

**DETERMINTION THE GENETIC RESISTANCE OF  
CERCOSPORA LEAF SPOT DISEASE in SOME  
SUGAR BEET CULTIVARS USING AGRONOMIC  
TRAITS AND MOLECULAR MARKERS by START  
CODON TARGETED (SCoTs)**

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**ABSTRACT**

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is root crop grown commercially for produce over 100 million tons per year of sugar (mainly sucrose) for worldwide consumption. Cercospora leaf spot is a foliar disease that caused by the fungus *Cercospora beticola* Sacc. can destroys the sugar beet yield. To identifying sugar beet cultivars with remarkable yield and disease resistance; six cultivars of sugar beet, namely Gergoria-KWS, BTS 2860, LP17B4011, MK 4199 (Emperor), Pintea, and Zeppelin were obtained. These six sugar beet cultivars were grown in experimental farm, Sakha Agriculture Research Station, Agriculture Research Center and examined under field growth conditions for two successive seasons, 2019/2020 and 2020/2021. Disease severity percentage, root and foliage-associated traits, % of sugar and total soluble solids (% TSS) content were measured. Novel start codon targeted (SCoT) markers via PCR-based applications using ten SCoT-specific primers was used and genetic similarity among cultivars were estimated. The results divided the studied cultivars to three classes; the first contains; Gerogoria-KWS and BTS2860 cultivars appear to have the lowest % disease severity (1.00-2.67 % and 0.83-5 %, respectively) and they have longer roots, higher biomass for foliage and root (on fresh and dry weight basis), higher content of TSS % and sucrose in the both seasons. The second class contains; Pintea, MK 4199 (Emperor), and LP17B4011 cultivars exhibited the highest % of disease severity, the lowest biomass and shortest roots. The third class include, Zeppelin cultivar had moderate of % disease severity and low TSS % content. In addition, the results of SCoT markers were showed that the average of

Polymorphism percentage was 46.37 and obtain 54 polymorphic bands; there were two bands from them with molecular weight 1056 bp and 1100 bp generated via SCoT3 and SCoT4 analysis, in respectively were distinguished as associations to Gergoria and BTS2860, which could be attributed to the high disease resistance phenotypes in those two cultivars and absent in the rest sensitive cultivars. The obtained values of genetic similarity ranging from 0.76 to 0.92, by which the highest was between Gerogoria and BTS2860 cultivars. Moreover, cluster analysis was conducted based on the genetic relationships illustrated a high degree of harmony between growth traits and results of PCR-based SCoT analysis. These results indicate the efficiency of SCoT markers in distinguish among the cultivars for the resistance or the sensitivity to this disease.

**Key Words:** *Beta vulgaris*, Cercospora leaf spot disease, SCoT PCR-based analysis, sugar and TSS content.

### INTRODUCTION

Sugar beet (*Beta vulgaris* subsp. *vulgaris*.) became the first sugar crop in Egypt and contributed to the total sugar yield, which amounted to 2.458 million tons (EMALR, 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 because these causing necrotic leaf lesions, causing 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). When an epidemic is severe, the foliage is initially destroyed, followed by regrowth, and the sugar content is significantly reduced from 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) are necessary to reduce the spread of the fungus from an infested crop (Sullivan *et al.*, 2021).

Resistance cultivars are the primary tool for the sustainable management of this disease and considered a significant challenge. Because resistance is quantitative, conferred by additive components, multiple genes and there were negative association between yield of sugar content and resistance (Skaracis *et al.*, 2010 and Stevanato *et al.*, 2019). The reaction to *Cercospora* leaf spot determines the resistance/susceptibility level of sugar beet cultivars 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 (BSA., 2000 and Görlich *et al.*, 2021). While environmental conditions usually influence physiological traits, biochemical expression variations, and the growth stage (Andrew *et al.*, 2010).

Molecular markers have been a powerful tool in determining the genetic variations among sugar beet cultivars (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, such as inter simple sequence repeats (ISSR), random amplified polymorphic DNA (RAPD), and sequence-related amplified polymorphisms (SRAP), are used to differentiate resistant from susceptible cultivars 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 Single Primer Amplification Reactions (SPAR) (Samuel, 2021). It has several advantages, including the utilization of universal primers in plants; it is less-expensive and straight forward technique; it results in a high percentage of polymorphism; and there is extensive genetic information available for SCoT (Gowayed and Moneim 2021).

Based on the above, the aim of the current study was aimed to determine the resistance ability of selected sugar beet cultivars to the Cercospora leaf spot disease and their agronomic performance. The susceptibility phenotypes were lined with the molecular markers using the novel SCoT-PCR technique.

## MATERIAL AND METHODS

### 1- Cultivars collection

Six cultivars of sugar beet; namely Gergoria-KWS, BTS 2860, LP17B4011, MK 4199 (Emperor), Pintea, and Zeppelin were obtained from the Sugar Crops Research Institute, Agricultural Research Center, Giza, Egypt to conduct this study. These sugar beet cultivars names, sources and category of disease severity are presented in Table (1). However, there was difficulty in obtaining the pedigree of the studied sugar beet cultivars, because they were imported from abroad, so the pedigree record was not written in Table (1).

**Table (1). Number of cultivars, cultivars name and source of sugar beet used in the present investigation.**

No.	Cultivar name	Source	Category of disease severity
1	Gergoria-KWS	Germany	Resistance
2	BTS 2860	Germany	Resistance
3	LP17B4011	France	Susceptible
4	MK 4199 (Emperor)	France	Susceptible
5	Pintea	Russia	Susceptible
6	Zeppelin	Iran	Resistance

### 2- Experimental Field

The experiments and data collection were performed in two successive growing seasons; 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 chosen of the experiments site to be an experiments field in this work due to the soil its old and cultivated for several years by many varied crops in summer and winter seasons. As well as, it is surrounded by many other farms, which makes it vulnerable to fungous pathogens that affect the leaves. These observations were confirmed by the owners' researchers of the farm themselves and the neighboring farmers as well as pervious research, which carried out in this zone farm (Ghazy *et al.*, 2020).

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 seeds sowing until the day of harvest (Table 2) by the weather unit located at the Research and Training Center of rice, Sakha, Kafr El-Shekh governorate.

### 3- Experiments Design

The randomized complete block design (RCBD) with three replications was used in all the measurements presented in the current study. The replication area was 10.8 m<sup>2</sup> consist of three rows (6.0 m long and 60 cm in width, with 20 cm apart between hills).

### 4-Agronomic Practices:

Sugar beet cultivars seeds were sown on October 10 in the first season (2019/2020) and on October 5 in the second season (2020/2021). The recommended agronomic practices such as ploughing, harrowing, thinning, manual weeding, irrigation... *etc.* for sugar beet plant in plots were done at the optimum time when it needs.

**Table 2. Meteorological data collected through the two growing seasons.**

Season	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
	Jan. 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.

### **5- Disease Severity and Classification of Environmental conditions**

The disease severity % of sugar beet plant was recorded, before harvest, according to **Walne and Reddy, (2022)**. At harvest, after 180 days of planting date, foliage fresh and dry weight/ plant (g), root fresh and dry weight/plant (g), 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 and Khan (2021)**, and Sucrose% (pol%) was evaluated according to the Association of Official Analytical Chemists, (**AOAC 1990 & 2005 and Khan et al., 2018**).

### **6- DNA extraction**

Two grams of young leaves collected from seedling of each studied cultivar after 10 days after planting for DNA extract using the CTAB method (**Lassner et al., 1989**) and their modification by **Torres et al., (1993)**. Total DNA was extracted by DNeasy Plant Kit (QIAGEN, Germany). The integrity of DNA was checked via agarose gel electrophoresis, and the concentration was measured by ultraviolet spectrophotometer (**Srivastava and Gupta, (2001)**).

#### **- PCR amplification and SCoT markers analysis**

Ten Start codon targeted (SCoT) primers were used in the detection of polymorphism (Table 3) (**Collard and Mackill, 2009**). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10 pmol), 3 µl template DNA (10 ng) and 7 µl dH<sub>2</sub>O (**Barnes et al., 2021**). PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 45s, an annealing step at 50°C for 50s, and an elongation step at 72°C for 1min. The primer extension segment was extended to 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000) (**Rohlf, 2000 and Elameen et al., 2021**). For SCoT analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between cultivars using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) was performed according to Euclidean similarity index using the PAST software Version 1.91 (**Hammer et al., 2001**). The DNA isolation and PCR were done at Central laboratory,

Agricultural Botany Department, Faculty of Agriculture. Ismailia Governorate, Egypt and Agriculture Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC).

**Table 3. SCoT PCR primers used in the current study**

Primer ID	Sequence (5' → 3')
SCoT-1	5'-ACGACATGGCGACCACGC-3'
SCoT-2	5'-ACCATGGCTACCACCGGC-3'
SCoT-3	5'-ACGACATGGCGACCCACA-3'
SCoT-4	5'-ACCATGGCTACCACCGCA-3'
SCoT-5	5'-CAATGGCTACCACTAGCG-3'
SCoT-6	5'-CAATGGCTACCACTACAG-3'
SCoT-7	5'-ACAATGGCTACCACTGAC-3'
SCoT-9	5'-ACAATGGCTACCACTGCC-3'
SCoT-10	5'-ACAATGGCTACCACCAGC-3'
SCoT-12	5'-CAACAATGGCTACCACCG-3'

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

## 7- Statistical analysis

The experimental data were analyzed according to the procedure of Randomized Complete Block Design (RCBD) using software MSTAT-C program, version 2.10, package 1991 (Hamed and Abdel-Monaim, 2016). The detected mean was at  $P < 0.05$  according to the LSD multiple range test by ANOVA (Marco *et al.*, 2022).

## RESULTS AND DISCUSSION

### 1- Analysis of Variance and Mean squares

Analysis of variance of all the studied characters of sugar beet cultivars during two seasons (2019/2020 and 2020/2021) is illustrated in Table (4). The results indicated that there were significant differences among the cultivars for all traits during both seasons. The existence of wide range genetic variability in characteristics of sugar beet cultivars for response to cercospora leaf spot disease indicates that conventional breeding programs and selection could improve these traits of sugar beet cultivars.

**Table 4. Mean squares of the studied traits recorded for sugar beet genotypes during 2019 and 2020 seasons.**

S.O. V	D.F	Disease severity		Root diameter		Root length		Foliage fresh weight		Foliage dry weight	
		2019/ 2020	2020/ 2021	2019/ 2020	2020/ 2021	2019/ 2020	2020/ 2021	2019/ 2020	2020/ 2021	2019/ 2020	2020/ 2021
Genotypes	5	520.01**	609.25**	3.74**	3.910**	26.70**	27.36**	28438.5**	15747.6**	806.32**	813.6**
Blook	2	59.013	29.55	0.77	0.407	4.71	6.84	628.03	836.51	18.48	46.17
Error	10	24.68	16.75	0.13	0.23	1.56	1.77	1137.14	579.8	16.59	27.70
Total	17	** significant at 5% level									
L.S.D		9.03	7.44	0.67	0.88	2.27	2.42	61.34	43.8	7.41	9.57

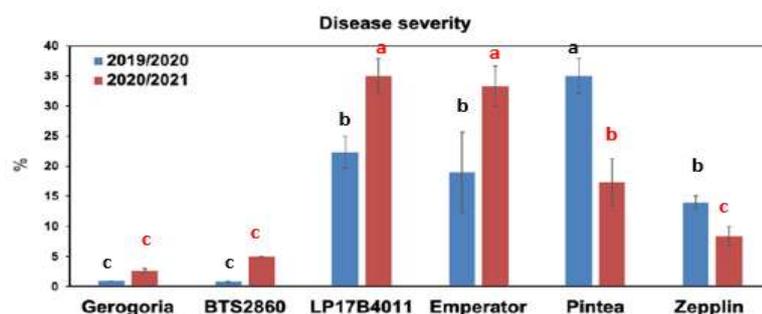
Genotypes

Table 4 Cont.

S.O. V	D. F	Root fresh weight		Root dry weight		TSS		S. content	
		2019/2020	2020/2021	2019/2020	2020/2021	2019/2020	2020/2021	2019/2020	2020/2021
Genotypes	5	382914.7**	159013.2**	2554.75**	3648.61**	29.84**	22.27**	11.67**	26.97**
Block	2	8802	30508.16	3739.06	46.98	0.193	0.28	0.058	0.10
Error	10	8592	8186.7	705.97	141.2	0.195	0.06	0.05	0.077
Total	17	** significant at 5% level							
L.S.D		168.64	164.60	44.29	21.62	0.80	0.45	0.44	0.50
Genotypes									

## 2- Disease severity

From the results of disease severity percentage, significant differences were found among the studied sugar beet cultivars. Gerogoria-KWS and BTS2860, cultivars were recorded with the lowest values 1.00–2.67 and 0.83–5, in respectively (**Fig. 1**). In addition, Zeppelin cultivar was recorded the moderate values 14.00 in the first season and 8.33% in the second season. While cultivars LP17B401, MK4199 and Pintea were recorded the highest values of disease severity % (22.33–35.00, 19.00–33.33 and 35.00 17.33% respectively). These results are in agreement with those reported by **Rangel et al., (2020)**.



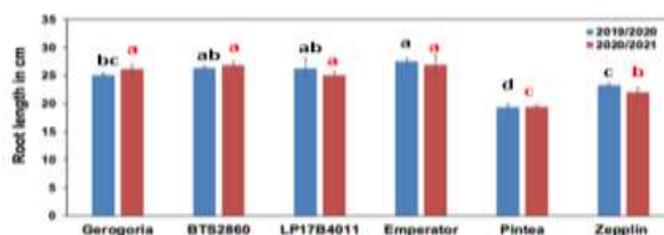
**Fig 1:** Percentage of disease severity of Cercospora leaf spot for the six sugar beet cultivars through the two growing seasons.

Symptoms of cercosporin leaf spot disease were recorded in the two growing seasons, and then analyzed in the current research. 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 percentage in some sugar beet cultivars were indicators of their disease resistance ability. In contrast, high values may indicate the cultivars' susceptibility or sensitivity to the disease. These findings are in agreement with **BSA, (2000)**, where sugar beet cultivars were 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 in higher % disease severity in the second season. Kaiser *et al.*, (2010) observed these differences, and accordingly suggested that the susceptible cultivars were different from resistant cultivars 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). While there is a fact that the diseases can reduce crop yield, whereas, farmers are interested in detecting plant diseases using sensors that can be mounted on aerial vehicles in order to select tolerant or resistant cultivars (Lawrence *et al.*, 2021).

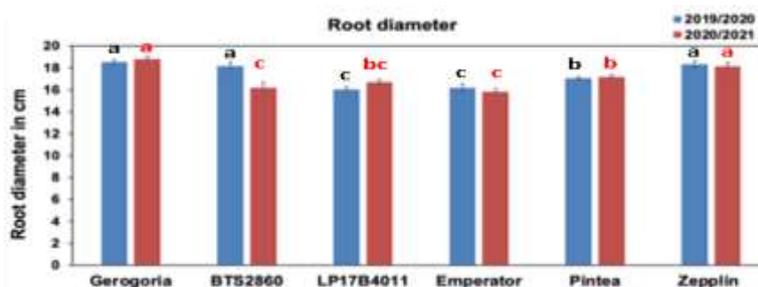
### 3- Growth traits of foliage and root

During the analysis of growth traits of foliage and root, Gergoria-KWS and Zeppelin cultivars owned the widest diameter roots with mean values of 18.53-18.80 and 18.31-18.14cm, in the two seasons respectively. In contrast, MK 4199 (Emperor) and LP17B4011 cultivars had the lowest diameter roots with mean values of 16.18-16.02 and 16-69 cm, respectively (Fig 2).



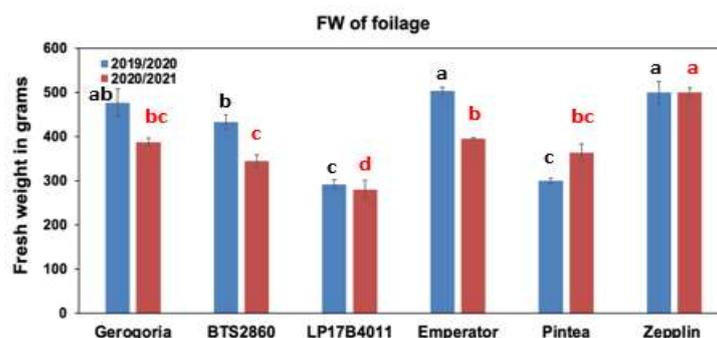
**Fig 2:** Mean values of root diameter for six sugar beet cultivars through two growing seasons.

For root length trait (Fig 3); MK 4199 (Emperor), Gergoria-KWS, and BTS 2860 cultivars had the longest roots (27.57-26.90, 25.07-26.13, and 26.50-26.83cm) in the two season respectively. While Pinteá cultivar had the shortest roots (19.36 and 19.47cm) comparing with other types.



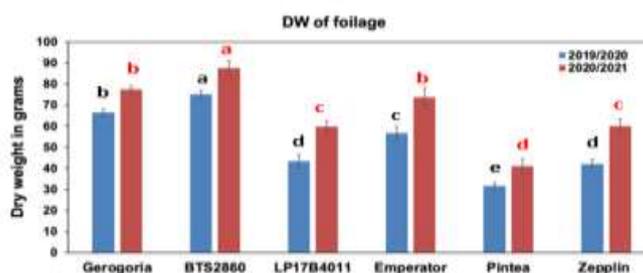
**Fig 3:** Mean values of root length for six sugar beet cultivars through two growing seasons.

On the other hand, MK4199 (Emperor) and Zeppelin cultivars owned the highest fresh weight of foliage 503.33-395 and 500-500gm in two growing seasons, respectively. While LP17B4011 and Pintea cultivars had the lowest fresh weight of foliage; 292-279 and 300-363g respectively (**Fig 4**).



**Fig 4:** Mean values of fresh weight of foliage (g) for six sugar beet cultivars through two growing seasons.

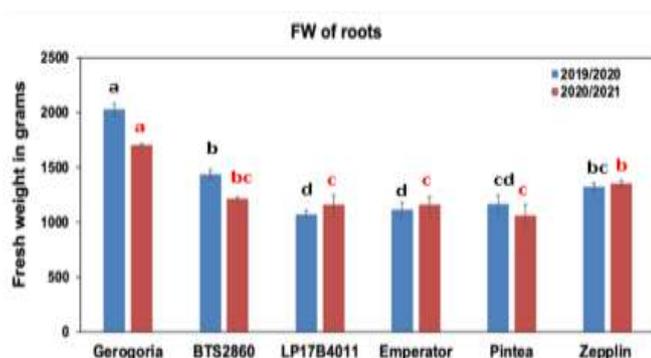
For dry weight of foliage disease (**Fig 5**); cultivars BTS2860 and Gergoria-KWS showed the highest mean values, 75.05-87.70 and 66.41-77.38g, respectively, in the two growing seasons. The second season showed increasing mean values of dry weight of foliage in comparison with the first season for all cultivars.



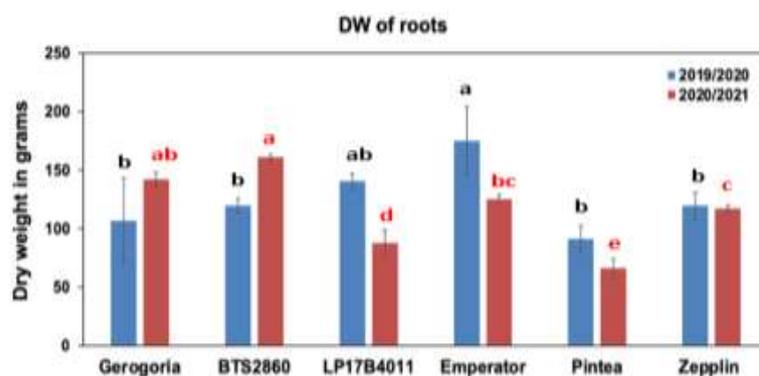
**Fig 5:** Mean values of dry weight of foliage (g) for six sugar beet cultivars through two growing seasons.

For fresh weights of root trait; Gergoria-KWS cultivar had the highest mean values 2031 g and 1702 g in the two growing seasons, respectively. While Pintea, MK 4199 (Emperor), and LP17B4011 cultivars had the lowest mean values of 1163.33 - 1060, 1116.66-1161, 1073.33-1158g respectively (**Fig 6**). At the same time; MK 4199 (Emperor) and LP17B4011 cultivars had the heaviest dry roots 174.91 g and 140.39 g respectively in the first season.

The Gergoria-KWS cultivar had the highest dry weight of roots (142.17 g) in the second season. Moreover, the Pintea cultivar had the lowest dry weight of root 91.35 and 66.15 g in the two growing seasons (Fig 7). There were significant differences between the results of dry weight of root in the two growing seasons for all studied cultivars. 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).



**Fig 6:** Mean values of fresh weight of root (g) for six sugar beet cultivars through two growing seasons.

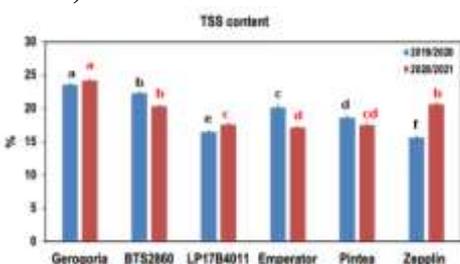


**Fig 7:** Mean values of dry weight of root (g) for six sugar beet cultivars through two growing seasons.

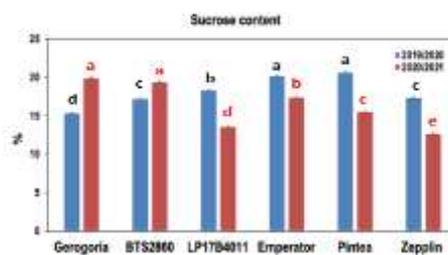
#### 4. TSS and sucrose content traits

According to % TSS and sucrose content determination, Gergoria-KWS and BTS2860 cultivars were observed a high content of TSS (23.60-24.23 % and 20.33-22.30 %, respectively) (Fig 8). In contrast, LP17B4011 cultivar revealed the lowest contents of 16.50 -17.63% in the two growing seasons and Zeppelin cultivar 15.63 in the first growing season. Results of

the % of sucrose content (**Fig 9**), showed that; Gergoria-KWS cultivar 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 of per cent was detected in the BTS2860 cultivar, which had the high content of 20.16- 19.37% in the two growing seasons, respectively. Penita cultivar owned the lowest range between the two growing seasons (15.33- 15.50%). The differences of sucrose content in each of each cultivar was due to the environmental conditions. Zpplen cultivar was remarkable by owning the lowest range of sucrose through the two growing seasons (17.30 and 12.63%), respectively. The highest 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 pressure is high, resistant cultivars produced white sugar compared to susceptible cultivars at the same disease level (**Skaracis et al., 2010 and Kaiser et al., 2010**).



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



**Fig 9:** Mean values of sucrose content (%) for six sugar beet varieties through two growing seasons.

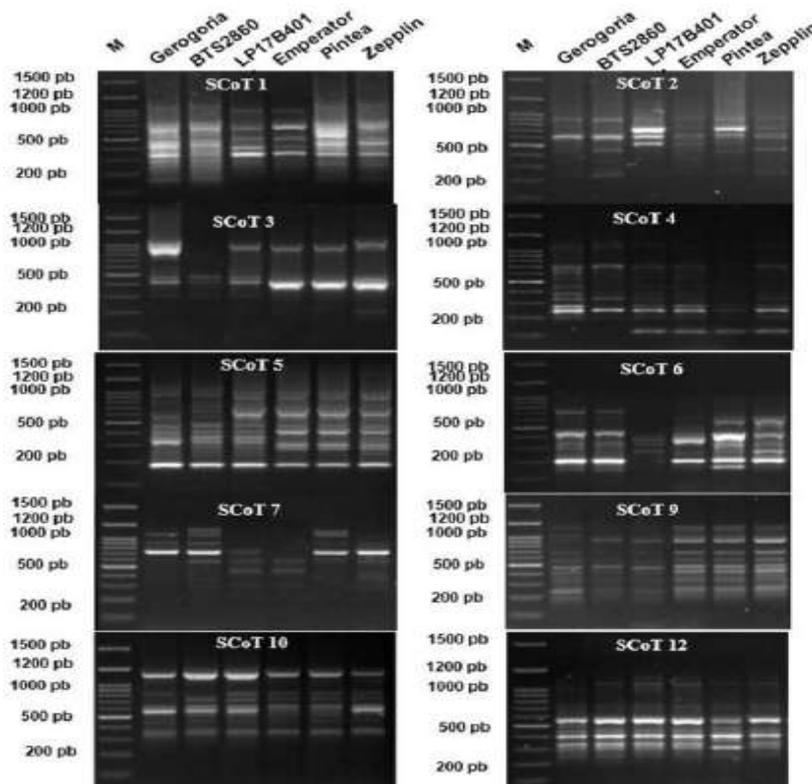
## 5. DNA analysis

### • SCoT-PCR analysis

Results of SCoT-PCR analysis showed that 112 total reliable bands were scored among studied sugar beet cultivars (**Fig 10 and Table 4**). Sixteen bands were the maximum number of bands produced by SCoT1 primer, whereas eight bands were the minimum number of bands produced by SCoT7. Fifty-three bands were monomorphic, ranging from eight bands (SCoT5 and SCoT9) to one band (SCoT7), with an average of 5.3 bands per primer. Fifty-four bands were polymorphic, ranging from ten bands (SCoT1) to three (SCoT6 and SCoT10), with an average of 5.4 bands per primer. Five unique bands were founded, ranging from two bands (SCoT3) to one band (SCoT4, SCoT5 and

SCoT10), with an average of 0.5 bands per primer. SCoT 3 primer was considered the most informative and had tremendous potential among the primers with two unique bands.

Polymorphism percentage ranged from sixty-seven (SCoT2, SCoT3 and SCoT4) to thirteen (SCoT7), with an average of 46.37 bands per primer. Two polymorphic bands; 1056 and 1100 bp were produced by SCoT3 and SCoT4 respectively presented in Gergoria and BTS2860, which were observed with low disease severity percentage of *Cercospora* leaf spot values. Therefore, they could be considered resistance cultivars. These band was absent in LP17B4011, Emperor, Pintea and Zeppelin cultivars, although they had the high values of disease severity percentage of *Cercospora* leaf spot. Therefore, they could be considered susceptible cultivars. Results are in harmony with **Guo *et al.*, (2012)** and **Gowayed and Moneim (2021)** who reported that the presence of Polymorphic bands are considered a good indicator of the SCoT technique's efficiency in differentiating among studied cultivars.



**Fig 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 cultivars are indicated as Gergoria, BTS2860, LP17B4011, Emperor, Pintea, Zeppelin in the lanes of Agarose gels. M indicated DNA Marker with sized shown as base pairs (pb).

**Table 5. SCoT polymorphism analysis.**

Primer Name	Total	# Monomorphic bands	# Polymorphic bands	# Unique bands	% Polymorphic	Mean of band frequency
SCoT 1	16	6	10	0	62%	0.78
SCoT 2	9	3	6	0	67%	0.70
SCoT 3	9	3	4	2	67%	0.70
SCoT 4	12	4	7	1	67%	0.71
SCoT 5	14	8	5	1	43.7%	0.71
SCoT 6	9	6	3	0	33%	0.89
SCoT 7	8	1	7	0	13%	0.60
SCoT 9	12	8	4	0	33%	0.84
SCoT 10	11	7	3	1	36%	0.83
SCoT 12	12	7	5	0	42%	0.81
Total	112	53	54	5	-	-
Means	11.2	5.3	5.4	0.5	46.37	-

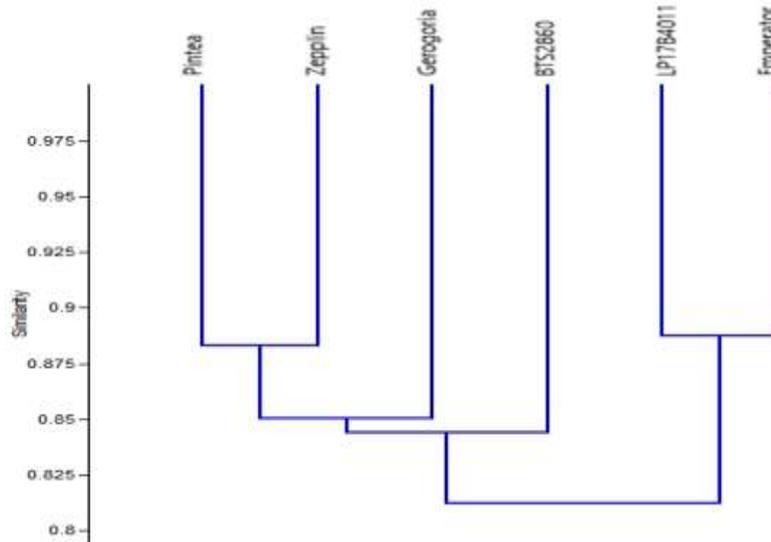
Data shown are the total number of PCR bands, monomorphic, polymorphic, unique PCR bands, % polymorphism, and mean of band frequency for each SCoT primer.

Genetic similarity values ranged from 0.89 to 0.79, as reported in **Table (6)**. The highest genetic similarity value was observed between LP17B4011 and Emperor, the two cultivars that share an ability to resist *Cercospora* leaf spot disease. Whereas the lowest value of genetic similarity was observed between each of (Gerogoria and LP17B4011), (BTS2860 and Emperor) and (LP17B4011 and Pintea).

**Table 6: Genetic similarity values of the selected six sugar beet genotypes based on SCoT analysis.**

	Gerogoria	BTS2860	LP17B4011	Emperor	Pintea	Zepplin
Gerogoria	1					
BTS2860	0.85	1				
LP17B4011	0.79	0.81	1			
Emperor	0.82	0.79	0.89	1		
Pintea	0.82	0.82	0.79	0.86	1	
Zepplin	0.88	0.87	0.80	0.85	0.88	1

The dendrogram based on the values of genetic similarity (**Fig 11**) showed that selected sugar beet cultivars were divided into two main clusters. The first cluster contained two cultivars; Emperor and LP17B4011, which are considered disease-susceptible cultivars, whereas the second cluster divided into three sub-clusters. The first sub-cluster contained BTS2860, the second sub-cluster contained Gerogoria (which are considered disease-resistance cultivars). While the third contained Zepplin and Pintea cultivars. Results indicated that the SCoT markers technique is reliable through divided sorts according to their genetic distance (Myles *et al.*, 2011 and Guo *et al.*, 2012). For fingerprinting of cultivars, SCoTs markers were more informative and efficient comparing with other markers based on the average polymorphism percentage (Gorji *et al.*, 2011; Xiong *et al.*, 2011; Hamid *et al.*, 2014 ; Gowayed and Moneim, 2021).



**Fig 11:** The phylogenetic tree of the six sugar beet cultivars, dividing them into two main clusters, two sub-clusters in one group and four sub-clusters in the second group.

### CONCLUSIONS

Gerogoria-KWS and BTS 2860 cultivars were remarkable by their lowest % disease severity values and observed the of greatest growth, yield, the highest content of both TSS and sucrose concentration unlike for the rest studied cultivars. Start codon and targeted (SCoT) molecular markers where novel molecular markers were highly efficient tool for differentiate between resistant and susceptible sugar beet cultivars for the Cercospora leaf spot disease. Two polymorphic bands; 1056 bp and 1100 bp were produced by SCoT3 and SCoT4 respectively in Gerogoria-Kws and BTS 2860 cultivars. These bands might be used as a molecular marker which used as an indicator resistance cultivar.

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### تعريف المقاومة الوراثية لمرض تبقع الاوراق السيركسبورى فى بعض اصناف بنجر السكر باستخدام الصفات المحصولية والمعلومات الجزيئية لكودونات البدء المستهدفة (SCoTs)

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يعد بنجر السكر من المحاصيل الجذرية التي تزرع بغرض انتاج السكر تجارياً، حيث يتعدى الانتاج العالمى المائة مليون طن سنوياً يستهلكها الانسان في تغذيته كما يعتبر مرض تبقع الاوراق السيركسبورى الذى يسببه فطر سيركوسبورا بيتيكولا *Cercospora beticola* Sacc من الأمراض المدمرة لمحصول بنجر السكر. لتعريف الأصناف ذات الانتاجية العالية و المقاومة للمرض تم الحصول على ستة أصناف من بنجر السكر هم Gergoria-KWS و Zeppelin و Pinte و MK 4199 (Emperor) و LP17B4011 و BTS2860 من معهد المحاصيل السكرية- مركز البحوث الزراعية - الجيزة - مصر ،وزراعتهم بالمرزعة

التجريبية بمحطة بحوث سخا بكفر الشيخ فى موسمى نمو متتاليين 2020/2019 و2021/2020 فى تصميم القطاعات الكاملة العشوائية ذات الثلاثة مكررات. تم فحص الأصناف الستة خلال موسمى الزراعة ثم تم تقدير نسبة شدة الإصابة، قياس الأوزان الغضة و الجافة لكل من المجموع الجذرى و الخضرى و طول و قطر الجذر. كما تم تقدير نسبة محتوى السكر و الذائبات الكلية. تم استخدام تقنية المعلمات الجزيئية PCR كودونات البدئ (SCoT) من خلال عشرة بوادئ متخصصة (primers) ، كما تم اجراء تحليل عنقودى للأصناف لتقدير نسبة التشابه الوراثى فيما بينهم. أظهرت نتائج الصفات المحصولية وجود ثلاثة فئات للأصناف المستخدمة : الفئة الأولى تحتوى على الصنفين Gergoria-KWS و BTS2860 الذين تميزوا بانخفاض نسبة شدة الإصابة فكانت 1-2.6 و 0.83-5 للصنفين على الترتيب فى موسمى النمو كما تميزت نباتاتهم بأوزان عالية للمجموع الجذرى و الخضرى و كذلك جذورها كانت ذات طول و قطر كبيرين وتحتوى على محتوى عالى من السكر و الذائبات الكلية. الفئة الثانية و ضمت الاصناف LP17B4011 و MK 4199 (Emperor) و Pintea تميزوا بارتفاع نسبة شدة الإصابة و كذلك انخفاض فى الصفات المحصولية ونسبة محتوى كل من السكر و الذائبات الكلية. أما الفئة الثالثة فاشتملت على الصنف Zeppelin الذى تميز بقيم معتدلة من شدة الإصابة و محتوى منخفض من السكر و الذائبات الكلية. وفيما يتعلق بنتائج تحليل الـDNA بتقنية SCoT أظهرت أن متوسط نسبة تعدد مظهرى 46.37% و 54 حزمة متعددة الاشكال منهم حزمتين ذات اشكال متعددة بأوزان جزيئية 1052bp و 1100bp ، حيث تم الحصول عليهم من البادئين SCoT3 و SCoT4 على الترتيب فى كلا الصنفين المقاومين للمرض Gergoria-KWS و BTS2860 و لم تظهر فى باقى الاصناف المدروسة وبذلك يمكن اعتبارهم معلمات جزيئية للأصناف المقاومة للمرض. تراوحت قيم نسبة التشابه الوراثى بين الاصناف 0.76-0.92 و تحققت القيمة العالية بين الصنفين Gergoria-KWS و BTS2860 . أشار تقسيم الاصناف الذى بنى على أساس العلاقات الوراثية من خلال التحليل العنقودى الى وجود توافق بين نتائج الصفات المحصولية و النتائج التى تم الحصول عليها من تقنية SCoT markers مما يؤكد فاعلية هذه التقنية فى التمييز بين التراكيب الحساسة و المقاومة لمرض التبغ الاوراق السيركسبورى.