Full Length Research Paper

Barley breeding for quality improvement in Tunisia

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This study was initiated to assess the effectiveness of three selection procedures applied in the early segregating generations of barley crosses for quality traits. The selection procedures were pedigree selection (PS), bulk selection (BS) and single seed descent selection (SSD). Selection was operated in F_2's crosses. Field trials were conducted with 19 F_3 crosses of barley in two different environments, a sub-humid environment at Beja and a semi-arid one at Kef. Heading date (HD), thousand kernel weight (TKW), protein content (PC), β-glucan content (BG) and husk percentage (HP) were evaluated. Data were subjected to an additive main effects and multiplicative interaction (AMMI) model to determine the efficiency of each method of selection for the proper environment. The results show that the AMMI model generated predictive optimizing selection method. The pedigree selection was more efficient in high input environment, while the bulk method was very effective for the selection of kernel weight in target environment.

Key words: Additive main effect and multiplicative interaction (AMMI) model, Barley, breeding methods, efficiency of selection, quality traits.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is, after wheat, the second major cereal crop in Tunisia. The barley improvement program was initiated late in 1893. The national program is based on the exploitation of the local and old cultivar populations, and on the introduction of foreign germplasm (Deghaïs et al., 1999). Until now, more barley research efforts in Tunisia have been invested in improving yield production than in improving quality product. Now, more than 80% of the barley produced in Tunisia is used for animal feed. The rest is used for food and malt. Local landraces are preferred for food preparations rather than the improved cultivars (Hamza et al., 2004; El Felah and Medimagh, 2005).

State of art showed that barley was a main food crop in North Africa for milliners, extending from Eastern African corn to North Africa, particularly to Southern Mediterranean countries (Newman and Newman, 2008). Early Neolithic civilization in Miner city of Gafsa and others Berber dynasty, Punic and Roman times contribute sensitively to make barley as a staple food in Tunisia (El Felah and Medimagh, 2005). Zohary and Hopf (1988) reported that barley took the status of "poor man's bread" comparably to many other food grains (wheat, rye, and oats) and played a significant role, as a sustaining food source in the evolution of humankind, by its evolution from traditional fermented foods to modern healthy human diet. In fact, barley contributes to health benefit and the most valuable component is β-glucan, as a native form in the grain (Wood, 2002, 2007).

In fact, barley flour fractions are rich in the non-starch polysaccharide family mixed-linkage (1→3) (1→4)-β-glucans. Many authors (Newman and Newman, 1991; Bhatty, 1999; Delaney et al., 2003) reported that β-glucan has beneficial health effects as hypocholesterolemic,

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Table 1. Origin, code and nature of parents.

Cultivar/landrace name	Code	Nature	Origin	Spike type	Quality type
Martin	A	Foreign local cultivar (introduced from Algeria on 1931)	Tunisia	6-row	Food/Feed
Taj	B	Improved cultivar	Tunisia	2-row	Food/Malt
Ardhaoui	C	Local Tunisian barley landrace	Tunisia	6-row	Food/Feed
Sahli	D	Local Tunisian barley landrace	Tunisia	6-row	Food/Feed
Rihane	E	Improved cultivar	Tunisia	6-row	Food
Salmas	F	Introduced improved cultivar	ICARDA	2-row	Malt

human glycemic response and insulin level and susceptibility to colon cancer. β-glucan in barley varies between 2 and 9% and a higher β-glucan level (>6%) is considered good for a healthy diet. The development of high β-glucan barley cultivars would be beneficial for the food industry (Bhatty, 1999). In recent years, β-glucan has become an important dietary fiber in the Tunisian barley breeding program.

Protein content in barley kernels plays the most important role during the breeding process with the aim of obtaining new forms for food industry. The cultivars bred for food industry should have high protein content.

Several selection methods were used to select adapted material with desirable properties. Different studies have been conducted on wheat to identify the efficient method of selection in Tunisia (Deghais, 1991; Deghais et al., 1999).

Pedigree method consists of selecting F_2 plants and practice head selection from F_3 to F_6 (Turcotte et al., 1980). Although bulks of heads will generate families with head-rows, homozygoty needs much more time as compared to the bulk and single seed descent selection (SSD) methods, where selection is shortly operated from the F5; however, early selection was practiced to speed-up the breeding work (Deghais, 1991; El Felah, 1998). The consistent difficulties observed in the breeding work are indicated to separate the additive from the non-additive effects in F_2 generation. As such, yield performances should be investigated only from the F_3 (Shakoor, 1983).

Noting that, in several studies, the application of additive main effects and multiplicative interaction (AMMI) has been limited to yield trials to provide interpretation of data from complex experiments (Gabriel, 1971; Ebdon and Gauch, 2002; Gauch, 2006). The AMMI model is effective for gaining accuracy in genotype x environment (GxE) studies (Gauch, 1992; Nachit et al., 1992; Hussein et al., 2000; Weikai and Tinker, 2005).

Our study investigates the merit of three breeding methods: pedigree selection (PS), bulk selection (BS) and SSD. AMMI analysis was used to estimate locations and genotypes which is the most efficient selection and breeding method adopted in high input or/and target environments. In our study, quality parameters were analyzed.

MATERIALS AND METHODS

Plant material and experimental design

A complete diallel cross (6 × 6) was done in 1999/2000 using six local/improved, two and six-row barley cultivars/landraces (Table 1). Parents were chosen on the basis of the following criteria: variety with a long history of cultivation; a newly improved successfully grown variety, local landrace, and introduced germplasm (Table 1). Martin is a long history grown six row cultivar introduced from Algeria in 1931, and mainly grazed by sheep and rarely for food.

Taj, improved cultivar (WI2198-Australia), was registered in Tunisia in 1985. It is a high protein, smaller kernels, adapted to low rainfall areas and mainly used for food and malt (El Faleh et al., 1985).

Early empiric selections generated a big diversity of local Tunisian barley populations such as Ardhaoui grown in the south and Sahli in center of Tunisia. The local landraces are six-rowed (Boeuf, 1911). Barley landraces can be used for food and feed.

Rihane, a six-rowed improved cultivar, was registered in Tunisia in 1987, and obtained through a cross between a local landrace Atlas 46 (As46), improved material Arrivat (Avt) and Athenaïs (Aths). It's now a widely grown variety (more than 40% of total barley cultivated areas in Tunisia).

Salmas, a two-rowed improved variety from International Center for Agricultural Research in the Dry Areas (ICARDA), plumbed grain and low protein content, is used mainly as a check in international ICARDA barley nurseries.

30 F_1's and six parental lines were sown at the National Agricultural Research Institute of Tunisia (INRAT) research station in Beja in November 2000 following a randomized three-block design under rainfed conditions. Plots had four rows, 1.5 m long with 0.25 m between rows. 19 F_2 derived crosses were selected within 30 bulked F_1 hybrids rows. They were replicated as F_2 offsprings in a randomized completely block design (RCBD) adding the six parents in two locations at Beja and Kef in 2001/02. In June and just before harvest, selection was applied on the F_2 lines at both Beja and Kef localities. Selection operated in F_2's crosses as BS, PS and SSD generated three sets of materials: 19 F_3 bulk inbred lines, 19 F_3 pedigree inbred lines and 19 F_3 SSD inbred lines. The three sets of material were grown in November 2002 in split plot design with three replicates to explore the genotype-environment interaction within the three breeding methods. F_3 inbred lines from a comparative screening trial are represented in Table 2. The seeding rate was a two-gram mixture of kernels per genotype for bulk method, a mixture of one spike kernels per genotype for pedigree method and 40 kernels of 40 spikes per genotype as one kernel by spike for SSD method viable seeds per each two lines of 2.5 m. The F_3 trial was conducted during 2002/03 harvest season at INRAT experimental stations of Beja (Lat 37° N, long 9° E, Alt 165 masl) and Kef (Lat 36°14' N, Long 08°27' E, Alt 518 masl). Beja INRAT experimental station is the main research site for INRAT having long term average annual rainfall of 600 mm

Table 2. F$_3$ inbred lines from the comparative screening trial at Beja and Kef locations.

Code	Spike morphology	Name/Cross
AB	2 / 6 row	Martin / Taj
AC	6 – row	Martin / Ardhaoui
AD	6 – row	Martin / Sahli
AE	6 – row	Martin / Rihane
AF	2 / 6 row	Martin / Salmas
BA	2 / 6 row	Taj / Martin
BD	2 / 6 row	Taj / Sahli
BE	2 / 6 row	Taj / Rihane
BF	2 – row	Taj / Salmas
CA	6 – row	Ardhaoui / Martin
DA	6 – row	Sahli / Martin
DB	2 / 6 row	Sahli / Taj
DE	6 – row	Sahli / Rihane
EA	6 – row	Rihane / Martin
EB	2 / 6 row	Rihane / Taj
ED	6 – row	Rihane / Sahli
EF	2 / 6 row	Rihane / Salmas
FA	2 / 6 row	Salmas / Martin
FB	2 – row	Salmas / Taj
A	6 – row	Martin
B	2 – row	Taj
C	6 – row	Ardhaoui
D	6 – row	Sahli
E	6 – row	Rihane
F	2 – row	Salmas

and a high input environment. It is situated in the sub humid area of Tunisia and characterized by a mild winter and a hot summer. Whereas, the Kef one, as a target environment, is situated on the semi arid area with a long term average annual rainfall of 450 mm and a cool winter (Medimagh et al., 2007). Plots were hand sowed and harvested at ripening.

Agronomic, phenologic and quality traits

Five parameters were evaluated for F$_3$ inbred lines:

a) Yield component: Thousand kernel weight (TKW),
b) Phenologic trait: earliness as heading date (HD),
c) Quality traits: protein content (PC), beta-glucan content (BG) and husk percentage (HP).

Thousand barley grains were measured by a seed counter Numigral X5. Thousand kernel weights were done in gram.
    For earliness determination, the international centers and national programs characterized earliness as 70% of germplasm heading (Valkoun et al., 1998). Indeed, earliness is the time in days from January 1st when at least 70% of the heads are half-way out of the boot. Grain protein content (in %) and beta-glucan content (in %) are determined by near infrared spectrometry NIR Systems Model 5000 scanning monochromator instrument.
    Hulled barley used for human food is pearled before being used (Jaby El-Haramein, 2005). Husk percentage (in %) is determined by pearling 20 g of grain in a seedburo barley pearler for 20 s and then

weighting the residual pearled kernels. The percentage loss in weight is recorded as the husk percentage. This includes both the husk and the bran. Barley quality traits were determined at ICARDA's cereal grain quality laboratory as described by Williams et al. (1988).

Statistical analysis

Statistical analyses were carried out using the GenStat - 7th Edition (GenStat, 2004). In our study, we have used selection methods as environments and entries as genotypes. Genotypes by selection methods interactions were partitioned according to a multivariate technique, particularly the AMMI model, as proposed by Zobel et al. (1988). AMMI model is a powerful statistical model which incorporates both analysis of variance (ANOVA) and principal component analysis (PCA) into a single model with additive and multiplicative parameters (Zobel et al., 1988). Principal components model, fitted to the residuals from the ANOVA, and the resulting scores called the interaction principle components analysis (IPCA) (I for interaction), are calculated for both the genotypes and the environments (Gauch, 1992). The magnitude of the selection method was also determined using AMMI model.

RESULTS

Analysis of yield components

Selection scores, for the three methods are presented in Table 3, mainly the first four AMMI selections per method for yield components at Beja and Kef locations. The results show that TKW was determinant for pedigree in sub-humid areas (Beja) and for bulk in semi-dry location (Kef). In Beja, pedigree method gave the high IPCA-AMMI scores for grain weight (2.363), whereas the bulk was more efficient in Kef with the best IPCA-AMMI scores (2.170). We noticed also that the genotype BA derived from (Taj × Martin) was the most selected genotype over all three selection procedures in favorable environment; but its reciprocal AB (Martin x Taj) has been selected as bulk promising genotype in target environment. This can be explained by the row material type (Taj, a two row cultivar) which can develop homogenous plump grains especially in sub humid areas. Martin has been selected as female parent for semi-dry areas following the bulk selection methodology.

Selection for earliness

Selection scores and parent/hybrid AMMI selections per method and location (Beja and Kef) are presented in Table 4.
    Results show that the pedigree strategy for high input environments (Beja) was the best method to screen for high yield potential and biotic stresses regarding to IPCA-AMMI scores as selecting early material expressed as short heading duration (1.854). In another hand, SSD strategy was more suitable for target areas (Kef) with an IPCA-AMMI score of 1.819; but the bulk method did cluster for AB and Rihane × Taj (EB) among the first four

Table 3. Scores of the first four AMMI selections within selection methods at Beja and Kef locations for 1000-kernel weight (TKW).

Location	Selection method[1]	Rank	Score	First four AMMI selections[2]			
	BS	3	-1.595	EB	BA	BE	BD
Beja	PS	1	2.363	BA	DB	B	AB
	SSD	2	-0.768	BA	EB	BE	DB
	BS	1	2.170	AB	EF	AF	BA
Kef	PS	2	0.372	AF	EF	EB	FB
	SSD	3	-2.542	AB	EB	AF	BE

[1]BS, Bulk selection; PS, pedigree selection; SSD, single seed descent selection. [2]A, Martin; B, Taj; C, Ardhaoui; D, Sahli; E, Rihane; F, Salmas.

Table 4. Scores of the first four AMMI selections within selection methods at Beja and Kef locations for heading date (HD).

Location	Selection method[1]	Rank	Score	First four AMMI selection[2]			
	BS	3	-2.170	B	AB	EB	BA
Beja	PS	1	1.854	FB	BA	AD	B
	SSD	2	0.316	B	AB	BA	BF
	BS	3	-1.952	AB	EB	B	BE
Kef	PS	2	0.132	EB	BE	FB	B
	SSD	1	1.819	B	AB	FB	BA

[1]BS, Bulk selection; PS, pedigree selection; SSD, single seed descent selection. [2]A, Martin; B, Taj; C, Ardhaoui; D, Sahli; E, Rihane; F, Salmas.

Table 5. Scores of the first four AMMI selections within the selection methods at Beja location for quality parameters.

Character	Selection method[1]	Rank	Score	First four AMMI selection[2]			
	BS	2	0.597	BA	BE	ED	AF
PC	PS	1	0.971	AF	FA	F	BF
	SSD	3	-1.568	EF	EB	DB	FA
	BS	3	-0.644	AB	AC	AF	AD
BG	PS	1	0.547	AD	BA	AC	CA
	SSD	2	0.097	AC	D	E	AF
	BS	3	-1.629	BD	AB	BA	AF
HP	PS	2	0.669	BE	BA	FA	AF
	SSD	1	0.960	BA	EB	ED	BE

[1]BS, Bulk selection; PS, Pedigree selection; SSD, single seed descent selection. [2]A, Martin; B, Taj; C, Ardhaoui; D, Sahli; E, Rihane; F, Salmas. PC, Protein content; BG, beta glucan content; HP, husk percentage.

selected genotypes including Martin, Taj and Rihane as parents. AMMI model suggested reliable selection model in both environments especially when we bulk all the changing environment variability within derived segre-

Table 6. Scores of the first four AMMI selections within the selection methods at Kef location for quality parameters.

Character	Selection method[1]	Rank	Score	First four AMMI selection[2]			
	BS	2	-0.456	BF	AF	F	EF
PC	PS	3	-0.958	AF	BF	AD	FB
	SSD	1	1.413	AF	F	FA	D
	BS	1	0.779	AF	DE	BA	AE
BG	PS	2	-0.195	BD	BA	AB	BE
	SSD	3	-0.584	CA	ED	BD	DA
	BS	3	-1.604	AB	BD	BE	AC
HP	PS	2	-1.221	EB	BA	CA	BE
	SSD	1	2.825	BE	EB	BA	AB

[1]BS, Bulk selection; PS, Pedigree selection; SSD, single seed descent selection. [2]A, Martin; B, Taj; C, Ardhaoui; D, Sahli; E, Rihane; F, Salmas. PC, Protein content; BG, beta glucan content; HP, husk percentage.

gating material.

Analysis of quality traits

Tables 5 and 6 show the selection methods scores and the first four AMMI selections per method at Beja and Kef locations respectively for protein content, beta glucan content and husk percentage.

AMMI analysis showed that the pedigree method has been efficient in high input environments (Table 5) for the selection of high quality genotypes essentially for protein and beta glucan content with IPCA-AMMI scores of 0.971 and 0.547, respectively and recommended in second rank after SSD selection for husk percentage.

For Beja location (Table 5), the cross Martin × Salmas (AF) and his reciprocal Salmas × Martin (FA) are the best for protein content selection. Also, the crosses Martin × Sahli (AD) and Taj × Martin (BA) were the more appropriate crosses to breed for beta glucan improvement.

For Kef location (Table 6), the following hybrids Martin × Salmas (AF), Sahli × Rihane (DE), Taj × Martin (BA) and Martin × Rihane (AE) were the best lines to breed for beta glucan improvement using the bulk method. Rihane as a male parent and Martin as female parent were the good combiners which could transfer their adaptative traits to derived lines.

For both Beja and Kef locations, the crosses Taj × Martin (BA), Rihane × Taj (EB) and Taj × Rihane (BE) have been the most selected genotypes to breed for husk percentage improvement as SSD method selected (Table 6).

DISCUSSION

Analysis of yield components

AMMI model has generated predictive optimizing selec-

tion method especially for yield components such as TKW, and for quality parameters such as PC and BG.

Analysis of earliness seems to be useful in semi-arid areas of Tunisia to escape drought in terminal filling period. Results show that SSD and pedigree methods could improve breeding methodologies compared to the bulks in early generation for qualitative traits. Bulk is essentially practiced in dry environments where drought is combined to heat and plant selection is usually done late in the F_5 generation (El Felah et al., 1991; El Felah, 1998).

Early materials are mainly needed in dry environments where drought and heat occur dramatically during critical crop stages especially at the filling period (Slafer et al., 2005). In semi-arid environments, the critical period coincides with the time between heading and maturity. This period should be as short as possible to escape from water stress. Desired genotypes are those that fill the grain very quickly, that is, have short heading-maturity duration.

Genotypes/treatments significant interaction indicated that barley genotypes responded differently to stress which affected significantly the plant traits, and earliness should be a positive trait for yield under stress conditions. Earlier genotypes may receive less water in total than the later ones (Vaezi et al., 2010). Late anthesis in barley is fully responsible of lower grain yields. For early heading material, plants can take profit of long grain-filling period (Mitchell et al., 1996). Drought affected seriously TKW as the predominant yield component. If drought occurs during grain filling period, the barley genotypes responses resulted in differences on individual grain weight, as indicator of stress tolerance.

Hamza et al. (2004) identified, using molecular and morphological markers, a cluster at genetic similarity (GS = 65%), splitted into two sub-groups, one regroup local accessions (Djerba, Gabes, Kairouan, Kebili and Kerken-

nah) and a fixed variety Martin. The second regroup a local accession Jendouba and a registered variety Rihane. Two-rowed barley cultivars (Faïz, Roho and Taj) and Manel variety, a six row type, clustered in the same group. Martin variety derived from Algerian local landrace collected in 1931 and selected following mass selection within spike epuration (Deghais et al., 2007). Martin genetic behavior similarity to local germ-plasm is mainly explained by the same evolutionary pressures effects and should have accumulated the same adaptive alleles and characters as our local germplasm (Chaabane et al., 2009). In this study, Martin, as male or female parent, is a good combiner that could transfer easily to its offsprings its adaptative traits.

Rihane pedigree showed Atlas 46 as a female parent, selected in a local population originating from Algerian/Tunisian bordering areas influenced by Atlas mountain chain from Tunisia to Morocco. Both Rihane and local Jendouba have Atlas Djebeli relatives. This could explain the rapid and easy diffusion of Rihane in the eighties of the late century, its wide adaptation and its contribution to barley production record years. These two genotypes were genetically very similar (GS=81%). In the first subgroup, Djerba and Kerkennah germplasm collections are genetically very similar (GS=90%) even though they are from two different island. Indeed these unique endemic landraces, which are morphologically divergent from continental collection, are considered to have evolved in specific environments that differ from the rest of mainland. This demonstrates that gene flow is limited due to the spatial isolation. Therefore it can result in rapid fixation of mutations and subsequent speciation (Barton, 1998). In addition, the lack of competition with other species and the possibility for colonization of new habitats may promote speciation on islands (Crawford et al., 1987).

Analysis of quality traits

This analysis revealed that Martin would be considered as the best combiner for quality traits followed by Salmas and Taj for protein content at both Beja and Kef locations and for beta glucan at Beja locality only. Advanced lines showed more adaptation to stressed environments within improved selection methodologies.

In harsh and difficult environments, SSD should be applied to select material for good protein content and husk percentage, whereas bulk is suitable to select for beta-glucan content.

Our study shows that Martin would be used as a good combiner for agronomic and quality traits in comparison to the improved barley cultivars; because Martin is well adapted to dry environments. Consequently, improvement project depends on the selection of suitable cultivar lines to be used in hybridization program.

Our results show that multivariate analysis is an effective tool in identifying of the best barley genotypes for

crossing and selection. We noted that the AMMI model has generated predictive optimizing selection method for agronomic characters and quality parameters especially beta glucan content. As compared to the bulk and SSD, the pedigree method of breeding is more efficient in favorable areas where predicted genotype offsprings should be developed toward large yields and including high quality of products. Complete diallel crossing strategy enables the breeder to decide on male versus female parents in the crossing program. Local material should be integrated in crossing blocks as gene donor for quality improvement.

Finally, the best selection criteria depend on selection methodologies suitable for the target environment. The best bulks were highly significant essentially for kernel weight in unpredictable semi dry location. Plant and head row selections within the best families were efficient procedures of pedigree method to achieve significant genetic gain in non-stressed locations. Single seed procedure did not carry all the environment variation in contrast to the pedigree and bulk methods.

In fact, head row and hulled/hull-less kernels can influence considerably the protein content and the quality traits, especially beta-glucan levels (Baidoo and Liu, 1998). Shape, size and endosperm color of barley kernels guide simultaneously the dynamic evolution of hordeum genus in the region within both local and em-piric vs. scientific knowledge (Ceccarelli et al., 2001a, b).

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