IDENTIFICATION OF NEW GENETIC SOURCES FROM SEA BEET TO IMPROVE SUGARBEET RESISTANCE TO CERCOSPORA LEAF SPOT

Chenggen Chu[1], Muhammad Massub Tehseen[2], Lisa S. Preister[1], Melvin D. Bolton[1], Peter Hakk[3], Emma Burt[4], Mohamed F.R. Khan[3], Mike Metzger[4], and Xuehui Li[2]

[1]USDA-ARS, Edward T. Schafer Agricultural Research Center, Fargo, ND 58102, [2]Department of Plant Science, North Dakota State University, Fargo, ND 58108, [3]Department of Plant Pathology, North Dakota State University & University of Minnesota, and [4]Minn-Dak Farmers Cooperative, Wahpeton, ND 58075

Introduction

Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is the most widespread foliar disease in sugarbeet (*Beta vulgaris* L.) and yield losses due to CLS can be as high as 42 - 50% (Verreet et al., 1996). Application of host resistance for CLS control would be more effective with a lower cost. Vogel et al. (2018) found that recent breeding efforts have made CLS resistant cultivars comparable to susceptible ones in terms of yield performance, consequently, the resistant cultivars thus have a relatively better economic performance since no fungicide needs to be applied.

Many studies were conducted to identify germplasms resistant to CLS and some accessions of *Beta vulgaris* spp. *maritima*, the wild ancestor of sugar beet, were found to have a high level of resistance and were used as a source of CLS resistance (Leuterbach et al., 2004). Our findings in the last year also indicated that a cluster of 355 *B. maritima* accessions showed a further genetic distance to sugarbeet and have much greater potential for improving CLS resistance and broadening the genetic base (Tehseen et al., 2023).

In this research, we analyzed genetic diversity of all available *B. maritima* accessions and selected 300 accessions as an association panel for identifying CLS resistance through genome-wide association study (GWAS), and this report summarized the evaluation in 2023 with preliminary results from association study.

Materials and methods

A total of 599 *B. vulgaris* L. ssp. *maritima* accession from NPGS (National Plant Germplasm System) and USDA-ARS sugarbeet genetics program at Fargo, ND (**Table 1**) were used for genetic diversity analysis, which led to the identification of 300 accessions planted in field nurseries at Fargo, ND, and Foxhome and Kent, MN to evaluate their resistance to Cercospora leaf spot. In addition, 30 sugarbeet lines were used as a reference cluster to indicate genetic distance between sea beet clusters with cultivated beets.

Table 1. List and origin of wild beet accessions used in the current study with their putative geographic regions.

Region	Countries (no. of lines)	Total
Africa	Egypt (26), Morocco (31), Tunisia (1)	58
Asia	China (1), India (2), Israel (1)	4
Northern Europe	Denmark (21), Ireland (49), Jersey Island (2), Unite Kingdom (108)	180
Southern Europe	Croatia (1), Cyprus (1), Greece (56), Italy (102), Portugal (6). Spain (8), Turkey (6)	182
Western Europe	Belgium (3), France (146), Germany (2), Netherlands, (2), Guernsey Island (1)	154
Eastern Europe	Poland (1), Russian Federation (3)	4
North America	California in the United States (15)	15

Field evaluation of CLS resistance was conducted as randomized complete block designs with two replications included. The two-row plots were 10 feet long, with 22-inch row spacing and 8 – 10 inches for plant space within a row. The trial was planted on May 31st at Foxhome, MN, and June 1st at Kent, MN in 2023. Inoculation was performed on July 18th and repeated after two weeks by spraying ground disease leaf mixed with Talca powder at the ratio of 1:3. Disease ratings were made on September 28th using a 0 – 9 scale with 0 as no CLS spots observed, 1 – 3 as resistant (a few scattered spots to some dieback on lower leaves), 4 - 6 as moderately resistant/susceptible

(increasing amounts of dead and disease tissue on several to most plants of the row), and 7 - 9 as susceptible (diseased leaf has 50 - 100% of area necrosed on most plants of the row) (Ruppel & Gaskill, 1971).

Genotyping in all accessions was previously done using GBS platform (Tehseen et al., 2023). Briefly, approximately 0.1 g of fresh leaf tissue was collected from 7 – 10 plants of each accession and was dried in a freeze drier 35EL (SP Scientific, Inc., Warminster, PA, USA) for 72 hrs. Dried tissues were ground using a 1600 MiniG SPEX homogenizer (SPEX, Inc., Metuchen, NJ, USA). Genomic DNA was extracted from dried tissue using a DNA purification system (KingFisher, Inc., Falls Church, VA, USA), and DNA samples were fragmented by co-digestion using restriction enzymes *NsiI* and *BfaI* to produce DNA fragments. Barcoded adapters were ligated to DNA fragments from each accession to identify fragments generated from each individual accession. GBS sequencing libraries were constructed according to Hilario et al. (2015) by PCR amplification of barcode ligated DNA using a 96-plex plate followed by purification and quantification of the PCR product before sequencing. An Illumina HiSeq 2000 sequencing system (Illumina, Inc., San Diego, CA, USA) was used to sequence about 150 base pairs at both ends of fragments. The obtained fragmental sequences were anchored to the reference sugarbeet genome sequence assembly EL10.2 of sugarbeet line EL10 (McGrath et al., 2022) and compared among accessions to identify genome-wide SNPs through reference-based Tassel pipeline (Glaubitz et al., 2014). Raw SNP data were filtered by removing SNPs with a missing data rate of over 20%, followed by genotype imputation through the computer program Beagle (v5.0) (Browning & Browning, 2007) that achieved a data-missing rate of 0% and only the bi-allelic SNPs were kept.

For analyzing population structure in the *B. maritima*, the computer program STRUCTURE v. 2.3.4 (Pritchard et al., 2000) and R package *adegenet* v.2.3.4 (Jombart and Ahmed, 2011) were both used. The analysis using STRUCTURE implemented model-based Bayesian cluster analysis to estimate the number of subpopulations in all *B. maritima* accessions. It uses ten independent replicated runs for each putative number of subpopulations ranged from $K = 2 – 10$ under the admixture model and assessed using a burn-in period of 5000 and 50,000 Markov Chain Monte Carlo (MCMC) replications. The best K value representing the optimum number of sub-populations was estimated based on Delta K (ΔK) changes between successive structure iterations calculated using Structure Harvester (https://taylor0.biology.ucla.edu/structureHarvester/). R package *adegenet* was used to conduct discriminant analysis of principal components (DAPC) to classify all *B. maritima* accessions into clusters, which verifies population structure estimated from the program STRUCTURE.

GWAS was carried out using a R package GAPIT (Genome Association and Prediction Integrated Tool) (Lipka et al., 2012). Briefly, a standardized mixed linear model (MLM) (Yu et al., 2006) was used as $y = X\beta + Qv + u + e$, where y is the vector of observed phenotypes, X is the vector of SNP markers, β is the marker fixed effects vector to be estimated, Q is the population matrix derived from PCA analysis, v is the vector of fixed effects due to population, u is random effects vector and e is the residual vector. The variance of u is estimated as Var $(u) = 2KVg$, where K is the kinship matrix derived from individuals based on the proportion of shared alleles and Vg is the genetic variance. K matrices were generated using TASSEL v 5.0 (Bradbury et al., 2007).

Results & discussion

Genotypic data

A set of 147,764 reliable SNP markers were previously obtained from GBS pipeline and marker distribution across all nine chromosomes was shown in Fig. 1. The maximum number of SNPs were observed on chromosomes 6 (19,140) and 5 (19,115), and chromosome 9 had the minimum SNPs (14,277). The average density of markers across the whole genome was 3.81 markers per kb. The lowest density was observed on chromosome 5 (4.07 marker/kb), whereas the highest density was on chromosome 1 (3.54 markers/kb).

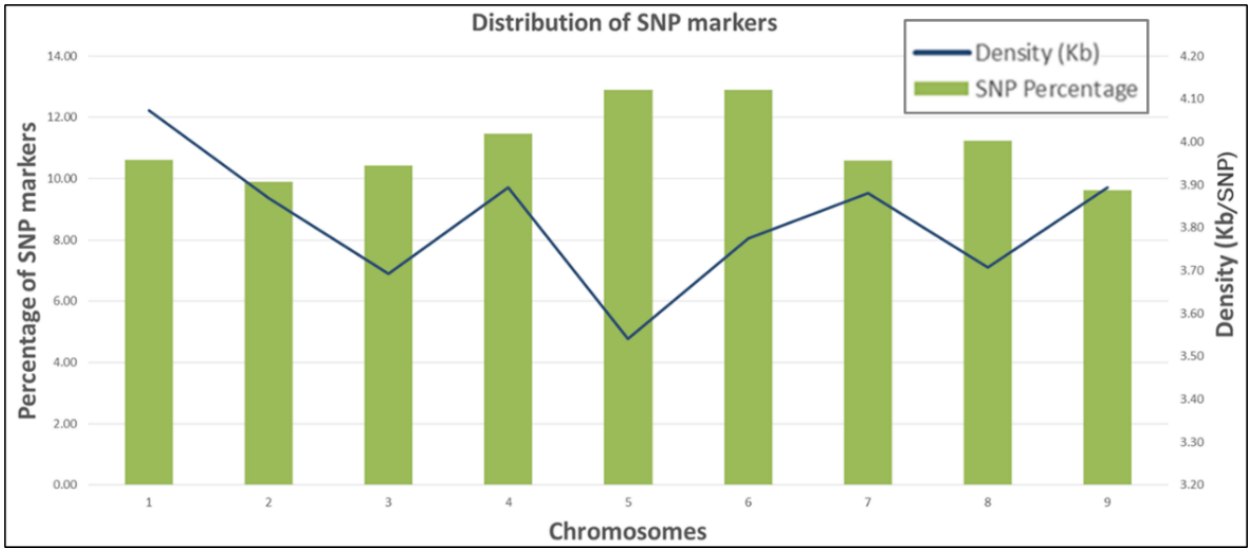

Fig. 1. Distribution of SNP markers across the genome in 599 *B. maritima* accessions.

Population structure

According to on ΔK assay using the STRUCTURE program, the 599 *B. maritima* accessions and 30 sugarbeet lines used in the current study likely contained 5 or 8 sub-populations (Fig. 2).

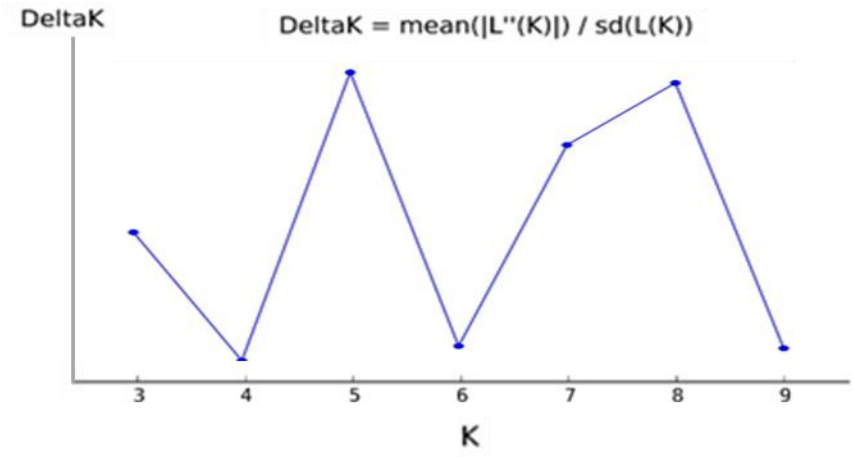

Fig. 2. Population structure analysis of materials included 599 wild beet accessions and 30 sugarbeet lines using the computer program STRUCTURE indicated 5 or 8 subpopulations in the collection.

The DAPC analysis proved eight clusters in the collection (Fig. 3) with cluster 1 mainly from northern and northwestern Europe, cluster 2 from southern and Western Europe, cluster 3 from Morocco and southwestern Europe, cluster 4 from southern Europe, cluster 5 from northern and western Europe, cluster 6 from sugarbeet lines, cluster 7 from Egypt and southern Europe, and cluster 8 from Morocco. Of those clusters, 2, 3 and 4 were very close to sugarbeet group, indicated their close genetic distance. Clusters 1, 5, 7 and 8 showed farther genetic relationship to sugarbeet with 7 is more distinct from the others.

The seven clusters of *B. maritima* accessions are strongly associated with geography location where the materials were collected, which also highly agreed with ocean current direction in north Atlantic Ocean and Mediterranean Sea (Fig.4) as *B. maritima* accessions are mostly grow along seashore and seeds were mainly spread by ocean current.

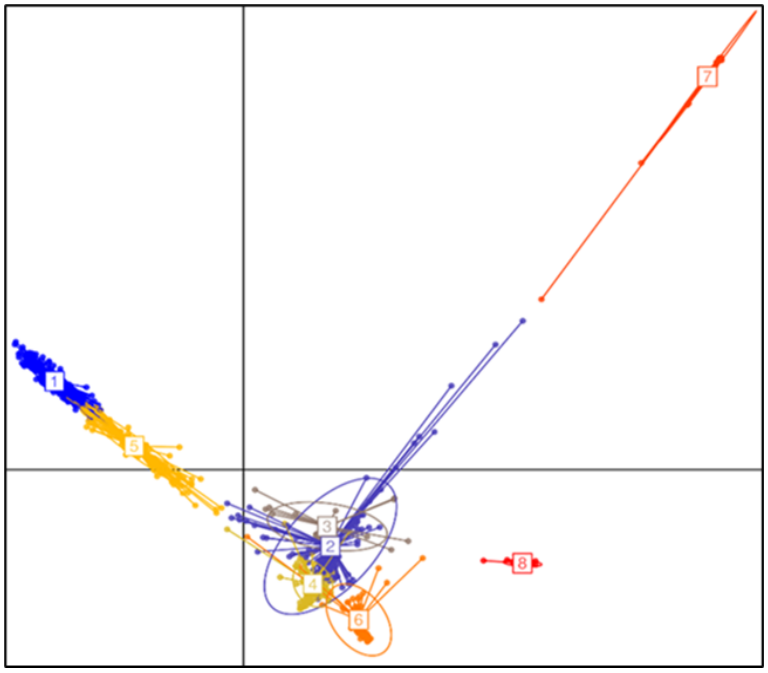

Fig. 3. Population structure analysis through discriminant analysis of principal components (DAPC) indicated eight clusters in the 599 *B. maritima* accessions and 30 sugarbeet lines. The numbers indicated cluster names, and the farther distance between clusters indicated the more distinct genetic difference.

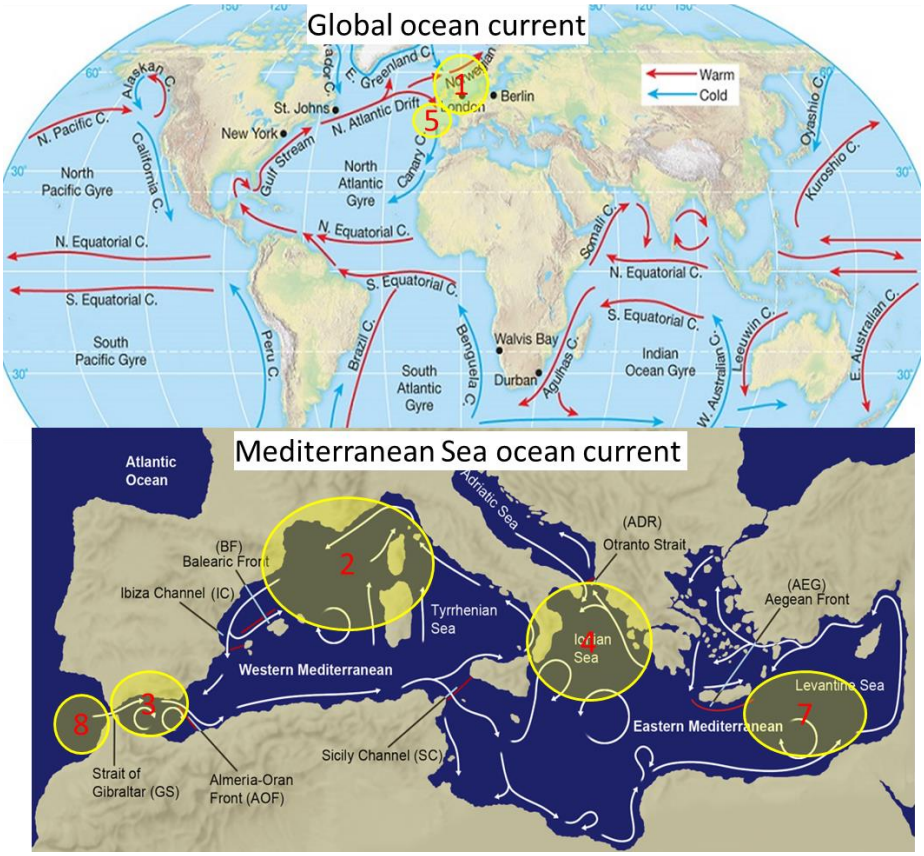

Fig. 4. The geographic location of *B. maritima* clusters determined by discriminant analysis of principal components (DAPC) and ocean surface current directions in global (above, Global Solo Challenge, 2023) and Mediterranean Sea (below, Pascual at al., 2017).

CLS evaluation

According to genetic diversity analysis, a set of 300 accessions was selected for CLS evaluation. Disease severity in field nursery at Fargo, ND is too light, and data were discarded for analysis. Disease at Foxhome location is severer than CLS at Kent location with much less accessions had rating less than 3 (Fig. 5). However, disease ratings in two replications at two locations showed the similar trend. Also, observation of the accessions with disease ratings over 7 suggested the high disease pressure at both locations though Foxehome is much higher, which due to the plenty of rainfall during inoculation at the beginning of August (data not shown). Overall, very small amount of the accessions with disease ratings as "1" in all experiments indicated that the high level of resistance was existed in the collection. This needs to be verified in future evaluations.

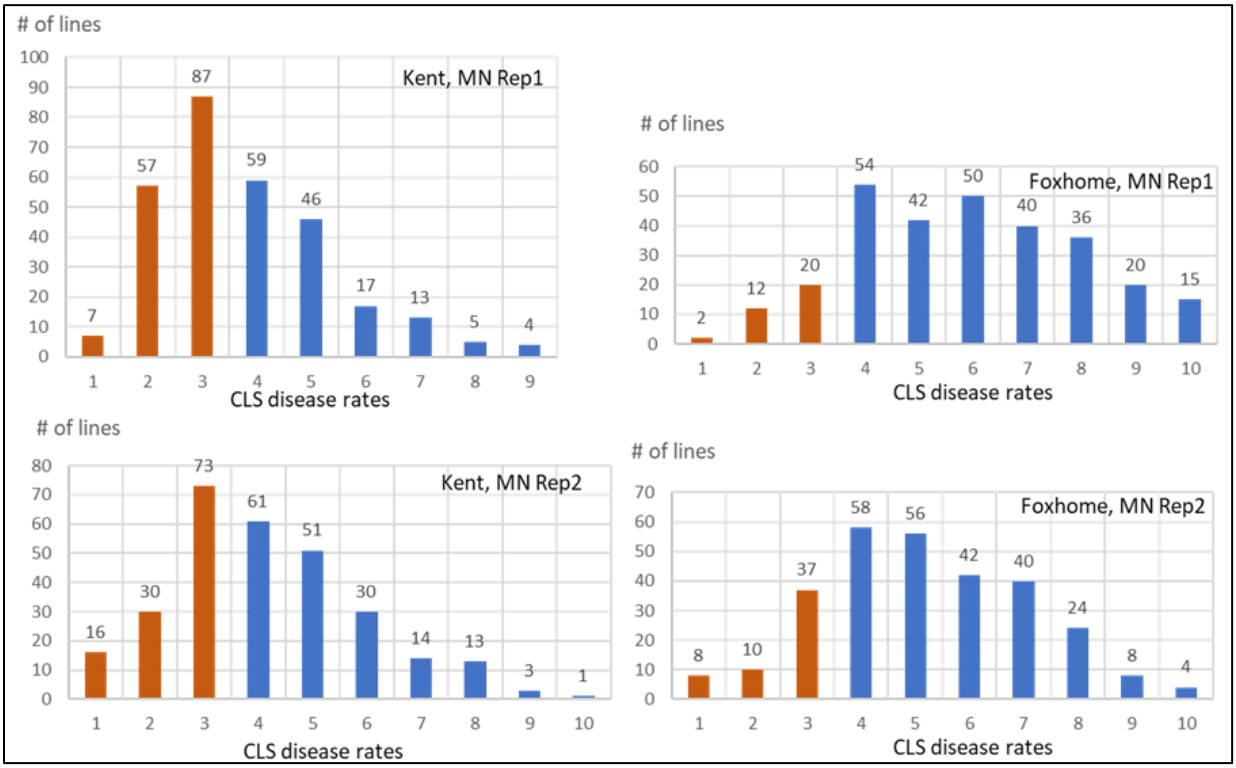

Fig. 5. Distribution of resistance to Cercospora leaf spot (CLS) in 300 *B. maritima* accessions evaluated in field nurseries located at Foxhome and Kent, MN in 2023.

Genome-wide association study (GWAS)

Association study indicated genomic regions on chromosomes 1, 2, 5, 8, and 9 are significantly associated with the resistance, which was repeatedly shown in at least two experiments (Fig. 6). This agreed that CLS resistance is likely a quantitative trait governed by 4-5 genes as indicated by Nielsen et al. (1997) and Smith & Gaskill (1970). Previously, Nilsson et al. (1999) reported five QTL on chromosomes 1, 2, 3 and 9 with phenotypic variability ranging from 7% - 18.3%. Schäfer-Pregl et al. (1999) detected seven QTL on chromosomes 2, 4, 5, 6, and 9. Setiawan et al. (2000) reported four QTL on chromosomes 3, 4, 7 and 9 and explained phenotypic variance ranging from 6.2% to 25.1%. Since this is an ongoing project and continuous evaluation will be conducted in future years to verify current results as well as detecting the new resistance associated regions.

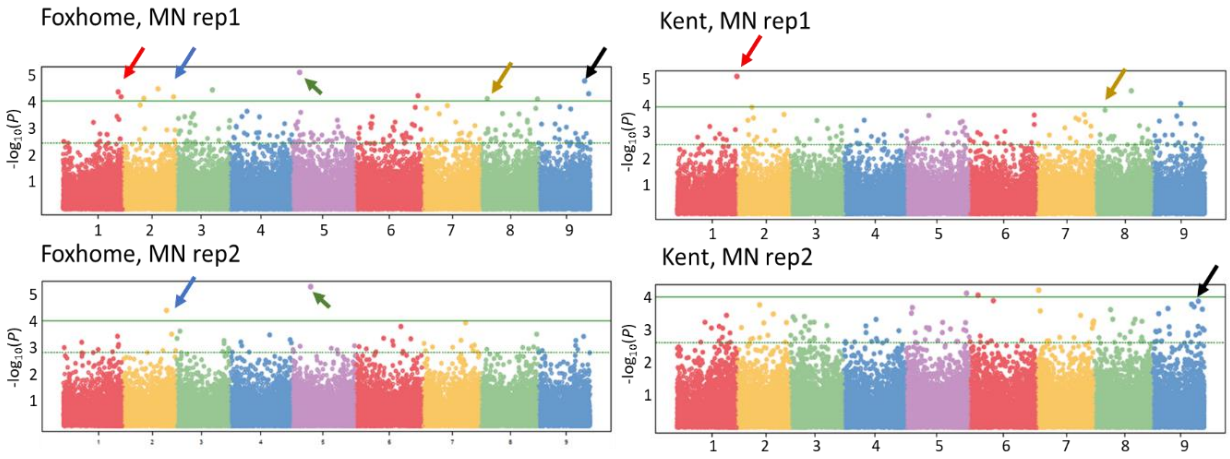

Fig. 6. Manhattan plots of GWAS showing genomic regions significantly associated with resistance to CLS in wild beet accessions.

Acknowledgements

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