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Exploiting landrace genetic diversity for germplasm enhancement in durum wheat breeding in Morocco

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Abstract. Traditional durum wheat farming communities have contributed for centuries to the evolution and enrichment of on-farm conservation of diverse wheat landraces, and to the development of farmer's seed exchange in order to ensure the continued evolution and diversification of these landraces especially under dry growing conditions. Landraces are genetically heterogeneous and have over many generations become adapted to the local environment and cultural conditions under which they are grown. However, during the last century, the introduction of high-yielding varieties, and the structural changes in wheat farming systems, led to the loss of genetic diversity of wheat landraces. In Morocco, landraces of durum wheat are still cultivated by farmers especially in marginal regions such as mountains and Saharan areas. Durum wheat landraces are highly appreciated for their adaptation to some abiotic stresses and mainly for their good grain and straw qualities. This paper summarizes some studies aiming to assess the amount of diversity of Moroccan landraces collected in two different agro-ecological areas of Morocco, and determine options for adding the value of these landraces. The evaluation of these landraces focused on agro morphological characters and specific quality parameters. The results showed a large genetic variability in this germplasm proving the possibility of using landraces as promising genepool in breeding program especially for improving grain quality. The results indicated also the possibility of improving on farm landraces productivity through "composite landraces" approach and low cost agricultural packages.

Keywords. Durum wheat landraces – Traits donors – Genetic diversity – Breeding – Adding value.

Exploiter la diversité génétique des variétés locales pour la valorisation du matériel génétique de blé dur au Maroc

Résumé. *La culture traditionnelle du blé dur par les communautés rurales a contribué au fil des siècles à l'évolution et à l'enrichissement de la conservation in situ de diverses variétés locales de blé, et au développement de l'échange de semences par les agriculteurs afin d'assurer l'évolution et la diversification continues de ces variétés locales, en particulier en conditions pluviales. Les variétés locales sont génétiquement hétérogènes et depuis de nombreuses générations, elles se sont adaptées à l'environnement local et aux conditions de culture. Cependant, au cours du siècle dernier, l'introduction de variétés à haut rendement, et les changements structurels dans les systèmes de culture du blé, ont conduit à la perte de la diversité génétique des variétés locales de blé. Au Maroc, les variétés locales de blé dur sont encore cultivées par les agriculteurs, en particulier dans les zones marginales telles que les montagnes et les régions sahariennes. Les races primitives de blé dur sont très appréciées pour leur adaptation aux stress abiotiques et principalement, pour la bonne qualité de leur grain et de leur paille. Dans cet article, on parcourt des études réalisées afin de mesurer la diversité des variétés locales marocaines collectées dans deux domaines agro-écologiques différents du pays et de déterminer les options possible pour accroître la valeur ajoutée de ces variétés locales. L'évaluation de ces races primitives est axée sur des caractères agro-morphologiques et des paramètres de qualité spécifiques. Les résultats ont fait ressortir une grande variabilité génétique de ce matériel indiquant ainsi la possibilité d'utiliser les variétés locales comme fond génétique prometteur dans des programmes de sélection, en particulier en vue d'améliorer la qualité du grain. Les résultats ont également confirmé qu'il est possible d'améliorer la productivité des variétés locales in situ par une approche "variétés locales composites" et des paquets agricoles à faible coût.*

Mots-clés. *Variétés locales de blé dur – Caractères des donneurs – Diversité génétique – Sélection – Valeur ajoutée.*

I – Introduction

The area planted with cereals in Morocco is about 5 million ha. Durum wheat (*Triticum turgidum* var. L. *durum*) is grown on over one million hectares. 45% of which are sown in the arid and semi-arid regions, 11% in high altitudes and 44% in more favorable areas (Nsarellah *et al*., 2011). The average durum wheat consumption is about 90kg/person/year. Morocco is ranked third in Mediterranean regions and first in North Africa and Middle East regions in term of durum wheat acreage. Because of the importance of this crop in Morocco, breeding program at Institute National de la Recherche Agronomique (INRA) has always provided the necessary efforts to release new varieties of durum wheat, that are productive, resistant to biotic and abiotic stresses, and of good quality. The official national catalogue lists over 35 durum wheat varieties. However, the agro-industries provide almost 99% of their supply from abroad. The release of durums with high quality standards is one of the priorities of breeding programs in the country in recent years. Landraces may provide new alleles for the improvement of commercially valuable traits.

Increasingly, the country is considered as centre of diversity for a number of cultivated crop plants and wild relatives. Indeed, Morocco constitutes one of the most important areas of diversity in the Mediterranean region. It is an important centre of diversity for global crops such as barley, faba bean, and wheat (Neal-Smith, 1955; Nègre, 1956 and Perrino *et al*., 1984). Morocco's crop diversity results from long-term adaptation to various local environmental conditions such as drought, cold and salinity (Sauvage, 1975; Graves, 1985). In many Moroccan traditional cropping systems, genetic diversity may be the only resource available to resource-poor farmers to cope with the environmental conditions and optimize their crop production. Moroccan durum wheat landraces represent an important source of valuable genetic resource (Sadiki *et al*., 2000). In fact, landraces of durum wheat are still cultivated especially by farmers in the mountains and arid regions of the country. They are highly appreciated by farmers for their adaptation to abiotic stresses and mainly for their good grain and straw qualities.

However, genetic diversity of the major crops including the durum germplasm has suffered an overall reduction over time as a consequence of their replacement by high-yielding varieties and urbanization (Zine el abidine *et al*., 1995). This genetic diversity is also facing the climate change threat. Durum wheat landraces have been largely replaced, in their centers of diversity by monocultures of pure genotypes. This genetic erosion resulted in significant loss of valuable genetic diversity of quality traits and resistance or tolerance to biotic and abiotic stresses. Also, durum wheat landraces from the mountainous areas of Morocco are known for their stem solidness which is an important trait for resistance to wheat stem sawfly (Damania, 1991).

Ex situ (gene bank) and *in situ* (on farm) conservation of these valuable genetic resources are ways to safeguard this genetic diversity from extinction for their present and future sustainable uses. Effective management and potential use of these genetic resources in breeding program require evaluation and description of the diversity in the gene pool, characterization of available accessions in order to detect the presence of variants of possible interest for breeding purposes.

II – Analysis of genetic diversity of Moroccan durum wheat landraces

The complexity of the population structure of wheat landraces may arise from a number of different homozygotes and the occurrence and frequency of heterozygotes in populations. The assessment of genetic diversity between and within wheat landraces is essential to utilize landraces as donors of traits in wheat breeding, and to identify priority areas for on-farm conservation.

Thirty nine landraces collected from oasis (Errachidia site) and nine from mountains (Taounate site) (Table 1) were characterized for the main agro-morphological traits according to descriptors suggested by Bioversity International; growth habit, plant height, spike characters (spike length and density, length and colour of awn, size and colour of grain, number of spikelets per spike and number of grains per spike.

Table 1. List of landraces collected from Errachidia and Taounate sites.

Site	Reference's landraces
Errachidia	CM98E1, CM98E2, CM98E3, CM98E4, CM98E5, CM98E6, CM98E7, CM98E8, CM98E9, CM98E10, CM98E11, CM98E12, CM98E13, CM98E14, CM98E15, CM98E16, CM98E17, CM98E18, CM98E19, CM98E20, CM98E21, CM98E22, CM98E23, CM98E24, CM98E25, CM98E26, CM98E27, CM98E28, CM98E29, CM98E30, CM98E31, CM98E32, CM98E33, CM98E34, CM98E35, CM98E36,CM98E37, CM98E38, CM98E39
Taounate	CM98T40, CM98T41, CM98T42, CM98T43, CM98T44, CM98T45, CM98T46, CM98T47, CM98T48

Variance analysis showed a high genetic diversity between and within all the landraces analysed in the two sites (Table2). Principal and factorial components analysis led to the classification of landraces into homogeneous groups characterized by specific traits (Figure 1 and Table 3).

Similar studies were done on Moroccan barley landraces and showed a high genetic variability between and within landraces (Rhrib and Taghouti 2001). Zarkti *et al*. 2010 measured genetic distance and diversity of seventeen Moroccan landraces through molecular markers analysis. The results revealed a high genetic diversity between the analyzed landraces; the hierarchical classification came up with five clusters related to earliness.

Table 2. Extent of genetic variability in Errachidia and Taounate landraces for the main agro-morphological traits.

Traits	Observed F Differences between landraces[1]	F test intra Errachidia accessions	F test intra Taounate accessions
Growth habit	3.19***	-	-
Height (cm)	4.25***	-	-
Awn length (cm)	6.56***	6.04***	4.19***
Awn color	105.33***	2.93***	13.35***
Spike length (cm)	13.42***	3.37***	2.17***
Spike density	8.62***	NS	4.13***
Number of sp kelets/spike	5.17***	2.06***	NS
Number of grains/sp kelet	4.65***	1.86***	2.10***
Thousand kernel Weight (g)	1.27NS	1.81***	2.21

*1 level of significance of F test: * significant at 0.05. ** significant at 0.01. *** Significant at 0.001, NS: Not significant.*

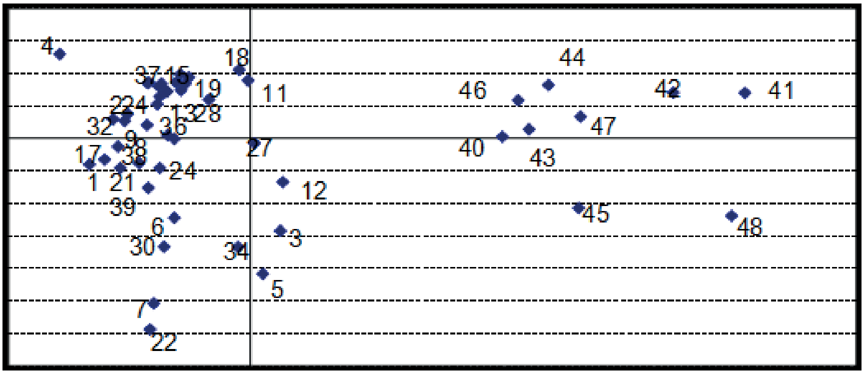

Figure 1. Representation of the Errachidia and Taounate Landraces in the graph formed by PCA1 and PCA2 axes.

Table 3. Groups of durum wheat landraces based on the multivariate analysis (FCA).

Groups	Traits	Traits level	Landrace origine
1	Grain color	Brown	CM98T40, CM98T41, CM98T42, CM98T43,
	Grain size	Intermediate	CM98T44, CM98T45, CM98T46, CM98T47,
	Spike density	Intermediate	CM98T48
	Number of grains/sp ke	25-43	
	Thousand kernel Weight (g)	22-44	
	Height (cm)	107-137	
	Growth habit	Low	
2	Number of grains/sp ke	45-53	CM98E1, CM98E8, CM98E14, CM98E16,
	Number of sp kelets/spike	21-23	CM98E19, CM98E20, CM98E23, CM98E25,
	Grain size	Large	CM98E28, CM98E29, CM98E31, CM98E32,
	Awn length (cm)	18-19	CM98E33, CM98E37, CM98E38
	Spike length (cm)	5.9-6.9	
	Height (cm)	137-153	
3	Grain size	Intermediate	CM98E 2, CM98E4, CM98E5, CM98E10, CM98E11
	Growth habit	strong	
4	Grain size	Large	CM98E 3, CM98E 21, CM98E22, CM98E24,
	Awn color	Black	CM98E27, CM98E30, CM98E34, CM98E39
	Awn length (cm)	16-18	
	Growth habit	medium	
5	Spike length (cm)	5.9-6.9	CM98E 15, CM98E25, CM98E7, CM98E33,
	Awn length (cm)	17-21	CM98E35.
	Number of sp kelets/spike	18-22	
	Growth habit	strong	

III – Adding value of durum wheat landraces

The local ecotypes are an important reservoir of genetic variability. Many authors were unanimous on the adaptation of landraces to their environment and confirmed that they outyielded the improved varieties in marginal areas and under low-input farming systems (Weltzein and Fishbek, 1990, Jaradat, 2011; Jaradat, 2013). Maintaining these landraces is therefore related to their utilization by farmers. The improvement of their productivity would surely contribute to their conservation.

On-farm conservation goal in Errachidia and Taounate sites is to encourage farmers to continue to maintain and manage durum wheat landraces. The primary method for achieving this goal is to Increase the value of durum wheat landraces on farm. Farmers on these two sites are still using traditional techniques in their parcels. The weeding is generally not practiced or if done manually it is too late. Seed borne diseases especially smut, bunt, and fusariose lead to the reduction of germination and thus of the yield.

Fungi seed treatment against seed borne diseases and chemical weeding of the crop are technologies that can increase the landrace productivity and ensure that they have a better added value. Furthermore, composite landraces made up of promising lines of selected landraces could be another technique for durum wheat landraces valorisation.

1. Influence of fungi treatment of seeds and chemical weeding on grain yield of durum wheat landraces

Five to ten kilogramms of seed lots of five landraces collected from five farmers of Errachidia and Taounate sites were divided into four lots. These seed lots were sown in plot on farmer field under four treatments; 1) fungi treated seeds in weeded plot, 2) fungi treated seeds in non-weeded plot 3) non fungi treated seeds in weeded plot and 4) non fungi treated seeds in non-weeded plot (check).

The analysis of variance (ANOVA) showed the significant effect of the fungi seed treatments and weeding; combined weeding and fungi seed treatment effect on grain yield of landraces was depending on the sites (Table 4). The combined effect of fungi treatment of seeds and weeding was significant in Taounate site and not significant in Errachidia site. Whereas, the individual effect of fungi treatment and weeding was significant in Taounate site and allowed an increase of yield of 33% and 17% respectively. But in Errachidia site, the only significant effect was fungi seeds treatment that allowed a grain yield gain of 34%. Similar study was conducted on Moroccan landrace of barley in Taounate site and showed the positive effect of fungi seeds treatment and weeding on grain yield (Rhrib and Amri, 2002).

Consequently, some farmers of the two sites were provided with simple manual machines for seed treatment (Photo 1). Farmers were taught how to operate and manipulate these machines and encouraged to integrate fungi seed treatment before sowing.

Table 4. Effects of fungi treatments of seeds and weeding on grain yield of durum wheat landraces in Taounate and Errachidia sites.

Treatments	Taounate site			Errachidia site		
	Test F[1]	Probability	Mean (kg/ha)	Test F	Probability	Mean (kg/ha)
Fungi seeds treatment and weeding combined	5.28	0.0052**	227.10	1.88	0.1732 NS	502.50
Fungi seeds treatment	4.50	0.0429*	246.70	5.06	0.038*	575.00
weeding	10.74	0.0038**	216.70	0.29	0.59NS	485.00

*1 level of significance of F test: * Significant at 0.05. ** Significant at 0.01.*

2. Evaluation of yield potential of composites durum wheat landraces

Three types of genotypes have been used:

- A composite landrace composed of a mixture of the most productive lines selected from the Errachidia landraces,
- One landrace originating from Errachidia site,
- An improved durum wheat variety; Oum Rabiaa.

This trial was conducted in a plot of $10m^2$ each with two replications in Errachidia farmer's fields. The measures recorded were grain and straw yield. The results showed that the composite population out yielded the original landrace. The yield gained by the mixture was about 8%

compared to the original landrace. Both of them have out yielded the improved variety Oum Rabiaa (Figure 2).

This result revealed the possibility to increase the productivity of durum landraces by the development of new landraces composed of a mixture of promising lines. The study done by Tesemma, (1996) showed that composite landrace of durum wheat outyielded the most common improved variety in Ethiopia by 40% and the original landrace by 37%. Similar studies have been done on barley (Ceccarelli and Grando, 2000; Rhrib and Taghouti, 2002) and bread wheat (Moghaddan *et al*., 1997).

Figure 2. Grain yields of the three types of genotypes (multilines, landrace and improved variety) used on farm.

IV – Potential value of landraces in durum wheat breeding programs

Landraces could act as donors of important characteristics, such as drought and cold tolerance, and mainly grain quality. In general, they represent significantly broader genetic diversity than modern varieties and, therefore, they could contribute to extend the genetic base of modern cultivars. Moroccan durum wheat landraces hold large genetic variability and considerable number of alleles with the probability of having some of these alleles associated with stress tolerance and yield (Nachit *et al*., 2004; Pagnotta *et al*., 2004). In Mediterranean countries, durum wheat landraces were largely used in breeding programs and contributed to the development of improved varieties in dry areas (Nachit, 1992). The identification of quality parameters such as protein content, gluten strength, yellow pigment and their integration in the improved varieties is a priority in research on durum wheat (Nachit *et al*., 1995). Mineral content in modern wheat cultivars has significantly decreased, including copper, iron, magnesium, manganese, phosphorus, selenium, and zinc. High levels of these nutrients can be found in landraces and old low-yielding varieties (Jaradat, 2011).

1. Agronomic evaluation of durum wheat local lines

Eight hundred lines derived from thirty-five landraces from Rif Mountains and pre-saharan regions of Morocco were evaluated. The lines were sown at the INRA experimental station of Merchouch in Augmented Design: 2 lines of 2.5m / line. Two improved varieties Oum Rabia and Karim were

2. Evaluation of quality traits of local durum wheat lines

One hundred and fifty lines selected from durum landraces already evaluated on experimental station, and fifty advanced durum wheat lines have been evaluated for some physio-chemical quality parameters: Gluten strength was estimated by SDS sedimentation test according to the standard Moroccan method NM 08.1.217 (Anonymous), The yellow pigment content representing carotenoid content extracted by n-butanol saturated with water and expressed in micrograms of beta carotene per gram of dry matter (ppm) was determined according to the standard Moroccan method NM .1.216 08 (anonymous). The protein content expressed on the dry matter was determined by the Kjeldahl method, based on the standard method (AFNOR, 1991) (anonymous).

Uni-variate analysis showed highly significant differences between local and advanced lines for all quality parameters. Local lines showed a genetic diversity higher than in advanced lines mainly for SDS sedimentation index and yellow pigments rate (Table 6).

Table 6. Mean values ± standard error, minimum and maximum values for advanced and local durum wheat lines for the studied characters.

Quality traits	Mean ± standard error	Minimum	Maximum
Advanced lines			
SDS volumes (ml)	38.10 ± 1.79	17	70
Yellow pigments (ppm)	6.85 ± 0.26	2.53	9.32
Protein content (%)	12.80 ± 0.14	11.07	14.96
Local lines			
SDS volumes (ml)	71.65 ± 1.12	40	94
Yellow pigments (ppm)	8.62 ± 0.12	4.18	12.55
Protein content (%)	12.31 ± 0.11	10.41	15.88

Factorial analysis also showed a wide range for quality traits studied. The hierarchical clustering tree has grouped the local durum wheat lines into five distinct branches (B1, B2, B3, B4, and B5) (Table 7). Within each branch, groups and subgroups were identified. Lines within each group were characterized by well-defined quality criteria.

Genetic diversity highlighted in the local germplasm could be used in breeding programs to improve the technological quality of durum wheat varieties. Thus, quality improving attributes in these varieties will be based on the choice among these groups of durum wheat landraces lines those with high levels of these quality criteria and included them as parents in the breeding program. For example, Group 4 of the branch 2 contains lines with a high rate of yellow pigments.

Table 7. Groupment of local lines based on factorial analysis on quality parameters

Branch	Group	Sub Group	Lines of landraces	Mean values of traits SDS (ml)	YP(ppm)	PC(%)
B1	G1	SG1	CM00E5(14), CM00E7(28,29), CM00E10(19,36), CM98T112(3).	62.8	8.07	12.55
		SG2	CM00E5 (13, 36, 42, 49), CM00E8(12).	62.2	8.58	13.28
		SG3	CM00E10(5, 18, 29, 48), CM00E5(5,12,23,29,34)	69.4	8.96	13.03
	G2	SG1	CM00E5(16), CM00E 12(3), CM00E 7(5,26,37), CM00E6(6), CM00E4(47).	71.8	7.45	12.39
		SG2	CM00E1(44), CM00E7(42), CM00E10(49).	73.0	8.71	14.97
		SG3	CM00E4(18), CM00E5(3,6,7,18,2,22,39) CM00E7(24, 49), CM00E10(30, 35, 46,47), CM00E11(48), CM98E105(8).	75.1	9.14	11.80

Branch	Group	Sub Group	Lines of landraces	Mean values of traits		
				SDS (ml)	YP(ppm)	PC(%)
B2	G1	SG1	CM98E10(3,4,48), CM00E12(4).	91.3	8.01	12.82
		SG2	CM00E5(1), CM00E7(38), CM00E10(42), CM00E12(8,11).	87.2	8.19	12.17
		SG3	CM00E5(41, 48), CM00E8(9), CM00E10(10,24,34)	89.3	9.85	11.45
	G2		CM00E5(38), CM00E7(48)	84.0	8.6	11.34
	G3	SG1	CM00E10(6), CM98E11(16).	79.0	6.67	11.74
		SG2	CM00E5(8), CM00E10(39), CM98E105(19).	80.3	10.06	11.74
		SG3	CM00E5(19,44), CM00E10(1,8,14,15,26,33).	79.3	8.70	11.85
	G4	SG1	CM00E5(9,30,47), CM00E7(25), CM98E10(2,7,11,16,20,32), CM98E18	82.4	9.21	12.20
		SG2	CM00E2(47), CM00E10(12).	82.0	11.73	11.54
		SG3	CM00E5(2,10,20,32,), CM00E7(23), CM00E10(17,31,43,47), CM98E19(5).	84.5	9.91	12.09
B3	G1	SG1	CM00E7(8,21).	71.0	6.35	12.37
		SG2	CM00E10(9).	83.0	12.52	10.41
		SG3	CM00E1(22), CM00E 7(9,22,30,47).	78.4	7.81	12.05
B4	G1	SG1	CM00E10(13,22,25,27), CM00E 11(6), Karim, Oum Rabiaa	53.6	8.52	12.27
		SG2	CM98T112(34), CM00E12(5).	57.5	6.17	12.10
	G2	SG1	CM00E2(15,40), CM00E6(31), CM00E10(40), CM00E12(10).	43.0	8.09	13.67
B5	G1	SG1	CM00E9(56), CM98T112(33).	66.0	6.45	11.62

V – Conclusions

Moroccan landraces analyzed displayed, as expected, a wide range of genetic diversity. This local germplasm forms an interesting source of favorable quality traits such as protein content, gluten strength and yellow pigments content useful to durum wheat breeders.

The persistent cultivation of durum wheat landraces in some Moroccan regions attests to their continued value to farmers, and to their competitive agronomic or nutritional advantage relative to modern varieties. Adding value of these landrace is the main motivating factor for their on- farm conservation. Fungi seed treatment against seed-born diseases and chemical weeding at the right time could improve the landraces productivity in a simple way.

Furthermore, Composite landraces made up of promising lines selected from landraces could be another way for durum wheat landraces valorization.

But, on-farm conservation of durum wheat genetic resources in Morocco could be more efficient provided that legislation changes are made that make it possible to market landraces as diversified genetic materials and encourage their consumption.

Moroccan durum wheat landraces have over many generations become adapted to the local environment and cultural conditions under which they are grown. Development of new varieties from landraces could be a viable strategy to improve yield and yield stability, especially under stress and future climate change conditions.

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