGAAGTTTGGAGAAACAGCG    1005
modified_recipientplant       GATCCCAATCTTGCAGATAAAATAAGACCAGAGTCCCTCAGGAAGTTTGGAGAAACAGCG    1005
                              **************** ************************************** ** *

sourceplant                   GTAAAATGTCTGGCTTTGTCTGGTAAAGATAGGCCATCAATGGGTGATGTGTTGTGGAAA    1073
recipientplant                GTAAAATGCTTAGCTTTGTCTAGTGAAGATAGGCCATCAATGGGTGATGTGTTGTGGAAA    1065
modified_recipientplant       GTAAAATGCTTAGCTTTGTCTAGTGAAGATAGGCCATCAATGGGTGATGTGTTGTGGAAA    1065
                              ********  * ********* ** ***********************************

sourceplant                   CTGGAGTATGCACTTTGTCTCCAAGAGTCTGTTATTTAAGATGATCCTGACTGCAAGCGG    1133
recipientplant                CTGGAGTATGCACTTCGTCTCCAAGAGTCTGTTATTTAAGATATTTTTGTTTTTCTGAGT    1125
modified_recipientplant       CTGGAGTATGCACTTCGTCTCCAAGAGTCTGTTATTTAAGATATTTTTGTTTTTCTGAGT    1125
                              *************** **************************  *  **  *    * *

sourceplant                   CCGCGGAGTCAGGATTTTCCTTAAGAATATTATTATGCTGCTAGCATCGGAGGAAGACAC    1193
recipientplant                TTTATATAGAAAGGTAAACTTTGAAAACTTGAAT-TGCTAT-ACCTGTGGATCCTTCTTT    1183
modified_recipientplant       TTTATATAGAAAGGTAAACTTTGAAAACTTGAAT-TGCTAT-ACCTGTGGATCCTTCTTT    1183
                                        * * *   * ** * **  * * * ****   * *   ***

sourceplant                   AACATCATCGATTTTTTGCAACCACTTTA-------CTAAACGTTCAACTTATGTCAAAA    1246
recipientplant                CATTTTATTAGGTGCGTCCGGCTGTTACACATATTGTATATGGTTCTTATTAAGTTGTTC    1243
modified_recipientplant       CATTTTATTAGGTGCGTCCGGCTGTTACACATATTGTATATGGTTCTTATTAAGTTGTTC    1243
                               *  * **    *   * *  *   *  *          *  ****   *** **

sourceplant                   TTTGATTTATATATTCAATGTAAGAGACAAGTGTAGCTCTGCCCTTGACTGCAAGTCTCA    1306
recipientplant                AGACATTTTCTTATTGTAAAGAGGCAAAAAGGAA-------------------------G    1278
modified_recipientplant       AGACATTTTCTTATTGTAAAGAGGCAAAAAGGAA-------------------------G    1278
                                  ****   ****  *   * *  * ***

sourceplant                   TTTGATGCTCAGATCTGGACGATCTTATCAAATCCGGATGATCTTTGTGCAGCTGGATAG    1366
recipientplant                TTTGCTGCTTTGA-----------------------------------------------    1291
modified_recipientplant       TTTGCTGCTTTGA-----------------------------------------------    1291
                              **** ****  **

sourceplant                   AGGGTTTTTAGCAATTGGTTAAGTCTGATGGTTGATCATTAAGTCAAAATGGTGCGTACC    1426
recipientplant                ------------------------------------------------------------    1291
modified_recipientplant       ------------------------------------------------------------    1291
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sourceplant                  ATCCTTAGTGTTTGTAAATTTCACTTTTGTTGATGGATCATATAAAGATATATTTTTTGT     1486
recipientplant               ------------------------------------------------------------     1291
modified_recipientplant      ------------------------------------------------------------     1291

sourceplant                  TTTTGCAAGAGTCTTCTGAATTTGATATAGAAAGTTAACATAGAAAACTTAAATTGCTCT     1546
recipientplant               ------------------------------------------------------------     1291
modified_recipientplant      ------------------------------------------------------------     1291

sourceplant                  ACCTGTGGATCCTTCTTTCATTTGATTAGGTGTGCCACATGTTGTATATGGTTTCTATTA     1606
recipientplant               ------------------------------------------------------------     1291
modified_recipientplant      ------------------------------------------------------------     1291

sourceplant                  AGTTCTTCATTCCATTGATGTCATGTTACTAATTCTTGGTGTATATATGTAAAAATTTTG     1666
recipientplant               ------------------------------------------------------------     1291
modified_recipientplant      ------------------------------------------------------------     1291

sourceplant                  CGCATGAAACTGCTGCAAAATGTTACTA   1694
recipientplant               ----------------------------   1291
modified_recipientplant      ----------------------------   1291
```

Deduced amino acid sequence alignment with polymorphic sites indicated:

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CLUSTAL O(1.2.4) multiple sequence alignment


sourceplant                  MKKVAIIILRQRQRQLHSKTCQSLLQISIALKFALSNTSRFIYVLMGSKYSKATNSISDA     60
recipientplant               ---------------------------------------------MGSKYSKATNSISDA     15
modified_recipientplant      ---------------------------------------------MGSKYSKATNSISDA     15
                                                                          ***************

sourceplant                  SNSFESYRFPLEDLEEATNNFDDKFFIGEGAFGKVYKGVLRDGTKVALKRQNRDSRQSIE     120
recipientplant               SNSFESYRFPLEDLEEATNNFDDKFFIGEGAFGKVYKGVLRDGTKVALKRQNRDSRQGIE     75
modified_recipientplant      SNSFESYRFPLEDLEEATNNFDDKFFIGEGAFGKVYKGVLRDGTKVALKRQNRDSRQGIE     75
                             *********************************************************.**

sourceplant                  EFGTEIEILSRRSHPHLVSLIGYCDERNEMVLIYDYMENGNLKSHLTGSDLPSMSWEQRL     180
recipientplant               EFGTEIGILSRRSHPHLVSLIGYCDERNEMVLIYDYMENGNLKSHLTGSDLPSMSWEQRL     135
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modified_recipientplant      EFGTEIGILSRRSHPHLVSLIGYCDERNEMVLIYDYMENGNLKSHLTGSDLPSMSWEQRL    135
                             ****** *****************************************************

sourceplant                  EICIGAARGLHYLHTNGVIHRDVKSSNILLDGNFVPKITDFGLSKTWHQLYQTHVSTNVK    240
recipientplant               EICIGAARGLHYLHTNGVMHRDVKSSNILLDENFVPKITDFGLSKTRPQLYQT---TDVK    192
modified_recipientplant      EICIGAARGLHYLHTNGVMHRDVKSSNILLDENFVPKITDFGLSKTWHQLYQT---TDVK    192
                             ******************:************ **************  *****   *:**

sourceplant                  GTYGYIDPEYVIRQKLTEKSDVYSFGVVLFEALCGRSTIEPSLPRDMVALADWAVKSHNN    300
recipientplant               GTFGYIDPEYFIKGRLTEKSDVYSFGVVLFEVLCARSAMVQSLPREMVNLAEWAVESHNN    252
modified_recipientplant      GTFGYIDPEYFIKGRLTEKSDVYSFGVVLFEVLCARSAMVQSLPRDMVNLAEWAVESHNN    252
                             **:*******.*: :****************.**.**::  ****:** **:***:****

sourceplant                  GQLEQIVDPNLAAKIRPESLRKFGEIGVKCLALSGKDRPSMGDVLWKLEYALCLQESVI     359
recipientplant               GQLEQIVDPNLADKIRPESLRKFGETAVKCLALSSEDRPSMGDVLWKLEYALRLQESVI     311
modified_recipientplant      GQLEQIVDPNLADKIRPESLRKFGETAVKCLALSSEDRPSMGDVLWKLEYALRLQESVI     311
                             ************ ************ .*******.:**************** ******
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(d) Information on the history of safe use of the plant-incorporated protectant.

(1) If the pesticidal substance is a known allergen or mammalian toxin/toxicant (*e.g.*, solanine), describe how conventional breeding practices are being used to ensure that it does not exceed human dietary safety levels in the recipient food plant (*i.e.*, ensure residues of pesticidal substance are not present in food at levels that are injurious or deleterious and are within the ranges of levels generally seen in plant varieties currently on the market and/or known to produce food safe for consumption).

N/A. The *pto* gene encodes a serine-threonine kinase which is not a known mammalian toxin or toxicant. Per AllergenOnline (Goodman et al., 2016), serine-threonine kinase does not have significant sequence identity to known allergens.

(2) If the source plant is a wild relative of the recipient plant, describe why the plant-incorporated protectant is not anticipated to pose a hazard to humans or the environment (*e.g.*, Are levels of the pesticidal substance produced in the recipient plant within the ranges of levels generally seen in plant varieties currently on the market and/or known to produce food safe for consumption? Is the pesticidal mode of action non-toxic? Does the plant-incorporated protectant lack sequence similarity to known mammalian toxins, toxicants, or allergens? Is the plant-incorporated protectant a commonly screened substance and therefore familiar to plant breeders?).

The identified polymorphisms in the *pto* gene are from a wild relative of tomato, *Solanum pennellii*. The toxicity of the *Solanum* genus is well described to be caused by glycoalkaloids (Milner et al., 2011). Serine-threonine kinase is not a glycoalkaloid, nor does it directly interact with a glycoalkaloid, and instead interacts with an endogenous gene to trigger a natural plant immune response. Therefore, there is no evidence to suggest that introduction of these polymorphisms into the cultivated tomato would pose a hazard to humans or the environment.

References

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