Revision of Genes in the Nitrogen Use Efficiency Pathway of *Zea Mays* for Agricultural Sustainability

Rija Asim, Jewel Larkins, Rebecca L. Seipelt-Thiemann
Biology Department, Middle Tennessee State University

Introduction

- Corn is the highest produced grain crop worldwide with important roles in world nutrition, agriculture, industry and energy uses (Feedgrains… 2019).
- Higher production of corn is necessary however corn depletes many nutrients from the soil that must be replaced. Therefore, it is essential to increase corn's sustainability to increase its use.
- Nitrogen is applied as a chemical fertilizer to enhance the growth and development of crops which in turn improves crop yield (Kumar et al. 2018).
- The overuse of nitrogen fertilizer leads to alarming increase in environmental costs and pollution and a decrease in nitrogen use efficiency (Chen et al. 2017)

Hypothesis

The goal of this project is to use actual biological evidence to revise computationally predicted gene models for genes involved in the nitrogen use efficiency pathway, which may then lead to production of more sustainable corn varieties and increased use of corn as biofuel without affecting the food supply.

Methods

- Selection of the nitrogen use efficiency pathway of genes in *Zea mays*
- Identification of the references sequences (REFSEQ) for each gene through the use of Gramene
- Leaf RNA extraction using Trizol and reverse transcription
- Resuspension of tubulin PCR primers
- PCR and small gel electrophoresis on cDNA and fragment sizes recorded
- Determined splicing patterns for the gene *Zm00001d052233* using Apollo
- Designed primers for the chosen region using the computational analysis
- Found appropriate annealing temperature for the primers through gradient PCR
- PCR and small gel electrophoresis on each cDNA (leaf, root and shoot) for gene *Zm00001d052233*
- Fragment sizes recorded and compared to expected splicing sizes
- Determine functionality of protein

Gene List Comparison Table

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Feature	Maize Genome ID	Gene name	Chromosome location:	Known transcripts	Number of exons for the REF SEQ
Gene 1	Zm00001d054060	nitrate transport1	Chromosome 4: 245,649,222-245,650,796 reverse strand.	1	1
Gene 2	Zm00001d054057	nitrate transport2	Chromosome 4: 245,638,263-245,639,837 forward strand.	1	1
Gene 3	Zm00001d011679	High affinity nitrate transporter 2.5	Chromosome 8: 158,415,720-158,417,282 forward strand.	1	1
Gene 4	Zm00001d017095	High affinity nitrate transporter	Chromosome 5: 185,262,626-185,263,851 reverse strand.	1	2
Gene 5	Zm00001d024587	nitrate transporter/peptide transporter family 1	Chromosome 10: 79,465,955-79,472,158 forward strand.	1	4
Gene 6	Zm00001d007255	Protein NRT1/ PTR FAMILY 4.4	Chromosome 2: 225,869,891-225,878,824 forward strand.	2	5
Gene 7	Zm00001d003208	Protein NRT1/ PTR FAMILY 4.2	Chromosome 2: 35,998,555-36,000,692 forward strand.	1	4
Gene 8	Zm00001d016982	Protein NRT1/ PTR FAMILY 6.4	Chromosome 5: 181,884,079-181,887,305 reverse strand.	1	4
Gene 9	Zm00001d013529	Protein NRT1/ PTR FAMILY 2.9	Chromosome 5: 13,659,653-13,664,953 forward strand.	2	3
Gene 10	Zm00001d038181	Protein NRT1/ PTR FAMILY 3.1	Chromosome 6: 150,745,127-150,748,423 reverse strand.	1	3
Gene 11	Zm00001d017249	ammonium transporter 2	Chromosome 5: 190,317,987-190,319,453 forward strand.	1	1
Gene 12	Zm00001d016771	ammonium transporter 2	Chromosome 5: 175,543,104-175,545,969 forward strand	2	3
Gene 13	Zm00001d038412	ammonium transporter 2	Chromosome 6: 156,654,389-156,657,185 forward strand	1	3
Gene 14	Zm00001d025831	ammonium transporter 1	Chromosome 10: 131,243,971-131,245,467 forward strand	1	1
Gene 15	Zm00001d002964	Probable sphingolipid transporter spinster homolog	Chromosome 2: 28,238,409-28,241,298 reverse strand	1	4
Gene 16	Zm00001d025894	Probable sphingolipid transporter spinster homolog 2	Chromosome 10: 133,222,842-133,243,011 forward strand	6	16
Gene 17	Zm00001d025015	Glucose-6-phosphate 1-dehydrogenase	Chromosome 10: 99,806,303-99,812,704 forward strand	5	10
Gene 18	Zm00001d020057	phosphoenolpyruvate carboxylase3	Chromosome 7: 89,268,035-89,273,487 reverse strand	5	3
Gene 19	Zm00001d052233	phosphoglycerate/bisphosphoglycerate mutase	Chromosome 4: 184,160,265-184,163,189 reverse strand	3	6
Gene 20	Zm00001d010321	pyruvate orthophosphate dikinase2	Chromosome 8: 109,459,050-109,469,711 forward strand	34	19

Figure A. Gene List and Overview
Using Gramene, *Zea Mays* Genes 1-20 associated with nitrogen use efficiency, are shown with number of known transcripts, chromosomal locations and reference sequence

Reference Sequence

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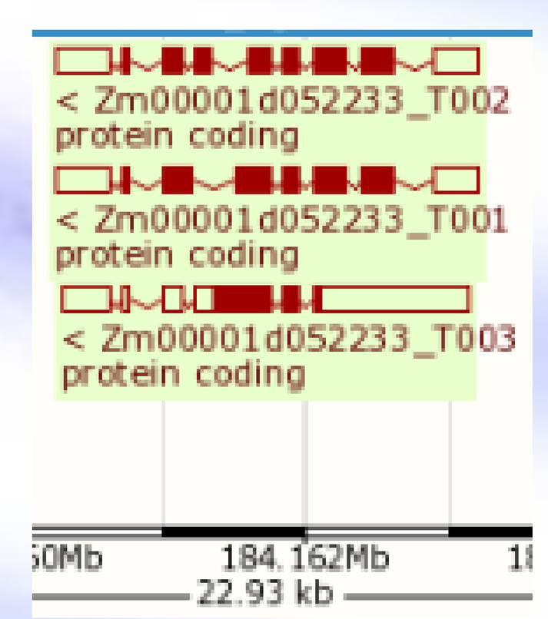
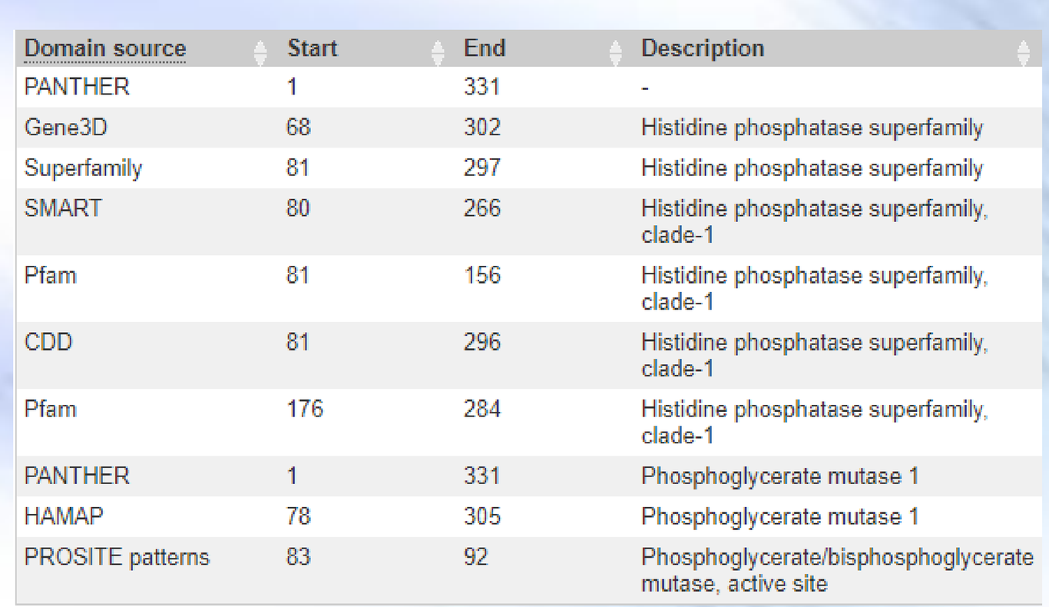


B



Domain source	Start	End	Description
PANTHER	1	331	-
Gene3D	68	302	Histidine phosphatase superfamily
Superfamily	81	297	Histidine phosphatase superfamily
SMART	80	266	Histidine phosphatase superfamily, clade-1
Pfam	81	156	Histidine phosphatase superfamily, clade-1
CDD	81	296	Histidine phosphatase superfamily, clade-1
Pfam	176	284	Histidine phosphatase superfamily, clade-1
PANTHER	1	331	Phosphoglycerate mutase 1
HAMAP	78	305	Phosphoglycerate mutase 1
PROSITE patterns	83	92	Phosphoglycerate/bisphosphoglycerate mutase, active site

C

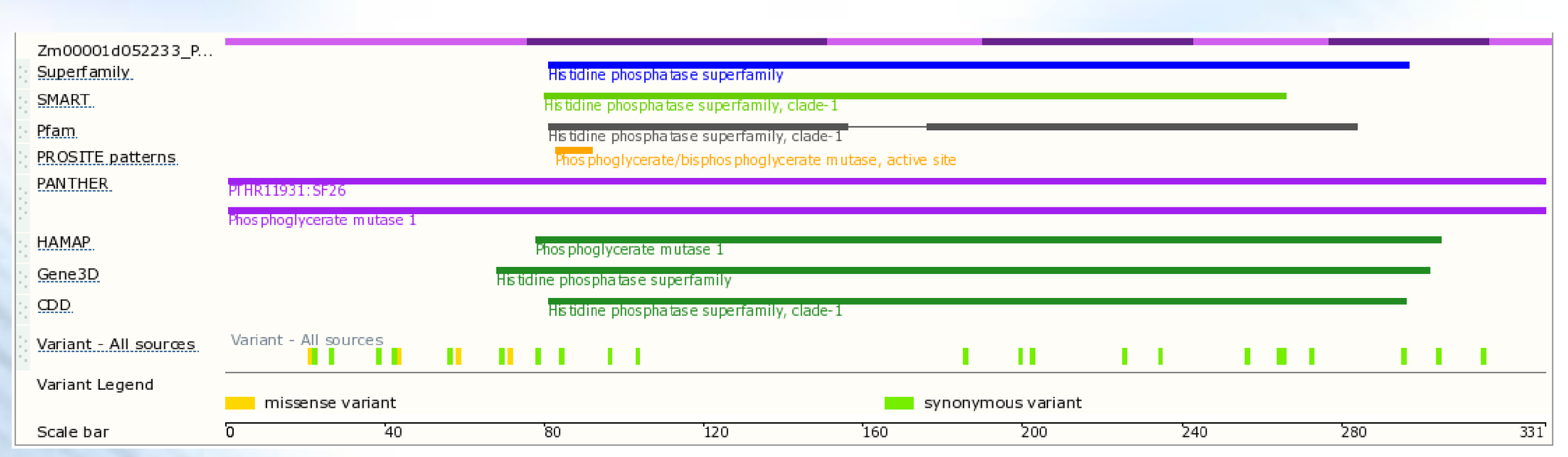


Figure A. Gramene Reference Sequence of *Zm00001d052233*
Figure B. Tabular Domain Evidence
Figure C. Protein Domain

Gene Models

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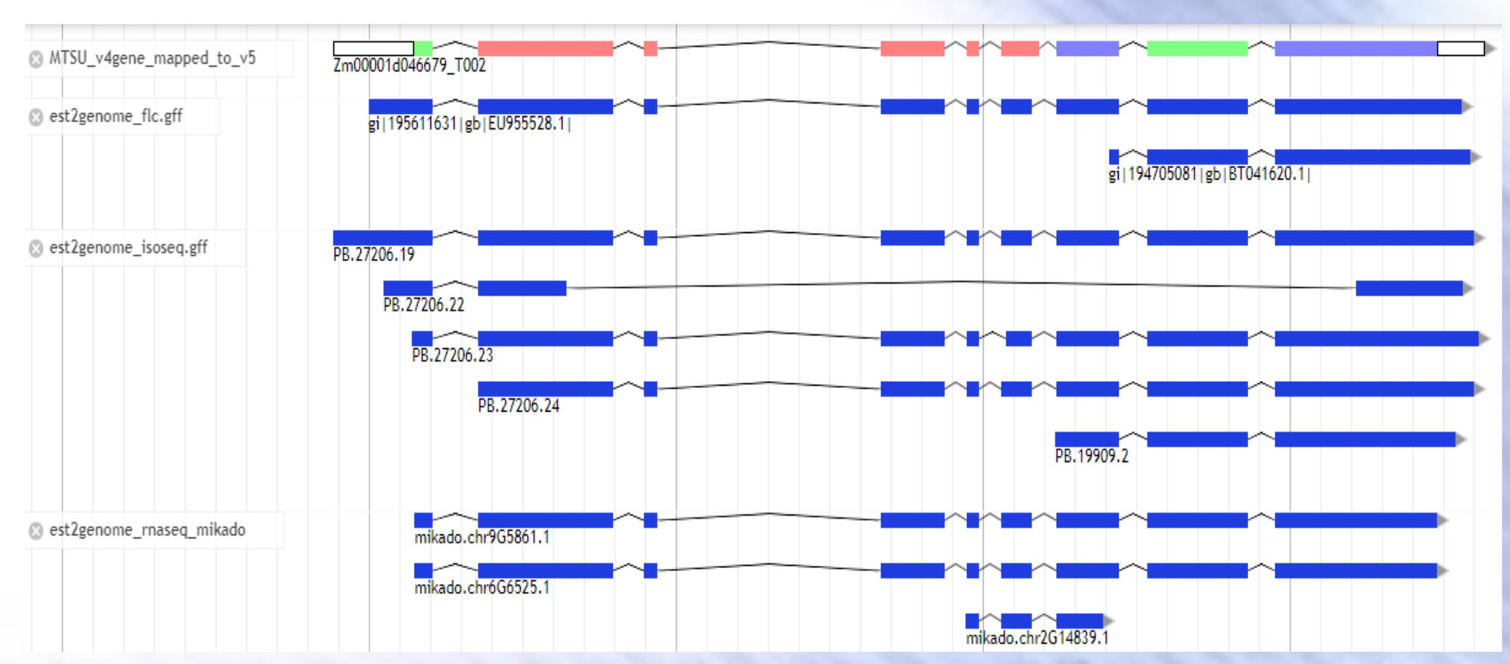

Figure A. Gene Structure from Apollo Annotator Version 5
Exons are solid blocks and introns are horizontal black lines. Each row is a proposed structure of the gene based on analysis of PCR

B

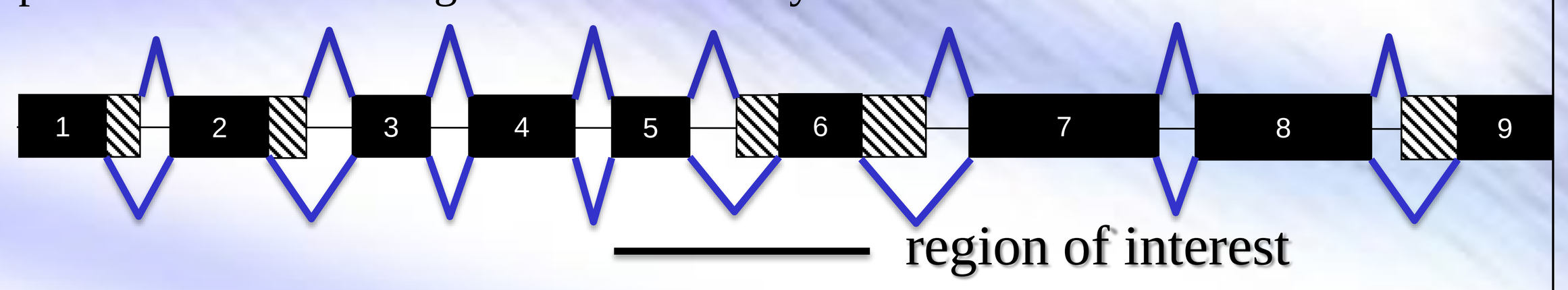

Figure B. Proposed Gene Structure for *Zea mays* Gene Zm00001d052233. Exons are shown as black boxes, introns are horizontal lines, hatched shows the alternative region. Horizontal lines represent introns. Angled lines above and below the image represent the constitutive splicing pattern. Horizontal line below the image shows the region of interest. The structure was designed using RNA evidences provided by the Apollo for Corn genome (unpublished data, Micklos, and Ware 2019).

C

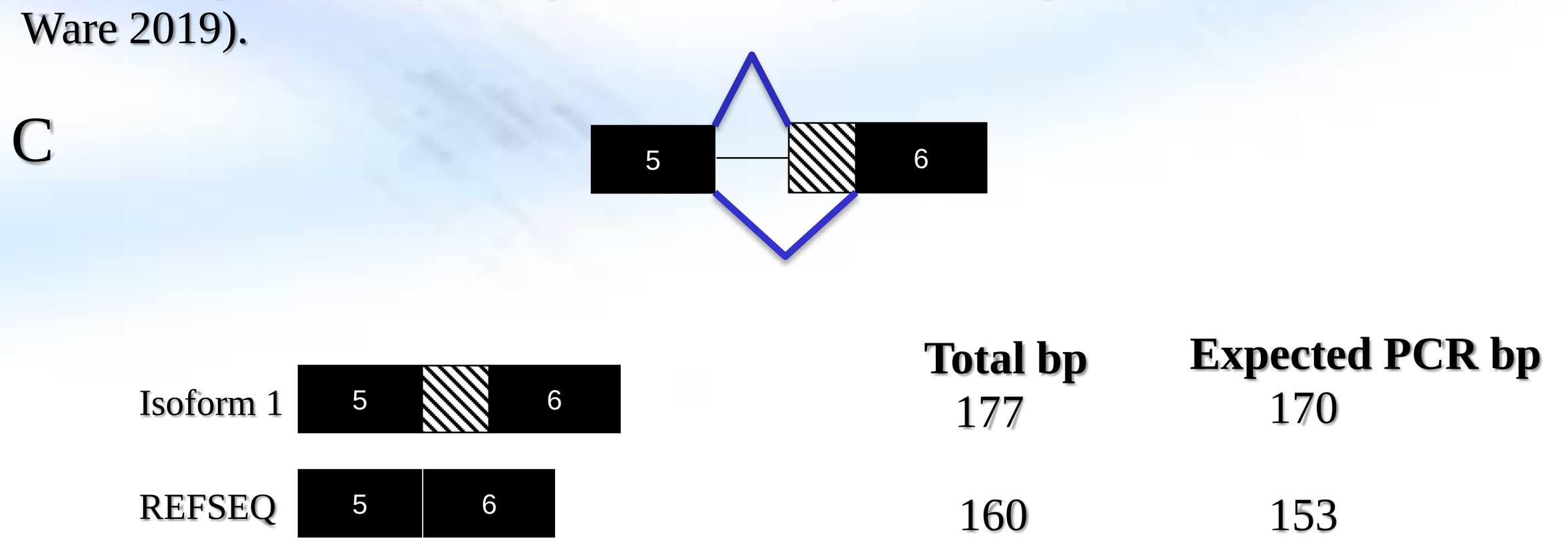

Figure B. Alternative Splices of Region of Interest Including Exon 5 and Exon 6. Expected RT-PCR base pair lengths in comparison to the total are noted at right in base pairs (bp) Splice pattern noted by the angled line above the structure produces isoform 1 transcript while the splice pattern below the structure produces the reference sequence transcript (REFSEQ). Primer to detect both transcripts were designed using Primer3Plus (Rozen et al. 2000).

RT-PCR

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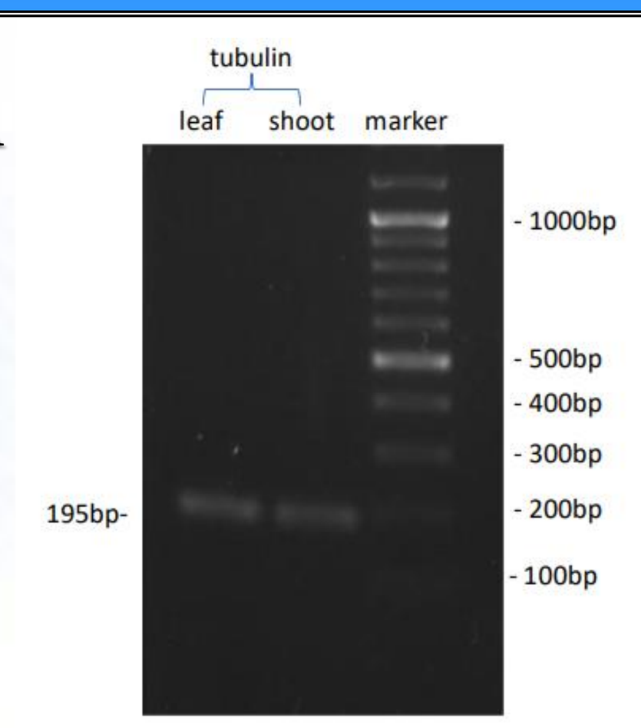

Figure A. Reverse Transcription PCR Test of RNA using Tublin Primers. Cassie's cDNA was used. Agarose gel electrophoresis was used to separate products of RT-PCR using corn RNA samples as noted above each well. Sizes of the marker are noted at right while the observed tubulin fragment size is noted at left. Expected size 195 bp (Ogene Ruler: Thermo Scientific).

B

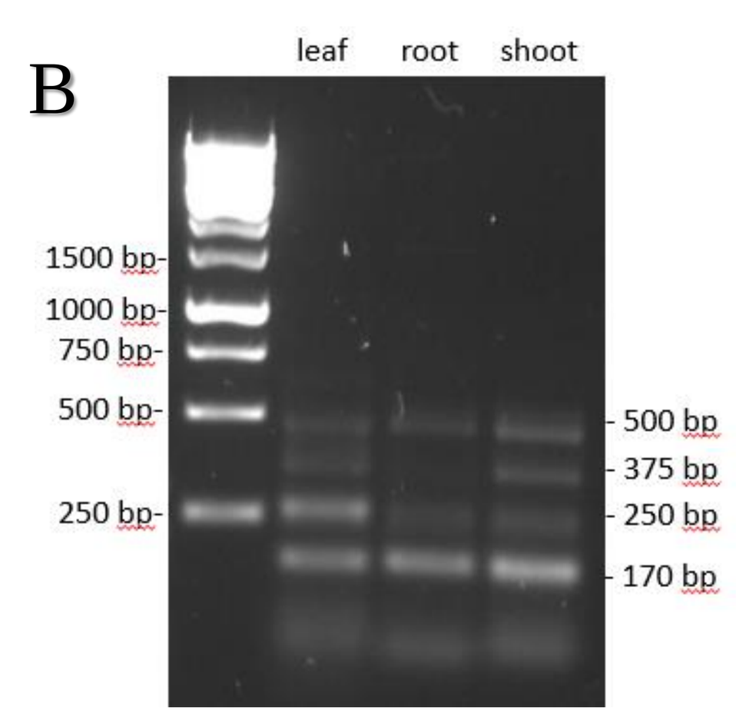

Figure B. Reverse Transcription PCR test of RNA Using Zm00001d2233 Primers at an Annealing Temperature of 57˚c. Agarose gel electrophoresis was used to separate RT-PCR products using corn RNA sample. Expected size for isoform 1 is 170 base pairs (bp) and 153 bp for isoform 2. Sizes of the OGene Ruler marker are noted at the left in bp and isoforms noted at the right in bp.

C

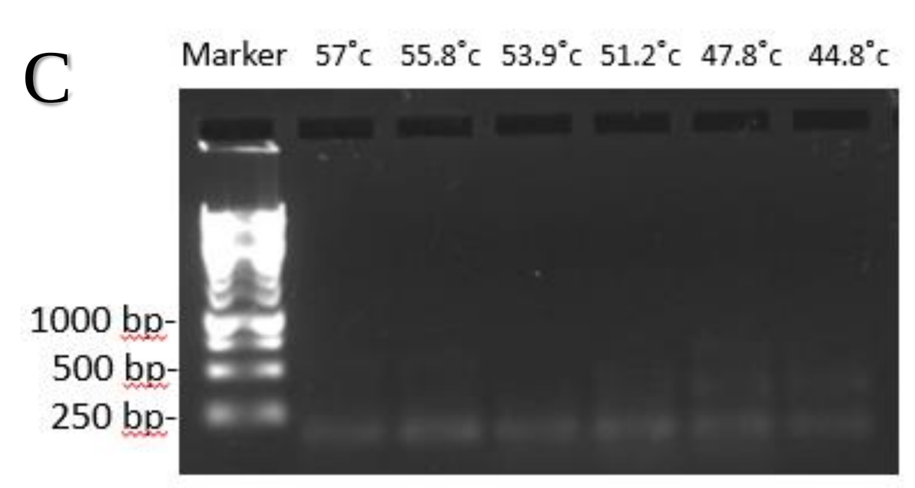

Figure C. Reverse Transcription RT-PCR of RNA using designed primers. Agarose gel electrophoresis was used to separate products of RT-PCR using leaf RNA annealed at various temperature as noted above each well. Marker (Ogene Ruler: Thermol Scientific) sizes are noted at left and observed product sizes are noted at the right.

Comparison Table

RT-PCR size (bp)	Transcript	Provides evidence for revision to gene models?	Expected and/or observed?	Domains likely missing	Likely functional?
170	Isoform 1	no	E, O	no	yes
153	Isoform 2	no	E	yes	no
250	Isoform 3	yes	O	unknown	unknown
375	Isoform 4	yes	O	unknown	unknown
500	Isoform 5	yes	0	unknown	unknown

Figure A. Analysis of RT-PCR of RNA using designed primers results.

Conclusions

- Leaf RNA extraction was unsuccessful and Jori's sample was used
- Reverse transcription was successful
- Primers were visible at all annealing temperatures and 57˚c was used
- Five RNA isoforms were present in all corn tissues
- We found isoform 1 to be expected and observed so its domains are likely not missing and is functional
- However, isoform 2 should have been observed so it likely has missing domains and is nonfunctional
- Three new isoforms (3, 4, and 5) were found observed but not expected have unknown domains and functionality so further research could be conducted
- Based on the results the gene structure should be revised

Future Directions

- Run new trials using our designed primers for the region of interest including exon 5 and 6 of the gene
- New fragments could be produced providing information supporting the current model
- New evidence of new splicing patterns that could support the revision of the gene model
- Run new trials on more tissues and all the newly found isoforms

Literature Cited

Chen P, Du Q, Liu X, Zhou L, Hussain S, Lei L, Song C, Wang X, Liu W, Yang F, et al. 2017. Effects of reduced nitrogen inputs on crop yield and nitrogen use efficiency in a long-term maize-soybean relay strip intercropping system. PLoS One [Internet]. [cited 2019 September 1]; 12 (9): 1-19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5598979/

Feedgrains sector at a glance [Internet]. USDOA; [updated 2019 Aug 20; cited 2019 Sept 11] Available from: https://www.ers.usda.gov/topics/crops/corn-and-other-feedgrains/feedgrains-sector-at-a-glance/

Kumar A, Pandeya A, Malik G, Sharma M, P HK, S AK, Gahlaut V, Gajula MNVP, Singh KP, Suravajhala P, et al. 2018. A web source for nutrient use efficiency- related genes, quantitative trait loci and microRNAs in important cereals and model plants. F1000Res [Internet]. [cited 2019 September 1]; (7):1-12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073097/

Untegrasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. [Internet] Primer3- new capabilities and interfaces. [cited fall 2019]. Available from: http://primer3plus.com/cgi-bin/dev/primer3plus.cgi