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Insights into a century of breeding of durum wheat in Tunisia: The properties of flours and starches isolated from landraces, old and modern genotypes

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ABSTRACT

The present work had a dual objective: to assess if the evolution of durum wheat from landraces to modern genotypes as function of breeding programs impacted proteins and starch fractions, as well as starch properties. Flours and starches isolated from Tunisian durum wheat landraces, old and modern lines were thoroughly characterized for their compositional, morphological, and gel properties. Statistical results showed significant ($p \leq 0.05$) differences among the studied set. Protein and starch fractions assessment revealed that modern genotypes had the highest total starch and albumin contents, old genotypes had the highest amylose and glutenin contents, and landraces had the highest protein and gliadin contents. Starch properties screening allowed several findings: no significant ($p > 0.05$) differences were found among starch granules morphology, significant ($p \leq 0.05$) differences were recorded in terms of technological properties, and old genotypes had the highest starch gel hardness. Overall, these results indicated that the influence of genotype on flour and starch properties was more relevant than breeding history.

1. Introduction

Wheat is one of the oldest and most extensively cultivated crops, and improving its quality and yield has been, and still is, one of the central focuses of national and international breeding programs. The quality of wheat grain is dependent on the characteristics of starch and protein (Singh, Nakaura, Inouchi, & Nishinari, 2008). The inner endosperm is primarily composed of starch granules and proteins, which account for about 65–75% and 8–20% of the grain dry weight, respectively (Huang, Lin, & Wang, 2007). Gluten proteins confer properties of elasticity and extensibility, which are crucial for functionality of wheat flours.

Starch contributes over 50% of the average caloric intake in the diet of Western countries and up to 90% in the developing world (Wang, Li, Copeland, Niu, & Wang, 2015). Starch is the major component of wheat endosperm, and it is composed by amylose and amylopectin. Amylose is a relatively long linear polysaccharide chain, containing α-D-glucopiranoside residues bound by 99% α-1,4 linkages and 1% α-1,6 linkages (Zhang, Dhital, & Gidley, 2015). Amylopectin is a bigger polysaccharide, composed of linear chains of α-D-glucopiranoside residues connected through α-1,4 linkages (94–95%) and α-(1,6) linkages (5–6%) (Zhang et al., 2015). Amylose and amylopectin have the same basic structure, but differ in their length and degree of branching, which ultimately affects the starch physicochemical properties (Sofi, Wani, Masoodi, Saba, & Muzaffar, 2013). The ratio of these two polymers and the structure of amylopectin influence the processing, cooking, organoleptic qualities, and digestibility of starch-based foods. Starch has diverse characteristics, such as amylose content, gelatinization temperature, gel consistency and texture, pasting viscosity, thermal properties, and tendency to retrograde, which are of great importance

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for its uses in food processing and other industries (Bao, Shen, & Jin, 2006). The variability in starch functionality derives from variability of structure, which is due to diversity in the genes that encode the starch biosynthetic enzymes and environmental factors that act on the genes and enzymes concerned during plant growth (Wang & Copeland, 2012). Starch biosynthesis in the cereal endosperm requires the coordinated activities of several enzymes (Jeon, Ryoo, Hahn, Walia, & Nakamura, 2010). Progress in identifying starch-biosynthesis genes and their functions will no doubt continue, given that the tools for constructing and analyzing diversity continue to improve and to be more accessible (Morell & Myers, 2005). Furthermore, it might be interesting to study the genetic progress via genotypic tools.

Tunisian local landraces were progressively abandoned from the first decades of the twentieth century and replaced by the improved and genetically more uniform modern varieties derived from international breeding programs. Indeed, Tunisian breeding history went through several phases from landraces (1910–1940), followed by old genotypes (1940–1970), and modern genotypes selected by CIMMYT (International Maize and Wheat Improvement Center) and ICARDA (International Center for Agricultural Research in the Dry Areas) (1970–1980) to the most recently genotypes made by INRAT. Such breeding programs were led by inducing changes and improving wheat characteristics. This work wants, therefore, to evaluate the effect of the breeding process on the protein and starch fractions of Tunisian wheat. Therefore, this work had a dual objective. The first objective was to assess the impact of the evolution of wheat germplasm from landraces to modern genotypes through the evaluation of proteins and starch fractions. The second objective was the characterization of the starch fraction isolated from Tunisian durum wheat genotypes, released during the last century and to determine starch properties.

2. Materials and methods

2.1. Plant material set up and climatic data

Durum wheat genotypes object of this study are presented in Table 1. The set included 6 landraces (Hamira, Jenah Khotifa, Richi, Biskri, Mahmoudi and Bidi 17), 3 old genotypes (Chili 931, Kyperounda and INRAT 69) and 6 modern genotypes (Karim, Khiar, Om Rabia, Nasr, Maali and Salim).

Table 1
Name, pedigree, origin and year of release of the durum wheat genotypes set, grown in 2014–2015 in semi-arid region in Tunisia (Deghais, Kouki, Gharbi, & El-Falah, 2007).

Code	Name	Pedigree	Origin and year of release
Landraces			
1	Hamira	Local landrace	Tunisia, 1908
2	Jenah Khotifa	Local landrace	Tunisia, 1915
3	Richi	Local landrace	Tunisia, 1908
4	Biskri	Local landrace	Algeria, 1925
5	Mahmoudi	Local landrace	Tunisia, 1908
6	Bidi 17	Local landrace	Algeria, 1937
Old genotypes			
7	Chili 931	Old cultivar	France, 1953
8	Kyperounda	Old cultivar	Cyprus, 1954
9	INRAT 69	Old cultivar (Mahamoudi981/ Kyperounda)	Tunisia, 1970
Modern genotypes			
10	Karim	New cultivar (Jori"S"/Anhinga"S"// Flamingo"S")	CIMMYT, 1982
11	Khiar	New cultivar (Chen"S"/Altar 84)	CIMMYT, 1992
12	Om Rabia	New cultivar (Jori C69/Haurani)	ICARDA, 1987
13	Nasr	New cultivar (GoVZ512/Cit//Ruff/Fg/ 3/Pin/Gre//Trob)	ICARDA, 1990
14	Maali	New cultivar (CMH80A.1016/4/ TTURA/CMH74A370/CMH77.774/3/ YAV79/5/Rassak/6/DACK"S"/ YEL3"S"//Khiar)	INRAT, 2003
15	Salim	New cultivar (ALTAR 84/FD8419-126-1-2/Razzak/3/Krf/Baladia Hamra)	INRAT, 2010

Wheat seeds were cultivated under rain fed conditions at the trial field of the graduate school of Kef (Tunisia), during the season 2014–2015. The experimental layout was a randomized complete block design with three replications. Plot size was 7.2 m^2 (6 rows, 6 m long, with 20 cm row spacing). The climatic characteristics of this growing season were 389 mm (average accumulated precipitation), 9.3 °C (average minimum temperature) and 22.1 °C (average maximum temperature).

2.2. Isolation of starch

Wheat starch was isolated from wheat flour following the procedure of Chen, He, Fu, and Huang (2015).

2.3. Chemical composition of wheat flour and starches

2.3.1. Starch

Total starch, amylopectin and amylose contents were determined using iodine colorimetric method as described by Jarvis and Walker (1993).

Starch was also classified based on the rate of hydrolysis from rapidly digested starch (RDS, digested within 20 min), slowly digested starch (SDS, digested between 20 and 120 min), to resistant starch (RS, undigested starch after 120 min) (Wang et al., 2014).

2.3.2. Protein

Albumin, globulin, gliadin and glutenin were sequentially extracted as described by Lookhart and Bean (1995) with some modifications. One hundred milligrams of flour were extracted with deionized water (500 μL) for 30 min and centrifuged for 5 min at 805g. The supernatant was recovered, and the pellet was vortexed with 400 μL of deionized water and centrifuged as before. The decanted supernatant was saved, and the pellet was vortexed again with 400 μL of deionized water and centrifuged. The three supernatants were poured off and recovered as albumin. The pellet from albumin was then extracted with an aqueous solution of 0.5 M sodium chloride (400 μL) for 30 min and centrifuged for 5 min at 805g. The supernatant was decanted and saved. This operation was repeated two times. The three supernatants were saved as globulin. The pellet from globulin was extracted with 70% aqueous ethanol (v/v, 400 μL) for 30 min and centrifuged for 5 min at 805g. The supernatant was decanted and saved. This step was redone two times. The three supernatants were poured off and saved as gliadin. Glutenins were extracted from the remaining pellet as described by Wieser, Antes, and Seilmeier (1998). The extraction was repeated twice with 1 mL of a solution containing 50% (v/v) propan-1-ol, 2 M urea, 1% (w/v) dithiothreitol and 0.05 M Tris-HCl (pH 7.5) and kept for 60 min at 60 °C. The suspensions were centrifuged for 20 min at 15,000g. The two supernatants were poured off and saved. Two determinations were performed for each sample.

RP-HPLC-UV characterization of protein fractions was performed following the method Li Vigni, Baschieri, Marchetti, and Cocchi (2013) with few modifications. In brief, the four protein fractions were analyzed separately. The samples were separated by a RP column (JUPITER 5 μm C4 300 Å 250 × 2 mm) in an HPLC-UV (HPLC Alliance 2695 with a dual λ absorbance detector model 2487, Waters, Milford, MA, USA) using a linear gradient. Phase A was trifluoroacetic acid 0.1% (v/v) in water; phase B was trifluoroacetic acid 0.1% (v/v) in acetonitrile. The applied gradient was: 0–40 min from 100% A to 100% B plus washing and reconditioning steps. RP-HPLC parameters were: flow 0.2 mL/min; column temperature 35 °C; injection volume was 25 μL for albumin, gliadin and glutenin, while it was 50 μL for globulin; acquisition λ = 214 nm.

An estimation of protein fractions concentration was obtained