Government of Saskatchewan
Ministry of Agriculture

**ADF Project Progress Report Format**

1. **Project title, ADF file number and reporting period.**

   Development of markers linked to oat crown rust resistance to help breed improved varieties for Saskatchewan producers.
   20180264
   January 1, 2019 – June 30, 2020

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4. **Abstract (Not more than 250 words).** *Describe in lay language the progress towards the project objectives over the last reporting period. Include any key findings and any interim conclusions. Include any deviations from the original methodology.*

Goodprogress on the objectives for project 20180264 were made in 2019-20, despite the significant slow down in the past 6 months due to COVID-19. Phenotyping of populations segregating for *Pc40* and *Pc46* were accomplished, genotyping of populations segregating for *Pc40*, *Pc46*, *Pc62* and *Pc67* were done and markers linked to *Pc98* were identified. New populations for mapping of *Pc46* and *Pc67* were created and are ready for phenotyping. Two adult plant resistance mapping populations were phenotyped in the field in 2019 and have been planted for a second year of phenotyping in 2020. Due to work being done on *Pc50* by a U.S. group, the McCartney group has decided to instead focus on mapping *Pc48*.The 6 month delay due to COVID-19 may impact our ability to deliver results by the current end-date of the project. However, we will not ask for an extension at the moment. Instead, we will see how much we can catch-up over the next year and we may ask for an extension at the time of the next progress report in July 2021.

5. **Introduction:** *Brief project background and rationale.*

Oat is considered a healthy cereal due to a number of nutritional compounds found within the grain, including β-glucan. β-glucan is a soluble fiber that has been shown to lower plasma cholesterol and reduce the risk of heart disease (Queenan et al. 2007; Liatisa et al. 2009). This has resulted in health claims being established in both Canada (Health Canada 2010) and the United States (U.S. FDA 1997). Oat grain also contains a number of antioxidant compounds, including the polyphenolic avenanthramides which have anti-inflammatory effects that may protect against coronary heart disease (Meydani 2009). Oat contains 12-20% protein which is rich in globulins and contains more lysine and threonine than other cereals, thus providing a better balance of essential amino acids (Klose and Arendt 2012). Finally, oat is able to be consumed by most people suffering from celiac disease and is thus considered to be gluten-free (Peraaho et al. 2004).

As a result of these desirable attributes, oat remains a significant Canadian crop that has been seeded on an average of 3.1 million acres over the past 5 years, with Saskatchewan accounting for 50% of these acres (Statistics Canada, 2018). Over the past 5 years, 50% of the 3.2 million metric tonnes (MT) of oat produced annually in Canada has been exported to the U.S., primarily for the food market. In addition to the export of raw oat, oat is critical to the domestic milling industry. Total annual domestic milling in Canada sits close to 750,000 MT, with Saskatchewan being home to Richardson Milling (Martensville), Grain Millers (Yorkton), Ardent Mills (Saskatoon), and Avena Foods (Regina), or about half of all milling production. Forecasts indicate that domestic milling will grow to just over 900,000 MT within the next 6 years (R. Strychar, Ag Commodity Research). Combined sales of oat

and milled oat products were valued at almost $750 million last year. In addition, farm-gate sales of oat represent an estimated value of $450 million to growers.

The largest on-going threat to oat production in Canada (and worldwide) is the fungal pathogen *Puccinia coronata* Corda f. sp. *avenae*Eriks which causes oat crown rust. In Canada, yield losses averaged 5.1% from 2001 to 2005 (Chong et al. 2008) with the highest loss reported at 11.2% (McCallum et al. 2007). Similarly, yield losses of 50% and 35% were reported in Minnesota and South Dakota in 2014 (USDA-ARS 2014). In addition, crown rust can weaken straw causing plants to lodge (Endo and Boewe 1958). While cultural control methods, such as crop rotation and early seeding, and fungicide application can reduce crown rust severity, incorporating genetic resistance is an important component to an integrated management strategy. Finally, the significant acreage devoted to organic oat production, second only to wheat at 150,000 acres (Guerra 2017), relies heavily on genetic resistance.

Nearly 100 crown rust resistance genes, the vast majority being seedling resistance genes, have been reported in oat (Gnanesh et al. 2014). Despite the effectiveness of these genes to combat crown rust, there has been a history of these genes being defeated with 10 years of widespread deployment in cultivars due to the development of new virulent races of the pathogen (McCallum et al. 2007, Nazareno et al. 2017). As such, gene pyramiding and use of adult plant resistance (APR) are considered viable methods to deal with this issue. For example, the APR present in the Minnesota line MN841801 has been effective since the 1970s (Leonard 2002).

In order to effectively and efficiently implement these approaches, it is necessary to genetically map the location of both seedling and APR genes within the oat genome and develop markers to these genes. Crown rust resistance associated with seedling genes, such as *Pc45* (Gnanesh et al. 2015), *Pc91* (McCartney et al. 2011) and the recently reported, but unnamed, gene derived from *A. strigosa* (Rines et al. 2018), as well as, APR genes from MN841801 (Lin et al. 2014), are examples of genes with mapped locations within the oat genome. The CDC oat breeding program is currently using markers linked to these genes in order to incorporate and select for crown rust resistance. This is not only cost and time efficient, but it also allows identification of individual genes within an oat line containing several resistance genes which normally would not be possible using crown rust nurseries (as the line would simply appear resistant) or because crown rust isolates with the appropriate virulence combinations may not exist to differentiate individual genes.

With the decreasing cost associated with genotype-by-sequencing (GBS) markers, the availability of a 6K Infinium HD SNP genotyping platform and a consensus oat map integrating both marker types (Bekele et al. 2018), it is now possible to not only develop markers linked to oat crown rust genes, but also understand their chromosome locations and more importantly, the allelic relationships among the vast number of reported genes. Such information will allow oat breeders to understand which combinations of resistance genes can be pyramided together and test the effectiveness of such pyramids. Although the presence of some genes has been known for decades, their current distribution within oat germplasm and effectiveness is unclear. For example, although the *Pc45* gene was reported in 1971 (Fleischmann et al. 1971) it remains a useful gene within regions of Western Canada and Ontario (J. Menzies, personal comm.). It is likely that other such useful genes exist within the current pool of reported genes.

To build on Saskatchewan's (and Canada's) position as a supplier of premium quality oat to current US markets (and developing markets in Mexico and China) requires developing varieties with improved crown rust resistance. This will provide value to growers, through improved yield and reduced input costs (i.e. reduced fungicide use) which will help oat remain a viable crop within a grower's rotation, and to millers/food processors, through higher select ability (i.e. good plumpness and test weight).

**6. Objectives and the *progress towards meeting each objective***

| **Objectives (*Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objective. A justification is needed for any deviation from original objectives)*** | **Progress (e.g. completed/in progress)** |
|---|---|

| | |
|---|---|
| a)Evaluatecrown rustreaction inbi-parentaloatpopulations. | Bi-parental oat populations segregating for seedling and adult plant resistance will beevaluated for reaction to crown rust in growth chamber and field nursery experiments. |
| b)QTLmapping ofcrown rustresistance. | Bi-parental oat populations will be genotyped and analyzed for QTL associated with crownrust resistance using the phenotypic data collected in Objective 1. |
| c)Developmentof highthroughputmarkerassays. | Markers underlying QTL identified in Objective 2 will be converted to high throughput markerassays (e.g. TaqMan or KASP) for use in oat breeding programs to produce varieties withimproved crown rust resistance. Such varieties improve grower returns, via greater yield peracre and lower cost of production, and increase returns to oat millers, via the maintenance ofgrain plumpness and test weight. |

7. **Methodology:***Specify project activities undertaken during this reporting period.Include approaches, experimental design, tests, materials, sites, etc. Please note that any significant changes from the original work plan will require written approval from the Ministry.*

Genetic mapping populations

Bi-parental populations segregating for seedling (PcXX genes) and adult plant resistance (APR) to crown rust have already been created for this project. The genes represent a mixture of eight older genes (Pc40-Pc67) and newer seedling genes (Pc98 and Pc101), and two sources of APR. Details on the populations to be used are outlined in Table 1. It is proposed that molecular markers will be developed for six of the eight seedling genes (to be determined during the course of the project based on disease reaction data obtained) and both APR genes. Approximately 120-150 progeny from each seedling resistance population and 150-250 progeny from each APR population will be evaluated for disease reaction. Crown rust isolates which are avirulent to each seedling resistance gene will be used to evaluate these populations, while a mixture of field collected isolates will be used to evaluate APR after ensuring the mixture is virulent against any seedling resistance genes carried in the parents of the two APR populations. Some populations will be used for mapping, meaning that the population will be used to identify QTL, while others will be used for confirmation, meaning that markers identified in the mapping population will be screened on the confirmation population to ensure that the marker accurately selects for resistance.

Table 1. Details on the oat genetic populations to be used for mapping of crown rust resistance.

| Population (R x S) | Generation | Pc Gene | Function |
|---|---|---|---|
| Pc40 x AC Morgan | F5 | Pc40 | Mapping |
| Pc40 x Kasztan | F2:3 | Pc40 | Confirmation |
| Pc40 x Bingo | F2:3 | Pc40 | Confirmation |
| Pc46 x AC Morgan | F6 | Pc46 | Mapping |
| Pc46 x Kasztan | F2:3 | Pc46 | Confirmation |
| Pc46 x Pc63 | F2:3 | Pc46 | Confirmation |
| Pc50 x Bingo | F2:3 | Pc50 | Mapping |
| Pc50 x Kasztan | F2:3 | Pc50 | Confirmation |
| Pc59 x Bingo | F2:3 | Pc59 | Mapping |
| Pc59 x Kasztan | F2:3 | Pc59 | Confirmation |
| Pc62 x AC Morgan | F5 | Pc62 | Mapping |
| Pc62 x Kasztan | F2:3 | Pc62 | Confirmation |
| Pc67 x AC Morgan | F2 | Pc67 | Mapping |
| Pc67 x Pc48 | F2:3 | Pc67 | Confirmation |

| | | | |
|---|---|---|---|
| Pc98 x Bingo | F2:3 | Pc98 | Mapping |
| Pc98 x Kasztan | F2:3 | Pc98 | Confirmation |
| Pc101 x Bingo | F2:3 | Pc101 | Mapping |
| Pc101 x Kasztan | F2:3 | Pc101 | Confirmation |
| CDC Dancer x AC Morgan | F4:7 | APR | Mapping |
| PI 184004 x AC Morgan | F6:7 | APR | Mapping |

Greenhouse phenotyping of seedling resistance

Screening of the three *Pc40* populations, the P*c46* x AC Morgan population, the *Pc62* populations and the *Pc67* populations will be conducted at the CDC. The *Pc46* x *Pc63* population will be screened at the USDA-ARS and the remaining seedling populations will be screened at AAFC-Morden. Seedlings from each segregating population will be grown in controlled environment chambers in root trainers potted with Sunshine Mix#3, comprised of 70-80% Canadian Spaghum peat moss, vermiculite and dolomite limestone (Sun Gro Horticulture Canada Ltd., Seba Beach, AB). Seedlings will be watered as needed and grown at a 22-23$^{o}$C daytime temperature, 18-20$^{o}$C night temperature and an 18-hour day length. Seedlings at the one leaf stage will be inoculated by spraying urediniospores (4 mg/450 µL) suspended in Bayol® on the leaves. Approximately 10 seedlings from each RIL in the F5/F6 RIL populations, and 20 F2:3 families from each of 200 F2 plants in each F2:3 population will be inoculated. The inoculated plants will be incubated in a humidity chamber (maintained at 100% relative humidity) overnight at 15-17°C and then returned to the previous growing conditions. Crown rust infection types (ITs) will be scored 12 days after inoculation on a 0 to 4 scale (Murphy 1935): 0 (immune), ; (fleck), 1 and 2, resistant; 3 and 4, susceptible. After rust scoring, infected leaves from F2:3 plants were trimmed and the plants were raised to the fourth leaf-stage for DNA collection.

Field phenotyping of adult plant resistance

Lines from populations segregating for APR will be grown in three-replicate lattice designs. Lines tested at AAFC-Morden Research and Development Centre (AAFC-MRDC, Morden MB) will be sown as 1 m rows with a row spacing of 0.34 m. Lines tested at the University of Saskatchewan (Saskatoon, SK) will be sown as hills with a row spacing of 0.3 m and spaced 0.6 m apart along the seed row. A spreader row of 'AC Morgan' (U of S) or 'Makuru' (AAFC-MRDC) will be sown to increase inoculum in the nursery.

Field nurseries will be inoculated with a mixture of field isolates collected from across eastern Saskatchewan and Manitoba the prior year. This mixture contains more than 100 races, and will be tested to ensure it is virulent on the seedlings of the parents. Two to three inoculations are done within 10 days when plants in the spreader rows reached the four-leaf stage. At each inoculation, 0.3 g of crown rust urediniospores were mixed with 300 ml light mineral oil (Bayol®, Esso Canada, Toronto, ON.) and sprayed onto spreader rows with a Herbiflex hand-held sprayer (Micron Sprayers Ltd., Bromyard, UK). At the U of S nursery water was sprayed onto the spreader rows after the Bayol had evaporated and covered with white plastic sheets to simulate dew formation and ensure germination of the urediniospores and infection of the spreader rows. The Morden field nursery is irrigated using overhead irrigation once a week, starting just before inoculation until the plants are assessed for crown rust reactions, to ensure dew formation leading to crown rust infection and spread. In Saskatoon, irrigation was applied once a week throughout the season and then 3 times per week in the week preceding rating.

Crown rust will be rated on flag leaves. Disease severity (DS) will be assessed using the modified Cobb scale (Peterson et al. 1948). Pustules will be defined by infection type (IT): resistant (R), moderately resistant (MR), moderately susceptible, and susceptible (S) (Stubbs et al. 1986). All ITs will be converted to a numerical value as follows: R = 0, RMR = 0.1667, MR = 0.3333, MRMS = 0.5, MS = 0.6667, MSS = 0.8333, and S = 1, such that a coefficient of infection (CI) can be calculated according to the formula: CI = (DS × IT) / 100.

DNA Extraction, Genotyping, QTL Mapping

DNA will be extracted from seedlings (coleoptile from RILs or 4 leaf stage leaves from F2:3 lines) using a CTAB extraction method (Procunier et al. 1991; Anderson et al. 1992) or from freeze-dried leaf tissue using the DNeasy Plant DNA extraction kit (Qiagen, Toronto, Canada). Mapping populations will be genotyped with the Oat 6K Infinium SNP Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA) or using a genotype-by-sequencing method as described by Huang et al. (2014). Genetic linkage maps will be generated and QTL mapping will be conducted using JMP Genomics 7 (SAS Institute Inc., Cary, NC) or a combination of MapDisto v. 1.7.7 and QGene v. 4.3.10. TaqMan and KASP assays will be developed and detected as described in Gnanesh et al. (2015).

J.A. Anderson, Y. Ogihara, M.E. Sorrells, and S.D. Tanksley, 1992. Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet., 83:1035-1043.

Y.-F. Huang, J.A. Poland, C.P. Wight, E.W. Jackson, N.A. Tinker, 2014. Using genotyping-by-sequencing (GBS) for genomic discovery in cultivated oat. PLoS ONE, 9: e102448.

H.C. Murphy, 1935. Physiologic specialization in *Puccinia coronata*f.sp. *avenae*. U.S. Department of Agriculture Technical Bulletin 433.

R.W. Stubbs, J.M. Prescott, E.E. Saari, H.J. Dubin, 1986. Cereal Disease Methodology Manual. Mexico: CIMMYT Publications.

R.F. Peterson, A.B. Campbell, A.E. Hannah, 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Canadian Journal of Research, 26c:496-500.

J.D. Procunier, X. Jie, K.J. Kasha, 1991. A rapid and reliable DNA extraction method for higher plants. Barley Genet Newsl, 20:74-75.

**8. Results and discussion:** *Describe research accomplishments during the reporting period under relevant objectives listed under section 6. The results need to be accompanied with tables, graphs and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

*Pc40*

The *Pc40* differential line (Pendek*2/CAV 4997) (Chong et al., 2000) was crossed with three crown rust susceptible cultivars: 'AC Morgan', 'Bingo', and 'Kasztan'. 'AC Morgan' is a high yielding Canadian cultivar that lacks any major Pc genes (Kebede et al., 2019). Bingo and Kasztan both are Polish cultivars used as *Puccinia coronata*f.sp. *avenae* P. Syd. & Syd. (Pca) susceptible checks in Poland (Paczos-Grzęda and Sowa, 2019). The F5 family of *Pc40* x 'AC Morgan' is comprised of 210 recombinant inbred lines (RILs), the F2 populations derived from *Pc40* x 'Bingo' and *Pc40* x 'Kasztan' consisted of 155 and 156 lines, respectively, and their F2:3 families contained 154 and 150 lines. These populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio.Pca isolate CR257 (BRBG-97) was used for evaluation of the populations.DNA was extracted from young leaves (one plant/line) of the *Pc40* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). As shown in Table 1, segregation ratios in all 5 populations indicate the presence of a single gene (i.e. *Pc40*) which governs resistance to crown rust (as assessed with Pca isolate CR257). The genotypic data was received in March 2020 and has not yet been analyzed in order to link markers to this gene.

Table 1. Disease reaction data for five*Pc40*populations evaluated with the Pca CR257 isolate.

| Population | Generation | Resistant | Segregating | Susceptible | Ratio | P-Value |
|---|---|---|---|---|---|---|
| *Pc40* x AC Morgan | F5 | 114 | – | 96 | 1:1 | 0.214 |
| *Pc40*xBingo | F2 | 120 | – | 35 | 3:1 | 0.575 |
| | F2:3 | 41 | 77 | 36 | 1:2:1 | 0.850 |
| *Pc40*xKasztan | F2 | 116 | – | 40 | 3:1 | 0.853 |
| | F2:3 | 42 | 68 | 41 | 1:2:1 | 0.520 |

*Pc46*

The *Pc46* differential line (Pendek*4/CAV 5115) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'.The F6 family of *Pc46* x 'AC Morgan'is comprised of 202 recombinant inbred lines (RILs), the F2 population derived from *Pc46* x 'Kasztan' consisted of 199 lines, and the F2:3 family contained 189lines. These populations were tested to determine the number of resistance genes by evaluating the resistant/segregating/susceptible segregation ratio. Pca isolate CR254 (LRBG) was used for evaluation of the populations. DNA was extracted from young leaves (one plant/line) of the *Pc46* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). As shown in Table 2, the segregation ratio forthe *Pc46* x 'Kasztan' F2 population is unclear as to the number of genes. The observed ratio was tested against both a dominant and recessive form of resistance with neither fitting. However, the F2:3 population clearly indicated the presence of a single gene (i.e. *Pc46*) which governs resistance to crown rust (as assessed with Pca isolate CR254). In the F2 population there were a large number of plants scored as a rating of 3 (i.e. susceptible). As this is close to the cut-off point between declaring a line to be resistant or susceptible, it may be that some of these plants could have been alternatively rated as a score of 2. The F2:3 population is a more reliable means to assess gene number as the data is based on 24 plants per family instead of a single F2 plant, and thus there is an opportunity to observe and discern the true interaction between the pathogen and the host. The F6 family of *Pc46*x 'AC Morgan' has not been phenotyped yet as a result of having to halt lab work due to COVID-19. The genotypic data was received in March 2020 and has not yet been analyzed in order to link markers to this gene.The *Pc46* x *Pc63*population created by the Klos group and described in Table 1 will be replaced by the *Pc46* x 'Ajay' (susceptible) population, also created by the Klos group. The F2 population will be phenotyped and DNA extracted by the Klos group in late 2020 or early 2021. As well, the F2:3 population from this cross will also be phenotyped in 2021.

Table 2. Disease reaction data for three *Pc46*populations evaluated with the Pca CR254 isolate.

| Population | Generation | Resistant | Segregating | Susceptible | Ratio | P-Value |
|---|---|---|---|---|---|---|
| *Pc46* x AC Morgan | F5 | | | Not rated yet | | |
| *Pc46*xKasztan | F2 | 88 | – | 111 | 3:1 | <0.0001 |
| | | | | | 1:3 | <0.0001 |
| | F2:3 | 55 | 96 | 38 | 1:2:1 | 0.21 |

*Pc50*

The *Pc50* resistance gene will not be mapped under this study as a group in the U.S. has taken the lead on mapping this gene. Instead, the McCartney group will focus on mapping *Pc48*.

*Pc59*

No work done on this gene to date.

*Pc62*

The *Pc62* differential line (Fraser*4/CAV 4274) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'.The F5 family of *Pc62* x 'AC Morgan' is comprised of 200 recombinant inbred lines (RILs), the F2 population derived from *Pc62* x 'Kasztan' consisted of 200 lines, and the F2:3 familyhas not been created yet. Pca isolate CR254 (LRBG) will be used for evaluation of the populations. DNA was extracted from young leaves (one plant/line) of the *Pc62* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). Populations segregating for *Pc62* have not been phenotyped yet as a result of having to halt lab work due to COVID-19. The genotypic data was received in March 2020 and has not yet been analyzed in order to link markers to this gene.

*Pc67*

The *Pc67* differential line (Harder, 1983) was crossed with crown rust susceptible cultivar 'AC Morgan' and will be crossed to 'Kasztan'.The F6 family of *Pc67* x 'AC Morgan' is comprised 200 recombinant inbred lines (RILs) and is currently being grown in the CDC oat breeding program field plots in Saskatoon in the summer of 2020. The population will be ready for evaluation in the fall of 2020.The*Pc67* x 'Kasztan' population will be started in the fall of 2020 and will be ready to screen by the summer of 2021. Instead of using the *Pc67* x *Pc48* population, the Klos group has made a cross between *Pc67* x 'Ajay' (susceptible). The F2 population has been genotyped and phenotyped, and the F2:3 population will be phenotyped in late 2020 or early 2021.

*Pc98*

Markers have been successfully developed for the *Pc98* gene. The work is described in Theoretical and Applied Genetics (2020) 133:1109–1122.

*Pc101*

The *Pc101* differential line was crossed with crown rust susceptible cultivars:'Bingo', and 'Kasztan'. Currently the *Pc101* x 'Bingo' is being advanced to the F5 generation in the CDC oat breeding programfield plots in Saskatoon in the summer of 2020. It will be ready for evaluation in fall 2021. The *Pc101* x 'Bingo' population will be screened in the winter of 2021. Pca isolate CR254 (LRBG) will be used for evaluation of the populations and the *Pc101* x 'Bingo' will be used for genotyping.

Adult Plant Resistance (APR)

The 'CDC Dancer' x 'AC Morgan' and PI 184004 x 'AC Morgan' populations were grown and evaluated at the AAFC-Morden (Menzies)and CDC nursery (Kutcher) field nursery in 2019. No data was received from the CDC nursery, despite the use of irrigation, due to the extremely dry conditions in 2019. Data was received from the AAFC-Morden nursery. Both populations have been planted in the nurseries again in 2020.The 'CDC Dancer' x 'AC Morgan' has also been genotyped. Analysis of marker-trait relationships will be reported once several field seasons of data have been obtained due to the quantitative nature of APR.

**9. Interim conclusions(If any).**

Work was progressing very well up to March 2020. Since that time I have been unable to obtain lab access for my staff members who are working on this project. As such, we have done no lab work (disease phenotyping or DNA extractions for genotyping) from Feb-July 2020. This 6 month delay may impact our ability to deliver results by the current end-date of the project. We will not ask for an extension at the moment. Instead, we will see how much we can catch-up over the next year and we may ask for an extension at the time of the next progress report in July 2021.

10. **Listany technology transfer activities undertaken in relation to this project:** *Include conference presentations, talks, papers published etc.*

    Markers linked to the *Pc98* resistance gene are reported in Theoretical and Applied Genetics (2020) 133:1109–1122.

11. **Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.**

    None.

12. **Appendices:** *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited, acknowledgments.*

    None.