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Behavior assessment of some Triticale (*Triticosecale wittmack*) Genotypes under Mediterranean Semi-arid Conditions

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ABSTRACT

Background: In Mediterranean climate, drought and heat stress are the main constraints for cereal production under rainfed conditions. In these conditions, the selection of suitable genotypes is crucial to improve production.

Methods: 20 advanced triticale lines were evaluated under semi-arid conditions in Setif (Algeria) during the 2019/2020 season using agronomic, morphological and physiological traits.

Result: The results obtained showed that lines with higher numbers of tillers, spikes, grains per spike, leaf area index and leaf chlorophyll content produced more grains and used water more efficiently when faced to drought and heat stress in post-anthesis phase. Also, high chlorophyll content, high relative water content and maintenance of cell membrane stability delayed senescence and extended the grain filling stage. Relative water content was also related to high grain protein content. This work evaluated this set of triticale lines and identified traits positively related to grain yield and water use efficiency in Mediterranean region.

Key words: Drought, Heat stress, Mediterranean climate, Selection, Semi-arid, Triticale (*Triticosecale wittmack*).

INTRODUCTION

Global warming resulting from climate change is affecting grain yields and increases food insecurity (Ortiz *et al*., 2008). One of the best strategies to mitigate the environmental consequences on cereal production, particularly in rainfed areas, is to cultivate adapted varieties with substantial yields. The changing environmental scenario, especially in the Mediterranean region, imposes an urgent need to develop genotypes that are either tolerant to terminal heat and drought stresses or mature early without yield loss (Halford, 2009). Selecting for these genotypes also requires screening for traits that could be used in breeding programs for heat and drought tolerance in cereals, including triticale.

Triticale (*Triticosecale wittmack*), a hybrid species created by crossing wheat and rye, combines the good quality, grain yield and stress tolerance from both its parents. The nutritional quality of triticale is comparable or even superior to that of wheat. Its higher lysine content improves protein digestibility and mineral balance, making it an excellent alternative or complement to other cereals in human nutrition and animal nutrition (Nefir and Tabãrã, 2011).

The success of this crop recently introduced in Algeria can reduce the local cereal production deficit, which currently covers only 30% of demand (FAO, 2021). This deficit is mainly caused by environmental stress. The adoption of adapted genotypes allows an efficient use of limited resources, resulting in significant and stable production.

This study evaluates the performance of 20 triticale advanced lines created by CIMMYT (Mexico) under Mediterranean semi-arid conditions in Setif (Algeria). The evaluation was based on agronomic, morphological and physiological traits.

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MATERIALS AND METHODS

Experimental design and trail conduction

A field experiment was conducted during the 2019-2020 cropping season at the experimental field of ITMA Setif in Algeria (5°20°E, 36°11°N) to evaluate 20 triticale advanced lines brought from CYMMIT (Mexico) (Table 1). The study used a completely randomized block design with three replications. On December 08th, 2019, the seeds were sown in plots of 6 rows of 05 m long, 0.17 m apart, at a seeding rate of 300 seeds per m².

Pre-plant granular phosphorus (P_2O_5) fertilizer (100 kg/ha) was incorporated into the soil and nitrogen urea (100 kg/h) was supplied at the tillering stage. Weeds were removed chemically by TOPIC (0.75 l/ha^{-1}) and GRANSTAR (15 g/ha).

Fig 1 presents an Ombrothermic diagram with the recorded precipitation and average temperatures, as well as an estimate of the water deficit during the crop cycle using the method of Doorenbos *et al*. (1979).

Measurements

At the flowering stage, the fresh biomass produced was weighed. The total chlorophyll of flag leaves was estimated using a SPAD meter. Relative water content was measured according to the method described by Barrs and Weatherley (1962). Cell membrane stability was measured according to the method of Sullivan (1972) and leaf area index was measured by dividing flag leaf area by its dry weight.

The 50% senescence rate at the flag leaf level and the velocity rate were measured by digital image analysis as described by Hafsi *et al*. (2000). In each plot, three flag leaves were photographed nine times from the flowering stage to the end of senescence. Images recorded in JPEG format were analyzed using Mesurim Pro 3.3 software, which includes automatic leaf area measurement.

The senescence rate was determined by the ratio of green to red in the image, in per cent. The sum of temperatures for 50% senescence was determined graphically. Velocity rate was calculated by dividing the percentage of senescence by the sum of the temperatures between each two successive senescence measurements. Grain protein was analyzed using the Kjeldahl method.

At maturity stage, grain yield, thousand-kernel weight, number of tillers per m^2, spikes per m^2, straw yield and the harvest index were measured. Also, water use efficiency was calculated by dividing the grain yield on the sum of precipitations during crop growth.

Data analysis

The obtained data was analyzed using the SAS 9.2 statistical analysis package. The analysis of variance was performed for the different measures. Then, means were compared by the least significant difference (LSD) at alpha ≤0.05 level. Linear correlation analysis was used to determine the relationships between the traits measured.

RESULTS AND DISCUSSION

Climate conditions

The cumulative rainfall during the 2019-2020 season was 355.3 mm, with only 11.4 mm during the entire 42-day post-anthesis period (Fig 1a). The weather data indicates a severe drought during the sensitive period of the grain filling period, where the available water, estimated using Doorenbos *et al*. (1979) method, by water covers one-third (1/3) of the plant needs (Fig 1b). This characteristic of the Mediterranean climate is the main threat of cereal production in the region, causing up to 80% of yield losses (Nachit *et al*., 1998). These conditions, on the other hand, represent a selective pressure on our set of lines, increasing the selection efficiency of adaptable genotypes to the Mediterranean conditions.

Agronomic traits

Triticale can be grown for forage, grain yield, or dual purposes (Garcia del Moral *et al*., 1995). For forage production, biomass at anthesis is more suitable for selection. For this trait, the difference between lines was significant and the lines 19, 18 and 16 outpaced other lines for forage production (Table 2). Furthermore, because of the low level of stress at the early stages of growth, the obtained results could be considered close to the potential forage production of the assessed lines.

In addition to forage, grain yield (GY) is the main objective of this by crop. Therefore, it was and still the most important trait used to select for productive and/or stress tolerant genotypes. Furthermore, the effectiveness of selection using other traits depends on the gain in GY resulting from this selection (Mwadzingeni *et al*., 2016).

The variance analysis (Table 2) showed a significant difference among genotypes for grain yield (GY) and water use efficiency (WUE). The difference between the lines was also significant for straw yield, tillers/m^2, spikes/m^2, grains/spike, harvest index and the thousand kernel weight (TKW).

The mean GY for all genotypes (34.71 q/ha) was associated with an important variation between genotypes. This important gap in GY between assessed genotypes shows variability in yielding ability under this particular environment and the superiority of the lines: 17, 10, 18, 13, 15 and 16 successively. The later line 16, in addition to the highest GY (69.2 q/ha), also had the highest value of WUE (47.1 kg/mm), number of tillers/m^2(742.2) and spikes/m^2 (546.7).The line 15 registered the highest number of grains/spike (58.4) and the highest TKW (42.88 g), while the line 20 had the highest harvest index (0.81).

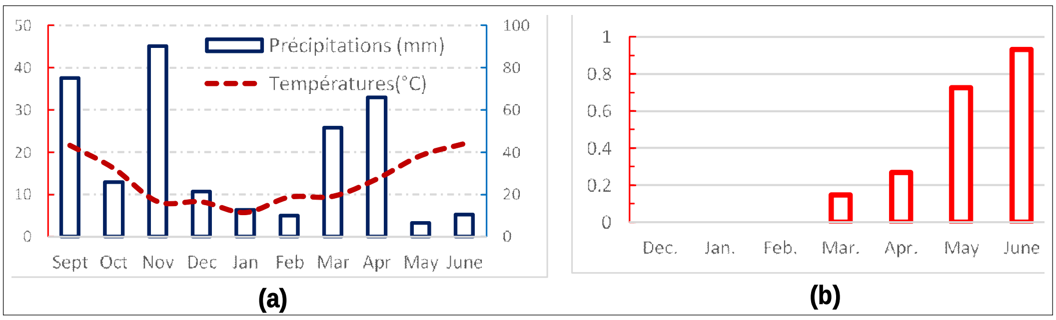

Fig 1: Rainfall and mean temperature during the crop season 2019-2020 in Setif (Algeria) (a) and monthly water deficit estimated according to Doorenbos *et al*. (1979) Method (b).

Table 1: The pedigree of the assessed lines.

Lines	Pedigree
01	FAHAD_8-2*2//PTR/PND T/3/GAUR_3/ANOAS_2//BANT_1/4/HARE_7265/YOGUI_1//BULL_2/5/…
02	SONNI_3*2//FARAS/CMH84.4414/6/RONDO/BANT_5//ANOAS_2/3/RHINO_3/BULL_1-1/5/DAHBI_6/3/…
03	PRESTO//2*TESMO_1/MUSK 603/4/ARDI_1/TOPO 1419//ERIZO_9/3/SUSI_2/7/FAHAD_4/…
04	08P166/9/T1505_WG//ERIZO_10/BULL_1-1/3/ERIZO_10/BULL_1-1/4COPI_1/5/ARDI_1/…
05	09P018-INT11/9/T1505_WG//ERIZO_10/BULL_1-1/4/COPI_1/5/ARDI_1…
06	08L129/4/CAAL/3/T1494_WG//ERIZO_10/2*BULL_BULL_1-1
07	08L129/8/POPPI_2/TAHARA/4/DAHBI_6/3/ARDI_TOPO 1419//ERIZO_9/7/CAAL/MANATI_1/5/…
08	08L129/4/CAAL/3/T1494_WG//ERIZO_10/2*BULL_1-1
09	08L129/9/BICEN/8/GAUR_2/HARE_HARE_3//JLO97/CIVET/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/…
10	08L129/9/BICEN/8/GAUR_2/HARE_3//JLO97/CIVET/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/…
11	08L129/8/DAHBI_6/3/ARDI_1/TOPO1419//ERIZO_9/5/804/BAT/3/MUSK/LYNX//…+
12	08L129/8DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/5/804/BAT/3/MUSX/LYNX//….
13	08L129/4/CAAL/3/T1494_WG//ERIZO_10/2*BULL_1-1
14	08L129/8/LIRON_2/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/MANATI_1/7/…
15	08L129/7/NILEX/3/BULL_10/MANATI_1//FARAS/CMH84.4414/6/HX87-255/5/PRESTO//…
16	08L129/8/POPP1_2TAHARA/4/DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/7/CAAL/MANATI_1/5/…
17	08L129/8/POPP1_2/TAHARA/4/DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/7/CAAL/MANATI_1/5/…
18	08L132/8/LIRON_2/5/DISB5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/MANATI_1/7/…
19	08L132/8/LIRON_2/5/DISB5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/MANATI_1/7/…CTSS14Y00043S-70Y-1M-2Y-2M-0B
20	08L132/7/NILEX/3/BULL_10/MANATI_1//FARAS/CMH84.4414/6/HX87-244/HX87-255/5/PRESTO//…

Table 2: Agronomic traits means and the significance of the genotypic effect (GE).

Lines	GY	SY	BA	NT	NS	GS	HI	TKW	WUE
01	7.7i	16.2bc	64.9gh	184.4f	75.6i	30.4e	0.32h	25.19gh	01.45i
02	30.8defg	22.7bc	72.4fgh	393.3abcd	253.3efg	31.0cde	0.57bcde	23.30h	11.14defg
03	35.7cdef	26.7bc	63.3fgh	400.0bcde	257.8efg	39.3de	0.57cde	30.86def	14.75cdef
04	21.3hi	13.3c	52.9h	333.3cde	217.8ghi	37.5e	0.62bcde	33.50cde	7.85hi
05	23.7ghi	44.2bc	43.6h	355.6bcde	228.9ghi	33.0bcde	0.35gh	31.90cdef	8.57ghi
06	29.6defgh	40.0abc	45.1gh	364.4bcde	231.1ghi	56.7a	0.44efgh	32.59cdef	10.65defg
07	41.5c	63.6a	77.8fg	424.4abc	308.9cd	46.7abcde	0.39fgh	37.03bc	17.15c
08	26.9fghi	29.3bc	65.3gh	342.2cde	237.8fgh	46.2abcde	0.71abc	37.01bc	10.81fghi
09	32.9cdefg	13.1c	92.9fgh	384.4abcde	273.3def	46.0abcde	0.46defgh	32.55cdef	13.97cdefg
10	48.0b	30.0bc	74.0fgh	448.9ab	348.9b	52.3abc	0.61bcde	27.98fgh	21.43b
11	38.8cd	39.3abc	129.6bcd	446.7ab	286.7cde	48.4abcde	0.51defg	33.66cde	14.08cd
12	30.9defg	29.6bc	48.9fgh	400.0abcd	213.3ghi	48.1abcd	0.50defgh	34.76bcd	09.25defg
13	60.5a	53.6ab	78.7ef	622.2a	451.1ghi	51.5abcd	0.54cdef	24.92gh	33.18a
14	22.6ghi	13.8c	102.0cde	297.8e	171.1hi	44.8bcde	0.63abcd	29.29efg	07.42ghi
15	62.0cde	16.4c	102.4cde	446.7ab	391.1a	54.0ab	0.80a	23.40h	31.14cde
16	69.2defg	54.0ab	115.6abc	742.2ab	546.7bc	46.8abcde	0.54cdef	26.04gh	47.07defg
17	23.0efgh	21.1c	95.6de	300.0e	213.3ghi	43.4bcde	0.54cdef	25.71gh	09.11efgh
18	59.8a	45.8abc	119.8ab	442.2ab	346.7b	58.4ab	0.55cdef	42.88a	26.36a
19	32.9cdefg	11.3bc	155.6a	313.3de	224.4cde	51.8abc	0.75ab	38.43b	13.22cdefg
20	37.9cd	8.9c	106.7h	395.6f	284.4cd	41.2bcde	0.80a	29.75defg	14.59cd
Mean	34.71	31.46	85.08	380.53	266.7	46.78	0.56	31.03	19.64
LSD 0.05	5.71	18.23	11.52	56.46	28.34	6.93	0.178	3.31	3.23
Gen. eff.	***	***	***	***	***	***	***	***	***

*: Significant at 0.05, **: Significant at 0.01, ***: Significant at 0.001, ns: Not significant, GY: Grain yield, SY: Straw yield, BA: Biomass at anthesis, NT: Tillers/m^2, NS: Spikes/m^2, NGS: Grains/spike, HI: Harvest index, TKW: Thousand kernel weight, WUE: Water use efficiency, Means with the same letter are not different according to LSD test (Alpha = 0.05).

The correlation analysis showed that GY was positively correlated to its components: number of spike m^2 (r=0.95) and the number of grain per spike (r=0.62). Grain yield was also related to number of tillers (r=0.88), straw yield (r=0.50) and WUE (r=0.96).

Tillering ability (TA) allows the crop to compensate for low density or plant losses and maximizes biomass and spike production, especially when the environmental conditions in the early stages favor crop development, as observed in our study. Genotypes with high TA also produced more spikes (r= 0.96), an important yield component, which explains the positive association of the number of tillers with GY (r= 0.88) and WUE (r= 0.92). These findings indicate that TA is an adaptation trait and could be used to select adaptive triticale genotypes for the Mediterranean region.

Similarly, the yield components: spike density and number of grains per spike had a positive relationship with GY (r=0.95 and r=0.62 respectively) and WUE (r=0.97 and r=0.50 respectively), validating their use as selection traits for productivity and drought tolerance in maximizing grain production, especially under terminal stress.

TKW, on the other hand, was not significantly correlated to GY, suggesting that terminal drought stress had a negative impact on grain filling. This is further supported by the non-significant relation between GY and the harvest index (HI), which expresses the translocation efficiency of assimilates from the vegetative biomass to the grain. These results reflect the drought effect on yield by reducing sink potential (Barnabás *et al*., 2008). Furthermore, the significant relation between HI and fresh biomass produced before the flowering stage (r= 0.54) indicates that the plant reserves in water and assimilates prior to drought exposure at grain filling stage contributed to the translocation efficiency. According to Blum (2014), the biomass produced before flowering can contribute up to 65% of winter triticale yield.

Morphological and physiological traits

In cereals, the flag leaf is the main source of photosynthates for grain filling and thus yield formation (Wardlaw, 1990). Therefore, it is used to assess the response of cereals to stress and its impact on yield. The flag leaf monitoring in our study consisted in measuring senescence rate (SR), Velocity rate (VR), sum of temperatures for 50% of senescence (S50), chlorophyll content (SPAD), cell membrane stability (CMS), relative water content (RWC) and leaf area index (LAI).

The analysis of variance (Table 3) revealed a significant difference between the tested lines for the means of RWC, LAI, SPAD, CMS, SR and VR. Both lines 07 and 17 had the highest LAI (0.338 cm^2/g). The line 01 had the highest RWC (0.63%) and CMS (90.73%), while the line 20 had the highest SR (42.89%) and VR (0.129).

Table 3: Physiological traits means protein content and the genotypic effect significance.

Lines	RWC	CMS	SPAD	SR	VR	LAI	S50	GPC
01	0.63a	90.73a	32.07defg	25.195hi	0.115cdef	0.164d	635.11	14.7
02	0.59a	90.24ab	31.05efgh	23.294i	0.11def	0.173bc	673.22	15.2
03	0.59ab	88.53abc	32.34defgh	30.861ef	0.12abcde	0.227bc	596.39	14.6
04	0.56ab	88.26abc	33.23cde	33.494cd	0.119abcde	0.181bc	571.40	14.7
05	0.57ab	88.29abc	28.37hi	31.906de	0.118abcde	0.17bc	559.58	13.5
06	0.56ab	84.25d	29.01ghi	33.569cd	0.12abcde	0.221bc	558.23	15.3
07	0.54ab	86.56c	30.31efgh	37.027b	0.126abc	0.338bc	502.17	14.9
08	0.56ab	82.5de	31.48efgh	37.001b	0.126abc	0.222bc	579.87	15.5
09	0.6ab	83.42d	34.25cd	32.553cde	0.122abc	0.262bc	558.01	14.9
10	0.59ab	88.71abc	32.22defg	27.993g	0.121abcd	0.217b	607.58	14.8
11	0.54ab	83.01d	34.55cd	33.667cd	0.117bcde	0.236bc	593.07	14.9
12	0.6ab	84.19d	27.4ij	34.71c	0.128a	0.185bc	593.34	14.9
13	0.6ab	87.68bc	32.33defg	24.929hi	0.107f	0.247a	667.30	14.9
14	0.52ab	88.11abc	35.07bc	29.297fg	0.12abcde	0.134bc	607.57	13.4
15	0.54ab	88.85abc	37.22bc	23.403i	0.11ef	0.324bc	671.80	14.8
16	0.55ab	86.36c	38.75a	26.033h	0.121abc	0.338b	631.64	13.7
17	0.48ab	88.8abc	32.86def	25.713h	0.12abcd	0.284cd	626.08	13.7
18	0.45ab	88.28abc	29.45fghi	29.752fg	0.121abcd	0.276bc	565.63	13.7
19	0.44b	80.6e	26.77j	38.426b	0.127ab	0.102bc	514.49	14.7
20	0.43b	80.63e	26.81j	42.891a	0.129a	0.161a	440.89	13.1
Mean	0.494	86.403	32.841	31.085	0.119	0.305	587.76	14.5
LSD 0.05	0.064	1.673	1.865	1.630	0.006	0.090	-	-
Genotype effect	***	***	***	***	***	***	-	-

*: Significant at 0.05, **: Significant at 0.01, ***: Significant at 0.001, ns: Not significant, LAI: Leaf area index, RWC: Relative water content, CMS: Cell membrane stability, SR: Senescence rate, VR: Velocity rate, S50: Sum of temperatures for 50% senescence, GPC: Grain proteins content, Means with the same letter are not different according to LSD test (Alpha = 0.05).

High frequency of heat and drought stress at the end of the winter cereal cycle is a characteristic of the Mediterranean climate. The significant correlation between mid-senescence and RWC (r = -0.55), which reflects leaves hydration, connects drought with the accelerated senescence process. The results also showed a significant correlation between SR and VR (r = 0.82), establishing the involvement of high temperatures in the increase of senescence rate. Heat stress reduces photosynthetic activity and its continuance by shortening different life cycle stages (Stone, 2001), as observed on grain filling stage in our case. While drought affects the photosynthetic activity and the assimilate translocation to the grain. The effect of heat and drought stresses on sink potential elucidates the lack of a significant correlation between TKW and HI with both GY and WUE.

By extending the grain filling stage, the flag leaf longevity is often related to GY, HI (Carmo-Silva *et al*., 2017) and single grain weight (Porter and Gawith, 1999). In contrast, our results showed a positive relationship of TKW with SR (r = 0.69) and RV (r = 0.65), implying that the decrease in SR during assimilates translocation was not sufficient to contribute significantly to GY due to the shortened grain filling period. Furthermore, the negative and significant correlation between TKW and S50 (r = -0.65) confirms that under severe stress, grain weight of the lines evaluated depends on filling rate rather than duration.

The results also showed that flag leaf longevity depends on maintaining cell membrane stability CMS (r_{SR}= -0.82, r_{VR}= -0.66, rS50= -0.66), having high SPAD values (r_{SR}= -0.60, r_{VR}= -0.47, rS50= 0.61) and high ability to absorb and/or retain water (r_{RWC}= 0.55). In addition to delaying senescence, our results showed that SPAD values, expressing chlorophyll content, were proportional to WUE (r = 0.47), indicating an association of SPAD values with productivity and water use efficiency under stress.

The LAI is associated with better growth, light reception and gas exchanges (Tang *et al*., 2022). This characteristic explains its positive relation with GY (r = 0.64) and WUE (r = 0.65) , in addition to its relation with SPAD values (r = 0.53). Also, the high heritability of LAI (Carmo-Silva *et al*., 2017) encourages its use in selecting for productivity and stress tolerance.

Nutritional value, including grain protein content (GPC), is a desired quality for better use of the grain. GPC in cereals, which is a quantitative trait, depends on nitrogen nutrition efficiency, protein synthesis and their translocation efficiency to the grain (Garcia del Moral *et al*., 1995). The GPC of assessed the lines varied between 13.5% and 15.5%, which are close to those of wheat. The results of many studies showed that water deficit usually increases GPC by reducing carbohydrate accumulation. However, a severe drought can affect nitrogen nutrition efficiency, which reduces GPC (Barati and Bijanzadeh, 2021). These results showed, in addition to the genetic variability, a significant association between GPC and RWC (r = 0.56) was noted, indicating that under severe drought, lines with the ability to maintain leaf hydration are more likely to produce grains with higher GPC.

CONCLUSION

The evaluation of 20 triticale lines under terminal drought and heat stress showed that genotypes capable of developing more tillers, spikes and kernels per spike are more tolerant to stress. High temperatures accelerate the senescence process and shorten life cycle stages, while drought affects photosynthesis and assimilates translocation to the grain. The effect of heat and drought stress on sink potential explains the lack of a significant correlation between grain weight and harvest index with yield and water use efficiency.

It is also important to mention that the productivity of the stressed lines was related to chlorophyll content and leaf area index. Moreover, high chlorophyll content, high relative water content and maintenance of cell membrane stability delayed senescence and prolonged grain filling stage and relative water content was also related to high grain protein content.

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Conflict of interest: None.

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