

# Portuguese Triticum aestivum

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Bread wheat (*Triticum aestivum*) is a major crop worldwide, and it is highly susceptible to heat. Genetic diversity is scarce as regards heat tolerance, and remains a major concern for breeders in Mediterranean region. Grain traits were evaluated in Portuguese *Triticum aestivum* germplasm after plants exposure to heat during grain filling stage. Seven ancient landraces and traditional varieties from INIAV wheat collection, Gentil Rosso, Grécia, Mocho de Espiga Branca (MEB), Mocho de Espiga Quadrada (MEQ), Transmontano 94 (T94), Restauração and Ruivo, and two commercial varieties, Ardila and Nabão, were considered in the study. Plants were grown in a semi-controlled greenhouse. Heat increased test weight (TW), thousand-kernel weight (TKW), soluble sugars, protein and essential fatty acids (C18:1 and C18:2) in some of the genotypes. From PCA analysis of traits, one commercial (Ardila) and two landraces (Restauração and Russo) showed clustering in approximate positions no matter the treatment and seem to be less susceptible to heat. In contrast, one landrace (MEQ) seems more differentially affected and hence less adapted to high temperatures during grain filling. Physiological responses to heat, particularly their impact on grain filling and quality, may assist breeding design of heat-adapted wheat in the scenario of predicted climate changes.

bread wheat

grain filling

heat stress

nutritional and quality traits

## 1. Introduction

Bread is a staple food in many parts of the world. Eating bread helps consumers to reach their everyday needs of many nutrients. In Europe, America, and Australia, the average per capita consumption in 2019 stood at 30.3, 20.0, and 18.7 kg, respectively [1]. Bread not only provides energy (primarily through starch) but also contains protein, dietary fiber, and a wide array of vitamins and minerals [1]. Global warming is an unavoidable ongoing phenomenon [2] and bread wheat (*Triticum aestivum* L.) production is extremely susceptible to heat stress [3][4]. High temperature can alter biochemical, physiological, and morpho-anatomical behavior in wheat, restraining pollen viability, duration of grain filling, and starch accumulation in the endosperm [4][5]. Modern wheat varieties have been selected for higher yields and to allow efficient agricultural practices. Landraces are a valuable source of genetic variability for breeding as regards adaptation to stresses and improved quality features.

## 2. Evaluation of Grain Nutritional and Quality Traits

Flour yield was evaluated through test weight (TW), that indirectly expresses quality of grain, giving information on intrinsic features related to milling—namely grain shape, texture of seed coat (integument), grain size, and weight [6]. High TW values correspond to a greater market acceptance and value [7]. This indicator of flour-yield potential is

greatly determined by high temperature during the grain filling. Under non-stress conditions, germplasm showed some variability in TW, but only Ardila presented values in the range of heavy wheat (ca. 78 Kg/hL) according to [8]. Despite a heat-induced TW decrease, Ardila remained amongst the genotypes presenting the highest values under stress. Nabão, Grécia, and Restauração presented higher TW with heat, suggesting an improved flour-yield potential of these varieties under high temperature.

Thousand-kernel weight (TKW) is mainly related to the size and density of the grain, but it also depends on the variety and environmental conditions (filling and maturation of the grain) [9]. Smaller grain size (lower TKW) may occur concomitantly to unaltered TW, suggesting the formation of well-shaped kernels and preservation of grain filling, which was the case in MEQ. In terms of the milling industry, the sieving of smaller grains implies larger grain waste, and may result in flour-yield reductions [6]. MEB showed considerably higher TKW under heat, as well as T94. Results for higher TW and TKW are consistent with increased grain production (g/plant), previously reported under high temperature [10]. Grain filling preservation during heat stress depends on photo-assimilate and stored carbohydrate reserves in vegetative organs, which can be remobilized into the grains, as suggested by others [3] [11]. Grain-yield reduction under heat may derive from inhibition of starch synthase, decreasing the conversion of sucrose into starch, or enhanced respiration leading to lowering of grain sugars and other stored compounds [3] [4] [12] [13] [14].

According to [15], concentrations of total sugars during grain filling were not significantly affected by high temperatures in wheat. However, some intraspecific genetic variability in levels of reducing sugars may indicate their involvement in tolerance signaling, possibly developmentally regulated, modulated by the sugar pool [6]. In this work, sucrose values presented some variation among genotypes, and also as a result of postanthesis heat. Heat induced an increase of total soluble sugars in Gentil Rosso, mainly due to augmented sucrose. MEQ variety presented the highest values of the most abundant sugars (sucrose, raffinose, and stachyose) in control and heat-stress plants, despite heat-induced total sugars decreasing. Nonstarch endogenous sugars may have beneficial effects for the bread-making industry, contributing as substrate in the yeast fermentation process [16].

Nitrogen accumulation in the grain is less negatively affected by high temperature than carbohydrate accumulation, because most plant nitrogen uptake generally occurs before anthesis [17]. Breeding programs are promoting the increase of protein content in wheat to satisfy the needs of the industry and the consumers [18]. Genotypes under study presented some diversity in protein content in control conditions, which varied between 20% (T94) and 17% (Nabão). Under heat, Ardila stood out for its heat-induced protein increase. In *T. aestivum*, bread-making quality depends on the amount and composition of gluten proteins in the endosperm, which determine the viscoelasticity properties of dough, evaluated through SDS [7]. This quality feature can be affected by high temperatures during grain filling [4] [15]. In this work, high SDS values (>45 mm) found in control plants of Gentil Rosso, Restauração, Nabão and MEB, indicate a higher gluten strength and potentially more suitable flours for bread-making purposes. SDS was unaltered by heat in most genotypes denoting unaffected gluten strength.

Color is an important rheological feature for assessing the quality of wheat grains or flour, reflecting the content of carotenoids, proteins, fibers, and the presence of impurities in the grind [19] [18]. It may be used to match specific

industry requirements related to grain end-use products, such as bread-making or pasta industry. Little variation was found between genotypes regarding ash content or color parameters.

Wheat kernels also contain relatively small quantities of lipids, usually from 2 to 3.5%, which nevertheless play an important role in cereal processing by affecting the properties of protein and starch [1][20]. The level and composition of grain lipids depend on the growing environment and the genetic background of the wheat, among other factors [1]. Under control conditions, genotype variability was present in total fatty acids (TFA) amounts, which express lipids content. The highest values (14–15 mg g<sup>-1</sup> DW) were observed in T94, Ruivo, and Ardila. Endogenous nonstarch lipids greatly affect fresh bread quality, being key players in bread leavening and crumb structure [21]. In this work lipid content under heat was unaltered in all genotypes except Ardila, that showed a TFA decrease. Results highlighted increased relative abundance of polyunsaturated C18:2 and monounsaturated C18:1 in Grécia under heat. Wheat grains are valuable sources of essential FAs (linoleate and α-linolenate), that are fundamental in human nutrition. A high proportion of unsaturated FAs compared to saturated ones in food protects against obesity, cardiovascular diseases, and inflammatory processes [1][22]. Variation in lipids and FAs in Italian durum wheat cultivars was used to set quality standards for wholegrain flours and products where the germ should be preserved, considering also the recent interest of industry and consumers for such foods [23].

The major lipids in wheat kernels are non starch endosperm lipids, consisting of nonpolar triacylglycerols, typically found in oil bodies or spherosomes in the germ and aleurone tissues. Starch lipids are located inside the starch granules and consist almost exclusively of phospholipids [24]. Lipids are unevenly distributed over the different structural parts of the grain. About 35% to 45% occur in the endosperm, 30% to 36% in the germ, 25% to 29% in the aleurone, and less than 4% in the pericarp [1]. Upon wheat milling into flour, kernels and kernel pieces are broken, further reduced in size and sieved to separate the endosperm from the bran (i.e., pericarp and aleurone) and the germ [24]. Lipids in the resultant flour include those of endosperm, but also a portion of those of aleurone and germ. In wholemeal flour, lipases are more abundant due to their presence in seed coat (integument), increasing the risk of lipoperoxidation [1][25]. In the germplasm under study overall lipid unsaturation, expressed as DBI index, was maintained under stress which could constitute a beneficial feature to reduce lipoperoxidation, that can lead to off-flavors during storage [25].

A reduction in unsaturated fatty acids was associated with selection during primary *Triticum* domestication [26][27]. Wheat-flour lipids indeed play important roles during bread making, and therefore, have great potential as targets for improving bread quality [1].

### 3. Main conclusions

The need to increase wheat productivity and quality in warmer climates is a main point for breeders in Mediterranean region, but genetic diversity for heat tolerance is scarce. This work was undertaken to evaluate nutritional and quality traits of Portuguese bread wheat germplasm, including ancient landraces and commercial varieties, under high temperatures conditions after anthesis (filling stage), which frequently occur in their natural environment. Results highlighted some variability in germplasm as regards grain nutritional and quality features

both under control and heat conditions. Several traits were negatively affected, while others were unaltered or even improved under stress. Cluster analysis of traits indicated a lower susceptibility to heat in Ardila, Restauração and Ruivo, reflecting a better adaptation to high temperatures in contrast to MEQ, that seems to be more differentially affected during grain filling.

Identification of genotypes with favourable features in response to stress may contribute to select parental lines for crossings and to obtain new varieties more adapted to changing climate, a major aim of Portuguese Cereal Breeding Program. Genotypes presenting interesting nutritional traits such as protein, sugars and FAs, might be further explored as a potential source of health-beneficial food products viewing new markets emerging from integrated farming systems.

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