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Evaluation of powdery mildew disease resistance of eight sugar beet varieties using agronomic traits and ISSR markers

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This research aimed to evaluate some sugar beet varieties (*Beta vulgaris* var. *saccharifera*, L.) to powdery mildew disease resistance. Eight sugar beet varieties namely 9K887 (Shantala)-KWS, Allanya-KWS, B8141 (BTS Smart 9175), Melodia, LP17B4011, FD18B4018, ST21801 (Mourad) and Zeppelin were planted under field condition during two seasons 2018/2019 and 2019/2020 at the Experimental Farm, Sakha Agriculture Research Station, Agriculture Research Center (ARC), Egypt. Growth and yield traits for foliage and roots were measured, economic traits; Total soluble solids (T.S.S. %) and sucrose content were estimated**.** Molecular differentiation was carried b**y** using six primers of inter simple sequence repeat (ISSR) markers. Results of studied traits showed significant differences among studied varieties. The varieties of B8141, 9K887 (Shantala) and Allanya had good characters; low percentage of disease severity, high mean values of foliage, root traits and high sucrose content in contrast with Zepplin, St21801, Mourad and FD18B4018 varieties. Results of Inter simple sequence repeat (ISSR) markers detect that technique was effectively differentiative produce 39 bands. Twenty-three bands were polymorphic with an average 58.97% polymorphism percentage. Polymorphic information content (PIC) ranged from 0.545 to 0.885 with an average of 0.772. Resistant varieties; Melodia, B8141, Allanya-KWS and 9K887 Shantala-KWS were distinct by presence of polymorphic band (165bp) produced by HB-8 primer. While sensitive varieties; Zepplin, ST21801, FD18B4018and LP17B4011 were distinct by the presence of two polymorphic bands; (175 and 945bp) produced by 89B primer. The highest value of similarity 0.97 was found between ST21801 and Zepplin. While the lowest value 0.66 was found between B8141 and FD18B4018 varieties. So, Cluster analysis classified the studied varieties into four clusters. There was great harmony between growth and DNA results.

Keywords: Evaluation, economic characters, growth, ISSR, Powdery mildew, Resistance, Sugar beet.

1. Introduction

Sugar beet (*Beta vulgaris* var. *saccharifera*, L.) is considered an important sugar crop which contributes with 40% from the total sugar production in the world. Powdery mildew disease caused by fungus (*Erysiphe betae Jacz*.), is the main foliar disease of sugar beet dispersed in dry and semi dry areas, causing destruction in plant growth and about 30% reduction in sugar production (**Grimmer *et al.*, 2007).** Genetic resistance is the best tool to control the disease dispersion and limit economic damage by reduces the needing of fungicides. The genetic base of the commercial sugar beet varieties has been narrowing for some time, mainly due to the small number of used parents in breeding programs so; The entrance of evaluated wild beet populations is necessary in the breeding programs because they are very rich in genetic terms (allelic richness) and have great ability to make physiological adjustment to changing environments (**Ribeiro *et al.*, 2016**). Recently, the studying of the genetic diversity of planted beet depends on morphological traits and molecular markers help in the management of genetic

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resources (**Abou-Elwafa *et al.*, 2020** and **Galewski** and **Mcgrath 2020**). The differences in the degree of resistance among genotypes become more pronounced when true leaves are formed. Phenotypic evaluations of genotypes were carried out in greenhouse as well as under field conditions with natural exposure to the disease. (J**anssen *et al.*, 2003** and **Taški-Ajduković *et al.*, 2017**). Molecular markers are an efficient means for linking genotypic and phenotypic variations because of they used to define the genetic variation in and among populations through providing precious information about genetic structure and gene flow without any effect of environmental factors (**Andrello *et al.*, 2015** and **Saidin *et al.*, 2016**). Several types of DNA markers have been used in genetic studies of sugar beet in parents' selection which have resistance alleles causing expedite cultivar improvement for disease resistance (**Teh *et al.*, 2017**). Inter simple sequence repeat (ISSR) which is a PCR- based technique that accesses variation in the numerous microsatellite regions (hypervariable), expressed as different variants dispersed into the nuclear genome and aid in characterizing individual loci (**Izzatullayeva *et al.*, 2014**). ISSR markers can be highly reliable for precise identification and genetic distance estimation among sugar beet populations. ISSR fingerprinting map could become a superior method for identifying genotypes of sugar beet. (**Qiaohong *et al.*, 2012** and **El Mouhamady *et al.*, 2021**).

Our study aimed to evaluate the diversity among some sugar beet varieties to identify and classify studied varieties according to their ability of resistance for powdery mildew disease and good economic traits by ISSR molecular markers and agronomic traits.

2. Materials and Methods

The experiment was performed during 2018/2019 and 2019/2020 seasons at the Experimental Farm, Sakha Agriculture Research Station, Agriculture Research Center (ARC), Egypt (30°3′N, 31°3′E) under field conditions. Eight sugar beet varieties namely 9K887 (Shantala)-KWS, Allanya-KWS, B8141 (BTS Smart 9175), Melodia, LP17B4011, FD18B4018, ST21801 (Mourad) and Zeppelin were used and obtained from Sugar Crops Research Institute, ARC, Giza, Egypt. A randomized complete block design (RCBD) with three replications was used. Plot area was 10.8 m^2, including 3 rows of 6.0 m long and 60 cm width, with 20 cm apart between hills. The other agronomic practices were conducted based on ministry of agriculture recommendation Parameters of assessment:

Parameters of assessment:

Visible signs of powdery mildew are the dusty white or whitish mycelium of the fungus on the upper leaf surface and later pale green to yellow leaves:

1- Disease severity of powdery mildew was determined according to **Whitney *et al.*, (1983).**

2- Foliage fresh and dry weights / plant (g), root fresh and dry weights /plant (g), root length and root dimeter/plant (cm) were determined.

3- Total soluble solids (T.S.S. %) was estimated in fresh roots of sugar beet, using hand refractometer according to **Ginnis** (**1982**).

4- Sucrose % (pol %) was polarimaterically determined using the pol method that described in Association of Official Analytical Chemists (**A.O.A.C.; 2005**). sucrose percent was measured by the aid of saccarometer.

Molecular analysis

- DNA isolation and PCR procedures were carried out at the Central Lab, Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Egypt. Young leaf tissues of the eight sugar beet genotypes were used to isolate DNA according to Cetyl trimethyl ammonium bromide (CTAB) method (**Doyle and Doyle, 1987**). Isolated DNA quality was checked on agarose gels (0.8%) and stained by ethidium bromide. A UV spectrophotometer at 260 nm was used to measure DNA quantity. Six ISSR primers (Table1) introduced from Operon Technology, Alameda, U.S.A. were used to amplify the template DNA.

- **The PCR reaction:** dH2O:11.7 µL, dNTP mix: 2.5 µL (2.5 mM each dNTP; Promega), 10X reaction buffer: 3.0 µL, MgCl2 (25 mM): 4.0 µL, ISSR primer: 2.0 µL, Taq DNA polymerase: 0.3 µL (5 U per µL; Promega) and1 µL of template DNA (50 ng per µL): 1 µL.

- **The PCR amplification thermocycle conditions are sequential for ISSR markers:** 94 °C for 3 min; 45 cycles at 92 °C for 1 min, annealing at 38- 44 °C for 1 min and at 72 °C for 2 min; at 72 °C; then one cycle of for 10 min , one cycle; a final hold at 4 °C. For each primer, the presence and absence bands of DNA for each genotype were scored in a binary matrix with (1) and (0) respectively. Genetic similarity: calculated according to **Dice (1945)**. A dendrogram was constructed according to (**Rohlf**, **2000)** based on values of similarity using NTSYS-pc version 2.11T. polymorphic information content (PIC) and Heterozygosity are calculated according to the formula **He** or **PIC** = 1- $\sum$ $pi^2$where pi is the frequency of the i*th* allele, and summation extends over n alleles **(Nei 1973)** and (**Weber, 1990**).

Table 1. List of ISSR primers and their nucleotide sequences.

Primer Name	Sequences
89B	5` CAC ACA CAC ACA GT 3`
89A	5` CAC ACA CAC ACA CA 3`
HB-8	5´ GAG AGA GAG AGA GG 3`
HB-10	5` GAG AGA GAG AGA CC 3`
HB-11	5` GTG TGT GTG TGT TGT CC 3`
HB-12	5` CAC CAC CAC GC 3`

Statistical analysis:

Statistical software package (MSTAT-C program version 2.10) was used to analyze the obtained data Anonymous, (1991). Analysis of variance (ANOVA) and comparisons among means were achieved according to **Duncan** (**1995**).

3. Results and Discussion:

Mildew disease is a primarily disease of young and actively growing tissue. Results showed that the range of disease severity percent was 0.00 to 15% in first season and 1 to 41% in the second season (Table 2). Powdery mildew disease is characterized by white dust-like colonies that grow over the surface of leaf. There is partial resistance in a wide range of varieties operates by slowing infection and appears to be under polygenic control (**Janssen *et al.*, 2003** and **Luterbacher *et al.*, 2004).**

Table 2. Disease severity (%) of powdery mildew for the eight studied sugar beet varieties during two growing seasons.

Varieties	Disease severity (%)	
	2018/2019	2019/2020
9K887(Shantala)-KWS	0.00 c	1.33 b
Allanya -KWS	0.00 c	2.00 b
B8141(BTS Smart 9175)	0.00 c	1.00 b
Melodia	0.00 c	3.67 b
LP17B4011	0.33 c	20.00 ab
FD18B4018	6.67 b	38.33 a
ST21801 (Mourad)	1.33 bc	41.67 a
Zeppelin	15.00 a	39.00 a
L.S.D	5.45	24.66

The varieties of B8141 (BTS Smart 9175), 9K887 (Shantala)-KWS and Allanya-KWS and Melodia had the lowest percent so would be used as sources for resistance. The resistance to powdery mildew is inherited as a single, dominant, major gene (**Lewellen** and **Schrandt** 2001). While in both seasons; some varieties had high percent of disease severity such as Zepplin, ST21801 (Mourad) and FD18B4018.

The heaviest fresh weight of foliage found in the varieties of Zeppelin, 9K887(Shantala)-KWS and Melodia (583.0 and 561.66); (589.66 and 530.00) and (533.00 and 552.33) in 2018/2019 and 2019/2020 seasons respectively **(Fig. 1).**

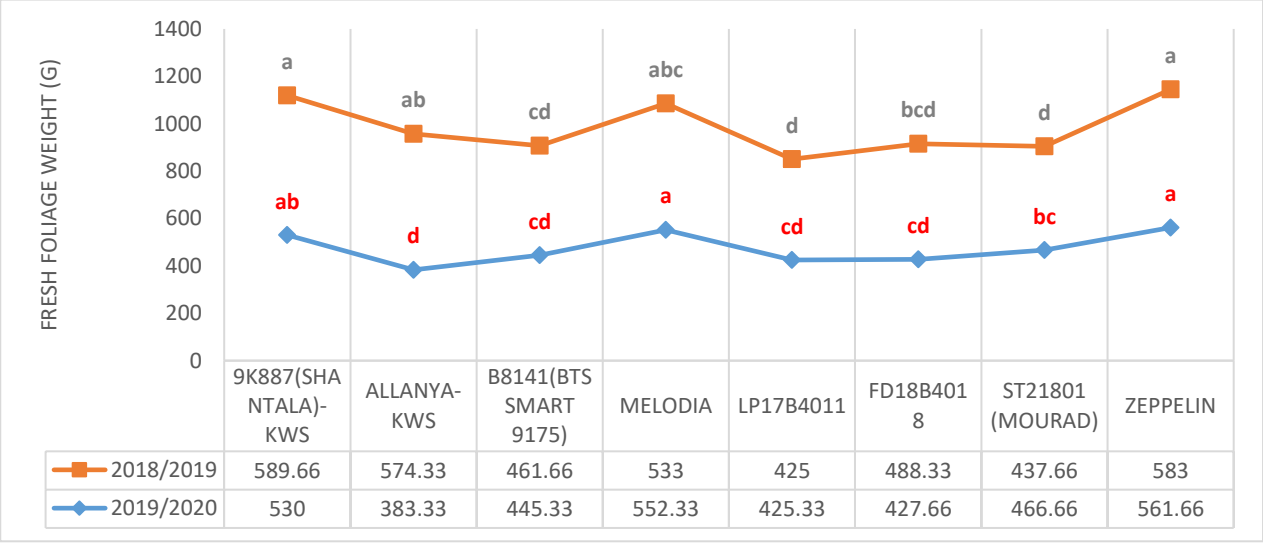
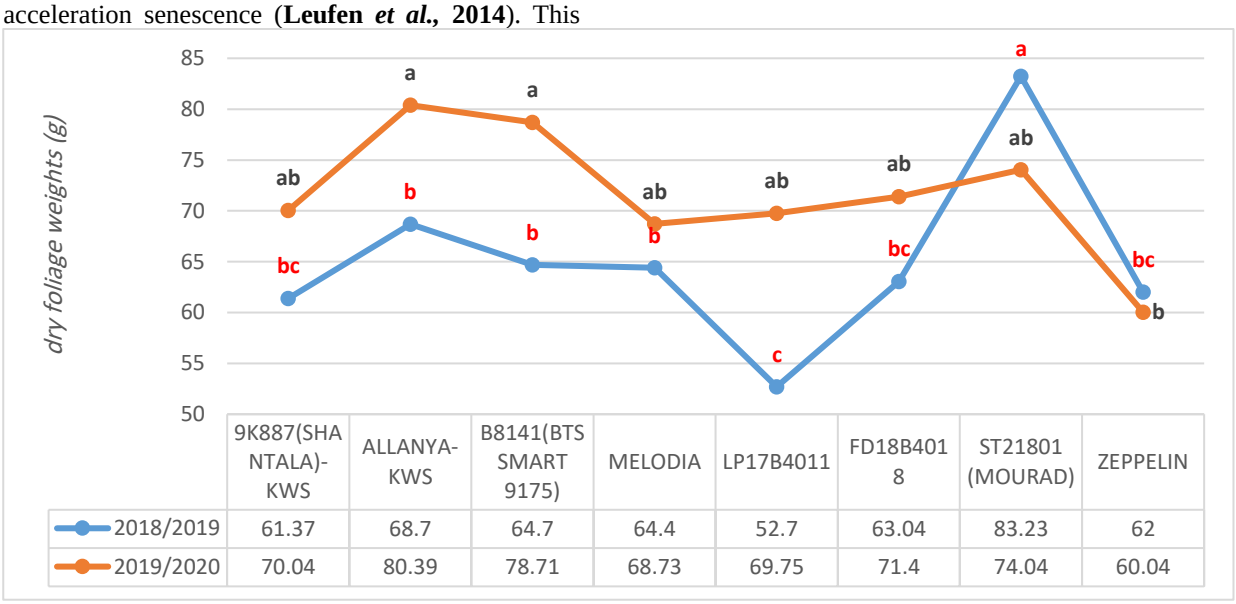


Fig. 2: Mean values of foliage fresh weight (g) for eight sugar beet genotypes during two growing seasons.

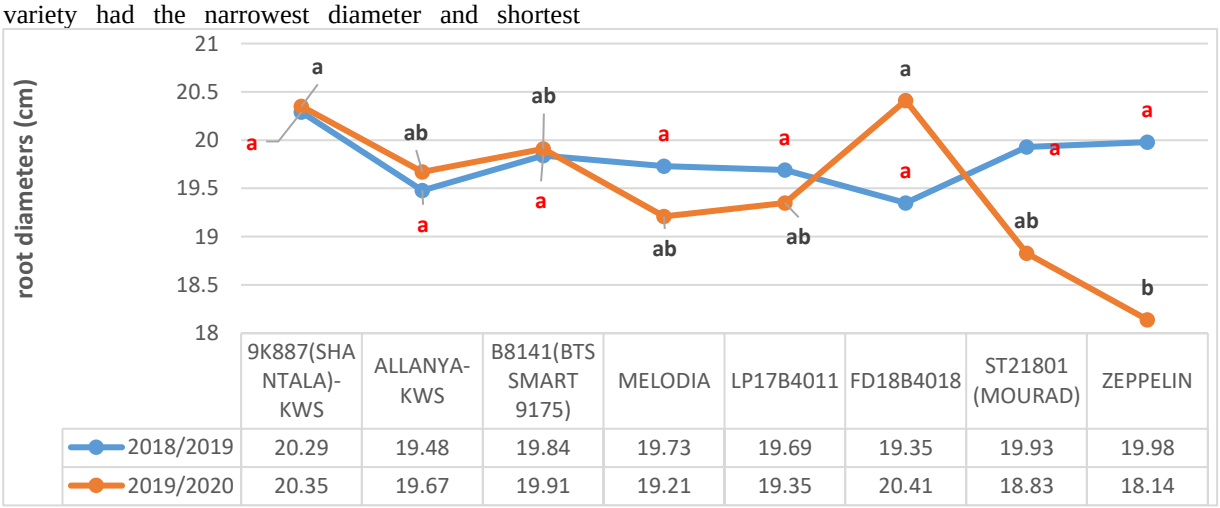
While the varieties of FD18B4018, B8141 (BTS Smart 9175) and LP17B4011 had low fresh weight of foliage (488.33 and 427.66) (461.66 and 445.33) and (425.0 and 425.33) in the first and second seasons respectively. The decreasing in weight of sensitive varieties might due to mildewed sugar beet leaves have great reduction in net photosynthesis as a result of the affected mesophyll conductance and acceleration senescence (**Leufen *et al.*, 2014**). This reduction caused limits the energy transfer between the photosystem II and I, and changing in the intensity of ChlF (**Bürling *et al.*, 2011**). varieties had high fresh weight showed decreasing in dry weight such as LP17B4011, 9K887(Shantala)-KWS and Zepplin in the first growing season **(Fig. 3).** While, the ST21801 (Mourad) variety had the highest dry weight.



	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	61.37	68.7	64.7	64.4	52.7	63.04	83.23	62
2019/2020	70.04	80.39	78.71	68.73	69.75	71.4	74.04	60.04

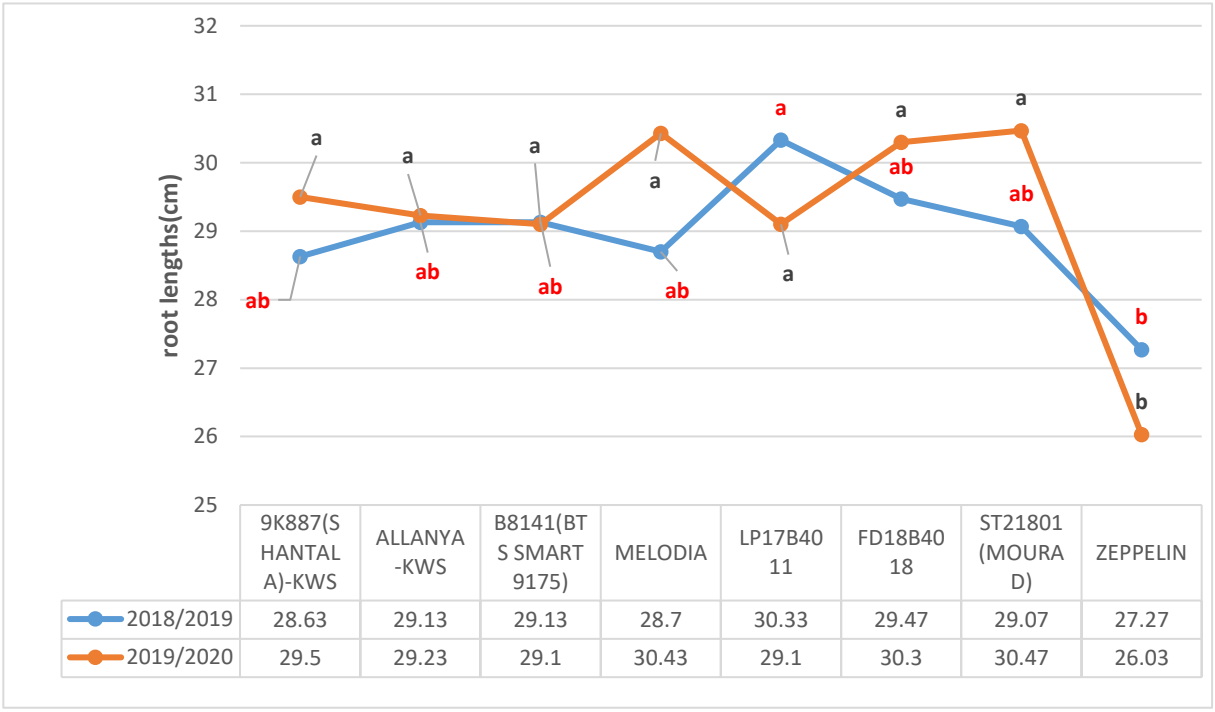
Fig. 4. Mean values of foliage dry weights (g) for eight sugar beet genotypes during two growing seasons.

Root characters; diameter, length, fresh and dry weight were estimated for all the studied varieties of sugar beet and presented as mean values in figures **(3, 4, 5 and 6).** Results of diameter were showed insignificant differences in the first season, but there were significant differences in the second season while length of root trait showed significant difference in the both seasons. The varieties of FD18B4018 and 9K887 (Shantala)-KWS had the highest mean values of root diameter while Zeppelin variety had the narrowest diameter and shortest length of root. Plants of LP17B4011 had the longest root; in the first season, comparing with the other varieties. Fresh and dry weight of root appeared significant difference among the studied varieties **(Fig. 5, 6);** The varieties of B8141 (BTS Smart 9175) and Allanya- KWS had the heaviest roots. Although FD18B4018 and LP17B4011 varieties had low fresh weight of root they had high mean value of root dry weight.



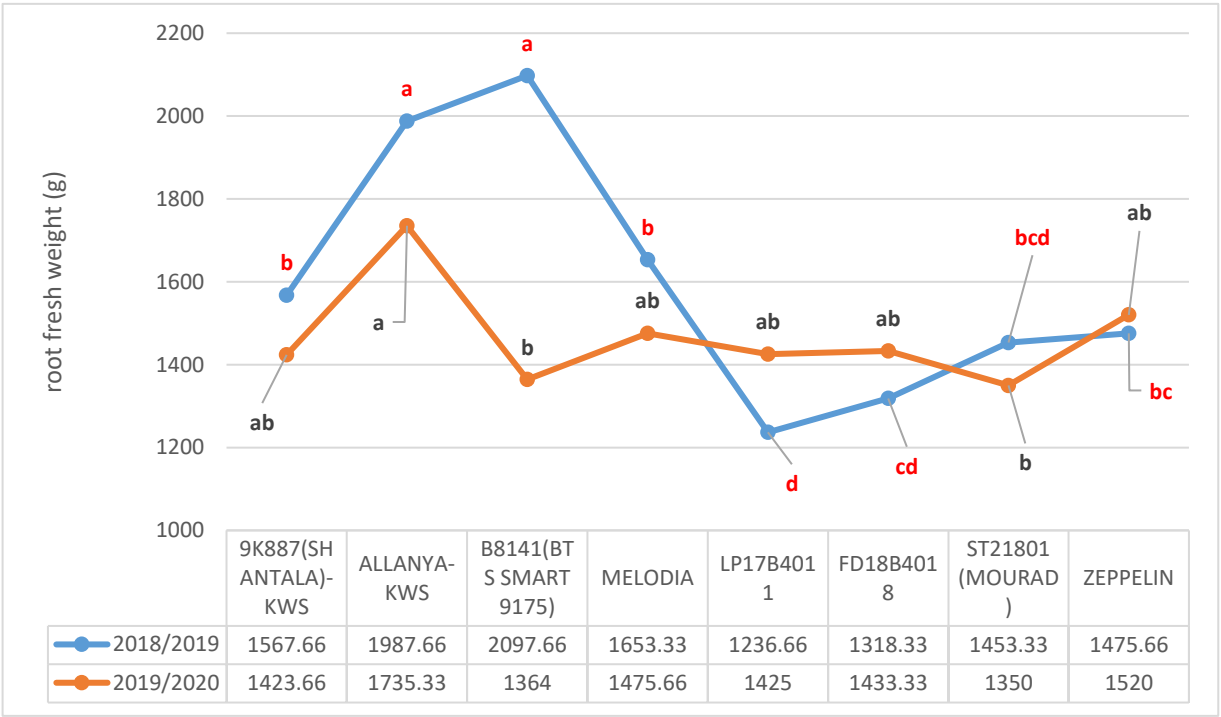
	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	20.29	19.48	19.84	19.73	19.69	19.35	19.93	19.98
2019/2020	20.35	19.67	19.91	19.21	19.35	20.41	18.83	18.14

Fig. 5. Mean values of root diameters (cm) for eight sugar beet genotypes during two growing seasons.



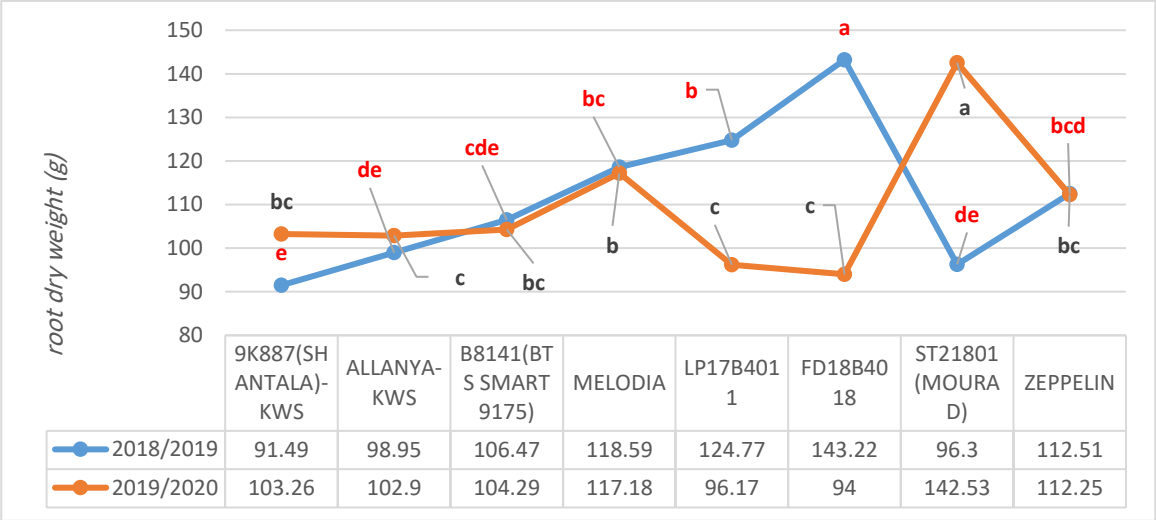
	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	28.63	29.13	29.13	28.7	30.33	29.47	29.07	27.27
2019/2020	29.5	29.23	29.1	30.43	29.1	30.3	30.47	26.03

Fig. 6. Mean values of root lengths (cm) for eight sugar beet genotypes during two growing seasons.



	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	1567.66	1987.66	2097.66	1653.33	1236.66	1318.33	1453.33	1475.66
2019/2020	1423.66	1735.33	1364	1475.66	1425	1433.33	1350	1520

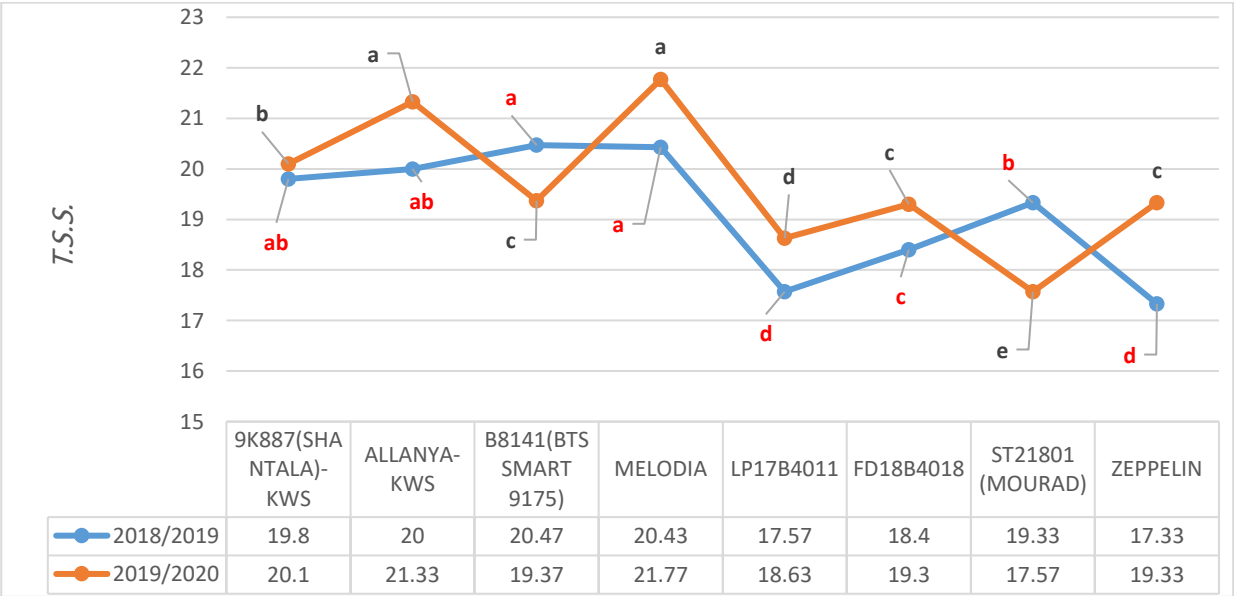
Fig. 7. Mean values of root fresh weight (g) for eight sugar beet genotypes during two growing seasons.



	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	91.49	98.95	106.47	118.59	124.77	143.22	96.3	112.51
2019/2020	103.26	102.9	104.29	117.18	96.17	94	142.53	112.25

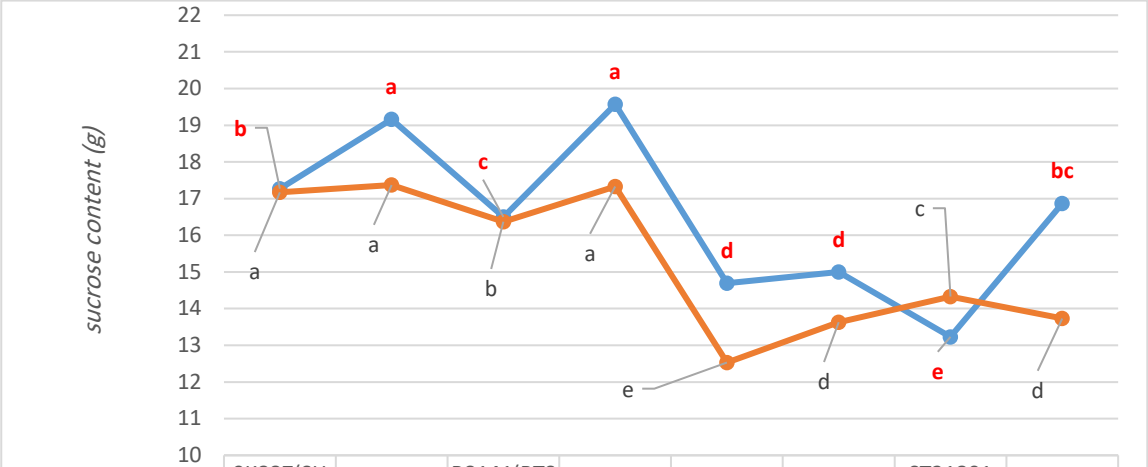
Fig. 8. Mean values of root dry weight (g) for eight sugar beet genotypes during two growing seasons.

Mean values of T.S.S. and sucrose content for the studied varieties are presented in **Fig. (7and 8).** Results showed significant difference among all the studied sugar beet varieties in the two seasons. Plants of Melodia, B8141(BTS Smart 9175), Allanya-KWS, 9K887 (Shantala) -KWS varieties had high content of T.S.S. and sucrose as well as low percent of disease severity which induced increasing in yield components traits such as weight and diameter of root, sugar content, T.S.S., and decreasing in the impurities. On the other hand, the varieties of Zeppelin, ST21801 (Mourad), FD18B4018 and LP17B4011 had low content. T.S.S. and sugar as a result of their sensitivity for this disease. The severe infection of the powdery mildew diseases causes abrupt root yield decrease; the juice purity and sugar content are negatively significant affected (**Gado 2013** and **Kikindonov *et al.*, 2016**). might due to the infection caused photosynthetic CO2 assimilation inhibition and alternate products that reduced the ability to form sucrose in infected plants. These changes are related with abnormalities in ultrastructure of chloroplast and decreasing in the enzyme's activity causing increasing in the organic acids production. Sucrose content is economic character, the aim of any breeding program and considered the net result of several process of metabolism related with the resistance ability of the genotype to any disease such as powdery mildew. Results illustrate great relationship between foliage and root weight of plant and all metabolism process which influence on net quality and quantity of yield.



	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	19.8	20	20.47	20.43	17.57	18.4	19.33	17.33
2019/2020	20.1	21.33	19.37	21.77	18.63	19.3	17.57	19.33

Fig. 9. Mean values of T.S.S. for eight sugar beet genotypes during two growing seasons.



	9K887(SHANTALA)-KWS	ALLANYA-KWS	B8141(BTS SMART 9175)	MELODIA	LP17B4011	FD18B4018	ST21801 (MOURAD)	ZEPPELIN
2018/2019	17.27	19.16	16.5	19.57	14.69	15	13.23	16.87
2019/2020	17.17	17.37	16.37	17.33	12.53	13.63	14.33	13.73

Fig. 10. Mean values of sucrose content (g) for eight sugar beet genotypes during two growing seasons.

DNA analysis:

Six ISSR primers generated bright amplified bands and polymorphisms **(Fig.9).** A total of 39 reliable fragments were obtained among eight sugar beet varieties **(Table3).** Bands number / primer ranged from 5 (89A, HB-11 and HB12 primers) to 9 (HB-8 primer) with an average number 6.5 bands. There are twenty-three polymorphic bands and polymorphism percent ranged from 40 to 71.42% / primer with an average 58.97%. polymorphic information content (PIC) ranged from 0.545 to 0.885 with an average of 0.772. Allele size ranged from 165 to 1185 bp and heterozygosity per locus ranged from 0.542 to 0.875. The obtained polymorphism percent was close to the percent was obtained by **El-Mouhamady *et al.*, (2021)** 72.18% among eight sugar beet varieties through using ISSR primers. While it was less than the percent that obtained by **Liu *et al.*,** (**2012a** and **2012b**) who recorded 86.3% and 87.5% polymorphism percent by six ISSR primers among ten and thirteen cultivars of sugar beet respectively. While **Izzatullayeva *et al.*,** (**2014**) found 97.2% polymorphism percent by 12 primes of ISSR markers in 42 sugar beet accessions. These differences in the percent of polymorphism might due to the number and sequences of primers in addition to the used sugar beet varieties in each study. The varieties of 9K887(Shantal)-KWS, Allanya-KWS, B8141 (BTS Smart 9175) and Melodia were distinct by the presence of the polymorphic band 165bp that produced by HB-8 primer and these varieties had heavy weight of root and foliage, high content of T.S.S and sugar which caused increasing in resistance for powdery mildew disease. On the other hand, Zepplin and ST21801 (Mourad) varieties were distinct from the other six studied varieties by presence of two polymorphic bands with molecular size of 175 and 945bp band that generated by 89B primer and the absence of these two bands (175 and 945 bp) from the other six genotypes might be related with more decreasing in traits of roots, foliage, T.S.S. and sugar content consequently the sensitivity to powdery mildew disease. Eight negative unique bands (1, 3, 3 and 1) were obtained from primers;89B, HB-8, HB-10 and HB-11 respectively. These unique markers can be used at the molecular level to identification sugar beet varieties. B8141 (BTS Smart 9175) variety had five unique negative bands; 520bp (89B primer), 860bp (HB-8 primer),1185,865bp (HB-10 primer) and 615bp (HB-11 primer). This genotype distinguished with high performance in growth, yield and resistance to powdery mildew disease.

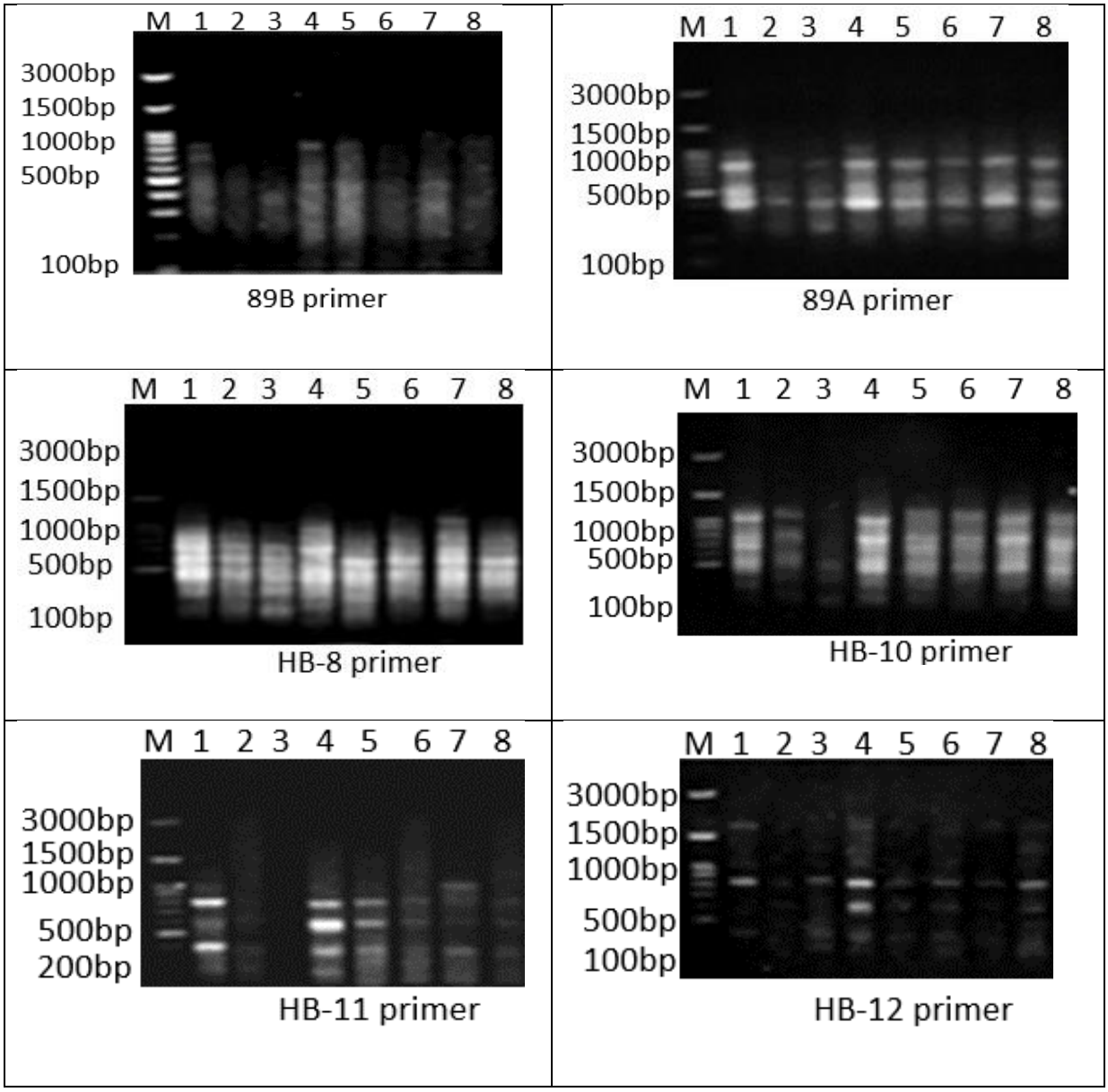


Fig. 9. The ISSR amplification profile of primers 89B, 89A, HB-8, HB-10, HB-11 and HB-12. M: Marker, 1: 9K887 (Shantala)-KWS, 2: Allanya-KWS, 3: B8141 (BTS Smart9175), 4: Melodia, 5: LP17B4011, 6: FD18B4018, 7: ST21801 (Mourad) and 8: Zepplin

Table 3. Total number of monomorphic, polymorphic and unique bands generated by six ISSR primers using the eight sugar beet genotypes, as well as percentage of polymorphism, heterozygosity per locus and polymorphic content information for each primer.

Primer	Total band	Monomorphic Band	Polymorphic band	Unique band	Polymorphism (%)	Alleles size range	Heterozygosity per locus	Polymorphic information content (PIC)
89B	8	3	5	1	62.50	175-945	0.542	0.545
89A	5	3	2	-	40.00	270-815	0.775	0.779
HB-8	9	3	6	3	66.66	165-860	0.875	0.885
HB-10	7	2	5	3	71.42	185-1185	0.847	0.847
HB-11	5	2	3	1	60.00	230-930	0.76	0.783
HB-12	5	3	2	-	40.00	415-815	0.785	0.793
Total	39	16	23	8				
Average					58.97			0.772

These results were in agreement with **Capistrano-Gossmann *et al.,* (2017)** and **Khatab *et al.*, (2019).** Moreover, **Qiaohong *et al.,* (2012)** and **El-Mouhamady *et al.,* (2021)** considered fifteen positive unique bands specific markers which were generated by ISSR primers for dividing taxonomic differences in sugar beet. Genetic similarity values among the eight studied sugar beet varieties ranged from 0.667 to 0.971 **(Table 4).**

Table 4. values of genetic similarity among the eight sugar beet genotypes.

	9K887 (Shantala)	Allanya-KWS	B8141 (BTS Smart9175)	Melodia	LP17B4011	FD18B4018	ST21801 (Mourad)	Zepplin
9K887 (Shantala)	1							
Allanya-KWS	0.82759	1						
B8141(BTS Smart 9175)	0.7451	0.8	1					
Melodia	0.90625	0.82759	0.70588	1				
LP17B4011	0.90625	0.82759	0.70588	0.90625	1			
FD18B4018	0.81967	0.8	0.66667	0.88525	0.88525	1		
ST21801(Mourad)	0.87879	0.8	0.67925	0.90909	0.87879	0.88889	1	
Zepplin	0.90909	0.8	0.67925	0.93939	0.87879	0.88889	0.97059	1

Values differed according to the degree of similarity between varieties. The highest value of similarity was 0.97 which were observed between ST21801 (Mourad) and Zepplin. These results were in harmony with results of growth, yield as these varieties were sensitive to powdery mildew disease. The lowest value of genetic similarity (0.667) was found between B8141 (BTS Smart 9175) FD18B4018 varieties. These values of similarity were higher than that obtained by **Liu (2012a), Izzatullayeva *et al.,* (2014)** and **El-Mouhamady *et al.,* (2021)** who found the range of similarity values were 0.43 to 0.83, 0.146–0.721 and 0.4 to 0.867 respectively among varieties.

The cluster analysis or phylogenetic tree divided all studied of sugar beet varieties into four clusters I, II, III and IV **(Fig.10)** the first cluster (I) contain two sub clusters one of them had three varieties two of them; ST21801 and Zepplin were sensitive to powdery mildew disease while the other one; Melodia is semi resistance to disease. The second sub cluster contain two varieties LP17B4011 which is sensitive and 9K887(Shantal)-KWS which is resistance to powdery mildew. Results illustrated that there was similar stock of these varieties although there was difference among them in some traits. On the other hand, there were three clusters each of them contains one variety and two of them were resistance to powdery mildew each cluster was differed. These results were in agreement with **Liu *et al.,* (2012a)** who reported that ISSR markers could be used as good tools to obvious the genetic distance among populations and moreover the dendrogram was illustrate the population character. **El-Mouhamady *et al.,* (2021)** found great divergent and genetic different between two clusters of beet genotypes. Differences were considered great chance to improve yield production of sugar beet in Egypt by insertion foreign genes through different methods of plant breeding. According to the genetic distance; distantly varieties are selected in breeding programs to improve sugar beet crop. (**Abou Elwafa *et al.,* 2020** and **Mehareb *et al*., 2021**).

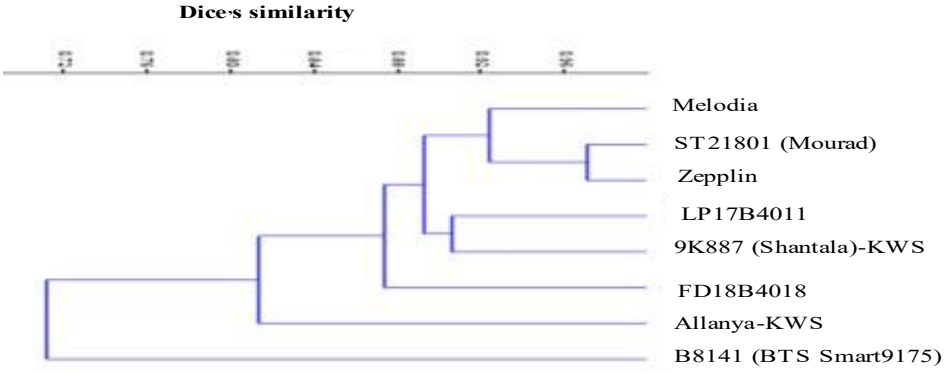


Fig. 10: Dendrogram of eight sugar beet genotypes based on data of the six ISSR primers

Conclusion

The increasing of T.S.S. and sucrose content were associated with decreasing of the degree of disease severity. ISSR technique was considered as a sensitive method to differentiate sugar beet varieties. The cluster analysis separated each resistant variety as single cluster. 9K887(Shantal)-KWS, B8141 and Allanya-KWS varieties are the best choice of plant breeders as they showed low percent of disease severity of powdery mildew as well as high performance of growth and yield characters.

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