



Screening for drought tolerance and molecular variability among some sugar beet cultivars

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Abstract

Drought is one of the significant abiotic stress factors that affect plant development and productivity. Screening and producing of more tolerant genotypes with higher yield capacity is the breeders' principal purpose. Therefore, this work was carried out to evaluate the performance of four sugar beet cultivars (Top, Hosam, Hercule and Kawamera) under water limitation. The drought stress experiment was applied based on three water regimes, 25%, 50% and 75% (severe, moderate and non-stress conditions, respectively) of relative water capacity (RWC). The study incorporated some productivity traits (roots and recoverable sugar yield) and quality parameters (pol%, sugar recovery% and quality index%). The results showed that drought stress has a significant effect on all studied traits. Whereas, increasing of water deficiency led to decrease of the productivity traits and increasing of quality parameters. Kawamera cultivar has superior performance in all the studied traits under all three different levels of water regime. Besides that, the four cultivars were assessed by both simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) molecular markers. SSR marker exhibited a higher polymorphism percentage (71.43%) than ISSR marker (44.26%). In addition, the mean polymorphism information content (PIC) value was higher for the SSR marker (0.25) than the ISSR marker (0.18) too. On the contrary, ISSR revealed a higher range of similarity (0.66-0.85). Moreover, the constructed dendrograms revealed that the SSR marker was able to separate the cultivars in line with according to their drought-tolerance, where the highest drought-tolerant cultivar (Kawamera) was classified alone in the main cluster. However, the superiority of Kawamera cultivar under drought stress indicated that it could be utilized in breeding programs for developing more drought-tolerant sugar beet cultivars.

Keywords: Agronomic traits; Drought; ISSR; Polymorphism; SSR; Sugar beet.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is the second source of sugar worldwide after sugar cane, as well as in Egypt too (CCSC, 2010). It is widely considered a temperate-zone crop, although it is generally spreading to subtropical areas. It has a growth time that is almost half that of sugarcane, but it produces much per unit of time and uses less water (Brar *et al.*, 2015). In Egypt, sugar beet produces about 59 % of overall sugar production (2.25 million tons per year, sugar crops and sugar production in Egypt, Sugar Crops Council Report, 2018). However, the annual sugar production in Egypt is not enough for it consumes and covered it via import from abroad. Sugar beet yield and quality are influenced by various of environmental and agronomic factors

(Wu *et al.*, 2016). Water limitation can affect the growth and activity of storage roots. Egypt suffers from water scarcity and drought, and this affects the productivity of sugar beets. To get the most productivity of sugar beet, the best cultivars for drought stress-tolerant must be chosen (Chaves *et al.*, 2003; Ren *et al.*, 2007; Farooq *et al.*, 2009; Hamed and Emara, 2019).

Screening for drought tolerance and diversity among different cultivars based on morphological characterization is not enough, as these traits are more sensitive to environmental changes (Fufa *et al.*, 2005). Therefore, the DNA molecular markers have been used as an accurate tool that are not influenced by environmental effects (Bachmann *et al.*, 2001; Tatikonda *et al.*, 2009). In addition, they can easily detect polymorphism that may result from nucleotide change or mutation in the genome loci (Hartl and Clark, 1997). However, among many PCR-based molecular markers, Inter Simple

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Sequence Repeat (ISSR), and microsatellites or Simple Sequence Repeat (SSR) have been widely used (Weising *et al.*, 1992; Zietkiewicz *et al.*, 1994; Bashandy and El-Shaieny, 2016; Bashandy *et al.*, 2020). ISSR (dominant) markers can detect multilocus markers along the genome, by using microsatellite sequences as primers. They can amplify different sizes of DNA segments in between SSR sequences. On the other hand, SSRs are co-dominant markers that can detect a variable number of tandem repeats that distribute at high frequency in the nuclear DNA of most organisms (Beckmann and Weber, 1992; Reddy *et al.*, 2002).

The main objective of this work is to evaluate four sugar beet cultivars for their performance under water limitations and assess the molecular variability among them using SSR and ISSR molecular markers.

2. Materials and Methods

2.1. Plant materials and growth conditions

Four multigerm sugar beet cultivars (Top, Hosam, Hercule and Kawamera) were used in this study. They were provided by Sugar Crops Research Institute, Agric. Res. Center, Giza, Egypt. The experiment was carried out during the 2017-2018 and 2018-2019 seasons in a private farm at Abnoub District, Assiut Governorate, Egypt. To mimic the drought stress conditions, three different water

regimes based on 25%, 50% and 75% (severe, moderate and non-stress conditions, respectively) of relative water capacity (RWC) were applied (Hamed and Emara, 2019). Seeds of the cultivars were sown on the 5th and 4th October 2017 and 2018, respectively. The experimental layout was split plot in the randomized complete block design with 6 replications, water regime treatments were assigned to the main plot and the four cultivars were arranged in sub-plots. Each plot included 5 rows of 3.50 m length, 60 cm spacing and 17- 20 cm spacing in rows. At the harvesting time, after removing the 1st and 5th rows of each plot and 0.5 m from both ends of each row, the area of 3 central rows was harvested. The soil has texture silty clay loam (1:2.50 soil: water) with 8.00 pH. Its salinity (ECe) was 1.08 m.mols /cm. To determine the RWC %, the soil was weighed, then dried at 105°C before being weighed again. After all, a Kopecky cylinder (100 cm³) was used to wet the soil to its maximum holding capacity before reweighing it. Relative water capacity was measured according to the formula of $RWC = 100 * (S_n - S_d) / (S_w - S_d)$. Where, S_n = weight of sampled soil, S_d = dry soil weight and S_w = soil with hundred percent RWC weight. The different agricultural practices were applied as commercial sugar beet production. The weather temperature of the two grown years is shown in Fig. (1).

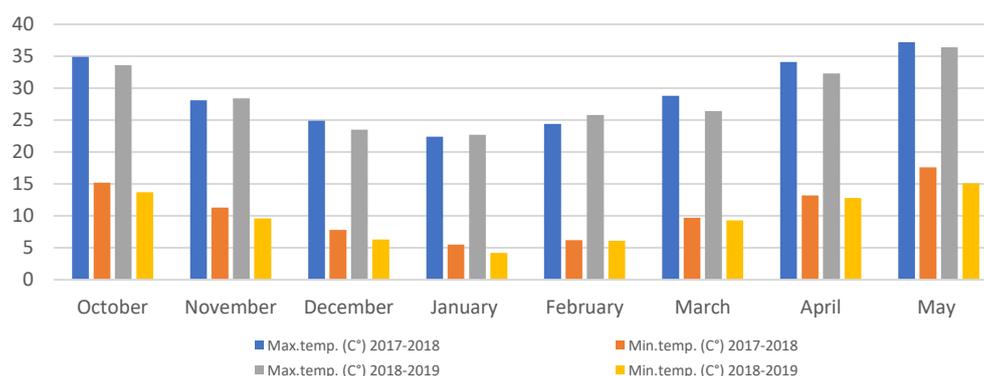


Fig. 1. Weather temperature at the private farm during carrying out experiments on sugar beet cultivars, 2017-2018 and 2018-2019 seasons (Meteorological station, Assiut, Assiut Governorate, Egypt).

At harvesting time (from sowing to harvest, it takes 195 days) a random sample of 12 healthy plants per treatment was harvested. Plants were separated into storage roots and leaves to determine the studied traits as following:

1- Root yield (ton/ hectare): Plants of all ridges from each subplot were harvested, cleaned, topped and weighed after that root yields (ton per hectare) were estimated and calculated for all experimental plots.

2- Recoverable sugar yield per hectare= root yield ton/hectare x recoverable sugar percentage (RS%).

3- Sugar content or pol%, sucrose was determined polarimetrically (ICUMSA, 2007). Sucrose concentrations for the samples obtained were expressed on a fresh weight basis.

4- Sodium, Potassium and Alpha amino nitrogen (millieq/ 100 g beet) were determined by using Analyzer according to A.O.A.C. (2005).

5-Recoverable sugar % (RS%) = Pol% - 0.29 - 0.343 (Na+ K) - 0.094 (alpha-amino-N), according to A.O.A.C. (2005), Where: Pol% = sucrose %, K, Na and α -amino N as millequivalent /100 g beet.

6- Quality index (QI) was calculated using the formula of QI= Sugar recovery % x 100 / pol %.

2.1.1. Statistical analysis

The data was statistically examined using the analysis of variance technique by using MSTAT-C statistical software program, and Fisher's test was used. The differences among treatment means were detected by the Least significant differences (LSD) test at a 5% level of probability and Duncan's letters (Gomez and Gomez, 1984).

2.2. Molecular characterization

2.2.1. DNA extraction

g-DNA was extracted from fresh young leaves of all cultivars using a DNA isolation kit (Favorgen

Biotech Corp. Cat.No. FAPGK001) according to the manufacturer's instructions.

2.2.2. PCR amplification and electrophoresis

PCR amplification of both SSR and ISSR was carried out in a reaction mixture of 25 μ l including 1x PCR buffer, four mM MgCl₂, 0.2 mM dNTPs, 2 μ l of 10 μ M primer in case of ISSR, while 2 μ l of 10 μ M each of forward and reverse primers for SSR marker, 2 units of Taq DNA polymerase and 2 μ l DNA (50 ng). In a thermal cycler (Labocon, U.K.) the reactions were carried out. Six SSR primers and Eight ISSR primers had been employed (Table 1), they were purchased from EZBiolab, USA. PCR amplifications were performed under control of program containing the preliminary denaturation for 5 min at 94°C, then 38 cycles: denaturation at 94°C for 1min, annealing temperature depending on the annealing temperature of each ISSR primer or the pair of SSR primers for 1min, extension at 72°C for 2min, with a final elongation at 72°C for 7min. Separation of PCR products was done using 1.5% and 2.5% agarose gels for ISSR and SSR markers, respectively at 5 V/cm. The 1xTBE (Tris-Borate-EDTA) running buffer was used. After that, gels were stained with ethidium bromide to make them visible.

Table 1. Primers ID and sequences of both SSR and ISSR used for molecular analysis of 4 sugar beet cultivars.

SSR primers	Sequence (5' to 3')	ISSR primers	Sequence (5' to 3')
Unigene24552	F:AACATCTCACTCATCCTTCTTC R:ATGATAGCAAACGACTAGCAG	UBC 807	(AG) ₈ T
Unigene16898	F:AGAACTTAGATTGTGACCTGCT R:GATGGGAAGAGAGAGATTAGTG	UBC 811	(GA) ₈ C
Unigene72402	F:TTAAGTACCAACTTCCAACAGC R:GCTGGCTAACGACATAAATTC	UBC 812	(GA) ₈ A
Unigene26319	F:CAGAATACACTTGGTGAGATGA R:TACTATGTTGTGCTGCTGTG	UBC 815	(CT) ₈ G
Unigene48657	F:TAAC TAAGGTTGGTGGAACA R:CTCTCATTCTCCCTATCTCTC	UBC 823	(TC) ₈ C
Unigene14118	F:AAGTCTAACACCAGAATCCAGA R:AACCAGAGAGAATATGAGGATG	UBC 826	(AC) ₈ C
		UBC 834	(AG) ₈ TT
		UBC 846	(CA) ₈ GT

Polymorphic information content (PIC) was determined using the formula of $1-p^2-q^2$ (Ghislain *et al.*, 1999), where p and q are the frequency of the present and the absent bands, respectively.

Each primer's resolving power (Rp) was estimated using the formula: $Rp = \sum Ib$ (band informativeness) according to Prevost and Wilkinson (1999). Whereas, the formula of $Ib = 1 - (2 \times |0.5 - p|)$ was used to calculate Ib, where p denotes the proportion of cultivars that have the band. Marker index (MI) was estimated according to Powell *et al.* (1996).

2.2.3. Data analysis

The identified bands were given a value of 1 (present) or 0 (absent). Genetic similarity was estimated using similarity coefficient of Jaccard (Jaccard, 1908). The unweighted pair group technique with arithmetic average (UPGMA) was used to create a dendrogram according to the matrix of similarity data, cluster analysis was performed using the software computational package MVSP 3.1.

3. Results and Discussion

3.1. Effect of drought stress on the productivity traits

Drought stress had a significant effect on the root and recoverable sugar yield, and they varied significantly among the cultivars during both seasons (Table 2). Concerning the root yield, under non-stress conditions, Kawamera had the highest value in the 1st and 2nd seasons (81.99 and 82.78 tons/hectare, respectively), while Hosam had the lowest values (68.24 and 69.83 tons/hectare, respectively). Also, Kawamera was superior over all the cultivars under moderate and severe stress conditions in both seasons. In the 1st season, it scored values of 69.35 and 54.74 tons/hectare, respectively, while in the 2nd season it had values of 70.95 and 57.31 tons/hectare, respectively. Drought stress had an antagonistic effect on the root yield of all the studied cultivars. Whereas the mean of root yield decreased with the increase of the drought level. This result is consistent with previous findings of Jozi and Zare Abyane (2015), Moosavi *et al.* (2017), Hamed and Emara (2019) and Khozaei *et al.* (2020). The reduction in root yield may be due to that drought stress can affect nutrient uptake that impairs leaves' growth and development and photosynthetic efficiency that ultimately leads to a decrease in yield (Khazaie *et al.*, 2007).

Table 2. Mean performance of root yield and sugar yield traits of the studied 4 sugar beet cultivars under the studied 3 water regimes during the two seasons.

Cultivar	Root yield (ton/hectare)			Sugar yield (ton/hectare)		
	Normal irrigation	Moderate stress	Severe stress	Normal irrigation	Moderate stress	Severe stress
2017/2018 season						
Top	74.42 c	68.86 a	48.22 c	10.88 b	10.80 b	7.81 b
Hosam	68.24 d	60.81 c	45.22 d	9.50 c	8.42 c	6.95 c
Hercule	77.78 b	63.67 b	51.24 b	10.72 b	9.45 c	7.82 b
Kawamera	81.99 a	69.35 a	54.74 a	12.92 a	11.50 a	9.24 a
F value	**	**	**	**	**	**
LSD 0.05	0.56	1.00	1.25	0.34	0.15	0.20
2018 / 2019 season						
Top	76.30 c	69.62 b	50.29 c	11.76 b	10.36 b	7.68 b
Hosam	69.83 d	64.14 d	46.81 d	9.89 c	8.71 d	6.64 c
Hercule	80.63 b	65.89 c	52.74 b	11.43 b	9.12 c	7.46 b
Kawamera	82.78 a	70.95 a	57.31 a	13.14 a	10.76 a	9.23 a
F value	**	**	**	**	**	**
LSD 0.05	1.27	1.27	1.64	0.40	0.19	0.23

** highly significant at 0.01 levels of probability; the different letters (in the same column) represent statistically significant differences between treatments ($p < 0.05$).

As shown in Table (2) the sugar yield trait differed significantly among the different levels of irrigation and the cultivars in the two seasons. In the first season of evaluation, Kawamera was the best one under normal, moderate and severe stress conditions, where scored the values of 12.92, 11.50

and 9.24 tons/hectare, respectively. Hosam cultivar had the lowest values of 9.50, 8.42 and 6.95 tons/hectare, respectively. In the second season, Kawamera and Hosam cultivars had the highest and the lowest value, respectively in all applied conditions. The superiority of Kawamera cultivar

may be due to it had the highest values of both root yield and sugar recovery %. The sugar yield trait was affected by the different levels of irrigation which agrees with the results of Mahmoodi *et al.* (2015). Nourjou (2008) reported that the decreasing of sugar yield resulted from decreasing in irrigation amount may be attributed to translocated metabolic products from leaves to root. On the contrary, Foroozesh *et al.* (2012) found that this trait did not differ significantly between different irrigation levels (normal and stress).

3.2. Effect of drought stress on the quality parameters

The pol% varied significantly among the cultivars under both stressed and non-stressed conditions (Table 3). In the first season of evaluation, Hosam cultivar had the lowest values of 16.10, 16.75 and 17.51% under normal, moderate and severe stress conditions, respectively. Whereas, the highest values (17.99, 18.71% and 19.05) were recorded for Kawamera cultivar under normal, moderate and severe stress conditions, respectively. Moreover, Hosam and Kawamera cultivars had the lowest and the highest values, respectively in the second season.

Concerning sugar recovery % trait, under normal conditions, its values ranged from 13.78% for Hercule to 15.75% for Kawamera cultivar in the first season (Table 3). Whereas, under moderate and

severe stress conditions, Kawamera cultivar had the highest values (16.58 and 16.89%, respectively). In the second season, Kawamera cultivar had the highest values (15.17, 16.12 and 15.87%) under normal, moderate and severe stress conditions, respectively.

For the quality index trait in the first season, Kawamera cultivar recorded the highest value (87.55%) under normal conditions and high significant than other genotypes, but the differences among the other cultivars were not significant. Moreover, Kawamera cultivar had the highest values (88.60 and 88.65%) under moderate and severe stress conditions, respectively, while Hosam produced the lowest value of 85.16% under moderate stress conditions. Whereas, in the second season the highest values (87.67 and 88.17%) under both the normal and moderate stress conditions, respectively was also obtained by Kawamera cultivar, while Hosam cultivar had the lowest values of 84.45 and 85.75%, respectively. Under severe stress, the differences among all cultivars were not significant.

Based on all the above results, drought stress had a significant effect on sugar content, sugar recovery and quality index. All these traits considerably increased as water deficiency increased. Similar findings were reported by Mahmoodi *et al.* (2015) and Hamed and Emara (2019).

Table 3. Mean performance of pol%, sugar recovery% and quality index traits of the studied 4 sugar beet cultivars under the studied 3 water regimes during the two seasons.

Cultivar	Pol%			Sugar recovery%			Quality index%		
	Normal irrigation	Moderate stress	Severe stress	Normal irrigation	Moderate stress	Severe stress	Normal irrigation	Moderate stress	Severe stress
2017/2018 season									
Top	17.19 b	17.92 b	18.58 b	14.62 b	15.69 b	16.21 b	85.03 b	87.54 b	87.23 b
Hosam	16.10 d	16.75 d	17.51 d	13.92 c	14.34 d	15.36 c	85.12 b	85.16 c	86.85 c
Hercule	16.60 c	16.92 c	17.77 c	13.78 c	14.84 c	15.26 c	85.11 b	87.71 b	86.69 c
Kawamera	17.99 a	18.71 a	19.05 a	15.75 a	16.58 a	16.89 a	87.55 a	88.60 a	88.65 a
F value	**	**	**	**	**	**	**	**	**
LSD 0.05	0.26	0.10	0.16	0.36	0.14	0.18	1.08	0.37	0.36
2018 / 2019 season									
Top	17.12 b	17.65 b	17.67 b	14.89 b	15.28 b	15.41 b	86.96 b	86.55 b	87.24
Hosam	16.09 d	16.54 d	16.52 d	13.58 d	14.18 c	14.17 b	84.45 c	85.75 c	85.34
Hercule	16.28 c	16.66 c	16.59 c	14.01 c	14.14 c	14.18 b	87.07 b	85.96 c	85.47
Kawamera	17.30 a	18.28 a	18.11 a	15.17 a	16.12 a	15.87 a	87.67 a	88.17 a	87.63
F value	**	**	**	**	**	**	**	**	ns
LSD 0.05	0.13	0.10	0.04	0.14	0.14	0.48	0.36	0.39	-

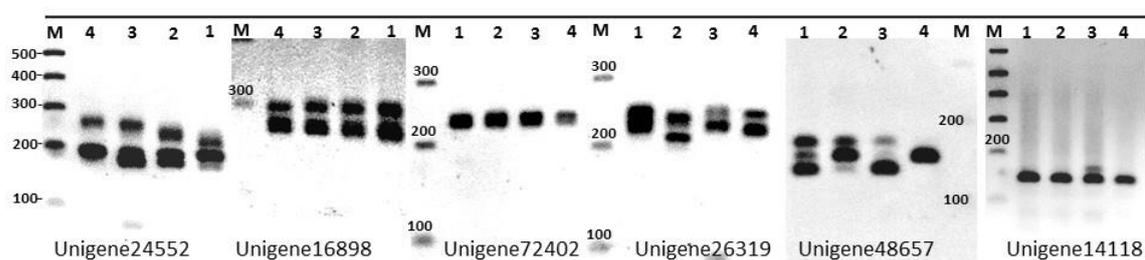
** and ^{ns} highly significant and non-significant at 0.01 and 0.05 levels of probability, respectively; the different letters (in the same column) represent statistically significant differences between treatments ($p < 0.05$).

3.3. SSR and ISSR marker analysis

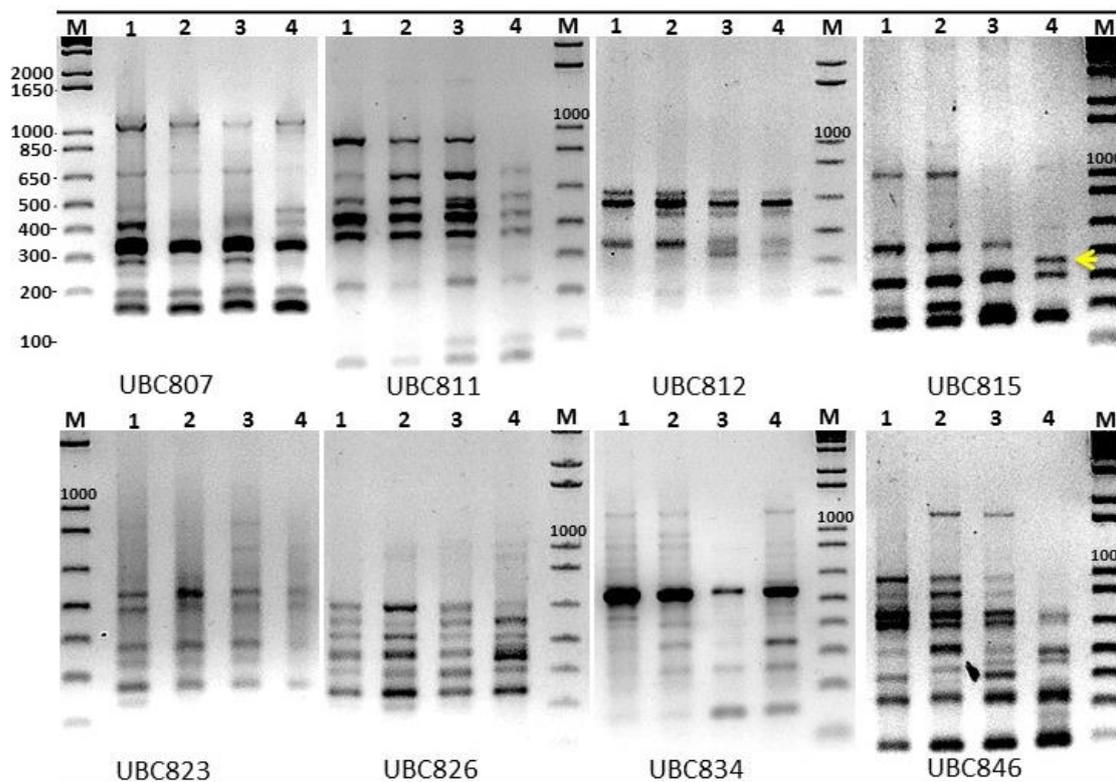
3.3.1. Polymorphism and variability evaluation

To study genetic diversity and relationship between the four different cultivars of sugar beet, two types of DNA molecular markers (SSR and ISSR) were utilized. Six SSR markers and eight ISSR markers were employed in the PCR amplifications (Fig. 2). Concerning the SSR marker, the sum of detected alleles varied from one primer to another. It was fluctuated from 2 alleles for both Unigene72402 and Unigene14118 primers to six for Unigene24552 primer (Table 4). A total of 21 bands were generated

having sizes ranged from 130 bp to 293 bp. Truly, 15 bands were polymorphic, with 71.43% polymorphism. However, ISSR markers produced 61 fragments with sizes varied from 125 bp to 1800 bp. Among them, 27 bands were polymorphic. Therefore, the polymorphism percentage was 44.26%. additionally, UBC815 primer was able to detect a unique band at size of 460 bp that only present in Hosam cultivar (the worst cultivar for most the evaluated traits). This band may be a negative specific band associated with drought tolerance.



a) SSR



b) ISSR

Fig. 2. SSR (a) and ISSR (b) pattern in the four sugar beet cultivars. M, kbp DNA marker; 1, Top; 2, Kawamera; 3, Hercule; 4, Hosam cultivar, yellow arrow shows a negative drought tolerance associated band.

Many researchers have used SSR and ISSR markers to detect the genetic diversity in sugar beet

(Izzatullayeva *et al.*, 2014, Abbasi *et al.*, 2015, Taški-Ajduković *et al.*, 2017 and Bogacheva *et al.*, 2019). As expected, the ISSR marker showed higher

both of average and the total number of alleles, which is in agreement with the results of Rawat *et al.* (2014) and Abdollahi-Mandoulakani *et al.* (2015). In contrast, the SSR marker detected a higher polymorphism percentage than the ISSR marker. Indeed, the level of polymorphism is reflecting the range of gene diversity, and its evaluation is based on DNA fragment length or repeats (Innan *et al.*, 1997). Similar results were exhibited by Hamza *et al.* (2013), Rawat *et al.* (2014) and Abdollahi-Mandoulakani *et al.* (2015). To better testing the competence of the markers for diversity detection, the PIC value was calculated (Table 4). The PIC value for SSR markers ranged from 0.00 to 0.42 with a mean of 0.25, but this value varied from 0.05 to 0.27 with the mean of 0.18. The estimated PIC value was higher for SSR than the ISSR marker. Thus, the SSR marker was more discriminative than the ISSR marker because of the high allelic diversity in the SSR sequences

(Abdollahi-Mandoulakani *et al.*, 2015). In a comparative investigation between ISSR and SSR Markers for characterization of Alfalfa populations, Abdollahi-Mandoulakani *et al.* (2015) revealed likewise the PIC value of SSR was higher than in ISSR marker. Furthermore, the resolving power (Rp) value was calculated to select the most distinguishable primer for the cultivars according to their genetic diversity (Table 4). In the SSR marker both Unigene24552 and Unigene26319 primers showed the highest value (3.0), while in the ISSR marker, UBC 846 primer had the highest value (4.8). Accordingly, all these three primers in both SSR and ISSR markers had the most capability for detecting and displaying the highest allelic variations (Sharma *et al.*, 2009). Furthermore, according to the estimated marker index (MI) values, SSR had more discriminative efficiency because SSR scored higher an average (0.88) in comparison to ISSR (0.68).

Table 4. Polymorphism (P%), polymorphic information content value (PIC), resolving power (Rp) and marker index (MI) obtained by SSR and ISSR markers in the four tested sugar beet cultivars.

Primers Name	Fragments size range bp	Fragments No.	Monomorphic fragments	Polymorphic fragments	P %	PIC	R p	MI
SSR								
Unigene24552	158-260	6	1	5	83.33	0.33	3	1.65
Unigene16898	260-293	3	3	0	0	0	0	0
Unigene72402	225-240	2	1	1	50	0.19	0.5	0.19
Unigene26319	211-250	5	0	5	100	0.4	3	2
Unigene48657	130-167	3	0	3	100	0.42	2	1.26
Unigene14118	135-155	2	1	1	50	0.19	0.5	0.19
Total	-	21	6	15	-	-	-	-
Average	-	3.5	1	2.5	63.89	0.25	-	0.88
ISSR								
UBC 807	327-635	7	6	1	14.29	0.05	0.5	0.1
UBC 811	145-890	10	7	3	30	0.11	1.5	0.33
UBC 812	425-667	5	3	2	40	0.18	1.5	0.36
UBC 815	230-980	6	2	4	66.67	0.27	2.5	1.08
UBC 823	240-895	7	3	4	57.14	0.21	2	0.84
UBC 826	165-665	9	4	5	55.56	0.21	2.5	1.05
UBC 834	125-825	6	4	2	33.33	0.13	1	0.26
UBC 846	195-1800	11	5	6	54.55	0.24	4.8	1.44
Total	-	61	34	27	-	-	-	-
Average	-	7.63	4.25	3.38	43.94	0.18	-	0.68

3.3.2. Polymorphism and variability evaluation

The relationships among the evaluated cultivars were further illustrated according to similarity coefficient of Jaccard based on SSR and ISSR data (Table 5). The SSR data displayed moderate similarity among the four cultivars ranged from 0.44 to 0.59. The highest similarity (0.59) was between Hercule and Top cultivar, while the lowest similarity (0.44) was noted between Hosam

and Kawamera cultivar. On the other hand, the ISSR data showed the highest similarity (0.85) was between Kawamera and Top cultivars, while the lowest similarity (0.66) was between Hercule and Hosam cultivar. Moreover, the dendrogram of genetic similarity in the SSR marker classified the four cultivars into two main clusters (Fig. 3a). The first one contained only the highest drought-

tolerant cultivar (Kawamera), while the second one was divided into two sub-clusters, Only more drought-sensitive cultivar (Hosam) was placed in one of them, but the moderate tolerant cultivars (Hercule and Top) gathered in the second sub-cluster. ISSR marker also grouped the four cultivars into two main clusters (Fig. 3b). The first cluster contained only Hosam cultivar, while the second cluster was divided into two sub-clusters. One of them contained only Hercule cultivar, but the other one joined Kawamera and Top cultivar. These results are consistent with that the SSR marker more efficient and associated with drought tolerance and some agronomic traits (Nachit *et al.*, 2000). Combined SSR and ISSR results showed that the total amplified bands were 81 bands, 42 out of them were polymorphic, with a 51.22% polymorphism (Table 4). Furthermore, the detected similarity ranged from 0.62 to 0.77. Like as shown by ISSR, the highest and the lowest

similarity was also between Kawamera and Top cultivars, Hercule and Hosam cultivar, respectively (Fig. 3c). Also, the dendrogram distributed the four cultivars as in the ISSR marker.

The results of molecular analysis, productivity and quality parameters were able to differentiate among all the cultivars according to their drought tolerance capacity.

Table 5. The similarity index among the four cultivars based on SSR, ISSR and combined.

Cultivars	Top	Kawamera	Hercule	Marker type
Kawamera	0.53			SSR
	0.85			ISSR
	0.77			Combined
Hercule	0.59	0.50		SSR
	0.74	0.80		ISSR
	0.71	0.73		Combined
Hosam	0.53	0.44	0.50	SSR
	0.67	0.69	0.66	ISSR
	0.64	0.63	0.63	Combined

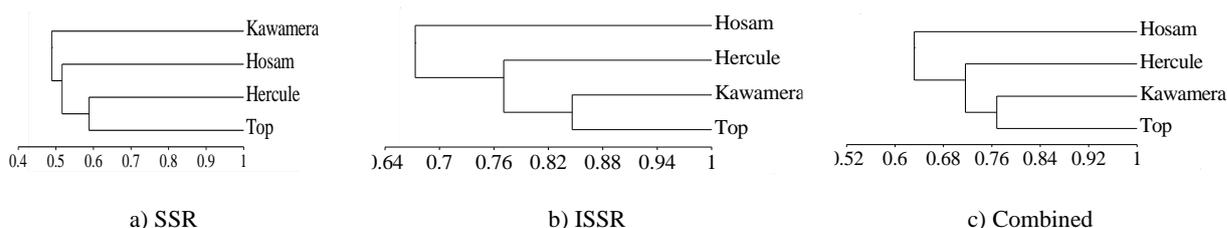


Fig. 3. The dendrograms of genetic similarity among the four sugar beet based on SSR, ISSR marker and combined.

4. Conclusion

In the present study, four sugar beet cultivars were evaluated for their performance under drought stress conditions and DNA molecular analysis. Significant differences were observed among them for the studied productivity and quality traits under normal and stressful conditions. Whereas, Kawamera cultivar showed the most superior performance in the studied traits. Moreover, both SSR and ISSR markers successfully differentiated among these cultivars. Furthermore, the SSR markers were more distinguishable according to the PIC value. In addition, they were able to classify the cultivars according to their drought tolerance, where the highest drought-tolerant cultivar (Kawamera) was separated in main cluster. However, the superiority of Kawamera cultivar under drought stress suggested that this

cultivar could be used in advanced breeding programs.

Acknowledgements

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