https://doi.org/10.48047/AFJBS.6.15.2024.10596-10625

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Contribution of morphological and SSR markers in durum wheat (*triticum turgidum l.)* Genetic diversity

HAMADA Youcef[1,2,3,*], DJEKOUN Abdelhamid[3,4], HAMIDECHI Mohamed Abdelhafid[1,2]

[1] Department of Molecular and Cellular Biology, Faculty of Natural and Life Sciences, Abbes Laghrour University, Khenchela 40000, Algeria
[2] Biotechnologies, Water, Environment and Health Laboratory, Abbes Laghrour University, Khenchela 40000, Algeria
[3] Genetic, Biochemistry and Plant Biotechnologies Laboratory, University Constantine 1, Algeria
[4] Pharmaceutical sciences research center, Constantine, Algeria
* **Correspondence**: HAMADA Youcef
Address: Abbes Laghrour University, BP 1252, road of 40004, Khenchela, Algeria
Tel : 00213.772.12.76.54
hamada.youcef@univ-khenchela.dz

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 25 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.10596-10625

Abstract:

This study aims to explore the genetic and phenotypic diversity of durum wheat varieties to improve their resilience and yield. Thirty-six durum wheat genotypes were studied in two sites with distinct agro-climatic conditions in Algeria: Constantine and Khenchela. The analysis of phenotypic traits focused on several parameters, such as leaf area, plant height, ear (spike) length, number of tillers per plant, number of grains per ear, a thousand grains weight and grain yield. The genotypes showed significant variations in grain yield, ranging from 2.5 to 5.8 tons per hectare. The plants height varied from 70 cm to 110 cm, while the thousand grains weight oscillated between 35 g and 50 g. Significant interactions between genotypes and environment were observed, highlighting the importance of local adaptation of varieties. At the same time, SSR molecular markers have been used to study the associations between phenotypic traits and genetic diversity. Fifteen SSR markers were applied for the amplification of genomic DNA of the studied genotypes. The results revealed correlations between some SSR markers and key phenotypic traits, which could facilitate marker-assisted selection in breeding programs. The Xgwm190 marker was found to be significantly associated with thousand grains weight and plant height. This study provides valuable information on the genetic diversity and adaptation of durum wheat varieties to varied environments. The results obtained support breeding efforts to improve tolerance to water stress and increase productivity of durum wheat, thus contributing to food security.

Keywords: Durum wheat, genetic diversity, phenotypic traits, SSR markers, tolerance of water deficit.

INTRODUCTION

Durum wheat, an essential element of global food security, plays a crucial role in the supply of basic foodstuffs (Reynolds and Braun; 2022). It is a crop of primary importance in Algeria, where it is widely cultivated and consumed. However, yields of this cereal remain variable due to various environmental and genetic factors (Lobell et al., 2011 and Tsenov et al., 2020). Understanding the genetic basis of phenotypic traits, such as drought tolerance, disease resistance and grain quality, is essential for improving durum wheat varieties (Ballesta et al., 2020).

In Algeria (https://www.fao.org/faostat/fr/#country/4), over the last decades, durum wheat production has experienced significant development, reflecting its growing importance in the food chain. In 2022, the harvested area of durum wheat reached 2929807 hectares, with a yield of 16104 x 100 g/ha, resulting in an annual production of 4718,203.91 tons. This remarkable progression contrasts sharply with the figures for the year 2000, where the harvested area was only 10581 84 hectares, the yield was of 8833 x 100 g/ha and the annual production was of 934656.39 tons. These data illustrate not only a substantial increase in cultivated area and productivity, but also a response to the growing needs of the national population (Yachi et al., 2021).

The study of associations between fluctuations in different phenotypic traits related to yield and/or tolerance to growing conditions and molecular markers is an important approach in the genetic improvement of cultivated varieties (Ibrokhin et al., 2008). Microsatellites, DNA fragments consisting of units of 1 to 4 nucleotides, are codominant, highly polymorphic and widely dispersed molecular markers throughout the genome (Najimi et al. 2003). These markers are not influenced by environmental fluctuations and are independent of the organ analyzed and the stage of plant development, which gives them a particular interest in such an approach. (Rôder et al. 1998 and Chabane et al. 2008)

In this study, we used SSR (Simple Sequence Repeats) markers to study the associations between phenotypic traits and genetic variations in durum wheat grown on two distinct sites located in eastern Algeria: Constantine (site 1) and Khenchela (site 2). These two sites present different agro-climatic conditions, providing a unique opportunity to examine the genotype-environment interaction. The objective of this work is therefore twofold: **(i)** We aim to highlight the biological behavior of 36 durum wheat genotypes studied depending on the environment, by cultivating them on the sites. This approach will allow us to understand how different genotypes respond to varied environmental conditions, providing

crucial information for crop adaptation and resilience. (ii) We will then study possible associations between phenotypic traits, particularly those linked to stress and performance, and SSR molecular markers. By identifying these correlations, we aim to provide genetic improvement of durum wheat in order to increase its resilience to environmental stresses and optimizing its yields. This approach is essential to develop more robust and productive durum wheat varieties, thus ensuring sustainable food security for future generations.

MATERIAL AND METHODS

1- Plant Material

36 genotypes of durum wheat (*Triticum turgidum* L. var. durum were studied as plant materiel of different origins, provided by INRA in Montpellier, 2n = 4x = 28 chromosomes, AABB genome). Their choice was made on the basis of two essential criteria: (i) tolerance and resistance to water deficit and (ii) good productivity.

- Four genotypes belonging to two different primitive tetraploid species are used for their tolerance to water deficit conditions:
  - Species 1: *T. Dicoccum*: The two "variety" genotypes (female parents) retained for this species are *noricium* (symbolized: Dico1; Origin Spain) and *semicanum* (symbolized: Dico3; Yemen).
  - Species 2: *T. Polinicum*: The two varieties used as parents are *pseudochrysospermum* (symbolized: Polo9; Hungary) and *hadrache* (symbolized: Polo1; Morocco).

- Two genotypes (varieties) belonging to the species *Triticum durum* Desf are used as male parents:
  - WAHA (Cham1), a variety selected by ICARDA (International Center of Agricultural Research in Dry Areas) in Syria. Introduced in Algeria since 1975/1976, it was selected and popularized by TIFC (Technical Institute of Field Crops) experimental station in Constantine, Algeria
  - OUM-RABÏ, symbolized MRB5: variety selected by ICARDA.

The other genotypes, numbering 30, used are hybrids resulting from these different interspecific crosses as shown in Tables 1 and 2.

Table 1: List and direction of crossings

Female parent	Abbreviation	Male parent	Abbreviation	Designation and direction of crossing
T. dicoccum		*T. durum*		
Noricium Korn	Dico1*	Waha	Cham 1**	C1 : Dico1 x Cham1
Semicanum Krause	Dico3*	OUM-RABÏ	MRB5**	C2 : Dico3 x MRB5
T. polinicum		*T. durum*		
Pseudochrysospermum	Polo9*	OUM-RABÏ	Cham 1**	C3 : Polo9 x Cham1
Pseudochrysospermum	Polo9*	Cham 1	MRB5**	C4 : Polo9 x MRB5
Hadrache	Polo1*	OUM-RABÏ	MRB5**	C5 : Polo1 x MRB5

(*) Very resistant to water deficit;
(**) Sensitive to water deficit.

2- Methods

The study was conducted on two different geographical sites:

i. Site 1: ITGC experimental station (El khroub, Constantine City), at 36.38° North and 4.17° East and at an altitude of 640 m.
ii. Site 2: Kaïs vocational training center (Khenchela city) at 35.29° North, 6.55° East and at an altitude of 934 m.

These two zones are characterized by a cold winter and a hot dry summer (dry and cold semi-arid climate). A cumulative rainfall of 373 mm/year was recorded at site 1 compared to 363.7 mm/year at site 2 (table 2).

The tests were conducted, under rainy conditions, according to an experimental design in **r**andomized **c**omplete **b**locks (RCB) (Federer 1956) with 3 repetitions. Each genotype is shown in five lines 1.5 m long spaced 25 cm apart, i.e. plot units of 1.5 m² spaced 1 m apart.

Several agro-morphological parameters were measured: leaf area (LA: cm^2), plant height (PH), ear (or spike) length (EL), beard length (BL) and neck length of the ear (NLE), all expressed in cm. Yield components including the number of plants per meter (NPM), the number of tillers per plant (NTP), the number of ears per plant (NEP), the number of spikelet per ear (NSE) and the number of grains per ear (NGE) were also recorded. The thousand grain weight (TGW), grain yield (GY) and aboveground biomass yield (ABY) expressed in grams were measured and the harvest index (HI) of each genotype was determined.

Table 2: Climatic conditions for carrying out the tests

Season	Month / Param.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Total	Region
Average.30 Years	Average T (°C)	21.9	17	11.8	8.2	7.2	8.5	10.1	12.4	16.4	22.1		CONSTANTINE
	Rainfall (mm)	31.6	44	44.2	72.8	61.9	58.9	66	46.9	39.2	16.6	482.1	
Average.30 Years	Average T (°C)	22.5	18	11.5	7.5	7.4	8.4	10.5	13	18			KHENCHELA
	Rainfall (mm)	73	48	32	36	61.9	58.9	48	50	59	-	466.8	

3- Genotyping

3-1 **Extraction of genomic DNA**

The fresh leaves collected from young seedlings of the different genotypes, at the three-leaf stage were freeze-dried for two days. A quantity of 30 mg of freeze-dried leaves was cut, then ground into a fine powder in a 2 ml tube containing a bead. The tubes were placed in balanced plates and the samples were ground using a Mixer Mill MM300® type shaker-grinder (Qiagen).

The extraction of genomic DNA was carried out using the CTAB (Cetyl Trimethyl Ammonium Bromide) technique described by Saghai-Maroof et al. (1984) and modified by Udupa et al. (1999).

- ***Quality test of the extracted DNA****:* The quality and quantity of the extracted DNA were verified by the integrity and intensity of the DNA bands obtained after electrophoresis on 1% agarose gel, stained with agarose bromide ethidium (BET) and visualized under ultraviolet (UV) rays.

3-2 **Amplification and use of SSR markers**

A set of 15 microsatellites (Table 3) distributed over the A and B genomes was chosen from the map of microsatellites developed in common wheat (Triticum aestivum L) by Röder et al. (1998) and also from molecular information available on the Graingeenes database (http://www.graingenes.org). These markers were divided into two groups: (i) a first group

composed of GWM (Gatersleben Wheat Microsatellite) type markers and (ii): a second group composed of WMC (Wheat Microsatellite Consortium) type markers.

Polymerase chain reactions (PCR) were carried out, according to Udupa et al. (1999), in a total volume of 10 µl, containing 1x PCR buffer (with 1.5 mM $MgCl_2$), 200 µl of each dNTP (Deoxynucleoside triphosphates), 10 pmol of each primer, 0.5 units of Taq DNA polymerization and approximately 50 ng of extracted genomic DNA. The amplification reaction was generated in an Eppendorf Master thermal cycler with:

- Initial denaturation for 5 minutes at 94°C;
- 35 (cycles) minutes including:
    - 30 seconds of denaturation at 94°C,
    - 30 seconds of hybridization at various temperature 55-60°C,
    - 45 seconds of elongation at 72°C,
    - Followed by a final elongation at 72°C for 6 minutes and
    - Cooling to 4°C for an indefinite period.

The amplifies obtained were separated on 8% polyacrylamide gels under denaturing (native) conditions

Table 3: Set of microsatellites for SSR amplification

Primer Name	Forward Primer (5’⟶ 3’)	% GC	Length	Reverse Primer (5’ ⟶ 3’)	% GC	Length	Localization	Repeated pattern	An. T. (°C)
XWmc24	GTGAGCAATTTTGATTATACTG	31.8	22	ATCCCTGATGCTGTAATATGTG	40.9	22	1AS	(GT) 28	55
XWmc420	ATCGTCAACAAAATCTGAAGTG	36.3	22	TTACTTTTGCTGAGAAAACCCT	38.3	22	2 and 4AS	(GT)26	50
XgWm319	GGTTGCTGTACAAGTGTTCACG	50	22	CGGGTGCTGTGTGTAATGAC	55	20	2BS	(CT)11 (N)23 (CT)6	55
Xstm773	ATGGTTTGTTGTGTTGTGTGTTAGG	41.6	24	AAACGCCCCAACCACCTCTCTC	59	22	2BS	ND	60
XgWm361	GTAACTTGTTGCCAAAGGGG	50	20	ACAAAGTGGCAAAAGGACACA	45	20	6BL	(GA)20	60
XgWm389	ATCATGTCGATCTCCTTGACG	52.4	21	TGCCATGCACATTAGCAGAT	45	20	3BS	(CT)14 (GT)16	60
XgWm193	GTAACTTGTTGCCAAAGGGG	50	20	ACAAAGTGGCAAAAGGACACA	42.9	21	6BS	(CT) 24	60
XgWm273	ATTGGACGGACAGATGCTTT	45	20	ACCAGTGAGGAAGGGGATC	57.8	19	1BS	(GA)18	55
XgWm344	CAAGGAAATAGGCGGTAACT	45	20	ATTTGAGTCTGAAGTTTGCA	35	20	7BL	(GT)24	55
XgWm513	ATCCGTAGCACCTACTGGTCA	52.3	21	GGTCTGTTCATGCCACATTG	50	20	5BS	(CA) 12	60
XgWm44	GTTGAGCTTTTCAGTTCGG	47.4	19	ACTGGCATCCACTGAGCTG	57.9	19	4AS	(GA) 28	60
XgWm146	CCAAAAAAACTGCCTGCATG	45	20	CTCTGGCATTGCTCCTTGG	57.9	19	7BL	(GA) 5 GG(GA) 20	60
XgWm247	GCAATCTTTTTTCTGACCACG	42.8	21	ATGTGCATGTCGGACGC	58.8	17	3A, 3BL	(GA) 24	55
Xgwm257	AGAGTGCATGGTGGGACG	61.1	18	CCAAGACGATGCTGAAGTCA	52.6	19	2BS	(GT) 30	60
XgWm533	AAGGCGAATCAAACGGAATA	40	20	GTTCCTTTAGGGGAAAACCC	45	20	3BS	(CT)18(CA)20	60

4- Statistical Analysis

4-1 Descriptive statistical parameters, normality tests of experimental data and Principal Component Analysis (PCA) were carried out using the computer program GraphPad PRISM v.10 (GraphPad Software, San Diego, California, United States). Results are given as mean ± standard deviation ($m \pm \sigma$). The normality of the distribution of the variables measured for all these characters was assessed using the Kosmogorov-Smirnov test by comparing the empirical cumulative distribution of the observed data to the theoretical cumulative distribution of a normal distribution. To corroborate the result of this test, a graph of the Gaussian distribution was made to assess the normality of the measured variables. At this point, we recall that, for the case of statistical tests, the decision method is as follows: if the p-value is less than α (α= 0.05), we reject the null hypothesis (H_0) and conclude that the two population parameters are not equal. Otherwise, if the p-value is large (i.e. greater than α), we do not reject H_0 and can then assume that the two parameters are the same. The parameters linked to stress tolerance and those linked to performance were compared using a Student test carried out on GraphPad Prism 10.0

4-2 **Principal component analysis**: was also carried out with GraphPad PRISM v.10 software (GraphPad Software, San Diego, California, United States). It is a dimensionality reduction technique that helps reveal underlying structures in our data, such as linear relationships between parameters that are not immediately apparent in the original variable space. We relied on reading the eigenvalues to indicate the amount of variance explained by each principal component. High values will mean that the component will explain a large part of the variance in the data. The eigenvectors (loadings) indicate the importance of each parameter for each principal component. For high values, the factor will contribute strongly to the principal component.

4-3 **Genotypic diversity**: The Shannon-Weaver index (H), is a measure of diversity based on frequency data. We used it to evaluate the geographic causes of diversity and to specifically determine the relative contributions of the two sites (Constantine and Khenchela) to the genetic resources of our durum wheat collection consisting of 36 genotypes

First, we determined the number of classes (k) for each of the two traits: Leaf surface and beard length; because these two factors significantly contributed to the construction of the two main components (PC1 and PC2 respectively). To that end, we used Sturges' formula:

$$k = 1 + 3.22 \log_{10}(N); \text{ with } N = 36 \text{ genotypes.}$$

The number of classes k is equal to six (k = 6) for both sites because N is 36 individuals in each site. The determination of the six classes was carried out by calculating the extent and amplitude:

Span (E) = LAmax – LAmin = 29.88 – 17.25 = 12.63 cm^2.

Amplitude (a) = E/k = 12.63/6 = 2.11 cm^2.

In the case of the Khenchela site, E = 22.28 – 14.24 = 8.04 cm^2 and a = 8.04/6 = 1.34 cm^2.

Using the class frequency distribution data, the Shannon-Weaver diversity index (H) was calculated for the two traits Leaf area and awn length according to the equation:

$$H = - \sum_1^s pi * log_2(pi)$$ (Jain et al., 1975).

Or :

- pi = the proportional abundance of the class within the trait (pi = ni/N).
- ni = the number of genotypes counted for an observed class.
- N = the total number of genotypes analyzed (N=36 for each Wilaya).
- S = the total number of classes present for a given trait (S=6).

Since different phenotypic classes have been recognized for the two traits, H was standardized by converting it to a relative index, H', by dividing it by Hmax = Ln (N) (Eticha et al., 2004).

$$H = - \sum_1^s pi * log_2(pi) / Ln(N)$$ (Jain et al., 1975).

Taking into account the classification proposed by Eticha et al. (2004), the relative diversity index (H') can be:

i. H' ≥ 0.60: High Diversity index;
ii. 0.40 ≤ H' < 0.60: Intermediate index;
iii. 0.10 ≤ H' < 0.40: Low index.

4-4 Logistic regression: was carried out on Prism 10.0 to model possible associations between explanatory variables (agronomic parameters measured on 36 genotypes) and a binary response variable (molecular markers). To that end, we tested the association between 15 molecular markers (alleles/SSR) and 14 phenotypes (Table 4), including five representatives of stress tolerance and nine associated with yield components.

Table 4: Explanatory variables (phenotypes) and binary variable (Alleles/SSR).

Binary response	Markers (n=15)	XgWm146, XgWm344, XgWm273, XgWm247, Xgwm257, XWmc24, Xgwm44, XWmc420, XgWm319 (Forward), Xstm773 (Forward), XgWm361 (Forward), XgWm389, Xgwm533, XgWm193, Xbarc263 (Forward)
Explanatory variables	Stress tolerance factors (n=5)	Leaf area (LA), Plant height (PH), Ear length (EL), Beard length (BL), Neck length of the Ear (NLE).
	Factors related to performance components (n=9)	Number of plants/linear meter (NPM), Number of tillers/plant (NTP), Number of ear per plant (NEP), Number of spikelet /ear (NSE), Number of grains/ear (NGE), a thousand grains Weight (TGW), aboveground biomass yield (ABY), harvest index (HI).

We used IBM SPSS Statistics software (v.20.0.2.0) to perform binary logistic regression between the markers (SSRs) and the different phenotypes. The SSR markers were coded as 1 and 0 to indicate respectively the presence of the band and its absence in a given genotype. The significance level was maintained at $\alpha \leq 0.05$.

RESULTS

1- Descriptive analysis of the measured parameters

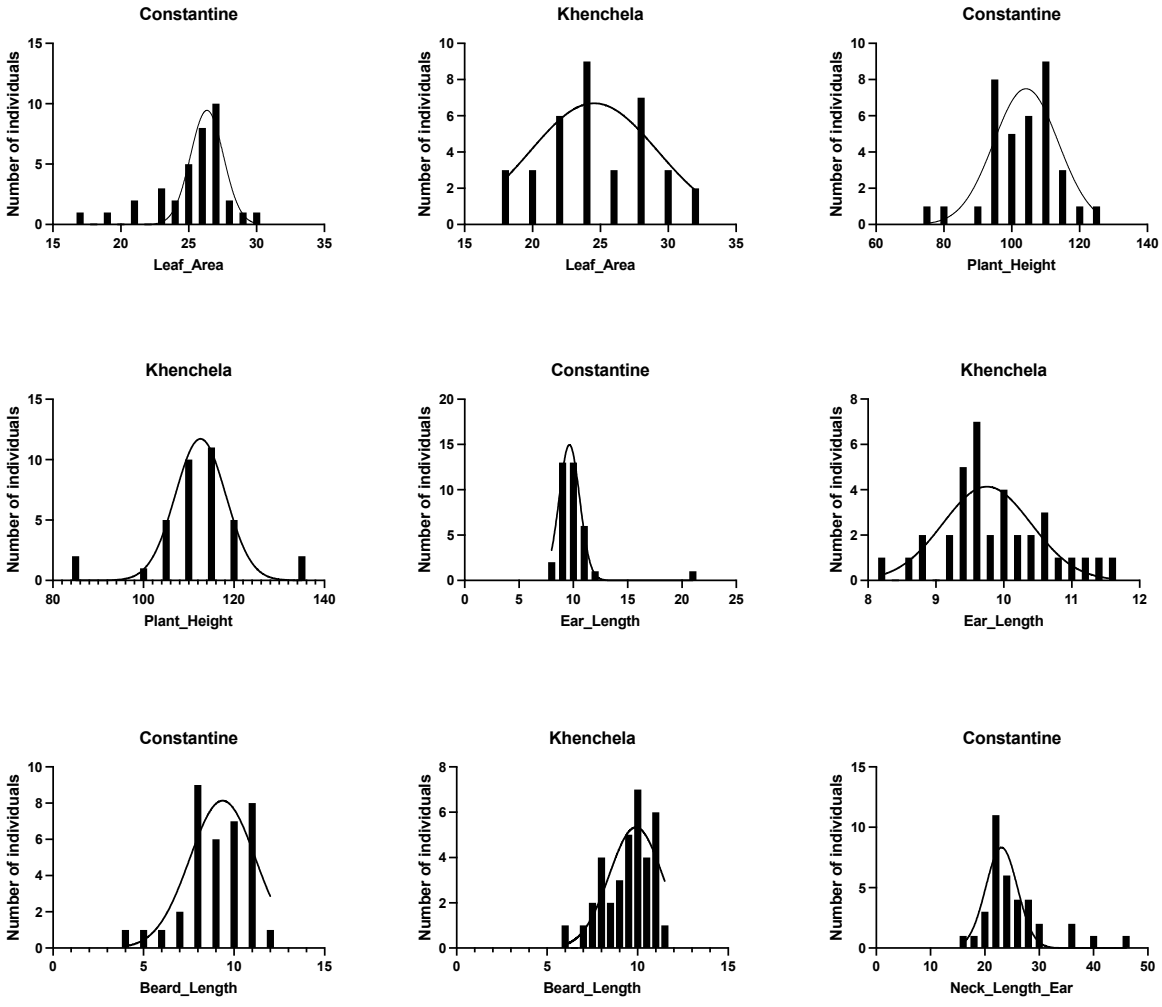
The different parameters of the descriptive statistics for the two sites (1: Constantine and 2: Knenchela) are shown in tables 5 and 6 (figure 1).

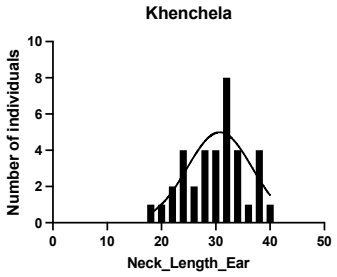
Table 5: Descriptive statistical parameters of phenotypes linked to water stress tolerance.

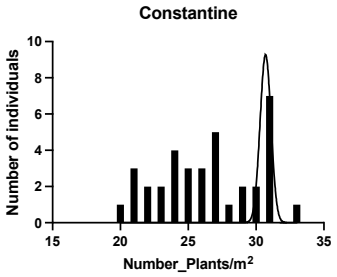
	LA		PH		EL		BL		NLE	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Minimum	17,25	17,03	77,06	85,80	7,944	8,189	4,056	6,013	15,00	18,32
25% Percentile	24,13	21,82	96,67	108,5	9,181	9,400	7,847	8,586	21,82	25,43
Median	25,94	24,06	103,8	112,4	9,756	9,734	9,289	9,807	23,64	31,23
75% Percentile	27,10	28,41	109,0	116,7	10,49	10,48	10,49	10,47	28,52	33,86
Maximum	29,88	32,39	123,3	134,5	21,28	11,58	11,52	11,34	45,98	39,24
Mean	**25,37**	**24,73**	**102,4**	**112,1**	**10,09**	**9,879**	**8,993**	**9,479**	**25,46**	**29,96**
Std. Deviation	2,665	3,969	9,963	9,470	2,112	0,7718	1,850	1,289	6,232	5,604
Std. Error of Mean	0,4442	0,6615	1,661	1,578	0,3520	0,1286	0,3084	0,2148	1,039	0,9339
Coefficient of variation (%)	10,51	16,05	9,726	8,450	20,92	7,813	20,57	13,59	24,48	18,71

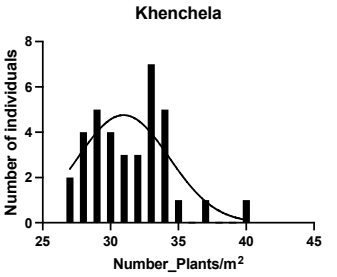
Table 6: Descriptive statistical parameters of performance-related phenotypes

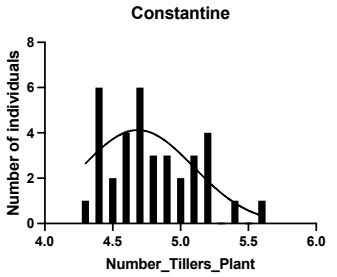
		Min	25% Percentile	Median	75% Percentile	Max	Mean	Std. Deviation	Std. Error of Mean	Coefficient of variation
NPM	C	20,22	23,67	26,39	29,86	32,67	26,39	3,564	0,5941	13,51
	K	26,89	29,25	31,39	32,89	39,89	31,43	2,858	0,4764	9,095
NTP	C	4,333	4,514	4,717	5,042	5,556	4,796	0,3105	0,0517	6,474
	K	3,833	4,458	4,806	5,097	5,444	4,770	0,3740	0,0623	7,840
NEP	C	3,333	3,625	3,833	4,042	4,667	3,850	0,2251	0,0558	8,705
	K	3,444	3,750	4,084	4,389	4,667	4,065	0,3737	0,0623	9,194
NSE	C	17,47	19,17	19,98	21,51	24,67	20,30	1,725	0,2875	8,496
	K	16,78	18,25	19,00	20,78	24,00	19,53	1,747	0,2911	8,943
NGE	C	21,78	23,47	25,61	27,44	28,78	25,51	2,069	0,3448	8,111
	K	27,56	31,22	33,17	35,14	39,78	33,42	2,830	0,4717	8,468
TGW	C	25,76	30,20	31,49	32,30	36,73	31,41	2,292	0,3820	7,298
	K	28,99	33,73	35,88	36,99	41,62	35,52	2,974	0,4956	8,371
GY	C	164,7	203,6	211,2	228,4	264,1	214,8	22,63	3,772	10,54
	K	281,2	358,2	393,5	413,5	585,6	389,8	58,08	9,680	14,90
ABY	C	690,6	789,0	839,3	934,0	1078	859,5	86,84	14,47	10,10
	K	1006	1229	1291	1410	1615	1307	128,2	21,37	9,807
HI	C	0,1960	0,2185	0,2460	0,2868	3,488	0,3411	0,5408	0,0901	158,6
	K	0,2080	0,2870	0,2990	0,3193	6,663	0,4751	1,061	0,1769	223,4

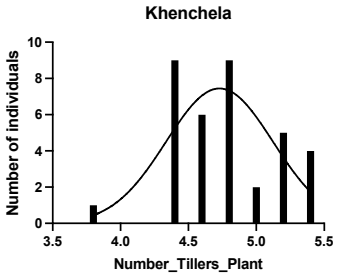


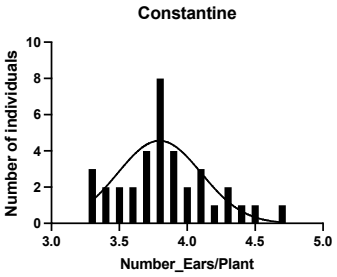

Khenchela
Number of individuals
Neck_Length_Ear

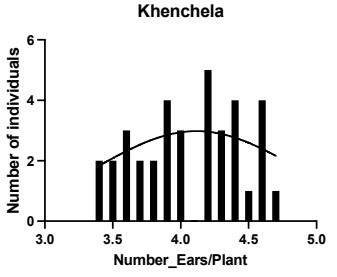

Constantine
Number of individuals
Number_Plants/m2

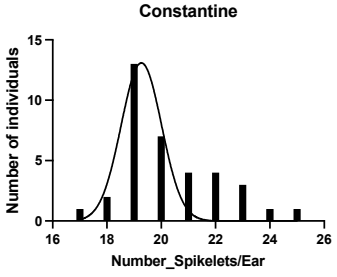

Khenchela
Number of individuals
Number_Plants/m2

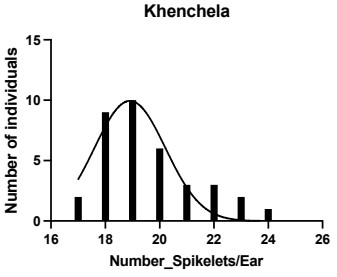

Constantine
Number of individuals
Number_Tillers_Plant

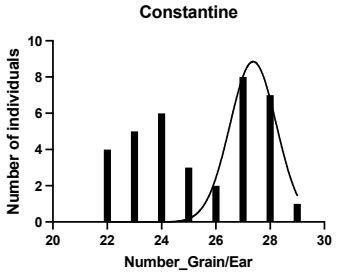

Khenchela
Number of individuals
Number_Tillers_Plant

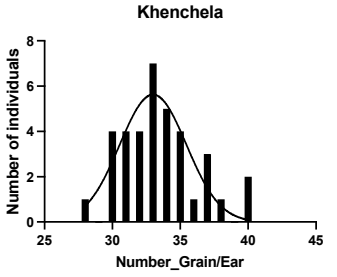

Constantine
Number of individuals
Number_Ears/Plant

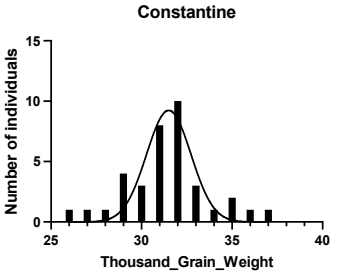

Khenchela
Number of individuals
Number_Ears/Plant

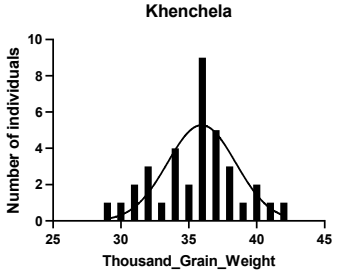

Constantine
Number of individuals
Number_Spikelets/Ear

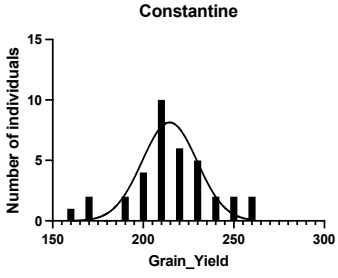

Khenchela
Number of individuals
Number_Spikelets/Ear

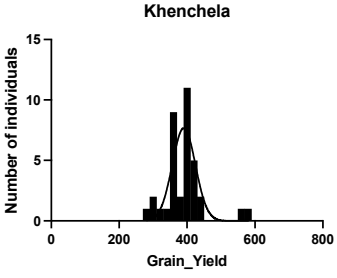

Constantine
Number of individuals
Number_Grain/Ear


Khenchela
Number of individuals
Number_Grain/Ear


Constantine
Number of individuals
Thousand_Grain_Weight


Khenchela
Number of individuals
Thousand_Grain_Weight


Constantine
Number of individuals
Grain_Yield


Khenchela
Number of individuals
Grain_Yield

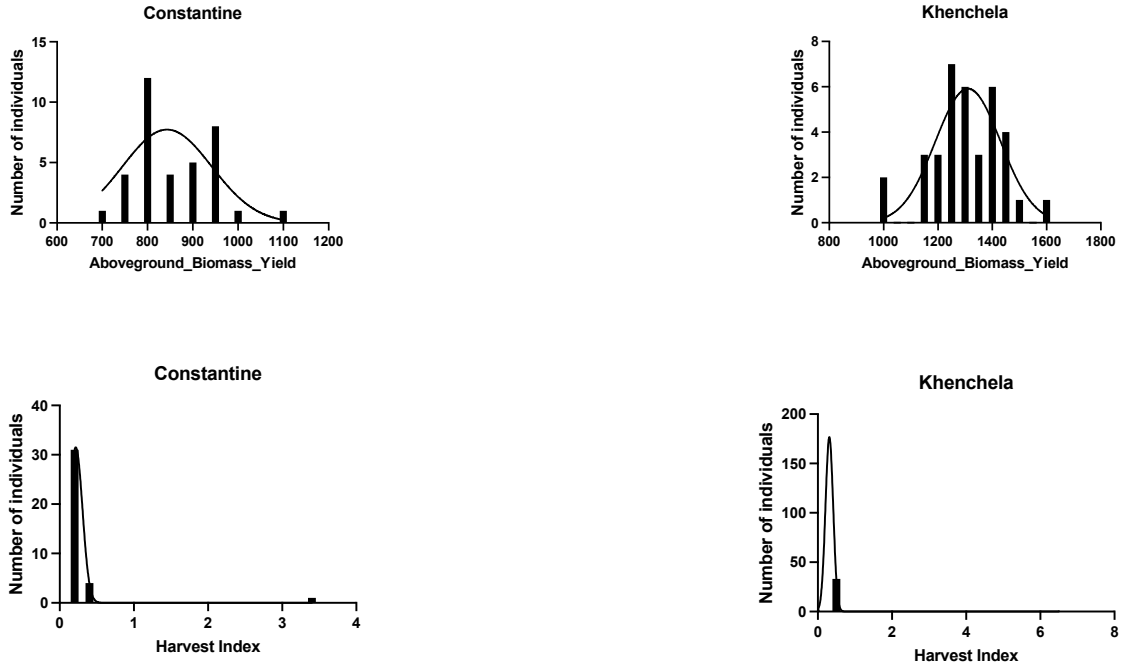


Figure 1: Gaussian distributions of the 14 traits measured on 36 genotypes

Our study revealed significant differences (Tables 7 and 8, Figure 2) between the genotypes grown in Constantine and those grown in Khenchela. In particular, genotypes grown in Constantine showed significantly greater leaf area and ear (spike) length than those observed in genotypes grown in Khenchela. However, this same study showed that the genotypes cultivated in Khenchela presented superior morphological characteristics compared to those cultivated in Constantine. Indeed, plant height and beard length were significantly higher in Khenchela genotypes. Alone, the NLE parameter did not show a significant difference between the genotypes of the two sites. Compared to the parameters related to yield, the number of plants (p=0.4036), grain yield (p=0.0970) and HI (0.2415) did not express significant differences between the two sites.

Table 7: t-tests for parameters linked to water stress

	LA	PH	EL	BL	NLE
t-Test	11,83	5,329	4,196	2,827	0,5540
p-Value	<0,0001	<0,0001	<0,0001	0,0061	0,5813
Significance	****	****	****	**	ns
p (Equality of variances)	0,2961	0,6985	<0,0001	0,9867	0,3969
Significance	ns	ns	****	ns	ns

ns: not significant ; ** : moderately significant ; **** : highly significant

Table 8: t_Tests for parameters linked to yield

	NPM	NTP	NEP	NSE	NGE	TGW	GY	ABY	HI
t-Test	10,89	0,8404	3,308	4,091	6,530	6,467	1,682	4,637	1,181
p-Value	<0,0001	0,4036	0,0015	0,0001	<0,0001	<0,0001	0,0970	<0,0001	0,2415
Significance	****	ns	**	****	****	****	ns	****	ns
p (Equality of variances)	<0,0001	0,0851	0,0288	0,2047	0,5660	0,0105	0,0626	0,3179	<0,0001
Significance	****	ns	*	ns	ns	*	ns	ns	****

ns: not significant ; * : weakly significant; ** : moderately significant ; **** : highly significant

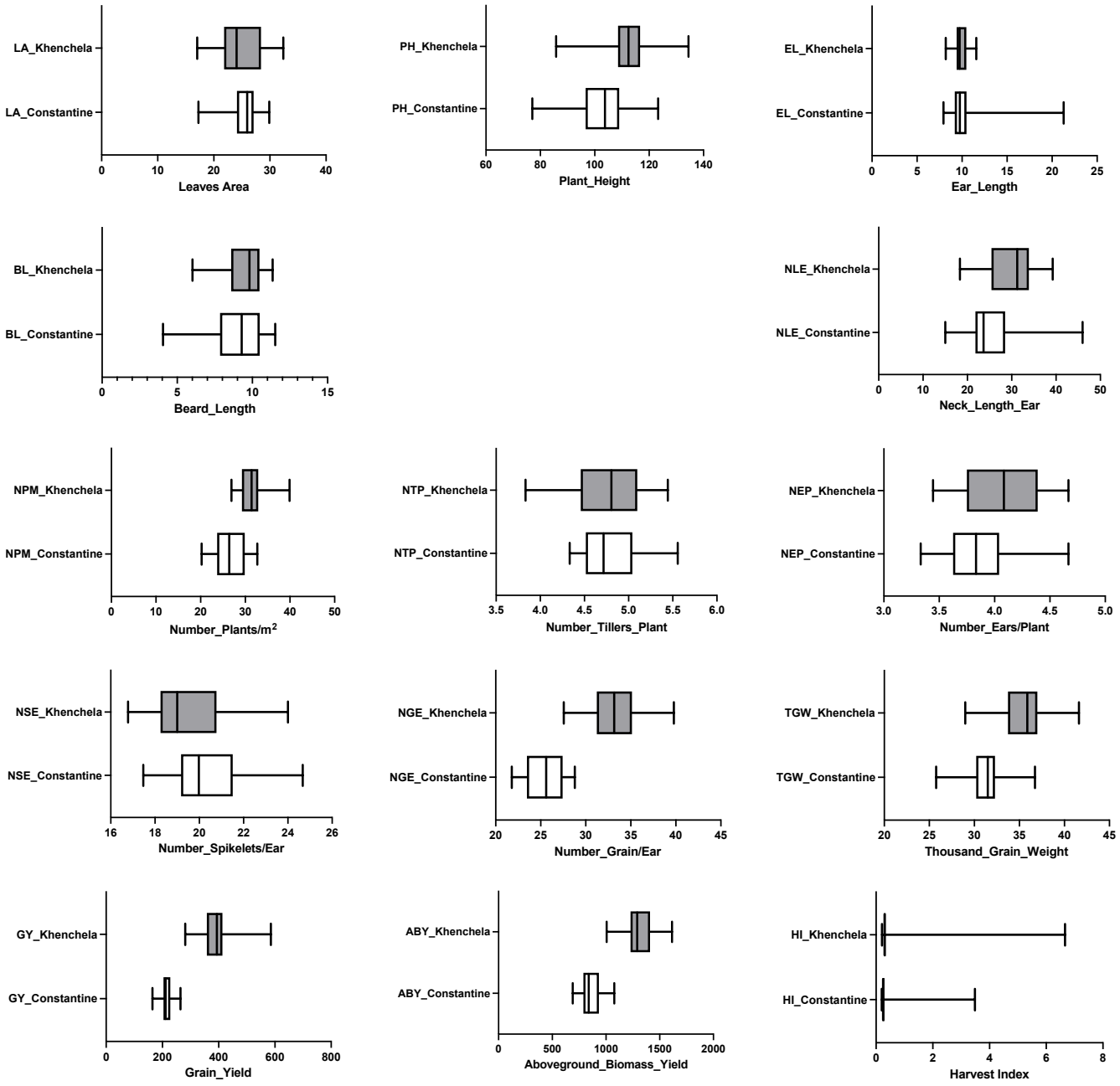


Figure 2: Box and whisker plots of parameters linked to water stress

2- Principal components analysis

PCA results indicate that the first three principal components explain a substantial proportion of the total variance in the data (Table 9, figure 3). The first principal component (PC1) has an eigenvalue of 4.199, which represents a significant portion of the variance. The second principal component (PC2) has an eigenvalue of 2.602, and the third principal component (PC3) has an eigenvalue of 1.912. Together, these three main components explain a large part of the total variance of the data, allowing an effective reduction of dimensionality while retaining the essential information. The values of the eigenvectors are shown in Table 10.

Table 9: Eigenvalues of the principal components

Principal component (CP)	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC9	PC10	PC11	PC12	PC13	PC14	PC15
Eigenvalues	4,199	2,602	1,912	1,232	0,863	0,757	0,611	0,442	0,343	0,299	0,260	0,199	0,163	0,118

Table 10: Values of the eigenvectors

	PC1	PC2	PC3
LA	**0,869**	-0,249	0,091
PH	0,849	0,271	0,161
EL	0,731	0,214	-0,095
BL	-0,001	**-0,803**	-0,001
NLE	0,517	0,651	0,310
NPM	0,469	-0,512	-0,303
NTP	0,165	0,536	-0,551
NEP	-0,368	0,403	-0,619
NSE	0,701	0,389	-0,099
NGE	-0,489	0,616	-0,127
TGW	0,665	-0,168	-0,180
GY	-0,033	-0,239	**-0,822**
ABY	0,605	-0,119	-0,313
HI	0,032	-0,097	-0,403

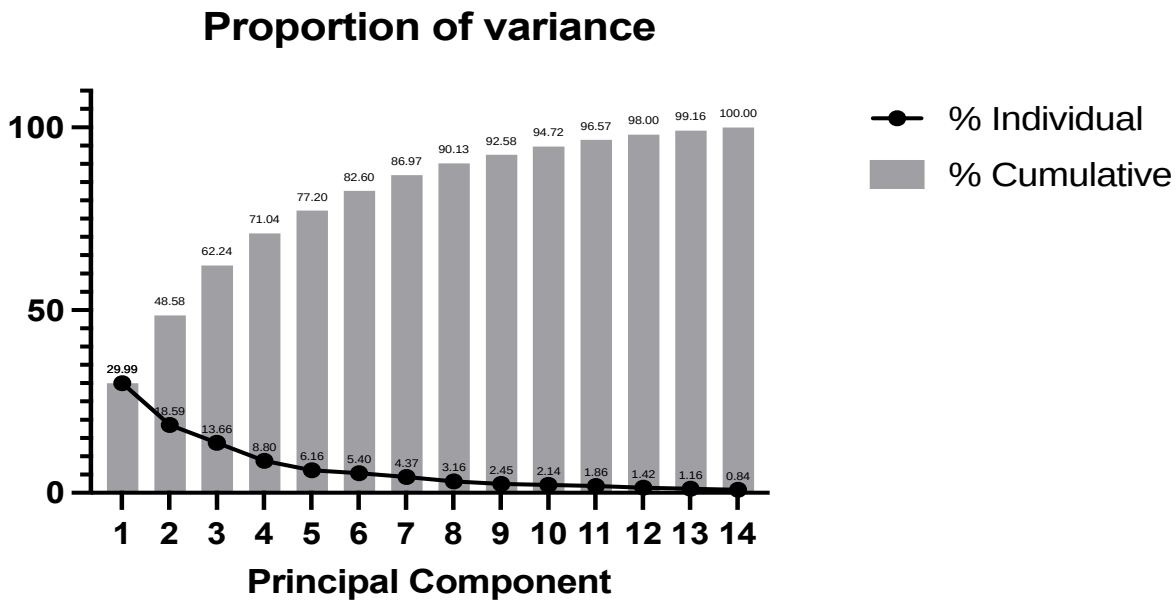


Figure 3: Proportion of variance

PC1 component is mainly influenced by leaf area (LA, 0.869), plant height (PH, 0.849), and ear (spike) length (EL, 0.0.731). These variables have high and positive coefficients, indicating that they are all positively correlated and are the most important for this component. PC1 could be interpreted as an overall measure of plant size. Furthermore, we note that variables NSE coefficients (0.701), TGW (0.665), and ABY (0.605) are all positive and relatively high. This means that they have a strong influence on the first component PC1 which can be interpreted as a general measure of productivity. This principal component captures variance related to the ability of genotypes to produce high quantity and quality of biomass and grains.

PC2 component is strongly influenced by the length of the beard (LB, -0.803) and the neck length of the ear (NLE, 0.651). PC2 captures a dimension of variation linked primarily to ear characteristics and awn length. The NPM, NTP and NGE parameters showed equally large correlation coefficients (-0.512, 0.536 and 0.616 respectively). CP2 can then be seen as a component measuring a balance or divergence between the density of genotypes and productivity per plant and per ear. CP2 makes it possible to distinguish variations due to growth strategies focused either on high density or on high individual productivity.

The correlation circle (figure 3) makes it possible to visualize the relationships between the parameters studied in order to interpret the biological meaning of the main components. The circle of correlations shows that PC1 is mainly influenced by variables related to plant size and productivity, while PC2 is influenced by variables related to the structure and density of ears and grains.

The analysis of this circle of correlations shows two main clusters. A cluster located on the positive part of CP1 and which varies between positive and negative values of CP2. Likewise, the second cluster is located in the negative part of CP1 and varies between positive and negative values of CP2.

The parameters strongly correlated with PC1 (LA, PH, EL, NSE, TGW and ABY) are main characteristics that differentiate these two clusters. Nonetheless, LB, NLE, NPM, NTP and NGE parameters play a secondary role in genotype differentiation.

The correlation matrix (table 11) indicates a significant link between plant height with neck length of the ear: NLE (r=0.681), with NSE (r=0.667) and with thousand grain weigh: TGW (r= 0.526). Nevertheless, the length of the ear is correlated with the parameters Aboveground biomass yield (r=0.576) and NSE.

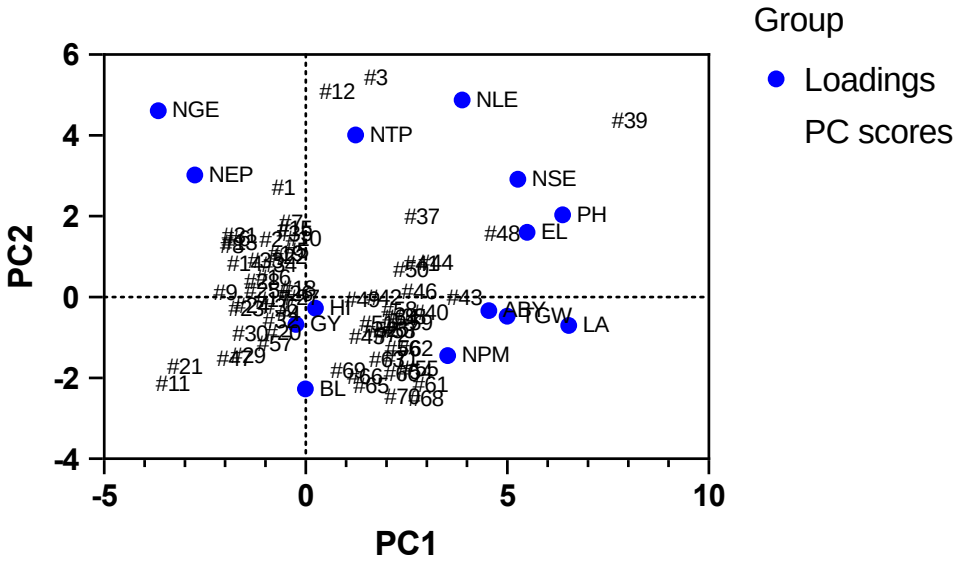


Figure 4: Circle of correlations and eigenvectors.

Table 11: Correlation matrix

	LA	PH	EL	BL	NLE	NPM	NTM	NEP	NSE	NGE	TGW	GY	ABY	HI
LA	1,000	0,674	0,556	0,138	0,245	0,517	0,002	-0,400	0,463	-0,620	0,521	-0,101	0,506	0,008
PH	0,674	1,000	0,515	-0,148	0,681	0,243	0,235	-0,259	0,667	-0,250	0,526	-0,200	0,348	-0,099
EL	0,556	0,515	1,000	-0,182	0,420	0,114	0,183	-0,123	0,512	-0,208	0,398	0,028	0,576	-0,021
BL	0,138	-0,148	-0,182	1,000	-0,449	0,292	-0,389	-0,247	-0,228	-0,403	0,182	0,205	0,084	-0,020
NLE	0,245	0,681	0,420	-0,449	1,000	-0,155	0,194	-0,152	0,563	0,148	0,225	-0,326	0,098	-0,081
NPM	0,517	0,243	0,114	0,292	-0,155	1,000	0,015	-0,263	0,249	-0,434	0,311	0,287	0,247	0,296
NTM	0,002	0,235	0,183	-0,389	0,194	0,015	1,000	0,521	0,274	0,136	0,095	0,156	0,149	0,050
NEP	-0,400	-0,259	-0,123	-0,247	-0,152	-0,263	0,521	1,000	-0,105	0,342	-0,155	0,309	-0,084	0,030
NSE	0,463	0,667	0,512	-0,228	0,563	0,249	0,274	-0,105	1,000	0,040	0,346	-0,042	0,311	0,184
NGE	-0,620	-0,250	-0,208	-0,403	0,148	-0,434	0,136	0,342	0,040	1,000	-0,388	0,110	-0,331	0,020
TGW	0,521	0,526	0,398	0,182	0,225	0,311	0,095	-0,155	0,346	-0,388	1,000	0,205	0,288	0,085
GY	-0,101	-0,200	0,028	0,205	-0,326	0,287	0,156	0,309	-0,042	0,110	0,205	1,000	0,320	0,233
ABY	0,506	0,348	0,576	0,084	0,098	0,247	0,149	-0,084	0,311	-0,331	0,288	0,320	1,000	-0,112
HI	0,008	-0,099	-0,021	-0,020	-0,081	0,296	0,050	0,030	0,184	0,020	0,085	0,233	-0,112	1,000

3- Shannon-Weaver diversity index

The different classes, their numbers and their respective frequencies (pi = ni/36) for the Constantine and Khenchela sites are shown in Table No. 12.

Table 12: Classes, numbers and frequencies for leaf area

Constantine			Khenchela		
Classes Ci	Numbers ni	pi	Classes Ci	Numbers ni	pi
[17,25 – 19,36[	2	0,056	[14,25 – 15,59[	2	0,056
[19,36 – 21,47[	2	0,056	[15,59 – 16,93[	7	0,194
[21,47 – 23,58[	3	0,083	[16,93 – 18,27[	10	0,278
[23,58 – 25,69[	9	0,25	[18,27 – 19,61[	6	0,167
[25,69 – 27,80[	16	0,444	[19,61 – 20,95[	3	0,083
[27,80 – 29,91]	4	0,111	[20,95 – 22,29]	8	0,222

The variability index (H) of the second factor (beard length) measured for the two sites gave the results summarized in Table 13.

Table 13: Classes, numbers and frequencies for beard length

Site 1			Site 2		
Classes Ci	Numbers ni	pi	Classes Ci	Numbers ni	pi
[17,25 – 19,36[	2	0,056	[14,25 – 15,59[	2	0,056
[19,36 – 21,47[	2	0,056	[15,59 – 16,93[	7	0,194
[21,47 – 23,58[	3	0,083	[16,93 – 18,27[	10	0,278
[23,58 – 25,69[	9	0,25	[18,27 – 19,61[	6	0,167
[25,69 – 27,80[	16	0,444	[19,61 – 20,95[	3	0,083
[27,80 – 29,91]	4	0,111	[20,95 – 22,29]	8	0,222

Concerning Constantine site, a calculated value of H equal to 2.134 is relatively high, which suggests considerable diversity in our sample. This means that the sample contains several categories of leaf areas, and that these categories are relatively well distributed in terms of abundance in Constantine region. This suggests good variability and possible resilience of the population studied. However, in Khenchela, this index is 2.416. The diversity of leaf area of genotypes is higher in Khenchela. Indeed, in terms of wealth, there are probably more LA categories represented in Khenchela compared to Constantine. In addition, we add that the distribution of leaf areas among the classes is more balanced in Khenchela.

The comparison of the Shannon indices for beard length of the 36 genotypes, between Constantine (H = 2.276) and Khenchela (H = 2.346), shows that Khenchela has slightly higher diversity. This suggests that the Khenchela site, in terms of richness, either has a slightly higher number of beard length classes, or that the classes are better distributed. Additionally, the distribution of beard lengths among categories is slightly more balanced in Khenchela.

The variability index (H) of the third factor (Grain yield) measured for the two sites gave the results summarized in Table 15.

Table 14: Classes, numbers and frequencies for grain yield

Site 1			Site 2		
Classes C_i	Numbers n_i	p_i	Classes C_i	Numbers n_i	p_i
[164,72 – 181,28[	3	0,083	[157,84 – 174,87[	5	0,139
[181,28 – 197,84[	3	0,083	[174,87 – 191,90[	11	0,306
[197,84 – 214,40[	13	0,361	[191,90 – 208,94[	6	0,167
[214,40 – 230,96[	10	0,278	[208,94 – 225,97[	2	0,056
[230,96 – 247,52[	3	0,083	[225,97 – 243,00[	7	0,194
[247,52 – 264,08]	4	0,111	[243,00 – 260,03]	5	0,139

At the population level of the 36 genotypes studied, the relative diversity index H' varies from intermediate, for the Leaf Surface trait (Constantine site, H' = 0.596), to high level

for the other traits (Beard Length and Grain Yield) in both sites (Table 15). The highest value is observed for the grain yield (GY) character in the Khenchela site (H'=0.728). Considering the average per site, the variability at Khenchela (H' = 0.686) is more pronounced than at Constantine (H' = 0.637).

Table 15: Relative diversity indices (H') for three characters measured in 36 durum wheat genotypes.

	LA	BL	GY
Constantine	**0,596**	0,674	0,680
Khenchela	0,674	0,655	**0,728**
F-Test	1,131	1,03	1,07
p-value	0,4479	0,4875	0,4713

The binary logistic regression gave (for the two sites) the results shown in the following tables 16 and 17.

Table 16: Statistical parameters of binary logistic regression in Constantine

				IC à 95% de Exp(B)	
Parameter	Marker	Sig.	Exp(B)	Inferior	Superior
LA	XgWm273 (R)	0,043	6,270	1,062	37,034
BL	XWmc24 (F)	0,026	2,040	1,088	3,826
	XgWm247 (R)	0,039	0,517	0,277	0,966
	XWmc420 (R)	0,030	0,511	0,278	0,936
NPM	XWmc24 (F)	0,023	0,694	0,506	0,951
	Xgwm44 (F)	0,037	0,622	0,398	0,972
	Xstm773 (F)	0,045	0,667	0,450	0,991
NTP	Xgwm44 (F)	0,099	38,507	0,501	2957,200
ABY	XgWm389 (F)	0,030	0,552	0,322	0,943

Table 17: Statistical parameters of binary logistic regression in Khenchela

				IC à 95% de Exp(B)	
Parameter	Marker	Sig.	Exp(B)	Inferior	Superior
PH	XgWm344 (R)	0,048	0,689	0,476	0,997
NTP	XgWm389 (R)	0,046	38,535	1,068	1390,257
NGE	XWmc24 (F)	0,014	3,944	1,315	11,830
	Xgwm44 (R)	0,015	3,941	1,304	11,909
	XWmc420 (R)	0,008	3,887	1,427	10,585
GY	XgWm344 (F)	0,029	2,319	1,091	4,927
ABY	XWmc24 (F)	0,040	0,875	0,770	0,994
	XgWm344 (F)	0,028	0,805	0,663	0,997
HI	XWmc24 (F)	0,050	0,000	0,000	1,316
	XgWm344 (F)	0,027	0,000	0,000	0,000

The binary logistic regression analysis was used to explore the associations between several molecular markers and phenotypic traits associated with water stress as well as yield components in *Triticum turgidum* L. var. durum. The results revealed significant associations between certain molecular markers and the phenotypes studied, highlighting their potential in modulating the organism's responses to water stress and determining yield.

The SSRs markers are widely used in genetic studies for their high polymorphism and their ability to reveal subtle genetic variations within and between populations (Schlötterer, 2004). Understanding how these markers are associated with phenotypic traits can provide valuable information for marker-assisted breeding and genetic improvement of crops.

Among the molecular markers studied, the 344 R (khenchela), 24 F, 247 R and 420 R (Constantine) alleles showed a particularly strong association with phenotypes linked to water stress. For example, the 344 R allele is strongly associated with the phenotypic parameter PH (plant height) with a significance threshold equal to 0.048 and an Exp(B) of 0.689. This value indicates that the presence of the 344 R marker is associated with an increase in the chances of having a plant height by a factor of 0.689 compared to its absence. In other words, the probability of having a spike with a good height is 6.89 times higher when the 344 R marker is present. Furthermore, the p-value of 0.048 is below the threshold of 0.05, which means that this association is statistically significant. There is less than a 5% chance that this association is due to chance. These results suggest a crucial role of this molecular marker in the regulation of the adaptive responses of *Triticum turgidum* to water stress.

Additionally, the analyses also highlighted associations between certain molecular markers and yield components, such as [insert yield components studied, e.g. grain yield, biomass weight, etc.]. These associations indicate a possible influence of metabolic pathways or biological processes regulated by these molecular markers on the overall performance of [plant/organism] in terms of yield, even under water stress conditions.

DISCUSSION

We observed that the genotypes grown in Constantine had a larger leaf area and a longer ear awn length compared to those grown in Khenchela. These differences can be attributed to several environmental and agronomic factors specific to each region.

The leaf area is a key indicator of plant photosynthetic capacity and is strongly influenced by environmental conditions such as water availability, nutrients and light exposure (Placide et al., 2015). In Constantine, climatic conditions may be more favorable for leaf growth due to better water availability and more nutrient-rich soil conditions. A study by Behouhou et al. (2022) showed that plants grown in rich soil conditions and with adequate irrigation develop a larger leaf area. Ear awn (beard) length is also an important agronomic trait that can be influenced by genetic and environmental factors. Bastos et al. (2020) on the one hand and Salmi et al. (2021), on the other hand, found that awn length is positively correlated with the plant's ability to capture light and carry out photosynthesis, which can be enhanced under optimal growing conditions. In Constantine, the genotypes could benefit from better growing conditions, thus favoring greater ear awn (beard) length.

The results of this analysis are consistent with those of Frantova et al. (2022), who observed that optimal environmental conditions, such as those found in regions with fertile soils and adequate water management, lead to an increase in leaf area and ear awn length. Furthermore, the work of Huzsvai et al. (2022) on regional variations in plant growth suggest that differences observed between two distinct geographic sites can be explained by local variations in climate and agricultural practices.

The significant differences in leaf area and ear awn length between genotypes grown in Constantine and Khenchela have important implications for the selection of the most suitable varieties for each region. Farmers and breeders could use this information to choose genotypes that maximize productivity under specific conditions, thereby improving agricultural yields and crop resilience (Placide et al., 2015).

Moreover, we observed a difference in the height of the plant and the length of the awn of the ear, which are greater in Khenchela compared to Constantine. This significant difference could be attributed to environmental, genetic and agronomic factors.

Plant height can be strongly influenced by environmental conditions such as water availability, soil quality, and temperature. Khenchela, located at a higher altitude than Constantine, benefits from a slightly different climate which may favor more vertical plant

growth (Placide et al., 2015). Studies have shown that moderate temperatures and good nutrient availability can increase plant height growth (Behouhou et al., 2022).

We sought, by performing a PCA, to determine which plant characteristics best explained the differences observed between the genotypes cultivated in the two regions (Constantine and Khenchela).

The results of this factor analysis revealed that the first two principal components (PC1 and PC2) together explained a substantial part of the total variance in the data, 41.99% and 26.02%, respectively. These first two principal components (PC1 and PC2) therefore together explain 68% of the total variance in the data, which is significant for reliable interpretation (Abdi & Williams, 2010). Variables such as leaf area (LA), plant height (PH) and ear length (EL) were strongly correlated with PC1, while ear beard length (BL) and number of grains per ear (NGE) were significantly correlated with PC2. These results indicate that PC1 could be interpreted as a measure of plant size and productivity, and PC2 as a measure of ear structure and density.

The first principal component (PC1) is strongly correlated with variables such as leaf area (LA), plant height (PH) and ear length (EL). These results suggest that PC1 can be interpreted as a measure of plant size and productivity.

The two distinct clusters of samples from Constantine and Khenchela probably correspond to the geographical and environmental differences between these two cultivation sites. The parameters linked to water stress tolerance and those linked to yield largely contributed to the distinction of these two clusters. Previous studies have also found that plant size and leaf area are key indicators of crop productivity (Smith et al., 2015). For example, Bastos et al. (2020) observed that variables related to plant size explained a significant part of the variance in their study on wheat varieties, which corroborates our results.

For the case of beard length, although the differences in Shannon diversity indices are not very marked, this can be attributed to environmental, genetic and agronomic management factors. These results may have implications for crop resilience and adaptability in these regions, as well as for variety breeding and crop improvement programs. Climatic differences (temperature, precipitation) can influence the phenotypic variability of beard length. Soil quality, as well as other edaphic factors, can also play a role in this difference.

In the study carried out by Dagnaw et al. (2023), analysis of phenotypic traits revealed a high Shannon diversity index (H′ = 0.78) among genotypes and indicated a high level of phenotypic variation. They specify that all phenotypic traits are highly polymorphic (H’ ≥ 0.60) except the two traits Days to maturity (H’=0.40) and vitreosity (H’=0.45).

The results of the comparison of the variances between the two sites (Constantine and Khenchela), calculated from the Shannon index for the three parameters (LA, BL and GY) showed p-values all greater than 0.05. This means that the variances of the Shannon indices between these two sites are identical for each of the parameters studied. These results also suggest that the two sites have similar variability in terms of diversity (measured by the Shannon index) for these parameters. The two sites are ecologically similar in terms of diversity for LA, BL and GY. Genotypic similarity between various geographic sites was also observed by Haile et al. (2013) where they suggest that the potential impact of their study is to indicate that durum wheat varieties marketed in Ethiopia over the past 43 years are genetically similar, highlighting the need to broaden the genetic base of varieties which will be marketed in the future.

The logistic regression was performed in this study to explore the relationships between various plant phenotypic parameters and a series of SSRs markers. The results of this logistic regression revealed several significant associations. For example, the SSR marker XgWm 273 (reverse) had a strong association with ear length ($p = 0.043$; $Exp(B) = 6.27$), while the SSR marker to the length of the beard ($p = 0.03$; $Exp(B) = 0.511$). In addition, certain SSR markers appeared to be associated with specific combinations of traits, suggesting a complex genetic influence on the observed phenotypic characters.

In the work of Khan et al. (2013), the study aimed to estimate the associations between yield and yield-related traits, and to identify the direct and indirect effects of traits on durum wheat grain yield. The study revealed significant variations between genotypes for all traits studied. Significant positive correlations were observed between grain yield and plant height, number of ears/m^2 and 1000 grain weight. Path coefficient analysis revealed that 1000 grain weight, days to maturity and number of ears/m^2 had significant positive direct effects on grain yield. In another hand, Mengistu et al. (2016), working on 210 phenotypic and agronomic traits of 89 durum wheat varieties in two different regions of Ethiopia, also observed significant genetic variation among the genotypes analyzed depending on the traits analyzed and the geographic region. For Marzario et al. (2023), genetic analysis of 123 durum wheat accessions grown in two regions Metaponto and Foggia, (Italy) using a range of SNPs and 33 phenotypic traits, revealed the presence of wide diversity regarding 10 traits showing significant differences between the two regions studied. Indeed, in their findings, local varieties are distinguished by the length and color of the seeds compared to modern varieties. They stated as well that among the quantitative characters, the height of the plant, the heading time, the length of the ears, the weight of grains per ear and the characters linked to the seeds

measured (area, length and width) had a significant weight on the differentiation of groups in the two environments. In addition, only phenol grain coloring contributes moderately in both regions.

In sum, our results demonstrate significant associations between certain molecular markers and phenotypic traits linked to water stress as well as yield components of our genotypes. These results provide new insights into the molecular mechanisms underlying drought stress adaptation and yield performance, paving the way for potential applications in crop improvement and resilience to changing environmental conditions.

CONLUSION

The analysis of phenotypic and molecular diversity highlighted the strong genetic variation of the durum wheat gene pool in two regions of Algeria (Constantine and Khenchela). The SSRs studied showed significant associations with certain phenotypic traits linked either to water stress tolerance or to yield components. As a result, favorable alleles of these SSRs can serve as markers for marker-assisted selection in durum wheat breeding programs, both to identify genotypes that can serve as parents for crossing and to group together desirable trait of agronomic characteristics and quality in a standard selection. This study highlights the usefulness of varieties for the development of cultivars, thus contributing to food security in Algeria.

RÉFÉRENCES

ABDI H. and WILLIAMS L. J. (2010). Principal component analysis. ***Wiley Interdisciplinary Reviews: Computational Statistics***. **2** (4) : 433-459. https://doi.org/10.1002/wics.101.

BALLESTA P., MORA F. and DEL POZO A. (2020). Association mapping of drought tolerance indices in wheat: QTL-rich regions on chromosome 4A. **Genetics and Plant Breeding**. Sci. Agric. 77 (2) : 1-8. http://dx.doi.org/10.1590/1678-992X-2018-0153.

BASTOS M. L., CARCIOCHI W., LOLLATO R. P., JAENISCH, REZNDE C. R. and SCHWALBERT R. (2020). Winter wheat yield response density as a function of yield environment and tillering potential: A review and field studies. ***Frontiers in Plant Science***. **11**: 1-17. https://doi.org/10.3389/fpls.2020.00054.

BEHOUHOU M. L., KERROUCHE I. and BAZRI K. (2022). Impact of organic matter on the morphological and physiological parameters of durum wheat (Triticum durum Desf.) in the southern region of the wilaya of Constantine (Algeria). **Doctoral Thesis**. Univ. Mentouri Constantine 1. Algeria

CHABANE K., VARSHNEY R.K., GRANER A. and VALKOUM J. (2008) Generation and exploitation of EST-derived SSR makers for assaying molecular diversity in durum wheat populations. ***Genetic resource and crop evolution***. 55 : 869–881. https://doi.org/10.1007/s10722-007-9292-8.

DAGNAW T., MULUGETA B., HALLESELASSIE T., GELETA M., ORTIZ R. and TESFAYE K. (2023). Genetic diversity of durum wheat (Triticum durum L. ssp. Durum, Desf) germplasm as revealed by morphological and SSR markers. ***Gene.*** 14 (6) : 1155. https://doi.org/10.3390/genes14061155.

ETICHA D., THÉ C., WELCKER C., NARRO L., STAß A. (2004). and HORST W.J. Aluminium-induced callose formation in root apices: inheritance and selection trait for adaptation of tropical maize to acid soils. ***Field Crops Research.*** 93 (2-3) : 252-263. https://doi.org/10.1016/j.fcr.2004.10.004.

FEDERER, W.T. (1956). Augmented (or Hoonuiaku) Designs. ***Hawaii Plr. Rec***., **55**: 191–208.

FRANTOVA N., BABEK M., ELZNER P., STREDA T., JOVANOVIC I. and HOLKOVA L. (2022). Different drought tolerance strategy of wheat varieties in spike architecture. ***Agronomy***. 12 (10) : 1-13. https://doi.org/10.3390/agronomy12102328.

HAILE J. K., HAMMER K., BADEBO A., NACHIT M. M. and RÖDER M. S. (2013). Genetic diversity assessment of Ethiopian tetraploid wheat landraces and improved durum wheat varieties using microsatellites and markers linked with stem rust resistance. ***Genet. Resour. Crop. Evol. 60*** : 513-527. https://doi.org/10.1007/s10722-012-9855-1.

HUZSVAI L., ZSEMBELI J., KOVACS E. and JUHASZ C. (2022). Response of winter wheat (Triticum aestivum L.) yield to the increasing weather fluctuations in a continental region of four-season climate. ***Agronomy***. **12** (2): 1-19. https://doi.org/10.3390/agronomy12102328.

IBROKHIM Y.A. and ABDUSATTOR A., (2008) Application of association mapping to understanding the genetic diversity of plant germplasm resources ***International J. of Plant Genomics***. 18 pages. https://doi.org/10.1155/2008/574927.

JAIN S. K., QUALSET C.O. and BHATT G. M., (1975) Geographical Patterns of Phenotypic Diversity in a World Collection of Durum Wheats. ***Crop Science.*** 15 (5) : 700-704. https://doi.org/10.2135/cropsci1975.0011183X001500050026x**.**

KHAN A. A., ALAM M. A., ALAM K. M., ALAM M. and SARKER Z. (2013). Genotypic and phenotypic correlation and path analysis in durum wheat (Triticum turgidum L. var. durum). ***Bangladesh J. Agil. Res. 38*** (2) : 219-225. https://doi.org/10.3329/BJAR.V3812.15885.

LOBELL D.B.; SCHLENKER W. and COSTA-ROBERTS J. (2011). Climate trends and global crop production since 1980. ***Science*** 333: 616-20. https://doi.org/10.1126/science.1204531.

MARZARIO S., SICA R., TARANTO F., FANIA F., ESPOSITO S., DeVITA P., GIOIA T. and LOGOZZO G. (2023). Phenotypic evolution in durum wheat (*Triticum durum* Desf.)

based on SNPs, morphological traits, UPOV descriptors and kernel-related traits**.** ***Front Plant Sci***. **14** : 1-20. https://doi.org/10.3389/fpls.2023.1206560.

MENGISTU D. K., KIDANE Y. G., FADDA C. and PÈ M. E. (2016). Genetic diversity in Ethiopian Durum Wheat (Triticum turgidum var durum) inferred from phenotypic variations. ***Plant Genetic Resources*** : 1-11. https://doi.org/10.1017/S1479262116000393.

NAJIMI B., El JAAFARI S., JLIBENE M. and JACQUEMIN J.M. (2003) Applications des marqueurs moléculaires dans l'amélioration du blé tendre pour la résistance aux maladies et aux insectes. ***Biotechnology, Agronomy, Society and Environment***, 7(1): 17-35.

PLACIDE R., SHIMELIS H., LAING M. and GAHAKWA D. (2015). Application of principal component analysis to yield and yield related traits to identify sweet potato breeding parents. ***Tropic. Agric.*** 92 (1): 1-15. https://doi.org/0041-3216/2015/010001-15.

REYNOLDS M. P., and BRAUN H. J. (2022). *Wheat improvement : food security in a changing climate* (p. 629). *Springer Nature*. http://dx.doi.org/10.1007/978-3-030-90673-3

RODER M.S., KORZUN V., WENDEHAKE K., PLASCHKE J., LEROV P. and GANAL M.W. (1998). A microsatellite map of wheat. ***Genetics.*** 149 (4) : 2007-2023. http://dx.doi.org/10.1093/genetics/149.4.2007.

SAGHAI-MAROOF M. A., SOLIMAN K. M., JORGENSEN*R. A.and ALLARD R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA. Vol. **81**: 8014-8018, December 1984. ***Population Biology.*** https://doi.org/10.1073/pnas.81.24.8014.

SALMI M., FELLAHI Z., BENBELKACEM A., BENMAHAMMED A. and BOUZERZOUR H. (2021). Inheritance of plant height, straw yield and flag area in MBB x Gaviota durum wheat (Triticum durum Desf.) cross. ***Acta agriculturae Slovenica***. 117 (4) : 1-10. http://dx.doi.org/10.14720/aas.2021.117.4.2119 .

SCHLÖTTERER, C. (2004). The evolution of molecular markers - just a matter of fashion? ***Nature Reviews Genetics***. 5 (1) : 63-69. https://doi.org/10.1038/nrg1249.

TSENOV N., GUBATOV T., and YANCHEV I., (2020). Correlations between grain yield and related traits in winter wheat under multi-environmental traits. ***Agricultural Science and Technology,*** 12 (4) : 295-300. https://doi.org/10.15547/ast.2020.04.047.

UDUPA S. M., ROBERTSON L. D., WEIGAND F., BAUM M. and KAHL G. (1999). Allelic variation at (TAA)n microsatellite loci in a world collection of chickpeas (Cicer arietinum L.) germplasm. ***Mol Gen Genet*** (1999) **261** : 354-363. https://doi.org /10.1007/s004380050976.