

Spanish Ancient Wheats: A Genetic Resource for Wheat Quality Breeding

Juan B Alvarez* and Carlos Guzmán

Departamento de Genética, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Edificio Gregor Mendel, Campus de Rabanales, Universidad de Córdoba, CeiA3, ES-14071 Córdoba, Spain

Abstract

Wheat (*Triticum* sp.) is one of the most important food crops worldwide. Di-, tetra- and hexaploid species are available, which along with the different subspecies of this genus have been used for food and feed. However, the genetic diversity of this crop has been seriously compromised, and only two main species; the durum and bread wheat, currently exist. The germplasm materials that were used in the past in Spain, have been regarded as ancient wheats, thus forming a group of neglected or underutilized crops that could be an interesting reservoir of variation for modern wheat breeding. In the last decade, our group has been evaluating and characterizing for the morphological and quality traits some of these Spanish ancient wheats. In these materials, using the SDS-PAGE technique and DNA sequencing, we detected an important variability for seed storage proteins, starch synthases and puroindolines, which are related with three important quality characteristics of wheat: gluten levels, starch levels and hardness, respectively. These novel variants could be used with the dual goal of genetic improvement and enriching the gene pool of these components in modern wheat.

Keywords: Diversity; Genetic resources; Morphological traits; Puroindolines; Seed storage proteins; Starch; Wheat

Wheat: Origin and Use

Among the first plants that were cultivated and domesticated, the characteristics associated with their easy transportation and storage was highly desired. The *Gramineae* species and most importantly, their domestic variants, including the cereals, included in this type of plants represent the staple food in different parts of the World. Their origin and domestication appear connected to the origin of the main civilizations. So, each one of the three main crops (rice, maize and wheat) have been associated with one important civilization: wheat with Sumer or Egypt in Mediterranean region; rice with Chinese civilization in Asia; and maize with the Olmec and Maya civilizations in America [1].

The wheat origin and domestication started in the Near East, in the well-known zone, regarded as the Fertile Crescent [2]. The wheat is a complex polyploid formed by multiple species of different ploidy levels, resulting from a combination of genomes that from different species of the *Triticeae* tribein the *Poaceae* family (Figure 1). Thus, diploid (2n=2X=14, AA), tetraploid (2n=4X=28, AABB), and hexaploid (2n=6X=42, AABBDD) species can be found.

The origin of the A and D genomes is well known. T. urartu Thum ex. Gandil (2n=2X=14, A^uA^u), a wild diploid species, has been proposed as the donor of the A genome in the polyploid species of wheat [3]. With respect to the D genome, several studies suggest that the donor of this genome is Aegilops tauschii Coss. (2n=2X=14, DD) [4,5], crossed with cultivated emmer (Triticum turgidum spp. dicoccum Schrank em. Thell., 2n=4X=28, A^uA^uBB), followed by chromosome doubling resulting in spelt (T. aestivum ssp. spelta L. em. Thell., 2n=6X=42, A^uA^uBBDD), the putative ancestor of bread wheat (*T. aestivum* ssp. aestivum L. em. Thell.), the species of the Triticum genus that is more important today. However, the origin of the B genome presents certain controversy. The currently accepted hypothesis suggests that T. urartu could have given rise to the wild tetraploid wheats, mainly in two different events. On one hand, a cross with an Aegilops species (section Sitopsis), probably Aespeltoides Tausch. (2n=2X=14, SS), generated a wild emmer (T. turgidum ssp. dicoccoides Korn. ex Asch. & Graebner em. Thell., 2n=4X=28, AuAuBB), from which the cultivated emmer was derived through domestication. The rest of the tetraploid wheat, including durum wheat (*T. turgidum* ssp. *durum* Desf. em. Husn.), was derived from this species, as well as the hexaploid wheat [6,7]. Similarly, the crossing of some other species of the section *Sitopsis* with *T. urartu* resulted in *T. timophevii* ssp. *armeniacum* Jakubz. em. Slageren (2n=4X=28, A"A"GG), whose domesticated form (*T. timophevii* ssp. *timophevii*) is restricted to western Georgia.

Wheat Food Quality

Wheat quality can be defined as the ability of a variety to produce flour suitable for a specific product. Consequently, this parameter is a variable that depends on consumer preferences, the product to be developed, and the process to be used in the preparation of the product. This trait is strongly associated with the grain components, and their physico-chemical properties.

Grain wheat is mainly composed of proteins (7-18%), lipids (1.5-2%) and carbohydrates (60-75%), together with other minor components such as certain vitamins and minerals [8]. Proteins and carbohydrates, especially starches, have considerable influence on three grain characteristics closely linked to the technical wheat qualities required for baking or pasta manufacture. These are gluten strength, starch properties and grain hardness or texture, which are associated with the endosperm storage proteins, starch synthesis enzymes and puroindolines, respectively.

Endosperm storage proteins

Two main groups (gliadins and glutenins) have been identified

*Corresponding author: Juan B Alvarez, Departamento de Genética, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Edificio Gregor Mendel, Campus de Rabanales, Universidad de Córdoba, CeiA3, ES-14071 Córdoba, Spain, E-mail: jb.alvarez@uco.es

Received January 23, 2013; Accepted February 13, 2013; Published February 17, 2013

Citation: Alvarez JB, Guzmán C (2013) Spanish Ancient Wheats: A Genetic Resource for Wheat Quality Breeding. Adv Crop Sci Tech 1: 101. doi:10.4172/2329-8863.1000101

Copyright: © 2013 Alvarez JB, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Alvarez JB, Guzmán C (2013) Spanish Ancient Wheats: A Genetic Resource for Wheat Quality Breeding. Adv Crop Sci Tech 1: 101. doi:10.4172/2329-8863.1000101



among these proteins according to their molecular characteristics [9]. Glutenins are also divided into high-molecular-weight (HMWGs) and low-molecular-weight (LMWGs) subunits [10,11]. The HMWGs are coded at the *Glu-1* loci located on the long arm of group-1 homoeologous chromosomes, whereas on the short arm are located the *Glu-3* loci that code for the LMWGs and the *Gli-1* loci that control the synthesis of ω -, γ - and some β -gliadins. On the short arm of group-6 homoeologous chromosomes are located the *Gli-2* loci that code mainly for components present in the α region and some β -gliadins [12].

Different studies have indicated that the HMWGs are the major group of gluten proteins that determine the bread-making characteristics of dough, whereas the variation in LMWGs would be more important in pasta production [13]. With respect to the latter, most qualitative evaluations of LMWGs have been performed on B-type subunits, as they are the most abundant and easiest to detect, while very little is known about the role of C- and D-type subunits [14].

Starch synthesis enzymes

The synthesis of starch in the endosperm occurs in the amyloplast, a plastid non- photosynthetic apparatus dedicated to this function. In this process, several enzymes act sequentially using ADP-glucose as a substrate by two different pathways to synthesize the starch components (amylose and amylopectin). These enzymes are ADPglucose pyrophosphorylase, starch synthases (SS) and branching enzymes (BE) [15].

The most studied of the enzymes involved in starch synthesis are GBSSI (granule bound starch synthase I) or waxy protein, which is the key enzyme in amylose synthesis. Depending on the ploidy level of each species of wheat, one, two or three waxy proteins are present. The latter is the case for common wheat where three waxy proteins are found encoded by the *Wx*-*A1* gene (chromosome 7AS), *Wx*-*B1* (chromosome 4 AL by translocation of a segment of chromosome 7BS) and *Wx*-*D1* (7DS chromosome) [16]. The other enzyme that has been studied is the

SGP-1 (SGP-A1, SGP-B1, and SGP-D1), which is coded by the *SSIIa-A*, *SSIIa-B* and *SSIIa-D* genes located on chromosome arms 7AS, 7BS and 7DS, respectively [17-19].

Puroindolines

The molecular-genetic basis of wheat grain hardness is now well established, being related to "friabilin", a Mr 15-kDa protein associated with the starch membrane, which is abundant in soft wheat starch, scarce in hard wheat starch, and absent in durum wheat [20]. The main components of "friabilin" are puroindolines a and b (PINA and PINB); and a third minor protein called the grain softness protein (GSP). These proteins are encoded by the *Pina-D1*, *Pinb-D1* and *Gsp* genes located in the locus Ha (Hardness), which is present in the short arm of the chromosome 5D of common wheat [21]. It is a complex locus formed by 10 closely linked genes [22], although only two loci (*Pina-D1* and *Pinb-D1*) have so far been associated to grain hardness [23-26].

When both puroindolines are present in its functional form, grain texture is soft; while the alteration of any of them, either by gene deletion or inactivation, causes the grain texture to become harder [21,23,26-28]. At the extreme, grain texture is very hard in durum wheat due to the deletion of both genes [29], which occurred during the development process in tetraploid wheat by partial removal of *Ha* locus from the A and B genomes [22].

Spanish Neglected and Underutilized Wheat Species

Archaeological evidences have shown that wheat has been cultivated in the Iberian Peninsula since the fifth millennium B.C. [30]. The archaeological remains found included both hulled and naked wheats. These denominations make reference to their glumes remaining intact or not adhering to the grain after threshing. Although the term «hulled» generate some controversy because it includes in the broad-sense many species within the genetic complex of the wheat as a species in the genus *Haynaldia, Aegilops* or *Agropyron* among others.

In a narrow-sense this denomination applies to three concrete crops: cultivated einkorn (*T. monococcum* L. ssp. *monococcum*), cultivated emmer (*T. turgidum* ssp. *dicoccum* Schrank em. Thell.) and spelt (*T. aestivum* ssp. *spelta* L. em. Thell.), which were cultivated in Spain some time ago, but currently could be catalogued as neglected or underutilized crops. Other wheat species that were cultivated in Spain in the past are: Polish wheat (*T. turgidum* ssp. *polonicum* L. em. Thell.), rivet wheat (*T. turgidum* L. ssp. *turgidum*) and club wheat (*T. aestivum* L. ssp. *compactum* Host. em. Mackey).

Wheat variability in Spain was studied in the 19th century by the Spanish botanists Mariano Lagasca and Simón de Rojas Clemente in their unpublished «*Ceres Hispanica*» herbarium [31]. This classic study permits comparison of the variability present in the 19th century when these crops were still widely used in Spain to the current situation where these plants are being conserved in Germplasm Banks (Table 1).

However, this great diversity presently has been widely reduced to two main species: durum and bread wheat, while the rest have lost or associated with traditional agriculture. Einkorn is considered to have practically disappeared in Spain. In fact, its use declined quickly with the introduction of tetraploid wheats, being exclusively used for animal feed; although its straw has been used for making cooking utensils and in certain regions as ceiling in huts.

In Spain, emmer and spelt form a complex group called *escanda* (Latin: "*scandŭla*"), although it is possible that other less-known species are also included in the group due to their similarity with some of the species in this complex. Emmer is also called *povia* or *pavida*; whereas spelt is called Asturian *fisga*. However, given that emmer is practically lost, the denomination *escanda* could be exclusive for spelt.

In Spain, both einkorn and emmer seem to have been present from the Neolithic period, although never as a main crop. Spelt, on the contrary, has been around since the Iron Age in some areas of northern Spain [30]. Later, references on its cultivation can be found around the Middle Age both in Christian texts as the "*Cronicon Albendense*" dated in 883 and in Arab agronomic treatises as the "Agriculture's Book" of Ibn Al Awam [32]. These crops were still identified by Lagasca and Rojas Clemente in the 19th century among the collections of *Triticum* species included in the *«Ceres Hispanica»* herbarium. These authors classified four botanical varieties of einkorn, ten of emmer and seven of spelt [31].

In the Twentieth Century, the percentage of hectares grown with hulled wheats was increased with time with an abrupt decrease during the Spanish Civil War (1936-39). After the 1930s, the popularity of hulled wheats increased until in the 1960s when the percentages dropped quickly. Dantín Cereceda [33] indicated that the crop area in Asturias was ~1050 ha; compared to more recently data that this area is approximately ~ 45 ha (36 of them in ecological system). This decline

in hectares corresponds with rural exodus, which was very important in many Spanish regions during the 60s and 70s, and mechanisation of agronomic tasks in many areas of Spain, together with the introduction of the semi dwarf wheats from International Center for Improvement of Wheat and Maize (CIMMYT), México. The progressive disappearance of these materials was in part stopped by their inclusion in Germplasm Banks. Nowadays, spelt survives in marginal farming areas of Asturias (North of Spain), where the farmers grow it by traditional farming systems for home consumption mainly [34,35].

Administratively, Asturias is divided into 78 *concejos* (municipalities). *Escanda* was cultivated in 37 of these at the beginning of the 20^{th} century [36], with an annual production of ~ 960 tonnes. Later on in the 1930s, staff of the Swiss Federal Research Station for Agroecology and Agriculture collected 50 populations in this region of Spain in only 23 *concejos*, which represents a clear reduction in the cultivation zone. Recently, one new collection mission was carried out by our group with the objective of collect hulled wheats in all places in the region where these crops are still cultivated. Between the end of July and the beginning of August 2004, 32 *escanda* populations were found in 31 localities from 14 *concejos* (Figure 2).

During the 2004 collection mission, we noticed the disappearance of the crop in many the localities indicated by the Swiss expedition of the 1930s. Only seven of these 23 *concejos* were common to both expeditions, which indicate a great decline in the cultivation of *escanda*, when compared to those indicated by Alvargonzalez [36].

Genetic Diversity of Spanish Ancient Wheats

Our group began the current research in 1999 with the aim of evaluating different types of neglected or underutilized Spanish wheats. This research focused on the species indicated in table 2.

The choice of each species was based on the objectives of the current research. First, these ancient wheats were evaluated and characterized as a source of new allelic variants for enlarging the genepool of modern wheat. Second, we evaluated their potential for use in creating new products as part of the revival in traditional foods that recently started in developed countries.

Einkorn

The lines we evaluated of einkorn can be grouped into four botanical varieties; three of which were not previously described in the *«Ceres Hispanica»* herbarium. The most abundant varieties were var. *tauricum* Drosd. and var. *monococcum*, awn colour being the only difference between both varieties, which was black for var. *tauricum* and white for var. *monococcum*. In minor frequencies were also found the var. *eredvianum* Zhuk., f. *punctatum* Stransk. and the var. *nigricultum* Flaksb. Both varieties showed shiny glumes which is black for the var. *nigricultum* and white for the var. *eredvianum* [37].

Disidu Javal	Species	Common name		
Piolog level	Species	English:	Spanish:	
Diploid	T. monococcum ssp. monococcum	einkorn escaña		
	T. turgidum ssp. dicoccum	emmer	povía o pavida	
Tetraploid	T. turgidum ssp. durum	durum wheat	trigoduro	
	T. turgidum ssp. polonicum	Polish wheat	trigopolaco	
	T. turgidum ssp. turgidum	rivet wheat	trigomoruno	
	T. aestivum ssp. compactum	club wheat	trigocabezorro	
Hexaploid	T. aestivum ssp. aestivum	bread wheat	trigoharinero	
	T. aestivum ssp. spelta	spelt	spelt espelta, escanda, fisga	

Table 1: Wheat species described by Lagasca and Clemente in the «Ceres Hispanica» herbarium.

Adv Crop Sci Tech

Page 3 of 7

Citation: Alvarez JB, Guzmán C (2013) Spanish Ancient Wheats: A Genetic Resource for Wheat Quality Breeding. Adv Crop Sci Tech 1: 101. doi:10.4172/2329-8863.1000101



Figure 2: Sites in which a collection mission was undertaken in 2004. The numbers indicate the *concejos* (municipalities) where some populations was identified: 1, Aller; 2, Belmonte de Miranda; 3, Cándamo; 4, Lena; 5, Morcin; 6, Oviedo; 7, Proaza; 8, Quiros; 9, Somiedo; 10, Teverga; 11, Villaviciosa; 12, Grado; 13, Pravia; 14, Salas.

Ploidy level	Common name	Species	Accessions
2X	Einkorn	T. monococcum ssp.monococcum	32
4X	Emmer	T. turgidum ssp. dicoccum	102
	Rivet wheat	T. turgidum ssp. turgidum	60
6X	Mexican Creole wheat	T. aestivum ssp. aestivum	105
	Spelt	T. aestivum ssp. spelta	489
Total			788

Table	2:	Wheat	species	from	Spain	analysed	by	our	group).
									J	

The variation in seed storage proteins in Spanish einkorn was high, with three allelic variants for the *Glu-A^m1* locus, six for the *Glu-A^m3* locus, seven for the *Gli-A^m1* locus and fourteen for the *Gli-A^m2* locus found among the evaluated accessions. Internal variability was detected in some of these materials, which could be related to their being landraces. Up to 48 different genotypes were identified based on their origin and seed storage protein composition [38].

In einkorn, waxy protein polymorphism was very low in the current collection, so electrophoretic analyses revealed only two alleles [37], although this was larger than that found by other researchers [39,40], who did not find any polymorphism in the collections of cultivated einkorn and *T. urartu*. One of the alleles ($Wx-A^m1a$) was previously described by Rodríguez-Quijano et al. [40]. The novel allele ($Wx-A^m1a'$) showed less mobility and was only found in one accession. Both alleles showed higher mobility than the Wx-A1a allele detected in common wheat (cv. Chinese Spring).

When the *Pina* and *Pinb* gene variability was evaluated in the einkorn collection, three *Pina* and five *Pinb* alleles were detected in the einkorn lines, including three novel alleles for the *Pinb* locus: *Pinb*- A^m1i , *Pinb*- A^m1i and *Pinb*- A^m1k [41].

Emmer

The most representative varieties of emmer wheat were *dicoccon* Körn. and *tricoccum* (Schübl.) Körn. These varieties, together with the *majus* Körn. variety, have white glumes. The difference between both varieties (*dicoccon* and *tricoccum*) is the awn length, which is long for *dicoccon* and short for *tricoccum*. The *macratherum* Körn. and *majus* varieties were less represented, whereas the presence of

the three varieties (*atratum* (Host.) Körn., *lagascae* Al. et Tell., nom. nud., and *pycnurum* Alef.) was very scarce [42]. Some of the varieties described by Lagasca and Rojas Clemente [31] did not appear among the materials analysed here (var. *inerme* Körn., var. *rufum* Schübl, and var. *pseudomacratherum* Flaksb.), which suggests that part of the diversity of this species in Spain could have lost in the first half of the twentieth century.

When the emmer collection was evaluated for HMWGs, four allelic variants were detected for the *Glu-A1* locus; one of which had not been previously described. For the *Glu-B1* locus, three of the nine alleles detected had not been previously identified [43]. Similarly, a high degree of variation was found for the LMWGs, where up to 23 different patterns were detected for B-LMWGs. When considering HMWGs and LMWGs collectively, 30 combinations were found among the evaluated lines. In a later study, we selected 31 lines that showed these allelic variations and evaluated them for morphological and quality traits [42,44]. Up to seven different botanical varieties were identified, which suggested a diversity of varieties, although lower than that for the 10 botanical varieties that had been historically described [31]. Furthermore, the allele data obtained for gluten strength also showed some of the alleles detected for HMWGs and LMWGs [44].

In contrast, emmer showed no polymorphism for the *Wx-A1* locus. All the accessions evaluated (87 accessions) had the *Wx-A1a* allele, which could be similar to the wild allele [45]. For the *Wx-B1* locus, three alleles were detected: *Wx-B1b* (*null* type) that lacks the protein; *Wx-B1c**, which had the same mobility as *Wx-B1c'* detected in the durum wheat cultivar 'Mexicali' but lower electrophoretic mobility than the *Wx-B1a* allele, and a new allele, called *Wx-B1g*. This new allele showed

Page 4 of 7

a slightly higher mobility than the *Wx-B1a* allele, but lower in the *Wx-B1d* allele found by Yamamori et al. [46]. The *Wx-B1g* allele was the most abundant, being detected in 85 accessions. The *null* type (*Wx-B1b* allele) and the *Wx-B1c** allele were only detected in one accession.

Because of the reduced variability for size among the different Wx alleles, the variability found was revaluated by PCR-RFLP and DNA sequence analyses [45]. These analyses showed that the Wx-A1a allele of emmer wheat is slightly different to the Wx-A1a allele of durum wheat, presenting two synonymous changes (silent mutations) in the eleventh exon that did not modify the predicted protein sequence.

For the Wx-B1 gene, the molecular data confirmed three alleles previously described by SDS-PAGE separation [45]. The deduced protein for two of them (Wx-B1b and Wx-B1g) was the same, although no protein could be detected for the Wx-B1b (null allele). Both alleles had 15 and 3 differences respectively, with the Wx-B1a and Wx-B1c'alleles detected in the durum wheat cultivars (Langdon and Mexicali) used as standards. The other Wx-B1 allele of emmer wheat (Wx- $B1c^*$) was also different from the standard alleles (Wx-B1a and Wx-B1c') with 13 and one amino acid changes, respectively, and two amino acid changes with the Wx-B1b and Wx-B1g alleles.

Rivet wheat

The analysis of the sixty accessions of Spanish rivet wheat of this collection showed high genetic diversity with sixteen botanical varieties (var. *buccale* Alef, var. *dinurum* Alef, var. *dreischianum* Körn, var. *gentile* Alef, var. *herrerae* Körn, var. *lusitanicum* Körn, var. *megalopolitanum* Körn, var. *melanatherum* Körn, var. *miscibile* Haçid, var. *nigro-glumaceum* Flaks, var. *nigroglumarum* Haçid, var. *pseudosalomonis* Papad, var. *rubroalbum* Flaks, var. *salomonis* Körn, var. *speciosum* Alef, and var. *triste-rubro-atrum* Al) and five additional botanical types, according to the criteria indicated by Lagasca and Rojas Clemente [31]. Additionally, five botanical types including seven lines were detected but these could not be associated to any botanical varieties described in the *«Ceres Hispanica»* herbarium.

Furthermore, up to 13 allelic variants (four alleles for the *Glu-A1* locus and nine alleles for the *Glu-B1* locus) were found in the evaluated lines, five of which were new (one for the *Glu-A1* locus and four for the *Glu-B1*). Also, thirty-four patterns were identified in the B-LMW glutenin subunits. In all cases, the data showed low values for the effective number of alleles as well as genetic diversity, which indicated that there is a danger of genetic erosion in these loci [47].

Spelt

The characterization of the spelt lines derived from the Germplasm Banks indicated that six out of the seven botanical varieties of Lagasca and Rojas Clemente [31] were present in the collection (var. *albivelutinum*, var. *arduini*, var. *caeruleum*, var. *duhamelianun*, var. *rubrivelutinum* and var. *vulpinum*); only the *album* variety (awnless and white glumes) was not found [48]. However, the collected populations showed certain genetic erosion with respect to the materials collected by the Swiss expedition in 1930s. Five of seven botanical varieties described by Lagasca and Rojas Clemente (var. *albivelutinum*, var. *arduini*, var. *caeruleum*, var. *rubrivelutinum* and var. *vulpinum*) for spelt were detected, together with two types not mentioned by previous authors (yellow spikes with glabrous and pubescent glumes).

In spelt, three allelic variants were detected for the *Glu-A1* locus. For the *Glu-B1* locus, two of seven alleles detected had not been found before while four of the nine alleles detected for the *Glu-D1* had not been described previously [49]. When the three loci were considered together, 25 combinations were found among the evaluated lines. For the LMWGs, extensive variation was found: 46 different patterns for the B-LMWGs and 16 for the C-LMWGs [50]. Concerning gliadins, 61 different patterns were found for the ω -gliadins, 44 for the γ -gliadins, 19 for the β -gliadins and 15 for the α -gliadins [51].

Within the spelt collection, the diversity of HMWGs was studied electrophoretically in 333 accessions, grouped in 50 populations, originally collected in Asturias (North Spain) during the 1930s. Inter and intra-population distribution of HMWGs alleles at the Glu-A1, Glu-B1 and Glu-D1 loci, together with the gliadin patterns, were investigated [51,52]. The results showed that genetic variation is mainly present within populations with the variation between populations being only 21%. The material analysed showed polymorphism for all seed storage proteins, although some allelic variants were clearly hegemonic while others appeared less frequently. This suggested the possibility that there was a loss of variability, even before the collecting mission, which could have deteriorated further with the subsequent reduction in the cultivated area of this species in Asturias. In fact, the materials collected during the collection mission to Asturias in 2004 [48], showed loss of nine HMWGs alleles that were detected in the old collection [49].

The electrophoretic analysis of the waxy proteins revealed polymorphism for the three *waxy* loci in the Spanish spelt lines that were evaluated [53]. For the *Wx-A1* locus, two alleles were found: *Wx-A1a*, similar to the allele present in cv. Chinese Spring, and *Wx-A1b* (null type), which lacks the protein, although subsequent studies showed that one of the *Wx-A1b* (null type) alleles showed low expression, being reassigned as *Wx-A1g* [54,55]. The sequence analysis of this allele showed the presence of a 160-bp insertion in the fourth intron that affects complete splicing of all the RNA molecules, thus reducing the yield of correctly processed RNA leading to a significant decrease in the final concentration of Wx-A1 protein.

For the *Wx-B1* locus, three different alleles were found: *Wx-B1a*, the *null* protein *Wx-B1b* and the *Wx-B1c'* allele, which showed less mobility than *Wx-B1a* allele. Both *Wx-B1b* and *Wx-B1c'* alleles were less common (11.67% and 12.86%) than *Wx-B1a*. The lines evaluated showed homogeneity for the *Wx-D1* locus, where 99.52% of the accessions contained the *Wx-D1a* allele. Two additional alleles were detected: the *null* allele (*Wx-D1b*), which was only present in one accession, and a novel *waxy* allele, not previously described, with a slightly lower electrophoretic mobility than the *Wx-D1a* allele. This new allele was provisionally named *Wx-D1g* [53].

Eight combinations were detected that showed variation in the *Wx-A1*, *Wx-B1* and *Wx-D1* loci, where there was a clear dominance by the combination *Wx-A1a*, *Wx-B1a*, *Wx-D1a*; appearing in 69.52% of the samples. In the other loci, three combinations with a relatively high frequency were observed. The combination *Wx-A1a*, *Wx-B1c'*, *Wx-D1a* appeared in 47 accessions, *Wx-A1a*, *Wx-B1b*, *Wx-D1a* in 44 and *Wx-A1g*, *Wx-B1a*, *Wx-D1a* in 23. The other four combinations were rare with frequencies of less than 5% [53].

Bread wheat (Mexican landraces)

In 2004, a collection of one-hundred and two lines of traditional common wheat landraces (Mexican Creole wheat) grouped into 15 landraces was received. These old wheat materials are the last trace of the common wheat carried by the Spanish to America during the 16th and 17th centuries. These materials are derived from old Spanish

Page 6 of 7

cultivars and landraces and their cultivation survived on small-scale traditional farms. They could represent the nexus between the old bread wheats cultivated in Spain and the traditional varieties stored in the Germplasm Banks and could contain genetic variability that is now missing from modern wheat.

Different morphological traits were measured with the purpose of evaluating how many of the 22 botanical varieties that Lagasca and Rojas Clemente established for common wheat in Spain throughout the 19th century still existed [31]. These Mexican Creole wheats were grouped into seven botanical varieties [56]. However, because there is no data on the botanical varieties taken to the Americas during the 16th and 17th centuries, the level of erosion undergone by these wheats could not be determined.

Up to 21 HMWG allelic variants (four, eight and nine alleles for the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively) were detected. The frequency distribution was very asymmetric: 90% of the lines had only two *Glu-A1* alleles, 88% had two *Glu-B1* alleles and only one *Glu-D1* allele was present in 81% of the lines. This suggests the possibility of erosion, mainly by genetic drift [56].

When grain hardness was measured, 16 lines were hard and 86 were soft-textured [57]. All hard lines could be explained by a mutation in either the *Pina-D1* or *Pinb-D1* genes. In six hard lines, there was no amplification of *Pina-D1*, suggesting that this gene was deleted (*Pina-D1b* allele). The remaining ten hard lines showed the presence of both *Pina-D1* and *Pinb-D1*. Sequencing the *Pinb-D1* genes of the hard lines revealed the presence of two different alleles (*Pinb-D1b* and *Pinb-D1e*). This latter allele has been described as rare, being found in only seven lines of bread wheat up to now, while those eight lines of Creole Mexican wheat appeared to carry the allele. Due to the origin of these wheat accessions, the presence of the rare puroindoline haplotypes may be explored in old local varieties of Spanish common wheat.

In general, the results substantiate the importance of very old Mexican landraces as potential sources of genetic diversity for key quality traits in the development of modern wheat cultivars.

Concluding Remarks

Wheat is an important crop that has been associated with human food for many centuries. It is the basis for a diverse range of products, mainly bread and pasta, and in some cases beer, which are present in most worldwide diets. In some cases, the same wheat type has been used for all of these three different products depending on the geographical or cultural area. For example, durum wheat was used to make bread in the Nile Valley, beer in the Euphrates Valley and pasta in the Yang-tsé Valley. In the Mediterranean region, wheat is linked to flour and its consumption.

From the mid 20th century, plant breeding, based on high-yielding cultivars, has contributed to the narrowing of the genetic base of crops, causing many modern cultivars to be closely related. Subsequently, it is necessary to search for and conserve plant genetic resources to enlarge the gene pool and avoid genetic uniformity, which makes crops vulnerable to biotic and abiotic stresses. In order to face the new challenges arising because of climate change, sustainable models of agriculture need to be found. Plant genetic resources can be used to enlarge the genetic background of the modern cultivars, although, in many cases, these ancient varieties are underutilized or neglected crops that could be reintroduced into agriculture.

In this review, the variability of several collections of Spanish

ancient wheat for three traits related to technological quality has been evaluated. In general, genetic diversity was high, although a large part of this diversity is at risk of erosion, given that the distribution of the combinations among the evaluated accessions was not random. There was an assumption that, in general, species distribution is conditioned by seed transference among farmers. This produces the homogenization of a crop or cultivar in any region after several generations, along with their irretrievable loss when these farmers stop farming the crop or cultivar.

The alleles found for these loci were different to those detected in cultivated wheat. The low frequencies of the new alleles confirmed the need to protect and conserve these accessions because it is unlikely that these alleles will be found in other crops. Therefore, the loss of these materials would be equivalent to the loss of these alleles. Although the revival of these old crops in modern agriculture is possible, it is more probable that the variation found in these species can be used by the plant-breeders for enlarging the genetic background of the modern wheat.

Acknowledgments

This research was supported by a grant AGL2010-19643-C02-01 from the Spanish Ministry of Economy and Competitiveness, co-financed by the European Regional Development Fund (FEDER) from the European Union.

References

- Harlan JR (1992) Crops and man. (2nd Edn), American Society of Agronomy, Madison, WI, USA.
- Zohary D, Hopf M, Weiss E (2012) Domestication of plants in the old world: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. (4th Edn), Oxford University Press, UK.
- Dvořák J, McGuire PE, Cassidy B (1988) Apparent source of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30: 680-689.
- McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its freethreshing hexaploid relatives. J Hered 37: 81.
- Kerber ER, Rowland GG (1974) Origin of the free threshing character in hexaploid wheat. Can J Genet Cytol 16: 145-154.
- Dvorák J, Zhang HB (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc Natl Acad Sci U S A 87: 9640-9644.
- Dvořák J, Zhang HB (1992) Reconstruction of the phylogeny of the genus *Triticum* from variation in repeated nucleotide sequences. Theor Appl Genet 84: 419-429.
- Matz SA (1999) Bakery technology and Engineering. (3rd Edn), Pan-Tech International, Taiwan.
- Aykroyd WR, Doughty J (1970) Wheat in human nutrition. FAO Nutr Stud: 1-163.
- Payne PI (1987) Genetics of wheat storage proteins and the effects of allelic variation on bread-making quality. Ann Rev Plant Physiol 38: 141-153.
- Singh NK, Shepherd KW (1988) Linkage mapping of genes controlling endosperm storage proteins in wheat. Theor Appl Genet 75: 628-641.
- Metakovsky EV, Novoselskaya AY, Kopus MM, Sobko TA, Sozinov AA (1984) Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis. Theor Appl Genet 67: 559-568.
- Wrigley CW, Békés F, Bushuk W (2006) Gliadin and glutenin: The unique balance of wheat quality. AACC International, St Paul, MN, USA.
- D'Ovidio R, Masci S (2004) The low-molecular-weight glutenin subunits of wheat gluten. J Cereal Sci 39: 321-339.
- James MG, Denyer K, Myers AM (2003) Starch synthesis in the cereal endosperm. Curr Opin Plant Biol 6: 215-222.
- 16. Yamamori M, Nakamura T, Endo TR, Nagamine T (1994) Waxy protein

deficiency and chromosomal location of coding genes in common wheat. Theor Appl Genet 89: 179-184.

- Li Z, Chu X, Mouille G, Yan L, Kosar-Hashemi B, et al. (1999) The localization and expression of the class II starch synthases of wheat. Plant Physiol 120: 1147-1156.
- 18. Li Z, Sun F, Xu S, Chu X, Mukai Y, et al. (2003) The structural organisation of the gene encoding class II starch synthase of wheat and barley and the evolution of the genes encoding starch synthases in plants. Funct Integr Genomics 3: 76-85.
- Shimbata T, Nakamura T, Vrinten P, Saito M, Yonemaru J, et al. (2005) Mutations in wheat starch synthase II genes and PCR-based selection of a SGP-1 null line. Theor Appl Genet 111: 1072-1079.
- Greenwell P, Schofield JD (1986)A starch granule protein associated with endosperm softness in wheat. Cereal Chem 63: 379-380.
- 21. Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol 48: 633-647.
- 22. Li W, Huang L, Gill BS (2008) Recurrent deletions of puroindoline genes at the grain hardness locus in four independent lineages of polyploid wheat. Plant Physiol 146: 200-212.
- Gautier MF, Aleman ME, Guirao A, Marion D, Joudrier P (1994) *Triticum* aestivum puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. Plant Mol Biol 25: 43-57.
- 24. Giroux MJ, Morris CF (1997) A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch-surface friabilin. Theor Appl Genet 95: 857-864.
- Giroux MJ, Morris CF (1998) Wheat grain hardness results from highly conserved mutations in the friabilin components puroindolinea and b. Proc Natl Acad Sci U S A 95: 6262-6266.
- Lillemo M, Morris CF (2000) Aleucine to proline mutation in puroindoline b is frequently present in hard wheats from Northern Europe. Theor Appl Genet 100: 1100-1107.
- Chen F, He ZH, Xia XC, Xia LQ, Zhang XY, et al. (2006) Molecular and biochemical characterization of puroindoline a and b alleles in Chinese landraces and historical cultivars. Theor Appl Genet 112: 400-409.
- Gautier MF, Cosson P, Guirao A, Alary R, Joudrier P (2000) Puroindoline genes are highly conserved in diploid ancestor wheats and related species but absent in tetraploid *Triticum* species. Plant Sci 153: 81-91.
- Rahman S, Jolly CJ, Skerritt JH, Wallosheck A (1994) Cloning of a wheat 15-kDa grain softness protein (GSP). GSP is a mixture of puroindoline-like polypeptides. Eur J Biochem 223: 917-925.
- Buxó i Capdevila R, Alonso N, Canal D, Echave C, Gonzalez I (1997) Archaebotanical remains of hulled and naked cereals in the Iberian Peninsula. Veg Hist Archaeobot 6: 15-23.
- Tellez-Molina R, Alonso-Peña M (1952) Los trigos de la Ceres Hispanica de Lagasca y Clemente. Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain.
- 32. Cubero JI (2000) El Libro de Agricultura de Al Awan. Edition and Commentaries on the Spanish translation of Josef Antonio Banqueri (1802) by Jose Ignacio Cubero. Empresa Pública para el Desarrollo Agrario y Pesquero de Andalucía, S. A., Málaga, Spain.
- Dantín Cereceda J (1941) Distribución geográfica de la escanda asturiana. Estudios Geográficos 5: 739-797.
- Peña-Chocarro L (1996) In situ conservation of hulled wheat species: the case of Spain: Hulled wheats; S. Padulosi, K. Hammer, J. Heller, Eds, International Plant Genetic Resources Institute: Rome, Italy. pp. 129-146.
- 35. the reference correct is: Alvarez JB, Martín MA, Caballero L, Martín LM (2010) The role of plant genetic resources in the Sustainable Agriculture: Sustainable Agriculture: Technology, Planning and Management; A. Salazar, I. Rios, Eds, Nova Science Publishers Inc., New York, USA, pp. 145-176.
- 36. Alvargonzalez C (1908) La escanda, su origen y su cultivo. Gijón, Spain.
- Guzmán C, Caballero L, Alvarez JB (2009) Variation in Spanish cultivated einkorn wheat (*Triticum monococcum* L. ssp.*monococcum*) as determined by morphological traits and waxy proteins. Genet Resour Crop Evol 56: 601-604.
- 38. Alvarez JB, Moral A, Martín LM (2006) Polymorphism and genetic diversity

for the seed storage proteins in Spanish cultivated einkorn wheat (*Triticum monococcum* L. ssp.*monococcum*). Genet Resour Crop Evol 53: 1061-1067.

- Urbano M, Margiotta B, Colaprico G, Lafiandra D (2002) Waxy proteins in diploid, tetraploid and hexaploid wheats. Plant Breed 121: 465-469.
- Rodriguez-Quijano M, Vázquez JF, Carrillo JM (2004) Waxy proteins and amylose content in diploid Triticeae species with genomes A, S and D. Plant Breed 123: 294-296.
- Guzmán C, Caballero L, Martín MA, Alvarez JB (2012) Molecular characterization and diversity of the *Pina* and *Pinb* genes in cultivated and wild diploid wheat. Mol Breed 30: 69-78.
- 42. Alvarez JB, Caballero L, Ureña P, Vacas M, Martín LM (2007) Characterization and variation of morphological traits and storage proteins in Spanish emmer wheat germplasm (*Triticum dicoccon*). Genet Resour Crop Evol 54: 241-248.
- 43. Pflüger LA, Martín LM, Alvarez JB (2001) Variation in the HMW and LMW glutenin subunits from Spanish accessions of emmer wheat (*Triticum turgidum ssp. dicoccum* Schrank). Theor Appl Genet 102: 767-772.
- 44. Caballero L, Martín LM, Alvarez JB (2008) Relationships between the HMWand LMW-glutenin subunits and SDS-sedimentation volume in Spanish hulled wheat lines. Czech J Genet Plant Breed 44: 114-117.
- 45. Guzmán C, Caballero L, Alvarez J (2011) Molecular characterization of the Wx-B1 allelic variants identified in cultivated emmer wheat and comparison with those of durum wheat. Mol Breed 28: 403-411.
- 46. Yamamori M, Nakamura T, Nagamine T (1995) Polymorphism of two waxy proteins in the emmer group of tetraploid wheat, *Triticum dicoccoides*, *T. dicoccum*, and *T. durum*. Plant Breed 114: 215-218.
- Carmona S, Alvarez JB, Caballero L (2010) Genetic diversity for morphological traits and seed storage proteins in Spanish rivet wheat. Biol. Plantarum 54: 69-75.
- Caballero L, Martín LM, Alvarez JB (2007) Agrobiodiversity of hulled wheats in Asturias (North of Spain). Genet Resour Crop Evol 54: 267-277.
- Caballero L, Martín LM, Alvarez JB (2001) Allelic variation of the HMW glutenin subunits in Spanish accessions of spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.).Theor Appl Genet 103: 124-128.
- Caballero L, Martín LM, Alvarez JB (2004) Genetic variability of the lowmolecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta* L. em Thell.). Theor Appl Genet 108: 914-919.
- Caballero L, Martín LM, Alvarez JB (2004) Genetic variability of the lowmolecular-weight glutenin subunits in spelt wheat (*Triticum aestivum* ssp. *spelta L. em Thell*). Theor Appl Genet 108: 914-919.
- Caballero L, Martín LM, Alvarez JB (2004) Variation and genetic diversity for gliadins in Spanish spelt wheat accessions. Genet Resour Crop Evol 51: 679-686.
- Guzmán C, Caballero L, Moral A, Alvarez JB (2010) Genetic variation for waxy proteins and amylose content in Spanish spelt wheat (*Triticum spelta* L.). Genet Resour Crop Evol 57: 721-725.
- Caballero L, Bancel E, Debiton C, Branlard G (2008) Granule-bound starch synthase (GBSS) diversity of ancient wheat and related species. Plant Breed 127: 548-553.
- Guzmán C, Caballero L, Yamamori M, Alvarez JB (2012) Molecular characterization of a new waxy allele with partial expression in spelt wheat. Planta 235: 1331-1339.
- Caballero L, Peña RJ, Martín LM, Alvarez JB (2010) Characterization of Mexican Creole wheat landraces in relation to morphological characteristics and HMW glutenin subunit composition. Genet Resour Crop Evol 57: 657-665.
- Ayala M, Guzmán C, Alvarez JB, Peña RJ (2013) Characterization of genetic diversity of puroindoline genes in Mexican wheat landraces. Euphytica 190: 53-63.

Citation: Alvarez JB, Guzmán C (2013) Spanish Ancient Wheats: A Genetic Resource for Wheat Quality Breeding. Adv Crop Sci Tech 1: 101. doi:10.4172/2329-8863.1000101