Short Communication

Unravelling the hidden inter and intra-varietal diversity of durum wheat commercial varieties used in Portugal

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Abstract
Assessing durum wheat genomic diversity is crucial in a changing environmental particularly in the Mediterranean region where it is largely used to produce pasta. Durum wheat varieties cultivated in Portugal and previously assessed regarding thermotolerance ability were screened for the variability of coding sequences associated with technological traits and repetitive sequences. As expected, reduced variability was observed regarding low molecular weight glutenin subunits (LMW-GS) but a specific LMW-GS allelic form associated with improved pasta-making characteristics was absent in one variety. Contrastingly, molecular markers targeting repetitive elements like microsatellites and retrotransposons – Inter Simple Sequence Repeat (ISSR) and Inter Retrotransposons Amplified Polymorphism (IRAP) – disclosed significant inter and intra-varietal diversity. This high level of polymorphism was revealed by the 20 distinct ISSR/IRAP concatenated profiles observed among the 23 individuals analysed. Interestingly, median joining networks and PCoA analysis grouped individuals of the same variety and clustered varieties accordingly with geographical origin. Globally, this work demonstrates that durum wheat breeding strategies induced selection pressure for some relevant coding sequences while maintaining high levels of genomic variability in non-coding regions enriched in repetitive sequences.

Keywords: genetic diversity, glutenins, microsatellites and retrotransposons

Introduction
Durum wheat (Triticum turgidum L.) represents the major ingredient of such an important food as pasta (Sissons, 2008). In the context of environmental changes, it is crucial to evaluate the genomic diversity of nowadays commercial varieties. Durum wheat varieties used in Portugal and previously assessed for heat stress response (Bento et al., 2017) were evaluated regarding the variability of coding sequences associated with technological traits and non-coding repetitive sequences.

Glutenins are polymeric gluten proteins determinant to dough elasticity (Sissons, 2008) among which low molecular weight glutenin subunits (LMW-GS) form intermolecular disulphide bonds creating polymers responsible for gluten structure and properties (D’Ovidio and Masi, 2004). Sequences coding for LMW-GS, a diverse protein family, were previously characterized in bread wheat (Zhang et al., 2011). LMW-1 and LMW-2 subunits were related to durum wheat gluten viscoelasticity, being LMW-2 associated with superior dough quality (Payne et al., 1984).

Genomic characterization using Inter Retrotransposons Amplified Polymorphism (IRAP) and Inter Simple Sequence Repeat (ISSR) techniques has been successfully used to identify several crop species and varieties (Smykal,
allelic form. The median joining network obtained from LMW-GS and LMW-1/LMW-2 concatenated data (Fig. 1 (a)) highlighted the low variability observed since besides Hélvio, which is the variety that accumulated more mutational steps, only variety DonDuro was distinguished. Low variability detected among coding regions in durum wheat varieties is not surprising and was previously reported in HMW-GS and LMW-GS composition of Spanish, Italian and Moroccan cultivars (De Vita et al., 2007; Subira et al., 2014; Henkrar et al., 2017). This lack of diversity regarding end-use traits can be a direct consequence of breeding programme strategies generally used (De Vita et al., 2007).

To further access genomic variability, we used ISSR and IRAP molecular markers targeting repetitive elements. These markers highlighted 57% polymorphic amplicons allowing the discrimination between varieties and even between individuals of the same variety (online Supplementary Tables S2 and S3). Even though the polymorphism level detected was lower than previously reported for durum wheat landraces using ISSR or IRAP (Pujar et al., 2002; Khan et al., 2015) it was significant considering only commercial varieties. Moreover, the separated characterization of multiple individuals of each variety, contrarily to previous studies (Pujar et al., 2002; Carvalho et al., 2008; Carvalho et al., 2012; Shirnasabian et al., 2014; Khan et al., 2015), allowed the determination of similarity coefficient values higher (0.60–0.92, online Supplementary Table S4) than those previously reported using ISSR in durum wheat landraces (Carvalho et al., 2009) or advanced genotypes (Zamanianfard et al., 2015). Additionally, these multiple individual analyses disclosed a much more obvious intravarietal diversity assessed through IRAP and ISSR in comparison with LMW.

Intravarietal diversity in repetitive genome regions was expected, but the ISSR/IRAP banding profiles and the presence of LMW-1 in one Marialva individual plant similar to Celta variety were not expected. Regarding the Marialva outlier genotype, we cannot completely discard the hypothesis of seed admixture, although all varieties were maintained by controlled self-fertilization during at least four consecutive years.

Importantly, combining ISSR and IRAP median joining network (Fig. (1b)) produced almost a unique profile for each individual (20 distinct profiles among 23 individuals). However, different individuals of the same variety were clustered, allowing inference of phylogenetic relationship between varieties. PCoA scatter plot (Fig. 2) with the first two principal components (51.9% variation) also grouped the first two principal components (51.9% variation) also grouped individuals by variety and separates varieties in three groups accordingly with their geographical origin.

Overall, our study revealed opposing scenarios coexisting in durum wheat genotypes regarding variability levels of coding sequences associated with important
technological traits and repetitive genome domains. The first, being a target of artificial selection, showed low variability in durum wheat commercial varieties, favouring the presence of particular alleles, while more labile and less scrutinized repetitive sequences retrieved high variability levels between and within the varieties studied.
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Supplementary material

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References


