

## **Productivity, heritability and stability analysis of a Moroccan sugar beet germplasm**

G. Tobi<sup>1,2,\*</sup>, Y.E. Bahloul<sup>1</sup>, S. Oumouss<sup>1</sup>, I. Rahmouni<sup>1</sup>, A. Birouk<sup>2</sup> and O. Benlhabib<sup>2</sup>

<sup>1</sup>Regional Center of Agricultural Research of Rabat, Research Unit of Plant Improvement Conservation and Development of Phytogenetic Resources, Avenue Mohamed Belarbi Alaoui BP 6570 – Instituts, 10101 – Rabat, Morocco

<sup>2</sup>Research Unit of Applied Biotechnologies in Agriculture, Agrobiodiversity and Local Products, Department of Plant Protection Production and Biotechnology, Hassan II Institute of Agronomy and Veterinary Medicine, Avenue Allal EL FASSI – Madinat Al Irfane – BP 6202 – 10101 Rabat, Morocco

\*Correspondence: [g.tobi@iav.ac.ma](mailto:g.tobi@iav.ac.ma)

Received: January 19<sup>th</sup>, 2021; Accepted: March 27<sup>th</sup>, 2021; Published: April 6<sup>th</sup>, 2021

**Abstract.** Progeny testing is the second part of maternal recurrent selection scheme adopted by INRA-Morocco for the national sugar beet breeding programme. The objective of this study is sugar beet germplasm productivity, heritability and stability analysis. The studied material concern 18 half-sib families (HSF) preselected initially for their seed production potential. Trials were conducted using randomised complete blocks designs during, 2013/14, 2014/15, 2015/16, 2017/18 campaigns in two experimental fields of INRA-Morocco; Sidi Allal Tazi (34° 30' N, 6° 19' W) and Larache (35° 11' N, 6° 09' W). Evaluated parameters concern the vigour, root weight (RW), leaf biomass yield (LBY), and sugar content (Sc). Data analysis by comparative procedures explores different accordance degrees of HSF versus controls. Good vegetative growth was observed, 85.6% closer to the maximal indicated scale level. The RW was significantly influenced by the genotype and reached a maximum of 1.06 kg versus 1.08 kg average recorded by controls. Sugar content recorded mean was 20.97% in HSF versus 21.39% in the controls. Most of HSF revealed mean values close to Z-type variety. Estimated heritability was 0.5 for RW, 0.2 for the LBY, and 0.02 for Sc. Sugar content was influenced by the environment and explained by the AMMI model (73.6%) versus 53.9% and 44.4% for root weight and leaf biomass yield respectively. The AMMI stability values showed F11, F12, F16, and F17 families as the most performing and stable HSF. Results demonstrate the relevance of the maternal recurrent selection scheme of the on-going national breeding programme.

**Key words:** half-sib family, heritability, germplasm, productivity, stability, sugar beet.

### **INTRODUCTION**

Sugar beet is a major economic crop that provides wide range of products, especially sucrose, pulp for animal feed, and bio products such ethanol (FAO, 2019). Sugar beet crop is mainly cultivated in temperate zones. At nationally level, sugar beet

covers more than 80% of the total sugar crops area. Morocco imports sugar beet seeds and sugar in large quantities every year to meet the national need. The local sugar beet germplasm enhancement will have significant contribution for sugar beet cropping extension and the development of locally adapted cultivars.

The average sugar beet root weight usually ranges between 0.5 to 1 kg and sugar content between 13–22 percent. Sugar yield as a combined trait between root yield and sugar content represents the most important parameter for farmer and breeder (Hoffmann et al., 2009). Sugar yield is strongly influenced by the environment and is highly correlated to root yield and sugar content (Powers et al., 1963; Schneider et al., 2002; Hoffmann et al., 2009). Several types of sugar beet varieties exist; they differ mainly in term of their vegetation season, root yield and sugar content. Sugar beet genotype has a significant effect on sugar yield. Z-type hybrids have better stability and sugar yield and a relatively shorter vegetation period (Curcic et al., 2018).

In plant breeding, crop productivity and heritable variation are key criteria in germplasm enhancement. Heritability for plant breeders and geneticists measures the precision of the experiments (Piepho et al., 2008), and evaluates the selection relevance (Schmidt et al., 2019). It measures the variance due to the genetic causes and predicts crop improvement progress (Songsri et al., 2008).

Multiple-sites trials are required to characterise and structure germplasm according to their behaviour in different environments. The environment and varietal effects produce substantial variation in the genotype expression between sites, which decreases the correlation between phenotypic and genotypic forces (Delacy et al., 1990). The genotype by environment interaction (GEI) is always acting in crop production by causing variation in the varietal performance and ranking under the environmental testing conditions (Ndhlela et al., 2014). The genotype x environment interaction is of foremost significance; it evaluates the environments' effect on the breeding genotypes performance and assesses their stability (Moldovan et al., 2000). Previous studies evaluate sugar beet germplasm stability through multi-variate analyses and AMMI (Additive Main effect and Multiplicative Interaction) model (Paul et al., 1993; Ranji et al., 2005). The AMMI model is the most used method; it interprets a major part of the total deviation of GEI and assesses the genotypes performance and stability (Ebdon & Gauch, 2002).

The present study aims to evaluate the HSF progeny for productivity, heritability, and stability of a Moroccan sugar beet germplasm. The evaluation corresponds to the second phase of three selection cycles performed on 18 half-sib families preselected initially for their seed production potential under local climate conditions. This study was performed to analyse the INRA-morocco breeding programme progress for sugar beet germplasm enhancement through the maternal pedigree selection method.

## **MATERIALS AND METHODS**

### **Studied material**

The plant material is composed of 18 sugar beet half-sib families of the national sugar beet germplasm collection received from USDA-ARS breeders. It is multigerm germplasm developed from a sugar beet population carrying a normal cytoplasm that confers several resistance genes to rhizomania. Adopting a maternal pedigree selection method, the 18 sugar beet half-sib families (HSF) were selected to constitute the initial material for the progenies evaluation. This evaluation represents the second phase of

three selection cycles, C1, C2, and C3. Seed production potential improvement (first part of a selection cycle) was based on several selection criteria, disease resistance, the vernalisation capacity, the plant vigour at the vegetative growth phase, and yield components performance. The half-sib progenies were duplicated in the field by open pollination in a polycross design between selected individuals belonging to 18 half-sib families. Advanced individuals are considered as potential parents and elected to improve sugar beet next generation. The preselected progenies were characterised through their seed production potential under local conditions during the first phase of the selection cycles (Table 1). The half-sib families were compared to three monogerm sugar beet varieties listed in the official catalogue by the National Office for Food Safety: VERDI type E, CANDIMAX type N and ELVIS type Z. E-type control variety has 230 days cycle duration, low sugar content, and high root yield. N-type control variety is intermediate for the cycle duration of 210 days, the root yield, and the sugar content. Z-type control cultivar is a short cycle (180 days), low root yield, and high sugar rate. These three controls are coded as TE, TN and TZ respectively. The control varieties above have good potential and plasticity under the Moroccan climate conditions, while the 18 half-sib families have wide genotypic variability for seed production.

### Experimental sites

Two experimental fields of the National Institute of Agricultural Research (INRA-Morocco) were targeted for this study. Sidi Allal Tazi's first site is situated at Gharb-Chrarda-Beni Hssen region (34° 30' N latitude, 6° 19' W longitude, and 10.5 m elevation). It has clay soil and sub-humid bioclimate types. It's annual rainfall averages 520 mm, and means minimum and maximum temperatures 4 and 34 °C respectively. Larache's second site is located in the Tangier-Tetouan-Al Hoceima region at 35° 11' N latitude, 6° 09' W longitude, and 38 m elevation and has sandy soil type. Its climate is sub-humid with an average annual rainfall of 630 mm and minimum and maximum temperatures of 9 and 28.3 °C.

The study was conducted during three selection seasons and in two sites considered as having independent environments. Therefore, these environments were symbolised as E1, E2, E3, E4, E5, and E6. The meteorological data during the vegetative growth phase

**Table 1.** Main characteristics of the half-sib families for seed production potential

Half-sib family	NdB (day)	NdM (day)	GY (g)	Gr (%)
F1	257.89	332.75	112.30	86.29
F2	256.00	327.45	102.56	89.18
F3	251.55	322.09	120.54	87.73
F4	250.86	324.00	136.08	88.29
F5	262.40	334.70	170.19	87.90
F6	252.86	324.71	114.11	89.71
F7	254.24	325.12	92.52	88.32
F8	266.33	333.94	115.28	87.17
F9	259.78	328.00	123.85	87.56
F10	255.50	320.38	88.51	88.50
F11	259.40	324.00	106.16	89.20
F12	254.86	328.64	79.20	83.86
F13	247.00	316.95	107.88	88.89
F14	247.74	319.87	124.74	88.91
F15	255.78	326.78	135.48	84.78
F16	248.82	324.18	122.41	84.64
F17	252.65	327.25	85.95	87.90
F18	263.56	330.11	255.28	89.00
Average	255.11	326.40	116.67	87.57

NdB = Number of days to bolting; NdM = Number of days to maturity; GY = Grain yield per plant in gram; Gr = Germination percentage.

are presented in Table 2 into three periods: P1 ‘Winter’ December to February, P2 ‘Spring’ March to May, and P3 ‘Summer’ June to July.

### Methods and techniques

Trials were conducted according to randomised complete block designs during 2013/14, 2014/15, 2015/16, 2017/18 cropping seasons. The studied HSF were distributed randomly in the elementary parcels. The sowing was carried out manually in December according to four simple flat rows spaced 0.80 m apart and 25 cm between individual seeds and placed at 3 cm depth. Since the HSF were multi-germ, excess plants were discarded at 2 to 3 true leaf stage. The roots harvest was carried out in July, 200 days after sowing, at approximately the average controls growth cycle duration. Once harvested, the plants in central rows were used to carry the measurements and the data collection and analysis.

### Data recording

Plant vigour (Vg) was evaluated 3 months after the sowing using a 1 to 5 scale. A score of 5 corresponds to the best vegetative growth and 1 to plants presenting the lowest. The yield components were measured at harvest: leaf biomass yield (LYB) and root weight (RW) were recorded in kilogrammes on 5 individual plants per plot. The sugar content was assessed using 6 randomly selected plants per plot and expressed in percentage. A field refract-metre (ATAGO) was used to assess the total extract, expressed in Brix degrees (°B) where 1 °B corresponds to a refraction index of 1% sucrose solution in water. This procedure was used as a comparative approach to the controls.

### Statistical analysis

Collected data were analysed using statistical software R, version 3.6.2, and the STATISTICA 6FR. For the root and sugar productivity analysis, the factorial ANOVA model was used to test the HSF and locality and their interactions’ main effects on the total variance. Tukey’s HSD test was performed to assess the significant differences between group means (Tukey, 1953). Dunnett’s test was carried out also in the HSF groups’ comparison to the controls (Dunnett, 1955). The independent factors considered in the heritability analysis are the HSF without the controls. The environments are represented by the combination of the experimental sites and the years.

**Table 2.** Rainfall means (mm), maximum temperatures mean (°C), minimum temperatures mean (°C) and relative humidity means (%) during the vegetative growth phase in 6 environments, E1, E2, E3, E4, E5 and E6

Environment	Period	Tmax (°C)	Tmin (°C)	Rainfall (mm)	Relative humidity (%)
E1	P1	18.3	8.3	33.3	78.3
	P2	21.7	12.3	42.3	77.7
	P3	26.5	17.5	2.0	72.0
E2	P1	17.7	8.3	45.3	78.7
	P2	20.7	12.0	66.3	80.0
	P3	26.5	17.5	4.5	73.5
E3	P1	21.3	11.3	27.3	75.0
	P2	22.7	13.3	23.0	71.0
	P3	30.0	20.5	4.0	65.0
E4	P1	20.7	11.0	35.3	79.0
	P2	21.7	12.7	38.0	74.0
	P3	30.0	21.0	6.5	64.0
E5	P1	19.0	9.0	36.3	78.7
	P2	24.7	14.3	26.0	69.7
	P3	30.5	21.0	8.0	63.0
E6	P1	19.7	9.0	35.0	78.3
	P2	24.7	14.7	19.0	68.3
	P3	28.5	19.5	6.0	66.0

P1 = From December to February; P2 = From March to May; P3 = From June to July; Tmax = Maximum temperature; Tmin = Minimum temperature.

The heritability ( $h^2$ ) in its narrow sense was calculated by extracting the variance components using the summer package, using the solving Mixed Model Equations in R (MMER) (Covarrubias-Pazarán, 2019). The core of this package represents the function, whereas the multivariate and the univariate mixed model were performed according to Maier et al. (2015). The heritability value is expressed as the fits rate (0 to 1) or as the percentage fits (0 to 100%).

The additive main effects and multiplicative interaction (AMMI) model (Gauch 1992) is used to select the best genotype and environment combinations with respect to the variables' response by analysis of variance. The non-additive residual that is attributed to the GEI (genotype-environment interactions) is analysed by the principal component analysis (Zober et al., 1988). The sum of squares of the GEI is divided into the Interaction of the Principal Component Axis (IPCA). The Biplot of the GEI (Gabriel, 1971; Bradu & Gabriel, 1978; Zobel et al., 1988) is used to explore and interpret the underlying structure and causes of interaction. The IPCA scores estimate the genotypes' stability; the best performing and stable genotypes have a high yield and an IPCA value close to zero. Yield stability was calculated using the AMMI stability value (ASV) as described by Purchase et al. (2000). The ASV represents the deviation from zero in the two-dimensional scattergram (IPCA 1, IPCA 2). The genotypes more stable in different environments record an ASV closer to zero.

## RESULTS

### Productivity and heritability analysis of the national sugar beet germplasm

As initial data analysis to identify sources of variation, factorial analysis of variance was performed to reveal the individual main effect of locality, HSF, and their interaction on yield components (Table 3). The locality has shown a significant effect on plant vigour and leaf biomass. The HSF revealed a significant effect on root weight and leaf biomass. There was no significant effect of the locality\*half-sib family interaction on the yield components. A post-hoc test was used to elucidate the productivity variation.

**Table 3.** Factorial analysis of variance of yield components versus two factors, locality and half-sib family

Variable	DF	Vigour			Root weight (kg)		Leaf biomass yield (kg)		Sugar content (%)	
		MS	F-test	MS	F-test	MS	F-test	MS	F-test	
Locality	1	2.39	4.80*	0.31	3.45	2.33	55.74***	17.39	2.23	
HSF	20	0.61	1.23	0.18	2.00***	0.08	1.81*	7.95	1.02	
Locality*HSF	20	0.42	0.85	0.09	0.98	0.03	0.73	5.55	0.71	
Error	290	0.50		0.09		0.04		7.79		

DF = Degrees of Freedom; MS = Mean Square; \* = significant at  $p < 0.05$ ; \*\*\* = significant at  $p < 0.001$ .

The Dunnett's test for pair comparisons of HSF versus controls exhibits significantly consistent values (Table 4). The first measurements reveal the good vegetative growth; the best vigour values (4.53) were recorded by two HSF, F2 and F7, which were closer to the control TE (4.58), and exceeded TN and TZ ones. All the HSF were vigorous at 100%, 88.8%, and 94.4% in comparison with TZ, TE, and TN respectively.

For the leaf biomass, the HSF and the controls showed only one group of means. The pair comparisons of HSF means versus the controls indicated significant correlations (Table 4); the similar HSF's values had leaf biomass mean of 0.48 kg. Three HSF had significantly higher values exceeding the controls; F1, F11, and F18 produced respectively 0.62, 0.54, and 0.57 kg per plant compared to the control average value of 0.22 kg.

**Table 4.** Multiple comparisons of means for the vigour and leaf biomass yield. Tukey's HSD test and Dunnett's test *p*-values for the pairs of means comparisons of the HSF versus a fixed control, TE, TN and TZ

HSF	Vigour			Leaf biomass yield (kg)				
	Mean	<i>p</i> -values for pair comparisons			Mean	<i>p</i> -values for pair comparisons		
		HSF vs. TE	HSF vs. TN	HSF vs. TZ		HSF vs. TE	HSF vs. TN	HSF vs. TZ
F1	4.31 <sup>a</sup>	0.97	1.00	1.00	0.62 <sup>a</sup>	0.01*	0.02*	0.11
F2	4.53 <sup>a</sup>	1.00	1.00	1.00	0.45 <sup>a</sup>	0.65	1.00	0.98
F3	4.31 <sup>a</sup>	0.97	1.00	1.00	0.56 <sup>a</sup>	0.11	0.02*	0.44
F4	4.44 <sup>a</sup>	1.00	1.00	1.00	0.53 <sup>a</sup>	0.33	0.96	0.80
F5	4.26 <sup>a</sup>	0.86	1.00	1.00	0.40 <sup>a</sup>	1.00	0.21	1.00
F6	4.42 <sup>a</sup>	1.00	1.00	1.00	0.41 <sup>a</sup>	0.99	1.00	1.00
F7	4.53 <sup>a</sup>	1.00	1.00	1.00	0.53 <sup>a</sup>	0.08	0.42	0.39
F8	4.42 <sup>a</sup>	1.00	1.00	1.00	0.52 <sup>a</sup>	0.10	0.62	0.45
F9	4.32 <sup>a</sup>	0.96	1.00	1.00	0.49 <sup>a</sup>	0.25	0.44	0.72
F10	4.44 <sup>a</sup>	1.00	1.00	1.00	0.42 <sup>a</sup>	1.00	0.05	1.00
F11	4.47 <sup>a</sup>	1.00	1.00	1.00	0.54 <sup>a</sup>	0.01*	0.80	0.10
F12	4.31 <sup>a</sup>	0.97	1.00	1.00	0.47 <sup>a</sup>	0.88	0.17	1.00
F13	3.62 <sup>a</sup>	0.00***	0.01*	0.15	0.52 <sup>a</sup>	0.15	0.45	0.49
F14	4.31 <sup>a</sup>	0.98	1.00	1.00	0.39 <sup>a</sup>	1.00	1.00	1.00
F15	4.15 <sup>a</sup>	0.65	0.92	1.00	0.50 <sup>a</sup>	0.31	1.00	0.75
F16	4.30 <sup>a</sup>	0.99	1.00	1.00	0.55 <sup>a</sup>	0.49	0.13	0.88
F17	4.30 <sup>a</sup>	0.99	1.00	1.00	0.57 <sup>a</sup>	0.33	0.16	0.74
F18	3.77 <sup>a</sup>	0.02*	0.08	0.44	0.57 <sup>a</sup>	0.03*	0.36	0.19
TE	4.58 <sup>a</sup>	-	1.00	0.98	0.34 <sup>a</sup>	-	1.00	1.00
TN	4.47 <sup>a</sup>	1.00	-	1.00	0.35 <sup>a</sup>	1.00	-	1.00
TZ	4.31 <sup>a</sup>	0.98	1.00	-	0.37 <sup>a</sup>	1.00	1.00	-

HSF = Half-sib family; \* = significant at  $p < 0.05$ ; \*\*\* = significant at  $p < 0.001$ ; Same lower letter (<sup>a</sup>) indicates a non-significant difference between half-sib families.

The root weight revealed two groups (Table 5) and the HSF average values fluctuated between 0.72 and 1.06 kg for F15 and F11 respectively. The HSF comparison with controls showed that all HSFs were closer to TZ type control, except F15 which had the lowest root weight.

For sugar content, there was no significant difference between HSF, and significantly comparable values with the controls TE, TN, and TZ (Table 5). The controls represented sugar content mean values of 21.23%, 20.60%, and 22.33% respectively, while the half-sib families' average values were between 19.47% and 22.07% recorded respectively by F8 and F15. The HSFs reveal a higher sugar content potential compared to new cultivated cultivars. The enhanced HSFs were likely closer to the TZ varieties with a high sugar content and lower root weight.

**Table 5.** Multiple comparisons of means for the root weight and sugar content; Tukey's HSD test and Dunnett's test *p*-values for the pairs of means comparisons of the HSF versus a fixed control, TE, TN and TZ

HSF	Root weight (kg)			Sugar content (%)				
	Mean	<i>p</i> -values for pair comparisons			Mean	<i>p</i> -values for pair comparisons		
		HSF vs. TE	HSF vs. TN	HSF vs. TZ		HSF vs. TE	HSF vs. TN	HSF vs. TZ
F1	0.88 <sup>ab</sup>	0.02*	0.01*	0.81	21.28 <sup>a</sup>	1.00	0.80	1.00
F2	0.84 <sup>ab</sup>	0.02*	0.01*	0.84	21.56 <sup>a</sup>	1.00	0.97	1.00
F3	0.86 <sup>ab</sup>	0.01*	0.01*	0.73	20.96 <sup>a</sup>	1.00	0.95	1.00
F4	0.89 <sup>ab</sup>	0.02*	0.02*	0.85	20.77 <sup>a</sup>	1.00	1.00	1.00
F5	0.74 <sup>b</sup>	0.00***	0.00***	0.23	20.66 <sup>a</sup>	1.00	1.00	0.99
F6	0.88 <sup>ab</sup>	0.07	0.04*	0.99	21.14 <sup>a</sup>	1.00	1.00	1.00
F7	0.86 <sup>ab</sup>	0.04*	0.02*	0.95	21.94 <sup>a</sup>	1.00	0.86	1.00
F8	0.90 <sup>ab</sup>	0.16	0.11	1.00	19.47 <sup>a</sup>	0.43	0.88	0.23
F9	0.90 <sup>ab</sup>	0.11	0.08	1.00	20.49 <sup>a</sup>	1.00	1.00	0.95
F10	0.83 <sup>ab</sup>	0.01*	0.00***	0.51	20.33 <sup>a</sup>	1.00	1.00	1.00
F11	1.06 <sup>ab</sup>	1.00	1.00	1.00	20.07 <sup>a</sup>	0.69	0.98	0.41
F12	0.94 <sup>ab</sup>	0.09	0.06	0.99	21.10 <sup>a</sup>	1.00	0.87	1.00
F13	0.80 <sup>ab</sup>	0.06	0.04*	0.94	20.65 <sup>a</sup>	0.99	1.00	0.86
F14	0.87 <sup>ab</sup>	0.22	0.16	1.00	21.10 <sup>a</sup>	1.00	1.00	0.99
F15	0.72 <sup>b</sup>	0.00***	0.00***	0.42	22.07 <sup>a</sup>	1.00	0.99	1.00
F16	1.00 <sup>ab</sup>	0.50	0.40	1.00	21.80 <sup>a</sup>	0.98	0.73	1.00
F17	0.96 <sup>ab</sup>	0.20	0.15	1.00	20.93 <sup>a</sup>	1.00	1.00	1.00
F18	0.86 <sup>ab</sup>	0.16	0.11	1.00	21.21 <sup>a</sup>	1.00	1.00	1.00
TE	1.14 <sup>a</sup>	-	1.00	0.76	21.23 <sup>a</sup>	-	1.00	1.00
TN	1.16 <sup>a</sup>	1.00	-	0.64	20.60 <sup>a</sup>	1.00	-	0.98
TZ	0.95 <sup>ab</sup>	0.76	0.64	-	22.33 <sup>a</sup>	1.00	0.98	-

HSF = Half-sib family; \* = significant at  $p < 0.05$ ; \*\*\* = significant at  $p < 0.001$ ; Lower case letters (<sup>ab</sup>) indicate a statistical difference ( $P < 0.05$ ) between half-sib families; Same lower letter indicates a non-significant difference between half-sib families.

Performing germplasm in a breeding programme should have significant heritability variation of the selection key traits. So, besides the variability analysis, the heritability was evaluated on the three main yield components, the root weight, leaf biomass, and sugar content. The controls barring in this analysis purpose was to disclose effectively the studied parameters heritability. Since the controls are monogerm registered improved cultivars, while the studied germplasm are basically multigerm still under genetic enhancement.

Variance components were assessed through the estimation of the heritability (Table 6). The Z-ratio represents the comparison score of the studied variables by standardising the distribution. The z-score is positive when the HSF score is higher than the average. The half-sib family had the largest effect on root weight in comparison with the two other variance components. The RW heritability reaches almost 0.5; which is the greatest value that confirms also the selection efficiency of this variable. The LBY has an  $h^2$  value of about 0.2. On the other hand, sugar content has the lowest heritability score of 0.02 since it's a trait significantly influenced by the environment. Such results are relevant for the selection purposes; root weight, then leaf biomass are valuable criteria for sugar yield selection. More analyses are needed to explain the sources of

variability, to detect the genotype-environment interaction effect on the behaviour of the studied germplasm and to discriminate the more stable and productive HSFs.

**Table 6.** Variance components and narrow sense heritability ( $h^2$ ) estimation

Variable	Variance component	Variance	Variance <i>SE</i>	<i>Z-ratio</i>	Heritability	
					Estimate	<i>SE</i>
Root weight (kg)	HSF	0.013	0.006	2.274	0.466	0.227
	Env	0.001	0.002	0.324		
	Env* HSF	0.006	0.003	1.810		
	Env*Block	0.000	0.000	0.000		
	Error	0.028	0.003	8.339		
Leaf biomass yield (kg)	HSF	0.006	0.003	2.143	0.196	0.152
	Env	0.004	0.005	0.777		
	Env* HSF	0.000	0.002	0.164		
	Env*Block	0.001	0.001	1.034		
	Error	0.024	0.003	8.268		
Sugar content (%)	HSF	0.235	0.172	1.363	0.017	0.025
	Env	8.191	5.398	1.518		
	Env* HSF	0.096	0.238	0.406		
	Env*Block	0.128	0.131	0.979		
	Error	3.515	0.362	9.700		

HSF = Half-sib family; Env = Environment; *SE* = Standard error.

#### Stability study by AMMI analysis of genotype × environment interaction

The variance analysis displayed significant effect of genotype-environment interaction on the yield component parameters which validates the genotypes differential performance between the environments (Table 7). The first interaction principal component IPCA1 gathered 53.9%, 44.4%, and 44.6% of the total variance for respectively the root weight, the leaf biomass, and the sugar content. The IPCA scores were used to explain the half-sib families' behaviour with regard to the environments accordingly throughout the three selection cycles, Recalling that, the HSF were analysed E1 and E2 for the third selection, E3 and E4 for a second selection cycle, and E5 and E6 for the first selection cycle. The climatic data were different mainly between seasons, winter and spring (Table 2). For E1, the average rainfall was 33.3 and 42.3 mm respectively during the winter and spring periods. For E2, the values were 45.3 and 66.3 mm respectively during winter and spring.

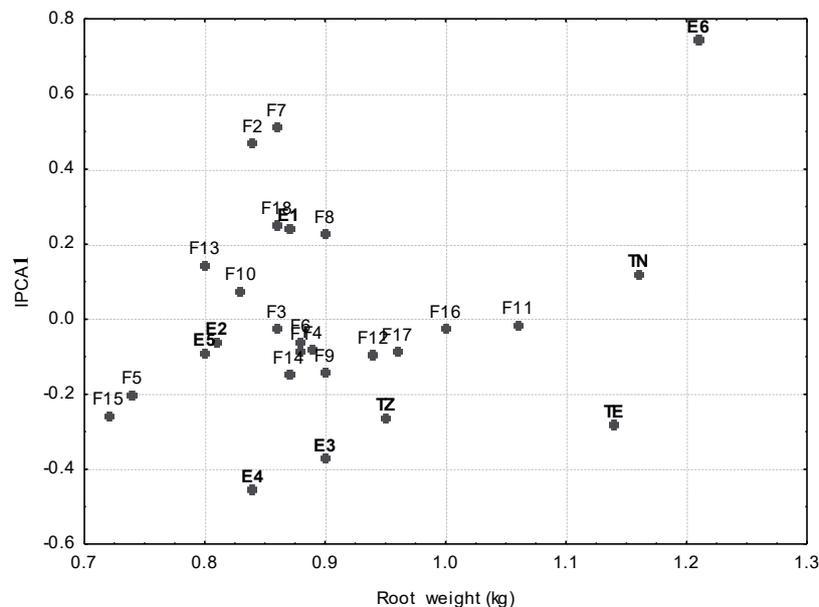
**Table 7.** Analysis of variance for the AMMI model for 18 half-sib families, three controls and six studied environments

Variable	DF	<i>SS</i>	<i>MS</i>	% explained	<i>F-test</i>
Env	5	5.47	1.09		1.86
Rep (Env)	13	7.64	0.59		12.93***
Génotype	20	4.25	0.21		4.67***
Env x Gen	78	5.44	0.07		1.53**
IPCA1	24	3.76	0.16	53.9	3.33***
IPCA2	22	1.44	0.06	20.7	1.44
Residuals	215	9.77	0.05		

Table 7 continued

Env		5	5.65	1.13		11.92***
Rep (Env)		13	1.23	0.09		3.76***
Génotype		20	1.88	0.09		3.72***
Env x Gen		78	2.76	0.03		1.40*
IPCA1	Leaf biomass yield (kg)	24	1.57	0.06	44.4	2.59***
IPCA2		22	0.85	0.04	24.1	1.53
Residuals		215	5.42	0.02		
Env		5	1,244.76	248.95		21.49***
Rep (Env)		13	150.53	11.58		3.57***
Génotype		20	146.92	7.35		2.26**
Env x Gen		78	362.19	4.64		1.43*
IPCA1	Sugar content (%)	24	205.69	8.57	44.6	2.64***
IPCA2		22	133.75	6.08	29.0	1.87*
Residuals		215	697.88	3.25		

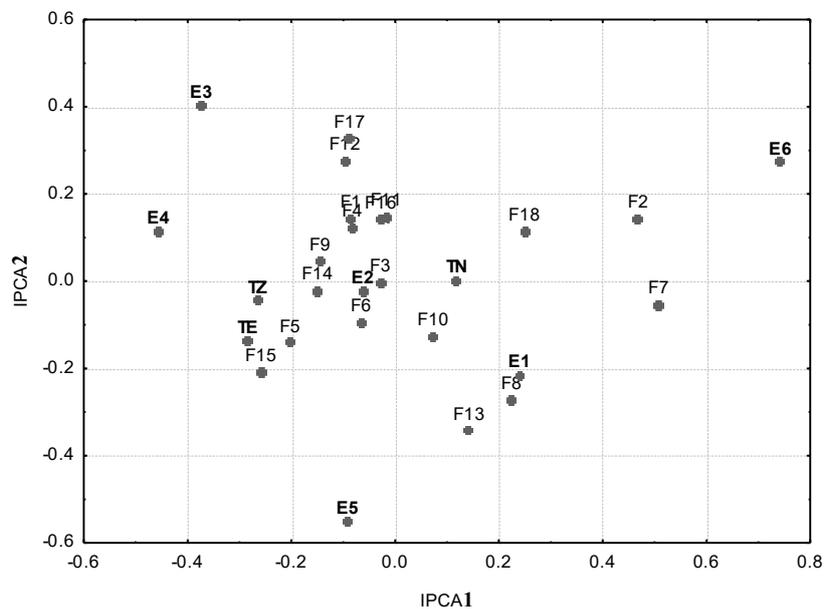
DF = Degrees of Freedom; MS = Mean Square; SS = Sum of Squares; Env = Environment; Rep = Repetition; Gen = Genotype; \* = significant at  $p < 0.05$ ; \*\* = significant at  $p < 0.01$ ; \*\*\* = significant at  $p < 0.001$ .



**Figure 1.** AMMI biplot for IPCA1 scores versus root weight (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

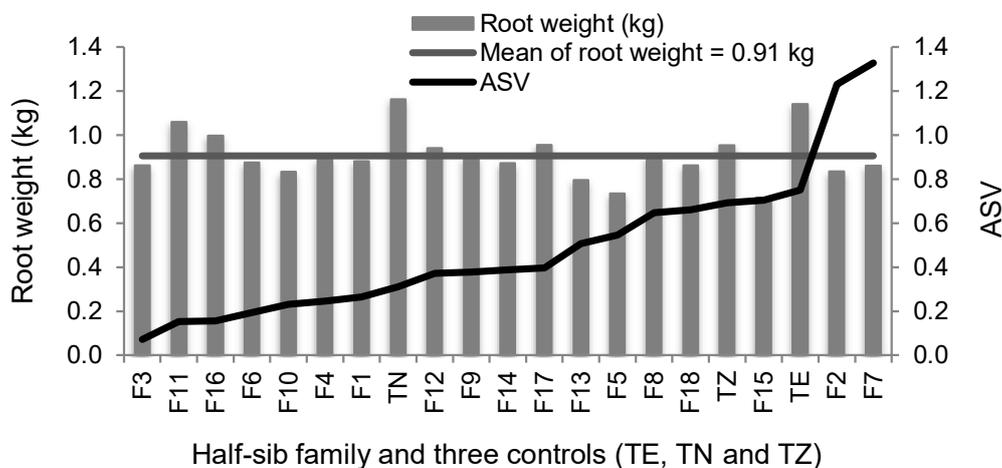
This analysis aims to discriminate the most performing stable HSFs and reveals the genetic progress lengthwise the selection cycles. For a better assessment of the data, every dependant variable is presented in scatter plots (IPCA1, dependant variable). Also, the scatter plots IPCA1 vs. IPCA2 are accomplished for the three yield components. High IPCA scores, negative or positive, indicate the good adaptation of the genotype to a special environment. The more stable is a genotype over the tested environments; the closer is the IPCA scores to zero. The biplot of the first interaction principal component (IPCA1) versus the root weight (Fig. 1) showed significant variation among the studied environments. Half-sib families F11, F12, F16, and F17 displayed better stability; their root weight ranked between 0.94 and 1.06 kg and IPCA1 scores between -0.017 and -

0.096. The four HSF have good adaptation and genetic flexibility to the experimental environments. The environments matching the third selection cycle E1 and E2 are less interactive as they recorded close IPCA1 scores of 0.24 and -0.062 respectively (Figs 1, 2).



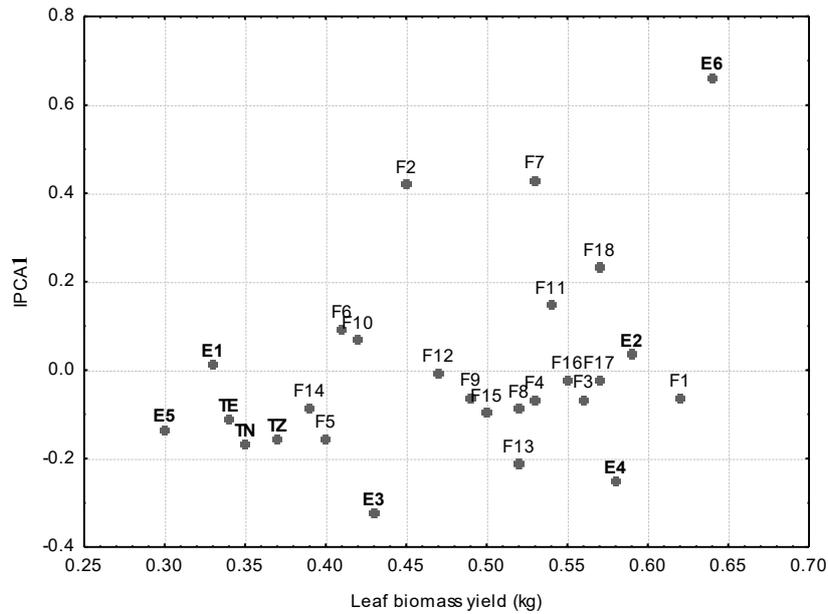
**Figure 2.** AMMI plotted IPCA1 and IPCA2 scores for root weight (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

Such data reveal that the improved germplasm heterogeneity has decreased via the panmictic crosses during the two first selection cycles. The root weight’s HSF ranking according to the AMMI stability values (ASV) is illustrated in Fig. 3. As shown, the more the ASV value is close to zero, the more stable is HSF across environments. Root weight and its corresponding ASV value reveal differences between half-sib families in terms of stability. The families F11, F12, F16 and, F17 were the most stable presenting ASV values stand between 0.15 and 0.39 and RW values of 1.06, 0.94, 1.0, and 0.95 kg respectively, exceeding the 0.91 kg overall average as are also the three controls.

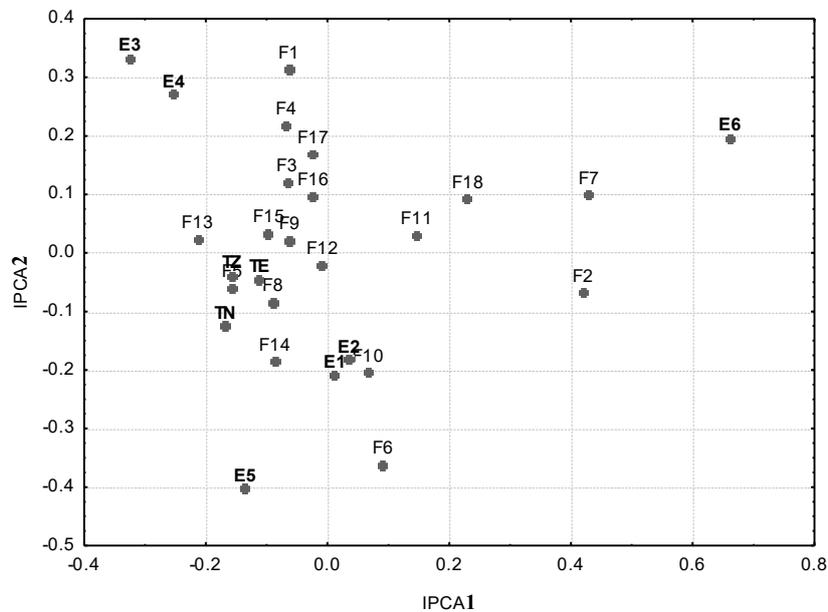


**Figure 3.** Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for root weight (kg).

The IPCA1 versus leaf biomass biplot (Fig. 4) shows that third selection cycle E1 and E2 environment data has IPCA1 scores close to zero, equal to 0.013 and 0.036 respectively and leaf biomass values of 0.33 and 0.59 kg correspondingly. The IPCA1 vs. IPCA2 elucidate further these results (Fig. 5). The most stable and adapted half sib-families are F17, F16, and F12; they present leaf biomass yields of 0.57, 0.55, and 0.47 kg respectively, and IPCA1 values between -0.008 and -0.0023. The HSF F14 and F5 are closer to the controls with means close to 0.40 kg.

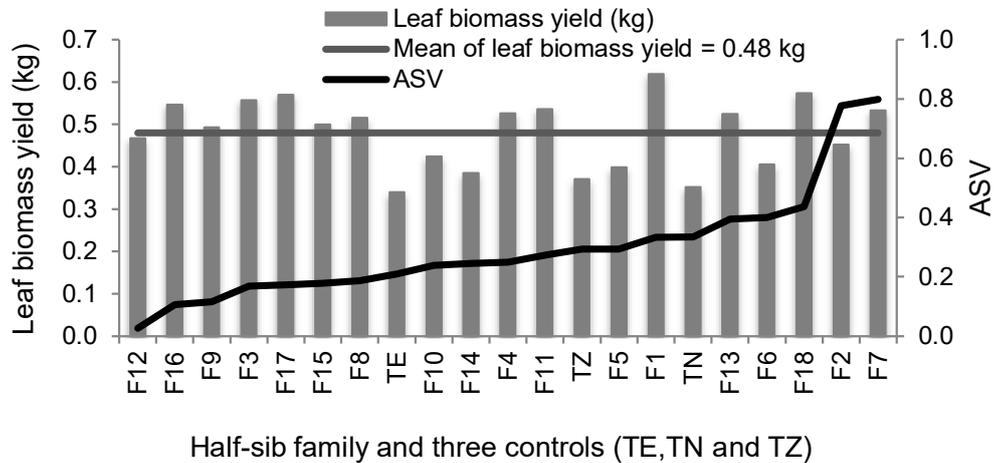


**Figure 4.** AMMI biplot for IPCA1 scores versus leaf biomass yield (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.



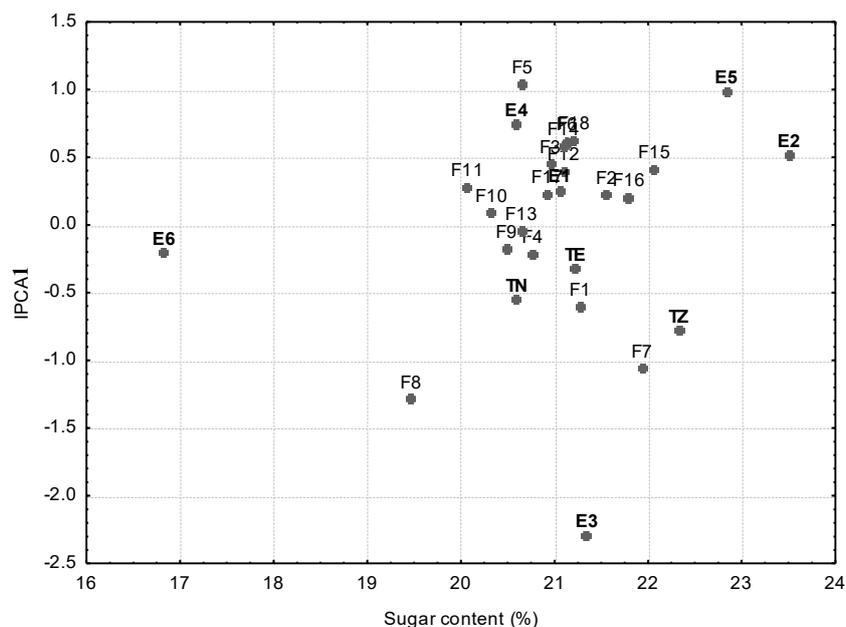
**Figure 5.** AMMI plotted IPCA1 and IPCA2 scores for leaf biomass yield (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

The foliar biomass stability values (Fig. 6) showed that the majority of the half-sib families have stability values as good as the controls and less than 0.3. The most stable families are F12, F16, F9, F3, F17, F15, and F8, all their ASV values less than 0.18 and biomass yield sizes between 0.47 and 0.57 kg.

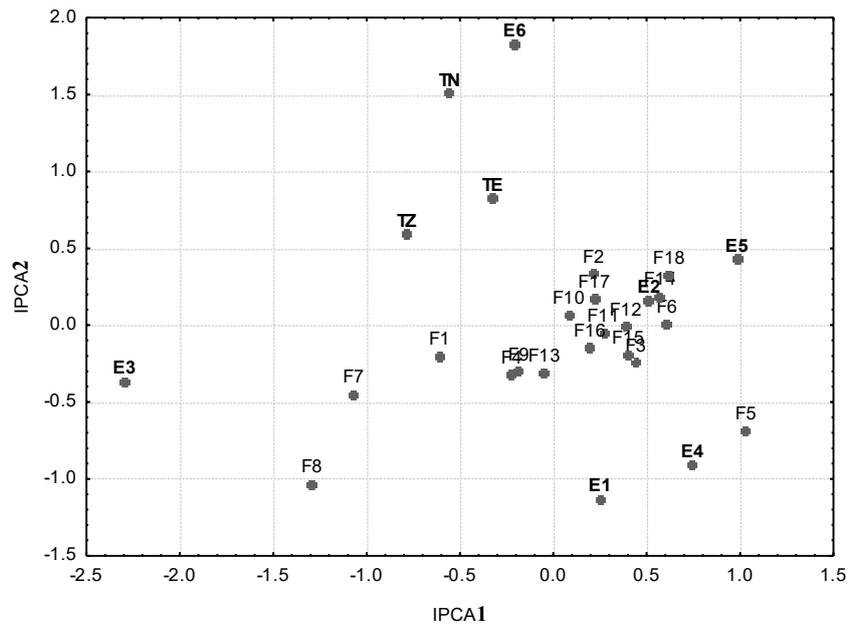


**Figure 6.** Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for leaf biomass yield (kg).

The sugar content vs. the IPCA1 biplot shows that the most stable and adapted HSF are F2, F4, F9, F13, F10, F11, F12, F16, and F17; their average sugar contents fit between 20.07 and 21.79%, and their IPCA1 scores between -0.047 and 0.271 (Fig. 7). The E1 and E2 IPCA1 values for sugar content are close to each other in comparison to the other environments. The IPCA1 vs. IPCA2 biplot adds more explanation (Fig. 8); IPCA1 scores for E1 and E2 are equal to 0.25 and 0.51 respectively.

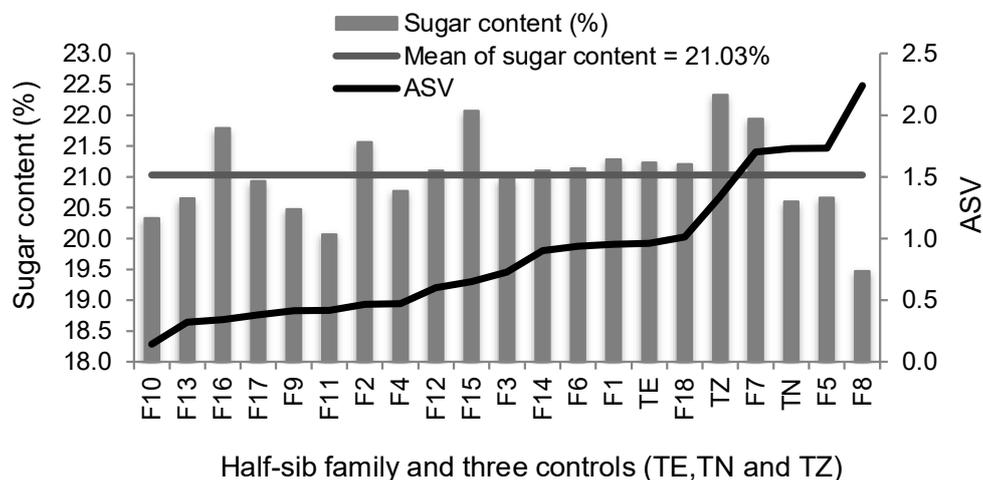


**Figure 7.** AMMI biplot for IPCA1 scores versus sugar content (%) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.



**Figure 8.** AMMI plotted IPCA1 and IPCA2 scores for sugar content (%) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

The sugar content's ASV ranking (Fig. 9) shows the HSF F16, F17, F2, F11, and F12 being the most stable; their ASV values are below 0.61. These HSFs recorded sugar concentration averages between 20.07 and 21.79% recorded respectively by F11 and F16. The F11 HSF, with 20.07% sugar content and 1.06 kg root weight, is the closest to the TN; the intermediate control variety which sugar level is 20.60% and root weight 1.16 kg.



**Figure 9.** Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for sugar content (%).

## DISCUSSION

The present study aims to analyse the selection efficiency of Moroccan sugar beet multigerm germplasm comparatively with three monogerm varieties used as controls, under local limited vernalising climate. The 18 preselected HSF standing for the analysed

plant material, were evaluated in two environments during three recurrent maternal selection cycles to gatherers multi-trials database. The HSFs did not show any specific adaptation to the experimental localities, Larache and Sidi Allal Tazi. The Locality-HSF interaction ANOVA values were not significant. The plants' vigour showed 95% significance in its correspondence to the controls. The seeds' quality and especially the germination rate, which are linked to the plants' vigour, seem to have an appealing effect on vegetative development. The maternal plants selected at the two first selection cycles exceeded a germination rate of 83% (Table 1). The plant vigour is assessed by using different methods at different stages of the vegetation. Among these methods, plantlet's rise is used as an indicator for seed vigour evaluation (Podlaski & Chomontowski, 2020). Low seed vigour is described to decrease root yield as seedling emergence is slower and less uniform compared to high vigour seeds. Generally, vegetative vigour is a robust indicator for seed quality. Improved germplasm with high and homogeneous vigour is a prerequisite in successful breeding programmes.

Root weight and leaf biomass are mainly influenced by the plant genotype; they both presented significant variability. The combined root weight and sugar content data enlightens TZ control type of the majority of the half-sib families and approves the negative correlation between sugar content and root weight as reported in many studies conducted on sugar beet (Curcic et al., 2018; Ahmadi et al., 2011; Biancardi et al., 2010; Hoffmann, 2010; Schneider et al., 2002). In sugar beet germplasm enhancement, when root weight is increased by the selection, sugar content tends to decrease, and vice versa (Biancardi et al., 2010). The 18 HSFs recorded significantly similar values with the two other controls, TE and TN. The HSFs' variability reported is principally due to the genotypes, and can be explained by the differences in the maturation stage and accordingly to the storage sugar content in the roots (Meier et al., 1993). Also, the trend towards an intermediate type variety will be optimal to provide flexibility for the harvesting schedule. Among the hybrid cultivars, there is the TZ- type that has balanced root yield and sugar content and is set for intermediate and late harvest (Ludecke, 1953; Bosemark, 2006). Harvest dates delayed up to 210 days is reported to increase significantly root weight and sugar content (Hussein et al., 2012).

The sugar-beet roots harvest was carried at 200 days, which is 10 and 30 days earlier to TN and TE controls growth cycle but was close to TZ control (180 days). Z-type sugar beet hybrids are considered more stable, having higher sugar yield and shorter vegetation periods (Curcic et al., 2018). Early harvesting is prized to avoid summer early drought and to seek high sugar yield and economic expenses. Such data proclaim opportunities to select and establish locally adapted cultivars. The variance decomposition reveals a large contribution of the half-sib families on the root weight and leaf biomass yield variability. Previous studies reported significant variability of root yield among several sugar-beet genotypes (Ulaković et al., 2015; Curcic et al., 2018). Leaves' size is influenced by the genotype, growth stage, or climatic conditions (Klotz, 2005). The heritability reaches 0.5 values for root weight and 0.2 for leaf biomass; both variables are slightly affected by the environment in comparison with sugar content which heritability value is as low as 0.002. Several research works validate sugar yield dependence on the environment and its high correlation to the root yield and sugar content (Powers et al., 1963; Schneider et al., 2002; Hoffmann et al., 2009). Genotype  $\times$  environment interaction studies on sucrose, as total dissolved solids in table beet, showed strong environmental effects and limited heritability (Goldman et al., 1996). The genotype-environment interaction

(GEI) analyses in the present study were relevant to interpret the variability and in structuring the sugar beet germplasm through their stability and sugar yield efficiency. The AMMI model shows high significant values for studied parameters exceeding 44%. Many studies record a significant effect of GEI in sugar beet field trials (Moradi et al., 2012; Hoberg et al., 2015; Al Jbawi et al., 2017). Significant GEI is valuable since it helps to discriminate the genotypes through their genetic and production potentials in different environments (Aghaee-Sarbarzeh et al., 2007). The stability explains the sugar content heritability recorded the highest significant AMMI value of 73.6% against 53.9% for root weight and 44.4% for leaf biomass. Significant genotype main effect can also be weighty for the sucrose (as total dissolved solids) in table beet; this happened when the genotype  $\times$  environment interactions are significant (Hanson & Goldman, 2019).

In our case, the environment E3, E4, E5, and E6 registered higher IPCA1 and IPCA2 scores than E1 and E2. E1 and E2 environments were less interactive and displayed the closest IPCA scores. These results express the heterogeneity declined at the third selection cycle through the open crosses between selected genotypes for seed production potential evaluation during early selection cycles. The most performing and stable HSFs were identified; F11, F12, F16, and F17 recorded high yield component values and were stable in comparison to the rest of HSFs. These analyses reveal the efficiency of the selection and the evaluation of the sugar beet genotypes. The present study reports promising HSFs and substantial germplasm advancement through three recurrent selection cycles.

## CONCLUSIONS

This comparative study confirms that the germplasm denotes a large yield components variability and constitutes a rich database for the national sugar beet breeding programme with probable wide and narrow adapted material. The studied Half-Sib Families recorded significant performing traits comparatively to the controls, especially for root yield and sugar content. Most HSFs are close to TZ control, highlighting the trend of the performing families that have a short cycle (200 days). The narrow-sense heritability values showed different levels for studied parameters; the root weight shows the highest values of 0.5, while sugar content reveals a value of 0.02 being as significantly influenced by the environment. The most performing and stable half-sib families are F11 (closer to TN control), F12, F16, and F17 (closer to TZ control) with high yield component values and high stability. This study is of great support to the national sugar beet breeding programme.

ACKNOWLEDGEMENTS. This research was performed in the National Institute of Agricultural Research of Morocco (INRA-Morocco) - Regional Centres of Agricultural Research of Rabat, Kenitra and Tangier.

## REFERENCES

- Aghaee-Sarbarzeh, M., Safari, H., Rostaei, M., Nadermahmoodi, K., Pour Siabidi, M.M., Hesami, A., Solaimani, K., Ahmadi, M.M. & Mohammadi, R. 2007. Study of general and specific adaptation in dryland advance wheat (*Triticum aestivum* L.) lines using GE biplot based on AMMI model. *Pajouhesh and sazanegi* 77, 41–48 (in Persian).

- Ahmadi, M., Majidi Heravan, E., Sadeghian, S.Y., Mesbah, M. & Darvish, M.F. 2011. Drought tolerance variability in S1 pollinator lines developed from a sugar beet open population. *Euphytica* **178**, 339–349.
- Al Jbawi, E., Al Huniesh, T., Al Jasem, Z., Al Mahmoud, N. & Al Zubi, H. 2017. Determining some stability adaptation parameters for sugar beet commercial varieties in summer sowing. *Syrian Journal of Agricultural Research* **4**, 171–182.
- Biancardi, E., McGrath, J.M., Panella, L.W., Lewellen, R.T. & Stevanato, P. 2010. Sugar beet. *Bradshaw J (ed) Handbook of plant breeding, tuber and root crops*. Springer, New York, **4**, pp. 173–219.
- Bosemark, N.O. 2006. Genetics and Breeding. In *Sugar Beet*, ed Draycott A.P. (Oxford: Blackwell Publishing Ltd.), pp. 50–88.
- Bradu, D. & Gabriel, K.R. 1978. Biplot as a diagnostic tool for models of 2-way tables. *Technometrics* **20**, 47–68.
- Covarrubias-Pazarán, G. 2019. Solving Mixed Model Equations in R. CRAN. DataGene (2017) 'Australian Dairy Herd Improvement Report 2016'. DataGene Limited.
- Curcic, Z., Ciric, M., Nagl, N. & Taski-Ajdukovic, K. 2018. Effect of sugar beet genotype, planting and harvesting dates and their interaction on sugar yield. *Frontiers in Plant Science* **9**, 1–9.
- DeLacy, I.H., Eisemann, R.L. & Cooper, M. 1990. The importance of genotype-by-environment interaction in regional variety trials. *Genotype-by-Environment Interaction and Plant Breeding (Ed. MS Kang)*. Louisiana State University, Baton Rouge, Louisiana, USA, 287–300.
- Dunnnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *Journal of the American Statistical Association* **50**(272), 1096–1121. doi:10.1080/01621459.1955.10501294.
- Ebdon, J.S. & Gauch, H.G. 2002. Additive main effect and multiplicative interaction analysis of natural turf grass performance trials. *Crop Science* **42**, 497–506.
- FAO (Food and Agriculture Organization). 2019. FAO – OECD Agricultural outlook, 2019–2028.
- Gabriel, K.R. 1971. The biplot graphic display of matrices with application to principal components analysis. *Biometrika* **58**, 453–467.
- Gauch, H.G. 1992. *Statistical analysis of regional yield trials: AMMI analysis of factorial designs*. Elsevier, Amsterdam, 278 pp.
- Goldman, I. L., Eagen, K. A., Breitbach, D.N. & Gabelman, W.H. 1996. Simultaneous selection is effective in increasing betalain pigment concentration but not total dissolved solids in red beet. *American Society for Horticultural Science* **121**, 23–26.
- Hanson, S.J. & Goldman, I.L. 2019. Genotype Is Primarily Responsible for Variance in Table Beet Geosmin Concentration, but Complex Genotype × Environment Interactions Influence Variance in Total Dissolved Solids. *American Society for Horticultural Science* **144**, 429–438. doi: 10.21273/JASHS04758-19
- Hoberg, F., Kenter, C. & Marlander, B. 2015. Genotype × environment interactions in sugar beet and implications for variety choice in Germany in consideration of Cercospora leaf spot. *Sugar Industry* **140**, 640–649.
- Hoffmann, C.M. 2010. Root quality of sugarbeet. *Sugar Tech.* **12**, 276–287.
- Hoffmann, C., Huijbregts, T., van Swaaij, N. & Jansen, R. 2009. Impact of different environments in Europe on yield and quality of sugar beet genotypes. *European Journal of Agronomy* **30**, 17–26.
- Hussein, S., Ling, A.P.K., NG, T.H., Ibrahim, R. & Paek, K.Y. 2012. Adventitious roots induction of recalcitrant tropical woody plant *Eurycoma longifolia*. *Romanian Biotechnological Letters* **17**, 7026–7035.
- Klotz, K. 2005. Anatomy and physiology. In: Genetics and breeding of sugar beet, eds. Biancardi, E, Campbell, LG, Skaracis, GN and De Biaggi, M., *Science Publishers Inc. Enfield. NH.* 9–18.
- Ludecke, H. 1953. *Sugar Beet Cultivation, A Guide to Practice*. Hamburg; Berlin: Verlag Paul Parey. (in German).

- Maier, R., Moser, G., Chen, G.B., Ripke, S., Coryell, W., Potash, J.B., Scheftner, W.A., ... & Lee, H. 2015. Joint analysis of psychiatric disorders increases accuracy of risk prediction for schizophrenia, bipolar disorder, and major depressive disorder. *American Journal of Human Genetics* **96**, 283–294.
- Meier, U., Bachmann, L., Buhtz, H., Hack, H., Klose, R., Märlander, B. & Weber, E. 1993. Phänologische Entwicklungsstadien der Beta-Rüben (*Beta vulgaris* L. ssp.). Codierung und Beschreibung nach der erweiterten BBCH-Skala (mit Abbildungen). [Phenological growth stages of sugar beet (*Beta vulgaris* L. ssp.). Codification and description according to the general BBCH scale (with figures)]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **45**, 37–41 (in German).
- Moldovan, V., Moldovan, M. & Kadar, R. 2000. Item from Romania. S.C.A. Agricultural Research Station. Turda, 3350, str. *Agriculturii 27 Jud Chuj, Romania*.
- Moradi, F., Safari, H. & Jalilian, A. 2012. Study of genotype × environment interaction for sugar beet monogerm cultivars using AMMI method. *J. Sugar Beet* **28**, 29–35.
- Ndhlela, T., Herselman, L., Magorokosho, C., Setimela, P., Mutimaamba, C. & Labuschagne, M. 2014. Genotype × environment interaction of maize grain yield using AMMI biplots. *Crop Science* **54**, 1992–1999. doi:10.2135/cropsci2013.07.0448
- Paul, H., Van Eeuwijk, F.A. & Heijbroek, W. 1993. Multiplicative models for cultivar by location interaction in testing sugar beets for resistance to beet necrotic yellow vein virus. *Euphytica* **71**, 63–74.
- Piepho, H.P., Möhring, J., Melchinger, A.E. & Büchse, A. 2008. BLUP for phenotypic selection in plant breeding and variety testing. *Euphytica* **161**, 209–228. doi:10.1007/s10681-007-9449-8
- Podlaski, S. & Chomontowski, C. 2020. Various methods of assessing sugar beet seed vigour and its impact on the germination process, field emergence and sugar yield. *Sugar Tech.* **22**, 130–136.
- Powers, L., Schmehl, W.R., Federer, W. & Payne, M.G. 1963. Chemical genetic and soils studies involving thirteen characters in sugar beet. *Journal of the ASSBT* **12**, 393–448. doi: 10.5274/jsbr.12.5.393
- Purchase, J.L., Hatting, H. & Vandeventer, C.S. 2000. Genotype × environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa. II. Stability analysis of yield performance. *South African Journal of Plant and Soil* **17**, 101–107.
- Ranji, Z., Mesbah, M., Amiri, R. & Vahedi, S. 2005. Study on the efficiency of AMMI method and pattern analysis for determination of stability in sugar beet varieties. In *Iranian Journal of crop sciences* **7**(1), 1–21 (in Persian, abstract in English).
- Schmidt, P., Hartung, J., Rath, J. & Piepho, H. P. 2019. Estimating broad-sense heritability with unbalanced data from agricultural cultivar trials. *Crop Science* **59**, 525–536. doi:10.2135/cropsci2018.06.0376
- Schneider, K., Schafer-Pregl, R., Borchardt, D.C. & Salamini, F. 2002. Mapping QTLs for sucrose content, yield and quality in a sugar beet population fingerprinted by EST-related markers. In *Theoretical and Applied Genetics* **104**, 1107–1113. doi: 10.1007/s00122-002-0890-8
- Songsri, P., Joglloy, S., Kesmala, T., Vorasoot, N., Akkasaeng, C. P. A. & Holbrook, C. 2008. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. In *Crop Science* **48**, 2245–2253.
- Tukey, J.W. 1953. *The problem of multiple comparisons*. In H. Braun (Ed.), *The collected works of John W. Tukey volume VIII, multiple comparisons: 1948–1983*, 300 pp.
- Ulaković, V., Glamočlija, N., Filipović, V. & Ugrenović, V. 2015. Mineral nutrition plants in function of stable sugar beet production. *Selekc Seminars* **21**, 39–49.
- Zober, R.W., Wright, M.J. & Gauch, H.G. 1988. Statistical analysis of yield trial. *Agronomy Journal* **80**, 388–393.