Variation and genetic diversity for gliadins in Spanish spelt wheat accessions

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Abstract

Gliadin composition has been analysed in 403 accessions of spelt wheat (Triticum aestivum ssp. spelta); 61 different patterns were found for the α-gliadins, 44 for the γ-gliadins, 19 for the β-gliadins and 15 for the α-gliadins. A subset of 333 accessions belonging to fifty populations from Asturias, North of Spain, showed high levels of genetic variation (A = 3.89, P = 0.88, Ne = 3.35 and He = 0.553), indicating that 82.5% of the genetic variation was within populations, and only 18.5% among populations. Thirty-five of these populations presented more of five accessions, in this new subset the values of genetic variation were higher that those of fifty populations (A = 4.49, P = 0.91, Ne = 3.80 and He = 0.595). The genetic variation within populations was 59.7% of the total, and 40.3% among populations, which could be associated to fixation effects of some alleles by genetic drift.

Introduction

Genetic erosion of the common wheat germplasm genetic base caused by frequent use of the same parental genotypes for breeding activities is becoming a serious problem (Porceddu et al. 1988). During the last decades, the interest for the ancient wheats has increased, thanks to their adaptability to poor soils, harsh climatic conditions, the low inputs (D’Antuono 1989), attractive nutritional attributes and demand for unconventional foods (Auricchio et al. 1982; Strehlow et al. 1991). In addition, the hulled wheats - einkorn (2n = 2x = 14, AA; Triticum monococcum L. ssp. monococcum), emmer (2n = 4x = 28, AAbb; T. turgidum ssp. dicoccum (Schrank) Thell.) and spelt (2n = 6x = 42, AABBDD; T. aestivum ssp. spelta (L.) Thell.) - constitute a useful gene reservoir for breeding programmes of both bread and durum wheat (see Padulosi et al. 1996 for a review).

In Spain, hulled wheats, mainly emmer and spelt, were widely cultivated during the first part of the 20th Century, to decrease towards the late 1960s, when the agricultural mechanisation started to occur in many areas of Spain. Spelt still survives in marginal areas of Asturias (Northern Spain), where is endangered (Peña-Chocarro and Zapata-Peña 1998).

Gliadins and glutenins are storage proteins of wheat endosperm. Among them gliadins, which are controlled by the Gli loci located on the short arms of chromosomes of the homoeologous groups 1 and 6 (Payne et al. 1982; Payne 1987) have so far received little attention. Each Gli locus codes for a group of gliadin polypeptides that are inherited as a block. Because of the multiple allelism at these loci, the different blocks generate an extremely complex gliadin pattern in hexaploid wheat (Sozinov and Poperelya 1980; Metakovski et al. 1984). Genes coding for most γ- and α-gliadins have been located on short arm of chromosomes 1A, 1B and 1D at the Gli-A1, Gli-B1 and Gli-D1 loci respectively, whereas genes coding for most α- and β-gliadins occur on short arm of group 6 chromosomes at the Gli-A2, Gli-B2 and Gli-D2 (Payne 1987).

Gliadins show the highest level of polymorphism when studied by standard method of acidic electrophoresis (Zillman and Bushuk 1979), and have
proved to be useful markers for assessing genetic variation (Lafiandra et al. 1990; Pflüger et al. 2001), and for genotype identification in different wheat species (Bushuk and Zillman 1978; Nevo and Payne 1987).

Investigations on seed storage protein composition have been rather frequent in bread and durum wheat, but not in hulled wheats. Seed storage proteins of a collection of Spanish emmer wheat have been analysed by Pflüger et al. (2001). Recently, both the variability for HMW glutenin subunits (Caballero et al. 2001) and their genetic diversity (Caballero et al. 2003) have been evaluated in a spelt collection collected in Northern Spain during the first half of the last century.

The main goal of the present study was to analyse the variability and the genetic diversity of gliadins in the same collection of spelt accessions.

Material and Methods

Plant material

Spelt wheat accessions (403), obtained from the National Small Grain Collections (Aberdeen, USA) and Centro de Recursos Fitogenéticos INIA (Alcalá de Henares, Spain), were analysed. Passport data on 333 of them, collected in Asturias (North of Spain) by personnel of Swiss Federal Research Station for Agroecology and Agriculture in 1939 (Dr. F. Weilsemann, pers. commun.), permitted to group them in fifty populations.

Polyacrylamide gel electrophoresis (A-PAGE) analysis

Gliadins were extracted with a 1.5 M dimethylformamide aqueous solution and fractionated by A-PAGE at 8.5% (C = 2.67%) with low catalyst levels (ferrous sulfate and hydrogen peroxide) for increasing the gel firmness (Khan et al. 1985). Electrophoresis was performed at 25 mA/gel at 18 °C for 45 min after the tracking dye (methyl violet) migrated off the gel. Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. Destaining was carried out with tap water.

Statistical analysis

The frequency of gliadin patterns was calculated at population and collection levels. Expected heterozygosity (He), proportion of polymorphic loci (P), average number of alleles per locus (A) and effective number of alleles per locus (Ne), were used to evaluate the genetic diversity within populations.

The gene diversity over all populations (Ht), together with the average allelic gene diversity within (Hs) and among (Dst) populations, was calculated according to Nei (1973). The relative magnitude of genetic differentiation among populations, Gst, was estimated as Dst/Ht.

Results and Discussion

Gliadin composition

Up to 72 different bands were detected, assuming that the bands with the same relative mobility represent the same subunit. These bands were grouped in to patterns at each of the four zones of gel (ω-, γ- β- and α-gliadins); each zone was considered as a single locus and the different patterns as allelic variants. A representative sample of the variation detected is shown in Figure 1.

Twenty-two different bands were found in the ω-gliadins zone, to form 61 different patterns. Diagrams of these patterns with indication of their frequencies are given in Figure 2. The number of bands present in these patterns varied from three to nine, the patterns in the ω-gliadins zone with six or seven bands being the most frequent (24.6% and 26.23 %, respectively). All patterns, except two (PI-348682 and PI-348744), had two slow bands. These two bands have been associated with the D genome in hexaploid wheat, but their absence does not indicate that the two accessions did not belong to the spelt groups. In fact, Caballero et al. (2001), by analysing HMW glutenin subunits of the same accessions confirmed that the D genome was present. Likewise, the complementary information on spike morphology confirmed that these accessions are spelt wheat.

The most frequent patterns were no. 1 and 2, which appear in 37 and 33 accessions, respectively. Other patterns with rather high frequency were 3, 4 and 5, detected in 27, 21 and 20 of the evaluated accessions. Conversely, 17 patterns were detected only in one accession.