Identifying genetic signatures of Cercospora beticola adaptation to CRplus sugar beet varieties.

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Management of Cercospora leaf spot (CLS) typically relies on a combination of cultural practices, moderate host resistance, and fungicide applications. Historically sugar beet resistance to *C. beticola* has been conditioned by four quantitative trait loci (QTL) (Smith and Gaskill, 1970; Nilsson et al. 1999; Setiawan et al. 2000; Taguchi et al. 2011). Wild sea beet is a reservoir of genetic diversity and has been a critical source of genetic diversity leveraged to introgress new traits into cultivated sugar beet (Smith and Campbell 1996). Due to the difficulty of developing durable *C. beticola* resistance, CLS is largely managed by the timely application of fungicides throughout the growing season.

C. beticola has shown a staggering ability to adapt to management practices largely due to its polycyclic nature and high sporulation rate (Rangel et al. 2020). The emergence of fungicide resistance in *C. beticola* populations serves as a primary example of the pathogen's ability to adapt quickly to management practices (Secor et al. 2010). Understanding the molecular basis of fungicide resistance can help with the understanding of how *C. beticola* adapts to management practices and can be used to inform future use of fungicides to avoid the resistance from developing.

In recent years, CLS resistant sugar beet varieties carrying the CR+ trait conditioned by the *BvCR4* resistance gene have increased in popularity. This new resistance has been a valuable tool for integrated disease management. However, like fungicides, there is a risk that *C. beticola* populations will adapt to this new resistance. Already, our group has obtained a large collection of *C. beticola* isolates that have been subjected to whole genome sequencing from each of 2016, 2017, 2021, and 2023. Preliminary analysis have identified genes under CR+ adaptation selection pressures and greenhouse assays show that isolates exhibit significant symptoms of CLS.

In 2024, reports of local CLS epidemics in fields planted with CR+ varieties were observed and this is the first severe outbreak reported on CR+ varieties in the RRV. Though unfortunate, isolates collected from these outbreaks present an opportunity to examine the genetic underpinnings of CR+ adaptation on a wide population level scale.

Objectives:

1. Sequence *C. beticola* isolates collected in the RRV in 2024
2. Perform population genetic analysis of populations collected in the RRV from 2016 – 2024 to characterize population shifts associated with CR+ adaptation.

Materials and Methods:

A clone corrected isolate population collected in 2024 will be subject to WGS through a prearranged sequencing contract with Novogene USA. Each sequenced isolate genome will be aligned to the currently available *C. beticola* reference genome to identify mutations prevalent in the population through already established bioinformatics pipelines designed by Dr. Wyatt.

Identified mutation will be used in population genetic analysis to identify regions of the genome undergoing selection pressures associated with CR+ adaptation. Preliminary findings from 2021 revealed a population bottleneck where isolates collected from CR+ sugar beet varieties represented a small subpopulation compared to the RRV population at large. From isolates belonging to the bottlenecked subpopulation, we identified genomic regions with genes potentially involved in CR+ adaptation. The addition of the already available sequencing data from 2023 and the addition of sequencing data from 2024 will provide the necessary power to narrow down the specific genetic associations underpinning CR+ adaption.

Results and Discussion:

To identify signatures of adaptation to CR+ mediated CLS resistance in the C. Beticola population present in the RRV, A collection of isolates from the 2024 growing season were selected for whole genome sequencing. Isolates were selected to evenly distribute sampled locations across the entire RRV growing region so as to avoid regional bias and potentially confounding population structure. A population consisting of isolates collected between the years of 2016 and 2023 was readily available with whole genome sequencing data already acquired.

Following whole genome sequencing, mutation identification was performed and identified over 1 million single nucleotide polymophisms that were filtered to a final set of 10,000 high confidence, unlinked genetic markers with an even distribution throughout the C. Beticola reference genome. A principle component analysis was performed to examine the population structure of isolates collected across the 2016-2024 period and population stratification using the year of collection to compare patterns of annual population structure distributions. Results from this analysis showed that in the years 2016 and 2017 the population structure was stable and produced a similar structural pattern. In 2021, PCA revealed a divergent lineage of C. Beticola isolates that correlated with the commercial deployment of sugar beet varieties containing the BvCR4 mediated resistance trait CR+. Subsequent sequencing efforts from 2023 and 2024 showed a strengthening of this population trend that further correlates with the wider adoption of the CR+ sugar beet varieties and potentially indicates that the population has experienced a major shift induced by the selection pressure imposed by the new sugar beet resistance (Figure 1).

Recent results from the Bolton and Wyatt Labs at the USDA-ARS Sugarbeet Research Unit have identified and partially characterized the C. Beticola gene AvrCR4 as the critical gene recognized by the CR+ resistance gene BvCR4. The loss of AvrCR4 from C. Beticola isolates allows the pathogen to circumvent CR+ resistance. Armed with this new knowledge we examined the whole genome sequenced populations collected from 2016-2024 and identified the proportion of isolates lacking AvrCR4 in each year. In 2016 and 2017, no isolates were identified that lacked the AvrCR4 gene, indicating that the populations present in the RRV in those years were not adapted to CR+ resistance. From 2021 -2024, we report an increasing frequency of C. Beticola isolates having lost the AvrCR4 gene and thus characterized as CR+ adapted isolates (Table 1).

Summary:

Through the use of whole genome sequencing of C. Beticola populations collected annually in the RRV, we successfully identified population structural shifts that corresponded to C. Beticola adaptation to the recently deployed CR+ sugar beet CLS resistance gene BvCR4. By sequencing isolates across the span of 2016 to 2024, we show that the frequency of adapted isolates increase over time and in response to the increased frequency of acreage planted with CR+ sugar beets. This work highlights the utility of leveraging molecular techniques to monitor pathogen populations to rapidly identify adaptation to management practices and future work will focus on rapidly acquiring this data to proactively forecast the impact of adapted pathogen populations.

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Table 1: Percentage of isolates collected and subject to whole genome sequencing that are adapted to the CR+ trait as determined by the loss of the *Cercospora beticola* effector AvrCR4.

Year	Percent
2017	0%
2016	0%
2021	11%
2023	37%
2024	76%

Figure1: PCA of C. beticola populations collected from 2026 to 2024 showing divergent lineages extending on the right side of the plot from isolates collected from 2021-2024 following the release of CR+ containing sugar beet varieties.