Suggested title: Molecular and Agronomic Characteristics of Sugar beet in Response to Cercospora Leaf Spot Disease Condition investigated by the PCR-based Start codon targeted (SCoT) marker

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Abstract

Sugar beet (*Beta vulgaris subsp. vulgaris*) is grown commercially mainly for sugar production, as it has a high sucrose concentration in the roots, and for manufacturing bio-oils. Sugar beet produces over 100 million tons per year of sugar (mainly sucrose) for worldwide consumption, and most of this production is directed to be utilized for human nutrition. Cercospora leaf spot is a foliar disease that destroys the sugar beet yield, which is caused by the fungus *Cercospora beticola* Sacc. Identifying sugar beet varieties with remarkable yield and disease resistance is a critical research interest. To achieve this goal, six types of sugar beet were examined and grown in two successive seasons, 2019/2020 and 2020/2021. Disease severity percentage, root and foliage-associated traits, sugar content, and percentage of total soluble solids (% TSS) were measured. Novel start codon targeted (SCoT) markers were identified, and the genetic variability between the sugar beet genotypes was assessed via PCR-based applications using SCoT-specific primers. Genetic similarity relationships among resistance and susceptible genotypes were estimated. The results indicated that Gerogoria-KWS and BTS2860 varieties appear to have the lowest % disease severity (1.00-2.67 % and 0.83-5 %, respectively) in the two growing seasons. The reduction in % disease severity in the two genotypes was associated with positive agronomic traits, including longer roots, higher biomass for foliage and root (on fresh and dry weight basis), higher TSS and higher sucrose content. Conversely, Pintea, MK 4199 (Emperator), and LP17B4011 genotypes exhibited the lowest biomass and shortest roots, and Zepplen genotype has low % disease severity and low % TSS content. Moreover, from PCR genotyping, ~1056 bp PCR fragment generated via SCoT3 analysis was distinguished as associations to Gergoria and BTS2860, which could be attributed to the high disease resistance phenotypes in those two genotypes. Furthermore, the genetic similarity analysis overall resulted in values ranging from 0.76 to 0.92, by which the highest was between Gerogoria and BTS2860 genotypes. Moreover, cluster analysis was conducted based on disease resistance properties and the genetic relationships between the tested genotypes. The results suggested a high degree of harmony between growth traits and finding from of PCR-based SCoT analysis. Altogether, the differentiation between resistant and susceptible sugar beet varieties to cercospora leaf spot disease can be investigated through the application of the reliable novel technique, SCoT.

Keywords: *Beta vulgaris*, Cercospora leaf spot disease, plant-pathogen interaction, SCoT PCR-based analysis

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1. Introduction

Sugar beet (*Beta vulgaris* var. *saccharifera*, L.) became the first sugar crop in Egypt. it contributed to production with 62.2% of the total sugar yield, which amounted to 2.458 million tons (Egyptian Ministry of Agriculture and Land Reclamation, Jan. 2021). *Cercospora* leaf spot, incited by *Cercospora baticola* fungus, is the sugar beet's most widespread foliar disease. The fungus spreads quickly from one region to another in the same country, causing necrotic leaf lesions, so there was a significant reduction in the photosynthetic capacity, and consequently, a reduction in root yield. The decline may reach 42% in sugar yield and an increase in the percentage of impurities, leading to considerable economic losses (Khan and Smith, 2005; Knight et al., 2019). In severe epidemic cases, the destruction of foliage in the first and progress to regrowth occurs, and there is a significant reduction in sugar content of 25 to 50% (Milijanka et al., 2020). Control strategies for this disease depend on growing resistant cultivars, applications of fungicide, and suitable crop rotation (2–3 years) is necessary to reduce the spread of the fungus from an infested crop (Sullivan et al., 2021).

Genetic resistance of genotypes is the primary tool for the sustainable management of this disease. It limits any economy, but the negative association between sugar yield and resistance is a significant challenge (Skaracis et al., 2010; Stevanato et al., 2019). The reaction to Cercospora leaf spot determines the resistance/susceptibility level of sugar beet varieties through two parameters; the first one before harvest is the infection of leaves based on a grading of disease severity; and the second parameter is the amount of loss in yield (B.S.A., 2000; Görlich et al., 2021). While environmental conditions usually influence physiological traits and biochemical expression variations and the growth stage (Andrew et al. 2010). Because the resistance is quantitative, meaning it is conferred by additive components and multiple genes and is unaffected by environmental factors, many traditional methods for breeding resistance beet genotypes to Cercospora leaf spot are used, such as backcrossing, mass selections, and family line selection (Skaracis et al. 2010).

Molecular markers have been a powerful tool in determining the genetic variations among sugar beet genotypes (Abbasi et al., 2014). Some advantages that render molecular markers useful in specific applications include the ease of use and their ability to target genes for the study's specific aims. Some PCR marker systems were used, such as inter simple sequence repeats (ISSR), random amplified polymorphic DNA (RAPD), and sequence-related amplified polymorphisms (SRAP), as molecular tools to differentiate resistant from susceptible cultivars and to precisely select parents for crosses in different breeding programs (Abd El-Fatah et al. 2020). Recently, start codon targeted (SCoT) became widely introduced in research as a new molecular marker based on SPAR (Samuel, 2021). It has several advantages, including the utilization of universal primers in plants; it is less-expensive and

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straightforward technique; it results in a high percentage of polymorphism; and there is extensive genetic information available for SCoT (Gowayed and Moneim 2021).

The current study aims to determine the resistance ability of selected sugar beet varieties to the cercospora leaf spot disease through studying their agronomic performance in response to the infection and linking their disease resistance or susceptibility phenotypes with molecular markers analyzed by using the novel SCoT-PCR technique.

2. Material and methods

2.1 Varieties collection

Six varieties (genotypes) of sugar beet; namely Gergoria-KWS, BTS 2860, LP17B4011, MK 4199 (Emperator), Pintea, and Zeppelin were selected to conduct this study. These varieties were obtained from the Sugar Crops Research Institute, Agricultural Research Center, Giza, Egypt.

2.2 Field Trials

The experiments and data collection were performed in two successive growing seasons (years); 2019/2020 and 2020/2021. The work was conducted at the Experimental Farm, Sakha Agriculture Research Station, Agriculture Research Center (ARC), Egypt under field growth conditions. The meteorological data was obtained from the Central Lab for Agricultural Climate, ARC. The data includes maximum and minimum air temperature, relative humidity (RH), pan evaporation, and quantity of rain per day. These data were recorded daily from the day of tuber sowing till the day of harvest (Table 1) by the weather unit located at the Research and Training Center of rice, Sakha, Kafr El-Shekh governorate. The randomized complete block design (RCBD) with three replications (*n* = 3) was used in all the measurements presented in the current study. The plot area was 10.8 m^2 of three rows (6.0 m long and 60 cm in width, with 20 cm apart between hills each).

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Table 1. Meteorological data collected through the two growing seasons

Seasons	Date	Air Temperature (C)		%RH		Wind velocity Km/24hr	Pan Evapo (inches)	Rain mm/day
		Max.	Min.	7.30 (AM)	1.30 (PM)			
First season 2019/2020	Sep. 2019	32.2	27.9	81.8	51.3	73.8	542.9	0.0
	Oct. 2019	30.3	26.7	87.3	54.3	56.6	383.7	14.3
	Nov. 2019	27.4	25.1	82.8	48.3	36.6	230.8	0.0
	Dec. 2019	21.4	13.4	86.9	58.9	38.5	265.6	10.3
	Jon. 2020	18.4	11.8	86.7	62.7	30.0	208.8	7.5
	Feb.2020	20.4	12.7	84.6	56.5	51.0	182.9	3.60
Second season 2020/2021	Seb.2020	34.6	27.1	86.7	47.7	93.3	624.2	0.0
	Oct.2020	31.5	24.6	84.8	47.1	72.7	412.3	0.0
	Nov.2020	25.0	17.5	86.7	56.8	46.9	228.3	2.47
	Dec.2020	22.9	13.7	87.7	55.7	44.9	248.7	4.70
	Jon.2021	21.0	13.5	86.7	59.5	99.2	256.8	3.51
	Feb.2021	29.5	12.5	87.5	55.9	58.3	355.6	0.0

Data shown are the maximum and minimum air temperature, % relative humidity (%R.H), wind velocity, pan evaporation, and quantity of rain per day.

2.3 Disease Severity and Classification of Environmental conditions

According to (Walne and Reddy, 2022), disease severity% was recorded before harvest. At harvest, after180 days of planting time, foliage fresh and dry weight/ plant (gm), root fresh and dry weight/plant (gm), root length, and root diameter/plant (cm) were determined. Total soluble solids (% TSS) were estimated in fresh roots of sugar beet using a hand refractometer according to Leilah et al., 2021, and Sucrose% (pol%) was evaluated according to the Association of Official Analytical Chemists, A.O.A.C in 1990 and 2005 (Khan et al., 2018).

2.4 DNA extraction

DNA extraction of Sugar beet was carried out in the central laboratory, Agricultural Botany Department, Faculty of Agriculture, Ismailia governorate, Egypt. Leaf samples were collected from six sugar beet varieties in the seedling stage. The integrity of DNA was checked via agarose gel electrophoresis, and the concentration was measured by ultraviolet spectrophotometer as by (Subidhya et al., 2020).

2.5 PCR amplification and SCoT markers analysis

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Start codon targeted (SCoT) marker procured from Biobasic Com. Amherst, New York, United States. Six primers of SCoT were designed (Table 2) (Collard and Mackill, 2009). PCR amplification was performed in a twenty µl reaction (1× PCR buffer, three mM $MgCl_2$, 2.5 µM primer, 50 ng of sample DNA, 200 µM of each dNTP and 1.5 unit of Taq DNA polymerase) (Barnes et al., 2021). All amplifications were carried out through the following conditions for 35 cycles: 94 °C for 3 min, 93 °C for 1 min, 48 °C for 1 min, 72°C for 2 min and finally, 72 °C for 10 min. PCR fragments were separated on agarose gels (1.5%), and Ethidium bromide was used to stain the bands. Scoring for the presence or absence of bands and cluster analysis were determined by the Unweighted pair group method of arithmetic averages (UPGMA) NTSYSpc program (Rohlf, 2000; Elameen et al., 2021).

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Table 2. SCoT PCR primers used in the current study

Primers ID	Sequences (5´→ 3`)
SCoT 3	5´ ACG ACA TGG CGA CCC ACA 3`
SCoT 5	5´ CAA TGG CTA CCA CTA GCG 3`
SCoT 6	5´ CAA TGG CTA CCA CTA CAG 3`
SCoT 9	5´ ACA ATG GCT ACC ACT GCC 3`
SCoT 10	5´ ACA ATG GCT ACC ACC AGC 3`
SCoT 12	5´ CAA CAA TGG CTA CCA CCG 3`

Data shown are the PCR primers used in SCoT analysis and their 5'-3' nucleotide sequences.

2.6 Statistical analysis

The obtained data were statistically analyzed using software MSTAT-C program, version 2.10, package 1991 (Hamed and Abdel-Monaim, 2016). ANOVA analysis was used to analyze variance, and the detected mean was at $P < 0.05$ according to the LSD multiple range test (Marco et al., 2022).

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3. **Results**

3.1 Disease severity

By examining the disease severity percentage, the low values were detected in Gerogoria-Kws, BTS2860, and Zepplin varieties, while the high values were detected in LP17B401, MK4199 and Pintea varieties (**Figure 1)**. Scercospora leaf spot disease symptoms were observed on primary leaves in the two growing seasons and the infection develops as necrotic spots spread and consolidated (**Rangel et al., 2020**). It was remarkable that the values of the disease severity percentage in the second season were more than that in the first season for all studied varieties (**Table 1**).

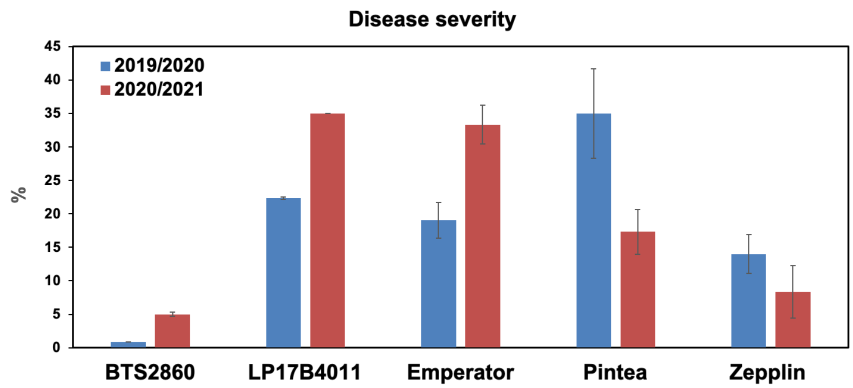

Figure 1: Percentage of disease severity of Cercospora leaf spot for the six sugar beet varieties through the two growing seasons

3.2 Growth traits of foliage and root

Gergoria-Kws and Zepplen demonstrated their ownership of the largest diameter among the mean values among the tested varieties (**Figure 2**). In contrast, MK 4199 (Emperator) and LP17B4011 demonstrated ownership to the smallest diameters. On the other hand, the root phenotypes were also affected in the tested varieties. The MK 4199 (Emperator), Gergoria-Kws, and BTS 2860 varieties had the longest lengths, whereas the Pintea varieties had the shortest roots among all the varieties (**Figure 3**).

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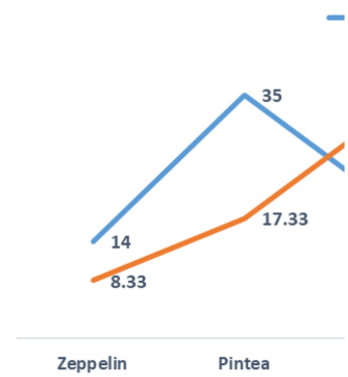

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Moreover, the weight of foliage was different between the sugar beet varieties, that MK4199 (Emperator) and Zepplen varieties owned the highest weight (**Figure 4**), in contrast, owned the lowest weight of foliage. Also, BTS2860 and Gergoria-Kws varieties owned the highest mean values (**Figure 5**). Also, during the analysis according to fresh weights of root, Gergoria-Kws variety had the highest mean values through the two growing seasons, While Pintea, MK 4199 (Emperator), and LP17B4011 genotypes had the lowest mean values (**Figure 6**). At the same time, Gergoria-Kws had the heaviest dry root among the second season (**Figure 7**). Among the analysis of the fresh weight of root parameter, Gergoria-Kws variety had the highest mean values of 2,031g and 1,702 g compared to other types through the two growing seasons, respectively**.** In contrast, Pintea, MK 4199 (Emperator), and LP17B4011 genotypes had the lowest mean values of 1163.33 - 11060, 1116.66-116 1073.33-1158g, respectively. MK 4199 (Emperator) and LP17B4011 varieties had the heaviest dry roots in the first season (174.91-125.32 and 140.39-87.83g). At the same time, Gergoria-Kws had the largest dry-weight roots (142.17 g) in the second season. Moreover, the Pintea variety had the lowest dry weight of root of 91.35 and 66.15 g in the two growing seasons. There were significant differences between the dry weight of root results in the two growing seasons for all studied varieties. Fresh and dry weight traits were essential characteristics for this crop. Depending on the fact that the root is the economic yield of this crop, the heavy root type was considered the aim of all plant breeding programs (**Cobb et al., 2019**).

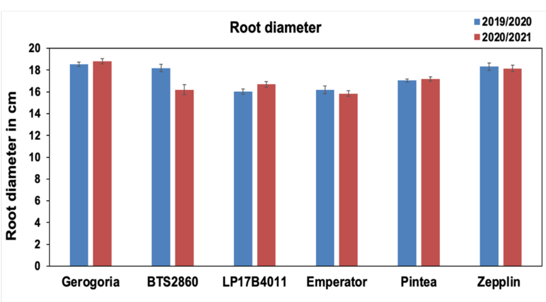

Figure 2. Mean values of root diameter for six sugar beet varieties through two growing seasons.

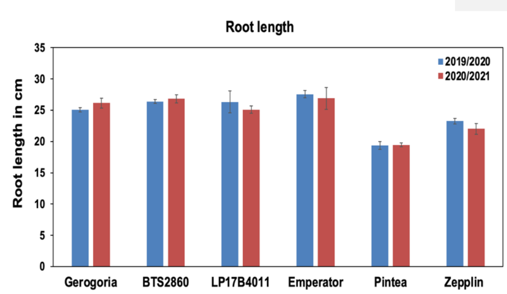

Figure 3. Mean values of root length for six sugar beet varieties through two growing seasons.

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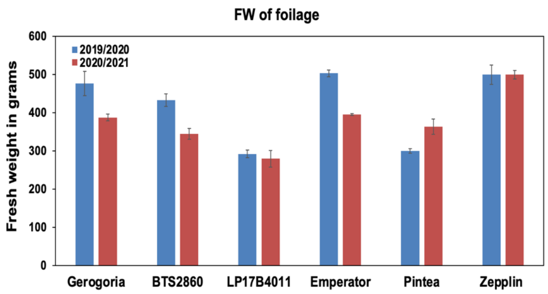

Figure 4. Mean values of fresh weight of foliage (g) for six sugar beet varieties through two growing seasons.

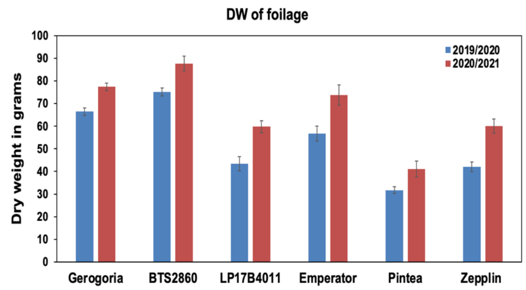

Figure 5. Mean values of dry weight of foliage (g) for six sugar beet varieties through two growing seasons.

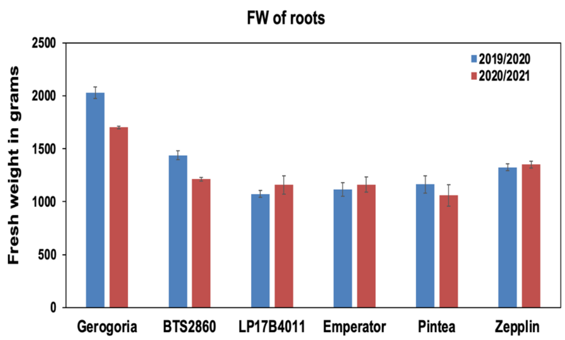

Figure 6. Mean values of fresh weight of root (g) for six sugar beet varieties through two growing seasons.

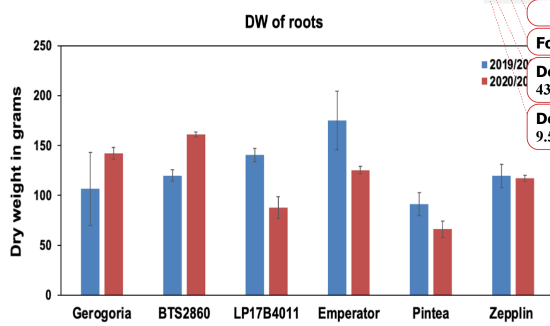

Figure 7. Mean values of dry weight of root (g) for six sugar beet varieties through two growing seasons.

3.3 TSS and Sucrose content traits

The total soluble solid (%TSS) and sucrose content traits were assessed in all the examined varieties. The highest levels of TSS contents were detected in Gergoria-Kws and BTS2860 varieties, while the lowest levels were detected in Zeppelin and LP17B4011 varieties in the two growing seasons. On the other hand, the highest level of sucrose in the two growing seasons was detected in Gergoria-Kws variety, while the lowest level was detected in Penita variety among the two growing seasons (**Figure 9**).

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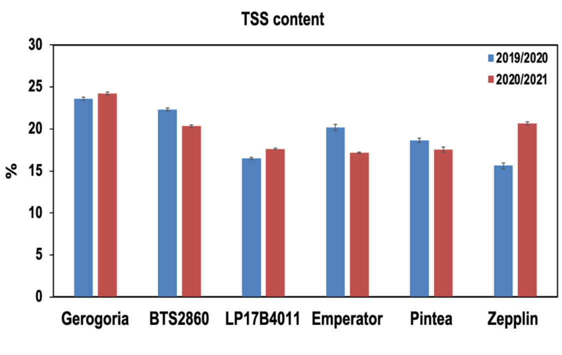

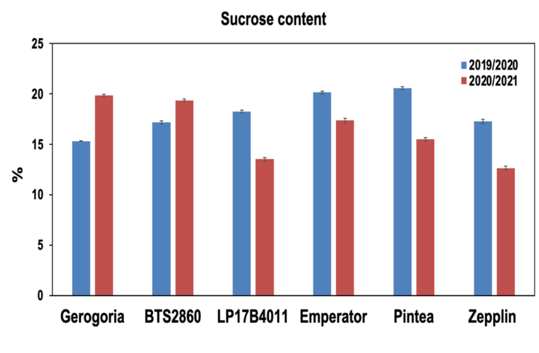

Figure 8. Mean values of %TSS for six sugar beet varieties through two growing seasons.

Figure 9. Mean values of sucrose content (%). for six sugar bee varieties through two growing seasons.

3.4 DNA analysis

Results of SCoT-PCR analysis showed that 38 total reliable bands were scored among studied sugar beet varieties **(Figure 10 and Table 3).** Eight bands are the maximum number of bands produced by each SCoT3 and SCoT10 primers, whereas four bands are the minimum number of bands produced by each SCoT5 and SCoT6. Eighteen bands were polymorphic, ranging from 2 (SCoT5 and SCoT6) to 4 (SCoT9 and SCoT12), with an average of 3 bands per primer. Genetic similarity values ranged from 0.76 and 0.92, as reported in **(Table 4).** The highest genetic similarity value was observed between Gerogoria and BTS2860, the two varieties that share a high ability to resist Cercospora leaf spot disease, whereas the lowest value of genetic similarity was observed between LP17B4011 and Zepplin.

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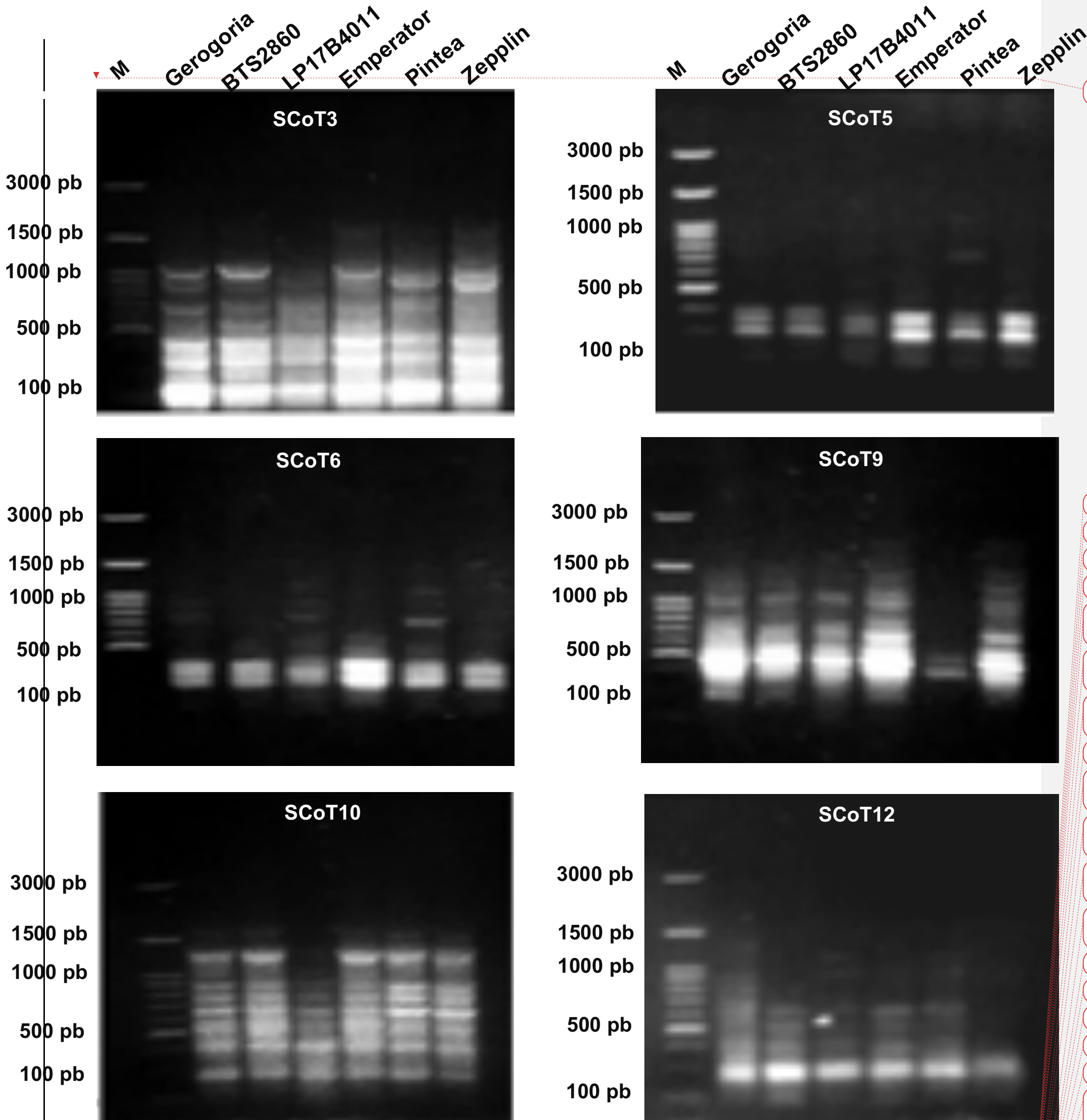

Figure 10. The SCoT amplification profile of SCoT primers. The PCR primers used for SCoT3 analysis are indicated as SCoT3, SCoT5, SCoT6, SCoT9, SCoT10, and SCoT12. The six sugar beet varities are indicated as Gerogoria, BTS2860, LP17B4011, Emperator, Pintea, Zepplin in the lanes of Agrarose gels. M indicateds DNA Marker with sized shown as base pairs (pb).

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Table 3. SCoT polymorphism analysis.

Primer Name	Total	# Monomorphic bands	# Polymorphic bands	# Unique bands	% Polymorphic
SCoT 3	8	5	3	-	37.5
SCoT 5	4	2	2	-	50
SCoT 6	4	2	2	-	50
SCoT 9	5	1	4	3	80
SCoT 10	8	5	3	2	37.5
SCoT 12	5	1	4	2	80
Total	38	16	18	7	47.36

Data shown are the total number of PCR bands, monomorphic, polymorphic, unique PCR bands, and % polymorphism for each SCoT primer.

Table 4. Genetic similarity values of the selected **six sugar beet varieties** based on **SCoT analysis.**

	Gerogoria	BTS2860	LP17B4011	Emperator	Pintea	Zepplin
Gerogoria	1					
BTS2860	0.92593	1				
LP17B4011	0.82353	0.81633	1			
Emperator	0.86792	0.90196	0.83333	1		
Pintea	0.83019	0.78431	0.79167	0.84	1	
Zepplin	0.80769	0.84	0.76596	0.89796	0.81633	1

All amplified SCoT fragments were used in cluster analysis through the UPGMA method to make the dendrogram, and accordingly the selected sugar beet varieties were divided into four clusters (Figure 11). The first cluster contained two genotypes; Emperator and Zepplin, which are considered disease-susceptible varieties, whereas the second cluster included two genotypes; BTS2860 and Gerogoria which are considered disease-resistance varieties. The third and fourth clusters contained one genotype, Pintea and LP17B4011. Results indicated that the SCoT markers technique is reliable through divided sorts according to their genetic distance (Myles et al., 2011; Guo et al., 2012).

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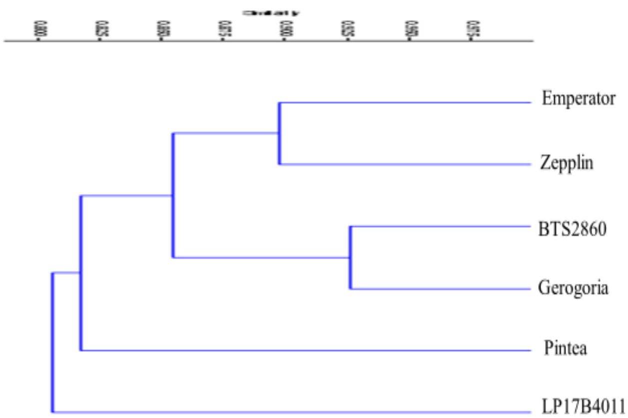

Figure 11. Dendrogram and divided studied varieties of sugar beet into four clusters.

4. Discussion

While there is a fact that the diseases can reduce crop yield, farmers are interested in detecting plant diseases using sensors that can be mounted on aerial vehicles in order to select tolerant or resistant genotypes (Lawrence et al., 2021). From the disease severity percentage, Gerogoria-Kws, BTS2860, and Zepplin varieties were recorded with the lowest values (1.00-2.67, 0.83-5, and 14.00-8.33% in both seasons, respectively), while LP17B401, MK4199 and Pintea varieties were recorded with the highest values of disease severity percent (22.33-35.00, 19.00-33.33 and 35.00 17.33% respectively). Symptoms of cercosporin leaf spot disease were recorded in the two growing seasons analyzed in the current research and these symptoms were observed on the primary leaves of which the pathogen develops as necrotic spots spread and coalesce (Rossi et al. 2000). Low values for % disease severity in some sugar beet genotypes were indicators of their disease resistance ability. In contrast, high values may indicate the genotypes' susceptibility or sensitivity to the disease. These findings are in agreement with (B.S.A., 2000), where sugar beet varieties are classified based on the level of resistance/openness before harvest in response to Cercospora leaf spot by estimating leaf infection according to the grading of disease severity. The differences between the values for % disease severity in the first growing season and the second growing season can be attributed to the difference in the environmental conditions, which results

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in higher % disease severity in the second season. Kaiser et al. (2010) observed these differences, and accordingly suggested that the susceptible varieties were different from resistant varieties in the highest values of % disease severity at the harvest stage and in the greater infection area sizes based on the disease progress curve (AUDPC).

During the analysis of growth traits of foliage and root, Gergoria-Kws and Zepplen varieties owned the widest diameter with mean values of 18.53-18.80 and 18.31-18.14cm, respectively. In contrast, MK 4199 (Emperator) and LP17B4011 varieties had the lowest diameters with mean values of 16.18-16.02 and 16-69 cm, respectively. Results appeared significant differences among six types of sugar beet through the two growing seasons. For root length trait, MK 4199 (Emperator), Gergoria-Kws, and BTS 2860 varieties had the longest (27.57-26.90, 25.07-26.13, and 26.50-26.83cm), respectively, while Pintea varieties (19.36 and19.47cm) had the shortest roots comparing with other types. On the other hand, MK4199 (Emperator) and Zepplen varieties owned the highest weight of foliage (503.33-395 and 500-500gm in two growing seasons, respectively, while LP17B4011 and Pintea genotypes owned the lowest weight of foliage; 292-279 and 300-363g respectively. BTS2860 and Gergoria-Kws varieties owned the highest mean values, 75.05-87.70 and 66.41-7738g, respectively, in two growing seasons. The second season showed increasing mean values of dry weight of foliage in comparison with the first season for all varieties.

According to % TSS and sucrose content determination, Gergoria-Kws and BTS2860 varieties observed a high content of TSS (23.60-24.23 % and 20.33-22.30 %, respectively (Figure 8). In contrast, Zeppelin and LP17B4011 varieties observed the lowest contents of 15.63-20.67 % and 16.50 -17.63% in the two growing seasons. Further, it was remarkable that Gergoria-Kws variety owned the highest range of sucrose through the two growing seasons with the score of 20.57% and 19.87%, in the first and second seasons, respectively. The next value was detected in the BTS2860 array which had the highest content of 20.16- 19.37% in the two growing seasons, respectively. Although, Penita variety owned the lowest range among the two growing seasons (15.33- 15.50%). The differences of sucrose content in each of each array was due to the environmental conditions. Zpplen variety was remarkable by owning the lowest range of sucrose through the two growing seasons (17.30 and 12.63%), respectively. The high TSS and some types of disaccharides had all been associated with the host resistance against *C. beticola* (Rangel et al., 2020). There was a significant reduction in photosynthetic potential due to Cercospora leaf spot disease infection. Moreover, the induction of vegetative regrowth occurred at the expense of sugar reserves in the root (Rossi et al., 2000; Khan and Smith, 2005; Ghazy et al., 2020). Moreover, the increasing rate of respiration due to the disease caused significant loss in Root storage. When disease

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pressure was high, resistant cultivars produced white sugar compared to susceptible cultivars at the same disease level (Skaracis et al., 2010; Kaiser et al., 2010).

Polymorphism percentage ranged from 37.5% (SCoT3 and SCoT10) to 80% (SCoT9 and SCoT12), with an average of polymorphism of 47.36%. Polymorphic band presence was considered a good indicator of the SCoT technique's efficiency in differentiating among studied genotypes. (Guo et al., 2012; Gowayed and Moneim, 2021). SCoT 9 primer was the most informative and had tremendous potential among the primers. One polymorphic band, 1056 bp, was produced by both SCoT3 presented in Gergoria and BTS2860, which was observed with low disease severity percentage of Cercospora leaf spot values. So, they could be considered resistance varieties. This band was absent in LP17B4011, Emperator, Pintea and Zepplin varieties, although they had the high values of disease severity percentage of Cercospora leaf spot. Therefore, they could be considered susceptible genotypes. There were seven unique bands observed, three bands (one positive and two negatives), two bands (negative), and two bands (one positive and one negative), were produced by SCoT9, SCoT10, and SCoT12, respectively.

For fingerprinting of varieties, SCoTs markers were more informative and efficient compering with other markers based on the average polymorphism percentage (Gorji et al., 2011; Xiong et al., 2011; Hamidi et al., 2014 Gowayed and Moneim, 2021). According to the growth and molecular identification results, Gergoria and BTS2860 varieties would be very helpful because they had disease resistance genes and could be used in a more sustainable sugar beet crop. Stevanato et al. (2019) reported that, to obtain enriched cultivars with desirable sustainability traits, different advanced molecular techniques transfer specific genes to domesticated cultivars to produce new lines with resistance to various diseases and high production.

Conclusions

In the current research, the characteristics of selected sugar beet varieties in response to the cercospora leaf spot disease were studied and the disease - resistant and susceptible varieties were identified through the morphological analysis coupled with molecular analysis using the reliable novel technique, SCoT.

The results indicated that Gerogoria-Kws and BTS 2860 varieties were remarkable by their lowest % disease severity values and observed the of greatest growth and yield. Zepplin variety ranked second regarding its performance in response to the disease, in which the values for % disease severity was relatively low (8.3 and 14%, in the first and second growing season, respectively), and it showed the lowest mean values of the most studied traits. Whereas, LP17B401, MK4199, and Pintea varieties were distinguished with the highest values of % disease severity as well as great growth and yield characters

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values. Moreover, Gergoria-Kws and BTS2860 varieties exhibited the highest content of both TSS and sucrose concentration, whereas Zepplen and LP17B401 varieties had the lowest contents. Furthermore, in the current research, to our knowledge, Start codon and targeted (SCoT) molecular markers were identified and confirmed in sugar beet. These novel molecular markers has the ability to differentiate between resistant and susceptible sugar beet varieties for the Cercospora leaf spot disease. Our findings indicated that SCoT is a highly efficient tool for determining the genetic variability among sugar beet genotypes. One polymorphic band (1056 bp) produced by SCoT3 distinct Gerogoria-Kws and BTS 2860 varieties might be used as a molecular marker used as an indicator resistance variety.

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