

## Durum wheat breeding before and after 1970 in Tunisia: Changes in yield components and quality attributes

**Ines Yacoubi<sup>1\*</sup>; Najla Kharrat<sup>2</sup>; Fatma Boukid<sup>1</sup>; Emna Khanfir<sup>1</sup>; Karama Hamdi<sup>1</sup>; Rhouma Sayar<sup>3</sup>; Yong Weon Seod<sup>4</sup>**

<sup>1</sup>Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax; B.P "1177", 3018, Sfax, Tunisia.

<sup>2</sup>Molecular and Cellular Screening Processes laboratory, Bioinformatics Group, Centre of Biotechnology of Sfax-University of Sfax, B.P "1177", 3018, Sfax, Tunisia.

<sup>3</sup>Tunisian Higher School of Agriculture of Kef, Boulifa, 7119 Kef, Tunisia.

<sup>4</sup>Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Anam-Dong, Seongbuk-Gu, Seoul 136-713, Republic of Korea.

**\*Corresponding Author: Ines Yacoubi**

Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax; B.P "1177", 3018, Sfax, Tunisia.

Tel: 21-602-039-8777; Email: inesbouchrityacoubi@cbs.rnrt.tn

### Abstract

This study highlights the changes in yield components and major quality traits of eighteen rain-fed Tunisian durum wheat genotypes, cultivated during the 20 century. The germplasm, which is of fundamental importance for the improvement of any species, was divided into three groups: G1: Landraces (breeding period before 1940); G2: Old varieties (breeding period between 1940-1970) and G3: Modern varieties (breeding period after 1970).

The obtained results spotlighted the impact of genotype and breeding programs on the most studied traits. In particular, the breeding after 1970 as much as had significantly enhanced grain yield, number of kernel by spike, total starch it had reduced significantly the thousand kernel weight, protein, wet and dry gluten concentrations. Furthermore, The PCA and clustering studies showed two distinct groups, where modern varieties (G3) was significantly estranged from landraces and old varieties (G1 and G2) which confirms the strong impact of the introduction of semi dwarf durum wheat lines generated by "green revolution" on Tunisian breeding programs. In particular, the example of Khiair from the advanced breeding varieties (G3), showing an interesting grain yield, strong gluten and highest yellow pigment concentration fully meets pasta end-use specifications. Though, Jnah-Khottifa, a North African landraces (G1), has shown interestingly both high thousand kernel weight and protein concentration which makes it an appealing genotype for "couscous" end-use.

**Keywords:** durum wheat; quality; yield; breeding.

Received: Nov 06, 2021

Accepted: Jan 14, 2022

Published: Jan 20, 2022

Archived: [www.bioaccent.org](http://www.bioaccent.org)

Copyright: © Yacoubi I (2022).

**Citation:** Yacoubi I, Kharrat N, Boukid F, Khanfir E, Hamdi K, et al. Durum wheat breeding before and after 1970 in Tunisia: Changes in yield components and quality attributes. BAOJ Biotechnology. 2022; 6(1): 1001.

**Abbreviations:** ICARDA: International Center for Agricultural Research in the Dry Areas; CIMMYT: International Maize and Wheat Improvement Centre; INRAT: Institut National de la Recherche Agronomique de Tunisie; GY: Grain Yield; NKS: Number of Kernel per Spike; TKW: Thousand Kernel Weight; P: Protein Concentration; WG: Wet Gluten Concentration; DG: Dry Gluten Concentration; GS: Gluten Strength; SI: Sedimentation Index; SDS: Sodium Dodecyl Sulfate; YP: Yellow Pigment Concentration; PCA: Principal Component Analysis; TS: Total Starch; ASE: Amylose; ISO: International Organization for Standardization; AFNOR: Association Française de Normalisation; POD: peroxidase; PO: Phenoloxidase; PPO: Polyphenol Oxidase; LOX: Lipoxygenase

## Introduction

Wheat (*Triticum* L. spp.) is the major staple of the human diet, supplying significant amounts of dietary carbohydrates and proteins. Global production of all wheat is about 750 mmt (International Grains Council, 2017). Durum wheat (*Triticum turgidum* L. var. durum) is an important food crop in the Mediterranean area, not only because of its large acreage but also for its importance in the human diet [1]. Durum wheat is most commonly used for the preparation of pasta, especially in European and North American countries, whereas in the Middle East and North Africa, it is also used for traditional bread and food as “couscous” and “bulghur”. The wheat grains consist of endosperm, bran and germ. The inner endosperm is primarily composed of starch granules and proteins, which account respectively for about 65–75% and 8–20% of the grain dry weight [2].

Assessment of wheat end-use potential begins with the grain quality aspects including visual appearance, weight of thousand kernels, non-durum contamination, starch, protein concentration and composition and Colour [3]. After milling, other parameters such as ash concentration, Colour, speck count, particle size distribution, milling yield and cooking quality are also important to consider for the final selection of genotypes for use in the manufacture of pasta and other wheat products [3]. Grain protein, starch and flour Colour are the major quality attributes of durum wheat. The suitability of durum wheat cultivars for pasta making is mainly determined by the characteristics of seed proteins and starch [4]. Indeed, pasta quality and cooking characteristics are dependent upon the protein-starch matrix of the extruded pasta product [5].

Wheat starch contains amylose and amylopectin, both of which are polysaccharides made up of homoglucans with  $\alpha$ -1,4-bond forming the main chain and  $\alpha$ -1,6-bond at branch points [6]. Normal wheat starches consist of 22–35% amylose and 65–78% amylopectin [7]. Functional attributes of starch are related to interactions between starch and water as influenced by temperature e.g., gelatinization, pasting, gelation, and retrogradation [8]. The ratio amylose/amylopectin determines the physicochemical properties of starch and, thereby, the end-use of the durum wheat cultivar. Durum Amylose molecules are largely responsible for the gelling and film-forming properties of cooked starch. Amylopectin molecules are associated with the crystallinity, gelatinization and swelling of starch. The amylose contributes to the protein network strength through its binding to a protein fraction, which may reduce leaching [9]. Although cooked faster, spaghetti made with semolina having a low concentration of amylose was too soft and did not resist overcooking [10]. Mutant genotypes, waxy, having an altered amylose/amylopectin ratio, a very high amylopectin level (up to 100%), were produced. A reduced amylose concentration in the endosperm was beneficial for making noodles with better eating quality and baked products with delayed staling [12–14]. The four major protein types present in wheat are the water-soluble albumins, the salt-soluble globulins, the alcohol-soluble gliadin

monomers, and the high and low glutenin subunits (HMM-GS and LMM-GS) [15]. The quantity and the quality of proteins present in the grain affect processing and quality of the final product. Gluten strength is a term used to describe the ability of the proteins to form a satisfactory network with the starch that promotes good cooking quality [16]. The key components of the endosperm are gluten ins and gliadins proteins, which form gluten following hydration [17]. The contribution of gliadins and glutenins to dough properties have been recognized and it has been suggested that glutenin polymers are responsible for the unique viscoelastic properties of wheat flour dough, while gliadins contribute to dough extensibility [18]. In particular, the effect of protein concentration in determining pasta quality has been largely discussed [38]. Overall studies that identified gluten strength as the prerequisite for getting pasta with a good texture. Also of note is that it has been clearly shown that the same concentration of protein may lead to contrasting rheology and cooking properties, thus indicating that characteristics other than gluten concentration are fundamental in pasta processing [38]. Cooked pasta made from high protein concentration and strong gluten is firm, non-sticky, resilient and retains its texture if overcooked. Pasta produced from low protein semolina is deficient in some or all of these characteristics [19].

Bright Yellow Colour is also an important quality parameter. Pasta Colour depends basically on the combination of yellowness and brownness [20]. The yellow Colour is mainly due to carotenoid pigments accumulation in pericarp and endosperm, although, during pasta processing, carotenoids can be affected by different enzyme activities such as lipoxygenase, peroxidase and polyphenol oxidase ones [21,22].

Most of the breeding programs aimed mainly the improvement of grain yield at the expenses of quality traits. Canadian durum breeding programs have selected simultaneously for grain yield, and quality traits as well as agronomic traits and disease resistance traits for more than 50 years [41]. The cultivation of local landraces in Mediterranean countries declined progressively from the first decade of the twentieth century, with the advent of new, high yielding modern varieties [14].

This present study is part of a joint project aiming towards a full characterization of Tunisian durum wheat germplasm. The objective of our study is to assess the variation and the Impact of National (INRAT) and international breeding programs (CIMMYT and ICARDA) on the yield components and major quality traits on a historical set of 18 durum wheat genotypes grown in Tunisia during the 20th Century.

## Materials & methods

### Plant material

A set of 18 durum wheat varieties were chosen for this study (Table 1). The germplasm was divided into three groups belonging to different breeding periods. The first period before 1940 primarily represented selections from landraces (Hamira, Jnah Khottifa, Richi, Biskri, Agili, Mahmoudi and Bidi). The second period from 1940 to 1970 was characterized by introductions

(Chili, Kyperounda) and the first Tunisian bred varieties (INRAT 69 and Badri). During the third period, after 1970 foreign genotype became more available and contributed to the development of high yielding varieties (Maghrebi, Karim and Khiar, released by CIMMYT; OmRabia and Nasr released by ICARDA; Maali and Salim released by INRAT).

### Experimental setup

The field experimental set design was grown during 2011-2012 season at the field of the Graduate School of Agriculture of Kef (Tunisia). The soil was composed of a clay-loam soil with mean values of 48% clay, 30% loam, 21% sand, 184 ppm total nitrogen (Kjeldahl method), 3.92 ppm available phosphorus (Olsen method,  $P_2O_5$ ), 1.22% organic matter (Walchey-Black method), 17%  $CaCO_3$  and 0.92 mmhos/cm electrical conductivity. This site is characterized as a semi-arid region. Experiments were conducted under rainfed conditions characterized by a sufficient and regular rainfall quantity.

Each experimental trial was arranged in a randomized complete block design comprising plots with three replications. Plot size was 7.2 m<sup>2</sup> (six rows, six m rows, spaced 0.20 m row spacing). Sowing density was 350 plants per m<sup>2</sup>. Weeds and diseases were controlled according to standard cultural practices. Plots were mechanically harvested at commercial maturity. After harvesting, the cleaned seed from each replication was bulked and stored at 4°C until analysis.

### Characterization of yield components

Grain yield was determined for each plot. The Number of Grains per Spike (NKS) was the average of the number of grains per spike of 10 random spikes. The Weight of Thousand Kernels (TKW) was determined according to Sakin et al., [23].

### Quality traits evaluation

Grain yield was determined for each plot. The Number of Grains per Spike (NKS) was the average of the number of grains per spike of 10 random spikes. The Weight of Thousand Kernels (TKW) was determined according to Sakin et al., [23].

Protein concentration was determined following the AFNOR method (NFV03-050). Gluten Strength (GS) was determined using the Sodium Dodecyl Sulfate (SDS) sedimentation test for wheat meals according to Pena et al [24]. The Sedimentation Index (SI) was computed as the ratio between gluten strength and protein concentration, expressed as ml per % protein unit [25].

Wet Gluten (WG) concentration was determined by hand washing of whole durum wheat flour according to the standard method (ISO 21415-1:2006). Dry Gluten (DG) was obtained following the standard method (ISO 21415-3:2006). Yellow Pigment Concentration (YP) was determined according to the standard method (ISO 11052: standard 1994). Polyphenol Oxidase (PPO) activity following the method of Kubo et al., [26], Phenoloxidase (PO) activity using Aparicio-Cuesta et al. protocol [27] and Lipoxigenase (LOX) activity according to Shiiba et al. [28].

Total starch (TS), amylose (ASE) was determined according to the iodine colorimetric method [22].

### Statistical analysis

All Tests were run in at least triplicate for all experiments analyzes. Standard one-way Analysis of Variance (ANOVA) was conducted for the comparison between means of the different studied parameters using Duncan test. Pearson's correlation

test was performed between parameters. Principal Component Analysis (PCA) was performed using the set of parameters generating the biplot of PCA. The biplot generated was used to project wheat cultivars into plane of the first and second principal components. The classification tree was obtained by clustering based on between group linkage method and squared Euclidian distance. The cut-off significance for all statistical tests was 0.05. Statistical analysis and the phylogeny tree study were carried out using the software SPSS version 13.0 (SPSS, Chicago, USA).

### Results and discussion

#### Genetic and breeding programs impacts on yield components and major quality attributes

Durum wheat has undergone intensive selection for certain desirable characteristics during domestication and the subsequent genetic enhancement programs, such as high and stable yields [29].

Yield and quality are among the primary goals of durum wheat breeding programs in the entire world and in the EU, where premiums have been established to promote the cultivation of high-quality cultivars [30]. The analysis of variance of means of the yield components showed that the genotype effect was significant for all measured parameters ( $p < 0.01$ , Table 1).

Tunisian landraces (G1; breeding period before 1940) and old cultivars (G2; breeding period from 1940 to 1970) showed a low average GY, 3.26tha<sup>-1</sup> and 3.38tha<sup>-1</sup>, respectively, which explains their abandonment. However, the improved cultivars (G3; breeding period after 1970), as expected showed higher average GY (5.09tha<sup>-1</sup>) with respect to the old one and landraces. Among the advanced breeding lines, Nasr, released by ICARDA, showed the highest GY, 7.3 tha<sup>-1</sup> in the rain-fed condition. Jnah-Khottifa, a very adapted Tunisian landraces to the semi-arid condition, had the highest GY among the studied landraces (5.2tha<sup>-1</sup>). Results of ANOVA showed a significant effect of breeding periods for the GY ( $p = 0.038$ ) and TKW ( $p = 0.032$ ) traits and no significant changes for NKS ( $p < 0.05$ ). Average values of the yield components (GY, NKS and TKW) for the three breeding periods are presented in table 1. The obtained results confirmed that breeding programs had increased the yield even under rainfed conditions (Figure 1). This result is consistent with previous studies on Mediterranean durum wheat. Indeed, Italian and Spanish landraces and old cultivars showed a low grain yield [17]. The superiority of modern durum wheat cultivars in terms of grain yield has been achieved by plant height reduction lowering the straw yield which resulted in an increased harvest index and increasing the kernel per square meter [31]. An important diversity in NKS and TKW was shown among the studied set. Indeed, TKW ranged from 37.43 g (Khlar) to 60.77 g (Kyperounda). A significant decrease in TKW and an increase in NKS were recorded during the third breeding period (Table 1 and Figure 1A). Most of the landraces are late maturity which implies a longer duration of grain filling [32]. The majority of high yielding varieties are small seeds varieties compared to landraces and old varieties.

Khlar and Karim, two advanced breeding varieties are actually among the most cultivated varieties in Tunisia [33,34]. The improvement of NKS can be attributed in part to the effect of dwarfing genes introduced in modern genotypes. Many authors have reported that modern cultivars have a lower indi-

vidual grain weight than older cultivars [35]. Tunisian breeding program as most of European programs has the main goal the increase grain yield and improving the grain quality traits was the second priority. However, the Canadian breeding program, since beginning focused on quality, yellow pigment content, protein content and particularly protein strength were emphasized.

The quality of durum wheat foods in terms of texture, Colour, flavour and appearance are determinant by the raw material composition [16]. The historical changes in the major quality attributes (protein, starch and colour parameters) of the durum wheat varieties are summarized in Table 1.

Average of TS and ASE concentrations for all the studied varieties are presented in Table 1. The mean values for TS and ASE concentrations ranged respectively from 58.5% to 71.93% and from 22.1% to 33.5%. Maali, advanced breeding variety, released by INRAT in 2003, had the highest TS concentration (71.93%) whereas Kyperounda recorded the lowest value (58.5%). Functional attributes of starch are related to interactions between starch and water as influenced by temperature, gelatinization, pasting, gelation, and retrogradation [8]. The ASE determines the physicochemical properties of starch and, thereby, the end-use of the durum wheat cultivar. Average values of the TS and ASE concentration for the three breeding periods are showed in Table 1. The majority of varieties developed after 1970 showed an important TS concentration (Table 1). Anova analysis demonstrated a highly significant effect of breeding periods on TS concentration ( $P < 0.01$ ) but no significant effect on starch composition ( $p > 0.05$ ).

Increased amylose in food is associated with increased resistant starch, which is important in obesity and diabetes prevention [36]. The amylose/amylopectin ratio is crucial for the varieties end-use. Mutant genotypes, waxy, having an altered amylose/amylopectin ratio, a very high amylopectin level (up to 100%), were produced. Low amylose content was detrimental to spaghetti cooking quality when starch-gluten blends with different amylose content were studied [37]. A reduced amylose concentration in the endosperm was beneficial for making noodles with better eating quality [11,38,39] and baked products with delayed staling [13,14,40].

Protein quantity (concentration) and quality (WG, DG, SI, and GS) exhibited significant variation among studied genotypes and across the twentieth century (Table 1 and Figure 1B). P, WG and DG exhibited a trend to decrease during the twentieth century, conversely, GS and SI showed a trend to increase (Table 1). Indeed P varied between 10.75% (Maghrebi) and 16.6% (Jnah-Kottifa). Jnah-Kottifa (local landraces) had the highest value. ANOVA analysis revealed that breeding programs significantly affected the protein concentration ( $p < 0.01$ ). The average of P was ( $14.57\% \pm 1.71$ ) for the first breeding period (landraces). A reduction in the P was noticed in second ( $13.13\% \pm 1.06$ ) and third breeding ( $11.70\% \pm 1$ ) periods. The majority of the studied landraces and old varieties are late maturity varieties which imply a longer filling time [32] with a reduced grain number per spike as detailed in Table 1. Cultivars, released by international breeding programs, were characterized by high yield and low P, conversely to the landraces. Protein concentration below 11% could lead to mediocre quality of pasta. Grain protein concentration showed a decreasing trend over time of release during the 20th century (Figure 1B) as in Italy [31]. In Canada, grain protein concentration tended to increase slightly over the same time period because of the requirement for minimum protein

concentration for cultivar release in Canada [41]. Recently, Mediterranean landraces showed higher protein content than modern but lower gluten strength [42].

Wet gluten values varied from 17.84% (Maali, high yielding variety) to 36.05% (Agili, landraces). Dry gluten ranged between 5.45% (Bidi, landraces) and 11.80% (Agili, landraces). The correlation between DG and WG ( $r = 0.78$ ;  $p < 0.05$ ) (Table 1) indicated that gluten hydration capacity is rather a measure of gluten quantity than gluten quality as reported by Pena [43]. Wet gluten is an important parameter for Couscous manufacturing that a paste product is made from mixing semolina with water. The durum landraces and old cultivars had a higher average DG and WG (respectively,  $30\% \pm 3.62$  and  $9.06\% \pm 2.54$ ,  $30.48\% \pm 2.05$  and  $9.97\% \pm 1.02$ ) compared to advanced breeding lines (respectively,  $22.88\% \pm 5.5$  and  $7.72\% \pm 1.54$ ).

Protein quality, particularly GS and SI are important factors in pasta manufacture and its cooking quality [44], and thus important selection criteria in cultivar development. When pasta is the final product, strong gluten is needed for firm and less sticky dough [16]. Overall, studies that identified gluten strength as the prerequisite for getting pasta with a good texture have shown that lines expressing  $\gamma$ -gliadin-42 exhibited inferior pasta texture. The durum wheat cultivars released after 1970 (currently grown in Tunisia) had the highest GS average ( $24.33 \text{ ml} \pm 5.76$ ) and the SI average ( $2.06 \text{ ml}/\% \pm 1.2$ ) compared to landraces (respectively,  $22.32 \text{ ml} \pm 2.41$  and  $1.56 \text{ ml}/\% \pm 0.31$ ). The improvement in gluten strength in the modern wheat cultivars counterbalanced the significant decrease in protein percentage resulting [25]. A minimum of 12-15% of protein content is required in manufactured pasta as it secures semolina with uniform particle size producing elastic, resilient, non-sticky and firm cooked pasta [45]. Petrova (2007) reported that durum wheat varieties, characterized by moderately strong gluten, have sedimentation volume ranged from 25 to 35 ml [36]. The majority of studied varieties belonged to medium quality class.

Sedimentation volume test indicated the high quality of proteins and the possibility for good pasta and bread-making quality [46]. Characteristics such as firmness, cooking loss, and stickiness of pasta may be due to the concentration of proteins [4,9] and gluten strength [10], as well as to the starch composition [11,12]. Khiar, released in 1992 by CIMMYT, having the highest GS (36.1 ml) and SI (2.68 ml/%), is considered suitable for pasta and bread making. Sensory evaluation showed no significant difference of cooked couscous made from weak or strong gluten durum cultivars. However, couscous stickiness appeared to be strongly influenced by protein quantity and it decreased as P increased [34]. The hydration of semolina particles is an essential step in couscous processing which leads to binding between particles for the formation of agglomerates. A positive correlation between water absorption and protein content whilst negative correlation to starch content were shown on a set of Syrian durum cultivars [5]. Tunisian landraces with higher protein content, wet gluten and lower starch concentration fully meet couscous end use specification. Toufeili et al. showed that bulgur (durum traditional food) quality was best when made from large and hard kernels durum wheat having low gluten strength which characterized some North African landraces [47].

Carotenoid concentration is one of the main criteria to assess the commercial and nutritional value of pasta products [36]. The bright yellow colour, a highly valued quality parameter in pasta product, arise from the pigments in the endosperm although some reduction in color can occur during pasta process-

ing due to enzymes such as lipoxygenase. Durum wheat varieties YP and enzymes activities LOX, POD and PPO are presented in Table 1. Average grain YP for the first, second and third breeding periods are shown in (Table 1). Results of Anova showed no significant impact of breeding program on the YP. The same result was obtained for the Italian breeding program; the carotenoid pigment content presented no trend over time passing from old to modern cultivars [31]. However, for the Canadian breeding program, the yellow pigment concentration increased with year of release, especially after the mid-1990s [41]. Indeed, in the last decade pasta colour has become an important aesthetic factor. According to the classification of Landi [48], the majority of studied varieties had YP above 5 ppm and belongs to high-quality class. Khiar cultivar (high yielding variety) had the highest YP (6.92 ppm) and Badri (old variety) had the lowest (3.43 ppm). The desirable yellow Colour in pasta products didn't only result from higher pigment concentration but also lower LOX activity [49]. A wide variability among genotypes was observed for enzymes activities.

Mahmoudi showed the highest LOX activity (7.31 U/mg) while Bidi showed the lowest one (0.45 U/mg). Taking into consideration both LOX activity and YP, among the studied landraces, Bidi had the highest YP and the lowest LOX activity. Relative to modern high yielding varieties, Khiar showed the lowest LOX activity and the highest YP.

The effects of POD and PPO, less documented than LOX effect, occur widely in plants and cause the enzymatic browning in food material through initial oxidation of phenol into quinones. Quinones readily undergo self-polymerization or condensation with amino acids or proteins via their amino groups to form complex brown polymers. Pasta products from durum wheat with a high peroxidase activity develop a brownish Colour during processing; the brown Colour tends to mask the yellow colour when it reaches substantial levels [50]. For the majority of the tested genotypes, POD and PPO activities were low, except for the two varieties, Bidi and Badri (respectively, 1.663 U/mg and 2.063 U/mg). Outer endosperm layers are generally very rich in POD activity and it is preferable to be eliminated during milling [21]. According to Kruger, due to the low level of PPO in semolina, its role in pasta brownness is unlikely but it may be the cause of the inherent brownness in semolina formed during grain maturation when PPO levels are much higher and they could oxidize the abundant phenols present in immature wheat [40].

#### Statistical interpretation of the obtained results

Table A1 (Supporting Information) illustrates the correlations between the yield components and major quality traits. In this study, GY showed a positive correlation to NKS ( $R^2=0.64$  and  $p<0.01$ ) and a negative correlation to TKW ( $R^2=-0.54$  and  $p<0.05$ ). Similar results have been observed in European breeding programs, confirming the improved yield was more closely associated with increased grain number than an increase in grain weight [31].

A significant positive correlation was also observed between NKS and TS ( $R^2=0.67$  and  $P<0.01$ ) whilst negative correlation was shown to WG ( $R^2=-0.550$  and  $P<0.05$ ). The concentration of the starch showed positive correlation to GY ( $R^2=0.509$  and  $P<0.05$ ) and NKS ( $R^2=0.67$  and  $P<0.01$ ), although negative correlation to TKW ( $R^2=-0.6$  and  $P<0.01$ ) and WG ( $R^2=-0.78$  and  $P<0.01$ ) were recorded. These results showed that the yield improvement is achieved essentially by increasing the starch concentration more than protein concentration, which is corroborated by previous reports [51].

The Principal Component Analysis (PCA) was carried out to highlight the impact of breeding program and genotype on the yield components and the quality attributes of a set of historical series of 18 Tunisian durum wheat cultivars. The two principal components explained 64.256% of the total variability. The first one (PC1) explained 46.024% of the variability and was positively correlated with GY, NKS, TS, SI, GS, YP and negatively associated with TKW P, WG and DG concentrations. The second component explained 18.231% and was negatively associated with the three yield components (GY, NKS and TKW). The PCA biplot (Figure 2A) confirmed the impact of the breeding program on Tunisian durum wheat and had showed two distinct groups, where the G1 group, containing high yielding varieties (Karim, Nasr, Maali, Salim and OmRabiaa) was significantly estranged from G2, formed by landraces (Hamira, JnahKhotifa, Richi, Beskri, Agili, Mahmoudi and Bidi) and old varieties (Chili, Kyperoundaand Badri). Indeed Tunisia is among countries continued to grow durum wheat landraces until the advent of the green revolution in late 1960. Khiar (high yielding variety) quite far from the rest of cultivars, is among the most cultivated varieties in Tunisia, forms the third group G3. With high YP, SI and GS, Khiar, fully meets the pasta end use specification. Moreover, the dendrogram (Figure 3) showed essentially two major clusters, validating the results obtained by the PCA. Cluster-I comprised all the landraces and old varieties. The Cluster-II, comprised only the high yielding varieties, and was divided in two sub-groups. One of the sub-cluster is composed by Khiar confirming the PCA obtained results.

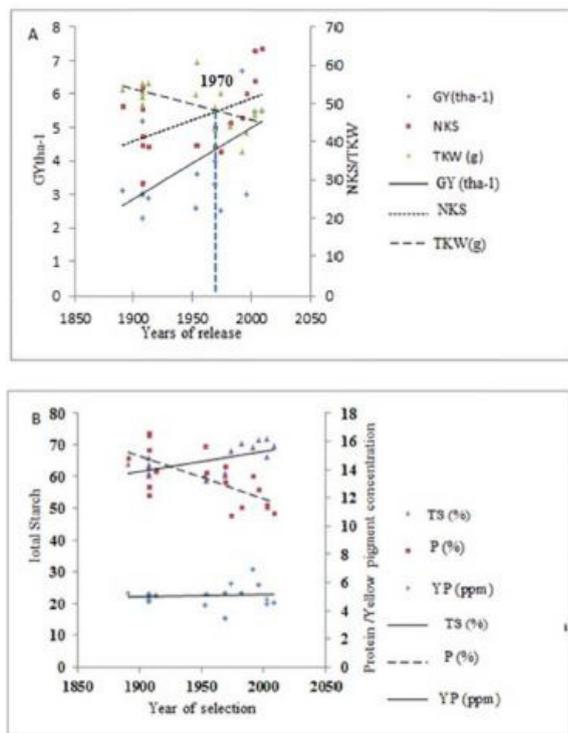
#### Conclusion

Durum wheat breeding programs are designed to improve firstly grain yield as well as value for cultivation and value for use. In Tunisia, the cultivation of local landraces declined progressively from the second half of the twentieth century, with the advent of new, high yielding modern varieties. Our study was carried on a historical set of Tunisian durum wheat, released during the 20th century. As a conclusion, Tunisia shared the Mediterranean durum wheat breeding's history. Indeed, breeding after 1970 had enhanced yield by increasing the number of kernel per spike and emphasized the gluten strength at expense of protein content. The statistical study illustrated the impact of dwarfing durum varieties, released during the world green revolution, on the Tunisian wheat history.

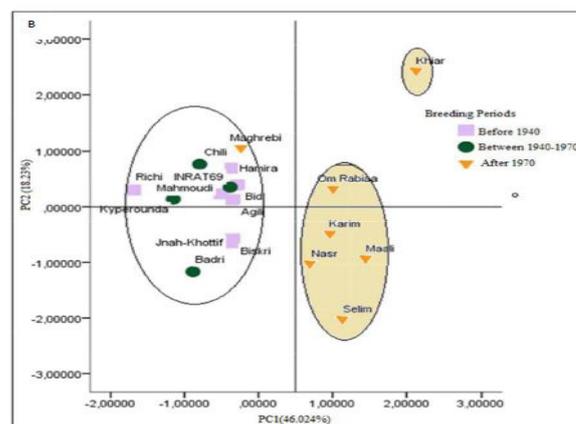
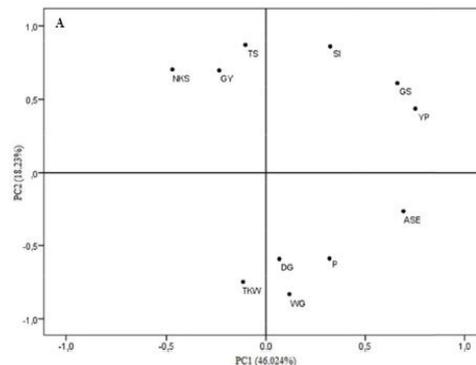
**Table 1:** List of studied durum wheat genotypes; their pedigree; their origin (landraces) and their breeding institute (High Yielding variety); Yield parameters: GY (tha-1); NKS; TKW; Starch parameters: TS(%), ASE(%); Protein parameters: P(%), WG(%), DG(%), S(ml/%), GS(ml). Color parameters: LOX (U/mg soluble protein); POD (U/mg soluble protein); PPO (U/mg soluble protein); YP (ppm). Data are expressed as mean from three replicates. Means of same column followed by different letters differ significantly ( $p < 0.01$ ).

Breeding Period	Variety name	Pedigree	Origin	Year of Selection/introduction/release	Yield parameters			Starch parameters			Protein parameters					Color parameters			
					GY (tha-1)	NKS	TKW (g)	Total starch (%)	Amylose (%)	P (%)	WG (%)	DG (%)	S (ml/%)	GS (ml)	LOX	POD	PPO	YP (ppm)	
Before 1940 (landraces)	Hamira	Selection from local Tunisian Landraces	Tunisia	1891	3, lab	49,2ef	53,73i	64,1e	32,23bcdef	14,77m	28,29cd e	8,45e	1,62cdef	23,96cdef	3,32h	0a	0,354c	5,2h	
	Inah-Khotifa	Selection from local Tunisian Landraces	Tunisia	1908	5,2 cd	54,33fg	51,6g	65,95cd	24,4a	16,6q	30,17efg	10,4g	1,29ab	21,42abcde	2,67g	0,313d	0,403d	5,02g	
	Richi	Selection from local Tunisian Landraces	Tunisia	1908	3ab	29,33a	53,07hi	60cde	29,5ab	16,42p	34,04ij	11,67i	1,09a	17,92a	1,56d	0,5f	0,487f	4,83f	
	Biskri	Selection from local Algerian Landraces	Algeria	1908	3ab	39,13bc	49,53f	62,79a	23,5defg	12,77g	26,63bcd	9,59f	1,64cdefg	21abcde	0,73b	0,163bc	0,195a	4,66de	
	Agili	Selection from local Moroccan landraces	Morocco	1908	3,3ab	48,66ef	51,72gh	61bc	24,6abcde	12,22e	36,05j	11,8i	2,03hi	24,83ef	3,89j	0a	0,942g	5,19h	
	Mahmoudi	Selection from local Algerian Landraces	Algeria	1908	2,3 ab	41,53bcd	55,19j	63,40ab	27,4ab	15,36n	26,9bcd	6,09a	1,47bc	22,58bcde	7,31k	0,16bc	0,245b	5,16gh	
Mean	Bidi	Selection from local Moroccan landraces	Morocco	1913	2,9 ab	38,8bc	55,37j	62,2ab	27,6a	13,87k	28,57cdef	5,45a	1,77defgh	24,5def	0,45a	1,663i	0,361c	5,03g	
					3,26	43,00	52,89	62,77	27,03	14,57	30,09	906	1,56	22,32	2,8	0,4	0,43	5,01	
SD					0,91	8,37	2,10	1,97	3,13	1,71	3,62	2,54	0,31	2,44	2,35	0,58	0,25	0,20	
					2,6 ab	39,2bc	60,77k	58,5 de	32,5 abcde	15,62o	32,26hi	8,78e	1,58cde	24,67ef	d	e		4,41b	
1940-1970 (old varieties)	Kyperounda	Introduction from Morocco	Cyprus	1953	3,6 ab	39,07bc	48,93ef	60,3a	24,7a	13,13h	30,75fgh	10,4g	1,49bcd	19,54ab	1,22c	2,063h	0,476f	3,43a	
	INRAT69	Kyperounda/ Mahmoudi 981	INRAT	1969	4bc	43,67bcde			27,4abcd	14,25l		11,13h	1,6cde	22,75bcde	d			5,24h	
Mean	Badri	Zenati/ Boufeille/Mamoudi/Marr D117	INRAT	1969	3,3 ab	38,67bc	48,93ef	60,3a	24,7a	13,13h	30,75fgh	10,4g	1,49bcd	19,54ab	1,22c	2,063h	0,476f	3,43a	
					3,38	40,15	51,32	60,06	28,5	14,19	30,48	997	1,53	21,74	1,11	0,66	0,32	4,55	
SD					0,59	2,36	7,32	1,06	3,28	1,06	2,05	1,02	0,07	2,41	0,42	0,95	0,15	0,83	
					2,5 ab	37,53b			33,5egh	10,75a	h	10,49g	1,91fghi	20,5abcd				5,92i	
After 1970 (high)	Maghrebi	GLL'S/3/BR180/LK/GZ/61.130	CIMMYT	1974	2,5 ab	37,53b			33,5egh	10,75a	h	10,49g	1,91fghi	20,5abcd				5,92i	
	Karim	D21563/AA'S'//Fg'S"	CIMMYT	1982	5,1 cd	44,93cde	44,03c	70,45f	26,4gh	11,32c	19,54a	6,73cd	1,86efghi	21abcde	1,4cd	0a	0,234b	5,22h	
Yielding varieties	Khlar	Chen'S'// Altar 84	CIMMYT	1992	6,7 ef	46,4de	37,43a	69,23f	29,5cdef	13,5i	18,57a	7,01d	2,68j	36,17g	0,9b	0,163bc	0,227b	6,92j	
	Om Rabiaa	Jori C69/Hau	ICARDA	1996	3 ab	52,73fg	42,57b	71,5fg	23,6h	12,62f	25,7bcd	8,58e	2,12i	26,75f	3,61i	0,22c	0,426e	5,85i	
Mean	Nasr	GdoVZ512/Cit//Ruff/Fg/3/Pin/Gre//Frob	ICARDA	2003	7,3f	56,00g	48de	66,24fg	24,9h	11,51d	29,12defg	8,49e	1,92ghi	22,13bcde	1,16c	0a	0,992h	4,8ef	
	Maali	CMH80A/1016/ATTURA/CMH91/370/CMH77.7/4/3/YAV79/5/Ra/ssaak/6/BACK'S'//VEL'S'//Khlar	INRAT	2003	5,5 de	63,93h	47,13d	71,93g	27,56efg	11,34c	17,84a	6,29bc	2,13i	24,17def	2,39f	0a	0,356c	4,5bc	
SD	Selim	Altar84/FP8419.126-1-2/KazzaK/3/Kir/BaladiaHamra	INRAT	2009	5,5 de	64,4h			22,1a	10,87b		6,45bc	1,8efgh	19,58ab		0,66g		4,55cd	
					5,09	51,65	45,73	69,61	26,79	11,70	22,88	7,72	2,06	24,33	2,18	0,17	0,63	5,39	
					1,77	10,83	4,88	1,96	3,84	1,00	5,50	1,54	0,30	5,76	1,2	0,2	0,65	0,89	

Color parameters: LOX (U/mg soluble protein); POD (U/mg soluble protein); PPO (U/mg soluble protein); YP (ppm). Data are expressed as mean from three replicates. Means of same column followed by different letters differ significantly ( $p < 0.01$ ).



**Figure 1:** Changes in A Yield components (GY-NK S-TKW); B Quality traits attributes (P %-TS %-YP %) mean values of 18 durum wheat varieties across the twentieth century. GY: Grain Yield; NKS: Number Of Kernel Per Spike; TKW: Thousand Kernel Weight; P%: Protein Concentration; T S%: Total Starch Concentration; YP%: Yellow Pigment Concentration.



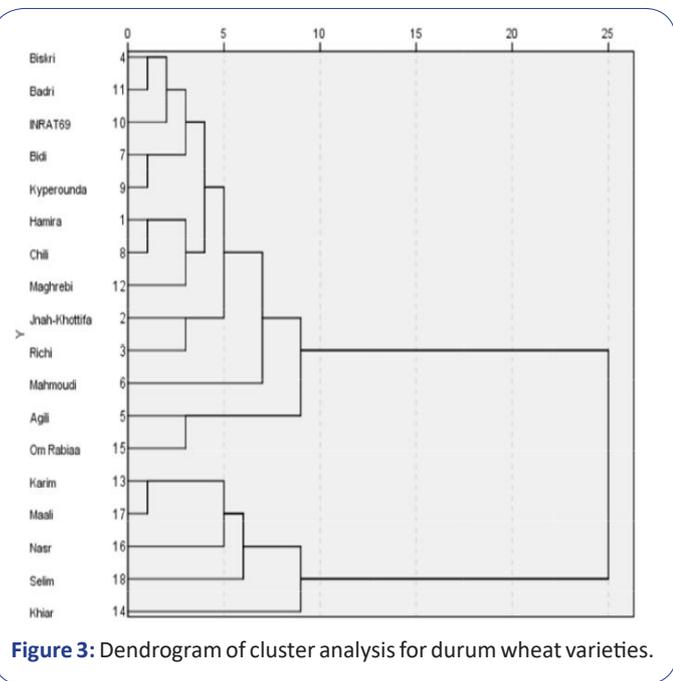
**Figure 2:** Principal Component Analysis. A Load plot of principal component analysis explaining 64.254 % of total variability; B Score plot with the wheat sample s projected onto the first two principal components. GY: Grain Yield; NKS: Number Of Kernel Per Spike; TKW: Thousand Kernel Weight; P%: Protein Concentration; TS%: Total Starch Concentration; YP%: Yellow Pigment Concentration; GS: Gluten Strength; ASE: Amylose; SI: Sedimentation Index; DG: Dry Gluten; WG: Wet Gluten.

**Declarations**

**Acknowledgements:** This work was supported by the Tunisian Ministry of Higher Education and Scientific Research (grant: TK/08/2012) in the frame of joint research program between Tunisia (Ministry of Higher Education and Scientific Research) and Korea (National Research Foundation) and by L’Oreal-Unesco for women in Science program- (Pan Arab Fellowship 2013). The authors would like to thank Pr. Ron DePauw; Science adviser, for constructive criticism of the manuscript and its improvement.

**References**

1. Flagella Z. Qualità nutrizionale e tecnologica del frumento duro. Ital. J. Agron. 2006; 1: 203-239.
2. Dupont FM, SB Altenbach. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of Cereal Science, 2003; 38: 133-146.
3. Corke H, CE Walker, C Wrigley. Encyclopedia of Grain Science, Three-Volume Set. Academic Press. 2004.
4. MacRitchie F. Physicochemical properties of wheat proteins in relation to functionality, in Advances in food and nutrition research. 1992; 1-87.
5. Samaan J, et al. Durum wheat quality: II. The relationship of kernel physicochemical composition to semolina quality and end product utilization. International journal of food science & technology. 2006; 41: 47-55.



**Figure 3:** Dendrogram of cluster analysis for durum wheat varieties.

6. McMaugh SJ, et al. Suppression of starch synthase I expression affects the granule morphology and granule size and fine structure of starch in wheat endosperm. *Journal of experimental botany*. 2014; 65: 2189-2201.
7. Zhang H, et al. Morphological features and physicochemical properties of waxy wheat starch. *International journal of biological macromolecules*. 2013; 62: 304-309.
8. Silverio J, et al. The effect of temperature cycling on the amylopectin retro gradation of starches with different amylopectin unit-chain length distribution. *Carbohydrate Polymers*. 2000; 42: 175-184.
9. D'EGIDIO M, et al. INTERACTION ENTRE L'AMIDON ET UNE FRACTION PROTEIQUE EXTRAITE DES SEMOULES DE T. DURUM. *Canadian journal of plant science*. 1984; 64: 785-796.
10. Vignaux N, et al. Quality of spaghetti made from full and partial waxy durum wheat. *Cereal Chemistry*. 2005; 82: 93-100.
11. Oda M, J Konishi, S Nemat-Nasser. Some experimentally based fundamental results on the mechanical behaviour of granular materials. *Geotechnique*. 1980; 30: 479-495.
12. Smits AL, et al. Ageing of starch based systems as observed with FT-IR and solid state NMR spectroscopy. *Starch-Stärke*. 1998; 50: 478-483.
13. Krog N, et al. Retrogradation of the starch fraction in wheat bread. *Cereal Foods World*. 1989.
14. Bhattacharya M, et al. Staling of bread as affected by waxy wheat flour blends. *Cereal Chemistry*. 2002. 79: 178-182.
15. DuPont FM, et al. Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. *Journal of Agricultural and Food Chemistry*. 2005; 53: 1575-1584.
16. Sissons M. Role of durum wheat composition on the quality of pasta and bread. *Food*, 2008; 2: 75-90.
17. Gupta R, I Batey, F MacRitchie. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem*. 1992; 69: 125-131.
18. Uthayakumaran S, et al. Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. *Cereal chemistry*. 1999; 76: 389-394.
19. Dexter J, NM Edwards. The implications of frequently encountered grading factors on the processing quality of common wheat. Canadian Grain Commission, Grain Research Laboratory. 1998:
20. Porceddu E. Durum wheat quality in the Mediterranean countries. *Durum wheat quality in the Mediterranean region*. 1995; 22: 11-21.
21. Aalami M, K Leelavathi, UP Rao. Spaghetti making potential of Indian durum wheat varieties in relation to their protein, yellow pigment and enzyme contents. *Food Chemistry*. 2007; 100: 1243-1248.
22. Blanco A, et al. Quantitative trait loci for yellow pigment concentration and individual carotenoid compounds in durum wheat. *Journal of Cereal Science*. 2011; 54: 255-264.
23. Sakin MA, A Yildirim. Induced mutations for yield and its components in durum wheat (*Triticum durum* Desf.). *Journal of Food Agriculture and Environment*. 2004; 2: 285-290.
24. Pena R, et al. Variation in quality characteristics associated with some spring 1B/1R translocation wheats. *Journal of Cereal Science*. 1990; 12: 105-112.
25. Subira J, et al. Breeding progress in the pasta-making quality of durum wheat cultivars released in Italy and Spain during the 20th Century. *Crop and Pasture Science*. 2014; 65: 16-26.
26. Kubo I, T Nitoda, K-I Nihei. Effects of quercetin on mushroom tyrosinase and B16-F10 melanoma cells. *Molecules*. 2007; 12: 1045-1056.
27. APARICIO-CUESTA M, M MATEOS-NOTARIO, J RIVAS-GONZALO. Sensory evaluations and changes in peroxidase activity during storage of frozen green beans. *Journal of food science*. 1992; 57: 1129-1143.
28. Shiiba K, et al. Purification and characterization of lipoxygenase isozymes from wheat germ. *Cereal Chem*. 1991; 68: 115-122.
29. Laidò G, et al. Genetic diversity and population structure of tetraploid wheats (*Triticum turgidum* L.) estimated by SSR, DArT and pedigree data. *PLoS One*. 2013; 8: e67280.
30. Royo, C, G Briceño-Félix. Spanish wheat pool. *The World Wheat Book*. 2011; 2: 121-154.
31. De Vita P, et al. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. *European Journal of Agronomy*. 2007; 26: 39-53.
32. Erchidi A, M Benbella, A Talouizte. Relation entre certains paramètres contrôlant les pertes en eau et le rendement grain chez neuf variétés de blé dur soumises au stress hydrique. *Options méditerranéennes, série A (Séminaires méditerranéens)*. 2000; 40: 279-282.
33. Déghaïes M, et al. Les variétés de céréales cultivées en Tunisie. *Institution of Scientific Agricultural Research and Education-National Institute of Agricultural Research of Tunisia, Tunis, Tunisia*. 2007; 250.
34. Saade ME. Adoption and impact of high yielding wheat varieties in Northern Tunisia. *CIMMYT Economics Working Paper*. 1996.
35. Slafer G, D Miralles. Fruiting efficiency in three bread wheat (*Triticum aestivum*) cultivars released at different eras. Number of grains per spike and grain weight. *Journal of Agronomy and Crop Science*. 1993; 170: 251-260.
36. Hazard B, et al. Registration of Durum Wheat Germplasm Lines with Combined Mutations in SBEII a and SBEIIb Genes Confering Increased Amylose and Resistant Starch. *Journal of plant registrations*. 2014; 8: 334-338.
37. Dexter J, R Matsuo. Changes in spaghetti protein solubility during cooking [Durum wheats and a hard red spring wheat]. *Cereal Chemistry*. 1979.
38. Miura H, et al. Genetic control of amylose content in wheat endosperm starch and differential effects of three Wx genes. *Theoretical and Applied Genetics*. 1994; 89: 276-280.
39. Zhao X, et al. A single genetic locus associated with starch granule properties and noodle quality in wheat. *Journal of Cereal Science*. 1998; 27: 7-13.
40. Starkweather W, et al. Alterations of erythrocyte lactate dehydrogenase in man. *Blood*. 1965; 26: 63-73.
41. Clarke J, F Clarke, C Pozniak. Forty-six years of genetic improvement in Canadian durum wheat cultivars. *Canadian journal of plant science*. 2010; 90: 791-801.
42. Roselló M, et al. Pasta-making quality QTLome from Mediterranean durum wheat landraces. *Frontiers in Plant Science*. 2018; 9: 1512.

43. Peña R. Durum wheat for pasta and bread-making. Comparison of methods used in breeding to determine gluten quality-related parameters. *Durum Wheat Improvement in the Mediterranean Region: New Challenges, Serie A: Séminaires Méditerranéennes*. 2000; 40: 423-430.
44. Dexter JE, PJ Wood. Recent applications of debranning of wheat before milling. *Trends in Food Science & Technology*. 1996; 7: 35-41.
45. Peña R, et al. Quality (end-use) improvement in wheat: compositional, genetic, and environmental factors. *Journal of crop production*. 2002; 5: 1-37.
46. Zečević V, D Knežević, D Mićanović. Variability of technological quality components in winter wheat. *Genetika*. 2007; 39: 365-374.
47. TOUFEILI I, H CHAMMAS, S SHADAREVIAN. Identification of key textural attributes of Arabic bread and their application to the assessment of storage and quality. *Journal of texture studies*, 1998; 29: 57-66.
48. Landi A. Durum wheat, semolina and pasta quality characteristics for an Italian food company. N. di Fonzo F, Kaan M, Nachit MM (editör), *Durum Wheat Quality in the Mediterranean Region*. Options, ICARDA, CHIEAM and CIMMYT. 1995; 11: 33-42.
49. Borrelli G, et al. Durum wheat lipoxygenase activity and other quality parameters that affect pasta color. *Cereal chemistry*. 1999; 76: 335-340.
50. Kobrehel K, MF Gautier. Variability in peroxidase isozymes in wheat and related species. *Canadian Journal of Botany*. 1974; 52: 755-759.
51. Motzo R, S Fois, F Giunta. Relationship between grain yield and quality of durum wheats from different eras of breeding. *Euphytica*. 2004; 140: 147-154.