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Genetic diversity and molecular characterization of puroindoline genes (*Pina-D1* and *Pinb-D1*) in bread wheat landraces from Andalusia (Southern Spain)

Marcela Ayala [a, b], Carlos Guzmán [c], Roberto J. Peña [c], Juan B. Alvarez [a, *]

[a] *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*
[b] *División de Fitomejoramiento, Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Paraguay*
[c] *Wheat Chemistry and Quality Laboratory, Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico*

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A B S T R A C T

Grain hardness is directly related to the damaged starch amount produced during the milling process and to dough water absorption and viscosity. This trait is controlled by the puroindolines genes (*Pin-D1*) located on the 5DS chromosome. Forty-five landraces from Andalusia (Southern Spain) were analyzed for grain hardness by NIR (predicted particle size index or PSI) and *Pin-D1* genes. Thirty-one accessions showed semi-hard texture (predicted PSI between 45 and 55%), while the rest was distributed in 13 hard accessions (predicted PSI < 45%) and one soft-textured (predicted PSI > 55%) accession. The wild alleles (*Pina-D1a* and *Pinb-D1a*) were found in all the soft and semi-hard texture accessions. However, only seven of the thirteen hard accessions could be explained by mutations in either *Pina-D1* or *Pinb-D1*. Two of them contain the null *Pina-D1b* allele. For *Pinb-D1*, three different alleles were detected, including *Pinb-D1b* and *Pinb-D1d*. The third allele, tentatively named *Pinb-D1ad*, was characterized by a C/T transition that led to the appearance of a premature stop codon, which is extremely rare. These results confirm the importance of these landraces, infrequently utilized in breeding programs, as potential sources of the genetic variability of *Pin* loci for developing wheat cultivars with different grain textures.

1. Introduction

Wheat is one of the most important crops in the world, occupying 219 million hectares for a global production of 715 million tonnes. Wheat based foods have different texture, shape, taste and aroma according to the geographical area where they are produced and consumed (leavened breads, flat breads, noodles, cookies, etc.). Although the conventional quality parameters of flour or semolina are usually sufficient to determine the potential end-use of wheat cultivars in industrial processing, these parameters should be used with precaution for the evaluation of traditional varieties, because this could undervalue the characteristics of these materials.

Grain quality is mainly determined by dough (gluten) viscoelastic properties and grain hardness. Grain hardness is the most important single trait determining wheat end-use quality (Pomeranz and Williams, 1990; Morris and Rose, 1996) and global markets classify wheat into three main hardness classes: very hard, hard and soft. The very hard class has been exclusively associated to durum wheat (*Triticum turgidum* ssp. *durum* Desf. em. Husn.; $2n = 4\times = 28$, **BBA^{u}A^{u}**); while the other two ones have been found in bread wheat (*Triticum aestivum* L. ssp. *aestivum*; $2n = 6\times = 42$, **DDBBA^{u}A^{u}**). Grain hardness is important because it is directly related to the amount of damaged starch produced during the milling process (hard grain produces more damaged starch than soft grain). Because of this, hard wheat is used for bread-making, which requires high water absorption for the correct development of fermented dough, while soft wheat is preferred for making cookies and pastries, which requires less hydrophilic flour, so that more water is available for the sugar to form syrup, resulting in greater dough spread and larger cookie diameter (Guttieri et al., 2001).

The *Ha* (hardness) locus located on the 5DS chromosome has been determined as the main responsible of the grain texture

* Corresponding author.
E-mail address: jb.alvarez@uco.es (J.B. Alvarez).

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(Morris, 2002). At this locus, the genes encoding the grain softness protein (*Gsp-1*) have been well characterized, together with those encoding for puroindolines (*Pina-D1* and *Pinb-D1*) considered to be mainly responsible for grain hardness (Morris, 2002). Puroindolines (PINA and PINB) are two basic grain proteins having a tryptophan-rich-hydrophobic domain (Gautier et al., 1994), which, when present in their wild form (*Pina-D1a* and *Pinb-D1a*), leads to soft wheat texture. The lack of either of them, together with most of the mutations described in either *Pina-D1* or *Pinb-D1*, leads to hard grain texture (Morris and Bhave, 2008). Some of the alleles leading to hard grain are associated to differences in the degree of hardness and in processing and end-use quality traits. For example, several studies have been done comparing the effects of the most common *Pin-D1* alleles associated with hard endosperm: *Pina-D1b* and *Pinb-D1b*. The genotypes with *Pina-D1b*/*Pinb-D1a* reported harder grains than in those with *Pina-D1a*/*Pinb-D1b* (Chen et al., 2013; Eagles et al., 2006; Giroux et al., 2000; Martin et al., 2001). The *Pinb-D1b* allele was also associated to higher milling yield compared to the allele *Pina-D1b* (Eagles et al., 2006; Martin et al., 2001). Chen et al. (2013) and Eagles et al. (2006) analyzed rheological properties and linked *Pinb-D1b* with higher dough extensibility than *Pina-D1b*, while Martin et al. (2001) showed better bread-making quality (higher loaf volume and better crumb) for *Pinb-D1b* than *Pina-D1b* too. These studies illustrate well the importance of *Pin-D1* genes not only for hardness but for processing and end-use quality.

Landraces and old cultivars possess variability for different genes that are not present in modern cultivars (Hammer et al., 1996) but could be useful to modern breeding programs generating materials with novel improved properties. In a previous study, a wide collection of bread wheat landraces from Andalusia were characterized for morphological characteristics and glutenin composition (HMWGs and LMWGs) (Ayala et al., 2016). Novel alleles for *Glu-A1*, *Glu-D1* and *Glu-B3* loci were detected, which indicated that this collection was diverse for quality traits and, therefore, could be a good source of novel alleles of other quality-related genes.

The aim of the current study was to evaluate the variability for puroindoline genes in a collection of common wheat landraces from Andalusia and to molecularly characterize the alleles found.

2. Material and methods

2.1. Plant materials

Forty-five accessions obtained from the Centro de Recursos Fitogenéticos INIA (Alcalá de Henares, Spain) and the National Small Grain Collection (Aberdeen, USA), were analyzed in this study. These accessions are landraces distributed throughout six of the eight provinces of Andalusia (Fig. 1). These materials were grown during 2012–13 in a 1-m, one-row plot of an un-replicated trial at the CIFA-IFAPA experimental station in Córdoba, Spain, using standard agronomic practices for the region (175 kg/ha N, 90 kg/ha P, and 90 kg/ha K).

2.2. Quality analysis

Quality analyses were performed using the methods established by the American Association of Cereal Chemists (AACC, 2000). Particle Size Index (PSI) (%) and grain protein content (%; 12.5 moisture basis) were estimated in whole grains using a near-infrared spectroscope NIR Systems 6500 (FOSS-Tecator, HillerØd, Denmark) previously calibrated for hardness by the Particle Size Index method (PSI; AACC method 55-30, 2000) and protein content by the Kjeldahl method (AACC method 46-11A, 2000). In PSI, soft wheat endosperm produces a higher proportion of fine particles which corresponds to higher percentages. Based on the predicted PSI data obtained by NIR, samples were classified as hard (30–44%), semi-hard (45–55%) and soft (>55%).

2.3. PCR amplification and sequencing of the Pina-D1 and Pinb-D1 genes

Genomic DNA was extracted from young leaves of single plants by the CTAB method (Stacey and Isaac, 1994).

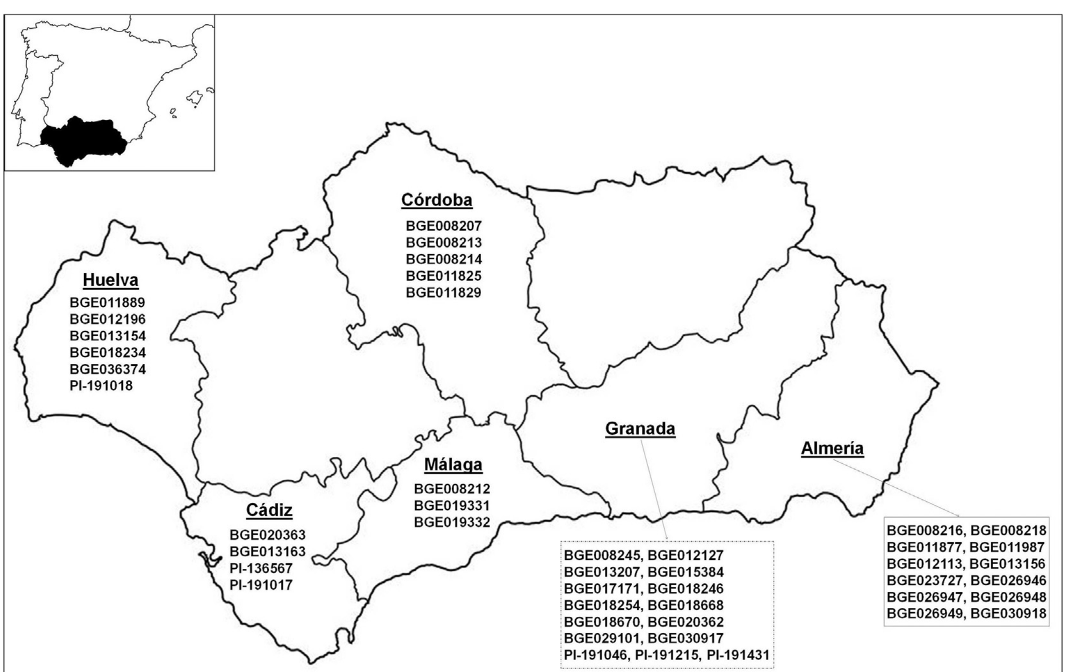

Fig. 1. Geographical origin of the bread wheat accessions evaluated in this study.