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

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Introduction

Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is the most widespread foliar disease in sugarbeet (*Beta vulgaris* L. [Carophyllales: Amaranthaceae]). Significant losses can occur under warm and humid environments (Lartey et al., 2010; Skaracis et al., 2010), with yield losses reaching 50% in addition to the negative impact on sugar extraction from roots of infected plants (Francis & Luterbacher, 2003; Rangel et al., 2020). Application of host resistance for CLS control would be more effective with a lower cost (Smith and Gaskill, 1970). For example, Vogel et al. (2018) found that breeding efforts have enabled recent CLS resistant cultivars to achieve comparable yield performance to that of susceptible varieties, and resistant cultivars therefore have relatively improved economic performance since reduced fungicides need to be applied. Therefore, it is vital to identify and pyramid novel resistance genes from different genetic sources to enable long-lasting resistance and maintain the sustainability of the crop.

Many studies were conducted to identify germplasms resistant to CLS (Nilsson et al., 1999; Smith and Gaskill, 1970; Ruppel et al., 1971; Schäfer-Pregl et al., 1999) 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). Genetic diversity analysis in Tehseen et al. (2023) also proved the potential of publicly available germplasm for improving sugarbeet resistance to CLS. Due to dynamic change of *C. beticola* isolates in field each year, identification and application of resistance from diverse genetic resources will lead to a long-last resistance.

In this research, we focused on identifying CLS resistance from both sugarbeet and wild sea beet from publicly available germplasm lines. We will then conduct genome-wide association study (GWAS) to detect genomic regions associated with CLS resistance and reveal if different resistance genes present in wild and cultivated *Beta vulgaris* germplasm, which will be used to determine strategy of combining CLS resistance for new breeding line development. In this report, we focus on reporting CLS evaluations conducted in past three years for both sugarbeet lines and *B. maritima* accessions.

Materials and methods

A total of 300 *B. vulgaris* L. ssp. *maritima* accession selected through genetic diversity analysis (Tehseen et al., 2024) were originally collected from 23 countries (Table 1) and 36 sugarbeet lines selected from previous years based on CLS resistance (Table 2) were used for this research. Materials were planted in field nurseries at Fargo, ND, and Foxhome and Min-Dak Farmer's Cooperative nurseries, MN to evaluate their resistance to Cercospora leaf spot.

Field evaluation of CLS resistance was conducted as randomized complete block designs with two replications included. The two-row plots were 15 feet long, with 22-inch row spacing and 8 – 10 inches for plant space within a row. Inoculation was performed in mid-July and repeated after three weeks by spraying ground disease leaf mixed with Talca powder at the ratio of 1:3. Disease ratings were made during late September to early October each year 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). Weed control was conducted by spraying non-glyphosate herbicides at micro-rate weekly during June to late August.

Table 1. Origin of 300 wild beet accessions used in the association panel.

Country	No. of accession	Country	No. of accession
Belgium	1	Morocco	32
Croatia	1	Portugal	6
Cyprus	1	Russian	1
Denmark	1	Sardinia	2
Egypt	19	Sicily	2
France	58	Spain	6
Germany	1	Tunisia	1
Greece	44	Turkey	5
India	2	UK	13
Ireland	11	USA	10
Israel	1	Unknown	1
Italy	81		

Table 2. List of 36 sugarbeet lines used for Cercospora leaf spot (CLS) resistance evaluation.

Entry	Description
25-1	Selection from SP 69550
25-2	Selection from EL44cms/SP 69550
25-3	Selection from FC712/C562cms
25-4	Selection from F1012/SP69550-01
25-5	Selection from CFM94/961009H2
25-6	Selection from SP69550-0/F1015
25-7	Selection from F1015/961009H2
25-8	Selection from SP69550-01/F1016
25-9	Selection from FC504cms/F1015
25-10	Selection from F1010/SP69550-01
25-11	Selection from F1015/951013
25-12	Selection from F1010 / SP69550-01 (cms)
25-13	Selection from FC607cms/F1001
25-14	Selection from SP69260/F1014
25-15	Selection from SP69550/L19
25-16	selection from SP8030-0
25-20	Selection from ND PI mixture
25-22	Selection from BW sugarbeet
25-23	Selection from FC709-2
25-24	Selection from SP69550-2
25-25	EL50
25-26	Selection from SP69550/L19
25-32	Selection from CL24-sugarbeet
25-33	Selection from CIM mix/Y577
25-35	Selection from FC607 cms/F1001
25-36	Selection from EL44CMS X SP69550
25-37	Selection from SP69269-01 X F1011
25-38	Selection from SP6926CMS X F1013
25-39	Selection from FC712/SP69550-01
25-40	Selection from F1015/961009H2
25-41	Selection from F1015/951013
25-42	Selection from F1015/SP69550-01 (cms)
25-43	Selection from F1010/SP69550-01 (cms)
25-44	Selection from F1013/SP69550-01
25-45	Selection from F1010/FC504cms
25-46	Selection from F1010/SP69559-01

For CLS evaluations, the categorical CLS ratings of each accession collected in one experiment (defined as one location in each year) were averaged, and the mean ratings from that experiment were treated as one replication for statistical analysis conducted using the R package *agricolae*. Accession was considered as a fixed effect and environments as a random effect. Histograms of CLS resistance and SBRM tolerance distributions in 300 *B. maritima* accessions were generated using Microsoft Excel.

Results & discussion

CLS evaluation in *B. maritima* accessions

Accessions of *B. maritima* showed phenotypic segregation when grow in field. Accessions were purified according to morphological traits such as leaf color, stem color, root color, etc. A total of 393 subgroups were obtained after regrouping according to morphological traits within accessions, and uniformity of plants within each plant type was obviously improved (Fig. 1).

Fig. 1. Example of regrouped subgroups from *B. maritima* accessions according to morphological traits. Each circle indicates one plant type.

Field plots at Foxhome in 2022 were seriously affected by drought, which led to light CLS infection, data of that environment were thus excluded from the analysis. CLS developed normally in 2023 and 2024 with severe infections in susceptible checks observed at all locations in Fargo, ND; Foxhome and Min-Dak Farmer's Cooperative nurseries, MN. In total, data were collected from six environments accordingly designated as Foxhome23, Mindak23, Fargo23, Foxhome24, Mindak24 and Fargo24. Disease severity distribution in each environment did not follow a normal distribution pattern, with over 50% of subgroups showing easily observed disease symptoms and receiving ratings of 6 or greater (Fig. 2). Variance analysis indicated that both B. maritima subgroups and environments were highly significant ($P < 0.001$) (Table 3) indicating that both host resistance and environmental conditions play significant roles during disease development.

Table 3. Analysis of variance for Cercospora leaf spot (CLS) and sugarbeet root maggot (SBRM) evaluation data collected in 388 *B. maritima* subgroups under multiple environments.

Source of variance	Degree of freedom	F-Value	Significance
Accessions	387	3.426	$P < 0.001$***
Environment	5	83.095	$P < 0.001$***
Residuals	1935		

When combining data from all six environments, a total of 42 accessions (comprised of 46 subgroups with 24 annual and 22 biennial) were considered resistant to CLS as they consistently showed fewer disease symptoms across all environments, with average severity ratings of 3 or lower (Table 4). According to *B. maritima* clusters defined using the method of non-model-based discriminant analysis of principal components (DAPC) in Tehseen et al. (2024), these accessions were found in almost all clusters except Cluster 7, which included accessions collected from the beaches of the east Mediterranean Sea in Turkey, Egypt and islands in southern Greece. Among the resistant accessions, 24 and 18 accessions were from clusters that were genetically close (clusters 2, 3, 4 and 6) and distinct (clusters 1, 5 and 8) to cultivated beets, respectively. Among the 18 accessions that were from genetically distinct clusters, 7, 6 and 5 were from clusters 1 (collected from northern and western Europe), 5 (collected from northern and western Europe) and 8 (collected from Morrocco), respectively.

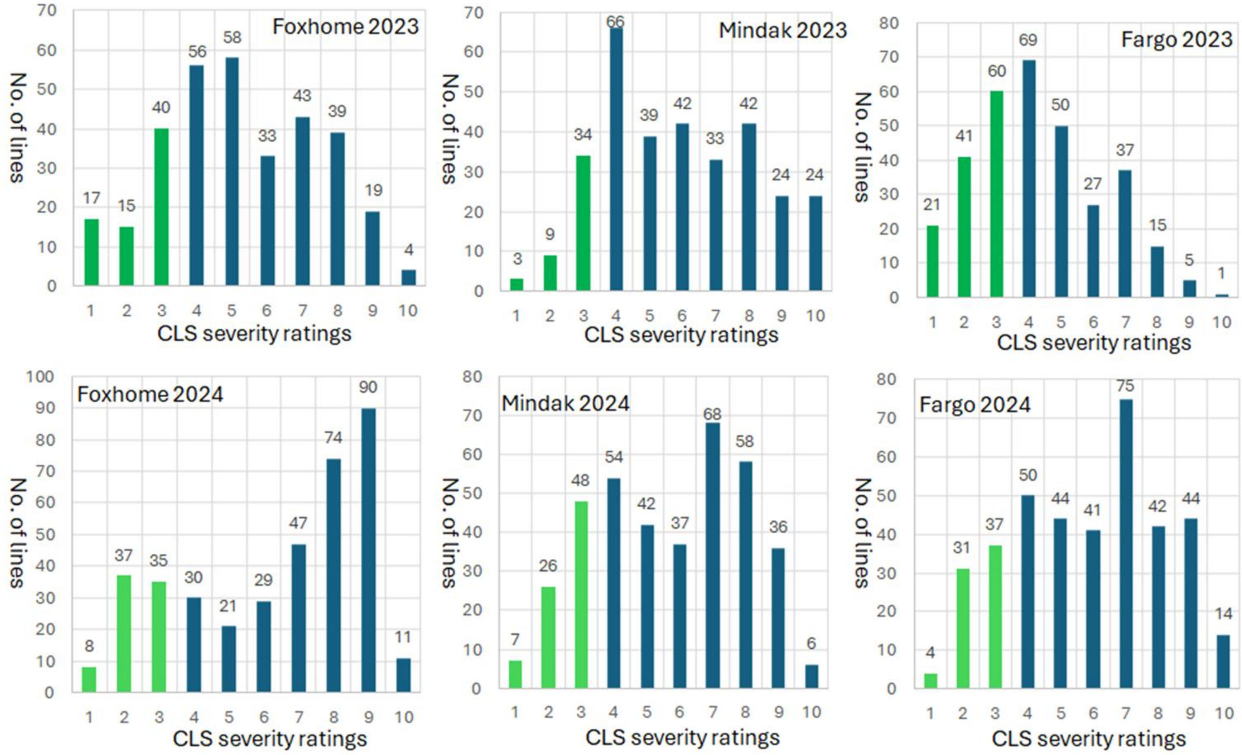

Fig. 2. Distribution of Cercospora leaf spot (CLS) ratings in 393 *B. maritima* plant types evaluated in field nurseries during 2023 to 2025. Light green color indicates accessions with CLS ratings of 3 or less.

Table 4. List of 42 *Beta maritima* accessions with resistance to Cercospora leaf spot (CLS) caused by *Cercospora beticola*.

Accession name[a]	Accession phenotype	Sub-population[b]	Origin	Average CLS ratings[c]	Standard Deviation
PI 540694	Biennial	1	France	2.9	±0.2
PI 518344	Biennial	1	United Kingdom	2.7	±0.6
PI 518312	Biennial	1	United Kingdom	2.7	±1.1
PI 518342	Annual	1	United Kingdom	2.6	±0.9
PI 540691	Annual	1	France	2.7	±0.9
PI 540625	Biennial	1	France	2.7	±0.5
PI 518398	Biennial	1	Ireland	2.9	±0.4
PI 504205	Biennial	2	Italy	2.8	±0.6
PI 504172	Annual	2	Italy	2.4	±0.7
PI 546399	Annual	2	Italy	2.9	±0.7
PI 546539	Biennial	2	Greece	2.6	±0.3
PI 546538	Annual	2	Greece	2.8	±0.5
PI 546538	Biennial	2	Greece	2.9	±0.2
PI 504253	Biennial	2	Italy	2.9	±1.0
PI 540565	Annual	2	France	3.0	±0.6
PI 504177	Annual	2	Italy	2.5	±0.9
W6 45840	Annual	3	Morocco	2.7	±1.0

PI 604540	Biennial	3	Spain	2.6	±0.4
PI 546393	Biennial	3	United States	2.5	±0.4
PI 504249	Annual	4	Italy	2.9	±0.7
PI 504254	Annual, Red root	4	Italy	2.8	±0.2
PI 504254	Annual, White root	4	Italy	3.0	±0.3
PI 504190	Biennial	4	Italy	2.9	±0.4
PI 504211	Annual	4	Italy	3.0	±0.5
PI 546424	Annual	4	Greece	3.0	±0.6
PI 540574	Biennial	4	France	2.4	±1.0
PI 504241	Biennial	4	Italy	2.8	±0.6
PI 504241	Annual	4	Italy	2.9	±0.2
PI 546431	Biennial	4	Greece	2.6	±0.8
PI 504201	Annual	4	Italy	2.9	±0.2
PI 504208	Annual	4	Italy	2.8	±0.6
PI 546519	Biennial	4	Greece	2.9	±0.2
PI 518320	Annual	5	United Kingdom	3.0	±0.4
PI 540609	Biennial	5	France	2.9	±0.2
PI 518315	Biennial	5	United Kingdom	2.5	±0.6
PI 540580	Biennial	5	France	2.8	±0.5
Ames 8448	Biennial	5	Ireland	2.9	±0.2
PI 540573	Annual	5	France	2.8	±0.2
W6 45825	Annual	6	Morocco	2.4	±0.7
W6 45823	Annual	6	Morocco	2.9	±0.7
PI 604528	Biennial	6	Spain	2.4	±0.8
W6 44496	Annual	8	Morocco	3.0	±0.5
W6 44497	Biennial	8	Morocco	1.9	±0.5
W6 44497	Annual	8	Morocco	2.8	±1.0
W6 44503	Annual	8	Morocco	2.9	±0.6
W6 44499	Annual	8	Morocco	2.8	±0.6

[a] Plant name of each accession was according to USDA-GRIN (Germplasm Resources Information Network).

[b] Sub-population structure of *Beta maritima* was according to Tehseen et al. (2024) that grouped accessions into 8 clusters with the cluster 6 mainly includes the cultivated beets, and clusters 2 and 4 have *B. maritima* accessions are genetically closer to the cultivated sugarbeet lines.

[c] Average disease severity ratings were calculated from data collected in six environments (Fig. 1) by using a 0 – 10 scale with 0 as immune (no CLS spots), 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 - 10 as susceptible (diseased leaf has 50 - 100% of area necrosed on most plants of the row) (Ruppel and Gaskill, 1971).

CLS evaluation in sugarbeet selections

Sugarbeet lines from previous selection all showed much slighter disease than the susceptible check (Fig. 4) though segregations were shown in some selections, indicated CLS resistance was mostly genotype-depended and selection based on resistance is an effective way to maintain the resistance. Therefore, the selected lines are very useful to form an association mapping panel to identify genetic factors controlling the resistance. If CLS resistance in wild sea beet is different from the resistance in cultivated sugarbeet, and cross between resistant plants from two sub-species will be conducted to pyramid resistance genes to let resistance stable and last longer. Genotyping of the sugarbeet lines and sea beet accessions are ongoing now and GWAS will be conducted right after genotyping. Combining resistance or introgression of resistance from sea beet into sugarbeet is on the schedule.

Fig. 4. Example of plants in selected sugarbeet breeding lines with excellent Cercospora leaf spot (CLS) resistance compared to the susceptible check. Photos were taken in 2025 at Foxhome, MN.

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