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Phenotypic characterization of recombinant inbred durum wheat lines segregating for resistance to Hessian fly (*Mayetiola destructor (Say)*,) in Morocco

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Abstract

Hessian fly, *Mayetiola destructor (Say)*, is one of the most destructive insect pests of wheat in North Africa. In Morocco, durum wheat yield losses caused by this pest in arid and semi-arid areas were estimated at 32%. Breeding for resistance has been the most practical and economical method for HF. control. The objective of this study was to agro-morphologically characterize, 180 recombinant inbred durum wheat lines derived from a cross between durum wheat lines with resistance to Hessian fly (CI115,Cando-H25) and susceptible lines adapted to dry areas (BZAIZ-AHF,CM829).The experiment were conducted during 2011-2012 and 2012-2013 in two experiments stations Sidi-El-Aidi (chaouia region) and Marchouch (Zaer region) in Morocco. Yield and yield components as well as agro-morphological and phenological traits were measured. The results indicate that all studied traits follow a quantitative, continuous and normal distribution. Data also showed a wide variation in traits related to yield and its components. Several statistically significant correlations between all the plant characteristics have been observed. Cluster analysis grouped agro-phenological traits, yield and its component into three clusters. These data indicate the usefulness of this population in genetic studies and in breeding programs aiming at improving economically important traits. Given the importance of Hessian fly in the dry areas, this population would be adequate for molecular studies (QTL) for adaptation in the temperate and H.F. infested dry lands.

Keywords: Genetic resistance, Hessian fly, durum wheat, yield components, agro-morphology, Marchouch, Sidi-El-Aidi, Morocco

Résumé

La cécidomyie (*Mayetiola destructor* (Say)) est l'un des insectes ravageurs les plus destructeurs du blé en Afrique du Nord. Au Maroc, les pertes en rendement dues à ce ravageur dans les zones arides et semi-arides ont été estimées à 32%. L'amélioration du blé pour la résistance génétique à la cécidomyie est la stratégie de lutte la plus pratique et la plus économique. L'objectif de cette étude est la caractérisation agro-phénotypique d'une population de 180 lignées recombinantes de blé dur issues de croisements entre des lignées résistantes à la cécidomyie (CI115, Cando-H25) et des lignées sensibles et adaptées aux conditions pédoclimatiques des zones arides et semi-arides (BZAIZ-AHF, CM829). Les essais sont conduits pendant les campagnes agricoles 2011-2012 et 2012-2013 dans deux domaines expérimentaux: Sidi El Aidi (région de chaouia) et Marchouche (région de Zaer). Le rendement et ses composantes ainsi que les caractères agro-phénologiques ont été mesurés. Les résultats obtenus montrent que tous les caractères étudiés suivent une distribution normale et ont une bonne variabilité quantitative. Outre, une grande variabilité du rendement et de ses composantes a été observée. Plusieurs corrélations statistiquement significatives entre caractères ont été observées. L'analyse du dendrogramme a permis de mettre en évidence trois groupes de caractères. Ces données indiquent l'utilité de cette population dans les études génétiques et dans les programmes d'élevage visant à améliorer les traits économiquement importants. Etant donné l'importance de la mouche de Hesse dans les milieux arides, cette population serait indiquée pour l'identification de QTLs pour l'adaptation dans les zones semi arides.

Mots-clés. Résistance génétique, cécidomyie, Blé dur, composantes de rendements, caractère agro-phénologique,Marchouch, Sidi El Aidi, Maroc

1. Introduction

Durum wheat is important in the Mediterranean basin and in several large cereal producing countries in the world. Durum wheat is an economically important crop because of its unique features related to grain end use products [1, 2, 3]. Among the stresses that wheat plants endure during development is infestation by Hessian fly: Mayetiola destructor (Say). In North Africa, especially in Morocco, durum wheat losses due to this pest have been estimated at 32% [4]. Genetic resistance is the only economical and practical means of controlling this insect. To date, 34 Hessian fly resistance genes identified so far [5]. This resistance is expressed as larval antibiosis and is controlled mostly by single genes that are partially or completely dominant [6].

New sources of Hessian fly resistance are necessary to incorporate into wheat breeding programs, especially where Hessian fly has the most diverse genetic variations and the greatest number of generations per year due to mild winter conditions [7-10]. Thereby, the incorporation of genetic resistance in wheat, followed by agro-phenological characterization contributes to a better understanding of genetic resources and diversity for pest resistance in durum wheat. The objective of this study was to characterize agro-morphologically and phenologically, 180 recombinant inbred durum wheat lines segregating for resistance to Hessian fly.

2. Materials and methods

2.1. Genetic materials, experimental design

A population of 180 of recombinant inbred lines (RILs) of durum wheat generated from a cross between durum wheat lines with resistance to Hessian fly (CI115/ Cando cross-H25) and susceptible lines adapted to dry areas (CM829//BZAIZ-AHF). The experiments were conducted at Sidi El Aidi (Chaouia region) and Marchouch (Zaer region) experiment stations of the National Institute of Agricultural Research (INRA) for two successive growing-seasons (2011-2012; 2012-2013). The sites general attributes are shown in table 1.). sites general attributes are shown in table 1. While Marchouch station is representative of moderately favorable rain fed. Both stations have high variability in climate.

Table 1.
Characteristics of the experiment sites [11]

Site name	Agro-ecological zones	Site characteristics.
Marchouch (MCH)	Mid favorable rainfed mid season length	Central region, heat, drought, leaf rust, root rot, tan spot, medium infestation by Hessian fly.
Sidi El Aidi (SED)	Dryland, short season Irrigable	West dryland, supplemental irrigation, Drought, heat, root rot, leaf rust, tan spot, high infestation by Hessian fly.

The lines were sown at the beginning of November in moist conditions in plots consisting of two rows of 1m length with a spacing of 0.3 m. Sowing is carried out at a depth of approximately 3 to 5 cm by hand. The experiment was designed as a three augmented blocks design and completely random with five checks. Evaluation of this collection was based partly on the observations that have been made throughout the vegetative cycle on phenological and agro-morphological traits. Then, determination of grain yield and yield components took place after harvest for Sidi-El-Aidi station in 2011-2012 growing season. The evaluation of Hessian fly resistance was made in a controlled infestation in the greenhouse. The resistance line was confirmed by the existence of dead larvae.

2.2. Parameters recorded

During the growth season the traits including number of days to heading (HD), plant height (PH), total plant's weight (PW), thousand kernels weight (TKW), number of days to maturity (MD), grain weight per line (GWL), grain weight per spike (GWS), number of grain per spike (NGS) and number of spikes per line (NSL), were measured in Sidi-El-Aidi station and number of days to heading (HD), plant height (PH) and number of days to maturity (MD) were measured in Marchouch experiment station.

2.3. Statistical analysis

Analysis of variance following an RCBD (augmented) design was performed on all traits measured. Frequency distributions were computed and normality was tested. The analysis of relationship between different measured traits by correlation was also conducted.

3. Results

3.1. Phenotypic characterization

At Sidi El Aidi and Marchouch experiment stations, there has been little to no attack and plants from all entries continued their growth and development normally. For all remaining materials and trials, the evaluation of the agro-phenological traits was made in good conditions.

3.1.1. Hessian fly resistance and fungal diseases

Evaluation of genetic resistance to Hessian fly under controlled conditions showed that 84 plants are resistant and 96 are susceptible. In addition, field evaluation of wheat plants showing symptoms of *Septoria* blotch and brown rust is reported in table 1. The *Septoria* infection response was different between lines while the majority of lines are susceptible to brown rust. The severity of leaf diseases and attacks by Hessian fly has not reached sufficient levels to cause significant losses to plants.

In addition, field evaluation of wheat plants showing symptoms of Septoria blotch and brown rust is reported in Table 2.

Table 2.

Frequencies of durum wheat lines according to their resistance to Hessian fly, Septoria blotch and brown rust

	S	**MS**	**MR**	**R**	**HR**
Septoria blotch	92	17	41	4	25
Brown Rust	147	16	8	8	1
Hessian fly	96	-	-	84	-

S: susceptible, MS: moderately susceptible, MR: moderately resistant, R: resistant, HR: highly resistant.

3.1.2. agro-morphologically and phenologically characteristics

3.1.2.1. Frequency distribution

Histograms showing frequency distributions of all phenotypic traits are presented in Figure 1, which suggest a quantitative, continuous and normal distribution. This assumption is true for both experiment sites.

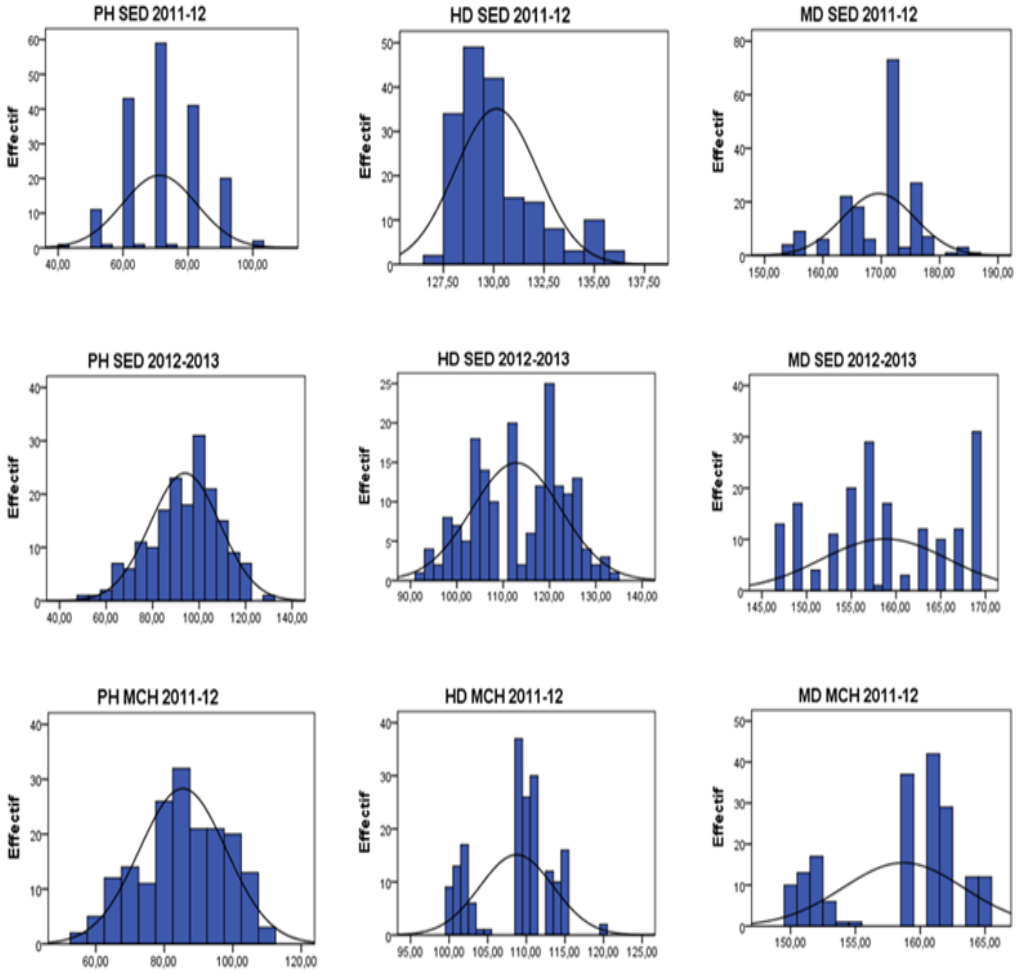

Figure 1**:** Frequency distribution of all phenological studied traits

(PH: plant Height, MD: number of days to Maturity, HD: number of days to heading, SED: Sidi-El-Aidi; MCH: Marchouch).

3.1.2.2. Descriptive statistics of agro-phenological characteristics

The results obtained for the plant height, number of days to heading and number of days to maturity, and their descriptive statistics are reported in Table 3. The means values of each character are close to those of the medians values; this seems to translate the dispersion around the mean is normal.

3.1.2.3. Variation of agro-phenological characteristics

Overall means, minimum and maximum values for the agro-phenological characters measured on 180 of RILs are presented in table 2. Data on plant height fluctuate between (40.50 cm and 100.50) and between (50 and 130 cm) for the Sidi-El-Aidi station during both 2011-2012 and 2012-2013 growing season respectively, and vary between 55 and 110 cm for Marchouch station during the 2011-2012 growing season. In addition, number of days to heading is prolonged over a period ranging from 127 to 136 and from 92 to 133 days respectively in Sidi-El-Aidi station during 2011-2012 and 2012-2013; and from 100 to 120 days in Marchouch station during 2011-2012. Number of days to maturity varies between (154 and 185 days) and between (147 and 169 days) in Sidi-El-Aidi station during 2011-2012 and 2012-2013 respectively; and 150 to 165 days in Marchouch station during 2011-2012. Coefficients of variation for these characters within the three sites were variable, where; the plant height was the most variable among all studied characters.

The differences between the four parent lines in terms of agro-phenological character are not important. This

population has been created to segregate for resistance to Hessian fly.

Table 3

Means, Max, Min, standard deviation, variance and Coefficient of variation for the all phenotypic traits

Station	SED 2011-2012			SED 2012-13			MCH 2011-12		
Trait	PH	HD	MD	PH	HD	MD	PH	HD	MD
Mean	71.22	130.13	169.55	93.94	112.85	158.76	85.41	108.73	158.69
Median	70.37	130.00	172.00	95.00	113.00	157.00	85.00	110.00	161.00
Standard deviation	11.46	2.045	6.208	15.004	9.621	7.123	12.68	4.753	4.653
Min	40.5	127.00	154.00	50.00	92.00	147.00	55.00	100.00	150.00
Max	100.5	136.00	185.00	130.00	133.00	169.00	110.00	120.00	165.00
CM829	90.5	130.00	176.00	100.00	117.00	157.00	95.00	115.00	161.00
Cando -H25	70.00	131.00	182.00	95.00	100.00	155.00	105.00	110.00	161.00
BZAIZ-AHF	70.5	130.00	176.00	100.00	119.00	159.00	70.00	114.00	165.00
CI115	60.25	130.00	164.00	100.00	121.00	169.00	75.00	109.00	159.00
Coefficient of variation	16.09	1.57	3.66	15.97	8.52	4.48	14.84	4.37	2.93

PH: plant Height, MD: number of days to Maturity, HD: number of days to heading; SED: Sidi-El-Aidi MCH: Marchouch

3.1.2.4. Analysis of variance components of agro-phenological characters:

Coefficients of variation for the studied traits are presented in table 3. The results indicate that plant height was most variable among all studied characters, with the coefficient of variation in the ranges of 14.84 to 16.09%. Number of days to maturity showed low coefficient of variation values among the three traits. The percentages of the contribution of genotype, sites factors and their interaction in the total variation are reported in table 4. These indices were calculated using the sum of squared deviations.

Table 4

Percentages of the contribution of genotype, sites factors and their interaction in the total variation.

	Site %	Genotype %	Site X genotype	Total variance
PH	0.19	10.74	89.07	259.77
HD	0.37	30.17	69.46	129.75
MD	0.22	80.79	18.99	67.10

PH: plant Height, MD: number of days to Maturity, HD: number of daysto heading

In terms of total variance, Plant height is the most variable characteristic followed by the number of days to heading and number of days to maturity. Furthermore, these traits are influenced by genetic factors and environmental factors (site). Number of days to maturity is affected by genotype (80.8%) and is very slightly affected by site (0.2%), while for the plant height, approximately 89% of the variation is due to the interaction site x genotype, and 10.7 % is due to the genotype. For number of days to heading, 69.5% of the variation is due to the interaction site x genotype and 30.1% is due to the genotype. In general, all traits are very slightly affected by site factor and are mostly affected by genotype and genotype by site interaction.

3.2. Analyze of yield and yield components

3.2.1. Frequency distribution

Histograms showing frequency distributions of yield and yield components are presented in figure 2, these histograms show that the distributions follows a continuous and normal distribution.

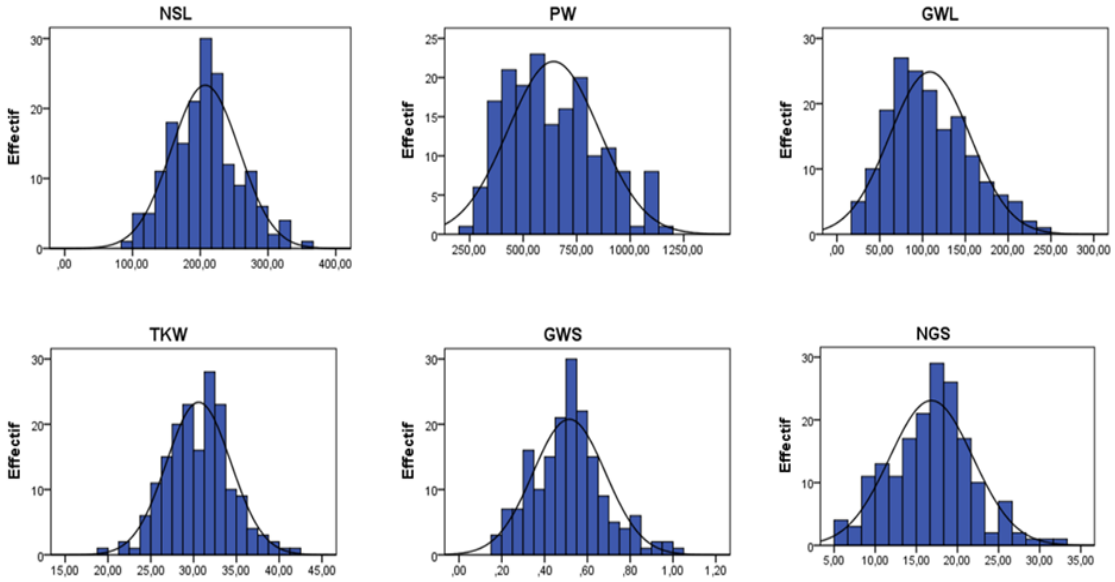

Figure 2: Frequency distribution of yield and its components evaluated at Sidi El Aidi station during 2011-2012 growing season
PW: plants weight; GWL: grain weight per line;; NGS: number of grains per spike, NSL: number of spikes per Line; TKW: thousand kernels weight; GWS: grain weight per spike

3.2.2. Variability of yield and yield components

The descriptive analysis of yield components indicate that the means values for all studied traits are close to the median values, conforming that the distribution around the mean is generally normal (Table 5). Also the gross variations in yield and its components are far superior to those agro-phenological characters. Thus, the values fluctuate between 86 and 352 spikes for number of spike per line, and between 261.50 and 1200 g for total plant's weight. In parallel, grain weight per line, Thousand kernels weight and grains weight per spike also ranged from (19 to 250 g), (19 to 41 g) and (0.16 to 1.03 g). Furthermore, number of grains per spike fluctuated between 5.58 and 32.95 grains. The coefficients of variation of yield components are therefore higher than those agro-phenological characters.

The coefficients of variation were classified from the highest to lowest as follow: 43.33 % for grain weight per line , 33.14 % for grain weight per spike, 33.06 % for total plant's weight , 30.13 % for number of grain per spike, 24.18 % for number of spikes per line and 12.27% for thousand kernels weight. These data show that the population used in this study has a great variability in components and in yield components. This greater variation in yield and yield components may be linked to resistance to Hessian fly.

Table 5
Means, Max, Min, standard deviation, variance and Coefficient of variation for the all Yield components studied.

Trait	NSL	PW	WGL	WTG	WGS	NGS
Mean	207.39	641.93	108.57	30.59	0.51	16.82
Median	207.00	600.90	102.40	30.65	0.508	17.01
Standard deviation	50.15	212.28	47.05	3.755	0.169	5.069
Min	86.00	261.50	19.40	19.40	0.16	5.58
Max	352.00	1200.00	250.00	41.80	1.03	32.95
Coefficient of variation	24.181	33.069	43.336	12.275	33.137	30.136

PW: plants weight; GWL: grain weight per line;; NGS: number of grains per spike, NSL: number of spikes per Line; TKW: thousand kernels weight; GWS: grain weight per spike

3.3. Phenotypic correlation among traits

Pearson's correlation coefficients were computed for all measured traits in Sidi-El-Aidi experiment site (Table 6). The results on the correlation matrix between the various traits studied, revealed that the number of spikes per line is perfectly and positively correlated with total plant's weight, grain weight per line, grain weight per spike and number of grains per spike. Furthermore, total plant weight is correlated with grain weight per line, grain weight per spike, number of grains per spike, plants height and number of days to maturity. Grain weight per line has positive and significant correlations with thousand kernels weight, grains weight per spike, number of grain per spike and total plant's height. Grain weight per spike is correlated number of grain per spike and plant height. Correlations exist between thousand kernels weight and grains weight per spike and between number of days to maturity and plant height .Therefore, these Associations are positive which shows that the individual characters are correlated to the same effect. Except number of days to heading had negative correlations with grain weight per line, grain weight per spike, number of grains per spike and plant height.

Table 6

Pearson's correlation coefficients for all studied traits at SED station during 2011-2012 growing season

traits	NSL	PW	GWL	TKW	GWS	NGS	PH	MD
PW	0.746**							
GWL	0.702**	0.687**						
TKW			0.303**					
GWS	0.195**	0.374**	0.812**	0.379**				
NGS	0.194**	0.349**	0.753**		0.919**			
PH		0.266**	0.281**		0.329**	0.315**		
MD		0.383**					0.165*	
HD			-0.230**		-0.276**	-0.304**	-0.243**	0.266**

PW: plants weight; GWL: grain weight per line;; NGS: number of grains per spike, NSL: number of spikes per Line; TKW: thousand kernels weight; GWS: grain weight per spike

3.4. Grouping traits according to similarities

To get a better view of traits association a clustering was performed based on the correlation method (Figure 3). The dendrogram showed three groups of characters: in the first cluster we found the number of spikes per line and total plant's weight. This first cluster is then tied to the number of grains per spikes, grain weight per spike, and grain weight per line. The last group to join the cluster is made of the number of days to heading and the number of days to maturity. Grouping traits according to similarities confirmsall previously observed associations in the correlation matrix.

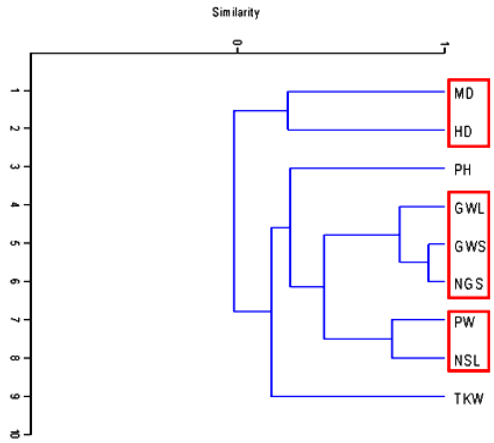

Figure 3: Dendrogram representing the clustering of the all measured characters

PW: plants weight; GWL: grain weight per line; NGS: number of grains per spike, NSL: number of spikes per Line; PH: plant height; TKW:thousand kernels weight; GWS: grain weight per spike, MD: number of days to maturity; HD: number of days to heading

4. Discussion

The principal character for which this population has been created is resistance to Hessian fly. It arises from the data collected that this character presented a normal variation because the relative frequencies of the resistant and susceptible lines are statistically not different. Concerning the comparison of gross variability of agro-phenological traits, plant height is the characteristic that varied the most, followed by the number of days to heading and the number of days to maturity. In addition, there are many variations between genotypes within stations and across stations. There is an effect of the stations and an effect of the interaction between stations and genotypes.

Significant positive correlations were observed between total plant weight, grain weight per line, weight and number of grains per spike. Total plant's weight has an important role in selection since it appears it is positively related to grain yield [12]. Furthermore, grain weight per line correlated with grain weight per spike, number of grain per spike and thousand kernels weight confirming to [13], and showed also that the number of grain per line and thousand kernels weight are the main components of yield; they are influenced by the genotypic characteristics. Similarly, [14] note that the number of grains per spike contributes directly to increase the grain yield in durum wheat. On the other hand, [15] found that the fertility is the most important performance of the component.

The positive correlation that was observed between grains weight per line and thousand kernels weight is confirming the findings of [16] that thousand kernels weight increased the yield directly. Also, plant height, was significantly and positively correlated with grain yield. [17] showed also that plant height has significant positive correlation with grain yield and suggested that these traits could be used as a direct criterion for improving yield of durum wheat. However, the number of days to heading was negatively and significantly correlated with the grain weight per line, grain weight per spike, number of grain per spike and with plant height. These correlations are also in agreement with [18-20], whom confirming the relationship between life-cycle plant development and yield. Therefore, our results are consistent with several studies showing that the performance consists of different components that are taking place successively or simultaneously during the development of culture [21, 22]. The fact these correlations and associations between characters of the plant were observed in this study indicates that recombinant inbred lines observed behaved according to the standards described in other studies.

5. Conclusion

The results have showed that in the studied recombinant inbred lines population; an important and normally distributed variation of trait was obvious. Some traits have shown more variation than others. It was also found that variability in yield and its components are far superior to those agro-phenological traits. Significant effects of genotype, site and their interactions were observed on the agro-phenological traits. Furthermore, there were positive and negative association between traits that were similar to the ones reported in the literatures.

This population would be useful in genetic studies and in breeding programs aiming at improving economically important traits. Given the importance of Hessian fly in the dry areas, this population would be adequate for identifying QTL for adaptation in the temperate and H.F. infested dry lands.

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