AgriScience Program - Projects Component

Final Performance Report

This template covers the annual performance reporting for the final year of the project and two additional questions to satisfy the final performance reporting requirements.

Section A: Annual Performance Reporting

This section is the same as previous Annual Reports completed to date, and is intended to capture only those results that were achieved during the final year of the project.

Name of Recipient: The Prairie Oat Growers Association (POGA)	
Project Title: Tuning the Oat Genome with CRISPR-based systems	
Project Number: ASP-061	**Final Period Covered by the Report** (2021/03/31 to 2022/03/31):
Project Start Date (2019/01/01):	**Project End Date** (2022/03/31):

1. Performance Measures – Project Level

In the performance measures table below, please provide the results and achievements that were finalized during this final reporting period, that combines all the CA and CRDA activities. Do not include results that are not final. Please see Annex A for a description of each performance measure.

	Performance Measure	**Results Achieved**	Provide a brief description of each final result achieved during the reporting period.
1.	Number of highly qualified personnel (HQP) working on funded activities (HQP refers exclusively to current Master and PhD students)	2	Two MSc students: 1. Thomas Donoso, MSc Plant Science, McGill University 2. Annis Fatmawati, MSc Plant Science, McGill University
2.	Training/knowledge transfer events		
	2.1 Number of training/knowledge transfer events organized by the recipient		
	2.2 Number of presentations made in training/knowledge transfer events	4	1. *CRISPR-Cas9 based genome editing technologies* (Zhou and Singh), Lincoln M. Alexander Secondary School, Brampton, Ontario, Dec 15, 2021 (delivered remotely). 2. *Gene editing through CRISPR: Possibilities of decoding and tuning of oat genes,* Prairie Oat Growers Association 24th annual conference, Dec 1, 2021, Fairmont Springs Hotel,

	Performance Measure	Results Achieved	Provide a brief description of each final result achieved during the reporting period.
			Banff, AB, Canada (**Keynote lecture**). 3. ***Decoding the oat genome***, Fifth International Scientific Conference, "Latest Achievements of Biotechnology", National Aviation University**,** Kyiv Ukraine, September 22-23, 2021 (**Plenary lecture**, delivered remotely due to Pandemic). 4. ***Technological advances that can accelerate oat improvement***, Speaking of Oats, Oat Global, University of Minnesota, USA, April 15, 2021 **(Webinar Keynote).**
3.	Number of participants at training/knowledge transfer events		
4.	Number of new knowledge transfer products developed		
5.	Number of papers published in peer reviewed journals	1	Mahmoud, M., Zhou, Z., Kaur, R., Bekele, W., Tinker, N., Singh J. 2022. Toward the Development of Ac/Ds Transposon-mediated Gene Tagging System for Functional Genomics in Oat (Avena sativa L.). *Functional and Integerative Genomics* (accepted).
6.	Number of new technologies (new products, practices, processes and systems) that are developed		
7.	Number of new technologies (new products, practices, processes and systems) that are assessed under research conditions	1	Gene editing – CRISPR-associated editing technique is a modern plant breeding tool which allows direct modification of genes precisely and efficiently. Gene editing has potential to generate mutations in the host genes without the need for transgenic organisms. In this project, we have successfully edited the oat gene in “Park” variety. This is the first ever gene-editing success in oat.
8.	Number of new technologies (new products, practices, processes and systems) that are demonstrated on-farm or in-plant		
9.	Number of new technologies (new products, practices, processes and		

Performance Measure		Results Achieved	Provide a brief description of each final result achieved during the reporting period.
	systems) that attain Intellectual Property (IP) protection.		
10.	Number of new technologies (new products, practices, processes and systems) that are utilized		

2. Activity-level Information

In this section, please complete one table for each activity. For activities with both a CA and CRDA component, please integrate the results into one table.

CA Activity Number: ____ / CRDA Activity Number: ___
Name(s) of Activity: Development of oat genome specific gene constructs for genome editing
Principal Investigator: Jaswinder Singh
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
Standardizing qRT-PCR-Based Analysis of *AsTLP8* homoeologs The sequence-based analysis led to interesting inferences into the role of *AsTLP8* homoeologs in beta-glucan regulation. However, we were interested in demonstrating if a similar relationship between beta-glucan and *TLP8* expression in oat as observed in barley (Singh et al. 2017). Primers for qRT-PCR were designed to target each homoeolog separately. Considering the beta-glucan content data available in the literature is typically measured by dry weight of the mature seed (Appendix A8), we looked at the relative expression of *AsTLP8* homoeologs within the mature seed. Although the means of certain varieties varied greatly, namely Terra and Goslin, the standard deviation was high between replicates (Figures 3.4-3.6). Further analysis using the Tukey test, a post hoc analysis of the ANOVA one-way test, determined that no statistically significant difference between the relative expressions was observed (Keselman and Rogan 1977). Therefore, it is difficult to infer the relationship between TLP expression and beta-glucan content and deserves further attention. Validation of genotypic and phenotypic relations could be discerned by generating and analysing gene specific mutants. In the next section, efforts have been made to edit each homoeologs of TLP8 in oat.

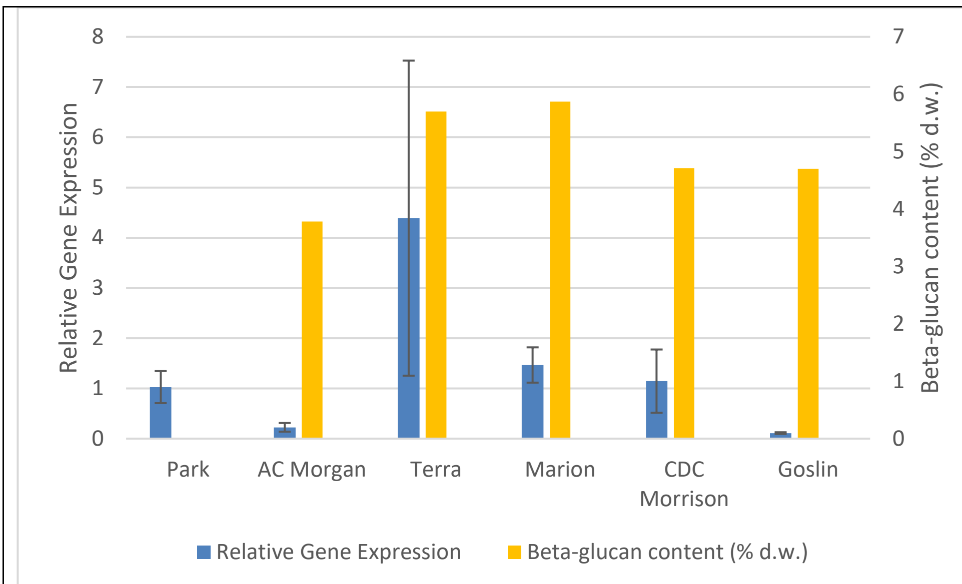

Figure 3. 1: Transcript abundance of *AsTLP8-A* in the mature seed of varieties with differing beta-glucan content. The relative gene expression was measured in the mature seed of each variety through qRT-PCR. The variety park was used as the control for relative gene expression. Error bars were measured by taking the standard deviation of biological and technical replicates of the relative expression. The beta-glucan content is derived from various sources (Appendix A8) and measures the percent dry weight within the mature seeds. None of the relative gene expression was shown to be significantly different based on the Tukey test with a $p < 0.05$ (Keselman and Rogan 1977).

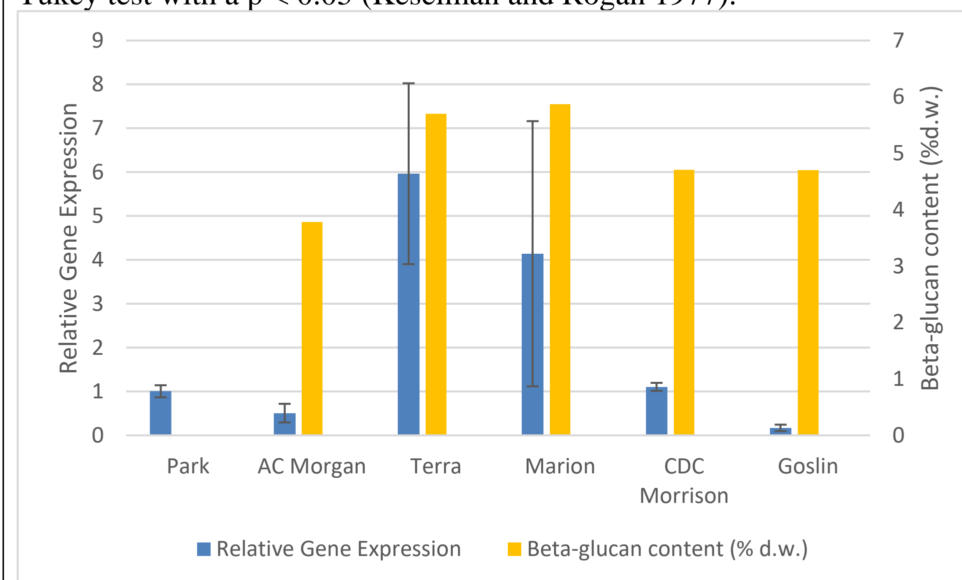

Figure 3. 2: Relative gene expression of *AsTLP8-C* in the mature seed of varieties with differing beta-glucan content. The relative gene expression was measured in the mature seed of each variety through qRT-PCR. The variety park was used as the control for relative gene expression. Error bars were measured by taking the standard deviation between the biological and technical replicates of the relative expression. The beta-glucan content was derived from various literary sources (Appendix A8) in which the dry weight beta-glucan content of the mature seed was measured. None of the relative gene expression was shown to be significantly different based on the Tukey test with a $p < 0.05$ (Keselman and Rogan 1977).

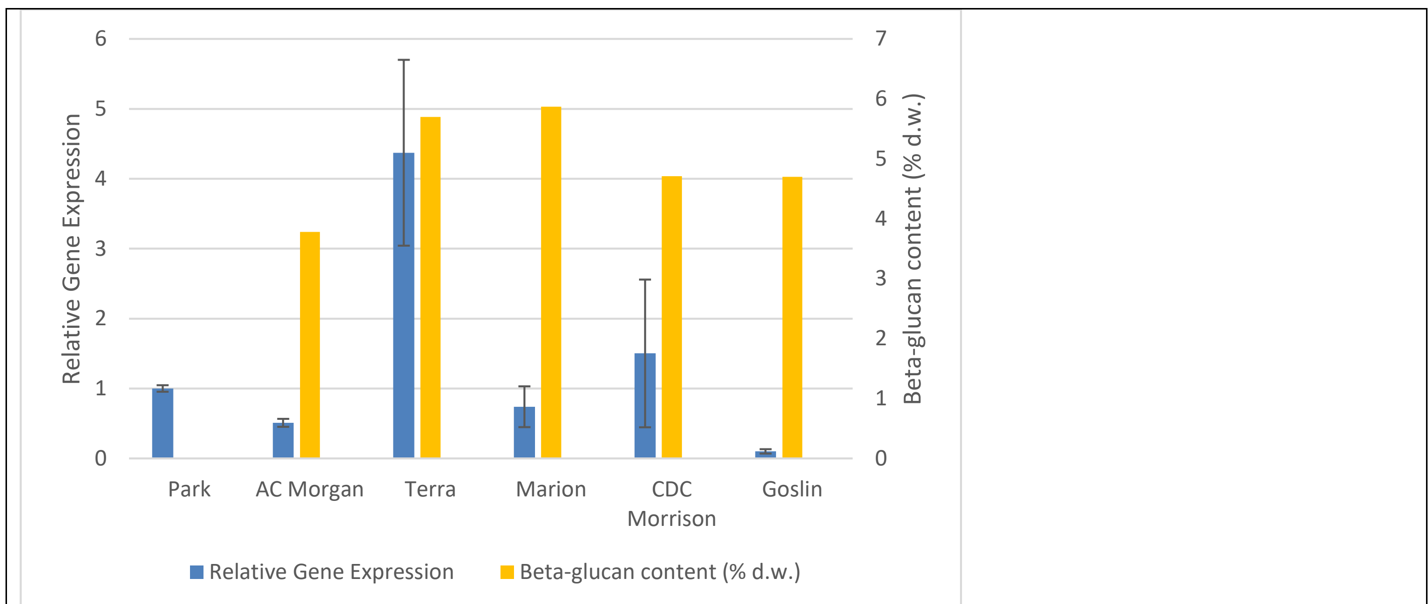

Figure 3. 3: Transcript abundance of *AsTLP8-D* mRNA in varieties with differing beta-glucan content. The relative gene expression was measured in the mature seed of each variety through RNA extraction, cDNA synthesis, and qRT-PCR. The variety park was used as the control for relative gene expression. Error bars were measured by taking the standard deviation between biological and technical replicates of the relative expression. The beta-glucan content was derived from various literary sources (Appendix A8) in which the dry weight beta-glucan content of the mature seed was measured. None of the relative gene expression was shown to be significantly different based on the Tukey test with a $p < 0.05$ (Keselman and Rogan 1977).

Transformation of oats using the *AsTLP8* gene-editing constructs

The three gene-editing constructs were introduced into the common oat (v. Park) via microprojectile bombardment. Considering the presence of Hygromycin phosphotransferase in each construct, Hygromycin was used to select for calli carrying the foreign DNA. A total of 210, 210, and 280 calli were bombarded for the *AsTLP8-A*, *AsTLP8-C*, and *AsTPL8-D* targeting constructs, respectively. Of the calli that survived Hygromycin selection, the A-genome targeting plasmid (pTAN) demonstrated the highest plantlet regeneration frequency at 87.5%. Meanwhile, the D- and C-genome targeting plasmids demonstrated a plantlet regeneration frequency of 65% and 66%, correspondingly. Regenerated plantlets were then PCR tested for transformation using gRNA within their respective constructs (Table3.1).

Table 3. 1: Regeneration frequency of gene-editing constructs.

Construct	Plates Bombarded	Calli pieces	Selection Medium	Calli Passed Selection	Plantlets Regenerated	Regeneration Frequency (%)
pTAN	6	210	Hygromycin	48	42	87.5
pTCN	6	210	Hygromycin	40	26	65
pTDN	8	280	Hygromycin	53	35	66

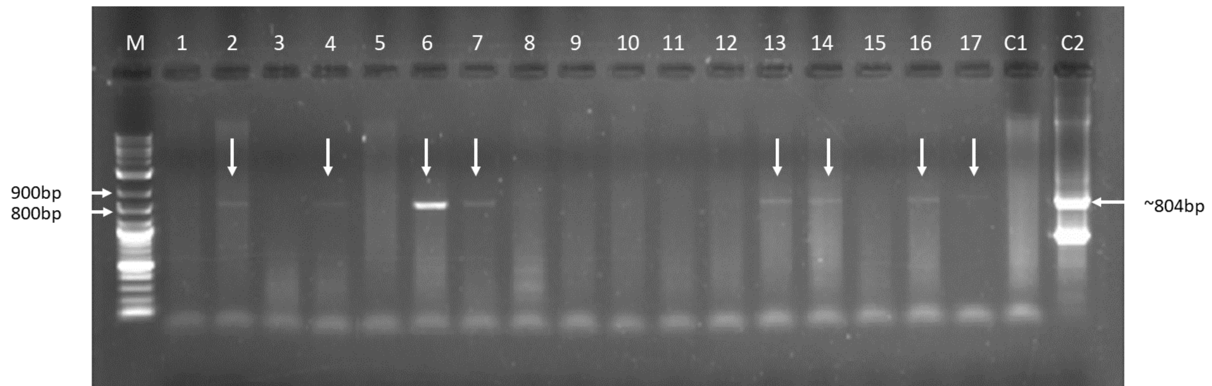

Figure 3. 4: PCR-based analysis of putative pTAN transformed plants. Samples were PCR amplified using the primers ASG1O1 and ASG3O2 (Table P). The M Lane contains a 1kb+ ladder (NEB). Lanes 1-17 denote genomic DNA of plantlets that survived Hygromycin selection and bombardment using the pTAN construct. Lane C1 contains the genomic DNA of the wild-type Park as a negative control, and C2 contains the pTAN construct as a positive control. Samples were run on a 0.8% agarose gel. Lanes 2, 4, 6, 7, 13, 14, 16, 17, and C2 contain a band approximately the size of the expected 804bp.

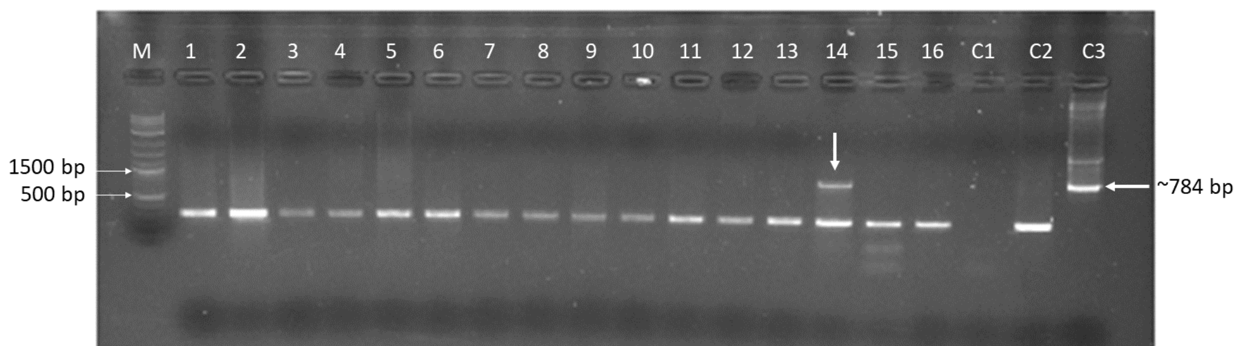

Figure 3.5: PCR-based analysis of putative pTCN transformed plants. Samples were PCR amplified using the primers CSG2O1 and CSG1O2 (Table P). The M lane contains a 1kb+ ladder (NEB). Lanes 1-17 denote genomic DNA of plantlets that survived Hygromycin selection and bombardment using the pTCN construct. Lane C2 contains the genomic DNA of the wild-type Park, and C1 contains water as negative controls. The C3 lane contains the pTCN plasmid as a positive control Samples were run on a 0.8% agarose gel. Lanes 14 and C3 contain a band approximately the size of the expected 784 bp.

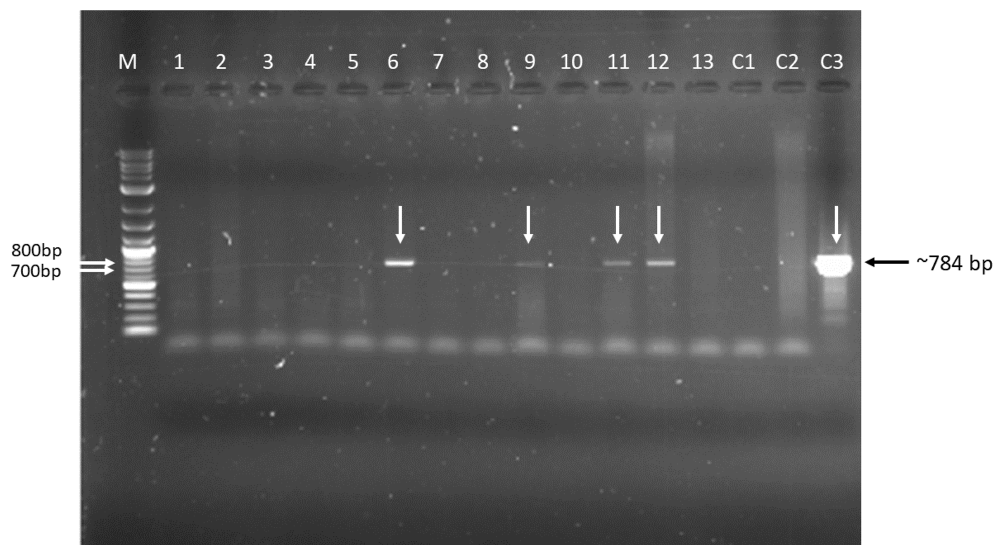

Figure 3.6: PCR-based analysis of putative pTDN transformed plants. Samples were PCR amplified using the primers DSG1O1 and DSG2O2 (Table P). The M lane contains a 1kb+ ladder (NEB). Lanes 1-13 denote genomic DNA of plantlets that survived Hygromycin selection after bombardment using the pTDN construct. Lane C2 contains the genomic DNA of the wild-type Park, and C1 contains water as negative controls. The C3 lane contains the pTDN plasmid as a positive control. Samples were run on a 0.8% agarose gel. Lanes 6, 9, 11, 12 and C3 contain a band approximately the size of the expected 784 bp.

The PCR-based analysis confirmed transformants in all three constructs in the T0 generation (Figures 3.4-3.6). The pTAN construct also boasted the highest transformation frequency at 5.23% (Table 3.2). The *AsTLP8-D* (pTDN) targeting construct had a transformation frequency of 2.86, while the *AsTLP8-C* (pTCN) targeting construct had a 0.47% frequency.

Table 3. 2: Transformation frequency of gene-editing constructs

Construct	Calli pieces bombarded	Transformants (PCR +)	Transformation Frequency (%)
pTAN	210	11	5.23
pTCN	210	1	0.47
pTDN	280	8	2.86

Analyzing the CRISPR/Cas9 efficiency in transgenic lines

Plants that were confirmed to be transgenic were continued into the next generation (T1). The T1 generation was then screened for deletions caused by the gRNA and Cas9 targeting *AsTLP8* homoeologs. Those transformed with the pTAN construct demonstrated no visible shift in band size (Figure 3.7). The pTCN transformed T1 generation had a notable size shift in one sample, which potentially indicates a deletion within the C-genome *AsTLP8*. However, this sample contained two bands, suggesting that this suspected mutation is in the heterozygous state (Figure 3.8). Lastly, small size shifts were observed in the T1 generation of confirmed pTAN transformed T0 lines (Figure 3.9)

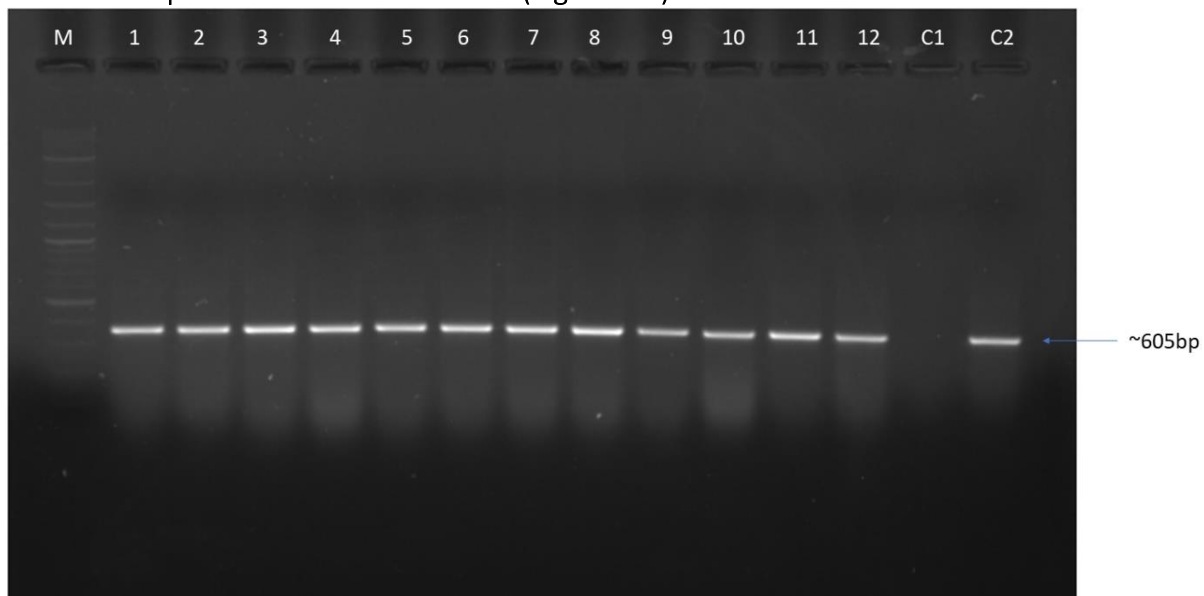

Figure 3. 7: Screening for CRISPR/Cas9 induced deletion within the *AsTLP8-A* gene of T1 transgenic lines. Samples were PCR amplified using the A-genome specific AsTLP8A-In2F and AsTLP8A-In3R primers (Table 3.1). Samples 1-12 indicate genomic DNA of the T1 generation of confirmed T0 transgenic lines. The C1 lane contains a negative water control, and the C2 lane contains the negative wild-type Park control. The M lane contains a 1kb+ marker. Samples were run on a 2% agarose gel. Samples 1-12 and C2 had a band of approximately 605bp with no noticeable shift in size. The water control contained no band.

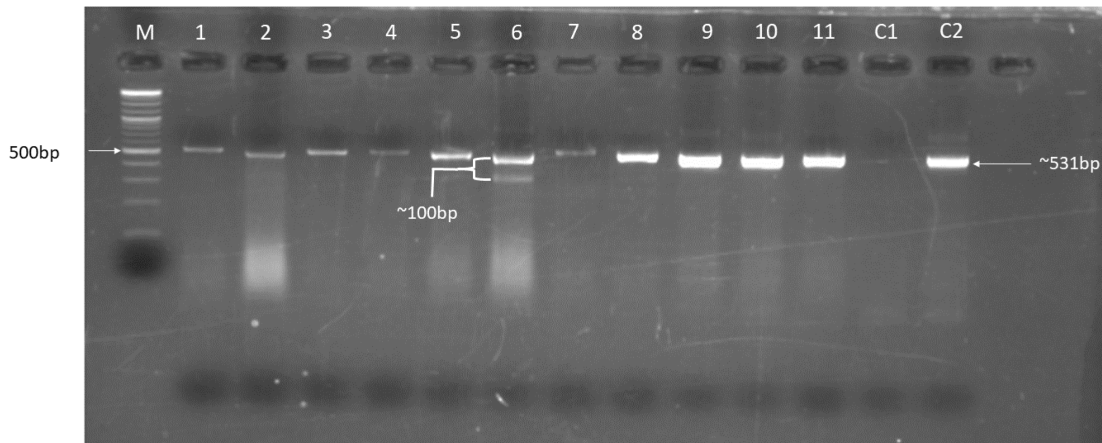

Figure 3. 8: Screening for CRISPR/Cas9 induced deletion within the *AsTLP8-C* gene of T1 transgenic lines. Samples were PCR amplified using the C-genome specific AsTLP8C-In2F and AsTLP8C-In2R primers (Table 3.1). Samples 1-16 indicate the genomic DNA of T1 generation of confirmed T0 transgenic lines. The C1 lane contains a negative water control, and the C2 lane contains the negative wild-type Park control. The M lane contains a 1kb plus (NEB) marker. Samples were run on a 2% agarose gel. Samples 1-12 and C had a band of approximately 531bp. The water control contained no band. Potential shifts in size can be seen in lanes 2, 5, 9, and 10. A clear shift can be seen in lane 6.

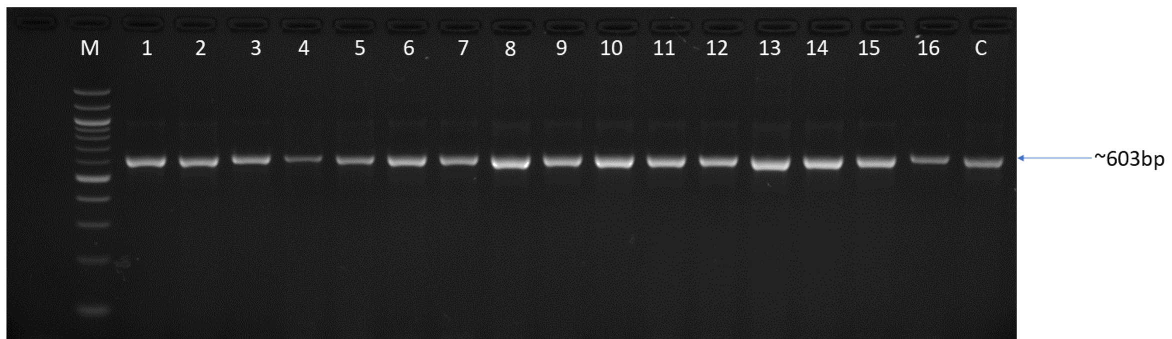

Figure 3. 9: Screening for CRISPR/Cas9 induced deletion within the *AsTLP8-D* gene of T1 transgenic lines. Samples were PCR amplified using the D-genome specific AsTLP8D-In2F and AsTLP8D-In1R primers (Table 3.1). Lanes 1-16 indicate the genomic DNA of the T1 generation of confirmed T0 transgenic lines. The C lane contains the negative wild-type Park control. The M lane contains a 100bp marker (NEB). Samples were run on a 2% agarose gel. Samples 1-12 and C had a band of approximately 603bp. The water control contained no band. Potential variations in size can be seen in lanes 1, 2, 6, 7, 13, and 14.

The T1 plants of the pTDN lines that demonstrated shifts were sent for Sanger sequencing. There were no changes at the site of the second gRNA within any of the sequences (Figure Se). At the site of the second gRNA, there were discrepancies between the expected sequence and the sequencing results. Specifically, in the D1 sequence there are ambiguous bases three nucleotides upstream from the PAM sequence (CGG). In the sequence D3, there is a one nucleotide base (T→G) in the same position. Evidence of gene editing was also visible within the site of the third gRNA. In two of the lines, a four base ambiguity is visible three nucleotides upstream of the PAM sequence (CGG). In the same position, D3 demonstrated a 3-nucleotide ambiguity. Overall, the *AsTLP8-D* targeting guide 1, 2, and 3 demonstrated a mutation frequency of 70, 0, and 80%, respectively (Table 3.7). These gene-edited lines are being pursued further to develop homozygous lines for downstream analysis for homoeologous specific TLP8 expression and beta-glucan content in mature and imbibed seeds.

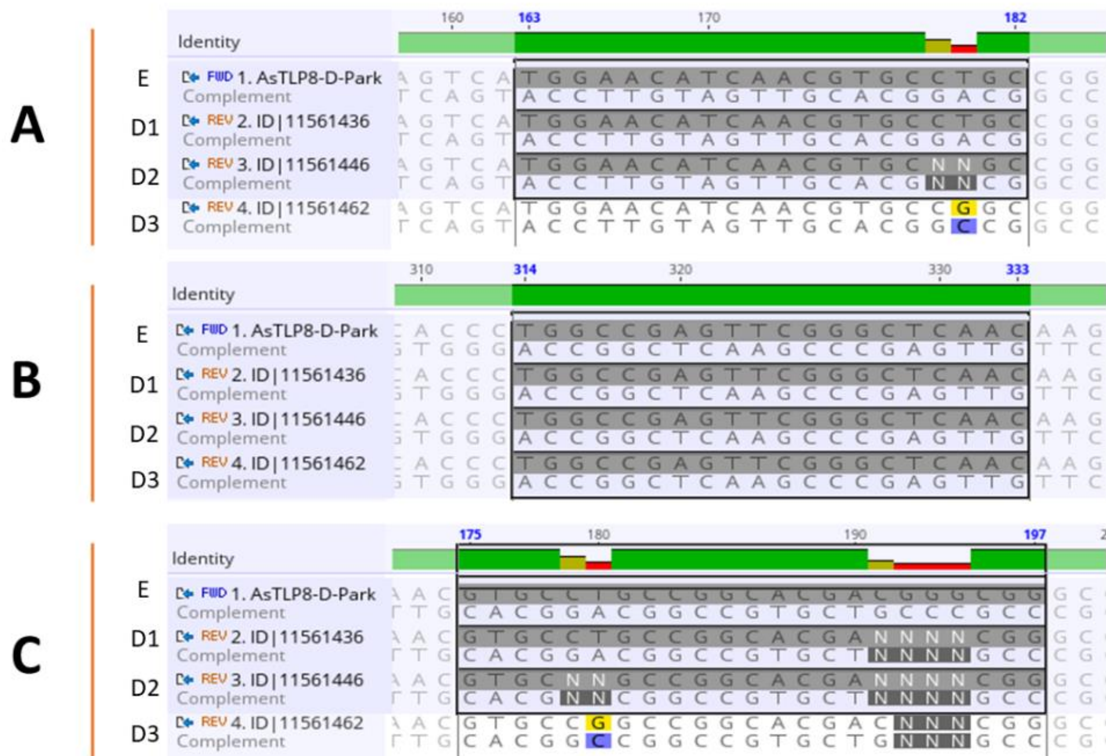

Figure 3. 10: Sanger sequencing of suspected gene-edited T1 plants. The E denotes the sequence of *AsTLP8-D* in oat variety Park. D1-3 are the sequences of *AsTLP8-D* of three suspected gene-edited lines. Sequences were aligned using the Geneious alignment tool. **(A)** The site of gRNA 1 within the *AsTLP8-D* sequence (Table SG). The D1 and expected have the same sequence based on the Sanger sequencing results. The sequences D2 and D3 vary from the expected sequence. D2 demonstrates ambiguous nucleotides three positions upstream of the PAM sequence (CGG). Likewise, the D3 sequence has one a nucleotide change (T →G) three positions upstream of the PAM sequence (CGG). **(B)** The site of gRNA 2 within the *AsTLP8-D* sequence (Table SG). The putative gene-edited lines, and expected all have the same sequence based on the Sanger sequencing results. **(C)** The site of gRNA 3 within the *AsTLP8-D* sequence.
The D1-3 sequences differed from the expected. The lines D1-2 demonstrated four ambiguous nucleotides three positions upstream of the PAM (CGG). The D3 line showed three ambiguous nucleotides in the same position. Letter N indicates the deletion of nucleotide.

Table 3. 3: Mutation frequency in *AsTLP8-D* of CRISPR/Cas9 by guide RNA.

Guide RNA	Transgenic T1 plants tested	Transgenic T1 plants with CRISPR/Cas9 induced mutation	Mutation frequency	Predicted Activity Score (Doench 2014)
AsTLP8-D Guide 1	10	7	70%	0.62
AsTLP8-D Guide 2	10	0	0%	0.866
AsTLP8-D Guide 3	10	8	80%	0.35

Issues

- Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome?
- Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?

Pandemic certainly influenced our project. The project was extended for additional year but without additional funding.
Key Achievements A key achievement represents a significant achievement or tangible result that could potentially be applied either by farmers or industry or the science community. In one to three paragraphs, please provide key achievements that meet one of the following criteria: 1) The item has commercial potential (all testing and piloting has been completed); 2) The item has been commercialized; or 3) The item has been adopted by the sector. Examples of tangible results could include increased sustainability (beneficial management practice), reduced costs, improved productivity or increased profitability. Please note that the information provided will be used for communication purposes only. If no key achievements have been realized at this stage, please leave this section blank.

CA Activity Number: ____ / CRDA Activity Number: ___
Name(s) of Activity:
Principal Investigator:
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
Issues • Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome? • Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?

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If no key achievements have been realized at this stage, please leave this section blank.

CA Activity Number: ____ / CRDA Activity Number: ___
Name(s) of Activity:
Principal Investigator:
Summary of Activity Please provide a high-level summary of this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Technical language can be used in this section.
Issues • Describe any challenges or concerns in achieving the results and deliverables of this activity during the reporting period. How were they overcome or how do you plan to overcome? • Describe any potential changes to the work plan and the budget during the reporting period. How were or how will they be managed?

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Please add additional tables here as required

Section B: Final Performance Reporting

The following three questions are supplemental to the standard APR questions, to gather additional information as required for the final year of performance reporting.

3. Results Variance

The table below presents the performance measure targets initially identified in the project's work plan, as well as the results achieved by this project as reported in previous Annual Performance Reports. The targets and results achieved include all the CA and CRDA activities. To easily see whether there is a variance between the targets set and the results achieved over the life of the project, you may add the value in the Previous Results column to the results achieved this year (as recorded in the table at the beginning of this document) and note the sum in the Total Results column.

	Performance Measure	**Targets** (as set out in the CA work plan)	**Previous Results** (as reported in previous APRs)	**Total Results** (previous results + results achieved this year)
1.	Number of highly qualified personnel (HQP) working on funded activities (HQP refers exclusively to current Master and PhD students)	1	2	2
2.	Training/knowledge transfer events			
	2.1 Number of training/knowledge transfer events organized by the recipient			
	2.2 Number of presentations made in training/knowledge transfer events		3	7
3.	Number of participants at training/knowledge transfer events			
4.	Number of new knowledge transfer products developed			
5.	Number of papers published in peer reviewed journals			
6.	Number of new technologies (new products, practices, processes and systems) that are developed			
7.	Number of new technologies (new products, practices, processes and systems) that are assessed under research conditions	1		1
8.	Number of new technologies (new products, practices, processes and systems) that are demonstrated on-farm or in-plant			
9.	Number of new technologies (new products, practices, processes and systems) that attain Intellectual Property (IP) protection.			
10.	Number of new technologies (new products, practices, processes and systems) that are utilized			

Please provide a brief explanation of the variance for any performance measures for which the **total results achieved** are less than the target set.

4. Knowledge and Technology Transfer (KTT)

What is your target audience for sharing information about the results of your project? Describe your strategy and success in reaching this target audience.
Target audience is oat reserchers, oat breeders, oat farmers, oat processors and information was shared at conferences, AGMs, workshops and symposia. The goal is to share information as broadly as possible. The project results were presented at the 2021 POGA AGM at Banff, AB. A peer reviewed research publication for scientific dissemination has been accepted for publication in the Journal "Functional and Integrative Genomics."

5. Gender-Based Analysis Plus (GBA+)

To the best of your knowledge, how many of the HQP who are working on the project meet the GBA+ categories outlined below? Please indicate the total number for each category. If a HQP fits in more than one category, please count them in as many of the categories as appropriate. Only indicate a number and not the names of the individuals.

	Female	Indigenous peoples	Visible minority	LGBTQ2+	Person with disability
Number of HQP	1				

Annex A

Performance Measures Table	
Performance Measures	**Description**
1. Number of highly qualified personnel (HQP) working on funded activities	This only includes individuals who are registered in Master or PhD programs and are working on activities that receive funding through the Canadian Agricultural Partnership. They are only counted in their first year working on projects. For each reported HQP, please provide the following: the name of the student, level of degree, field of study and name of the academic institution.
2. Training/knowledge transfer events	
2.1. Number of training/knowledge transfer events organized by the recipient	This includes events completed in the reporting year that were organized under the project to share results of the activities with audiences who may use that knowledge in the future. Examples could include training events, scientific meetings, symposia, conferences, workshops, industry meetings, field days or webinars. Annual General Meetings do not normally qualify for this category as they are considered to be part of normal day-to-day business. For each reported item, please provide the following: name of the event, name of the organizer and organization, location, and year/month/day.
2.2. Number of presentations made in training/knowledge transfer events	This includes oral presentations and poster presentations at events that are not organized by the recipient, for example, conferences, symposiums or training events. For each reported item, please provide the following: name of presenter, title of presentation, name of the event, location, and year/month/day.
3. Number of participants at training/knowledge transfer events	This includes individuals who attend the events listed and who may use that knowledge in the future.
4. Number of new knowledge transfer products developed	New knowledge could include, but is not limited to: 1) newly acquired knowledge that differs significantly from previously acquired knowledge; 2) existing knowledge that is enhanced to meet different requirements; 3) existing knowledge that is applied in different situations. These are knowledge transfer materials created under the project that have been disseminated to transfer information to audiences who may use that knowledge in the future. Examples could include brochures, factsheets, flyers, guides, articles in trade magazines, technical bulletins and social media items. Only the number of products developed should be reported, not the number of copies that were printed and disseminated. For each reported item, please provide the following: author(s), title of the item, type of the reported item (e.g. brochure), name of the trade magazine/publisher and page number(s) if applicable, and year/month/day.

5. Number of papers published in peer reviewed journals	This includes scientific papers that are published in peer reviewed journals. Papers that are not yet published (ex. manuscripts in preparation, under review or accepted) should not be reported. For each reported item, please provide the following: author(s), year of publication, article title, title of journal, volume (issue), and page number(s). If the item is a book or a book chapter, add name of publisher. If the item is an article for conference proceedings, add title of published proceedings, location, and year/month/day.
6. Number of new technologies (new products, practices, processes and systems) that are developed	A new technology could include, but is not limited to: 1) a newly created technology that differs significantly from existing technologies; 2) an existing technology that is modified to meet different requirements; 3) an existing technology that is tested in different situations. New products are goods and services that differ significantly in their characteristics or intended uses from products previously produced and used. Examples could include equipment, software, novel foods or consumer goods. New practices are new agronomic techniques or methods that can be applied directly by producers. New processes are the set of operations performed by equipment in which variables are monitored or controlled to produce an output in labs or processing facilities. New systems are the set of detailed methods, procedures and routines created to carry out a specific activity, perform a duty, or solve a problem. Development consists of the creation of a new product, the generation of a new practice, or the demonstration of utility of a new process or system. This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.
7. Number of new technologies (new products, practices, processes and systems) that are assessed under research conditions	See the definition of new technologies under #6. Are assessed: when new technologies are evaluated or tested under research conditions. This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category.

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8. Number of new technologies (new products, practices, processes and systems) that are demonstrated on-farm or in-plant	See the definition of new technologies under #6. Are demonstrated: when new technologies are presented to the sector by experiments, prototypes, examples or pilot on-farm or in-plant. This category does not include new varieties. New varieties are only reported under 'Number of new technologies that attain Intellectual Property protection' and/or 'Number of new technologies that are utilized'. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.
9. Number of new technologies (new products, practices, processes and systems) that attain Intellectual Property (IP) protection	See the definition of new technologies under #6. Examples for IP protection could include, but are not limited to: plant breeder rights, patents filed, registered trademarks and copyrights, and registered germplasms and released varieties (excluding breeding lines and gene sequences). For each new variety, please provide the registration number, the variety name, and year/month/date.
10. Number of new technologies (new products, practices, processes and systems) that are utilized	See the definition of new technologies under #6. Are utilized: when new technologies are adopted or implemented for use within the sector. Examples may include, but are not limited to: a signed license agreement, a signed letter of intent, a new product that is available on the market, and a new practice which is adopted by farmers. Gene sequences, breeding lines and populations are not eligible under this category. To avoid duplication, for any new technologies, only set a target that represents the last stage in the innovation process. For example, a new technology is either developed, or assessed, or demonstrated or utilized.