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Change on morphometry of lodicules, grains and spikes of barley landraces (Hordeum vulgare L.) under soil and climate conditions of three agroclimatic environments

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Abstract

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Barley is a strategic crop worldwide predominantly cleistogamous. This automatic self-pollination favorable among others for spike resistance to Fusarium and to Ustillago nuda is essentially assured by lodicules in grain. The study of the impact of environment conditions on the expression of lodicule traits beside other grain and spike parameters and their relationships can be useful for breeding programs. The principal aim of this study was to test four barley cultivars under three different agroclimatic conditions using some traits related to lodicules, grains and spikes.

Except for thousand grain weight for which interaction genotype x environment was no significant, for the other traits namely spike length, grains per spike, awn length, grain length, grain width and in lodicules (length and width of principal lobe, length of higher hair of principal lobe and length of lateral lobe), interaction between the genotype x environment was significantly very high, significantly high or at least significant. The morphometric traits explaining the greatest variation among genotypes through the locations were: thousand grain weight, spike length, grain width, width of principal lobe and length of lateral lobe in lodicules. The pedoclimatic parameters influencing the variation among genotypes were in descending level of importance: soil pH followed by average temperature, rainfall and by the soil electrical conductivity.

Some interesting correlations among others were found. It was the case for the width of principal lobe of lodicules which was negatively correlated with the thousand grain weight and with the spike length. These later were positively and strongly correlated between them and were correlated positively with grain width. The number of grains per spike was positively correlated with rainfall. Heading date was positively very highly correlated with the soil pH and strongly but it was negatively correlated with temperature and negatively significantly with rainfall. Also pH of soil and grain ash content correlated negatively with rainfall.

Key words: Barley; Lodicules; Grains; Spikes; Morphometry; Variation; Localities

Introduction

Barley is one of the oldest domesticated food sources and currently a widely adopted cereal crop (Verstegen et al., 2014).

Lodicules are the diminutive paired bodies lying between the base of the lemma (flowering glume) and the ovary and palea in the grass floret (Heslop-Harrison & Heslop-Harrison, 1996). The paired lodicules are situated next to the embryo near the base on the dorsal side of the grain (Sparks & Malcolm, 1978).

According to Kosina (2011), lodicules in grasses play a main role in cleisto- or chasmogamy behaviour of florets and one can also accept their role as storage organs for developing caryopses. Cleistogamy is the production of flowers that do not open and undergo self-fertilization "in the bud" (Lord, 1981). Barley is predominantly self-pollinated (Von Bothmer & Kumatsuda, 2011). Zhang et al. (2019) concluded that cleistogamy may be a favorable trait for self-pollination crops (such as rice, wheat and barley) to avoid failure of pollinating caused by adverse weather conditions, while it may prevent transgenes from spreading into the environment by reducing out-crossing rates. Otherwise, according to Honda et al. (2005), cleistogamy in barley shows increased resistance to Fusarium head blight, which induces high economic damage to the kernel. On his side, Neergaard (1977) mentioned that barley cultivars having closed flowers are much less affected by Ustilago nuda than cultivars with open flowers. There is much evidence that the flowering habit of barley is closely related to the size of lodicules (Neergaard, 1977) and morphologically cleistogamous flowers have smaller lodicules than chasmogamous flowers (Peter & Jonhson, 2006).

Zhang et al. (2019) reported that the identity of lodicules is mainly determined by B-class genes and also by two MADS-box genes which regulate the development of the morphological characteristics of the lodicules.

Connor (1979) indicated that Tateoka (1960) and Lenlsek & Jozifova (1968) discussed lodicule structure and morphology, and brought these organs into prominence for taxonomy following on the foundation provided by Stebbins (1956). According to Carson & Horne (2013), the value of the lodicules in the classification and identification of barley varieties was pointed out towards the end of the nineteenth century by several authors.

Many authors like (McNeal & Ziegler, 1975; von Bothmer & Jacobsen, 1979; Von Bothmer et al., 1982), have studied morphology of lodicules.

Selection for many traits is not only being complicated by their quantitative nature, but also by the interaction between genotype and environment (Kraakman, 2005). Barley landraces (LANs) represent an important source of breeding material in unfavourable environments, as they are often grown in stressful environments with zero to limited agronomic input (Ceccarelli & Grando, 2000).

In the literature, little or no work exists on the variation of the size of lodicules in different environments and their relationships with other useful agro-morphological characters whether concerning barley or other species.

Thus, understanding modifications of morphological parameters of lodicules with change of environment conditions and their correlations with other traits related to grains and spikes can guide breeding work in favor of agronomical interesting traits. That was the principal objective of our work taken on barley landraces tested at three different agro-ecological conditions in Algeria.

Materials and Methods

In presence of an approved Algerian variety named Tichedrett (33), the three landraces considered in this study were collected from Algerian Sahara: P14 (barley with two rows from Béchar, Western Sahara of Algeria), P17 (barley with six rows from Touggourt, Eastern Sahara of Algeria), P20 with local name of "Ras El Mouch" (barley with six rows from Adrar, Western Sahara of Algeria). Three different locations were studied; the first was located in Mitidja (INRAA's experimental station of Mehdi Boualem (MB) which is a subhumid region, Latitude: 36, 68; Longitude: 3, 11; altitude: 18 m). The second region was Ain El Hadjel (ALA) (semi-arid climate with hot summer; Latitude: 35.674, Longitude: 3.88176, altitude: 544 m). The third location was Relizane (REL) at H'Madna (eastern city located 28 km from Relizane with a semi-aride climate and is at 35°54' N of latitude, 0°47' E of longitude and 48 m as altitude).

Characteristics of the locations on soil texture, soil pH, soil electrical conductivity, average rainfall and average temperature of experimental periods are presented in table 1.

All experiments were set up in November 2017. In each location, two randomized plots were taken. Each cultivar of each plot was represented by two lines with 2 m length and 1 m interline. The seedling dose was 50 kg per hectare. Experiments were carried out without fertilization and pesticides.

Biometric analysis

At maturity, in order to measure the size of the lodicules localized around the embryo, each grain was detached from its lower lemma using a scalpel. The lodicules were then removed (fig. 1 and fig. 2) and measured by a compact Greenough stereomicroscope stemi 305 Zeiss, with a camera connected to a screen. For this parameter, grains per cultivar were randomly chosen per location. Measurements were taken using the graduations at the stereo microscope with objective three.

Traits considered in lodicules were: length of principal lobe (LPL) (mm), width of principal lobe (WPL) (mm), length of higher hair of principal lobe (LHH) (mm) and length of lateral lobe (LLL) (mm). Also at maturity, morphological traits related to spikes and grains were studied: thousand grain weight (TGW) in grams (g), spike length (SPL) (cm), awn length (AWL) (cm), grains per spike (GRS), grain length (GRL) (mm) and grain width (GRW) (mm). For thousand grain weight, they were two replications by one random sampling per plot and per location. For the other traits, twenty random replications per cultivar and per site were taken.

In addition of these traits, ash content of grains (ASH) in cultivars was determined at maturity. Expressed in dry weight basis (percentage), the grain ash content was obtained according to the AFNOR (1985) method. For each cultivar per site, one random sample taken from the two plots was considered and then ground to obtain 3 g of powder to be analyzed. The heading date (number of days from sowing date to emergence of 50% of spikes) was studied (HEA) per site for each cultivar.

Statistical analysis

Analysis of variance was taken using GENSTAT Discovery version 3 (GENSTAT, 2008) for grains and spikes traits and for lodicules characters via three different agro-ecologic locations. Ash content and heading have not been considered in analysis of variance.

Multivariate analysis (principal component (PCA)) was taken with means of all these traits: TGW, ASH, SPL, AWL, GRS, GRW, GRL, LPL, WPL, LHH, LLL and HEA through three locations (Table 3). In addition, the following parameters were also considered as supplementary variables: the average of the cumulative monthly rainfall (RAF) and mean temperature (TAV) (°C) of the experiment period, the pH and the electrical conductivity of soils (CE) per location.

Correlations and PCA were performed in STATISTICA version 6 (STATISTICA, 2001). Pearson correlations were taken through the locations with means of the following parameters: TGW, ASH, SPL, AWL, GRS, GRW, GRL, LPL, WPL, LHH, LLL, HEA, RAF, TAV, pH and CE.

Results

Lodicules disposition

Lodicules can be frontal or clasping. As reported by Della (1985), small lodicules are referred to as 'small', 'bib', 'parvisquamose' or 'frontal' but bigger lodicules which cover the embryo like a collar, are referred to as 'large', 'collar', 'latisquamose' or 'clasping' (fig. 3, according to UPOV, 2016).

At maturity, the disposition of the lodicules was studied for each cultivar tested in each location. The results showed that the lodicule dispositions for all cultivars and in all environments were the same and consisted of the clasping "collar" type.

Table 1. Characteristics of test locations

Location	Average rainfall (mm) (test period)	Average temperature (test period)	Soil pH	Soil CE	Soil texture
Mehi Boualem	82.6	14.86	7.6	0.17 dS/m	Sandy clay loam
Ain El Hadjel	17.8	14	8.69	0.87 dS/m	Sandy clay loam
Relizane	45.26	17.83	7.32	2.1 dS/m	Silty clay

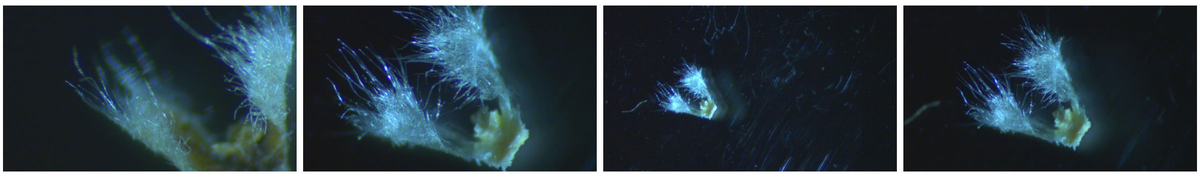

Fig. 1. Aspect of lodicules after removing from grain embryo of cultivars

Fig. 2. Parts of the lodicules detached for measurements

Fig. 3. Frontal lodicules (figure left) and Clasping lodicules (figure right)

Analysis of variance

The analysis of variance showed a significant ($P<0.001$) genotype effect in terms of agro-morphological and lodicule traits investigated except for grain length for which the genotype effect was highly significant ($P<0.01$) showing existence of genetic differences among cultivars for these traits (Table 2).

The effect of environment (location) was very highly significant for spike length, grains per spike and grain width; highly significant for grain length but no significant for the rest of characters related to lodicule traits, awn length and thousand grain weight.

Moreover, results for traits studied in spike, grains and lodicules for cultivars across the three regions revealed a strong interaction between genotype x environment (location) for characters like this: very high significant effect genotype x environment for spike length, awn length, grains per spike and grain width; high significant for length of principal lobe in lodicules and significant for the rest of traits mainly grain length, width of principal lobe, length of higher hair of principal lobe and length of lateral lobe. These findings demonstrated significant changes at level of genotypes from one location to another for these traits. However, only for the thousand grain weight, the effect of environment x genotype was not significant (Table 2). Thus, this important yield parameter did not change significantly with change in environmental conditions for the cultivars studied.

Correlations

The study of correlations showed interesting links between certain traits and in relation with some factors of environment.

We found that thousand grain weight and spike length were negatively significantly correlated with one of lodicules traits studied which was the width of principal lobe (WPL). This last was positively significantly correlated with LLL (length of lateral lobe) and LLL had high positive correlation with LPL (length of principal lobe). Thousand grain weight was positively very highly correlated with spike length and positively highly significantly correlated with grain width. The number of grains per spike had a positive significant correlation with average rainfall. In contrary, ash grain content highly negatively correlated with rainfall beside heading and pH of soil which had negative significant correlations with rainfall.

Heading very highly positively correlated with pH of soil but negatively highly correlated with temperature average. This last environmental parameter (temperature average) very highly positively correlated with electrical conductivity of soil but highly negatively correlated with pH of soil (Table 4).

Multivariate analysis

Principal component analysis was down (Table 5, fig. 4) to evaluate the greatest agro-morphological traits explaining the differences between cultivars tested at three different environments beside environmental parameters considered as supplementary variables. Based on Eigen values greater than 1, four components were considered in the principal component analysis with a percentage total variation rate of 82.8%. Characters that are correlated with the first component that absorbs the largest percentage of variation (33.74%) were in descending order of their correlation coefficients: TGW, SPL, GRW, WPL, LLL and GRL. The second component representing 21.71% of the total variation correlated with the following traits: NGE, AWL, LHH and LPL. The following characters: pH, HEA, TAV, RAF and ASH correlated to the third component, which absorbs 15.74% of variation. Finally, CE correlated to the fourth component with the lowest percentage of variation (11.65%).

Discussion

The identification of biological mechanisms underlying the development of complex quantitative traits, including those that contribute to plant architecture, yield and quality potential, and seed dispersal, is a major focus in the evolutionary biology and plant breeding (Ntakirutimana & Xie, 2019). Lodicules located around the embryo of grain play a critical role in taxonomy. They also had important part in cleistogamy which is useful for barley.

Our study revealed that lodicules disposition was maintained as clasping “collar” type for all cultivars tested at different environments. According to NIAB (2008), cleistogamy (closed flowering) is due to the clasping ‘collar’ type lodicule disposition, and results in an alost entirely autogamous habit due to the release of pollen within the closed flower.

In our case, beside thousand grain weight, spike length, grain width and grain length, the width of principal lobe and the length of lateral lobe in lodicules were among the traits explaining the big variability among the genotypes through the different environments. The largest value in length of principal lobe (1.48 mm) was almost twice the smallest average value (0.88 mm). For the width of principal lobe, the highest mean value was 1.05 mm and the lowest was 0.65 mm. The width of lodicules studied by Wang et al. (2015 at green anther stage on barley cultivars showed a variation between a minimum of 0.35 mm and a maximum of 0.85 mm, so lower values than we found in our study at maturity. In wheat, accession to accession variation in lodicule size was considerable and the largest was more than twice the size of the smallest in the study of Ning et al. (2013). Note that in our study all traits related to lodicule size were correlated to the two first components explaining the greatest percentage of variation (55.45 %). Recall that existence of variability is promising for breeding works. Otherwise, we noted very high interaction genotype x environment for traits related to size of lodicules showing that lodicules varied for the traits studied with variation of environments in our tests. According to Zhang et al. (2016) in angiosperms, vegetative and reproductive organs show a differential plastic development between varied environments, with a low plasticity or high robustness for flower formation, but little is known about its intrinsic mechanism. Otherwise, Cheplick (2007) concluded that relative allocation to Cleistogamy and Chasmogamy varies among species and populations, and is influenced by ontogeny and environment.

For other traits like spike length, awn length, grains per spike, grain width and grain length, significant interaction effect was showed between genotype and environment in our study but no significant effect was found for thousand grain weight. In barley lines studied by Hanifi-Mekliche et al. (2011), significant environment × genotype interaction was found for number of tillers/m², grain yield, thousand-grain weight, straw height and ear length. For twelve barley varieties tested in four locations, Welu (2014) found significant interaction genotype x environment for days to heading, days to maturity, biomass harvest index, thousand kernel weight, grain yield, plant height, spike length, number of kernels per spike, Tillers/plant and spikelets per spike.

Our study revealed a fairly interesting negative and significant correlation between the width of the principal lobe in lodicule and the thousand grain weight. This link between this lodicule trait and one of the most important yield parameters in barley is very promising and prompts more investigations, in particular of a genetic level, in order to derive possible profits for this crop or can to be for others. Similarly, we found a negative significant correlation between the spike length and the width of principal lobe of lodicules. In the literature, work relating to the relationships of traits of lodicules with certain parameters such as the thousand grain weight or others is almost absent. Remember here that various authors accepted the lodicules simply as structural components of the floret, as indicated by Heslop-Harrison & Heslop-Harrison (1996). In wheat and barley, Miralles et al. (2000) found under controlled conditions that an increased duration of the late reproductive phase from terminal spikelet to anthesis resulted in heavier spikes and more fertile florets per spike. Otherwise, according to Rodriguez et al. (2016), the reproductive organs in the Poaceae (grasses) family are the basic units determining grain yield in cereal crops.

In our case, principal component analysis has shown that the environmental parameter having the greatest effect on variation between cultivars was pH of soil followed by average temperature, rainfall and lastly by electrical conductivity of soil.

Table 2. Analysis of variance for ten traits in barley cultivars at three locations

Source	df.	SPL	AWL	GRS	GRL	GRW	TGW	WPL	LPL	LHH	LLL
E.	2	41.08***	0.503ns	4533.58***	27.27**	4.41***	3.632ns	0.02ns	0.04ns	0.039ns	0.015ns
G.	3	703.99***	275.43***	3372.37***	28.9**	7.07***	707.61***	0.098***	0.02***	0.73***	0.09***
G. x E.	6	6.65***	7.69***	469.8***	13.68*	1.07***	15.05ns	0.034*	0.07**	0.156*	0.04*

E.: Environment; G.: Genotype; G. x E.: Genotype x Environment; df. – Degree of freedom, *P < 0.05, **P < 0.01, ***<0.001, ns: non-significant

Table 3. Means of twelve traits studied per cultivar and per location

Traits / G.	L.	SPL	AWL	GRS	GRL	GRW	WPL	LPL	LHH	LLL	TGW	ASH	HEA
P14	MB	9.32	7.8	27.55	9.99	3.3	0.65	1.23	1.56	0.76	58.5	2.4	122
P14	REL	6.95	8.3	18.4	9.33	3.09	0.78	1.13	1.63	0.87	52.55	3.44	116
P14	ALA	7.3	7.34	17.8	10.65	3.65	0.7	1.2	1.87	0.75	54.5	3.99	147
P17	MB	7.22	11.26	40.7	11.18	3.2	0.75	1.0	1.01	0.7	46.4	3.12	124
P17	REL	5.7	11	35.4	9.67	2.74	0.86	1.25	1.2	0.75	48	3.42	120
P17	ALA	6.3	11.55	37.05	11.15	3.08	1,0	1.19	0.9	0.8	53	3.59	147
P20	MB	3.42	7.75	46.2	8.9	2.09	1.02	1.17	1.3	1.0	28	3.31	123
P20	REL	3.4	8.2	30.45	9.06	2.52	0.82	1.42	1.82	0.93	30.9	3.58	118
P20	ALA	3.66	9.35	22.5	9.22	2.96	1.05	1.48	1.33	1.0	30.8	3.55	148
P33	MB	5.2	12.54	48.8	12.06	3.1	0.95	1.22	1.77	1.0	47.6	2.85	127
P33	REL	3.4	12.4	29	9.46	2.82	0.78	0.88	1.4	0.63	42	3.09	117
P33	ALA	4.44	11.7	31.6	9.5	3.3	0.82	1.18	1.57	0.75	46	3.44	158

G.: genotype; L.: location; MB: Mehdi Boualem; REL: Relizane; ALA: Ain El Hadjel

Table 4. Pearson correlations between 16 parameters in barley genotypes from three locations

	SPL	AWL	GRS	GRL	GRW	TGW	LPL	LHH	LLL	WPL	HEA	ASH	TAV	CE	pH
AWL	-0.24														
GRS	-0.23	0.48													
GRL	0.46	0.46	0.4												
GRW	0.67*	0.09	-0.45	0.54											
TGW	0.86***	0.11	-0.24	0.55	0.76**										
LPL	-0.16	-0.41	-0.17	-0.22	-0.12	-0.34									
LHH	-0.03	-0.39	-0.36	-0.12	0.21	0.03	0.26								
LLL	-0.38	-0.3	0.25	-0.12	-0.45	-0.55	0.67*	0.24							
WPL	-0.61*	0.23	-0.44	-0.04	-0.53	-0.59*	0.38	-0.38	0.68*						
HEA	-0.04	0.12	-0.18	0.17	0.48	0.08	0.28	-0.06	0.01	0.26					
ASH	-0.31	-0.21	-0.38	0.2	0.02	-0.22	0.26	0.04	0.08	0.24	0.46				
TAV	-0.21	0.01	-0.15	-0.42	-0.42	-0.12	-0.18	0.15	-0.16	-0.25	-0.78**	-0.04			
CE	-0.3	0.03	-0.47	-0.49	-0.21	-0.09	-0.01	0.15	-0.23	-0.14	-0.33	0.38	0.84***		
pH	0.02	0.01	-0.25	0.18	0.49	0.1	0.3	-0.09	0.03	0.27	0.98***	0.49	-0.8**	-0.34	
RAF	0.21	-0.03	0.6*	0.21	-0.31	-0.03	-0.28	-0.02	0.15	-0.14	-0.68*	-0.76**	0.13	-0.44	-0.69*

Df=10; α0.05=0.576; α0.01=0.708; α.0.001=0.823; *: significant at 5 % level; **: highly significant at 1 % level; ***: very highly significant at 0.1 %; no significant at p>0.05

Table 5. Principal component analysis (PC) of barley genotypes based on sixteen parameters and three locations

Parameter	PC 1	PC 2	PC3	PC4
Eigen values	4.04	2.60	1.88	1.39
% of variance	33.74	21.71	15.74	11.65
Cumulative %	33.74	55.45	71.2	82.84
		Characters		
SPL	0.833	0.124	-0.031	0.347
AWL	0.116	-0.783	0.307	-0.211
NGE	-0.295	-0.817	0.004	0.336
GRL	0.508	-0.462	0.453	0.435
GRW	0.826	0.243	0.447	0.064
TGW	0.922	0.003	0.120	0.152
LPL	-0.490	0.526	0.322	0.424
LHH	0.052	0.626	-0.172	0.325
WPL	-0.779	-0.305	0.447	0.092
LLL	-0.732	0.166	0.142	0.597
ASH	-0.268	0.451	0.490	-0.474
HEA	0.036	0.201	0.884	-0.164
*CE	0.108	-0.271	0.285	-0.532
*TAV	0.0996	-0.021	0.693	0.249
*RAF	-0.032	0.453	0.618	-0.552
*pH	-0.052	-0.256	-0.875	0.149

*Supplementary variables

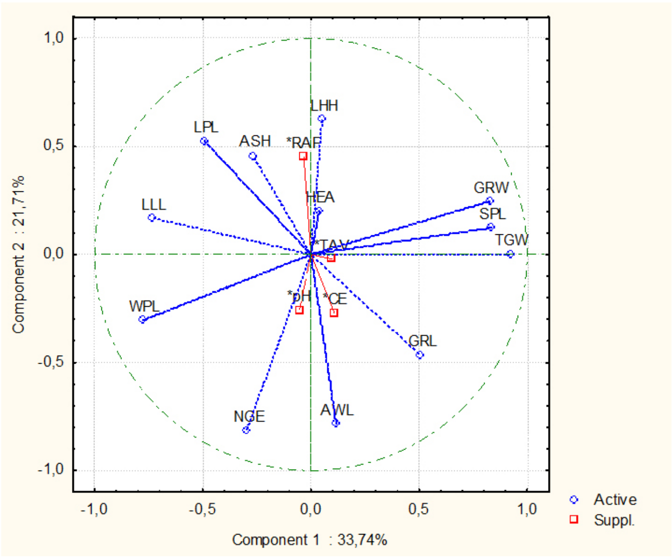

Fig. 4. Projection of active and supplementary variables on first two components

According to Zhang et al. (2019), studies have shown that soil pH can influence crop yields, soil nutrient release, and soil microbial activity to a large extent. Otherwise, the study taken by Peprah (2014) on plantain, maize, cassava, cocoyam, rice and yam for 14 years concludes that temperature explains the larger portion of the crop yield variation than rainfall.

In our study, soil pH was highly negatively correlated with temperature and negatively correlated with rainfall. These results agree strongly with those of Cheng-Jim et al. (2014) who reported that pH has an obviously negative correlation with mean temperature and mean precipitation. Many studies indicated that temperature and precipitation are important factors that control soil pH as indicated by Zhang et al. (2019).

A strong correlation positive and very highly significant was found in our study between the average temperature and the electrical conductivity of soil. These results are consistent with those of Campbell et al. (1949) and Bai et al. (2013).
We found that heading date was negatively significantly correlated with rainfall and negatively highly correlated with temperature. According to Calderini et al. (2001), higher temperatures, also called heat stress, significantly affect floret growth rate during anthesis and heading. In our case, the number of grains per spike was positively correlated with rainfall. In barley, Van Oosterom et al. (1993) found that season rainfall was positively significantly very highly correlated with grain yield.

Ash content is an important parameter in crops. According to Bogale & Tesfaye (2011), the accumulation of minerals (measured as ash content) in both vegetative tissue and kernels has been proposed an inexpensive and simple way to predict drought adaptation and yield in cereals. On their side, Rodehutscord et al. (2016) indicated that characterization of variations in the nutritional value of cereal grains that result from such factors may help to define appropriate breeding objectives for improving the value of cereal grains for nutrition.

In our study, we noted the presence of a high negative correlation between ash grain content and rainfall. In the study taken by Högy et al. (2013), amount reduction of precipitation increased the concentrations of several minerals and amino acids. Otherwise and according to several authors, the grain mineral content of crop species is affected by both genotype and environment (Pontieri et al., 2014).

Conclusion

Landraces known by their resiliency are strategic face the climate change. Study of parameters related to lodicules and other useful traits, their stability and their relationships in barley landraces and/or varieties could provide good opportunities for breeding programs. Our study taken on four barley cultivars through three different agroclimatic conditions showed interesting results notably concerning existing of significant correlations between thousand grain weight representing an important yield parameter and the width of principal lobe in lodicules. This last was also correlated negatively and significantly with the spike length. Except for thousand grain weight with no significant change via environments in cultivars, significant effect genotype x environment was found for all the morphometric traits related to grains, spikes and lodicules. Otherwise, the variability noted for all the agro-morphologic traits among barley cultivars could be in favour with breeding works. The disposition of lodicules was clasping “colar” type for all cultivars and has not change through environments. This type of disposition is in favour with cleistogamy which provides more resiliency for barley. Finally, interactions found between environmental parameters of soil and climate in liaison with different sites were interesting and were consistent with research findings worldwide. These relationships can help improve crop production by limiting negative environmental impacts.

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