INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

A novel approach for wheat germplasm evaluation: bridging high temperature tolerance and grain quality

Diana Raquel dos Santos Tomás

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE (PhD) IN BIOLOGY

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À minha filha
Aos meus avós
Ao homem da minha vida

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Abstract

Wheat is an essential crop for food and feed, due to its nutritional value and unique aptitude to produce gluten and their derived food products. It is thus essential to understand how increasingly common extreme weather events like heatwaves, defined as short periods of high temperatures (HT), affect wheat grain production and quality, and transcriptomic modulation. Thus, in order to identify wheat varieties with increased tolerance to HT, the objective of this work focused on the characterization of the already referred parameters in plants of several commercial varieties recommended to be used nowadays in Portugal and traditional varieties, submitted to high temperatures during grain filling.

Using molecular markers, we showed that commercial genotypes have predicted good grain technological quality, based on the allelic composition of genes related with grain composition. Most commercial and traditional genotypes showed negative effects, induced by heatwave-like treatment, revealed by a decrease in grain number and weight, while protein content was increased. Also, through attenuated total reflection Fourier transform infrared (ATR-FTIR) analysis, we denoted the occurrence of alterations in grain polysaccharide composition induced by HT. Additionally, HT increased protein content variability in landraces and reduced it on commercial varieties.

Regarding transcriptomic profiles assessed immediately after the HT treatment, traditional varieties revealed a significantly higher number of differentially expressed genes (DEGs), that include genes coding for heat shock proteins and cupins, and more similar HT responses than commercial varieties. Furthermore, Bancal and landraces DEGs appear to be more associated with several metabolic pathways, while in Antequera DEGs were preferentially related with transcription modulation and RNA and protein synthesis.

Keywords: bread wheat, genetic diversity, heatwave, grain composition, grain transcriptomics

Resumo

O trigo é uma cultura com relevância na alimentação humana e animal devido ao seu valor nutricional e à capacidade de produção de glúten e alimentos derivados do mesmo. Considera-se essencial compreender como eventos climáticos extremos, tais como ondas de calor definidas como períodos curtos de altas temperaturas (AT), influenciam a produção e qualidade do grão e a modulação da transcrição. Assim, de forma a identificar variedades de trigo com maior tolerância às AT, o objetivo deste trabalho focou-se na caracterização dos referidos parâmetros em plantas de variedades comerciais presentemente recomendadas para uso em Portugal e de variedades tradicionais, submetidas a AT durante o enchimento do grão.

Através de marcadores moleculares para alelos específicos de genes relacionados com a qualidade dos grãos, foi possível inferir que as variedades comerciais apresentam características de boa qualidade tecnológica. Comprovou-se igualmente que a maioria das variedades comerciais e tradicionais são afetadas pelas AT, apresentando uma diminuição do número e peso de grãos associado a um aumento do teor proteico. Através da análise de espectroscopia no infravermelho por transformada de Fourier com reflectância total atenuada (ATR-FTIR), detetaram-se alterações na composição em polissacarídeos dos grãos. Adicionalmente verificou-se um aumento da variabilidade do conteúdo proteico em variedades tradicionais e uma redução dessa variabilidade em variedades comerciais, em resposta às AT.

Paralelamente, a análise dos perfis transcriptómicos logo após o tratamento de AT, revelou nas variedades tradicionais um número superior de genes diferencialmente expressos (GDEs), sendo de se realçar genes que codificam proteínas de choque térmico e de cupinas, e respostas mais semelhantes à AT em relação ao observado nas variedades comerciais. Para além disso, os GDEs em Bancal e nas variedades tradicionais parecem estar mais associados a diferentes vias metabólicas, enquanto em Antequera perecem estar preferencialmente relacionados com a modulação da transcrição e a síntese de RNA e proteínas.

Palavras-chave: trigo mole, diversidade genética, onda de calor, composição do grão, transcriptómica do grão

Resumo Alargado

O trigo mole é um dos cereais mais produzidos no mundo, sendo a cultura mais relevante em regiões temperadas. Esta cultura assume um importante papel na alimentação humana pelo seu elevado valor nutricional, fornecendo cerca de 20% da proteína e hidratos de carbono consumidos diariamente pela população. Para além disso, dadas as caraterísticas tecnológicas, o trigo mole permite a produção de múltiplos alimentos como o pão, bolos e bolachas, amplamente consumidos em todo o mundo.

Na zona Mediterrânica, as alterações climáticas têm sido responsáveis pelo aumento da temperatura média afetando as fases mais sensíveis do desenvolvimento das plantas nomeadamente desde a floração até à maturação do grão, com relevante impacto na produção e na qualidade do grão. Neste contexto, são particularmente prejudiciais eventos extremos como as ondas de calor - períodos curtos com altas temperaturas - cuja frequência se prevê que venha a aumentar.

Os processos de melhoramento associados ao cultivo do trigo fomentaram a utilização de um limitado número de genótipos, causando um fenómeno de erosão genética e uma perda de heterozigocidade, pelo que as variedades tradicionais ou landraces assumem especial importância devido à elevada diversidade genética existente que poderá estar até associada a melhores características nutricionais e a maior tolerância a stresses bióticos e abióticos.

O objectivo deste trabalho focou-se no estudo dos efeitos de altas temperaturas (AT) em parâmetros de produtividade e qualidade de trigo mole, avaliando as respostas de plantas de variedades comerciais recomendadas para utilização em Portugal e de variedades tradicionais provenientes da coleção estabelecida em 1933 pelo Professor Vasconcellos a tratamentos simulando ondas de calor, durante sete dias consecutivos com uma temperatura máxima de 40ºC.

Inicialmente procedeu-se à utilização de marcadores moleculares associados a características do grão essenciais para a definição da qualidade tecnológica tais como a qualidade do glúten, a quantidade de amilose e a dureza do grão, através da identificação de alelos específicos de genes codificantes de *High Molecular Weight (HMW) Glutenins*, *Granule Bound Starch Synthase* e Puroindolinas Em todas as variedades comerciais detetou-se a presença de alelos associados a boa qualidade tecnológica.

Nas plantas desenvolvidas em condições controlo observou-se uma marcante diversidade intervarietal em relação ao rendimento, tendo como base características fenotípicas tais como o número e o peso de grãos. Nas variedades comerciais, a exposição das plantas ao tratamento da AT induziu respostas distintas, apesar de se verificar uma tendência geral de redução no número e peso dos grãos, excetuando-se a ausência de alterações significativas em Bancal e o aumento de ambos os parâmetros em Pata Negra. Em relação às variedades tradicionais, verificou-se uma grande diversidade nos parâmetros fenotípicos, destacando-se a variedade Ardito que apresenta diversas características semelhantes às das variedades comerciais, como a altura da planta e a data de floração precoce. A avaliação dos parâmetros de plantas tratadas com AT demonstrou uma redução

significativa do peso de grão e uma maior diversidade intervarietal em relação à altura e área das plantas, bem como ao número de espigas por planta, sendo de salientar o maior crescimento vegetativo e o aumento do número de espigas por planta observado na variedade Magueija.

Para a avaliação qualitativa dos efeitos do tratamento de AT na composição do grão utilizou-se a técnica de Espectroscopia no infravermelho por transformada de Fourier com Reflectância Total Atenuada (ATR-FTIR). Esta técnica revelou-se muito vantajosa, pela forma rápida e não destrutiva associada ainda à possibilidade de análise de reduzidas quantidades de farinha. Consonantes com os resultados de rendimento, as respostas das variedades comerciais às AT foram distintas, observando-se aumentos ou reduções de intensidade de bandas do espectro atribuídas a grupos químicos relacionados com proteínas, amido e gorduras. Paralelamente, observaram-se espectros mais intensos na farinha de grãos provenientes de plantas das variedades tradicionais submetidas a AT relativamente a amostras de grão controlo, podendo então sugerir-se um aumento de todos os componentes do grão em detrimento do amido. No entanto, é de se realçar que tanto nas variedades comerciais como nas tradicionais, se identificaram alterações nos padrões dos espectros obtidos, indicadoras da ocorrência de variações quantitativas e qualitativas na composição do grão. Dada a importância nutricional do teor proteico do grão de trigo, estabeleceu-se um modelo de predição dos valores de azoto com base nos espectros obtidos por ATR-FTIR. Esta quantificação permitiu confirmar os resultados obtidos através dos espectros, revelando na maioria das variedades uma redução no teor proteico dos grãos induzida pelo tratamento com AT. Através da análise das diferentes frações proteicas do grão, detetaram-se também em algumas variedades alterações ao nível da razão gliadinas/gluteninas, relevante na determinação de características tecnológicas.

Tendo presente que os mecanismos envolvidos na tolerância das plantas a altas temperaturas são regulados por diversos genes que envolvem distintas vias de sinalização procedeu-se à análise do transcriptoma global em quatro variedades. Estas foram selecionadas através das alterações fenotípicas contrastantes apresentadas após os tratamentos com AT, nomeadamente em relação ao rendimento e qualidade do grão. Assim, das variedades comerciais foram selecionadas Antequera e Bancal, tendo presente que a primeira demonstrou boas características de qualidade do grão, embora o tratamento de AT tenha induzido uma redução no número, no peso e no teor em proteína dos grãos, contrastando deste modo com a variedade Bancal que não apresentou alterações significativas após o tratamento com AT. Relativamente às variedades tradicionais, selecionaram-se Ardito e Magueija pois a primeira apresenta características fenotípicas semelhantes às variedades comerciais, como altura da planta e floração precoce, bem como teores de proteína elevados tanto em condições controlo como após o tratamento de AT, e Magueija, como uma variedade em que a AT induz um aumento de crescimento vegetativo e conteúdo proteico do grão, e na qual os valores de peso de grãos se mantêm mais elevados após tratamento de AT que nas restantes variedades, ainda que inferiores aos obtidos em condições controlo.

A análise de transcriptomas de grãos imaturos de plantas controlo e de plantas tratadas logo após o final da última exposição a AT, permitiu a identificação de genes diferencialmente expressos (GDEs) em cada uma das variedades estudadas. Assim, verificou-se que após o tratamento com AT as variedades tradicionais apresentam uma menor diversidade intervarietal ao contrário do observado nas variedades comercias. Globalmente, identificou-se um maior número de GDEs associados a respostas a altas temperaturas e a vias metabólicas de síntese de hidratos de carbono, aminoácidos e lípidos, embora Antequera apresente preferencialmente GDEs envolvidos na modulação da síntese proteica. Paralelamente, observou-se também um efeito do tratamento de AT na alteração da expressão de genes codificantes de enzimas envolvidas na síntese de compostos determinantes da qualidade do grão, nomeadamente amilopectina, gliadinas e cupinas. Por último, é interessante realçar o reduzido número de GDEs detetado na variedade comercial Bancal, potencialmente indicador de uma maior tolerância a condições de alta temperatura, tendo igualmente presente a maior estabilidade fenotípica identificada em relação ao rendimento e à qualidade do grão após a exposição das plantas ao tratamento com AT.

As AT estão descritas como prejudiciais para características de rendimento e qualidade do grão de trigo, tornando-se por isso essencial a procura de variedades com distintas capacidades de lidar com as alterações induzidas por estas condições restritivas. É igualmente fundamental desenvolver estudos de transcriptómica que permitam compreender os mecanismos moleculares responsáveis por essas características diferenciadoras. Assim, parâmetros como peso do grão, conteúdo de proteína e perfis de transcrição de genes de resposta ao calor detetados nos genótipos tradicionais estudados preconizam a necessidade de estender a avaliação realizada às restantes variedades tradicionais da coleção Vasconcellos. Adicionalmente, os estudos efetuados sugerem a variedade comercial Bancal como um genótipo promissor no contexto do aquecimento global.

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Abbreviations

AGPase - ADP-glucose pyrophosphorylase

ANOVA Analysis of Variance

AT - Altas temperaturas

ATR-FTIR - Attenuated total reflection Fourier transform infrared

Bp - Base pairs

C – Control

cDNA – Complementary DNA

cm - Centimeter

CRISPR/Cas9 - Clustered Regularly Interspersed Short Palindromic Repeats

Ct - Threshold cycles

Daa - Days after anthesis

DBE - Starch-debranching enzyme

DEGs - Differentially expressed genes

DNA - *Deoxyribonucleic acid*

dNTP - Deoxyribonucleotide TriPhosphate

Dpa - Days post anthesis

DTT - Dithiothreitol

G, mg μg- Grams, milligrams, micrograms

GBSSI - Granule-bound starch synthase I

GEDs - Genes diferencialmente expressos

GO - Gene enrichment

Gsp-1 - Grain Softness Protein

h, min - Hour, Minute

Ha - Hardness locus

HMW-GS – High Molecular weight - glutenin subunit

Hsf - Heat shock factors

HSP - Heat shock protein

HT – High temperature

KEEG - Kyoto Encyclopedia of Genes and Genomes

L, ml, μL—Liter, Milliliter, Microliter

LMW-GS - Low Molecular weight – glutenin subunit

MgCl - Magnesium chloride

M, mM, µM -Molar, Millimolar. Micromolar

N - Nitrogen

ºC - degree Celsius

p - p value

padj - p value adjusted

PCA - *Principal Component Analysis*

PCR - Polymerase Chain Reaction

Pina - Puroindoline a

Pinb - Puroindoline b

PLS - Partial least squares

QTL - Quantitative trait *loci*

R^2 - Coefficient of determination

RMSECV - Minimum root-mean-square error of cross-validation

RMSEP - Random mean square error of prediction

RNA - Ribonucleic *acid*

RPD - Ratio of performance to deviation

RP-HPLC - Reversed-phase high-performance liquid chromatography

RT- qPCR – Quantitative *reverse transcription* PCR

SBE - Starch-branching enzyme

SDS-PAGE - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SNP - Single nucleotide polymorphism

SS - Starch synthase

SSS - Soluble starch synthase

WT - Wildtype

wx - Waxy *loci*

Chapter I

General Introduction

1 Introduction

1.1 Wheat relevance for food and feed and the global warming risks

Wheat is the third most produced and consumed cereal worldwide (©FAO, 2018a, 2018b). The two main species for food chains are durum wheat (*Triticum turgidum* spp. *durum*), a tetraploid species (2n = 28), and bread wheat (*T. aestivum* L.), an hexaploid species (2n = 42). Bread wheat is approximately responsible for 90-95% of worldwide wheat production, as well as in Portugal, where wheat is the third most produced cereal, after maize and rice (INE, 2019). The three largest wheat-producing countries in the world are China, India and Russia (Shewry and Hey, 2015).

In temperate countries wheat is a dominant crop, being used for human food and livestock feed, mainly for two reasons. The first is its great nutritional value, contributing for human diet with about 20% of energy intake of carbohydrates, an average of 20% of protein daily consumption, as well as fiber and minor components including lipids, vitamins, minerals, and phytochemicals which contribute to a healthy diet (Shewry and Hey, 2015). Moreover, for feed wheat is also relevant since metabolizable energy per unit of dry matter is similar to corn, and higher than other major grains (reviewed in Yang and Shen, 2018). The second main reason of wheat importance as food is the fact that it possesses unique technological properties for the production of food goods like breads cakes and biscuits, breakfast cereals, pasta and noodles. In Portugal wheat is the main source of carbohydrates and the second one for protein diary intake (INE, 2016).

Besides the current trends in population growth and consumption patterns, global warming constitutes a threat to wheat production (Ray et al., 2013; Gaupp et al., 2019). Global warming is characterized by shifts in weather with an increase of the frequency and magnitude of extreme events (Semenov and Shewry, 2011). Modeling studies predict that warming will affect yield gains on the majority of wheat-growing regions, leading to a reduction on global wheat production estimated as 6% for an increase of 1°C, becoming more variable over space and time (Semenov and Shewry, 2011; Asseng et al., 2014; Liu et al., 2016).

Climate changes already affect wheat productivity (kg/ha) in Portugal (Olesen et al., 2011) where a substantial decrease has been observed in the last years (INE, 2019). Particular extreme high temperature events as heatwaves, defined by World Meteorological Organization (2015) as five or more consecutive days with daily maximum temperatures at least 5°C higher than the average maximum temperature, are foreseen to be intensified onward especially in Portugal (Cardoso et al., 2019). Since heatwaves, have detrimental effects in wheat development and yield (Nuttall et al., 2018) it is urgent to evaluate their potential effects in distinct wheat

genotypes. This project is therefore focused on the study of commercial varieties used in Portugal as well as traditional landraces to identify genotypes with superior grain quality and enhanced heat stress tolerance more able to face predicted global warming.

The selection of stress tolerant varieties with increased quality and productivity is extremely important taking into consideration predicted climate alterations and the huge food and feed national demand.

1.2 Wheat grain development, yield and composition

Wheat is an annual plant which development is subdivided in ten phases by Zadoks decimal growth scale: germination, seedling growth, tillering, stem elongation, booting, head emergence, anthesis (flower development), milk development, dough development and ripening (Zadoks et al., 1974). Wheat grain development begins following anthesis and is divided in three distinct phases: grain enlargement (0-14 days post anthesis - dpa, Zadok's scale: Z69-Z75); grain filling (15-35 dpa, Z75-Z87); and physiological maturity (36-50 dpa, Z87-Z92). The duration of each phase varies with the genotype and the environmental conditions, namely temperature and water and nitrogen supply (Bowden et al., 2007). For the majority of wheat development stages, optimal temperature is between 20 and 22 ºC, although this species has the ability to grow in a broad temperature range as the upper and lower limits of temperature lethality are 47.5 ± 0.5 ◦C and −17 ± 1.2 ◦C, respectively (Porter and Gawith, 1999).

One of the most important wheat traits is grain yield which results from the interaction of several factors as plant height, total biomass, number of productive tillers, grain number per spike, spike length, grain number and weight per spike, thousand grains weight and harvest index, and physiological parameters as canopy temperature, chlorophyll content, photosynthetic rate and water-soluble carbohydrates (reviewed in Tshikunde et al., 2019).

In the Green Revolution period (from 1940s to late 1960s), the utilization of natural semi-dwarf genotypes led to a significant increase in cereal production, due to mutations in the *Reduced height-1 (Rht1*) gene, affecting gibberellin signaling and metabolism (reviewed in Hedden, 2003). Nowadays, improving wheat yields is again a priority as global production needs can double by 2050 to meet the demands of predicted population growth and changes in consumption patterns (Ray et al., 2013).

Genetic gains in grain yield are likely to be obtained through the search of important yield-related agronomic and physiological traits in genotypes collections, for further introduction in breeding programs. Although several works are presently oriented to predict yield genotype performance under different conditions (He et al., 2016; Huang et al., 2018; Juliana et al., 2018), the complex inheritance of so many traits with gene expressions modulated by environmental interactions does hamper genotypes selection.

Michel *et al.* (2019) combined genomic selection for both yield and baking quality showing that protein quality has a much lower trade-off with grain yield than protein content, suggesting that a simultaneous improvement in baking quality and grain yield is feasible. Also, Kumar et al. (2019) identified 43 genomic regions related with three quality traits (flour extraction, grain protein content and grain hardness), three yield traits (grain yield, grains per spike and spikes per square meter), and two agronomic traits (days to heading and plant height). Modeling studies also highlighted relevant key traits, related with canopy structure and phenology, root water intake and drought tolerance, to improve wheat yield potential under predicted climatic changes (Semenov and Halford, 2009; Senapati and Semenov, 2020; Senapati et al., 2020).

To better understand wheat yield it is also important to have present the constitution of wheat grain caryopsis, with fruit and seed coats (pericarp and testa, respectively) surrounding the embryo also referred as germ and the endosperm. At physiological maturity, bread wheat grain has a length of about 5 mm with an oval shape with embryo positioned at one extremity, and a bundle of hairs, usually referred as *brush*, at the opposite one.

Wheat grain embryo represents 2-3% of seed dry matter, (Šramková et al., 2009) containing 26%-35% of proteins, 17% of sugars, 10%-15% of lipids, 1.5-4.5% of dietary fibers, 4% of minerals, besides other bioactive compounds, and is removed prior to milling due to its susceptibility to oxidation and unfavorable baking properties. Due to high percentage of lipids, wheat germ is a suitable raw material for oil production (Brandolini and Hidalgo, 2012). Most of the seed dry matter corresponds to the endosperm (80-85%) which comprises two tissues: the central starchy endosperm and the outer aleurone layer. Starchy endosperm is the major grain tissue and is rich in starch (60-70%) and storage proteins (10-15%). On the other hand, aleurone is represented by a single layer of cells with high content of dietary fibers, minerals, vitamins, phytochemicals, storage lipids and globulin proteins (González-Thuillier et al., 2015). Wheat bran, mainly composed by fibers (around 50%), carbohydrates and proteins (around 16% each) and a great percentage of minerals, corresponds to 13-17% of global seed dry matter. The embryo and bran are removed during milling, leaving the starchy endosperm as the principal contributor to white flour, in opposition to wholemeal flour, resulting from whole grain milling (Šramková et al., 2009).

High temperature effects on wheat plants differ significantly with temperature intensity and exposure duration, the genotype and the growth stages facing the stress (Balla et al., 2019). Some high temperature effects in plant morphology and growth are common to several genotypes, with impairing in seed germination and emergence, accelerated plant development, leaf senescence and abscission and reduced biomass (Rahman et al., 2009; Essemine et al., 2010; Balla et al., 2019). Also, physiological responses to thermal stress were reported, as plant dehydration and respiration rate increase, reduction of photosynthetic capacity (Almeselmani et al., 2011),

alteration in hormone proportions and production of oxidative reactive species. Additionally, the synthesis of heat shock proteins and accumulation of osmoprotectants and solutes (reviewed in Akter and Islam, 2017) were also observed.

Heat stress has higher detrimental effects when is imposed during the reproductive phase as it can cause severe grain yield and quality losses, mainly because it reduces grain number and shortens the duration of grain-filling (Altenbach et al., 2003; Dupont and Altenbach, 2003). The required optimum temperature for wheat anthesis and grain filling is from 12 to 22 ºC (Tewolde et al., 2006) and each degree Celsius increase reduces wheat yield by 4.1% to 6.4% (Asseng et al., 2014; Liu et al., 2016). Temperatures above 20 °C between spike initiation and anthesis speed up the development of the spike but reduce the number of spikelets and grains per spike. These effects are related with the decrease of florets differentiation period in the double ridge stage, and higher temperatures may cause complete sterility, as viability of anthers and pollen are affected as well as ovary growth (Prasad and Djanaguiraman, 2014). Additionally, recently formed kernels abortion, related with insufficient carbohydrates supply, was reported (Yang et al., 2002).

1.3 Wheat grain quality parameters and high temperature effects

Wheat quality is mainly related with protein and starch content, which are differentially affected by high temperatures. Seed storage proteins are responsible for gluten strength and grain hardness which are essential characteristics for milling and baking quality of wheat.

1.3.1 Grain proteins fractions

Protein is one of the most important nutrients for human and animal diets. Wheat grain protein generally vary between 10% - 15% of total seed dry matter, and its nutritional value is based on the balance of essential amino acids present in wheat flours (wholemeal and white flour) (Shewry and Hey, 2015). Since protein content is a key parameter on market grading and classification several studies have been using traditional varieties and wild relatives to develop wheat cultivars with higher- contents (Uauy et al., 2006; Fahima and Distelfeld, 2008). Besides its contents, the type of wheat flour protein fractions is also known to be crucial in relation to breadmaking quality. Protein fractions were initially classified accordingly to their solubility by Osborn (1924) in four major groups: water-soluble albumins; salt-soluble globulins; and prolamins group composed by the alcohol-soluble gliadins and alcohol-insoluble glutenins. Later on, amino acid sequences analysis of prolamin groups representatives lead to a redefinition of their classification based on structural and evolutionary relationships, dividing the prolamins into

three distinct groups of proteins, namely the S-rich, S-poor and HMW prolamin fractions (Shewry et al., 1986).

Non-gluten proteins, albumin, and globulin, which account for approximately 20% of total wheat flour proteins, have also a great nutritional value due to higher lysine and methionine contents as compared to the other protein fractions (Shewry and Hey, 2015). The most common albumins and globulin proteins are α-amylase/trypsin, serpins and purothionins which have dual roles as nutrient reserves, during seed the germination, and as inhibitors of insects and fungal pathogens prior to germination (reviewed in Dupont and Altenbach, 2003). A more recent study mapped albumin + globulin fraction of recombinant inbread lines of bread wheat and successfully identified distinct families of Heat Shock Proteins, beta-amylases, UDP-glucose pyrophosphorylases, peroxydases and thioredoxins (Merlino et al., 2009).

Glutenins and gliadins have been intensively studied as they confer viscoelasticity to doughs (reviewed in Goutam et al., 2013). When hydrated, gliadins contribute mainly to the viscosity and extensibility of dough, and glutenins are mainly cohesive and elastic and responsible for dough strength (Wieser, 2007). Together both proteins form a heat resistant network, called gluten, with the capacity to retain gas during bread leavening process. The ratio between these two fractions is associated with parameters like dough resistance and loaf volume, considered essential for breadmaking quality (Shewry, 2002). Gluten is also important for a range of other uses including making unleavened breads, cakes, biscuits and noodles, and as a binder in processed foods (Shewry et al., 2009; Kucek et al., 2015).

Accordingly to their molecular weight, glutenins are classified as high molecular weight (HMW) and low molecular weight (LMW) and although HMW glutenins are less abundant (around 12% of total seed storage proteins), they are determinant for gluten strength and good bread making performance (reviewed in Shewry et al., 2003).

Hexaploid bread wheat has six HMW subunits encoded by genes located at *Glu-1 loci* on the long arms of the group 1 chromosomes (1A, 1B, 1D). Each *locus* encodes two subunits of x-type (larger) and y-type (smaller) originating 1Ax, 1Ay; 1Bx, 1By and 1Dx, 1Dy. Subunits 1Bx, 1Dx and 1Dy are present in most bread wheat cultivars while 1By and 1Ax are only present in some wheat cultivars and the gene coding 1Ay generally remains silent in most bread wheat cultivars (Halford et al., 1989). Each subunit exhibits allelic variation and the presence of different alleles have different impacts on dough characteristics (Payne et al., 1987). It is generally agreed that *Glu-D1* has more significant effects on processing flour quality than *Glu-A1* and *Glu-B1* (reviewed in Rasheed et al., 2014). The *Glu-D1d* allele (Dx5 and Dy10 subunits pair), *Glu-A1a* and *Glu-A1b* alleles (Ax1 and Ax2* subunits) and *Glu-B1b*, *Glu-B1c* and *Glu-B1i* alleles (that encode for the Bx7 + By8, Bx7 + By9, Bx17 + By18 subunits pairs, respectively) represent the combinations previously associated with stronger dough and superior end-use quality (Payne et al., 1987).

LMW glutenins represent about one third of total storage proteins and initially were considered as only encoded by genes located on *Glu-A3*, *Glu-B3* and *Glu-D3 loci* on the short arms of chromosomes 1A, 1B and 1D, respectively, although it is presently known that three other *loci Glu-2*, *Glu-4* and *Glu-5* located on chromosomes 1B, 1D and 7D, are also involved (reviewed in Rasheed et al., 2014). A classification based on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) mobility classified LMW-GS in three types: B, C and D (D'Ovidio and Masci, 2004), with the B type further divided into three classes, LMW-m, LMW-s and LMW-i, based on the first amino acid residue being methionine, serine and isoleucine, respectively (Muccilli et al., 2010). The characterization of allelic variation for LMW-GS among cultivars and its relationship with end-use quality has been a key area of research on wheat quality improvement. *Glu-B3* plays more significant roles in dough properties than *Glu-A3* or *Glu-D3* being *Glu-A3d* and *Glu-B3d* considered associated with better quality (He et al., 2005). The LMW-GS are difficult to identify because of their complexity, heterogeneity, and similarity to each other and to some gliadin components. The identification of LMW alleles is several times inferred through the characterization of gliadin alleles due to genetic linkage as for instance *Glu-3 loci* have a close genetic linkage to the *Gli-1 locus* (Rasheed et al., 2014).

Gliadins are monomeric proteins with impacts both on processing and nutritional quality although less significant than HMW- and LMW-GS glutenins. Gliadins are classified as α-, β-, γ- and ω-gliadins, according to their electrophoretic mobility in SDS-PAGE, with the first two being encoded by *Gli-2 locus* on the short arms of 6A, 6B and 6D chromosomes and the last two encoded by *Gli-1 locus* on the short arms of group 1A, 1B and 1D chromosomes. Cysteine residues in gliadins facilitate interchain cross links with glutenins, influencing flour characteristics (reviewed in Rasheed et al., 2014). In a recent study of a set of 1060 cultivars and lines bred in the last century, 182 alleles of Gli *loci* were revealed, and the polymorphisms detected demonstrated that common wheat germplasm is differentiated and structured by country or region and cultivar type (Metakovsky et al., 2018).

Characterization of wheat cultivars regarding gluten proteins is very important since it is well established that glutenin and gliadin allelic composition of each genotype is directly related with different technological characteristics of distinct flours characteristics (Gupta and MacRitchie, 1994; Gupta et al., 1994; D'Ovidio et al., 1997; D'Ovidio and Masci, 2004; Hu et al., 2013). SDS-PAGE, Reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis are used in several works to discriminate gluten proteins (reviewed in Rasheed et al., 2014). However, these methodologies are costly, have low discrimination ability of some proteins, and are not suited for large amounts of samples due to quite time-consuming procedures (reviewed in Goutam et al., 2013). Functional molecular markers based on alleles polymorphisms of nucleotide sequences are therefore usually used to identify HMW and LMW glutenin subunits and gliadin encoded genes in wheat breeding programs (Ahmad, 2000; Ma et

al., 2003; Lei et al., 2006; Wang et al., 2009, 2010). Recently, 81 European cultivars of spring wheat were surveyed to determine the frequency of occurrence of HMW glutenin subunits encoded by the *Glu-1 locus*, and it was observed that most cultivars present alleles associated with increased dough extensibility, elasticity, viscosity and consistency, predicting appropriate technological characteristics for the food industry (Nucia et al., 2019).

High temperature stress usually increases grain protein contents (Corbellini et al., 1998; Daniel and Triboi, 2001; Spiertz et al., 2006), although end-use quality parameters seem to be negatively influenced as heat reduces gluten strength. Glutenin macro polymer particle size distribution, responsible for positive dough mixing properties, is altered by high temperature stress (Corbellini et al., 1998; Spiertz et al., 2006), due to increases in HMW-GS and ω-gliadins and some α-gliadins increased while LMW-GS and a minor γ-gliadin decreased (Hurkman et al., 2013). Changes in grains gliadin and glutenin ratios, usually result in weaker doughs (Blumenthal et al., 1993), with detrimental effects in lactic acid retention capacity and mixograph peak time (Li et al., 2013), as well as sedimentation index (Dias et al., 2008).

1.3.2 Grain starch composition

Mature wheat grain comprises 85% (w/w) of carbohydrates, being starch the most abundant carbohydrate in the endosperm (80%). Starch is composed of two distinct polymers; amylopectin, which consists of long chains of (1–4)-linked a-D-glucopyranosyl units with extensive branching resulting from (1–6) linkages, and amylose, which is a relatively linear molecule of (1–4)-linked a-d-glucopyranosyl units (reviewed in Shewry and Hey, 2015). Starch is packaged into starch granules categorized as A-type and B-type. These categories differ in size and morphology, with the A-type being >10 μm and with a lenticular shape, and the B-type <10 μm having a spherical shape, as well as in polymer composition and structure, with B-type granules containing a lower proportion of amylose than the A-type (Shinde et al., 2003). Some studies report the existence of a third granule type, the C-type, which seems to appear latter in grain development, being usually included in B-type due to its small size (Zhang et al., 2010; Tanaka et al., 2017).

Starch synthesis is catalyzed by several enzymes responsible for the elongation of both amylose and amylopectin chains, which are also related with the dynamics of the starch granule size distribution (Zhang et al., 2010). However, whereas a number of starch synthases are thought to catalyze amylopectin synthesis, as ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch-branching enzyme (SBE) and starch-debranching enzyme (DBE), granule-bound starch synthase I (GBSSI) is considered to be the unique starch synthase responsible for amylose formation (Singletary, 2000). GBSSI is known as Waxy protein and is encoded by three homeologous genes located on *Waxy loci* (*wx*) on wheat chromosomes 7A (*wx-A1*), 4A (*wx-B1*)

and 7D (*wx-D1*). *Wild-type* wheats have the three waxy proteins present, but in partial waxy wheats, originated by the presence of null alleles, one or two are absent. In waxy wheats none of the proteins are present resulting in reduced amylose content (Nakamura et al., 2002). Amylose and amylopectin occur in mature wheat grain at a ratio of 1:3 amylose:amylopectin and waxy wheats, characterized by reduced or lacking amylose production, are usually chosen by food industry as they produce doughs with increase viscosity and water-retention and reduce stalling in flour products increasing shelf-life time (Shewry and Hey, 2015).

Codominant markers for *Wx-A1*, *Wx-B1*, and *Wx-D1* on chromosome 7AS, 4AL, and 7DS were designed to identify Wx *null* alleles and allow a prediction of genotypes amylose contents (reviewed in Saito et al., 2010). Besides the great allelic diversity detected in those l*oci*, the *wild type* allele revealed to be the one responsible for the higher amylose contents (Guzmán and Alvarez, 2016).

High temperatures during grain filling stage (after anthesis and until grain maturity) have a greater impact in seed reserves, reducing grain dry matter (Spiertz et al., 2006), resulting in shrunken kernels and affecting its quality and weight (reviewed in Akter and Islam, 2017). Such phenotypic changes are mainly caused by the reduction in starch synthesis and the alteration in size and distribution of starch granules in the mature grain (Liu et al., 2011). There is an alteration in the activities of enzymes in the starch biosynthetic pathway, especially soluble starch synthase (SSS), which activity is the most decreased (Jenner, 1994; Hurkman et al., 2003).

1.3.3 Grain hardness

Grain hardness or texture is another grain important characteristic and is also related with starch granule size and shape and the amount of starch damage generated during the milling process (which strongly affects the water absorption of dough). Based on grain hardness, bread wheat is classified in hard or soft, determining its end-use and technological utilization since hard grains are used for bread production and the soft ones for pastry and cookies. Soft wheats are more friable, require less energy to mill and produce flours with reduced particle-size distribution, including many free starch granules. Hard wheat flours are coarser, with more broken and damaged starch granules, although flowing and bolting more easily (reviewed in Morris, 2002). Two proteins, Puroindoline a (Pina) and Puroindoline b (Pinb), encoded by genes located at the *Hardness* (*Ha*) *locus* located on the small arm of chromosome 5D, are the major determinants of grain texture (reviewed in Morris, 2002). The *wild-type* (WT) alleles of *Pina* and *Pinb*, confer soft kernel texture, although mutations caused by either large deletions in the *Ha locus*, resulting in the loss of *Pina* and/or *Pinb* genes, or SNPs in coding regions, can lead to proteins with amino acid substitutions or truncated forms, which enhance grain hardness. When genes encoding Puroindolines are absent, as is the case of durum wheat, a third very hard phenotype is observed

(Giroux and Morris, 1997, 1998; Lillemo and Morris, 2000). Soft wheats contain *Pina wild-type* allele (*pinA-D1a* allele) and a functional or *wild-type* allele of *Pinb* (*pinB-D1a* allele), whilst hard wheats present either a deletion of the *Pina* gene (*PinA-D1b* allele) or one of several mutant forms of *Pinb* (Giroux and Morris, 1998). McIntosh (2013) listed 19 alleles at *Pina-D1* (*Pina-D1b~t*) and 28 alleles at *Pinb-D1* (*PinbD1b~ac*). A tightly linked gene, *Gsp-1* encodes for the Grain Softness Protein, which is also related with grain hardness while the known allelic variants did not produce significant effects on grain texture, suggesting a reduced role in grain hardness (Tranquilli et al., 2002).

In order to characterize grain texture, the presence or absence of the *Pina* gene, which have a highly conserved sequence between cultivars, may be determined using gene-specific Polymerase Chain Reaction (PCR) to discriminate *pinA-D1* alleles *a* and *b* (Gautier et al., 1994), as well as *pinB-D1a* and *b* alleles (Giroux and Morris, 1997), being therefore useful markers to screen bread wheat collections for such alleles (Lillemo et al., 2006; Ayala et al., 2013; Chen et al., 2013; Shaaf et al., 2016).Two quantitative trait *loci* (QTL) related with grain hardness were also detected in chromosome 5D , indicating that puroindoline content alone does not explain grain hardness (Igrejas et al., 2002).

1.3.4 Grain lipidic composition

Wheat kernels typically contain 2.0% to 2.5% lipids with large structural diversity and comprising neutral (acylglycerols and free fatty acids) and polar (glycolipids and phospholipids) components. As in most seed tissues, triacylglycerols are the main storage lipids present in subcellular organelles called oil bodies (González-Thuillier et al., 2015). Although lipids are minor components of wheat flour, they can have significant impacts on flour and dough functionality during breadmaking, due to interactions with gluten proteins and starch which affect gas retention (reviewed in Pareyt et al., 2011).

1.4 Wheat grain transcription patterns and its modulation by high temperature

Several studies have been oriented to unravel the mechanisms of wheat grain development intending to identify candidate genes involved in the functional and nutritional properties of wheat, as well as the determinants of yield and quality. One of the major difficulties on studies in bread wheat is its allopolyploid nature, comprising three diploid homeologous chromosome sets (A, B and D), with the possibility that each gene being represented by three homeologous copies. Past genomic rearrangements resulting from the "genomes shock" has moreover originated sequences changes or loss of a large fraction of homeologous sequences leading to marked alterations in expression or even its total absence (reviewed in Liu et al., 2015).

Even so, either gene expression quantification of specific genes in different developmental stages (Altenbach et al., 2002, 2003; Altenbach and Kothari, 2004; Wan et al., 2008), as well as the identification of important genes for grain development using Next Generation sequencing techniques (Gillies et al., 2012; Mayer et al., 2014; Pfeifer et al., 2014; Yu et al., 2016; Rangan et al., 2017; Chi et al., 2019) was already achieved.

Pfeifer et al. (2014) showed that gene expression profiles of wheat grains vary more between tissues than between developmental stages. Moreover, it was also shown that there is a large genome-specific variation in the number and abundance of genes related with dough quality, which implies the presence of genomic asymmetry with a preferential expression of B and D genomes.

In starchy endosperm, most transcripts represent the activity of genes involved in carbohydrate metabolic processes associated to the generation of precursor metabolites responsible for energy production. Contrastingly, in aleurone (the first bran layer closer to the endosperm) transcripts correspond to genes coding proteins responsible for lipids, carbohydrates and amino acids metabolic processes (Gillies et al., 2012). Accordingly with gene functions, transcripts profiles vary in each tissue accordingly with specific developmental phases. Transcripts encoding storage proteins like glutenins and puroindolines accumulate throughout endosperm development. On the other hand, transcripts from genes encoding for proteins involved in signal transduction, a variety of metabolic processes (as carbohydrate metabolisms and glycolysis), in protein processing (synthesis, folding, transport and turnover) are predominant in early development, and transcripts coding several proteins involved in defense only appear in the middle of the developmental process and last until the latter phases (Altenbach and Kothari, 2004).

Guan et al. (2019) identified genes from the B genome with differential expression between the 5th and 14th days post anthesis and also disclosed candidate genes related to grain-size being involved in starch and sucrose metabolism, hormone signal transduction, glycolysis/gluconeogenesis metabolism and protein processing in the endoplasmic reticulum. Rangan et al. (2017) compared expression profiles of immature grains at the 14th and 30th days post anthesis and demonstrated that transcripts exclusively detected at 14 days post anthesis are related with carbohydrate metabolic processes, photosynthesis, cellular component organization, response to stress, and cellular protein modification process. Contrastingly, 30 days post anthesis a lower number of transcripts are identified as unique and are related with cellular oxidant detoxification, glyoxylate metabolic process, hydrogen peroxide catabolic process, sucrose metabolic process, and negative regulation of endopeptidase activity.

Global transcriptome analyses have also been used to identify genes responsive to heat stress, unraveling mechanisms of heat tolerance. Tolerance to heat stress is a complex phenomenon controlled by multiple genes and the implication of several signaling pathways have

been revealed (Abhinandan et al., 2018). During grain development, transcription levels of individual HMW-GS and LMW-GS encoding genes share the same temporal regulation being anticipated by high temperature, but were not substantially altered (Altenbach et al., 2002; Altenbach and Kothari, 2004). Global transcriptome analysis intent not only to identify genes responsive to heat stress but also to compare responses between different genotypes to recognize those with potential interest to be included in breeding programs. Rangan et al. (2019) analyzed gene transcripts of three genotypes with different heat tolerance in two grain developmental stages and identified a cluster of genes, including, *6-phosphogluconate dehydrogenase* (*pgd3*), *S6 RPS6-2 Ribosomal protein*, *Peptidylprolyl isomerases*, *Plasma membrane proton ATPase*, *heat shock cognate-70*, *FtsH protease* and *RuBisCO activase B* playing a crucial role in imparting heat stress tolerance in addition to known *Heat shock proteins.* A recent study identified and characterized 753 *HSP* genes expressed in bread wheat seedlings, revealing their roles and the developmental stage and stress situation at which they are responsive (Kumar et al., 2020). Kino et al. (2020) compared RNA-Seq data obtained from whole grains submitted to a post anthesis high temperature treatment, against existing sequence data from individual pericarp and endosperm tissue and observed an anticipated down-regulation of genes associated to cuticle formation, suggesting that high temperature induces modifications in pericarp expansion which may constrain endosperm expansion, ultimately limiting final grain size and weight.

1.5 In course routes for wheat improvement facing predicted climatic changes

Efforts on developing innovative breeding strategies, and the exploitation of present molecular tools able to find new genes/alleles to improve wheat productivity, are essential to reduce vulnerability and enhance nutritional quality of this so relevant crop (reviewed in Chenu et al., 2017). Fleitas et al. (2020) screened in distinct field environmental conditions, bread wheat commercial genotypes released during the last 50 years searching for genotypes capable of maintaining grain yield and quality under HT stress. Two groups with different yield performances across distinct environments were identified, as well as genotypes with stable grain quality traits under stress conditions, particularly those focused on bread-making. That study also demonstrates that heat-stress substantially affects grain filling duration and grain yield components in both groups, although leading to an increase in grain protein content, dough extensibility and strength and loaf volume in both sets. The authors moreover caveat that most of the lines that produced high yields in optimal conditions, maintaining similar performances under high temperature, were also able to preserve and even improve grain quality characteristics.

CRISPR/Cas9 (Clustered Regularly Interspersed Short Palindromic Repeats) method of gene editing, already used as a tool to enhance yield and biotic and abiotic stress tolerance in

several crops, has also been exploited on wheat breeding (reviewed in Hussain et al., 2020). Due to wheat genome complexity, studies on genome editing were performed in protoplasts, as their transient expression system is an effective and simple method to test the specific genome editing capacity. Shan *et al*. (2014) successfully demonstrated the application of CRISPR/Cas9 in wheat or through the knockout of *TaMLO* gene (Mildew resistance locus O) conferring resistance to powdery mildew disease. Later on, Kim *et al*. (2018) have reported the use of the same technique to silence two abiotic stress-related genes namely, *wheat dehydration responsive element binding protein 2* (*TaDREB2*) and *wheat ethylene responsive factor 3* (*TaERF3*). More recently Wang et al., (2018) used a multiplex gene editing, knocking out three genes: *TaGW2* (a negative regulator of grain weight), *TaLpx*-1 (lipoxygenase, which provides resistance to *Fusarium graminearum*) and *TaMLO,* which resulted in a substantial increase of seed size and thousand grain weight parameters, confirming moreover the transmission of the mutated alleles to the next generation.

Besides the potential relevance of gene editing on future wheat breeding strategies, the search for new genotypes as sources of genetic diversity is very important, since domestication and breeding induced genetic bottlenecks resulting in significant loss of diversity in modern cultivars (Gregová et al., 1999; Caballero et al., 2001; Srinivasan et al., 2003). Recently, Reynolds et al. (2017) screened a great number of genotypes from diverse sources, focusing on yield and other agronomic traits, to select the ones with increased biomass. The main goal of that study is to cross the selected genotypes with lines with good harvest index, kernel number per m^2, thousand kernel weight and grains per spike (sink) to boost genetic gains. Part of the genotypes screened by Reynolds et al. (2017) were landraces which showed superior yield and biomass. Landraces appear as crucial germplasm pool that can be used to improve diverse wheat traits such as disease resistance, improved nutritional quality and abiotic stress adaptation (Newton et al., 2010; Lopes et al., 2015). Landraces are defined as dynamic populations of cultivated species lacking formal crop improvement, often being genetically diverse, locally adapted and associated with traditional farming systems (reviewed in Villa et al., 2005). Diversity of bread wheat landraces have been characterized using markers for morphological, agronomic, physiological, biochemical and technological traits (reviewed in Newton et al., 2010). Crossa *et al*. (2016), studying the genomic prediction of heat and drought tolerance in a panel of wheat landraces, identified a large number of genotypes with high value for improvement of elite varieties. Likewise, Sehgal et al. (2015) investigated sequence polymorphisms in a panel of landraces well adapted to heat and drought conditions unraveling novel alleles for drought and heat tolerance, and also for vernalization and glutenin genes. Allelic variation related with specific wheat traits, such as improved thousand kernel weight, biomass, and photosynthesis, has also been identified in landraces (Lopes et al., 2015), as well as for the puroindoline *loci*, in a panel of wheat Mexican landraces (Ayala et al., 2013).

Landraces can therefore enrich breeding programs since present significantly broader genetic diversity than modern varieties, possess several traits of interest for breeding strategies and have closer affinity with modern cultivars than wild species. This great potential of landraces will then be essential to extend the genetic base of modern cultivars, helping to overcome present productivity constrains (Ray et al., 2013). An old collection of Portuguese wheat landraces collected by Vasconcellos (1933) represent a valuable genetic diversity resource. Both bread and durum wheat genotypes from this collection revealed to possess HMW and LMW subunits associated with better dough quality as well as promising responses to high temperature and drought stress (Ribeiro et al., 2011; Scotti-Campos et al., 2011, 2014, 2015; Bento, 2014)

1.6 Objective

Taking into consideration the major relevance of wheat as human food as well as its importance in animal feed, and the high potential risks of present and predicted climatic changes, the main goal of this project is the identification of bread wheat genotypes more tolerant to heat stress temperature during grain filling. With that purpose, we will evaluate how grain quality of commercial varieties recommended to be used in Portugal as well as that of some Portuguese Old landraces, is affected by a high temperature treatment mimicking a heatwave imposed ten days after anthesis. Alterations in the transcriptomic patterns will be evaluated in immature grains immediately after high temperature treatment, while grain quality parameters will be evaluated in the end of plant cycle. To accomplish these goals, we will initially characterize the technological quality of commercial varieties, through genomic and transcriptomic analysis of genes associated with grain technological characteristics, as well as grain protein fractions relative contents. Further comparisons of high temperature effects on different genotypes will be performed through the quantification of macro components composition in mature grains, using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. Previous approaches will ultimately allow the selection of genotypes with distinct responses to heat temperature treatment, to finally identify changes in gene expression on developing grains just after the stress exposure. With this transcriptomic analysis, aimed to unravel genes and pathways involved in high temperature responses, we expect to further enrich the needed tools to amplify wheat breeding strategies.

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Chapter II

Effects of Post-Anthesis Heatwaves on the Grain Quality of Seven European Wheat Varieties

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Effects of Post-Anthesis Heat Waves on the Grain Quality of Seven European Wheat Varieties

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Abstract: Wheat is undoubtedly one of the most important crops worldwide and it is essential to study how the distinct varieties answer to heat waves associated with climatic changes, in order to design adequate wheat breeding strategies. To assess high temperature (HT) impact in wheat grain characteristics, seven commercial varieties, which have been recommended for production in Portugal, were submitted for one-week HT treatment ten days after anthesis. Firstly, predicted grain technological quality was determined by giving high scores for all varieties studied, based on the allelic compositions of genes encoding high molecular weight glutenins, granule-bound starch synthase and puroindolines. The effects of HT on transcription levels of those genes were, for the first time, evaluated in distinct wheat genotypes, in comparison with control plants. Finally, protein fraction content in mature grains were also estimated in untreated and treated plants. Immature grains from plants, maintained in control conditions, showed significant intervarietal differences in transcription levels of genes associated with grain quality traits, a variability that was significantly reduced in grains from HT treated plants. On the other hand, the influence of HT in mature grain protein-fractions and in gliadin/glutenin ratios revealed intervarietal diversity, even with opposite effects in some varieties. The present study, therefore, discloses marked variability in parameters associated with flour quality between the wheat varieties analyzed, which are differentially affected by HT treatments, similar to heat waves frequently observed in climate change scenarios.

Keywords: Bread wheat; heat waves; quality related genes; genetic and transcription diversity; grain protein fractions

1. Introduction

Hexaploid wheat (*Triticum aestivum* L.) is the third most produced cereal worldwide and provides nearly 20% of the world's daily food supply based on calorie intake [1]. Wheat aptitude in producing unique food products, like bread depends on grain quality, and determined by parameters, such as protein and starch composition, grain hardness and flour color. Wheat grain storage proteins are classified into three main classes, based on their solubility: Albumins, globulins and prolamins. Albumins and globulins constitute 10 to 22% of total flour protein and have high nutritional value although minor importance in baking quality [2]. On the other hand, prolamins comprise monomeric gliadins and polymeric glutenins, responsible for wheat dough extensibility, and elasticity, respectively, and their ratio is associated with parameters, like dough resistance and loaf volume. Glutenins are crucial for the establishment of interchain disulphide bonds to form the gluten matrix, a protein network that entrains air bubbles during dough fermentation and confers elasticity to the dough. Glutenins account for 30–40% of the total grain protein and are classified as high molecular weight (HMW) and low molecular weight (LMW). Although, HMW constitutes only 7% to 15% of gluten

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The starch fraction, comprising about 70% of wheat grain total dry matter also greatly affects end-use quality and the nutritional value of wheat products. Starch comprises two macromolecules, amylose and amylopectin, and its biosynthesis requires the coordinated activities of several enzymes. Granule-bound Starch Synthase I (GBSSI), also called waxy protein, is a key enzyme in amylose synthesis in the endosperm tissue (Guzmán and Alvarez, 2016). Hard or soft wheat kernel textures are also determinants for milling properties and wheat end-use quality, since soft wheat kernels result in finer flour suitable for cookies, cakes and pastries, while hard wheats are used in breads leavened by yeast. Grain hardness is mostly controlled by Puroindolines A and B (Morris, 2002).

A significant decrease in wheat productivity is expected due to climate changes and temperature stress in Europe (Semenov and Shewry, 2011). Wheat grain yield, as well as flour technological and nutritional qualities, although genetically determined, are also strongly modulated by environmental conditions. Several studies demonstrated that high temperature stress, during grain filling accelerates, and compresses key events during wheat grain development, like storage protein and starch synthesis in endosperm (Blumenthal et al., 1993; Ashraf, 2014). Altogether, it is presently consensual that high temperature has great impact on grain composition, and expression patterns of key quality-related genes at an early stage of seed development (Altenbach et al., 2002; Hurkman et al., 2003; DuPont et al., 2006; Yang et al., 2011; Zhang et al., 2017). Heatwaves, defined by the World Meteorological Organization as five or more

consecutive days of heat in which the daily maximum temperature is at least 5 °C higher than the average maximum temperature (WMO, 2015), have been recently predicted to be particularly frequent and severe in Portugal (Cardoso et al., 2019). In the present work, the impact of heatwaves, particularly frequent in Southern Portugal wheat fields during grain filling, was comparatively assessed in seven varieties recommended for use in Portugal in relation to the transcription levels of genes associated with grain technological characteristics and grain protein fractions relative content.

2.3 Materials and methods

2.3.1 Plant material and high temperature treatments

In this work, the seven bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) commercial varieties were used, which were recommended to be used in Portugal, based on phenological, agronomic and technological traits (ANPOC et al., 2014). These seven varieties were Almansor, Antequera, Bancal, Estero, Nabão, Pata Negra and Roxo. Chinese spring was also used as reference lines for allelic composition analysis. The seeds used in this work were obtained after two years of controlled propagation of material, gently supplied by INRB/INIAV Portugal (National Institute of Biological Resources) and ANSEME, Portugal (National Association of Seed Producers and Traders), Portugal. Twenty seeds from each commercial variety were germinated and grown in controlled conditions with 16h light 25 °C/8h dark 20 °C and three-week old plants were then transferred to soil pots and maintained in greenhouse conditions. Fresh young leaves of 1-month-old plants were collected and stored at −80 °C for DNA extraction to be used in allelic composition identification.

When the first anther was observed (anthesis) in the first spike, the plants were transferred to growth chambers with controlled conditions with 16h light at 25 °C/8 h dark at 20 °C. Ten days after anthesis (daa) subsets of ten plants were submitted to two different growth conditions for one week. While ten control plants were maintained in 16h light at 25 °C/8 h dark at 20 °C, ten other plants were submitted during seven days to a daily high temperature (HT) treatment, simulating a heatwave. This treatment consisted in a gradual increase of temperature, at the end of the dark period, from 20 to 40 °C during 6 h, followed by an exposure to 40 °C for 4 h during daylight period and a subsequent gradual decrease to 20 °C during 6 h. In the last day of the treatment, immediately after the period of 4 h at higher temperature (40 °C for treated plants and 25 °C for control) two immature grains from the middle of each first spike of each plant were collected and stored at −80 °C for posterior RNA extraction. After treatments plants were again transferred to greenhouse

and maintained until the end of lifecycle. All further analysis in mature grains were restricted to seeds from the first spike to guarantee identical developmental stage during HT treatments.

2.3.2 Genetic variability analysis

DNA was extracted using the Citogene® DNA Cell&Tissue Kit (Citomed, Lisbon, Portugal) and its concentration and integrity were evaluated in microplate reader Synergy HT (Biotek, Winooski, Vermont) using the software Gen5TM (Biotek, Winooski, Vermont). For the identification of allelic composition of genes encoding High Molecular Weight Glutenin Subunits (HMW-GS), Waxy and Puroindolines, specific primers and PCR amplification conditions were used (primers and references in Supplemental Table 2.1). PCR mixture included 1x PCR buffer, 1.5 mM MgCl2, 0.25 mM dNTP's, 1 mM each primer, 0.5 U Taq polymerase and 50 ng DNA template in a total volume of 20 microliter. PCR products were separated in agarose gel electrophoresis detected with ethidium bromide and photographed using a Bio-Rad GEL DOC 2000 (Bio-Rad Laboratories, Inc., Hercules, California). For each sequence and variety studied, at least three individual plants were analyzed in three technical replicates each.

2.3.3 Comparative transcription analysis

The total RNA was individually extracted from immature grains collected from five plants of each condition (control and heat treated) immediately after the one week HT period (17 daa), using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, Inc, St.Louis, Missouri), and following manufacturer's instructions. After RNA concentrations and integrity verification executed as described above, 2µg of total RNA was used for RQ1 RNase-Free DNase digestion (Promega, USA) and first strand cDNA synthesis using RevertAid H Minus Reverse Transcriptase with Oligo(dT)18 primer (Thermo Fisher Scientific Inc, Waltham, Massachusetts). Quantitative Real-time PCR (RT- qPCR) was performed using BIO-RAD IQ5 Multicolor Real-Time PCR detection System with the SsoFastTM EvaGreen® Supermix (Bio-Rad Laboratories, Inc., Hercules, California, CA, USA). Each 20µL PCR mix consisted in 5µL SsoFast EvaGreen supermix, 1µL of forward and reverse gene- specific primers (10nM each) and 1µL of cDNA (1:20 dilution). PCR amplification conditions followed those established in the reference of each primer pair, presented in Supplemental Table 2.2 and monitored via intercalation of Eva-Green. In all quantification experiments, four endogenous reference genes with stable expression across a wide range of developmental and environmental conditions (Paolacci et al., 2009) were used: *ADP-ribosylation* factor, *Ubiquinol-cytochrome C reductase iron-sulfur subunit, Superoxide dismutase [Cu-Zn]* and *Glyceraldehyde 3-phosphate dehydrogenase*. Each run was completed with a melting curve analysis and PCR products separation by electrophoresis, as previously described

to confirm single amplification products. Quantification analysis was performed using threshold cycles (Ct), equilibrated with mean of the four housekeeping genes previously tested for transcription stability under HT conditions, to calculate ΔCt (ΔCt = Ct gene of interest − Ct mean of reference genes). The measured gene transcription levels (ΔCt) obtained for the seven varieties studied were fitted to a linear model (ANOVA with one factor with fixed effects) and analyzed through multiple means comparison test (Tukey test). The individual effect of HT treatment in each variety in relation to the control was evaluated using a t test. Models were fitted in R using *aov* and *Tukey.HSD* functions. Differences were considered significant for p-value < 0,05. For each gene and variety studied, five individual plants were analyzed in three technical replicates each.

2.3.4 Protein fractions quantification

Mature grains from the first spike of control and high temperature treated plants from each variety were grounded individually using Cryomill (Retsch GmbH, Haan, Germany) and protein fractions were then separated accordingly with modified Osborne method (Lookhart and Bean, 1995). This procedure is based in the successive extraction of different fractions (albumins, globulins, gliadins and glutenins), according to their solubility in water or in 0,5N sodium chloride aqueous solution, 70% ethanol and 50% 1-propanol + 1% dithiothreitol (DTT), respectively. Each fraction was obtained in the supernatant, after 30 min of vigorous shaking, followed by centrifugation for 5min at 8000g.

Comparative quantification of protein extracts was spectrophotometrically performed in triplicates for each extract through Bradford method (Bradford, 1976), using Bradford Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Inc., Hercules, California) and measured at 595nm after 10min incubation.

2.4 Results and discussion

2.4.1 Genomic analysis revealed high similarity on genes related with grain quality

In order to support intervarietal comparisons of transcriptional patterns of genes associated with grain quality parameters, an initial evaluation of predicted breadmaking values of the seven varieties analyzed was performed, based on allelic characterization (Table 1) estimated using specific primers (Supplemental Table 2.1). The inbred line Chinese Spring was used as reference due to its well established allelic composition. At least three distinct plants from each variety were analyzed and no intravarietal variability was detected for any loci.

Table 2.1. High Molecular Weight glutenin subunits, waxy and puroindolines allelic composition and correspondent predicted flour technological characteristics.

		Almansor	Antequera	Bancal	Estero	Nabão	Pata Negra	Roxo	Chinese Spring
Glu-1 loci	***Ax***	2*	1	2*	1	2*	1	2*	Null
	Bx + By	17 + 18	7 + 8	7 + 9	17 + 18	7 + 8	17 + 18	7 + 8	7 + 8
	Dx + Dy	5 + 10	5 + 10	5 + 10	5 + 10	5 + 10	5 + 10	5 + 10	2 + 12
	Glu-1 Score	10	10	9	10	10	10	10	8
Waxy loci	***Wx-A1***	WT	WT	WT	WT	WT	WT	Null	WT
	Wx-B1	Null	WT	WT	WT	WT	WT	WT	WT
	Wx-D1	WT	WT	Null	WT	WT	Null	WT	WT
	Waxy type	Partial waxy	Wild Type	Partial waxy	Wild Type	Wild Type	Partial waxy	Partial waxy	Wild Type
Hardness locus	***Pina-D1a***	+	-	-	-	-	-	-	+
	Pinb-D1a	-	+	-	+	-	+	-	+
	Hardness	Hard	Hard	Hard	Hard	Hard	Hard	Hard	Soft

Note: ***Glu-1 loci:*** Numbers correspond to the allele present in each subunit (x and y) of each *Glu-1 locus* (*Glu-A1*, *Glu-B1* and *Glu-D1*) for each variety. The resulting score ranges between 1 and 10, corresponding higher values to better predicted breadmaking quality accordingly to Payne et al. (1987). ***Waxy locy*****:** WT and Null indicates the presence of the wild-type or mutated allele form, respectively, in each encoding waxy *locus*. Waxy type results from the combination of wild and null alleles, related with the expected flour amylose content. ***Hardness locus*****:** (+) Indicates the presence and (-) the absence of wild-type alleles, *Pina-D1a* or *Pinb-D1a*. Hardness is the expected endosperm texture, accordingly to the allelic combination (Morris, 2002).

HMW glutenin subunits are encoded by *Glu-1* genes located in the long arms of homoeologous chromosomes 1A, 1B and 1D (named *Glu-A1, Glu-B1,* and *Glu-D1*, respectively). Each HMW glutenin locus harbors two adjacent genes coding for x-type and y-type subunits with high and low molecular weights, respectively. The subunits encoded by the *Glu-D1* have a predominant effect on technological properties of bread wheat and the *Glu-D1d* allele with 1Dx5 and 1Dy10 subunits, previously associated with stronger dough and superior end-use quality (Payne et al., 1987), was detected in all varieties studied.

Concerning *Glu-A1 locus*, only *Glu-Ax* gene is usually active since *Glu-Ay*-type is silent in common wheat (reviewed in Payne et al., 1987). Ax1 and Ax2* subunits (*Glu-A1a* and *Glu-*

A1b alleles, respectively) which are positively correlated with better breadmaking quality (Payne et al., 1987), are differentially present in the varieties here analyzed since Ax2* was identified in Almansor, Bancal, Nabão and Roxo while *Ax1* was detected in the other three varieties.

The Bx7 + By8, Bx7 + By9, Bx17 + By18 subunits pairs encoded by genes located on *locus Glu-B1* (*Glu-B1b*, *Glu-B1c* and *Glu-B1i* alleles, respectively) were also correlated with superior end-use quality (reviewed in Payne et al., 1987). The analysis of this *locus* disclosed however some intervarietal diversity as Almansor, Estero and Pata Negra present Bx17 + By18 subunits, whereas Antequera, Nabão and Roxo are characterized by Bx7 + By8 subunits and Bancal by subunits Bx7 + By9. The first two combinations are related with better breadmaking quality while the Bx7 + By9 subunit combination confers intermediate quality characteristics for bread production (Rasheed et al., 2014).

According to Payne et al. (1987) *Glu-1* quality scores, relating HMW subunits with quality evaluated through Sedimentation test (SDS), most varieties analyzed presented the maximum value of 10, excepting Bancal with a *Glu-1* score of 9, due to the subunit pair Bx7 + By9 encoded by *Glu-B1* locus.

Since amylose content is very relevant for wheat grain technological characteristics and nutritional value, genotypes are usually classified according with the prediction of amylose content based on the presence of mutations on *waxy* genes. GBSSI encoding genes are located on *Waxy loci* (*wx*) on chromosomes 7A (*wx-A1*), 4A (*wx-B1*) and 7D (*wx-D1*) and in wild type wheats the three *waxy* genes coding Waxy proteins are present, in partial waxy one or two are absent (Types 2 to 7) and in waxy none of the proteins are present (type 8) (Nakamura et al., 2002). The analysis of genes coding Waxy proteins revealed wild type genotypes (*Wx-A1a, Wx-B1a and Wx-D1a)* for varieties Antequera, Estero and Nabão and partial waxy genotypes for the other varieties with one *null* allele, resulting in the loss of one GBSSI enzyme isoform (types 2, 3 or 4, depending on which *locus* has the *null* allele, Table 5.1). Since the absence of Wx-A1 or Wx-D1 proteins is not relevant for grain amylose content, the starch characteristics of the varieties analyzed are predicted to be very similar, except Almansor, which has *Wx-B1 locus null* allele, associated with lower amylose content (Nakamura et al., 2002). However, it was very interesting to identify type 4 varieties, as Bancal and Pata Negra, since the absence of the Wx-D1 protein is rare in germplasm collections (reviewed in Ashraf, 2014), exposing their putative importance in breeding programs oriented to select waxy or partial waxy wheats with applications in industry.

Grain texture (hardness or softness of the grain) is an important attribute of breadmaking quality mainly controlled by two *Puroindoline-D1* genes, *Pina* and *Pinb*. Soft kernel texture is only associated with wild type alleles of both Puroindoline genes (*Pina-D1a* and *Pinb-D1a*), while mutations or deletions in their coding regions result in hard textured grain more apt for bread production (Morris, 2002). To predict the grain texture of the varieties studied, we used primer pairs

that are designed to detect the wild type alleles. *Pina-D1a* allele was detected only in Almansor and *Pinb-D1a* allele was observed in Antequera, Estero and Pata Negra whereas both wild type alleles were absent in Bancal, Nabão and Roxo. Therefore, all varieties studied correspond to hard grain phenotype usually associated with superior breadmaking quality.

Overall the present study revealed intervarietal similarity in the predicted grain quality based on the allelic composition of genes associated with the most important technological traits. The transcription levels of those genes were further evaluated in immature grains, collected immediately after HT treatment period, in both treated and untreated plants.

2.4.2 Transcription levels of flour quality related genes in immature grains vary between wheat varieties

The allelic composition and the transcription levels of glutenin genes are associated with dough properties (Rasheed et al., 2014). Thus, transcriptional levels of genes associated with wheat grain quality were evaluated in five plants per variety 17 daa - milk stage kernel, when carbohydrates and proteins are deposited (Bowden et al., 2007). RT-qPCR was performed for the five active HMW-GS genes (Ax, Bx, By, Dx, Dy), for both puroindoline genes (Pina and Pinb), as well as for gene encoding Granule bound starch synthesis (GBSSI). Contrary to the specific primers previously used to discriminate alleles, the primers used to evaluate genes transcription levels were those already well-established to amplify conserved regions of such genes (Supplemental Table 2.2). All primer pairs used amplified a single product with the expected size in all samples analyzed, independently of the allelic composition.

The quantification of gluten protein genes transcription in control conditions disclosed several differences between varieties (Figure 2.1). Bancal presents the lower *Ax* gene expression value, significantly different from Almansor, Estero, Nabão and Roxo. Regarding *Glu-B1* gene, no significant differences were detected in Bx subunit while By presented a significantly higher expression level in Roxo in comparison with Antequera, Bancal, Nabão and Pata Negra. Transcription levels of *Glu-D1* gene only show significant differences in Dx subunit between Estero and Nabão and no significant differences were detected regarding Dy.

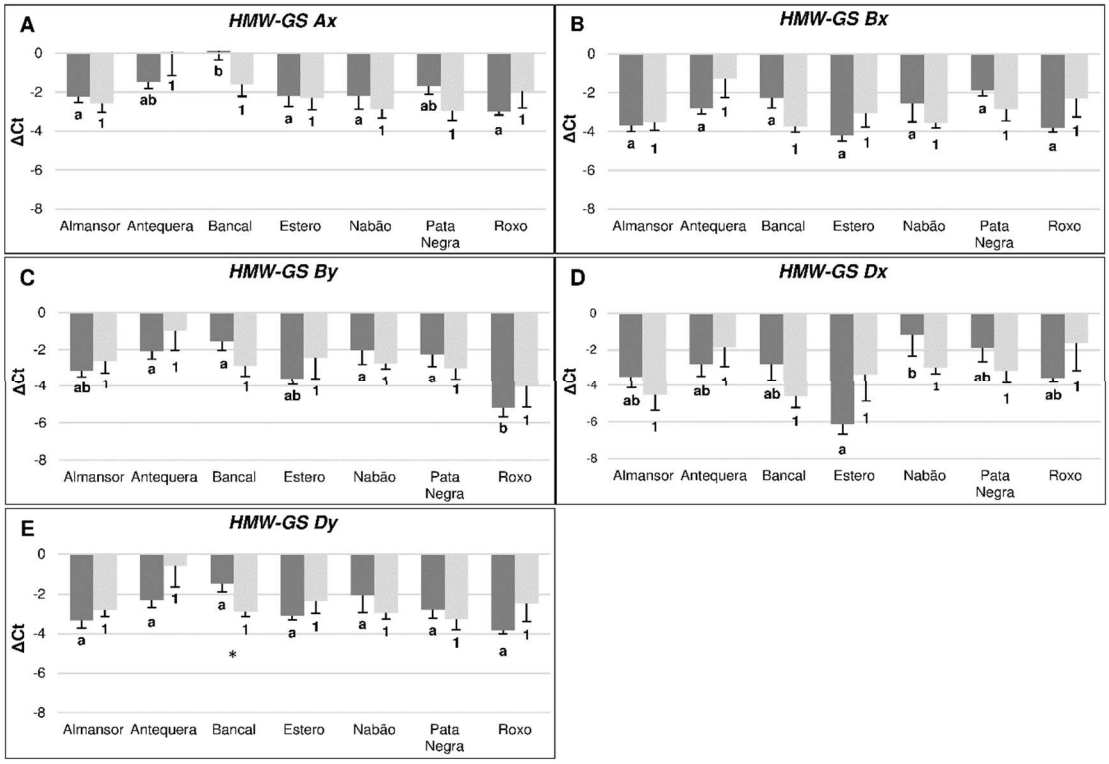


Figure 2.1 Transcription levels of High Molecular Weight Glutenin Subunits (HMW-GS) encoding genes: **A** *HMW-GS Ax*, **B** *HMW-GS Bx*, **C** *HMW-GS By*, **D** *HMW-GS Dx*, **E** *HMW-GS Dy*. Dark bars represent control conditions and light bars represent high temperature treatment. Negative ΔCt values result from their marked higher expression in relation to reference genes. Means ± SE from five biological replicates. Different letters (control) and numbers (treatment) indicate significant differences between varieties and (*) show statistical differences between control and treatment values ($p < 0,05$).

Genomic evaluation of puroindoline genes indicate that all commercial varieties used in this work are expected to possess hard kernel, although differences in *Pin* genes transcript levels may be responsible for differences in grain texture characteristics (Igrejas et al., 2001). Assessed quantification of *Puroindoline a* and *b* genes transcripts in control conditions are summarized in Figure 2.2A and 2.2B and the results shown that *Pina* is significantly more expressed in Almansor in comparison with all other varieties. Whereas, *Pinb* transcription levels were significantly higher in Almansor and Estero only in comparison with Pata Negra.

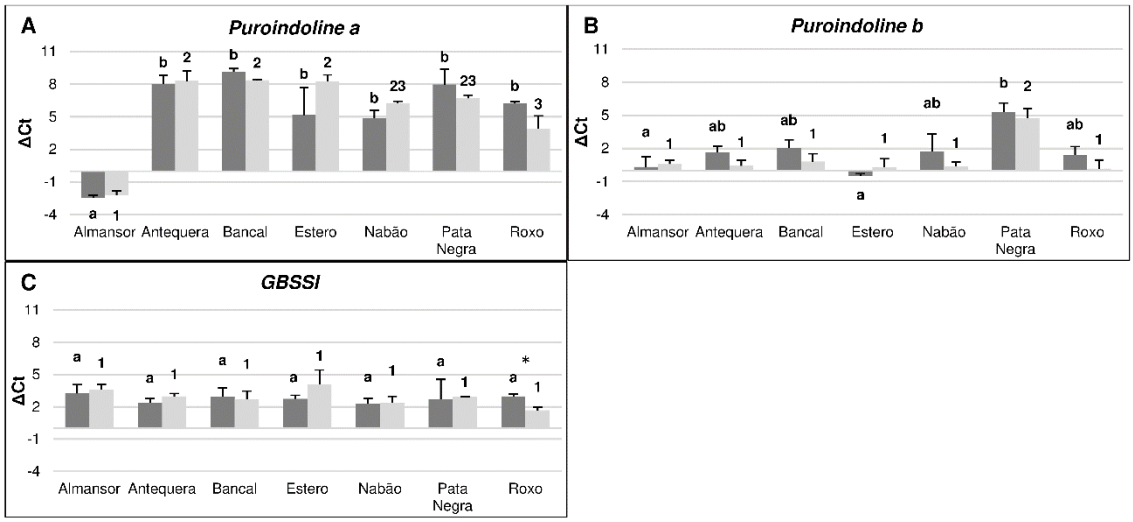


Figure 2.2 Transcription levels of **A** Puroindolines a, **B** puroindolines b and **C** GBSSI encoding genes. Dark bars represent control conditions and light bars represent high temperature treatment. Negative ΔCt values result from their marked higher expression in relation to the reference genes. Means ± SE from five biological replicates. Different letters (control) and numbers (treatment) indicate significant differences between varieties and (*) shows statistical differences between control and treatment values ($p < 0,05$).

Granule Bound Starch Synthase I expression levels, related to amylose synthesis in grains, were additionally evaluated and the results are presented in Figure 2.2C. No significant differences were identified between varieties in the transcription level of this gene which is in accordance with the similar amylose content predict through the genetic characterization of the varieties studied as wild type and partial waxy types 2, 3 and 4 (Nakamura et al., 2002).

Altogether these results clearly show that, although no intervarietal diversity was predicted, based on the allelic composition of grain quality related genes, and significant differences were detected, for the first time, in their transcriptional levels between the seven varieties studied.

2.4.3 High temperature differentially affects the transcription of flour quality related genes in immature grains

Predicted increasing temperatures can affect several aspects of wheat production, and high temperature peaks during grain filling phase can be particularly detrimental (reviewed in Farooq et al., 2011). Thus, we further evaluated the expression levels of *HMW-GS*, *GBSSI* and *puroindolines* genes in immature grains of plants exposed to HT during grain filling from the 10th to the 17th daa. The average transcription levels in treated plants of each variety are summarized in Figures 2.1 (*HMW-GS*) and 2.2 (*puroindolines* and *GBSSI*).

Although, the comparative analysis between treated and control plants of each variety seem to reveal differential HT effects in the transcript levels of each of the five *HMW-GS* genes

analyzed, only in Bancal the increase on *HMW-GS Dy* gene transcription level induced by HT treatment is significant. The results obtained show moreover that, after HT treatment, the *HMW-GS* genes transcripts levels become similar between varieties, markedly contrasting with the variability previously observed in control plants.

Transcription levels of several starch biosynthetic enzymes were shown to be affected by high temperature exposure from anthesis to maturity, reducing starch content, granule size and distribution and duration of starch accumulation (Hurkman et al., 2003). In the present work, however, the HT treatment, which was restricted to one week during grain filling, only induced a significant increase on *GBSSI* gene transcription in Roxo variety. Nevertheless, as observed in control plants, no significant differences for GBSSI were observed between varieties after HT. Also, Altenbach et al. (2002) and Hurkman et al. (2003) detected small changes on the levels of *HMW-GS* and *GBSS* transcripts and the anticipation of transcripts accumulation period in wheat plants from the variety Butte 86 submitted to high temperatures (37/28 °C).

In relation to *Pina* genes associated to grain hardness, transcripts levels of Almansor after HT maintain significantly higher than the ones observed in the other varieties, as observed in control plants. Also, Antequera, Bancal and Estero keep significantly lower transcription levels in relation to those detected in Roxo. Finally, HT treatment in Antequera, Bancal, Nabão and Roxo induces *Pinb* gene expression levels significantly superior in relation to Pata Negra, that presented significantly lower value in comparison with all other varieties.

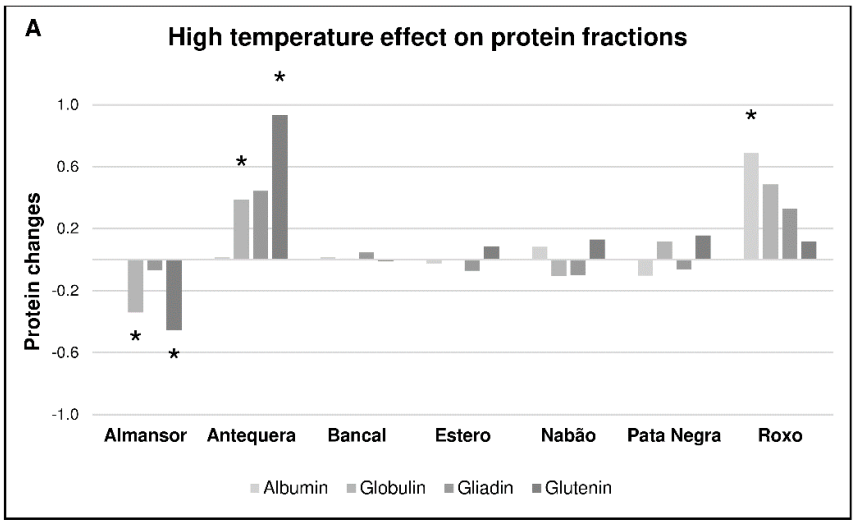
This work showed that a short period of high temperature treatment, similar to a heatwave during grain filling, attenuates the differences between varieties observed in transcription levels of *HMW glutenin* genes in normal conditions. These results were obtained at the end of HT treatment period, which does not invalidate any differences that may occur during the treatment period and must be further studied.

2.4.4 Comparative contents of protein fractions in mature grains vary between varieties and with HT treatment

Wheat grain protein fractions—albumins, globulins, gliadins and glutenins, determinant for grain quality, were quantified spectrophotometrically using Bradford reagent (Bradford, 1976) in mature grains from control and HT treated plants.

It has been previously reported that different environmental conditions during grain filling induce alterations in the time course of grain development, modulating final grain weight, protein and starch contents, as well as gluten protein composition. However, these former studies were based on the evaluation of only one bread wheat variety (Altenbach et al., 2002; Hurkman et al., 2003; DuPont et al., 2006; Yang et al., 2011; Zhang et al., 2017). Our results, obtained in different varieties, disclosed heat response variability in terms of seed storage proteins composition. In fact,

comparisons of control and HT treated plants (Figure 2.3A) revealed significant alterations in Almansor and Antequera, which presented significant differences in globulins and glutenins contents, although with opposite effects, both increasing in Antequera and decreasing in Almansor. In turn, Roxo grains from HT treated plants have significantly higher albumin levels.



B

gli/glu	Almansor*	Antequera*	Bancal	Estero	Nabão	Pata Negra	Roxo
Control	0,94 [a]	2,23 [b]	2,35 [b]	1 [a]	0,99 [a]	0,95 [a]	0,89 [a]
HT	1,16 [1]	1,14 [1]	2,29 [2]	0,97 [1]	0,84 [1]	0,82 [1]	1,01 [1]

Figure 2.3 Grain protein fractions comparative evaluation. **A** Changes in protein fractions levels in mature grains of plants exposed to high temperature treatment during grain filling stage in comparison with plants kept in control conditions. **B** Gliadin/glutenin ratios for control and treated plants. Different letters (control) and numbers (treatment) indicate significant differences between varieties and (*) shows statistical differences between control and treatment values ($p < 0,05$).

It must be emphasized that flour quality for bread making depends on the balance between gliadins and glutenins for the required equilibrium between dough viscosity and elasticity/strength (Dhaka and Khatkar, 2015). The value of the gliadin/glutenin (gli/glu) ratio is, therefore, particularly important, being negatively related with dough development and stability (Barak et al., 2013). Antequera and Bancal varieties presented significantly higher gli/glu ratios (2.23 and 2.35, respectively), corresponding to poorer dough stability (Figure 2.3B). The remaining varieties presented significantly lower ratios ranging from 0.89 and 1. Thus, despite of the similar genomic prediction of high quality for all varieties analyzed, both gene transcription levels and protein fraction content disclosed significant differences between varieties.

Several reports have shown that heat stress induces a decrease in glutenin fraction and a relative increase in gliadins (reviewed in Branlard et al., 2001). However, our results demonstrate that HT only induces a significant increase in gli/glu ratio in Almansor while in Antequera an

inverse effect was detected. Thus, it is expected a reduction of Almansor technological quality after temperature stress and, in turn, an enhancement in Antequera. Balla et al. (2011) reported similar diversity between varieties in gli/glu ratio changes caused by diary 8 h at 35 °C during 15 days, imposed 12 days after anthesis. After HT treatment, Bancal is the only variety with a significant higher gli/glu ratio, being the one with predicted inferior quality regarding dough development, compared with all other varieties. Although, it is clearly a marked intervarietal variability in mature grains composition induced by short HT treatments. These results are not in accordance with transcriptional analysis of HMW immediately after HT treatment. This apparent inconsistency suggests that HT may affect other features of plant development, like grain filling duration and particularly HMW-GS accumulation period, as previously proposed (Altenbach et al., 2002; Hurkman et al., 2003).

2.5 Conclusions

Contrary to most previous works developed using only a single bread wheat variety, submitted in continuous long-lasting heat stress (Altenbach et al., 2002; Hurkman et al., 2003; DuPont et al., 2006; Yang et al., 2011; Zhang et al., 2017), in the present work the impact of heatwaves was comparatively assessed in seven bread wheat varieties recommended to be produced in Portugal. Besides the similarity of the allelic composition of genes associated to flour technological qualities of the genotypes analyzed, both transcription and protein fraction content evaluations disclosed considerable diversity in heat stress response of the varieties studied. Transcription patterns variability detected between distinct varieties in control conditions was significantly reduced in HT treated plants. On the other hand, the heat impact on mature grain protein-fractions content and in gliadin/glutenin ratios revealed higher intervarietal diversity. The novelty of the present work contributes to the development of an integrative portrait of the complexity of plant response to thermal constraints, which are essential for planning breeding programs oriented to face the climatic changes. Moreover, the present work leads to future work focused in the development of methodologies to expeditiously evaluate grain quality traits, with limited grain quantity, in order to consolidate the effects heatwaves assessed.

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2.7 Supplemental material

Supplemental Table 2.1. Primer used for genomic analysis

Sequence	Primer (5' → 3')	Fragment size	Reference
HMW-GS subunits encoding genes			
AxNull	CGAGACAATATGAGCAGCAAG CTGCCATGGAGAAGTTGGA	920 bp	(Lafiandra et al., 1997)
Ax2*	ATGACTAAGCGGTTGGTTCTT ACCTTGCTCCCCTTGTCTTT	1319 bp	(Ma et al., 2003)
Bx6	CACTGAGATGGCTAAGCGCC GCCTTGGACGGCACCACAGG	246 bp	(Schwarz et al., 2004)
Bx7	CAAGGGCAACCAGGGTAC AGAGTTCTATCACTGCCTGGT	670 and 770 bp	(Butow et al., 2003)
Bx17	GGGCAATCGGGGTACTTCC CCCTTGTCTTGGCTGTTGTC	534 bp	(Xu et al., 2008)
By8	TTAGCGCTAAGTGCCGTCT TTGTCCTATTTGCTGCCCTT	527 bp	(Lei et al., 2006)
By9	GCAGTACCCAGCTTCTCAA CCTTGTCTTGTTTGTTGCC	2 bands for By9 3 bands for By8, By8*, By18 No band for By16, By20, By-null	(Lei et al., 2006)

Supplemental Table 2.1. (continued)

By16	GCAGTACCCAGCTTCTCAA CCTTGTCTTGTTTGTTGCC	3 bands for By16 2 bands for By8, By8*, By9, By18 and By15 No band for By20 and By-null	(Lei et al., 2006)
Dx5	GCCTAGCAACCTTCACAATC GAAACCTGCTGCGGACAAG	450 bp	(Ahmad, 2000)
Dy10	GTTGGCCGGTCGGCTGCCATG TGGAGAAGTTGGATAGTACC	576 bp for Dy10 612 bp for By12	(Ahmad, 2000)
Granule-bound Starch Synthase I encoding genes			
Waxy-A1	TCGTGTTCGTCGGCGCCGAGATGG CCGCGCTTGTAGCAGTGGAAGTACC	*Wx-A1a: 389* *Wx-A1b: 370*	(Nakamura et al., 2002)
Waxy-B1	CTGACGTCCATGCCGTTGACGA CTGGCCTGCTACCTCAAGAGCAACT	*Wx-B1a: 410* *Wx-B1b: -*	
Waxy-D1	CTGTTTCACCATGATCGCTCCCCTT CTGGCCTGCTACCTCAAGAGCAACT	*Wx-D1a:* 2307 *Wx-D1b:* 1731	
Puroindoline encoding genes			
Pina-D1a	CATCTATTCATCTCCACCTGC GTGACAGTTTATTAGCTAGTC	520 bp	(Ayala et al., 2013)
Pinb-D1a	ATGAAGACCTTATTCCTCCTA CTCATGCTCACAGCCGCC	239 bp	(Giroux and Morris, 1997)

Note: Chinese Spring allelic composition for HMW-GS: *AxNull*; *Bx7+By8*; *Dx2+Dy12* (Shewry et al., 1992); waxy genes *Wx-A1a*, *Wx-B1a* and *Wx-D1a* (Murai et al., 1999), and puroindolines a and b (*pina-D1a*, *pinb-D1a*) (Simeone et al., 2006).

Supplemental Table 2.2. Primers used for Quantitative Real-Time PCR gene expression analysis

Gene	Primer Sequence 5' → 3'	Fragment size	Reference
Housekeeping genes			
ADP-ribosylation factor	GCTCTCCAACAACATTGCCAAC GCTTCTGCCTGTCACATACGC	165 bp	(Paolacci et al., 2009)
Ubiquinol-cytochrome C reductase iron-sulfur subunit	CCTGCCCCGTACAACCTTGAG CACCGTTGCGATAGTCCTGAAAC	185 bp	
Superoxide dismutase [Cu-Zn]	TGAGCAAGAGCACTGGAAAC CGTTGGTCGGCGAAGATG	88 bp	
Glyceraldehyde 3-phosphate dehydrogenase	GTTGAGGGTTTGATGACCAC TCAGACTCCTCCTTGATAGC	290 bp	
HMW-GS subunits encoding genes			
Glu-Ax	AGCGGTTGGTTCTTTTTGC CTTTGTTGGAGTTGCTGTGG	263 bp	(Altenbach et al., 2002)
Glu-Bx	AGCAACTCCGAGACGTTAGC TGGCCTGGATAGTATGACCC	209 bp	
Glu-By	AGCAGCTCCGAGATGTTAGC CTGAGGAGAACTTACGCTTGG	205 bp	
Glu-Dx	GCGGTTAGTCCTCTTTGTGG TGCGGACAAGTTACACTTGG	335 bp	
Glu-Dy	AGCAGCTCCGAGATGTTAGC TGGCCTGGATAATATGACCC	228 bp	
Granule-bound Starch Synthase I encoding genes			
Waxy	GACACTATCGTGGAAGGCAAG TTGACCATCTCATGGTACGC	152 bp	(Wang et al., 2014)

Supplemental Table 2.2. (continued)

Puroindoline encoding genes			
Pina -D1	CCACATGAAGGCCCTCTTCCT ACTGCCAACAACTTCGCTATATTG	82 bp	(Amoroso et al., 2004)
Pinb – D1	GAAAACATGAAGACCTTATTCCTCCTA CCTGCGGACATTGTTGAGAAC	112 bp	

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Chapter III

Assessment of High Temperature Effects on Grain Yield and Composition in Bread Wheat Commercial Varieties

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Tomás, D., Rodrigues, J. C., Viegas, W., and Silva, M. (2020b). Assessment of High Temperature Effects on Grain Yield and Composition in Bread Wheat Commercial Varieties. *Agronomy* 10, 499. doi:10.3390/agronomy10040499. https://www.mdpi.com/2073-4395/10/4/499

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Assessment of High Temperature Effects on Grain Yield and Composition in Bread Wheat Commercial Varieties

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Abstract: Wheat is one of the most important cereals for food and feed, and it is, therefore, necessary to determine the effects of short-term high temperature events (heatwaves) during grain filling. These heatwave events are increasingly common, especially in Portugal. In this work, seven commercial varieties recommended for production in Portugal were submitted to one-week high temperature (HT) treatment ten days after anthesis to evaluate heat effects on grain yield and quality. Grain yield parameters, such as grain number and weight, were evaluated as well as grain composition through attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. Variation in HT response between varieties was detected. Grain number and weight tended to decrease in most varieties analyzed. However, two varieties proved to be more resilient since grain number and weight remain unaltered in the Bancal variety, which is the one with better yield results, and even increased in the Pata Negra variety. Regarding grain composition, the comparison between ATR-FTIR spectra of milled grains from control and HT plants revealed alterations in peaks assigned to polysaccharides and proteins. Additionally, a model was built based on nitrogen elemental analysis to predict protein content in flour samples through spectral data that corroborated the differences identified by spectra profile comparison. Moreover, both analyses showed that the intervarietal diversity observed in control conditions was significantly reduced in HT treated plants. The results obtained highlight the intervarietal diversity of wheat response to HT, regarding grain yield parameters, grain composition, and particularly, protein content.

Keywords: bread wheat; heatwave; yield; grain composition; protein content

1. Introduction

Wheat (*Triticum aestivum* L.) represents 25% of the world's cereal production and constitutes one of the main food sources of carbohydrates, proteins, fibers, amino acids, and vitamins, providing 20% of the calories and 25% of proteins consumed worldwide on a daily basis [1,2]. Although being produced worldwide under diverse environmental conditions, the required optimum temperature for wheat anthesis and grain filling is from 12 to 22 °C [3]. Each degree Celsius increase reduces wheat yield by 4.1% to 6.4% [4]. Several yield parameters are affected by high temperatures as vegetative weight and grain number and weight, as reviewed in [5]. Grain number is strongly affected by high temperatures, especially between spike initiation and anthesis [6]. Grain mass is reduced with high temperature after anthesis, particularly when the treatment is imposed in early stages [7,8]. Heat stress also shortens grain filling duration, as reviewed in [9], affecting starch and storage protein

3 Assessment of High Temperature Effects on Grain Yield and Composition in Bread Wheat Commercial Varieties

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3.1 Abstract

Wheat is one of the most important cereals for food and feed, and it is, therefore, necessary to determine the effects of short-term high temperature events (heatwaves) during grain filling. These heatwave events are increasingly common, especially in Portugal. In this work, seven commercial varieties recommended for production in Portugal were submitted to one-week high temperature (HT) treatment ten days after anthesis to evaluate heat effects on grain yield and quality. Grain yield parameters, such as grain number and weight, were evaluated as well as grain composition through attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. Variation in HT response between varieties was detected. Grain number and weight tended to decrease in most varieties analyzed. However, two varieties proved to be more resilient since grain number and weight remain unaltered in the Bancal variety, which is the one with better yield results, and even increased in the Pata Negra variety. Regarding grain composition, the comparison between ATR-FTIR spectra of milled grains from control and HT plants revealed alterations in peaks assigned to polysaccharides and proteins. Additionally, a model was built based on nitrogen elemental analysis to predict protein content in flour samples through spectral data that corroborated the differences identified by spectra profile comparison. Moreover, both analyses showed that the intervarietal diversity observed in control conditions was significantly reduced in HT treated plants. The results obtained highlight the intervarietal diversity of wheat response to HT, regarding grain yield parameters, grain composition, and particularly, protein content.

Keywords

Bread wheat; heatwave; yield; grain composition; protein content

3.2 Introduction

Wheat (*Triticum aestivum* L.) represents 25% of the world's cereal production and constitutes one of the main food sources of carbohydrates, proteins, fibers, amino acids, and vitamins, providing 20% of the calories and 25% of proteins consumed worldwide on a daily basis (©FAO, 2018a, 2018b). Although being produced worldwide under diverse environmental conditions, the required optimum temperature for wheat anthesis and grain filling is from 12 to 22 ˚C (Tewolde et al., 2006). Each degree Celsius increase reduces wheat yield by 4.1% to 6.4% (Liu et al., 2016). Several yield parameters are affected by high temperatures as vegetative weight and grain number and weight, as reviewed in (Akter and Islam, 2017). Grain number is strongly affected by high temperatures, especially between spike initiation and anthesis (Farooq et al., 2011). Grain mass is reduced with high temperature after anthesis, particularly when the treatment is imposed in early stages (Gibson and Paulsen, 1999; Castro et al., 2007). Heat stress also shortens grain filling duration, as reviewed in (Altenbach, 2012), affecting starch and storage protein deposition. During grain filling, the activity of starch synthesis enzymes is moreover reduced with temperatures above 30 ˚C (Jenner, 1994; Hurkman et al., 2003), decreasing even more starch content. On the other hand, during grain filling, high temperature has been reported to increase protein grain content, as kernel size is smaller, and this augment seems to be higher when high temperatures are imposed in early stages of grain filling (Corbellini et al., 1998; Daniel and Triboi, 2001; Castro et al., 2007).

The study of wheat grain composition is fundamental since it could be associated with variations in breadmaking performance and nutritional quality. However, classical analytical methods are usually time consuming and laborious. Infrared spectroscopy, on the other hand, is a rapid, non-invasive methodology that can detect a range of functional groups and changes in molecular structure. Chemical mapping using ATR-FTIR (attenuated total reflection Fourier transform infrared) spectra have clear and easily identifiable peaks that correspond to specific bonds and functional groups and has been successfully applied to a wide range of cereals and food and feed products (Che Man et al., 2005; Syahariza et al., 2005; Philippe et al., 2006; Antunes et al., 2016; Sujka et al., 2017; Prates et al., 2018). This technique was already used in wheat to assess endosperm cell-wall composition, grain infection, and flours quality control (Philippe et al., 2006; Toole et al., 2007; Singh et al., 2017; Sujka et al., 2017).

Wheat is one of the crops most affected by the increase in mean temperature during the growth season (Semenov and Shewry, 2011; Teixeira et al., 2013). Climate changes enhance the frequency of extreme heat events in Portugal (Cardoso et al., 2019). Thus, it is becoming urgent to acquire a deeper understanding of their effects in yield and nutritional parameters, such as protein content of wheat varieties, to enrich breeding programs. In the present work, we aim to evaluate the effect of a short-term high temperature, impose at the initial stages of grain filling, on grain

yield and quality in distinct bread wheat varieties recommended to be produced in Portugal. Comparative analysis of both yield and ATR-FTIR spectra provide evidence of intervarietal diversity in high temperature response, with alterations on grain number, weight and macro components, such as starch and protein. In addition, a model based on ATR-FTIR was established to allow the expeditious estimation of protein content based on elemental analysis data.

3.3 Materials and methods

3.3.1 Plant material

The bread wheat (*Triticum aestivum* L., 2*n*= 6x = 42, AABBDD) commercial varieties studied in this work were selected from the List of Bread Wheat Varieties Recommended for Portugal (ANPOC et al., 2014). This list was established, considering the phenological, agronomic, and technological traits. Varieties used were Almansor, Antequera, Bancal, Estero, Nabão, Pata Negra, and Roxo, and seeds were gently supplied by INRB/INIAV Portugal (National Institute of Biological Resources) and ANSEME, Portugal (National Association of Seed Producers and Traders). Twenty seeds (obtained after two years of controlled propagation) from each variety were germinated and grown in control conditions—8 h of darkness at 20 ˚C and a 16 h light period divided into 6 h with increasing temperature to 25 ˚C, 4 h at 25 ˚C, and 6 h decreasing to 20 ˚C. Three-week old plants were transferred to soil pots and maintained in greenhouse conditions.

When the first anther was observed in the first spike (anthesis), plants were again transferred to growth chambers with the previously described conditions. Ten days after anthesis (daa) subsets of ten plants each were submitted to two different growth conditions for seven days. Ten plants were maintained in the same (control) conditions, and another ten were submitted to a high temperature (HT) treatment in which the 16 h daylight period was initiated by a gradual increase in temperature from 20 to 40 ˚C during 6 h, followed by exposure to 40 ˚C for 4 h, and a subsequent gradual decrease to 20 ˚C during 6 h (Supplemental Figure 3.1). After treatments, all plants were transferred to the greenhouse and maintained until the end of the growing cycle. All further analyses were performed only in seeds from the first spike to guarantee identical developmental stages during HT treatments. For grain ATR-FTIR spectra analyses and nitrogen content quantification, the embryo was removed, simulating germen industrial removal procedure for flour production.

3.3.2 Yield evaluation

Yield parameters were evaluated in all plants of the seven varieties studied in both control and treatment conditions. The number of grains/spike and grain weight/spike (g/spike) were assessed in the first spike of each plant. The average weight of 10 grain (g/10 kernels) was deduced from the last two.

3.3.3 ATR-FTIR spectroscopy

Before ATR-FTIR spectra acquisition, grains were previously weighted, ball-milled in a Cryomill (Retsch GmbH, Haan, Germany), and lyophilized overnight. Spectra were acquired on a minimum of eight single kernels per variety and per condition (control and high temperature treated).

Single kernel flours FTIR spectra were recorded with a Bruker-P Alpha spectrometer (Bruker, Ettlingen, Germany) equipped with a single reflection diamond ATR accessory. The spectra were obtained between 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}. Each spectrum was the average of 24 scans. Processing of the spectra was performed with OPUS software V. 8.0 (Bruker Optics, Ettlingen, Germany). For comparison of the average spectra by variety, spectra were Min-Max normalized between the minimum at 1800 cm^{-1} (set to zero) and the maximum (set to 2) bellow 895 cm^{-1}.

Partial least squares (PLS) regression models were calculated by regressing the vector normalized spectra information against nitrogen concentration for 42 samples (calibration), using OPUS/QUANT V 8.0 (Bruker Optics, Ettlingen, Germany). Vector normalization normalizes a spectrum by first calculating the average intensity value and subsequent subtraction of this value from the spectrum. Then the sum of the squared intensities is calculated, and the spectrum is divided by the square root of this sum. The vector norm of the resulting spectrum is always 1. The number of principal components was selected according to the minimum root-mean-square error of cross-validation (RMSECV) by the "leave one out" method, i.e., each sample is left out of the model formulation and predicted once. The nitrogen content of the remaining samples was predicted, and 14 samples, covering the range of predicted nitrogen values, were selected for further reference analysis and model validation. The quality of the model was assessed by the statistics of the validation including the coefficient of determination (R^2), the random mean square error of prediction (RMSEP), and the residual prediction deviation or ratio of performance to deviation (RPD), calculated as the ratio of two standard deviations; the standard deviation of the reference data for the validation set (Williams and Sobering, 1993). The nitrogen content values obtained were used to calculate the protein content using the conversion factor of 5.7.

3.3.4 Elemental analysis

The nitrogen content was quantified in the flour of three individual grains per variety/condition, at the REQUIMTE@UCIBIO-FCT-UNL analytical laboratory using a Flash EA1112 CHNS analyzer (Thermo Finnigan CE Instruments, Milan, Italy) equipped with a gas chromatography column and a thermal conductivity detector.

3.3.5 Data analysis

To compare yield parameters and protein content between varieties, values were fitted to a linear model (ANOVA with one factor with fixed effects) and analyzed through a multiple means comparison test (Tukey test). The individual effect of the HT treatment in comparison with control condition for each variety was tested using *t*-test. Models were fitted in R using aov and Tukey. HSD functions.

3.4 Results and Discussion

Yield and grain composition parameters were comparatively evaluated between the varieties for control and high temperature treated plants.

3.4.1 HT treatment effects on grain yield parameters disclosed intervarietal diversity

The number of grains/spike, grain weight/spike (g/spike), and the average weight of ten grain (g/10 kernels) of both control and treatment plants are presented in Figure 3.1.

The mean number of grains/spike in control conditions revealed no significant differences between varieties, with values ranging between 10 (Pata Negra) and 14.43 (Nabão). On the other hand, under control conditions, first spike grain weight revealed significant differences between varieties. Bancal presented a value significantly higher (0.49 g) than Antequera (0.22 g) and Pata Negra (0.21 g), and Bancal ten grains' weight (0.32 g) was also significantly higher than Antequera (0.19 g), and Pata Negra (0.21 g).

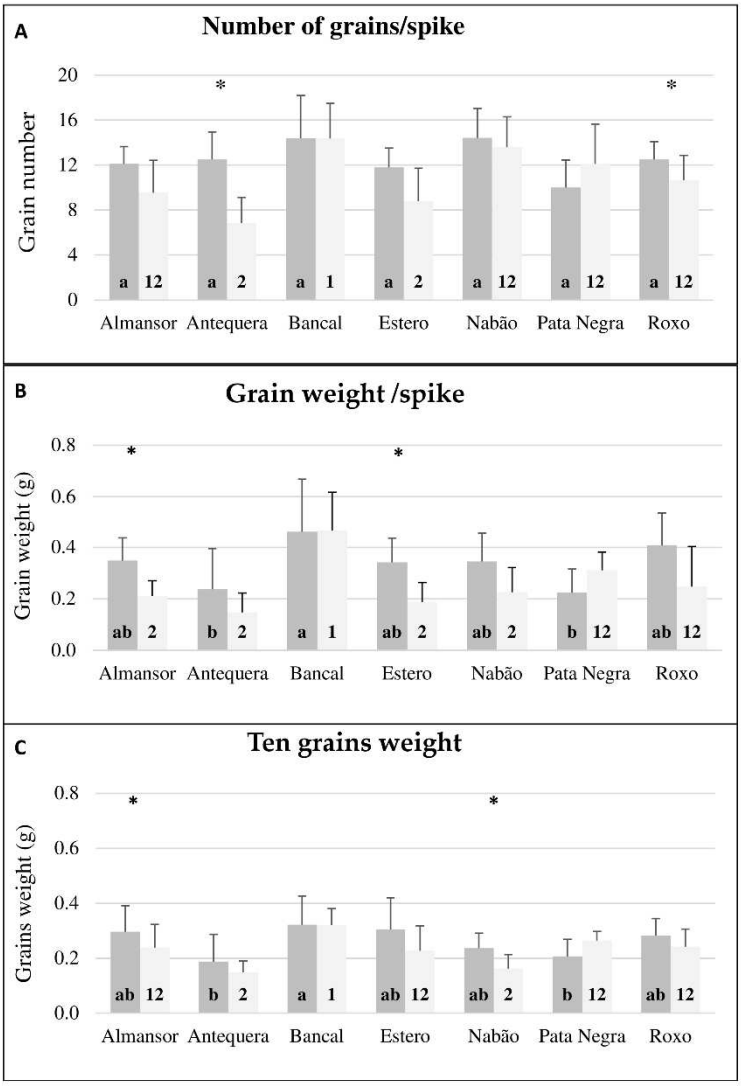

Figure 3.1 Grain yield parameters. A -Number of grains/spike **B** -grain weight/spike and **C** -ten grains weight of plants, kept in control conditions (dark gray) and high temperature treatment (light gray). Means ± standard deviation (represented as bars). Different letters (control) and numbers (treatment) inside bars indicate ANOVA significant differences between varieties detected by multiple means comparison test. (*) indicates t-test statistical differences between control and treatment in each variety ($p < 0.05$).

The evaluation of high temperature treatment effects on each variety revealed that the number of grains per spike was not significantly affected ($p < 0.05$) in most of the varieties analyzed except in Almansor and Roxo that denoted a decrease. Previous works indicated that major differences in grain number are caused by heat treatments before or during anthesis, as reviewed in (Farooq et al., 2011), as they affect meiosis and fertilization. In fact, grain abortions and reduction in grain number resulting from heat before and during anthesis were documented for a few cultivars (Stone and Nicolas, 1995; Hays et al., 2007). However, our results suggest that the effect of heat stress on grain number depends not only on the developmental phase affected

but is also influenced by the differential tolerance of each variety to heat stress. Regarding grain weight parameters, significant differences were observed in grain weight/spike of Almansor and Estero and ten grains weight of Almansor and Nabão. Interestingly, both grain weight parameters evaluated consistently increased in HT treated Pata Negra plants.

The comparison between varieties after imposition of high temperature revealed that Bancal was the least affected variety. It recorded higher values in all the three parameters assessed in comparison with all the other varieties. The mean number of grains/spike of Bancal (14.38) was significantly higher than Antequera (6.86) and Estero (8.8). In addition, Bancal grain weight/spike (0.49 g) was significantly higher than Almansor (0.21 g), Antequera (0.11 g), Estero (0.19 g), and Nabão (0.23 g). Regarding ten grains weight, Bancal also exhibited a value (0.32 g) significantly higher than Antequera (0.15 g),heat and Nabão (0.16 g). Although in most varieties, the values of the grain yield parameters studied tended to diminish, different responses to HT treatment were revealed. Bancal and Pata Negra seem to be the most promising varieties for wheat breeding strategies considering high temperature conditions since the former presented superior yield parameters in such conditions in comparison with all other varieties and the latter presented higher grain yield after HT treatment.

3.4.2 ATR-FTIR comparison of control and HT treated grains revealed complex responses

In this work, we have performed single kernel ATR-FTIR spectroscopy spectra from at least eight kernels per variety. Figure 3.2 shows the average spectrum in the wavenumber region 3700–780 cm^{-1} from Roxo variety control samples. All the spectra from the remaining varieties in both conditions presented the same bands with variations in intensities.

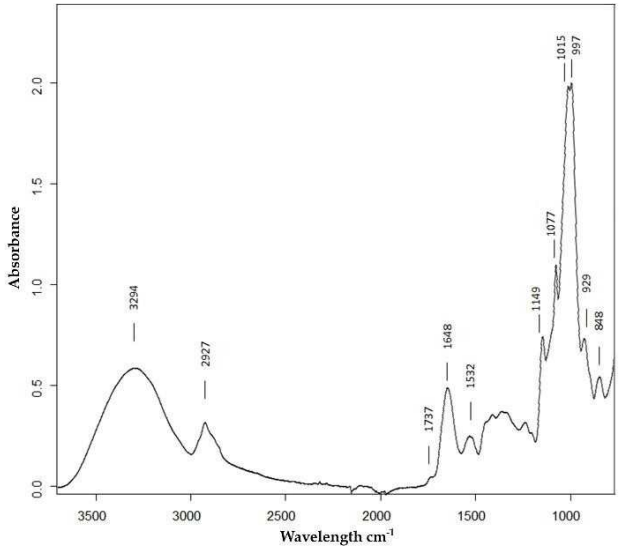

Figure 3.2 Average attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrum between 3700 and 780 cm^{-1} of the Roxo variety of milled grains in control conditions with the assignment of relevant bands.

The wheat flour spectra obtained were dominated by a large envelope with two intense bands with maxima at 1015 and 997 cm^{-1}, arising from C–O valence vibration in starch, and lower intensity ones at 1149, 1077, and 929 cm^{-1}, all typical saccharide bands arising mainly from starch that is the main component (60%–75%) of the wheat grains (Shewry, 2009). The band with a maximum at 2927 cm^{-1} assigned to the stretch vibration of CH_2 is also mainly from starch. The contribution of the proteins, the second most important component of wheat grain (Shewry, 2009), is clearly seen in the spectrum as two bands with maxima at 1648 and 1532 cm^{-1} from amide I and amide II, respectively. The contribution of N-H stretching from proteins molecules was detected around 3200, but in our spectra was masked by the broad band with a maximum at 3294 cm^{-1}, mainly assigned to O–H stretching from the starch polymer. A very weak band, in some cases only a shoulder, located at 1737 cm^{-1} could be from C=O stretching from lipids that, if present, would be in a small percentage.

The comparison of the intensity maxima at selected wavenumber bands between the average spectra of treatment vs. control for each variety is shown in Table 3.1. For the majority of varieties, spectra were normalized at 997 cm^{-1} (maximum) with three exceptions from control groups (Antequera, Estero, and Pata Negra), where maximum (normalization) occurred at 1015 cm^{-1}.

Table 3.1 Comparison between peaks' high of control and treated average spectra of wheat milled grains after Min-Max normalization.

Variety	Condition	Wavelength (cm^{-1})										
		Protein/Saccharides	**Fat/Saccharides**	**Fat**	**Amide**	**Amide**	**Saccharides**					
		O−H; N−H	**CH_2**	**C=O**	**I**	**II**	~1149	~1077	C−H	C−H	~929	~848
		~3294	**~2927**	**~1737**	**~1648**	**~1532**			~1015	~997		
Almansor	C	−	+	+	+	+	+	+	+	= 2	+	+
	HT	+	−	−	−	−	−	−	−	= 2	−	−
Antequera	C	−	−	~	++	++	+	+	= 2	++	−−	−−
	HT	+	+	~	−−	−−	−	−	−−	= 2	++	++
Bancal	C	=	+	+	+	+	=	+	+	= 2	~	~
	HT	=	−	−	−	−	=	−	−	= 2	~	~
Estero	C	+	+	+	++	++	~	+	= 2	~	~	+
	HT	−	−	−	−−	−−	~	−	~	= 2	~	−
Nabão	C	−	−	~	−−	−−	−	−	+	= 2	−−	−−
	HT	+	+	~	++	++	+	+	−	= 2	++	++
Pata Negra	C	+	+	+	+	+	−	~	= 2	~	−	−
	HT	−	−	−	−	−	+	~	~	= 2	+	+
Roxo	C	−	~	~	~	~	−	−	−	= 2	−−	−−
	HT	+	~	~	~	~	+	+	+	= 2	++	++

Note: (+) and (–) refers to higher and lower intensity, respectively (more than one symbol means bigger difference of intensities); =2 identifies the maximum at which the spectrum was normalized (1015 or 997 cm^{-1}).

Since the spectra were normalized for the most intense starch bands (1015 or 997 cm^{-1}), it was expected that changes would occur in the two bands from proteins, the second most abundant component of wheat grain (Shewry, 2009). In fact, differences between control and HT for each variety occurred in the intensity of amide I and II bands (1641 and 1532 cm^{-1}, respectively), particularly a reduction in Antequera, Estero, Almansor, Bancal, and Pata Negra, was observed although less intense in the last three varieties (Figure 3.3A). The opposite pattern was observed in Nabão, presenting more intense bands in samples from HT plants than in samples from control. For Roxo, no discernible difference was observed between control and HT spectra (Table 3.1 and Figure 3.3B and C).

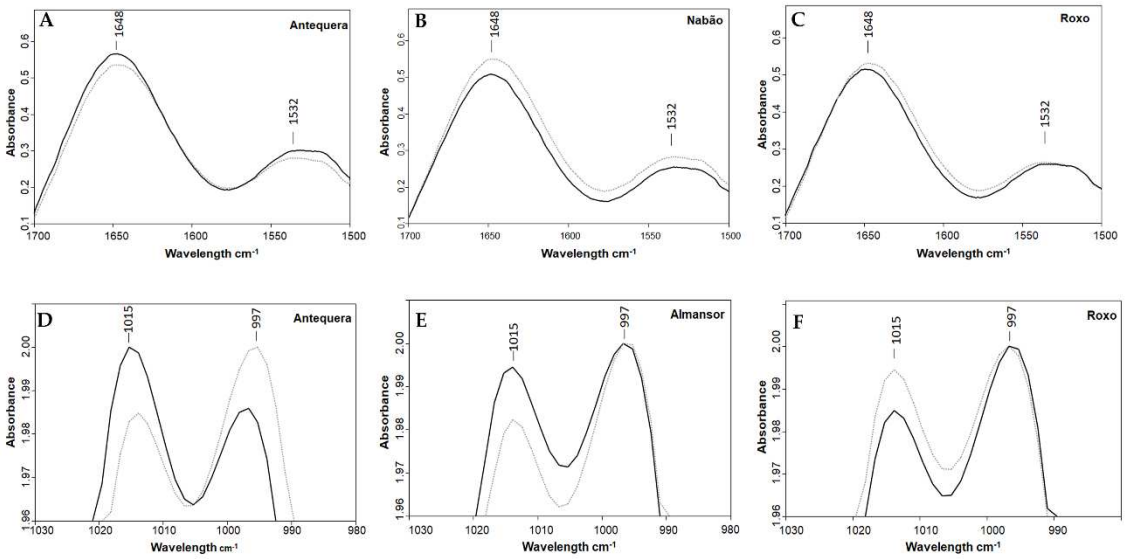

Figure 3.3 ATR-FTIR spectra of wheat milled grains representing different variations between control (dark lines) and HT treated (light lines) plants obtained in **A-C** amide I and II bands associated with protein, and in **D-F** 1015 and 997 cm−1 bands associated with starch.

Considering starch bands from spectra, in Almansor, Bancal, Nabão, and Roxo, the maximum of both control and HT samples spectra was in 997 cm−1 (Table 3.1, Figure 3.3E, F). On the other hand, in Antequera, Estero, and Pata Negra, it was possible to observe a shift between both conditions in the maxima of the spectra, in which the maximum was in 1015 cm−1 in control samples. In treated grains, the maximum was in the 997 cm^{-1}, and this variation was more pronounced in Antequera, as observed in Figure 3.3D. These results represent the absence of intervarietal differences in polysaccharides composition previously observed in control conditions, clearly indicating a marked effect of HT in polysaccharides synthesis. This was already obtained in transcription levels of genes associated with grain quality traits and protein fractions evaluations after high temperature treatment during grain filling developmental phase (Tomás et al., 2020). Furthermore, bands with maxima at 929 and 848 cm−1 have higher relative absorbance in grains from HT plants of Antequera, Nabão, Pata Negra, and Roxo in comparison to the ones obtained from

control plants, while the opposite was observed in Almansor and one Estero peak. On the other hand, bands with the maxima at 1149 and 1077 cm^{-1} were only slightly affected by HT, since relative absorption of one or both peaks diminished in Almansor, Antequera, Bancal, and Estero and was enhanced in Nabão, Pata Negra, and Roxo. Using those peaks as an inference for the amount of starch, we can speculate that HT induced in Nabão and Roxo an unexpected increase in these grains' constituent. This novel result obtained through ATR-FTIR spectra analysis and predicted protein content contrasts with Hurkman et al.'s (2003) report of starch content decrease induced by longer periods at 37 ºC in only one wheat variety, or a slower deposition rate observed in plants of different varieties submitted to similar temperatures (Jenner, 1994).

It is also possible to see the bands that are indicative of the presence of lipids, namely, the C–H stretch at ~2927 cm^{-1} and a peak from the ester linkage at ~1737 cm^{-1} (Warren et al., 2015). Both peaks presented lower relative absorption in samples from treated grains of Almansor, Bancal, Estero, and Pata Negra, while in Antequera and Nabão, the 2927 cm^{-1} peak was more intense in treated plants spectra, and the 1737 cm^{-1} peak was very similar in both conditions (Table 3.1). Again, Roxo appeared to be less affected, having both spectra very similar relative absorptions. The contribution of these lipids fraction peaks should be negligible as it constitutes 3%–4% of the whole grain. Moreover, the embryo, which is responsible for one-third of the wheat grain lipid fraction (Wrigley et al., 2009), was removed before grain milling. Even so, four varieties presented less intense peaks in treated grains. Interestingly, as already observed in amide I and II protein peaks, no significant variation was detected in Roxo lipids fraction peaks revealing higher stability coping with high temperatures treatments.

3.4.3 Calibration and validation of the model for nitrogen content based on ATR-FTIR spectra

High correlations were obtained between spectral data and nitrogen content both for cross-validation and validation (Figure 3.4). The nitrogen content values obtained by elemental analysis (Supplemental Table 3.1) for calibration set ranged between 1.7 and 2.7 mg of N/100 mg of flour. The model obtained had good statistics: R^2 = 0.89, RMSECV = 0.10, RPD =3.5. Validation set samples, comprising twelve single kernels, were selected covering the entire range of nitrogen values predicted by the model and additionally four outsider samples with values above the range in the model. The predicted nitrogen values ranged from 1.8 to 3.2 mg of N/100 mg of flour. The validation statistics (R^2= 0.96, RMSEP= 0.10, and RPD=4.3) show that the model correctly predicts the nitrogen content, including the four outsider samples. Mean protein values of each variety (Figure 3.5) and conditions were obtained from nitrogen values predicted by the model established multiplied by the conversion factor of 5.7x (Caporaso et al., 2018).

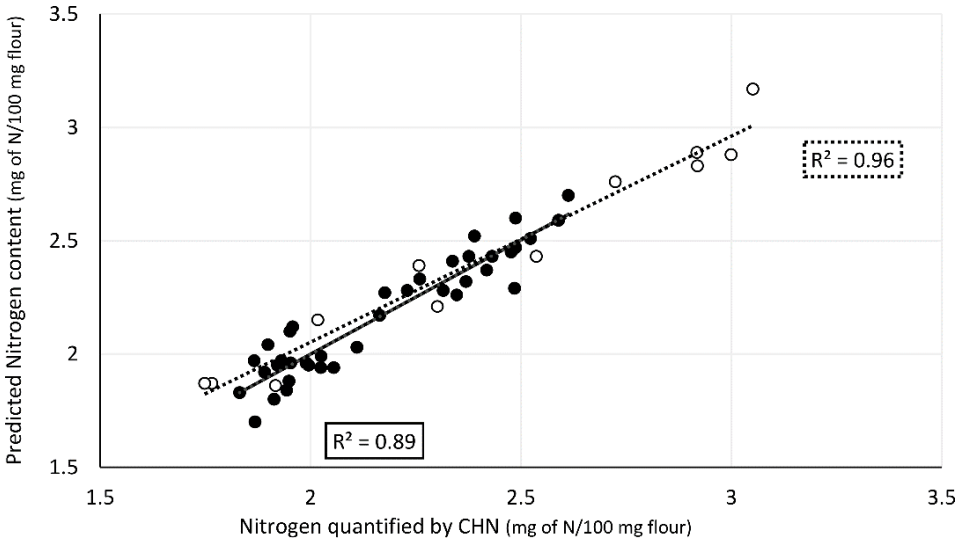

Figure 3.4 Correlation between predicted nitrogen content in flour from single wheat kernels using ATR-FTIR spectral region 1800–500 cm^{-1} and nitrogen content determined by elemental analysis. Calibration dataset (n = 42) in black and validation dataset (n = 12) in white.

Protein content values obtained in all samples analyzed in this study (control or HT treated) ranged between 9.5 and 21.4%. These values are in accordance with a recent report (Caporaso et al., 2018) that surveyed protein contents ranged from 6.2 to 19.8% in samples from the UK, Canada, France, Italy, Germany, and Eastern Europe grown under a wide diversity of agronomic and climatic conditions. The global results obtained from plants control or HT treated (Figure 3.6, dark grey and light grey bars, respectively) revealed a tendency to average protein content reduction induced by HT (from 13.4% and 12.9%, respectively).

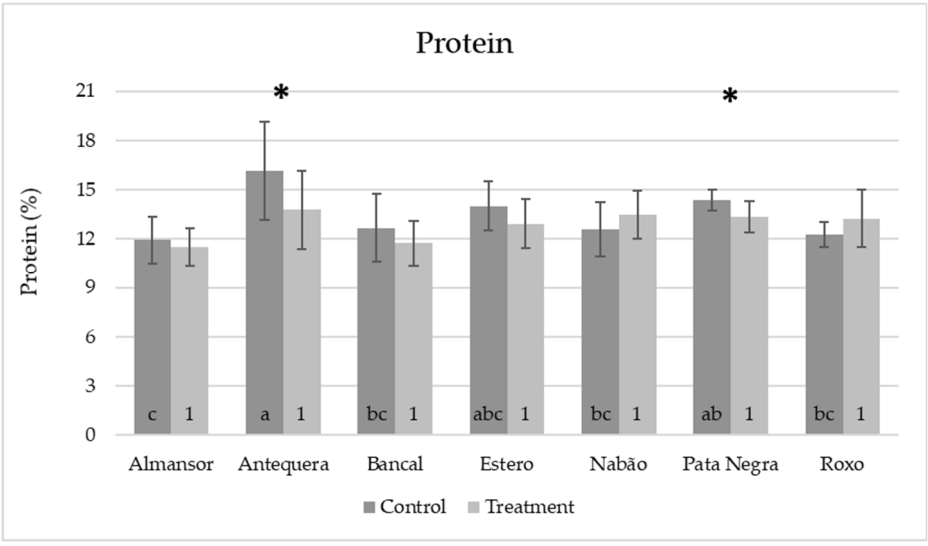

Figure 3.5 Mean values of predicted protein contents in single milled grains from control (dark gray) and high temperature treated (light gray) plants. Means and standard deviation values (represented as bars). Different letters (control) and numbers (treatment) inside bars indicate ANOVA significant differences between varieties detected by multiple means comparison test. (*) indicates t-test statistical differences between control and treatment in each variety (p< 0.05).

The comparative analysis between varieties maintained in control conditions (Figure 3.5, dark grey bars) revealed that the protein content of Antequera was significantly higher than the ones obtained in Almansor, Bancal, Nabão, and Roxo. In Almansor, this value was also significantly lower than Pata Negra. However, as can be seen through the analysis of light gray bars in Figure 3.5, such significant intervarietal differences were no longer observed after HT treatment, which was also evidenced by the lower range of average protein content per variety in grains from treated plants (2.0%) in comparison with the range observed in control ones (4.2%). Again, the comparison of all control vs. HT values obtained, regardless the genotype presented in Figure 3.6, show that the dispersion of protein content values was lower in the treatment dataset, as well as the standard deviation values obtained, which diminished from 2.20 in control condition to 1.76 in HT treated plants.

The comparison between the average protein content of each variety control and HT samples showed significant variation in Antequera and Pata Negra varieties corresponding to a decrease in the predicted protein contents (from 16.3% to 13.4% and 14.7% to 13.7%, respectively, Figure 3.5). These results were in accordance with the ones obtained for amide I and II bands peaks described above, validating ATR-FTIR comparative assessment consistency. Moreover, single-seed analysis possible with this methodology unraveled intervarietal quality diversity that may be valuable across variable climatic conditions, as suggested by (Mitchell et al., 2016).

Considering the relation between changes in protein content and grain weight, previous works suggested that rising temperature during grain-filling results in shrunken grain with increased protein content (Corbellini et al., 1998; Daniel and Triboi, 2001; Castro et al., 2007).

However, we only observed significant variation in both parameters in the Pata Negra variety that revealed an increase in grain weight and a decrease in protein content. This result highlights once again the novelty of the present work disclosing intervarietal diversity in plant response to cope with heat stress.

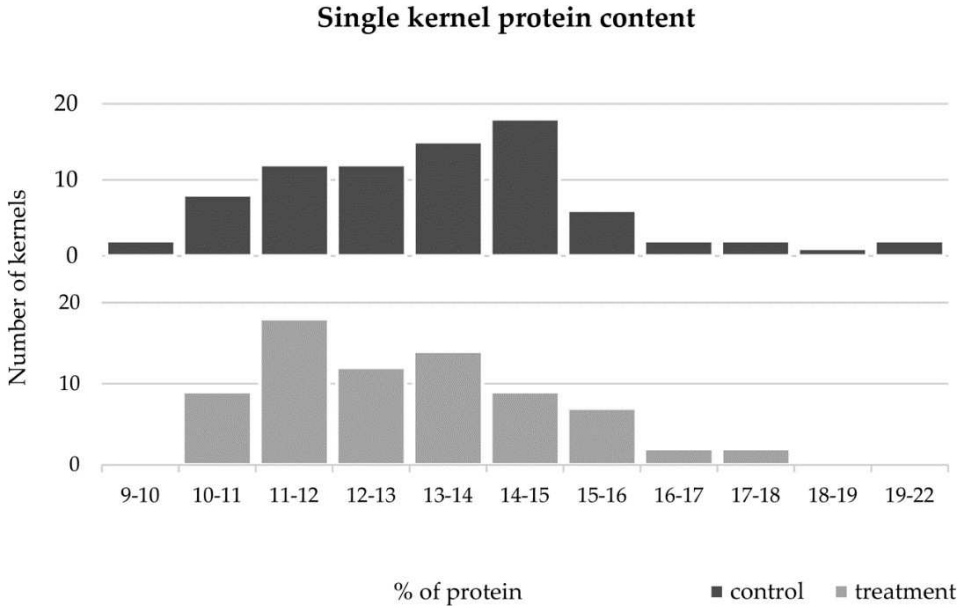

Figure 3.6 Distribution of single grains protein content from untreated (black) and high temperature treated (light gray) plants.

3.5 Conclusions

This work contributes to understanding the distinct response of different varieties to heatwave events that are increasingly common and intense in Portugal. Altogether our results clearly unravel that HT treatment impact on grain composition parameters leads to lower intervarietal diversity. A similar effect of short-term HT treatment, imposed during grain filling, was previously reported not only regarding transcription levels of genes related to grain quality but also in the proportions of distinct protein fractions (Tomás et al., 2020).

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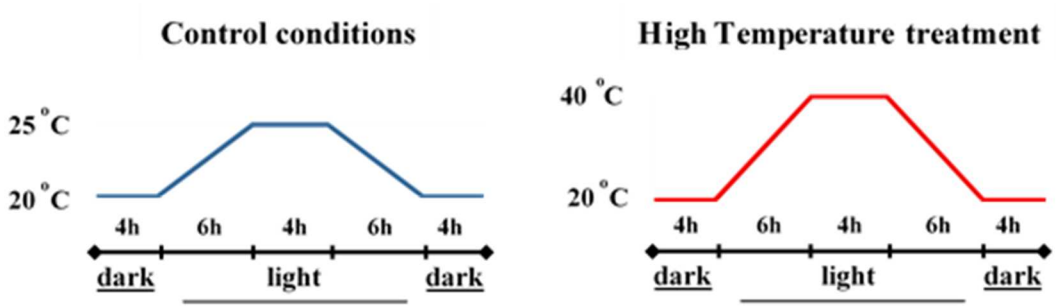
3.7 Supplemental material



Notes: One-week High Temperature (HT) treatment, mimicking a heatwave, was performed ten days after anthesis in growth chambers with controlled conditions at 8h dark / 16h light cycle. During the 16 h light, a progressive temperature increase from 20 ºC to 40 ºC was implemented (20ºC to 25 ºC in control), temperature was maintained 4h at 40ºC and then progressive decreased back to 20 ºC.

Supplemental Figure 3.1 Wheat plants growth conditions.

Supplemental Table 3.1 Single grain nitrogen content quantification by elemental analysis in mature grains of three plants of each genotype in both conditions.

	Control			HT Treatment		
	1	2	3	1	2	3
Almansor	1.97	1.92	1.7	1.96	1.81	1.94
Antequera	2.28	2.43	2.37	2.52	2.12	1.8
Bancal	1.95	2.04	2.28	1.83	1.88	1.83
Estero	2.71	1.96	2.03	2.29	2.60	2.59
Nabão	2.43	2.33	1.97	2.47	2.51	2.27
Pata Negra	2.15	2.32	2.7	2.45	2.26	2.41
Roxo	1.94	1.95	1.99	2.1	1.84	2.17

Notes: Values presented were obtained in the REQUIMTE@UCIBIO-FCT-UNL analytical laboratory using a Flash EA1112 CHNS analyzer (Thermo Finnigan CE Instruments, Italy) equipped with a gas chromatography column and a thermal conductivity detector. The three values presented per variety / temperature condition correspond to three biological replicates.

Chapter IV

Assessment of Four Portuguese Wheat Landrace Diversity to Cope With Global Warming

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Tomás, D., Coelho, L. P., Rodrigues, J. C., Viegas, W., and Silva, M. (2020a). Assessment of Four Portuguese Wheat Landrace Diversity to Cope With Global Warming. *Front. Plant Sci.* 11, 1803. doi:10.3389/fpls.2020.594977.

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Assessment of Four Portuguese Wheat Landrace Diversity to Cope With Global Warming

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Wheat is a dietary staple consumed worldwide strongly responsible for proteins and carbohydrate population intake. However, wheat production and quality will scarcely fulfill forward demands, which are compounded by high-temperature (HT) events as heatwaves, increasingly common in Portugal. Thus, landraces assume crucial importance as potential reservoirs of useful traits for wheat breeding and may be pre-adapted to extreme environmental conditions. This work evaluates four Portuguese landrace yield and grain composition through attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, particularly protein content, and their responses to HT treatment mimicking a heatwave. Landraces showed distinct yield traits, especially plant height and first spike grain number, and a similar pattern in FTIR spectra, although revealing differences in grain components' proportions. Comparison between spectra band intensity indicates that Ardito has the highest protein-related peaks, contrary to Magueija, which appears to be the landrace with higher lipid content. In plants submitted to 1 week of HT treatment 10 days after anthesis, the first spike grain size and weight were markedly reduced in all landraces. Additionally, it was observed that a general increase in grain protein content in the four landraces, being the increment observed in Ardito and Grécia, is statistically significant. The comparative assessment of control and HT average FTIR spectra denoted also the occurrence of alterations in grain polysaccharide composition. An integrated assessment of the evaluations performed revealed that Ardito and Magueija landraces presented diverse yield-related characteristics and distinct responses to cope with HT. In fact, the former landrace revealed considerable grain yield diminution along with an increase in grain protein proportion after HT, while the latter showed a significant increase in spikes and grain number, with grain quality detriment. These results reinforce the relevance of scrutinizing old genotype diversity seeking for useful characteristics, particularly considering HT impact on grain production and quality.

Keywords: bread wheat, landraces, heatwave, yield, grain composition, protein content

4 Assessment of Four Portuguese Wheat Landrace Diversity to Cope With Global Warming

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4.1 Abstract

Wheat is a dietary staple consumed worldwide strongly responsible for proteins and carbohydrate population intake. However, wheat production and quality will scarcely fulfill forward demands, which are compounded by high-temperature (HT) events as heatwaves, increasingly common in Portugal. Thus, landraces assume crucial importance as potential reservoirs of useful traits for wheat breeding and may be pre-adapted to extreme environmental conditions. This work evaluates four Portuguese landrace yield and grain composition through attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, particularly protein content, and their responses to HT treatment mimicking a heatwave. Landraces showed distinct yield traits, especially plant height and first spike grain number, and a similar pattern in FTIR spectra, although revealing differences in grain components' proportions. Comparison between spectra band intensity indicates that Ardito has the highest protein-related peaks, contrary to Magueija, which appears to be the landrace with higher lipid content. In plants submitted to 1 week of HT treatment 10 days after anthesis, the first spike grain size and weight were markedly reduced in all landraces. Additionally, it was observed that a general increase in grain protein content in the four landraces, being the increment observed in Ardito and Grécia, is statistically significant. The comparative assessment of control and HT average FTIR spectra denoted also the occurrence of alterations in grain polysaccharide composition. An integrated assessment of the evaluations performed revealed that Ardito and Magueija landraces presented diverse yield-related characteristics and distinct responses to cope with HT. In fact, the former landrace revealed considerable grain yield diminution along with an increase in grain protein proportion after HT, while the latter showed a significant increase in spikes and grain number, with grain quality detriment. These results reinforce the relevance of scrutinizing old genotype diversity seeking for useful characteristics, particularly considering HT impact on grain production and quality.

Keywords

bread wheat, landraces, heatwave, yield, grain composition, protein content

4.2 Introduction

Wheat (*Triticum aestivum* L.) is a major cereal consumed worldwide on a daily basis (FAO, 2017). However, the global mean growth rate of wheat is not sufficient to cover the production predicted to be necessary in 2050 (Ray et al., 2013), and one of this limitation causes is the progressive global warming (Gaupp et al., 2019). In fact, the increase in mean temperature during wheat development was predicted to reduce grain production (Asseng et al., 2014; Wang et al., 2019).

Major effects of high temperature (HT) on wheat plants include decrease in pollen viability, plant cycle shortening, as well as deterioration of chlorophyll and reduction of photochemical efficiency with consequent grain number diminution and kernel shrinkage (reviewed in Akter and Islam, 2017). Temperatures above 30°C after anthesis, in the early stages of grain filling, accelerate plant development leading to smaller and shrunken grains (Altenbach et al., 2002, 2003). This reduction in grain development time caused by heat decreases starch and protein deposition, affecting grain composition and final quality (reviewed in Farooq et al., 2011). Several reports suggested that a HT induces higher grain protein content as kernel size is smaller, and this augment seems to be more pronounced when HTs are imposed in early stages of grain filling (Corbellini et al., 1998; Daniel and Triboi, 2001; Castro et al., 2007). However, distinct stress responses were registered in different wheat genotypes commercially available with a reduction in both kernel weight and protein content in some varieties (Tomás et al., 2020a). In this context, it is particularly relevant to comparatively assess the variability of distinct commercial varieties (Pradhan et al., 2019) and also, more importantly, the old and traditional landraces, considering the eroded genetic pool of commercial varieties that resulted from decades of homogenization through breeding.

Landraces provided notable successes in crop improvement (reviewed in Dwivedi et al., 2016). Wheat landraces, defined as traditional varieties with potential higher tolerance to biotic and abiotic stresses, present better yield stability under low input agricultural system (Zeven, 1998). Thus, landraces may constitute extremely valuable agrobiodiversity pools assuming a prominent role in the actual unpredictable weather conditions (Lopes et al., 2015; Alipour et al., 2017).

The effects of extreme heat events particularly frequent in Portugal, like heatwaves (Cardoso et al., 2019), defined as five or more consecutive days of heat in which the daily maximum temperature is at least 5°C higher than the average maximum temperature (WMO, 2015), have been studied in wheat commercial varieties. Those reports showed that HT treatments mimetizing heatwaves during grain filling leads to lower intervarietal diversity in transcription levels of genes related to grain quality and in the proportions of distinct protein fractions (Tomás et al., 2020b), as well as in grain polysaccharide composition and global protein content (Tomás et al., 2020a). Thus, it is crucial to assess the biodiversity enclosed in landraces to cope with a broad range of environmental conditions. The objective of this work was to assess the effect of a short period of HT during grain filling in distinct Portuguese landraces on grain yield and composition with special focus on protein content as one of the most determinant parameters of grain quality. Our results

revealed distinct responses to HT treatment concerning most yield parameters, except grain weight, and a concordant general increase in protein content and reduction in starch amount. Additionally, landraces presenting distinct responses to HT treatment imposed during grain filling were identified.

4.3 Materials and methods

4.3.1 Plant material

Bread wheat (*T. aestivum* L., 2n = 6 = 42, AABBDD) old Portuguese landraces from Vasconcellos collection, established in the 1930s of the last century (Vasconcellos, 1933), were used in this work—Ardito, Grécia, Magueija e Ruivo. These landraces were selected considering a previous study of photosynthetic rate and thousand grain weight (Scotti-Campos et al., 2011). The seeds used were obtained after 2 years of controlled propagation under equal environmental conditions of material gently supplied by EAN Germplasm Bank (Oeiras, Portugal, PRT005). Twenty seeds from each landrace were simultaneously germinated and grown in controlled conditions—8 h of dark at 20°C followed by 16 h of light period divided into 6 h increasing to 25°C, 4 h at 25°C, and 6 h decreasing to 20°C. Three weeks after germination, plants in the growth stage between 1.3 and 1.4 Zadoks code (Zadoks et al., 1974) were transferred individually to 7-L soil pots and maintained in greenhouse conditions.

When the first anther was observed in the first spike (anthesis), plants were transferred to growth chambers with the previously described conditions. A HT regime with a daily plateau of 40°C maximum temperature (Supplemental Figure 4.1) was imposed to subsets of 10 plants (independent biological replicates) of each landrace, 10 days after anthesis beginning–anthesis complete (Zadoks decimal code 61) (Zadoks et al., 1974) in each plant, thus occurring in distinct dates (flowering times presented in Supplemental Table 4.1) for each wheat landrace/plant evaluated. After treatments, plants were kept in the greenhouse until the end of the lifecycle. All yield and grain composition analyses were performed exclusively in seeds from the first spike to guarantee identical developmental stages during HT treatments. For grain composition analyses through ATR-FTIR spectroscopy and elemental analysis, the embryo was removed from each kernel, simulating germen industrial removal procedure for flour production.

4.3.2 Yield evaluations

Yield parameters were evaluated in all plants of all varieties in both control and treatment conditions after the plants reached harvest maturity, corresponding to at least eight independent biological replicates for each genotype/condition. The parameters evaluated per plant were height, area,

and number of spikes; and in the first spike were length, number of grains, and grain weight. The average weight of 10 grains (g/10 kernels) was deduced from the two later data.

Plant area was calculated through the analysis of mature plant images (Supplemental Figure 4.2). At the end of the growing cycle, the plant shoot system was photographed with a

Nikon D90 camera using a black background for easier software segmentation, with constant light conditions and image capture parameters (exposure time, aperture, and ISO speed). Raw images were quantified using ImageJ software (United States) with Fiji platform (Schindelin et al., 2012).

4.3.3 ATR-FTIR spectroscopy

For attenuated total reflection Fourier transform infrared (ATR- FTIR) spectra acquisition, four grains of the first spike were pooled from each plant, and a minimum of eight independent biological replicates per variety and per condition (control and HT treated) were evaluated. Grains were ball-milled in a Cryomill (Retsch GmbH, Haan, Germany) after embryo removal, and all samples obtained were lyophilized overnight. Flours ATR- FTIR spectra were recorded with a Bruker-P Alpha spectrometer (Bruker, Ettlingen, Germany) equipped with a single-reflection diamond ATR (attenuated total reflection) accessory. The spectra were obtained between 4,000 and 400 cm^{-1} with a resolution of 4 cm^{-1}, and each spectrum was the average of 24 scans corresponding to technical replicates. Processing of the spectra was performed with OPUS software Vsn. 8.0 (Bruker Optics, Ettlingen, Germany). The average spectra were calculated per landrace and condition and subsequently Min–Max normalized between the minimum at 1,800 cm^{-1} and the maximum between 1,800 and 895 cm^{-1}. For the nitrogen (N) prediction model, the partial least square (PLS) regression model obtained previously (Tomás et al., 2020a) was used to predict the landrace samples. After prediction, 10 samples covering the obtained N range were selected for nitrogen content quantification by elemental analysis. The spectra and values obtained (Supplemental Table 4.2) were included in the model, and a new model (further on referred as adjusted model) was obtained and further used to predict N content, which was then used to calculate protein content using the conversion factor of 5.7x (Caporaso et al., 2018).

4.3.4 Elemental analysis

The nitrogen content was quantified in flour of 10 samples (obtained as described for ATR-FTIR analysis) at the REQUIMTE@UCIBIO-FCT-UNL analytical laboratory using a Flash EA1112 CHNS analyzer (Thermo Finnigan CE Instruments, Italy) equipped with a gas chromatography column and a thermal conductivity detector.

4.3.5 Data analysis

To compare the yield parameters and protein content between varieties, values were fitted to a linear model (*ANOVA* with one factor with fixed effects) and analyzed through multiple means comparison test (*Tukey test*). The individual effect of HT treatment in comparison with control condition for each variety was tested using *t-test*, and χ^2 test was used to compare frequency distributions. Models were fitted in R using *aov* and *Tukey.HSD* (*agricolae* package) and *chisq.test* functions, respectively.

The principal component analysis (PCA) and clustering analysis (dendrogram) were made based on yield quantification data in RStudio using *prcomp* and *HCPC* functions, and *FactoMineR* and *factoextra* packages.

4.4 Results

In this work, plants of landraces Ardito, Grécia, Magueija, and Ruivo were submitted to HT treatment simulating a heatwave for 1 week during grain filling stage. Yield parameters were comparatively evaluated in the end of the lifecycle in these plants and in plants kept in control conditions. The results obtained were used to compare between landraces in each condition and to evaluate the HT effects on each landrace.

4.4.1 Landraces revealed different responses to HT treatment in yield parameters

The yield parameters considered are the following: (i) per plant— height, area, and spike number; (ii) in the first spike—length, grain number, grain weight, and 10 grains weight. The results obtained are summarized in Figure 4.1.

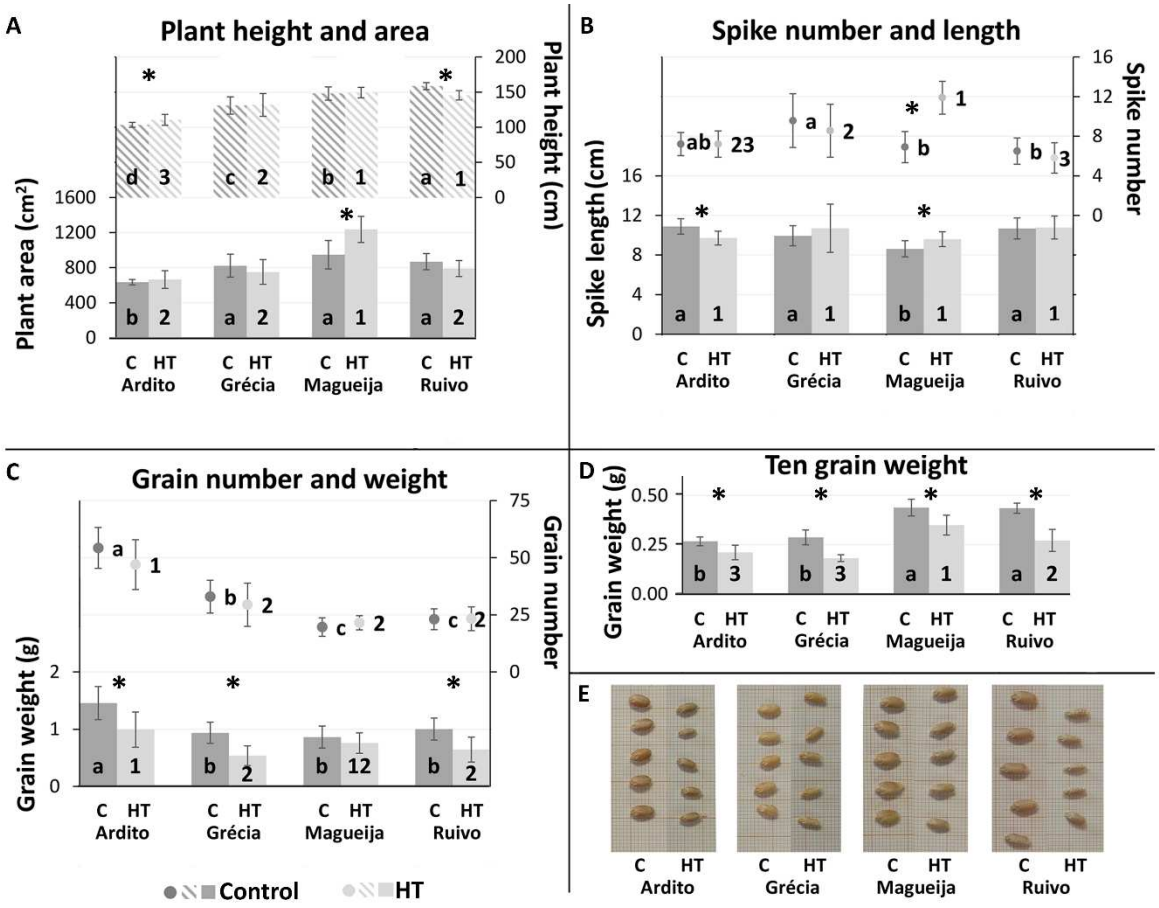

Figure 4.1 Yield parameter evaluation. A plant height (listed columns) and area (full columns). **B** Number of spikes per plant (dots) and first spike length (columns). **C** First spike number of grains (dots) and grain weight (columns). **D** First spike 10-grain weight. Mean values of plants kept in control conditions (dark gray) and high temperature (HT) treatment (light gray) ± standard deviation (represented as bars). Different letters and numbers indicate *ANOVA* significant differences between varieties, in control and high-temperature (HT) conditions, respectively. **t-test* statistical differences between control and treatment in each variety ($p < 0.05$). **E** Grains from the four landraces' plants kept in control conditions or HT treated.

Plant height and area, often used as predictor of the plant biomass (Armonienė et al., 2018), calculated for control condition, revealed that Ardito was the landrace with significant lower average values in both parameters (103.42 and 549.94 cm^2, respectively) in comparison with the other landraces (Figure 4.1A). However, only plant height values are significantly different among all landraces. On the other hand, HT treatment influenced significantly Ardito and Ruivo plant height, although in inverse ways. Ardito HT treated plants are 6.9% taller (110.52 cm), and Ruivo plants are 8.3% shorter (145.42 cm). Magueija HT treated plants showed an average area significantly higher (31%) than the control ones and the comparison between landraces submitted to HT conditions indicates that this value (1,166.37 cm^2) is significantly higher than other landrace plant areas (Figure 4.1A). Considering the average number of spikes per plant kept in control conditions,

Magueija and Ruivo presented the lowest average values (6.9 and 6.5, respectively), significantly different than the highest average number (9.6) shown by Grécia (Figure 4.1B). The comparison of the average number of spikes between control and HT-treated plants revealed a significant difference only in Magueija, with a remarkable increase of 72%, (from 6.9 to 11.9). Also, the comparison between landraces submitted to HT treatment revealed that Magueija plants showed a significantly higher number of spikes in comparison with all other genotypes (Figure 4.1B). The number of spikes was moreover influenced by the appearance of new tillers after the HT treatment period, during ripening, with subsequent additional spikes (Figure 4.2A). These late spikes were observed in all landraces, although not in all plants, and their average number per plant as well as the percentage of plants with late spikes are presented in Figure 4.2B. In control conditions, the average number of late spikes per plant observed ranged between 2 in Ardito and 1 in Ruivo, but no significant differences were observed between landraces. On the other hand, Ardito was the landrace in which we detected a lower percentage of control plants with late spikes (20%), and Magueija was the landrace with higher percentage (50%) (Figure 4.2B). In HT-treated plants, only Magueija landrace presented a significant increase in the average number of late spikes per plant compared with the control ones, from 1.6 to 3.6. Regarding the percentage of plants with late spikes, it was observed a significant increase in all the landraces except Grécia and all HT treated Magueija plants presented late spikes.

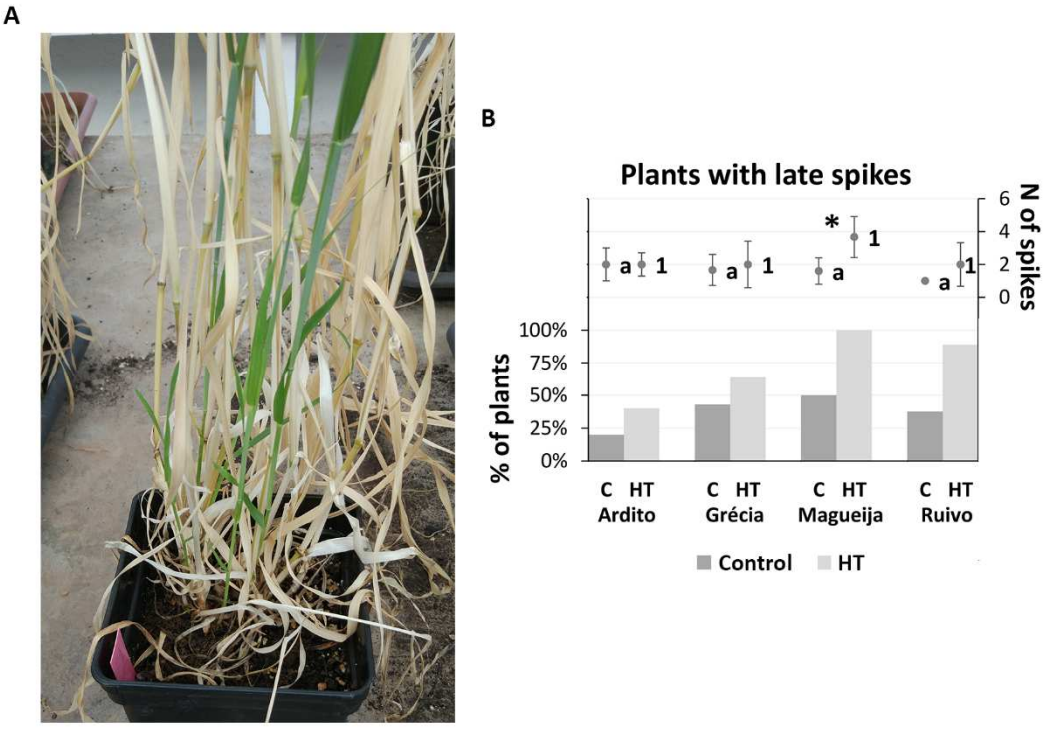

Figure 4.2 Late spike evaluation. **A** HT-treated plant of Magueija landrace presenting four late spikes. **B** Mean number of late spikes per plant in control (dark gray dots) and HT treated (light gray dots) plants ± standard deviation (represented as bars) and percentage of plants with late spikes in control (dark gray columns) and high temperature (HT) conditions (light gray columns). Different letters and numbers next to dots indicate *ANOVA* significant differences between landraces in both control and treatment conditions, respectively, and *indicates *t-test* statistical differences between control and treatment in each variety ($p < 0.05$)

Spike length and grain parameters (number and weight) were measured only in the first spike. Magueija plants kept in control conditions revealed to have the smallest spike with an average length of 8.6 cm, significantly lower than the other three landraces (Figure 4.1B), though it was the only landrace revealing a significantly larger spike in HT-treated plants in comparison with the control ones. Ardito HT-treated plants, on the other hand, showed a significant decrease in average spike length in comparison with the control plants. HT-induced alterations reduced the intervarietal variability observed regarding spike length since no significant differences were observed between landraces after HT treatment.

In accordance with the spike length, both grain number and grain weight/spike were also lower in Magueija plants maintained in control conditions (19.7 and 0.86 g, respectively) comparative to the other landraces (Figure 4.1C). On the other hand, Ardito was the landrace with significantly

higher values in these two parameters (54.3 and 1.46 g). Although HT treatment showed no significant effect in grain number, it induced a grain weight/spike decrease in all the varieties that was statistically significant in all landraces except in Magueija. The comparison between landraces submitted to HT treatment showed that Ardito has the significantly higher number of grains/spike of all landraces (47) and a higher grain weight/spike (0.99 g) than Grécia and Ruivo. Ten grain weight (Figure 4.1D) allows a more accurate assessment of the distinct developmental conditions' effects in plants' yield. In plants kept in control conditions, Magueija and Ruivo have higher values (0.44 g) in comparison with Ardito (0.27 g) and Grécia (0.29 g). This yield parameter was significantly lower in HT-treated plants of the four landraces (between 0.17 g in Ruivo and 0.06 g in Ardito), and Magueija remains the variety with significantly higher ten grain weight in plants submitted to HT treatment. This result is clearly illustrated by the comparison of grain size presented in Figure 4.1E since grains from treated plants are smaller in all the landraces.

4.4.2 HT impact in grain composition revealed by attenuated total reflection fourier transform infrared spectra

The spectra in the wavenumber region between 4,000 and 400 cm^{-1} obtained for the four landraces studied in each condition show no evident pattern differences, but the same bands presented intensity variations (Figure 4.3). The most intense bands in the region of 1,150 and 800 cm−1 are mainly from starch, including the most intense band of the spectra, with a maximum close to 997 cm^{-1}. The band with a maximum at 2,927 cm^{-1} assigned to the stretch vibration of CH_2 is also essentially from starch with a small contribution from proteins and lipids. The protein contribution, the second most important component of wheat grain, is clearly seen as two bands with maxima close to 1,648 and 1,532 cm^{-1} from Amide I and II, respectively. The broad band with maximum close to 3,294 cm^{-1} from O–H stretching of the starch polymer masks completely the NH band from proteins. A very weak band, in some cases only a shoulder, located at 1,745 cm^{-1}, could be from C = O stretching from lipids that if present at all would be in a very small percentage.

The comparison between landraces' min–max normalized spectra obtained for control conditions, regarding the maxima intensity at selected wavenumber bands, was performed (Figure 4.3A). This analysis unravels that Ardito has a more intense spectra than Magueija and Ruivo in half of the selected bands, including the band with maximum at 3,294 cm^{-1} assigned to starch polymer and Amides I and II bands. On the contrary, the average spectrum of grains from Magueija control plants was the most intense at the 1,745 cm^{-1} band (probably related with fat), and 2,927, 929, and 848 cm^{-1} starch-related bands. Globally, only for the Amide I, the four spectra are clearly separated, while for the other selected bands, at least two spectra have similar absorbance intensities.

The comparison of maximum intensity at selected wavenumber between the average spectra of grains from control and HT treated plants, after min-max normalization is shown in Table 4.1.

Overall, the spectra of grains from HT-treated plants are more intense than the ones obtained from grains of control plants for all four landraces. The only exceptions to these were the more intense Grécia and Magueija control spectra in the band with a maximum at 3,294 cm^{-1}, mainly assigned to O–H stretching from the starch polymer. Amide I and II proteins bands are the ones that revealed more relevant differences between control and HT. In fact, the intensity of HT Ardito and Grécia spectra is much higher than the control ones in both Amide bands, as well as in Ruivo regarding Amide I band. This increase in protein content is expected to be associated with a proportional reduction in starch grain content, as these are the main components of wheat grain, and the spectra normalization was done by the more intense band at 997 cm^{-1}, associated with starch. Although Ardito HT spectrum is also quite more intense than the control one in bands with a maximum at 1,149 and 1,077 cm^{-1}, and Grécia presented a greater difference between the control and HT spectra in 1,077 and 929 cm^{-1} bands, in both cases, the bands are associated with starch. These alterations suggest that the proportions of distinct polysaccharides may also be altered after HT treatment. Last, based on the 1,745- cm^{-1} band, the lipid fraction increases slightly in grains from HT-treated plants of Ardito, Grécia, and Ruivo.

Table 4.1 Comparison between peaks' high of average spectra of grains from control and treated landraces plants after min-max normalization.

Landrace	Condition	Wavelength (cm^{-1})										
		Starch/ Proteins	**Starch/Fat**	**Fat**	**Proteins**		**Starch**					
		OH, NH ~3294	**CH ~2927**	**C=O ~1745**	**Amide I ~1648**	**Amide II ~1532**	**C-O_C ~1149**	**1077**	**1015**	**997**	**929**	**848**
Ardito	**C**	**-**	**-**	**-**	**--**	**--**	**--**	**--**	**-**	=1	**-**	**-**
	HT	**+**	**+**	**+**	**++**	**++**	**++**	**++**	**+**	=1	**+**	**+**
Grécia	**C**	**+**	**-**	**-**	**--**	**--**	**-**	**--**	**-**	=1	**--**	**-**
	HT	**-**	**+**	**+**	**++**	**++**	**+**	**++**	**+**	=1	**++**	**+**
Magueija	**C**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	=1	**-**	**-**
	HT	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	=1	**+**	**+**
Ruivo	**C**	**-**	**-**	**-**	**--**	**-**	**-**	**-**	**-**	=1	**-**	**-**
	HT	**+**	**+**	**+**	**++**	**+**	**+**	**+**	**+**	=1	**+**	**+**

Note: C – Control plants, HT – High temperature treated plants, (+) and (−) higher and lower intensity, respectively, (=1) maximum at which the spectra were normalized.

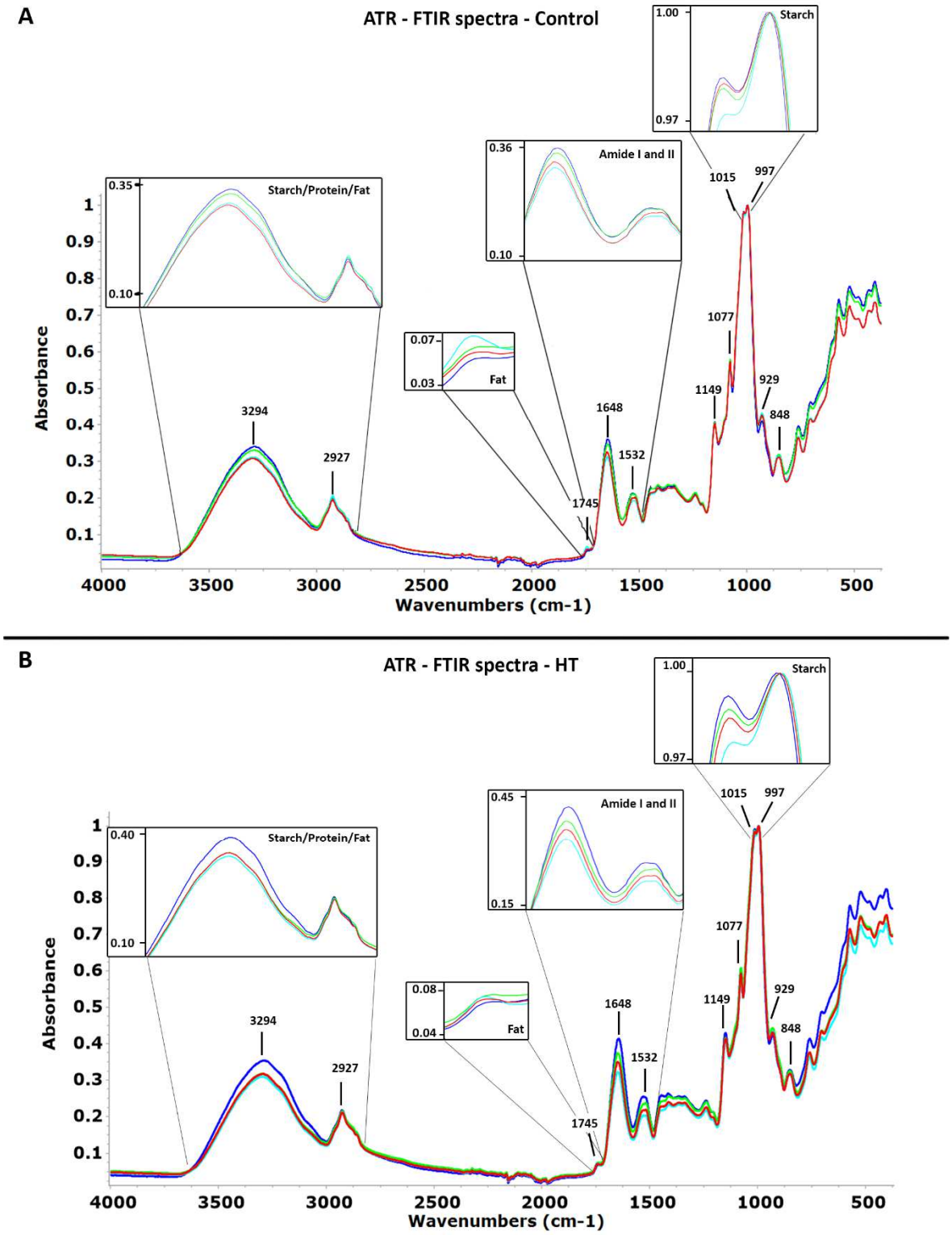

Figure 4.3 ATR-FTIR average spectra of Ardito (dark blue), Grécia (green), Magueija (light blue) and Ruivo (red) **A** control and **B** high temperature (HT) samples with the assignment of relevant bands. Insets magnify peaks related with starch, protein and fat showing differences between landraces in each condition.

Intensity differences between spectra obtained from grains of HT-treated plants are presented in Figure 4.3B and showed that Ardito spectra are the most intense in all wavenumber range, except for the 929-cm^{-1} band. In the wavenumber region most related to fat with a peak at 1,745 cm^{-1} as

well as two other regions more related to starch with peaks at 2,927 and 997 cm^{-1}, the intensities are similar for all landraces. As for control conditions, Magueija is the landrace with lower intensity in eight of the selected spectra bands. Compared with the control, it is possible to observe more differences between the spectra of grains obtained from HT-treated plants, indicating more dissimilarities between landraces under this abiotic stress condition.

4.4.3 Grain protein content increase is a common response to HT treatment

Protein content was predicted using spectra acquired from grain of control and HT-treated plants of the four landraces using the model calibrated in Tomás et al. (2020a) adjusted with N content values of landrace grains (Supplemental Table 4.1). The adjusted model to predict nitrogen content had very good statistics (R^2 = 0.92, RMSECV = 0.14), and the predicted nitrogen values for all control and HT samples ranged from 1.8 to 4.5% (mg of N/100 mg of flour). These values were used to infer protein content using a conversion factor of 5.7 (Caporaso et al., 2018). The average protein content of grains from control and HT-treated plants of the four landraces studied are summarized in Figure 4.4. Considering the values obtained from plants kept in control conditions for each landrace, Magueija samples are the ones with the lower mean protein content (12%), significantly different from Ardito and Grécia with 15.3 and 14.8%, respectively (Figure 4.4). Grains of HT-treated plants showed higher protein content in all landraces analyzed in comparison with control being this augment significant in Ardito (20.1%) and Grécia (17.9%). The comparison between landraces of mean protein content obtained in HT-treated plants showed a higher value in Ardito, which is significantly different from those of Ruivo (15.4%) and Magueija (13.3%) (Figure 4.4).

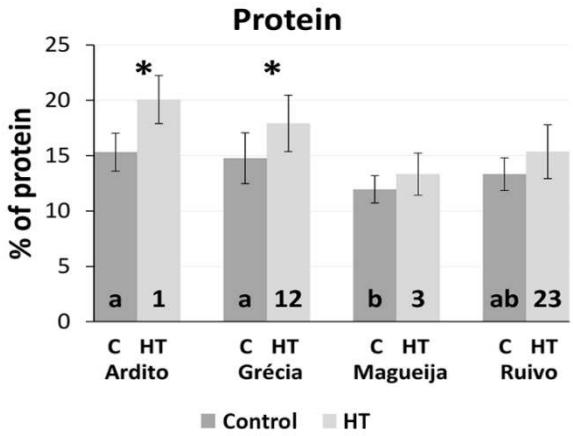

Figure 4.4 Mean protein content of plants kept in control conditions (dark gray) and high temperature (HT) treated (light gray) and respective standard deviations (represented as bars). Different letters and numbers inside columns indicate *ANOVA* significant differences between varieties in control and treatment conditions (HT), respectively. (*) indicates *t-test* statistical differences between control and treatment in each variety (p<0.05).

A global perspective of protein content in all analyzed samples is presented in Figure 4.5 that represents the division by classes of protein content of all individual samples from control or HT-treated plants analyzed (dark and light bars, respectively), independently of the genotype. It shows that control grains presented a lower number of classes (nine classes with values ranging between 10.3 and 19.7%) than HT-treated samples (13 classes, with values ranging between 10.2 and 25.4%). Likewise, this result representation substantiates the lower average protein content of the control samples (13.8%) in comparison to the average value of the treatment samples (16.8%).

On the other hand, an integrated assessment of the four landraces studied can be performed through the PCA of all yield parameters and protein quantification presented in Figure 4.6. In this PCA, the two represented dimensions explain 63% of the variation found between samples. The first, that clearly separates Ardito and Magueija, is defined by five of the eight parameters used (plant area, spike length, grain number, grain weight, and protein content). On its turn, Ardito responds to HT privileging plant growth, increasing plant height, but reducing grain yield, with spike length, and both grain number and weight reduced in treated plants. Concerning grain composition, a significant increase in protein content was observed and, allied to the reduction in grain size and weight, foresee a reduction in grain starch amount. Also, in Magueija, the responses to HT increase plant biomass, spikes in both number and length, and grain quantity.

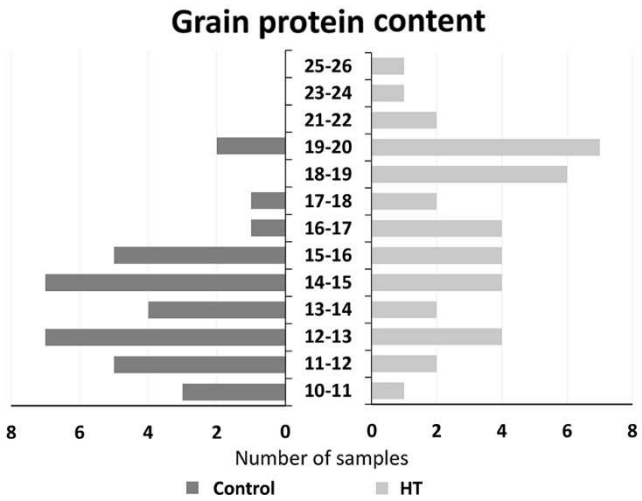

Figure 4.5 Grain protein content. Distribution of all grain samples protein content from control (dark grey) and high temperature treated (HT, light gray) plants of the four landraces studied.

4.5 Discussion

Landrace variability may assume special relevance due to commercial varieties reduced genetic diversity, constituting valuable agrobiodiversity pools potentially more adapted to local conditions where they have been cultivated for long periods (Alipour et al., 2017). Facing a global warming scenario, these advantages are even more relevant for essential crops like bread wheat considering the projections of insufficient cereal production to meet the demand in a few decades (Ray et al., 2013; Gaupp et al., 2019). In this context, the invaluable resource encompassed in the wheat old traditional landraces collected in the 1930s of the last century by Vasconcellos (1933) in Portugal fields assume special relevance. In this work, we studied four of these bread wheat landraces evaluating their yield and grain quality modulation by a HT treatment mimicking a heatwave during grain filling. This particular extreme heat event was predicted to be intensified onward especially in Portugal (Cardoso et al., 2019). Yield parameters and grain composition were comparatively evaluated in landrace plants kept in control conditions and HT treated.

The evaluation of the four landraces showed considerable intervariety diversity since significant differences were detected in all yield parameters analyzed in control plants, especially in the number of spikes, grain number, and plant height, the latter being significantly different between all the landraces. The variability disclosed in the number of grains per spike contrasts with the complete homogeneity observed in this parameter of grain yield observed in bread wheat commercial varieties (Tomás et al., 2020a). On the other hand, the diversity disclosed in the number of spikes contrasts also with the lack of diversity reported in commercial genotypes (Khan and Naqvi, 2011). Ardito landrace stands out as the one with the lower plant height and area along with the higher grain number and weight in the first spike, characteristics close to the desired for commercial varieties (Khush, 1999). Globally, the yield parameters are similar to other European landraces previously studied (Dotlaèil et al., 2003). Unexpectedly, two landraces (Magueija and Grécia) 10

grain weight was higher (0.44 g) than the higher value reported for commercial varieties recommended to be used in Portugal (0.38 g) assessed in similar assays (Tomás et al., 2020a). Concerning the average protein content, the values obtained in the landraces studied, ranging from 10.3 to 19.7% (control condition), were similar to the ones assessed in commercial varieties through the same methodology (between 9.5 and 21.4%, Tomás et al., 2020a). It is relevant that although these landraces were not submitted to breeding programs, their values of protein content are very acceptable and similar to the ones reported for commercial varieties. Recently, the screening of Pakistani wheat landraces also found several traditional genotypes with high storage protein concentration, pointing out their potential to improve the nutritional quality of modern wheat commercial genotypes (Mughal et al., 2020).

On the other hand, different landraces studied in our work revealed distinct responses to HT traduced even in opposite effects in most yield parameters evaluated. The evaluation of the plant height of Ardito and Ruivo, and the area of Magueija revealed significant differences between the control and HT plants. As plant area is often used as plant biomass predictor (Armonienė et al., 2018), our results indicate that Magueija increase in biomass may compromise grain filling, as in this plant development phase, all plant resources should be directed to grain. Both number of spikes per plant and first spike length showed a significant difference between the control and HT plants in Ardito and Magueija, although HT induced differences in the first spike length in opposite ways in these two landraces. Additionally, the increase in Magueija number of spikes induced by HT treatment was mainly due to the appearance of new tillers with spike during ripening. The appearance of these late tillers was observed in all the landraces, in both control and HT- treated plants. It must be emphasized that late spikes were never observed in commercial varieties previously assayed in similar conditions (Tomás et al., 2020a). Moreover, extemporaneous tiller appearance was described in some wheat varieties but only until the beginning of stem elongation (Bowden et al., 2007). We speculate that this phenomenon can constitute a strategy to assure descendance in extreme conditions.

The less affected yield parameter was grain number since it was the only one that did not reveal significant differences between the control and HT-treated plants in any of the genotypes assessed, in opposition to 10-grain weight, which was significantly lower in HT-treated plants of all four landraces. This is in accordance with some previous works that reported that grain number is mostly affected by HT treatments imposed before fertilization, while elevated temperature occurring during grain filling is known to shorten developing period and lead to shrunken grains (Stone and Nicolas, 1995; Farooq et al., 2011; Talukder et al., 2014; Tao et al., 2018). Although an increase in assimilate supply was reported in this phase, it was not sufficient to fully compensate the shorter duration of grain filling period (Lobell et al., 2012). Contrary to this uniform effect on grain weight observed in all landraces analyzed, some previous works revealed different HT effects in grain weight between distinct genotypes (Scotti-Campos et al., 2011; Tomás et al., 2020a). Globally, the comparative evaluation of yield-related traits between genotypes in the control and HT treatment

plants showed that parameters determinant for grain yield like spike number and 10-grain weight presented high variability in both developmental conditions assayed.

Concerning grain composition evaluated by ATR-FTIR, all spectra here obtained were similar to the ones already described in Tomás et al. (2020a) for commercial wheat varieties and were concordant with the main components of wheat grain— starch and proteins (Shewry, 2009). The balance between starch and the other components suggests that grains from Ardito plants have higher protein content than the other four landraces, especially due to the contribution of Amide I (1,648 cm^{-1}). Lipid fraction constitutes only 3–4% of the whole grain (Wrigley et al., 2009), and in our work, it is negligible as the embryo, which is responsible for one-third of the wheat grain lipid fraction that was removed before grain milling. Nevertheless, the comparison between average spectra shows that Magueija grains have the higher fat amount.

Overall, spectra from HT-treated samples were more intense than the control ones in all the landraces, indicating an increase in protein content and a decrease in starch. These results are in accordance with model predicted protein content, which shows a significant increase in Ardito and Grécia grains from HT-treated plants and with previous works (DuPont et al., 2006; Zhang et al., 2017; Tao et al., 2018). Also, an increase in protein content should be related with a decrease in starch content and this is in accordance with the decrease in 10-grain weight and grain size previously observed and with other studies showing that HTs affect the starch synthesis in wheat grain (Hurkman et al., 2003; Tomás et al., 2020b). A shift between landrace spectra in bands mainly assigned to starch suggests that also the proportions of different polysaccharides are altered as the effect of HT treatment. This effect was also observed in commercial genotypes submitted to similar HT treatments (Tomás et al., 2020a).

After HT treatment, the significant increase in Ardito protein amount was also reflected in the greater distance between this landrace and the other ones regarding maximum intensity at Amide I and II bands. Also, the range of maxima intensity values in each spectra band is bigger indicating differences between landraces in HT-treated plants not observed in control ones. This is also corroborated by the increased dispersion of HT sample protein values as shown in Figure 4.5. In fact, associated with the global increase in protein content induced by HT treatment, a higher range of protein content values was obtained after HT (15%) in comparison to control samples (9%). Even more important was the increase in protein observed in HT-treated plants that corroborate the relevance of identifying variable wheat genotypes more adapted to global warming, particularly concerning the major determinant of grain quality—protein content (Asseng et al.,2019). Additionally, the comparison of protein content range in grains from plants submitted to heatwave like the treatment here observed in landraces (10.2–25.4%) and reported in commercial genotypes (10.1– 17.6%, Tomás et al., 2020a). This diversity, together with the higher average protein content observed in landraces after HT treatment, supports the relevance of old traditional genotypes as a source of useful variability breeding focused in wheat nutrimental quality.

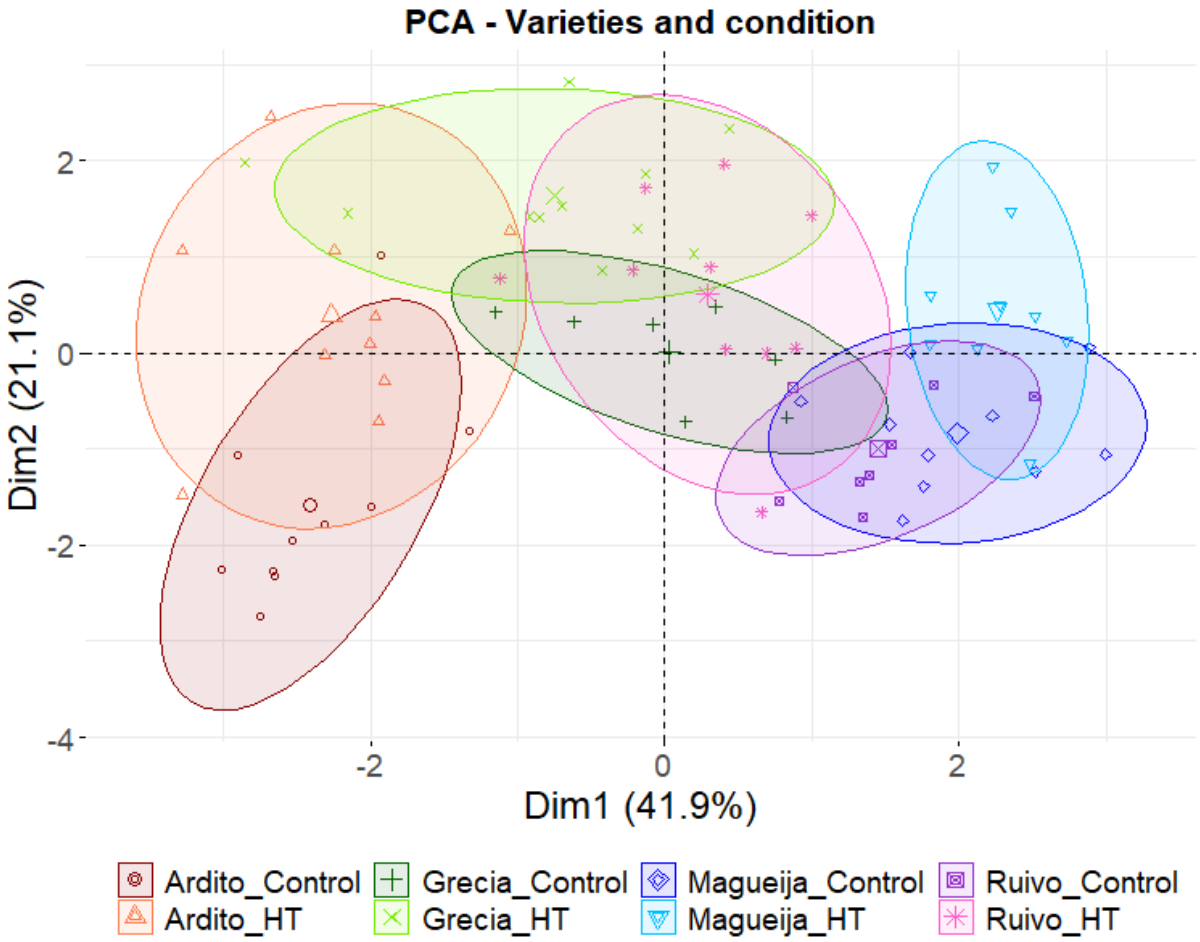

Figure 4.6. **Principal component analysis** using yield parameters (plant height and area, number of spikes, first spike length and grains number and weigh) and grain protein content of Ardito (red), Grécia (green), Magueija (blue) and Ruivo (pink) plants kept in control conditions (darker colors), or HT treated (lighter colors).

Altogether, the four landraces studied presented clear distinct pathways in HT response testifying once again the diversity enclosed in the old varieties studied. Grécia and Ruivo are both affected in vegetative growth and yield, with a reduction, although not always significant, in almost all the parameters. The other two landraces - Magueija and Ardito - showed opposite behaviors, as unraveled by the PCA of all yield parameters and protein quantification (Figure 4.6). Magueija plants seem to be less affected by heatwave-like treatment in terms of yield as after HT, 10-grain weight is higher even when compared with commercial varieties. However, no significant increase in grain protein content was induced by HT, suggesting that the increase in tillers' number may reduce the allocation of resources to the grain filling per spike, ultimately resulting in worst flour quality (Li et al., 2016; Yang et al., 2019). On the other hand, Ardito not only revealed the higher protein content in the control condition but also disclosed a significant increase in this grain quality parameter after HT treatment. Moreover, Ardito is the earlier landrace (Supplemental Table 4.1), with a number of days from germinations to flowering similar to the ones previously observed (not published) in

commercial varieties studied in Tomás et al. (2020a), which may be determinant to avoid heat stress conditions.

The overall diverse outcomes induced by a heatwave-like treatment in distinct landraces contrasts with the reduced diversity observed in wheat commercial varieties submitted to a similar treatment previously reported (Tomás et al., 2020a,b). This superior variability, unraveled under extreme thermal conditions, highlights the potential usefulness of the biodiversity enclosed in old traditional wheat genotypes facing climate changes already sensed. Moreover, the integrative assessment of this work outcomes suggests that both Magueija and Ardito genotypes should be further evaluated seeking for attractive genotypes for wheat breeding plans.

4.6 References

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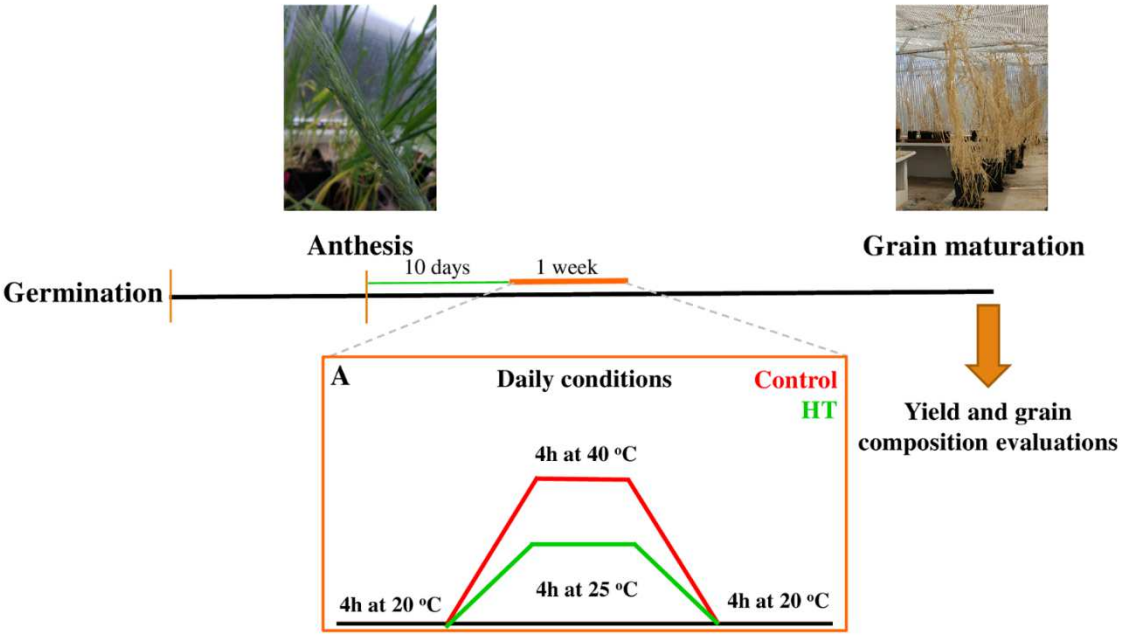
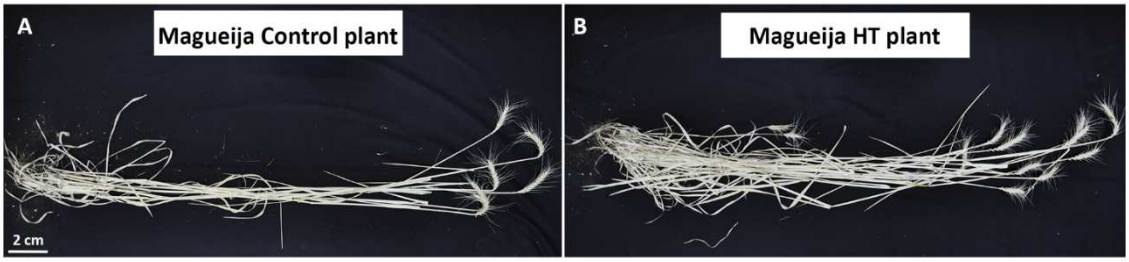
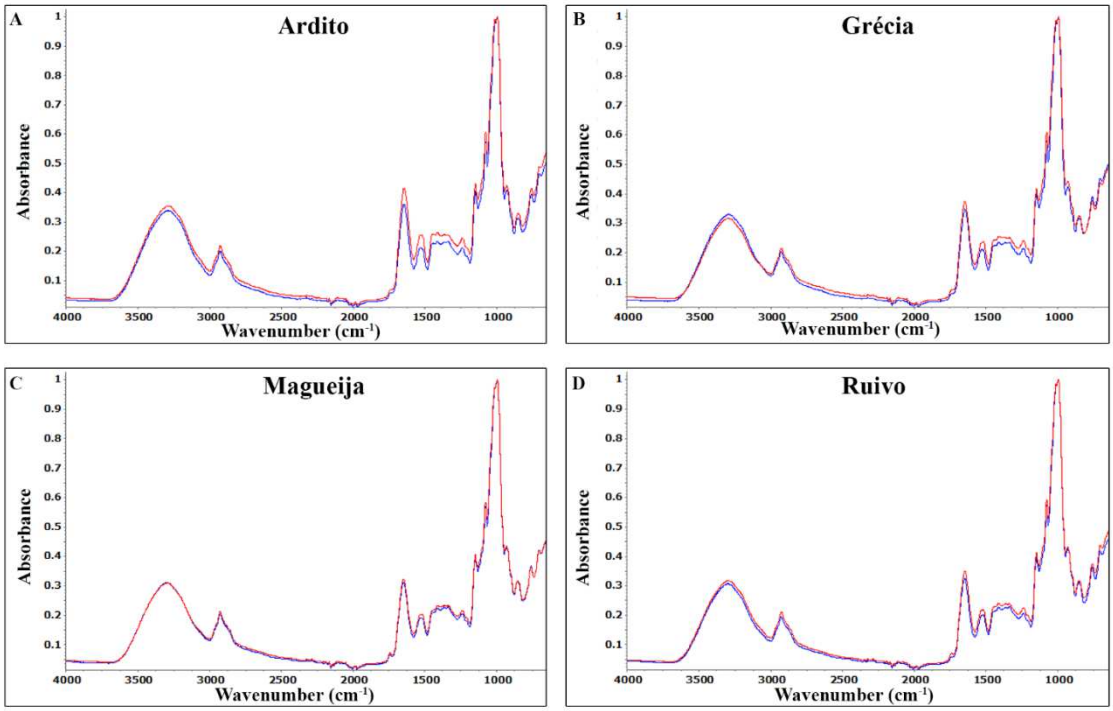
4.7 Supplemental material



Supplemental Figure 4.1 Plant developmental conditions and (A) temperature regimens schematic representations.



Supplemental Figure 4.2 Magueija mature plants (A) kept in control conditions (20ºC/25ºC) and (B) high temperature treated (20ºC/40ºC).



Supplemental Figure 4.3 ATR-FTIR average spectra of control (blue) and HT treated (red) plants of (A) Ardito, (B) Grécia, (C) Magueija and (D) Ruivo.

Supplemental Table 4.1 Flour samples nitrogen content quantified by elemental analysis in mature grains of plants kept in control conditions (20ºC/25ºC) or exposed to high temperature treatment (20ºC/40ºC).

Sample	**Nitrogen content (mg of N/ 100 mg of flour)**	
	Predicted by model	Quantification by elemental analysis
Ardito HT	4.7	4.5
Ardito HT	4.2	3.8
Grécia HT	3.9	3.4
Ardito HT	3.7	2.9
Ardito Control	3.5	2.8
Ardito Control	3.2	2.6
Grécia HT	3.1	2.7
Magueija HT	2.8	2.5
Ruivo HT	2.4	2.1
Magueija HT	2.0	2.1

Chapter V

Grain Transcriptome Dynamics Induced by Heat in Commercial and Traditional Bread Wheat Genotypes

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5 Grain Transcriptome Dynamics Induced by Heat in Commercial and Traditional Bread Wheat Genotypes

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5.1 Abstract

High temperature (HT) events have negative impact on wheat grains yield and quality. Transcriptome profiles of wheat developing grains of commercial genotypes (Antequera and Bancal) and landraces (Ardito and Magueija) submitted to heatwaves-like treatments during grain filling were evaluated. Landraces showed significantly more differentially expressed genes (DEGs) and presented more similar responses than commercial genotypes. DEGs were more associated with transcription and RNA and protein synthesis in Antequera and with metabolism alterations in Bancal and landraces. Landraces upregulated genes encoded for proteins already described as HT responsive, like heat shock proteins and cupins. Apart from the genes encoding HSP, two other genes were upregulated in all genotypes, one encoding for Adenylate kinase, essential for the cellular homeostasis, and the other for ferritin, recently related with increased tolerance to several abiotic stress in Arabidopsis. Moreover, a NAC transcription factor involved in plant development, known to be a negative regulator of starch synthesis and grain yield, was found to be upregulated in both commercial varieties and downregulated in Magueija landrace. The detected diversity of molecular processes involved in heat response of commercial and traditional genotypes contribute to understand the importance of genetic diversity and relevant pathways to cope with these extreme events.

Keywords

Bread wheat, commercial varieties, landraces, heatwave, grain transcriptome, RNA Sequencing

5.2 Introduction

Wheat is the third most produced and consumed cereal worldwide on a daily basis (©FAO, 2018) and hexaploid bread wheat (*Triticum aestivum* L, 2n = 42) represents 90-95% of this production. However, the current growth rate of wheat production is not sufficient to cover the predicted global demand in 2050. Specifically in European countries, the stagnation of wheat yield increase is related with the progressive global warming (Brisson *et al.*, 2010; Ray *et al.*, 2013; Gaupp *et al.*, 2019). The increase of mean temperature during wheat development affects grain yield and quality, due to reduction in lifecycle, pollen abortion, kernel shrinkage and decrease in seed reserves (Asseng *et al.*, 2014; Nuttall *et al.*, 2018; Wang *et al.*, 2019). The required optimum temperature for wheat anthesis and grain filling ranges from 12 to 22 ºC (Tewolde *et al.*, 2006) and the overall acceleration of grain development observed under high temperature regimes is associated with the speed up of transcriptomic events (Altenbach and Kothari, 2004; Wan *et al.*, 2008).

Transcription modulation of genes encoding heat shock proteins (HSPs) is the most studied molecular response under heat stress (Wahid *et al.*, 2007). A recent study identified and characterized 753 HSP genes expressed in bread wheat, revealing the developmental stage and stress situation at which they are responsive (Kumar *et al.*, 2020). *HSPs* transcripts were also differentially detected after one hour and one day at 40 ºC using Wheat Genome Array profiles in seedlings of two genotypes with contrasting thermotolerances (Qin *et al.*, 2008). The same work also detected transcription factors and genes involved in phytohormone biosynthesis/signaling, calcium and sugar signal pathways, RNA metabolism, ribosomal proteins, primary and secondary metabolisms synthesis, and biotic and abiotic stress responses. Chauhan et al (2011) identified heat responsive genes, after two hours of heat stress treatments (34 and 40 ºC), implicated in metabolites and protein synthesis in seedling shoot, flower tissues and developing grain through subtractive hybridization.

Whole transcriptome sequencing of wheat seedlings reported similar transcripts profiles after heat, drought and their combination treatments of one and six hours (Liu *et al.*, 2015). The main biological groups associated with upregulated genes were stress response, hormone stimulus response and nutrient metabolic processes, while downregulated genes were mainly enriched in photosynthesis and nutrient biosynthesis pathway. A more recent study used RNA Sequencing data obtained from developing grains of genotypes with distinct thermotolerances that underwent post anthesis heat stress for three days, identified different clusters of genes unique to tolerant and susceptible genotypes (Rangan *et al.*, 2019). This work also refers that most genes uniquely expressed in tolerant genotype during heat stress are detected in both early and late grain filling reinforcing their role in heat stress response. Other work from Kino et al (2020) compared RNA Sequencing data obtained from whole grains after post anthesis high temperature treatment (35 °C during two to twelve days) against existing sequence data from individual pericarp and endosperm tissue. A significant down-regulation of pericarp genes with a known role in regulation of cell wall

expansion was observed. For that reason, the authors suggested that heat treatment induces reduced expansion capability of the pericarp, which may result in a physical constrain of endosperm growth.

Several studies shown increasing genetic erosion caused by the replacement of diverse old landraces by comparatively few and homozygous modern cultivars (Gregová *et al.*, 1999; Caballero *et al.*, 2001; Srinivasan *et al.*, 2003). Landraces are dynamic populations of cultivated species lacking formal crop improvement, locally adapted and often genetically diverse (reviewed in Villa et al., 2005). Thus, landraces provide notable successes in crop improvement (reviewed in Dwivedi et al., 2016) as sources of nutritional and technological quality traits and marginal environment tolerance (reviewed in Newton et al., 2010). They are considered extremely valuable agrobiodiversity pools in changing environmental conditions (Trethowan and Mujeeb-Kazi, 2008; Lopes et al., 2015) that may constitute a key resource facing extreme heat events like heatwaves. Heatwaves are defined by World meteorological organization (2015) as five or more consecutive days of heat in which the daily maximum temperature is at least 5°C higher than the average maximum temperature. These adverse environmental events are foreseen to be increasingly frequent (Cardoso *et al.*, 2019). The main goal of this work was to evaluate whole transcriptomic alterations induced by heatwave-like treatment during grain filling. This study was comparatively performed in two commercial varieties and two Portuguese landraces, chosen based on previous evaluations of high temperature (HT) responses regarding yield and grain composition (Tomás et al. 2020a, b, c).

5.3 Materials and methods

5.3.1 Plant material and high temperature treatment

The genotypes studied in this work comprehend two bread wheat (Triticum aestivum L., 2n = 6x =42, AABBDD) commercial varieties recommended to be used in Portugal (ANPOC et al., 2014), Antequera and Bancal, and two old Portuguese landraces from Vasconcellos collection, established in the 30s of last century (Vasconcellos, 1933), Ardito and Magueija. Seeds of commercial varieties were gently supplied by ANSEME (Portugal) and seeds of traditional landraces by EAN Germplasm Bank (Oeiras, Portugal, PRT005). Twenty seeds from each genotype obtained after two years of controlled propagation were germinated and grown in control conditions - eight hours of dark at 20 ºC and a 16 hours light period divided in six hours increasing to 25 ºC, four hours at 25 ºC, and six hours decreasing to 20 ºC. Three-week old plants were transferred individually to seven liters soil pots and maintained in greenhouse conditions.

When the first anther was observed in the first spike (anthesis), plants were transferred to growth chambers with the previously described control conditions. Ten days after anthesis (daa) subsets of ten plants (biological replicates) of each genotype were submitted to two different growth conditions for seven days: control conditions above described or high temperature (HT) regime with

a daily plateau of 40 ºC maximum temperature (Supplemental Figure 5.1). Immediately after the period of four hours at maximum temperature in the last day of the treatment, two immature grains from the middle of each first spike of each plant were collected (17 daa) and stored at -80 ◦C for posterior RNA extraction.

5.3.2 RNA extraction, library preparation and sequencing

Total RNA was individually extracted from control and heat-treated immature grains using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, Inc, Spain) and following manufacturer's instructions. For the RNA sequencing three biological replicates were analyzed per condition and genotype. Each sample 100ng of RNA and was composed by a pool with equal contribution of three immature grains.

Both library preparation and sequencing were performed and optimized by the Genomics Unit of the Instituto Gulbenkian Ciência, Oeiras. mRNA-libraries were prepared using the SMART-seq2 protocol adapted from Macaulay et al. (2016) Illumina® libraries performed used the Nextera protocol adapted from Baym et al. (2015). The libraries quantification and quality verification were done using the Agilent Fragment Analyzer in combination with HS NGS Kit (Agilent Technologies, Santa Clara, California). Libraries were sequenced in the NextSeq500 Illumina® Sequencer using 75 SE high throughput kit (Illumina, San Diego, California) and 937302653 reads were obtained from the 24 samples.

5.3.3 RNA sequencing data processing and differential gene expression analysis

Bioinformatic analysis from quality assessment to differential expression analysis were performed by BioData.pt. Quality control was evaluated on raw reads using FastQC (Andrews *et al.*, 2010). Raw reads were then trimmed using fastp (Chen *et al.*, 2018*a*) to the longest continuous segment of Phred-quality (threshold of 30 or above) in order to improve overall base quality, and remove the Illumina® Smart-Seq2 adaptors from sequencing. A new quality control with FastQC was performed. The trimmed reads were mapped to *Triticum aestivum* genome (ftp://ftp.ensemblgenomes.org/pub/plants/release-48/fasta /triticum_aestivum/dna/Triticum_aestivum.IWGSC.dna.toplevel.fa.gz) using hisat2 with default parameters (Kim *et al.*, 2015). Quality control of the mapping procedure was accessed with Qualimap (Okonechnikov *et al.*, 2016).

Read assignment to genomic features and gene expression quantification were made using featureCounts (Liao *et al.*, 2014). Differential gene expression was tested using DESeq2 (Love *et al.*, 2014) between transcript sets of control and HT treated samples. Manual search of gene ID and encoding products was made in Ensemble Plants BioMart (Kinsella *et al.*, 2011).

R software (Team R Core, 2018) was used to integrate all the analysis and obtain multi-dimensional scaling analysis (MDS) plot (to show the general relationship between the samples) and, hierarchical clustering of samples for all varieties and conditions represented as an heatmap and Venn diagrams (showing the relationships between the differential expressed genes lists of all varieties and conditions).

5.3.4 Gene ontology enrichment analysis

Gene enrichment (GO) analysis was done in AgriGOv2 (http://systemsbiology.cau.edu.cn /agriGOv2/index.php) web-based tool (Tian et al., 2017). AgriGO SEA parameter settings were as follows: Fisher test, with Bonferroni multi-test adjustment method, 0.05 significance level, five minimum mapping entries, and complete gene ontology. The GO database (http://geneontology.org) was used to analyze GO terms enrichment of DEGs, and the Kyoto Encyclopedia of Genes and Genomes (KEEG) database (http://www.kegg.jp/kegg) was used to identify the enriched metabolic pathways, as well as the enzymes involved.

5.4 Results and discussion

In this work plants of four wheat genotypes, Antequera, Ardito, Bancal and Magueija were submitted to HT treatment simulating a heatwave, for one week during grain filling stage. Transcriptome profiles of immature grains collected immediately after treatment period (17 days after anthesis) from control and treated plants were analyzed.

5.4.1 Traditional genotypes presented a more similar HT response than commercial varieties

The reference genome used to map transcripts was the IWGSC RefSeq v1.0 assembly (the first version of the reference sequence obtained from the bread wheat variety Chinese Spring). Overall, about 90% of the transcripts were mapped against the reference genome, and from these 37% mapped to multiple sites and the other 53% mapped specifically to one site in the genome. From the mapped transcripts an average of 68% of the reads aligned to exonic regions, 29% to the intergenic regions and only 3% to intronic regions. The great percentage of transcripts mapped to intergenic domains is probably due to the incomplete genome annotation. Interestingly, commercial varieties Antequera and Bancal presented a significantly ($p<0.05$) higher percentage (71.5%) of transcripts mapped to the exonic regions than the traditional ones Ardito and Magueija (61%). Concomitantly, the number of transcripts mapped to both intronic and intergenic regions is higher in the landraces. This may be explained by the fact that old traditional genotypes, collected in the 1930s, are more distinct from the reference genome than commercial varieties. Lastly, the results

summarized in Figure 5.1A indicate that most reads (between 73% and 82%) were assigned as protein coding regions, the next most found class was nontranslating - coding sequence (between 18 and 27%), and in a very small amount ribosomal DNA (less than 3%).

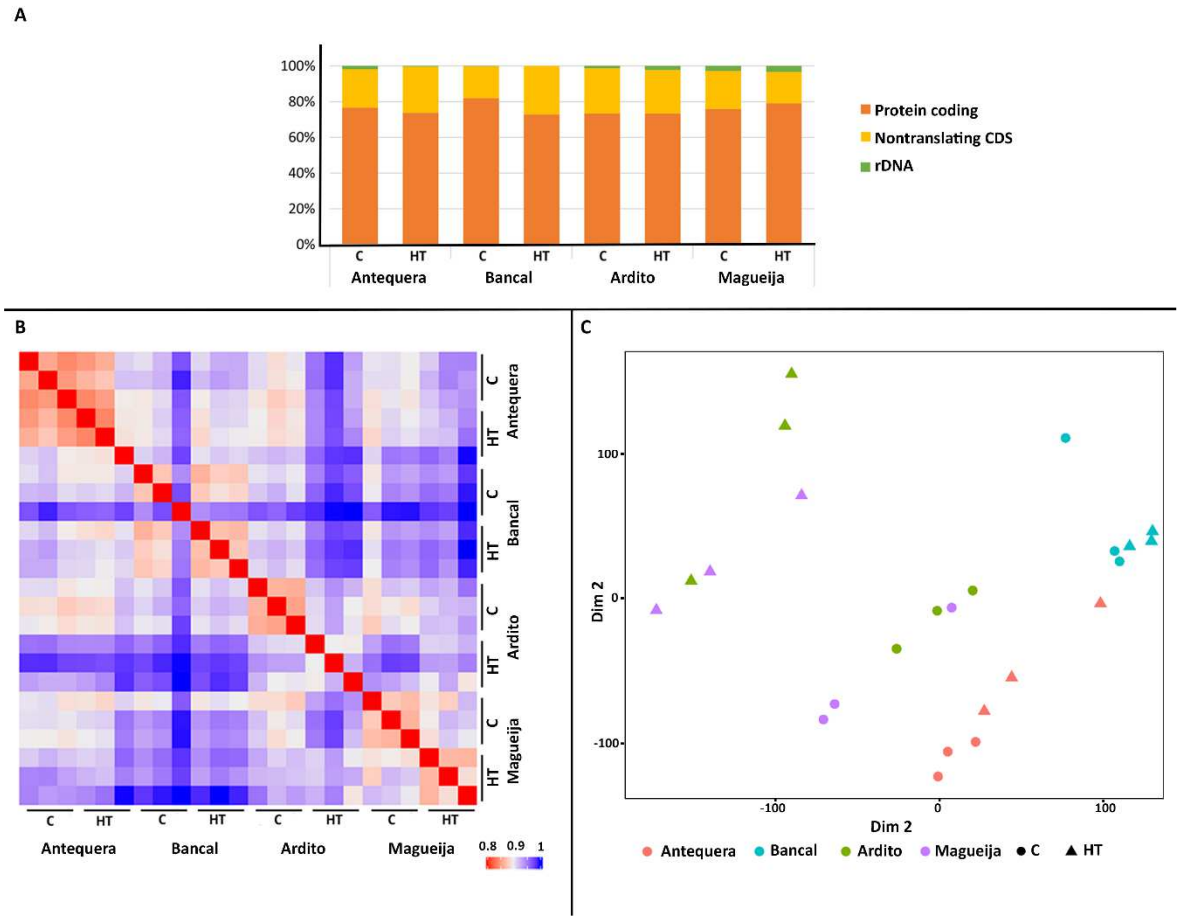

Figure 5.1 Reads assignments by gene type and relationship between samples. **A** Read assignments and relative abundance of reads per type of gene. **B** Hierarchical clustering of sample-to-sample correlations based on Pearson correlations (right). **C** Multi-dimensional scaling (MDS) plot showing similarity between all samples. The comparisons were made between control (C) and high temperature (HT) reads sets of commercial varieties Antequera and Bancal and landraces Ardito and Magueija.

Also a hierarchical clustering (Figure 5.1B) of sample-to-sample correlations revealed great intravarietal similarity between Antequera and Bancal samples, independently of the treatment, while for Ardito and Magueija, the similarities were greater between samples of the same condition (control/ HT). In fact, the MDS (multi-dimensional scaling) plot grouping (Figure 5.1C) shown that five of the six samples of each commercial varieties are closer to each other, while in landraces a clear separation between control and treatment samples was observed.

Differential expressed genes (DEGs) between transcriptomes of immature grains from plants kept in control conditions and submitted to high temperature were considered significant with and adjusted *p-value* (*padj*) < 0.05 for all genotypes. Up and downregulated genes were obtained filtering the $\log^2$foldchange absolute value higher than 1. For the four genotypes analyzed, a total of 10366

DEGs were identified, 86% of them referent to Ardito and Magueija traditional genotypes, showing that they have a greater response to high temperature treatment. In a similar study done recently (Rangan *et al.*, 2019), grain transcriptomes of three genotypes showed a higher number (more than 80%) of downregulated genes in susceptible genotypes, comparing with tolerant ones (2% of the DEGs were downregulated). Thus, the HT response of our landraces was similar to susceptible genotypes, as they present a higher number of DEGs.

The number of DEGs is significantly different between all genotypes ($p<0.05$), although these differences were less accentuated between Ardito and Magueija (Figure 5.2A). Particularizing to each genotype, the commercial variety Bancal presented the lower number of DEGs of all varieties studied, 129 in total, 69 upregulated and 60 downregulated. A ten times higher number of DEGs were identified in Antequera (1298), 339 upregulated and 959 downregulated. Considering the work above referred (Rangan et al 2019) the higher number of DEGs detected in Antequera is in accordance with the previously reported worst heat response of this genotype in comparison to Bancal regarding grain protein content and grain yield (Tomás *et al.*, 2020*b*). In Ardito 4375 DEGs were identified, 2397 upregulated and 1978 were downregulated. The genotype with greater number of DEGs in response to high temperature treatment was Magueija, 4564, 2661 downregulated and 1903 upregulated.

Our first approach was to investigate if any of A, B and D genomes or distinct chromosomes were particularly affected by high temperature treatment, since is already documented that chromosomes 3A and 3B harbor genes involved in high temperature response (reviewed in Ni *et al.*, 2018). Although no significant differences were detected between genomes neither between chromosomes (Supplemental Figure 5.2).

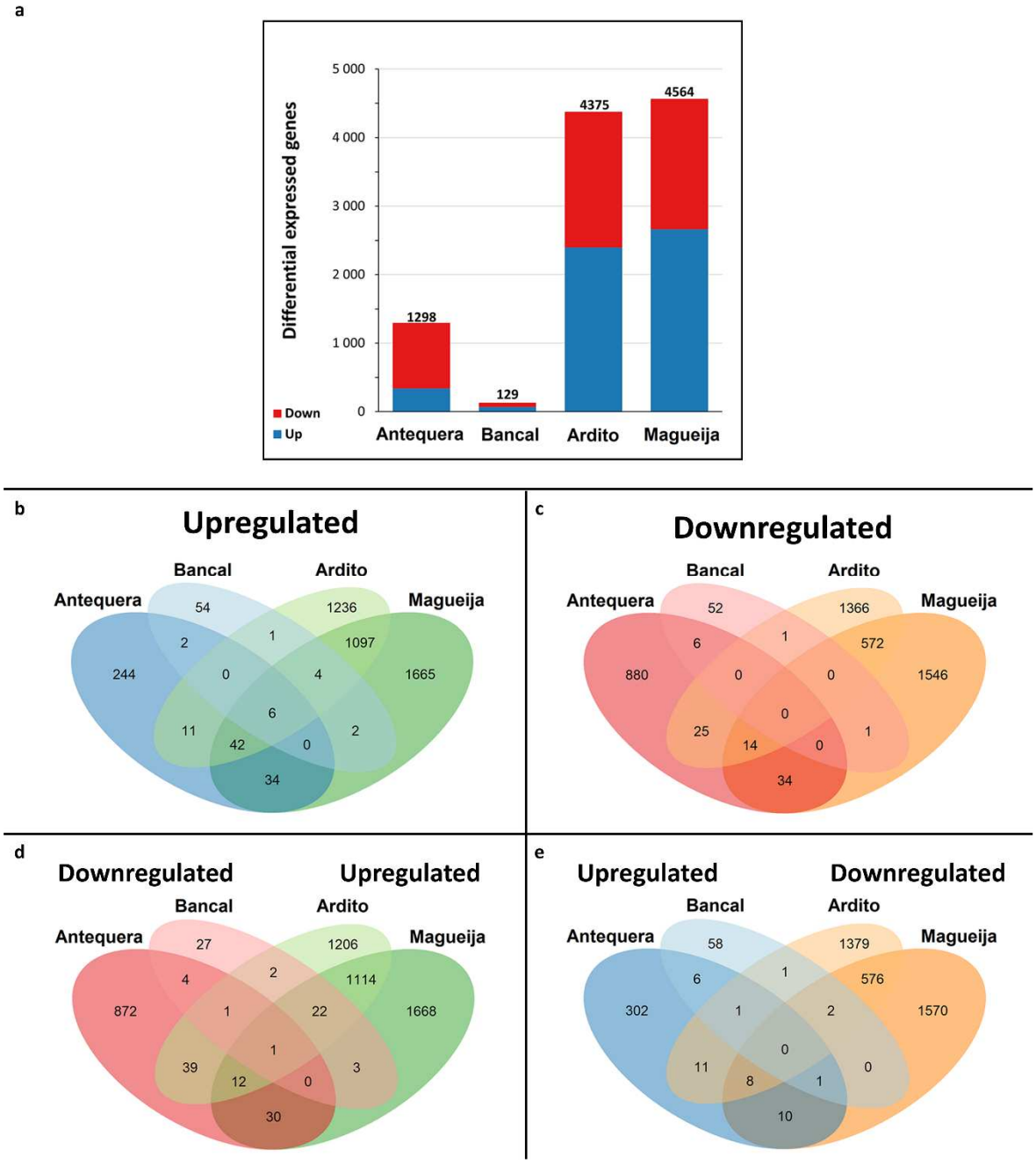

Figure 5.2 Differentially expressed genes (DEGs). **A** Number of DEGs between control and high temperature treated samples of commercial varieties Antequera and Bancal and landraces Ardito and Magueija. Red and blue indicate down and upregulated genes, respectively. **B-E** Venn diagrams of differential expressed genes in commercial varieties Antequera and Bancal and landraces Ardito and Magueija: **B** upregulated in all genotypes; **C** downregulated in all genotypes; **D** downregulated in commercial varieties and upregulated in landraces; **E** upregulated in commercial varieties and downregulated in landraces. Non-overlapping regions represent the number of genes exclusive to one genotype. Overlapping regions indicate the number of genes common to two, three or four genotypes.

The results presented in Figure 5.2B shown that from the 1199 upregulated genes common to more than one genotype, only six genes were common to Antequera, Bancal, Ardito and Magueija.

These six upregulated genes common to all genotypes (Table 5.1) encompassed annotated genes encoding three small heat shock proteins HSP20, one adenylate kinase, a BAG domain proteins and a ferritin. The adenylate kinase catalyzes a reversible transphosphorylation reaction that converts adenine nucleotides (ADP to ATP and AMP) and is critical for many processes in living cells (Pradet and Raymond, 1983), as for example abiotic stresses (Komatsu et al., 2014). BAG domain proteins are responsible for the modulation of chaperones activity as they bind to HSP70 proteins and promote the substrate release. Lastly, ferritin is a protein that function in the iron storage in a soluble, non-toxic, readily available form. A recent study (Zang et al., 2017) showed that the overexpression of a gene encoding a ferritin (TaFER-5B) functionally complemented the heat stress-sensitive phenotype of a ferritin-lacking mutant of Arabidopsis enhancing heat, drought, oxidative and excess iron stress tolerance associated with the ROS scavenging, as well as leaf iron content. Thus, the present work not only identified genes commonly modulated by HT in distant related hexaploidy wheats, but also pointed out an upregulated one that seem to be involved in HT response not only of wheat genotypes but also of dicot plants like Arabidopsis.

Table 5.1 Upregulated genes common to all genotypes analyzed.

Gene ID	Encoded protein
TraesCS3B02G155300	Adenylate kinase
TraesCS4D02G086200	Small heat shock protein (Hsp20 family)
TraesCS4A02G092900	Small heat shock protein (Hsp20 family)
TraesCS5A02G548000	BAG domain
TraesCS4B02G089800	Small heat shock protein (Hsp20 family)
TraesCS7D02G428200	Ferritin

There is a great difference between the number of upregulated genes common to both commercial varieties and the number of these genes common to both traditional ones, as can be seen in Figure 5.2B. Only 2 of the 418 (0.48%) HT upregulated genes were common in both commercial varieties. These genes encode for a protein induced by water deficit or abscisic acid stress and ripening, and a NAC transcription factor involved in plant development (NAC019-A1). A recent work revealed that this transcription factor is known to be a negative regulator of starch synthesis, kernel weight, and kernel width in wheat developing grains (Liu et al., 2020). In fact, our previous analyses of mature grains of these genotypes subjected to HT during grain filling revealed a reduction of starch amount in both commercial varieties and an increase in both landraces (Tomás et al., 2020b,a). On the other hand, a much higher proportion of upregulated genes, 1097 of the 5058 (21.7%) are shared by the landrace genotypes. These genes are associated with 1747 biological processes gene ontologies, being the most represented terms related with protein folding and metabolic process.

Concerning downregulated genes, no one was commonly detected in all genotypes and it was also observed a much higher number of genes common to both traditional landraces (572 - 87.6%) than between commercial varieties (6 - 0.92%) (Figure 5.2C). These results reinforce the already referred suggestion that traditional genotypes have a more similar response to the HT treatment than commercial ones.

Among the 110 genes downregulated in commercial genotypes and upregulated in landraces (Figure 5.2D) there were genes encoding for several HSP of different classes, related with HT response proteins involved in nitrogen metabolism and seed storage proteins, that are mainly involved in the seed quality. On the other hand, only 34 genes were upregulated in commercial varieties and downregulated in traditional genotypes (Figure 5.2E), and the gene products are very diverse, encompassing proteins involved in DNA binding, zinc finger domains, transport proteins, and several No Apical Meristem (NAM) proteins, referred before as negative regulator of starch synthesis (Liu et al., 2020).

Looking forward to unravel if there was any HT common response related with the more affected genes, we analyzed the ten most up and downregulated genes of each genotype (Supplementary Table S1). It was possible to note that in the commercial varieties upregulated genes encode for diverse products, several involved in the RNA processing. For example, pentatricopeptide-repeat-containing proteins (PPR) were encoded by these upregulated genes in both commercial genotypes. They are known to influence the expression of several organellar genes by altering RNA sequence, turnover, processing, or translation (Barkan and Small, 2014). Also PPR proteins have crucial roles in response to different abiotic stresses in rice and were found as miRNAs target genes associated with thermotolerance in wheat (Tan et al., 2014; Chen et al., 2018b; Ravichandran et al., 2019). On the other hand, 60% of landraces upregulated genes encode for products involved in heat shock response, as heat shock proteins or heat shock factors, well documented as high temperature responsive genes (reviewed in Kaur et al., 2019). One of the Magueija up regulated genes is the already identified in leaves and roots TaHsfA6f , associated with increased thermotolerance (Xue et al., 2015; Bi et al., 2020) and to our knowledge it is for the first time identified in developing grains. As for the downregulated genes, the only characteristic that stood out was that in Antequera 7 out of the 10 downregulated genes encode for products related with protein synthesis and regulation, which can be related with the reduction in grain protein content observed in this variety after HT treatment (Tomás et al., 2020b).

5.4.2 Functional annotation and gene ontology mapping of high temperature DEGs

In a more global perspective regarding each genotype response to high temperature treatment, functional annotation of DEGs of each genotype was made through the assignment of gene

ontologies (GO) for biological processes, molecular functions and cellular components (Figure 5.3 and Supplemental Table 5.2). Figure 5.3 indicates the percentage of up and downregulated genes of each genotype, assigned to second and third levels of categories associated with each ontology. For all categories of the three ontologies the proportions of up and downregulated genes associated are very similar, being the classes with higher and lower number of genes the same in both cases for the four genotypes. This may indicate that, although the number of altered genes may be different in distinct genotypes (Figure 5.3), the functional roles in which the DEGs are involved constitute a common feature in wheat heat stress response.

In biological processes for both up and downregulated genes, the most represented categories are biological regulation (GO:0065007), cellular process (GO:0009987), metabolic process (GO:0008152) and response to stimulus (GO:0050896). It was also notorious that Bancal had a great percentage of downregulated genes assigned to several categories. For molecular functions ontology, catalytic activity and binding, mainly protein (GO:0005515) and organic cyclic compound binding (GO: 0097159) were clearly highlighted as compared with the other classes. Regarding cellular component GO, the most represented class was organelle (GO:0043226), with more than 50% of the DEGs in almost all the genotypes, and the other was membrane (GO:0016020) with half of this amount.

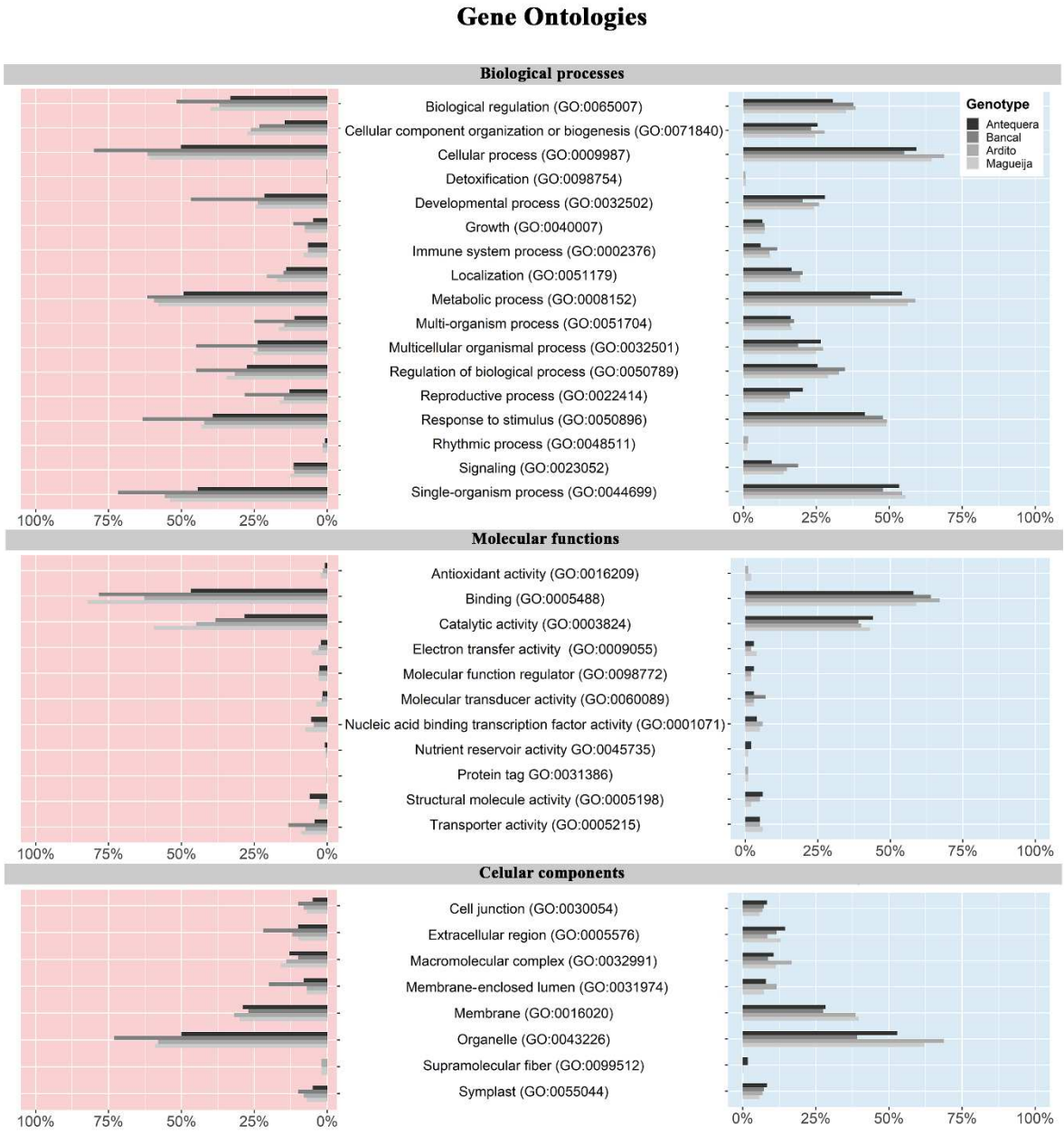

Figure 5.3 Gene ontology percentage of up and downregulated genes in commercial varieties Antequera and Bancal and landraces Ardito and Magueija assigned to second and third levels of Biological processes, Molecular functions and Cellular component gene ontologies. Red and blue indicate down and upregulated genes, respectively.

Several GO terms were significatively represented in each genotype (Supplementary Table S2), except for Bancal up regulated genes, that were only significantly enriched in 16 molecular function ontologies. In total 395, 129 and 154 distinct ontologies were identified for biological processes, molecular function and cellular components, respectively. Ardito and Magueija present a closer response as several common ontologies were significatively represented, namely some categories of response to stress, establishment of cell polarity, protein complex biogenesis, de novo protein folding and carbohydrate catabolism for upregulated genes and DNA metabolism, regulation of gene expression and protein complex biogenesis for downregulated genes. We also found common

categories in which Antequera and both landraces upregulated genes were enriched, such as protein folding, response to light and reactive oxygen species and heat acclimation biological processes and peroxisome and microbody cellular components. On the other hand, categories significantly enriched by downregulated genes common between commercial and landraces were only identified in cellular components, for instance related to nuclear lumen and thylakoid.

Particularly, from all the DEGs engaged in high temperature treatment response, 512 were assigned to response to heat category (GO:0009408), most of them presenting increased expression levels in the traditional varieties while in the commercial ones only a small part was affected (Supplementary Table S3). These genes are related with other 106 biological processes, being the most represented protein folding (16%) and transcription regulation (8%), 41 cellular components with nucleus and integral component membrane being associated with a greater number of genes (14% each), and 105 molecular functions, being the most represented ATP binding (12.6%), protein binding (8.7%) and unfolded protein binding (7.3%). As expected, a great number of genes in this category encode heat shock proteins (Hsp) and heat shock factors (Hsf). About 30% of the genes encode for the different Hsp20, Hsp40 (DNAJ domain), Hsp70 and Hsp90 and the great majority were upregulated in the traditional genotypes and remain unaltered or downregulated in the commercial ones. Also in the traditional genotypes, 12 genes encoding Apetala 2 proteins were identified as upregulated. Several proteins of this class were involved in grain and spike morphology, plant height, and spike emergence time determination, and play a key role in growth and development, including regulation of plant architecture and yield-related traits (Li et al., 2016; Zhao et al., 2019).

To further disclose biological functions of DEGs and determine if any pathway have a significant involvement in heat tolerance, we investigated DEGs involved in Kyoto Encyclopedia of genes and Genomes (KEGG) pathways and 749 DEGs were assigned related with 107 KEEG pathways (Figure 5.4 and Supplemental Table 5.4). Antequera was the genotype with less DEGs associated with these pathways (0.5%), the traditional genotypes revealed 9% each, and Bancal was the genotype that presented the higher percentage (57%).

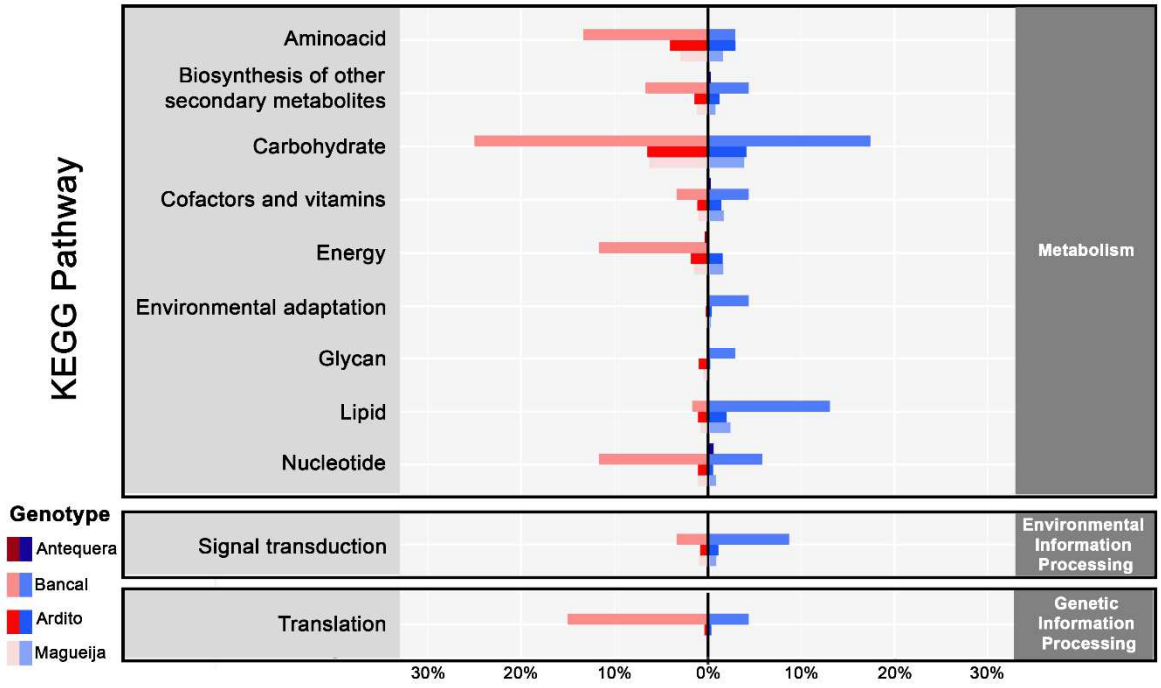

Figure 5.4 KEGG Pathways enrichment percentage of up and downregulated genes in commercial varieties Antequera and Bancal and landraces Ardito and Magueija associated with metabolic, environmental and genetic information processing pathways. Red and blue indicate down and upregulated genes, respectively.

Analyzing the pathways associated with products encoded by downregulated genes, the ones related with carbohydrate metabolism were the most influenced in Bancal and both traditional genotypes. The only carbohydrate pathway associated with downregulated genes of all four genotypes were the Glycolysis / Gluconeogenesis pathway, although neither the genes nor the encoded enzymes were common. Although, inside this category, the majority of Bancal downregulated genes encoded for enzymes involved in pentose and glucuronate interconversions pathway and in landraces encoded for starch and sucrose metabolism, with the majority of encoded enzymes associated with glucose synthesis. Some of the enzymes categorized in the pentose and glucuronate interconversions were pectinesterases known to be involved in cell wall remodeling that occurs during high temperature response (Wu *et al.*, 2018). Kino *et al* (2020) reported also a downregulation of genes involved in pericarp cell wall expansion due to high temperatures exposure during post anthesis, and speculate that this can be related with the reduction in grain weight observed after this stress. Our work also corroborated this suggestion since the majority of DEGs encoding pectinesterases were downregulated in landraces in which a reduction in grain weight was observed (Tomás *et al.*, 2020*a*). The second most affected pathways were the ones involved in amino acid metabolism, with the majority of DEGs assigned to cysteine and methionine metabolic pathways for Bancal and both landraces. This was an unexpected result as the accumulation of this amino acid was reported in high temperature conditions (Tao *et al.*, 2018). Again, only one pathway, the glycine,

serine and threonine metabolism, was identified as being associated with downregulated genes in all the genotypes, but also again none of these genes was common to all the genotypes. An interesting result was the percentage of Bancal downregulated genes encoding for Aminoacyl-tRNA synthetases (nine different genes encode for six different synthases), classified in the translation pathways. This was not an expected result as several works in distinct species report an increase in different enzymes of this family in abiotic stress situations (Giritch *et al.*, 1997; Thimm *et al.*, 2001; Kobayashi *et al.*, 2005; Baranašić *et al.*, 2021). Lastly several downregulated genes in Bancal were associated with nucleotide metabolism, more specifically with purine metabolism, and encoded for Adenosine triphosphatase (ATPase).

Upregulated genes were also associated with the majority of the mentioned pathways for downregulated genes. In fact, the encoded products were in some cases the same as for downregulated genes, suggesting that they include several cases of different enzyme isoforms or homologous genes with different functions, as already reported (Liu *et al.*, 2015; Kaushik *et al.*, 2020). Carbohydrate pathways include the greater number of associated upregulated genes for Bancal and both landraces. Particularizing, starch and sugar pathway was the most common, and glycolysis was the second. For Bancal nucleotide metabolism was again the pathway with the higher percentage, although the encoded enzymes were involved in the dephosphorylation of ATP molecules, as well as translation pathways, in which were detected transcripts for enzymes involved in glutamate and tryptophan tRNA synthesis. Upregulated genes of Bancal and both landraces encoded also for enzymes in lipid metabolism. Specifically involved in cutin, suberine and wax biosynthesis, glycerolipid metabolism, fatty acid elongation, fatty acid biosynthesis, being the latter two related only with upregulated genes in landraces. This may indicate an alteration in lipids proportions in response to high temperature as previously reported (reviewed in Abdelrahman *et al.*, 2020).

5.4.3 HT effects in storage proteins encoding genes

Gluten is determinant for the wheat suitability to produce bread as it is a protein network that entrains air bubbles during dough fermentation. It is composed by two classes of storage proteins, glutenins, responsible for the dough strength and elasticity, and gliadins which confer extensibility and viscous properties to gluten required for dough development. Gliadin/glutenin ratio is determinant for rheological characteristics (Dhaka and Khatkar, 2015), being for that reason important to access if these proteins encoding genes' are affected by high temperature treatment. Storage proteins encoding genes are classified in the nutrient reservoir activity ontology and the expression levels of DEGs associated with this category are presented as heatmap in Figure 5.5. None of the genes presented altered expression in Bancal genotype. Additionally, about 60% of the DEGs were related with two protein families, Cupins and Gliadins.

Figure 5.5 Differential expressed genes involved in nutrient reservoir activity ontology in commercial varieties Antequera and Bancal and landraces Ardito and Magueija. Red and blue indicate down and upregulated genes, respectively, and color intensity are related with the degree of gene expression alteration; gray represents unaltered genes.

The results obtained revealed 12 gliadin encoding genes differentially expressed, mostly upregulated in Magueija and Antequera, that may have implications in grain quality. In fact, several studies showed that an increase in gliadin fraction has a detrimental effect on the technological characteristics of wheat. Flours with higher gliadin content presents weaker gluten quality and dough, with increased viscosity and stickiness (Barak *et al.*, 2015). Additionally, Antequera and Ardito presented 6 and 2 downregulated genes encoding for Cupins, respectively, while three genes were upregulated, 1 in Ardito and 2 in Magueija. Cupins were already described as heat responsive proteins with an unusual thermostable character which facilitates their accumulation in a number of heat-stressed organisms (Dunwell *et al.*, 2001). A more recent work shows that these proteins are

preferentially accumulated when protein synthesis components are generally decreased during heat stress, suggesting that they may provide valuable insights into improving the protein content of wheat (Wang *et al.*, 2018). A significative reduction of protein content was previously observed in Antequera mature grains after heat stress treatment (Tomás *et al.*, 2020*b*).

Expression levels of genes encoding high molecular weight glutenin subunits (HMW-GS), GBSSI and puroindolines A and B were already evaluated in commercial varieties (Tomás *et al.*, 2020*c*). Through annotation overlapping genes we have investigated if these genes are differentially expressed as assessed by RNA Sequencing analysis (Supplemental Table 5.5). The results obtained revealed that only PinB-D1 was downregulated in Ardito, with all the other studied genes being undetectable in DEGs data. In fact, this is in accordance with our previous work (Tomás *et al.*, 2020*c*) in which no significant differences were observed in any of the HMW-GS encoding genes, except the increase of HMW-Dy in Bancal. Altogether, these results show that gliadins are more affected by high temperature treatment than glutenins in both wheat commercial varieties and landraces and reinforce the need to investigate the cupins role in heat stress response.

The results obtained in this work substantiate the advantages of germplasm exploitation to understand the intricate wheat stress response and outline new research strategies to identify bread wheat genotypes with increased heat tolerance

5.5 References

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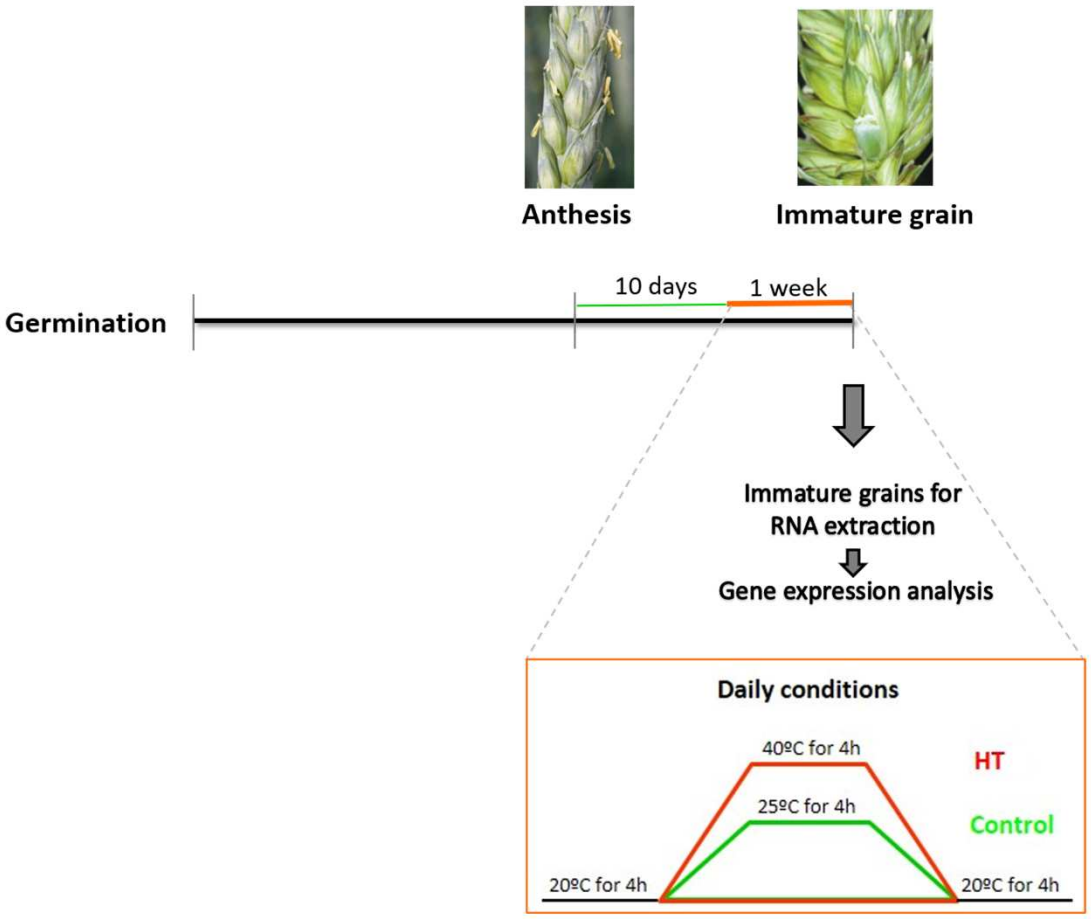
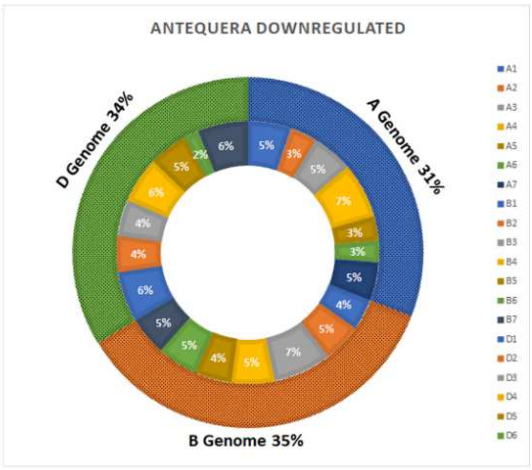
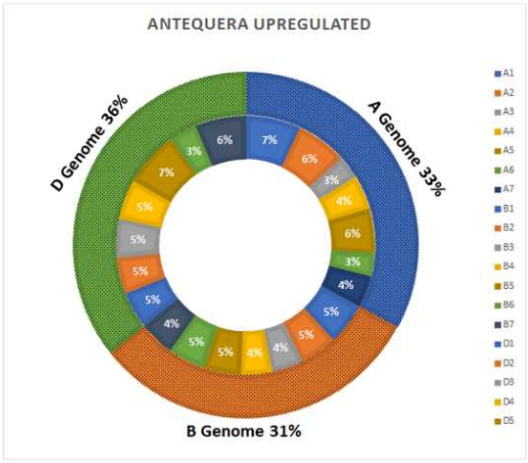
5.6 Supplemental material



Supplemental Figure 5.1 High temperature assay scheme - representation of assay conditions and immature grains collection timepoint for further RNA extraction (adapted from Tomás et al., 2020a).


ANTEQUERA DOWNREGULATED
A Genome 31%
B Genome 35%
D Genome 34%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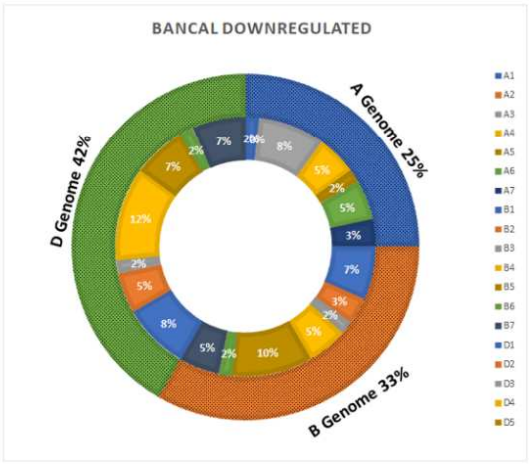
B Genome 31%
D Genome 36%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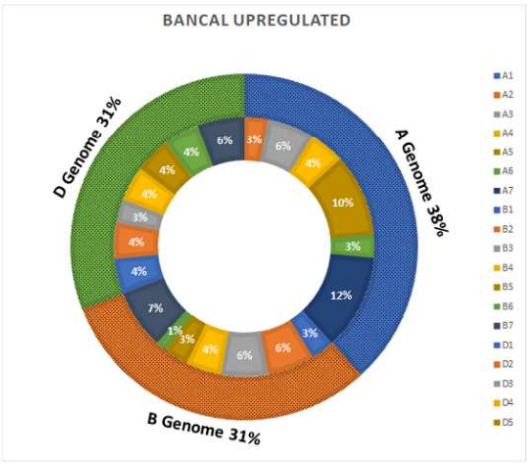
B Genome 33%
D Genome 42%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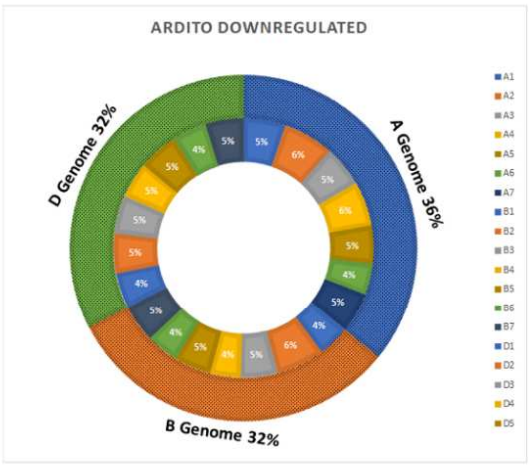
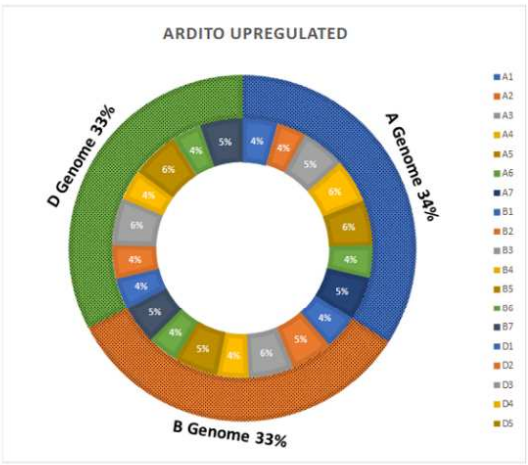
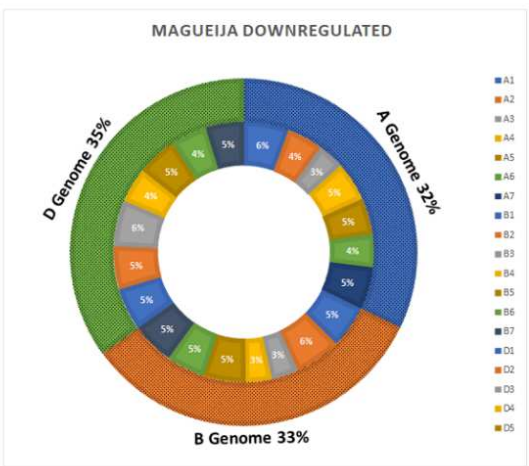
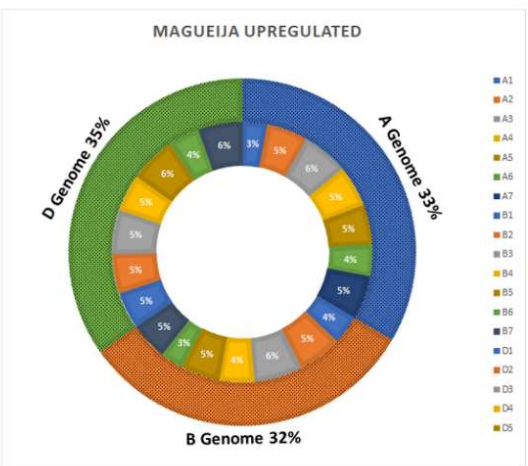
B Genome 31%
D Genome 31%


ARDITO DOWNREGULATED
A Genome 36%
B Genome 32%
D Genome 32%


ARDITO UPREGULATED
A Genome 34%
B Genome 33%
D Genome 33%


MAGUEIJA DOWNREGULATED
A Genome 32%
B Genome 33%
D Genome 35%


MAGUEIJA UPREGULATED
A Genome 33%
B Genome 32%
D Genome 35%

Supplemental Figure 5.2 Genome/chromosome locations of differentially expressed genes - Schematic representation of the genomic position of DEG on all chromosomes of commercial varieties Antequera and Bancal and landraces Ardito and Magueija. The three genomes (A, B and D) are displayed in the outer circle, and the chromosomes 1 to 7 of each genome are displayed in the inner circle. In both cases the percentage of associated DEGs is displayed.

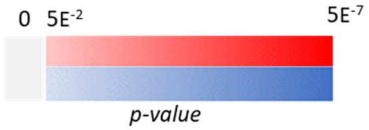
Supplemental Table 5.1 Supplemental Table 5.1 List of ten more up and downregulated genes with families and functions of encoded products, for commercial varieties Antequera and Bancal and landrace Ardito and Magueija. Red and blue indicate categories associated down and upregulated genes, respectively, and color intensity is related with the log_2 fold change value; gray represents unaltered genes.

Genotype	Gene ID	log2 fold change	Protein family	Protein function	Reference
			Upregulated		
Antequera	**TraesCS5D02G452700**	7.315389391	Protein of unknown function		
	TraesCS5B02G034200	6.886886615	Importin	Transport protein molecules into the nucleus by binding to specific recognition sequences, called nuclear localization sequences (NLS)	Görlich et al 1996
	TraesCS4A02G387800	6.857236125	Pentatricopeptide-repeat-containing proteins	Binds to RNA influencing folding, splicing, and degradation as well as RNA-processing events such as cleavage or editing	Barkan and Small 2014
	TraesCS3D02G032300	6.773634772	Uncharacterized protein		
	TraesCS7B02G101300	6.582944366	NADPH-dependent oxidoreductases - aldo-keto reductase family	Reduce aldehydes and ketones to their respective alcohols using NADPH, or rarely NADH as cofactor, apart from reducing other substrates, including monosaccharides, steroids, prostaglandins and polycyclic hydrocarbons	reviewed in Sengupta 2015
	TraesCS7A02G081200	6.497478805	Pentatricopeptide-repeat-containing proteins	Binds to RNA influencing folding, splicing, and degradation as well as RNA-processing events such as cleavage or editing	Barkan and Small 2014
	TraesCS7D02G069700	6.407908889	SNF2-related domain	ATPase component of the SNF2/SWI multisubunit complex, which utilises energy derived from ATP hydrolysis to disrupt histone-DNA interactions, resulting in the increased accessibility of DNA to transcription factors	Eisen et al 1995
	TraesCS2D02G107900	6.35716813	Haem peroxidase	Enzymes that use hydrogen peroxide as the electron acceptor to catalyse oxidative reactions	reviewed in Pandey 2017
	TraesCS5D02G523700	6.142205966	Pentatricopeptide-repeat-containing proteins	Binds to RNA influencing folding, splicing, and degradation as well as RNA-processing events such as cleavage or editing	Barkan and Small 2014
	TraesCS7B02G294500	6.093562545	B3 DNA binding domain - reproductive meristem (REM).	Involved in the organization of reproductive meristem	reviewed in Elisson 2009
Bancal	TraesCS3B02G155300	9.391024852	Adenylate kinase	Catalizes the phosphotransfer reaction for the interconversion of adenine nucleotides (ATP, ADP, and AMP), maintaining the cellular energy homeostasis	Raveneau et al 2017
	TraesCS2B02G124600	8.682008059	Haem peroxidase	Enzymes that use hydrogen peroxide as the electron acceptor to catalyse oxidative reactions	reviewed in Pandey 2017
	TraesCS5A02G480900	8.525371926	NB-ARC domain	A nucleotide-binding adaptor shared by plant resistance gene products and animal cell death regulators	van der Biezen and Jones 1998
	TraesCS4D02G272800	8.436610812	Uncharacterized protein		
	TraesCS7A02G045600	8.285082879	NB-ARC domain	A nucleotide-binding adaptor shared by plant resistance gene products and animal cell death regulators	van der Biezen and Jones 1998
	TraesCS5D02G360300	8.154722903	Pentatricopeptide-repeat-containing proteins	Binds to RNA influencing folding, splicing, and degradation as well as RNA-processing events such as cleavage or editing	Barkan and Small 2014
	TraesCS3B02G240600	8.082115961	Cleavage and polyadenylation specificity factor (CPSF) A subunit region	Involved in mRNA polyadenylation, binds the AAUAAA conserved sequence in pre-mRNA and also participate in splicing of single-intron pre-mRNAs	Li et al 2001
	TraesCS6D02G080900	8.072071161	Uncharacterized protein		
	TraesCS1B02G261100	8.057952176	ABA-induced Wheat Plasma Membrane Polypeptide-19 (AWPM-19-like) family	Roles in seed development, dormancy and stress responses. Promotes freezing tolerance of the wheat suspension-cultured cells by ABA treatment.	Koike et al 1997
	TraesCS2B02G615300	7.89531638	Proton-dependent Oligopeptide Transporter (POT) Family	Implicated in proton dependent oligopeptide transport.	Paulsen and Skurray 1994
Ardito	**TraesCS4D02G086200**	10.75617929	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2017
	TraesCS3A02G112900	10.26439025	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2018
	TraesCSU02G194600	10.25820435	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2019
	TraesCS3D02G114700	10.08682357	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2020
	TraesCS5D02G266000	10.06968499	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS4A02G275400	9.940190135	Pre-mRNA-splicing factor 18	Prp18 is required for the second step of pre-mRNA splicing annd appears to be primarily associated with the U5 snRNP.	Jiang et al 2000
	TraesCS5D02G269400	9.768157451	EF-hand domain	Calcium binding protein	Feng et al 2011
	TraesCS4D02G212500	9.443271922	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS3D02G115200	9.199020905	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS4B02G038900	8.953985386	Pre-mRNA-splicing factor 18	Prp18 is required for the second step of pre-mRNA splicing annd appears to be primarily associated with the U5 snRNP.	Jiang et al 2000
Magueija	**TraesCS4D02G086200**	11.59853083	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS4D02G134900	10.91989502	AAA ATPases	Involved in cell-cycle regulation, protein proteolysis and disaggregation, organelle biogenesis and intracellular transport	Latterich and Patel, 1997
	TraesCS4D02G035800	10.73327133	Pre-mRNA-splicing factor 18	Prp18 is required for the second step of pre-mRNA splicing annd appears to be primarily associated with the U5 snRNP.	Jiang et al 2000
	TraesCS7B02G267300	10.31228805	Heat shock factors (HSF)	Activate the expression of heat shock protein (Hsp) genes and thermotolerance-related genes by binding to HS responsive elements (HSEs) within promoters	Guo et al 2016
	TraesCS2D02G070500	10.04237693	Haem peroxidase	Enzymes that use hydrogen peroxide as the electron acceptor to catalyse oxidative reactions	reviewed in Pandey 2017
	TraesCS5D02G452700	9.92446874	Protein of unknown function		
	TraesCS3B02G048600	9.909770059	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS3B02G131300	9.761593979	Small heat shock protein (Hsp20)	ATP-independent molecular chaperones that prevent protein aggregation during biotic and abiotic stress conditions	Muthusamy et al 2021
	TraesCS7A02G360400	9.758280868	Heat shock factors (HSF)	Activate the expression of heat shock protein (Hsp) genes and thermotolerance-related genes by binding to HS responsive elements (HSEs) within promoters	Guo et al 2016
	TraesCS6A02G091400	9.728454938	Uncharacterized protein		

Genotype	Gene ID	log2 fold change	Protein family	Protein function	Reference
Downregulated					
Antequera	TraesCS2D02G458700	-10.79786182	Ubiquitin - Ribosomal protein L40	Controls the degradation of many proteins in the cells and affects a range of cellular processes	reviewed in Sharma et al 2016
	TraesCS5D02G333800	-10.09768349	Aminoacyl-tRNA synthetase, class II	Responsible for the linkage of a specific amino acid to the cognate tRNA	reviewed in Perona and Gruic-Sovulj
	TraesCS4A02G248900	-9.411113229	RNA recognition motif	Involved in all post-transcriptional events: pre-mRNA processing, splicing, alternative splicing, mRNA stability, RNA editing, mRNA export, pre-rRNA complex formation (nucleolin), translation regulation and degradation	reviewed in Maris et al 2005
	TraesCS2A02G458100	-9.268596228	Ubiquitin - Ribosomal protein L40	Controls the degradation of many proteins in the cells and affects a range of cellular processes	reviewed in Sharma et al 2016
	TraesCS7D02G474200	-8.992208273	Ubiquitin - Ribosomal protein L40	Controls the degradation of many proteins in the cells and affects a range of cellular processes	reviewed in Sharma et al 2016
	ENSRNA050013898	-8.815573474	5.8S ribosomal RNA	Ribosomal RNA component of the large subunit of the eukaryotic ribosome involved in protein production	reviewed in Sáez-Vásquez and Delseny 2019
	ENSRNA050016813	-8.592714639	5.8S ribosomal RNA	Ribosomal RNA component of the large subunit of the eukaryotic ribosome involved in protein production	reviewed in Sáez-Vásquez and Delseny 2019
	TraesCS6B02G217200	-8.585916933	Pyridoxal 5'-phosphate dependent enzymes	Pyridoxal 5'-phosphate is the active form of vitamin B6. These enzymes are involved in crucial cellular metabolic pathways in most of living organisms	reviewed in Liang et al 2019
	ENSRNA050017144	-8.554796881	Eukaryotic large subunit ribosomal RNA	Involved in protein production; it contains the peptidyl transferase site, the site at which peptide bonds are formed.	reviewed in Sáez-Vásquez and Delseny 2019
	TraesCS2B02G121200	-8.514646733	eIF2B_5 domain-containing protein	Protein with translation initiation activity	
Bancal	TraesCS1B02G348500	-8.127448738	O-methyltransferase domain	Responsible for both small and macromolecules metilation for different functional and regulatory processes transversal to all organisms.	reviewed in Ibrahim et al 1998
	TraesCS4A02G414700	-8.10551659	Protein Kinase	Catalyse the transfer of the gamma phosphate from nucleotide triphosphates (often ATP) to one or more amino acid residues in a protein substrate side chain, resulting in a conformational change affecting protein function	reviewed in Wei and Li 2019
	TraesCS3D02G517400	-7.667184306	ABA/WDS induced protein	Has chaperone-like activity for direct plant protection under abiotic stress	Yacoubi et al 2021
	TraesCS2D02G119400	-7.536894558	Xylanase inhibitor C-terminal	Terminal of xylanase inhibitor proteins that tpgether with the N-terminal to create the catalytic pocket necessary for cleaving xylanase, which in turn are responsible for arabinaxylan degradation.	reviewed in Juge 2004
	TraesCS1D02G221100	-7.114309099	Sugar major facilitator transporter	Also called uniporters, catalyze diffusion of the sugar down it electrochemical gradients	reviewed in Yan 2015
	TraesCS4D02G075200	-7.00541421	Pentatricopeptide-repeat-containing proteins	Binds to RNA influencing folding, splicing, and degradation as well as RNA-processing events such as cleavage or editing	Barkan and Small 2014
	TraesCS1D02G288500	-6.917605864	uncharacterized protein		
	TraesCS2B02G247100	-6.470345565	Calponin-homology domain	Intermediate in the connection of filaments to the actin cytoskeleton	reviewed in Bañuelos et al 1998
	TraesCS3A02G105900	-6.331131857	tRNA (Uracil-5-)-methyltransferase	Catalyze the formation of 5-methyl-uridine at position 54 (m5U54) in tRNA and may have a role in tRNA stabilisation or maturation	reviewed in Bañuelos et al 1999
	TraesCS7D02G466800	-5.976330778	Ureide permease	Transports ureide, a heterocyclic nitrogen compounds may serve as nitrogen sources or nitrogen transport compounds in plants.	reviewed in Tegeder and Masclaux-Daubresse 2017
Ardito	TraesCS5B02G488400	-8.987238471	Ribosome inactivating protein	Inhibit the translocation step in protein synthesis through the removal of a single adenine residue from a universally conserved stem-loop structure in the large-subunit large rRNA.	Massiah and Hartley 1995
	TraesCS4B02G381200	-8.591674887	Natural resistance-associated macrophage protein (NRAMP)	Metal transporters	Peng et al 2018
	TraesCS5B02G488700	-8.5421327	Ribosome inactivating protein	Inhibit the translocation step in protein synthesis through the removal of a single adenine residue from a universally conserved stem-loop structure in the large-subunit large rRNA.	Massiah and Hartley 1995
	TraesCS2A02G353400	-8.430797491	Uncharacterized protein		
	TraesCS2B02G125800	-8.108522756	Haem peroxidase	Enzymes that use hydrogen peroxide as the electron acceptor to catalyse oxidative reactions	reviewed in Pandey 2017
	TraesCS4B02G381900	-8.064565459	Protein phosphatase 2C	Mg+ dependent protein dephosphorylases involved in various signaling cascades including phytohormone signaling networks like abscisic acid (ABA), salicylic acid (SA)-ABA crosstalk, and developmental processes like mitogen-activated protein kinase (MAPK) signaling, and CLAVATA (CLV) signaling pathway	Yu et al 2019
	TraesCS3D02G133100	-8.041255782	Glutathione S-transferase	Catalyzes the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification	Wang et al 2019
	TraesCS5D02G173900	-7.73486749	Indole-3-glycerol-phosphate synthase	Catalizes the synthesis of indole-3-acetic acid biosynthesis in the tryptophan biosynthetic pathway	Ouyang and Li 2000
	TraesCS5A02G254500	-7.667617053	F-box protein	Mediates ubiquitination of proteins targeted for degradation by the 26S proteasome.	An et al 2019
	TraesCS4B02G234100	-7.602576658	Cytochromes P450	Heme-containing membrane-bound enzymes that can perform oxidation-reduction reactions, involved in plant defence and secondary metabolite biosynthesis	Reviewed in Morant et al 2003
Maguecija	TraesCS7D02G135000	-7.922314356	Myb-like DNA-binding domain	Domain present in Myb proteins family that act as transcription factors and are involved in controlling various processes, including responses to biotic and abiotic stresses, development, differentiation, and metabolism	Reviewed in Ambawat 2013
	TraesCS6A02G148800	-7.63497813	Fe(II) 2-oxoglutarate-dependent dioxygenase	Catalyse the oxidation of an organic substrate using a dioxygen molecule, and is involved in DNA demethylation, proline hydroxylation and formation of plant hormones and pigments	Reviewed in Kawai et al 2014
	TraesCS3B02G602400	-7.3469429	Protein Kinase	Catalyse the transfer of the gamma phosphate from nucleotide triphosphates (often ATP) to one or more amino acid residues in a protein substrate side chain, resulting in a conformational change affecting protein function	Reviewed in Wei and Li 2019
	TraesCS6D02G021000	-7.310180397	Glycosyltransferase	Enzymes that are involved in the biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates	Reviewed in Breton et al 2006
	TraesCS6D02G340200	-6.896463331	Uncharacterized protein		
	TraesCS5B02G053800	-6.883288711	Protein kinase domain-containing protein	Catalyse the transfer of the gamma phosphate from nucleotide triphosphates (often ATP) to one or more amino acid residues in a protein substrate side chain, resulting in a conformational change affecting protein function	Reviewed in Wei and Li 2019
	TraesCS5B02G251600	-6.729837226	Fasciclin-like domain	Present in arabinogalactan proteins are Implicated in cell adhesion and may link the cell membrane and cell wall	Nirmal et al 2017
	TraesCS6B02G024900	-6.684866195	Cytochrome b5	Electron transfer protein with a redox potential	Vergères and Waskell 1995
	TraesCS4A02G460700	-6.492804587	Plant invertase/pectin methylesterase inhibitor	PME catalyses the demethylesterification of galacturonic acid units of pectin, generating free carboxyl groups and releasing protons	Giovane et al 2004
	TraesCS1D02G387000	-6.487021223	OPT oligopeptide transporter protein	cell membrane proteins that play a critical role in the transport of small peptides, secondary amino acids, glutathione conjugates, and mineral uptake	Kumar et al 2019

Supplemental Table 5.2. Significant enriched gene ontology terms, (all levels), associated with DEGs in commercial varieties Antequera and Bancal and landraces Ardito and Magueija. Red and blue indicate categories associated down and upregulated genes, respectively, and color intensity are related with the degree of significance; gray represents unaltered terms.

Legend:



Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
Biological process									
GO:0006457	Protein folding								
GO:0009644	Response to high light intensity								
GO:0009642	Response to light intensity								
GO:0010286	Heat acclimation								
GO:0009416	Response to light stimulus								
GO:0000302	Response to reactive oxygen species								
GO:0009768	Photosynthesis, light harvesting in photosystem I								
GO:0018298	Protein-chromophore linkage								
GO:0009765	Photosynthesis, light harvesting								
GO:0006412	Translation								
GO:0006518	Peptide metabolic process								
GO:0043603	Cellular amide metabolic process								
GO:0043604	Amide biosynthetic process								
GO:0043043	Peptide biosynthetic process								
GO:0009769	Photosynthesis, light harvesting in photosystem II								
GO:0009637	Response to blue light								
GO:0010218	Response to far red light								
GO:0010187	Negative regulation of seed germination								
GO:0001510	RNA methylation								
GO:0010029	Regulation of seed germination								
GO:0043457	Regulation of cellular respiration								
GO:0090332	Stomatal closure								
GO:0010114	Response to red light								
GO:0051241	Negative regulation of multicellular organismal process								
GO:0048581	Negative regulation of post-embryonic development								
GO:0043462	Regulation of atpase activity								
GO:0051093	Negative regulation of developmental process								
GO:0050896	Response to stimulus								
GO:0009735	Response to cytokinin								
GO:0080037	Negative regulation of cytokinin-activated signaling pathway								
GO:0010033	Response to organic substance								
GO:0043467	Regulation of generation of precursor metabolites and energy								
GO:0042221	Response to chemical								
GO:0009725	Response to hormone								
GO:0006624	Vacuolar protein processing								
GO:0019684	Photosynthesis, light reaction								
GO:0009719	Response to endogenous stimulus								
GO:0009628	Response to abiotic stimulus								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description
GO:0006091	Generation of precursor metabolites and energy
GO:0009750	Response to fructose
GO:0033993	Response to lipid
GO:0009737	Response to abscisic acid
GO:0097305	Response to alcohol
GO:1901700	Response to oxygen-containing compound
GO:0080167	Response to karrikin
GO:0009791	Post-embryonic development
GO:0010196	Nonphotochemical quenching
GO:1990066	Energy quenching
GO:0010501	RNA secondary structure unwinding
GO:0009314	Response to radiation
GO:0048316	Seed development
GO:1901566	Organonitrogen compound biosynthetic process
GO:0009845	Seed germination
GO:0097577	Sequestering of iron ion
GO:0006880	Intracellular sequestering of iron ion
GO:0009408	Response to heat
GO:0042542	Response to hydrogen peroxide
GO:0006979	Response to oxidative stress
GO:1900140	Regulation of seedling development
GO:0010035	Response to inorganic substance
GO:0009266	Response to temperature stimulus
GO:0010118	Stomatal movement
GO:2000026	Regulation of multicellular organismal development
GO:0048580	Regulation of post-embryonic development
GO:0050793	Regulation of developmental process
GO:0090351	Seedling development
GO:0048573	Photoperiodism, flowering
GO:0009648	Photoperiodism
GO:0051239	Regulation of multicellular organismal process
GO:0044403	Symbiosis, encompassing mutualism through parasitism
GO:0044419	Interspecies interaction between organisms
GO:0042742	Defense response to bacterium
GO:0044764	Multi-organism cellular process
GO:0007275	Multicellular organism development
GO:0009615	Response to virus
GO:0044767	Single-organism developmental process
GO:0048856	Anatomical structure development

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0010228	Vegetative to reproductive phase transition of meristem								
GO:0032502	Developmental process								
GO:0016032	Viral process								
GO:0034976	Response to endoplasmic reticulum stress								
GO:0061077	Chaperone-mediated protein folding								
GO:0006458	'De novo' protein folding								
GO:0006984	ER-nucleus signaling pathway								
GO:0006950	Response to stress								
GO:0051084	'De novo' posttranslational protein folding								
GO:0051085	Chaperone mediated protein folding requiring cofactor								
GO:0008380	RNA splicing								
GO:0051131	Chaperone-mediated protein complex assembly								
GO:0000398	Mrna splicing, via spliceosome								
GO:0033554	Cellular response to stress								
GO:0000375	RNA splicing, via transesterification reactions								
GO:0042026	Protein refolding								
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile								
GO:0046686	Response to cadmium ion								
GO:0006970	Response to osmotic stress								
GO:0009987	Cellular process								
GO:0006006	Glucose metabolic process								
GO:0006090	Pyruvate metabolic process								
GO:0019637	Organophosphate metabolic process								
GO:0046939	Nucleotide phosphorylation								
GO:0006397	Mrna processing								
GO:0016071	Mrna metabolic process								
GO:0019318	Hexose metabolic process								
GO:0006396	RNA processing								
GO:0006757	ATP generation from ADP								
GO:0006096	Glycolytic process								
GO:0071840	Cellular component organization or biogenesis								
GO:0006094	Gluconeogenesis								
GO:0009135	Purine nucleoside diphosphate metabolic process								
GO:0009179	Purine ribonucleoside diphosphate metabolic process								
GO:0046031	ADP metabolic process								
GO:0009185	Ribonucleoside diphosphate metabolic process								
GO:0010038	Response to metal ion								
GO:0006165	Nucleoside diphosphate phosphorylation								
GO:0009132	Nucleoside diphosphate metabolic process								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0044724	Single-organism carbohydrate catabolic process								
GO:0030010	Establishment of cell polarity								
GO:0019319	Hexose biosynthetic process								
GO:0016485	Protein processing								
GO:0046364	Monosaccharide biosynthetic process								
GO:0051604	Protein maturation								
GO:0009117	Nucleotide metabolic process								
GO:0071826	Ribonucleoprotein complex subunit organization								
GO:0006753	Nucleoside phosphate metabolic process								
GO:0006626	Protein targeting to mitochondrion								
GO:0072655	Establishment of protein localization to mitochondrion								
GO:0070585	Protein localization to mitochondrion								
GO:0005996	Monosaccharide metabolic process								
GO:0016043	Cellular component organization								
GO:0072594	Establishment of protein localization to organelle								
GO:0070646	Protein modification by small protein removal								
GO:0046496	Nicotinamide nucleotide metabolic process								
GO:0019362	Pyridine nucleotide metabolic process								
GO:0009651	Response to salt stress								
GO:0006996	Organelle organization								
GO:0006839	Mitochondrial transport								
GO:0090407	Organophosphate biosynthetic process								
GO:0051716	Cellular response to stimulus								
GO:0022618	Ribonucleoprotein complex assembly								
GO:0006983	ER overload response								
GO:0045471	Response to ethanol								
GO:0006081	Cellular aldehyde metabolic process								
GO:0072524	Pyridine-containing compound metabolic process								
GO:0007163	Establishment or maintenance of cell polarity								
GO:0050821	Protein stabilization								
GO:0000038	Very long-chain fatty acid metabolic process								
GO:0006986	Response to unfolded protein								
GO:0044723	Single-organism carbohydrate metabolic process								
GO:0046034	ATP metabolic process								
GO:0032781	Positive regulation of atpase activity								
GO:0055086	Nucleobase-containing small molecule metabolic process								
GO:0031647	Regulation of protein stability								
GO:0033365	Protein localization to organelle								
GO:0043933	Macromolecular complex subunit organization								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0034641	Cellular nitrogen compound metabolic process								
GO:0018208	Peptidyl-proline modification								
GO:0048193	Golgi vesicle transport								
GO:0009205	Purine ribonucleoside triphosphate metabolic process								
GO:0009144	Purine nucleoside triphosphate metabolic process								
GO:0009123	Nucleoside monophosphate metabolic process								
GO:0009161	Ribonucleoside monophosphate metabolic process								
GO:0006139	Nucleobase-containing compound metabolic process								
GO:0045041	Protein import into mitochondrial intermembrane space								
GO:0010467	Gene expression								
GO:0009167	Purine ribonucleoside monophosphate metabolic process								
GO:0009126	Purine nucleoside monophosphate metabolic process								
GO:0009199	Ribonucleoside triphosphate metabolic process								
GO:0046685	Response to arsenic-containing substance								
GO:0016579	Protein deubiquitination								
GO:0016052	Carbohydrate catabolic process								
GO:0080158	Chloroplast ribulose bisphosphate carboxylase complex biogenesis								
GO:0009141	Nucleoside triphosphate metabolic process								
GO:0071277	Cellular response to calcium ion								
GO:0070544	Histone H3-K36 demethylation								
GO:0007005	Mitochondrion organization								
GO:0046128	Purine ribonucleoside metabolic process								
GO:0003032	Detection of oxygen								
GO:0070483	Detection of hypoxia								
GO:0042278	Purine nucleoside metabolic process								
GO:0043335	Protein unfolding								
GO:0022613	Ribonucleoprotein complex biogenesis								
GO:0070076	Histone lysine demethylation								
GO:0042255	Ribosome assembly								
GO:0018158	Protein oxidation								
GO:0018171	Peptidyl-cysteine oxidation								
GO:0000413	Protein peptidyl-prolyl isomerization								
GO:0006816	Calcium ion transport								
GO:0019253	Reductive pentose-phosphate cycle								
GO:0006325	Chromatin organization								
GO:0051276	Chromosome organization								
GO:0006334	Nucleosome assembly								
GO:0006333	Chromatin assembly or disassembly								
GO:0034728	Nucleosome organization								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0031497	Chromatin assembly								
GO:0006323	DNA packaging								
GO:0065004	Protein-DNA complex assembly								
GO:0071824	Protein-DNA complex subunit organization								
GO:0071103	DNA conformation change								
GO:0006342	Chromatin silencing								
GO:0045814	Negative regulation of gene expression, epigenetic								
GO:0070828	Heterochromatin organization								
GO:0016458	Gene silencing								
GO:0040029	Regulation of gene expression, epigenetic								
GO:0045892	Negative regulation of transcription, DNA-templated								
GO:0010629	Negative regulation of gene expression								
GO:1903507	Negative regulation of nucleic acid-templated transcription								
GO:1902679	Negative regulation of RNA biosynthetic process								
GO:0008283	Cell proliferation								
GO:0051253	Negative regulation of RNA metabolic process								
GO:0006595	Polyamine metabolic process								
GO:0045934	Negative regulation of nucleobase-containing compound metabolic process								
GO:0006598	Polyamine catabolic process								
GO:0061647	Histone H3-K9 modification								
GO:0051567	Histone H3-K9 methylation								
GO:0016570	Histone modification								
GO:0016569	Covalent chromatin modification								
GO:0006275	Regulation of DNA replication								
GO:0016572	Histone phosphorylation								
GO:2000113	Negative regulation of cellular macromolecule biosynthetic process								
GO:0006730	One-carbon metabolic process								
GO:0046500	S-adenosylmethionine metabolic process								
GO:0010605	Negative regulation of macromolecule metabolic process								
GO:0010558	Negative regulation of macromolecule biosynthetic process								
GO:0006306	DNA methylation								
GO:0006305	DNA alkylation								
GO:0044728	DNA methylation or demethylation								
GO:0006304	DNA modification								
GO:0051172	Negative regulation of nitrogen compound metabolic process								
GO:0031327	Negative regulation of cellular biosynthetic process								
GO:0006261	DNA-dependent DNA replication								
GO:0009892	Negative regulation of metabolic process								
GO:0006479	Protein methylation								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0008213	Protein alkylation								
GO:0006260	DNA replication								
GO:0042398	Cellular modified amino acid biosynthetic process								
GO:0006542	Glutamine biosynthetic process								
GO:0009890	Negative regulation of biosynthetic process								
GO:0051225	Spindle assembly								
GO:0016571	Histone methylation								
GO:0007051	Spindle organization								
GO:0018022	Peptidyl-lysine methylation								
GO:0034968	Histone lysine methylation								
GO:0050667	Homocysteine metabolic process								
GO:0006461	Protein complex assembly								
GO:0051726	Regulation of cell cycle								
GO:0070271	Protein complex biogenesis								
GO:0071822	Protein complex subunit organization								
GO:0019252	Starch biosynthetic process								
GO:0006346	Methylation-dependent chromatin silencing								
GO:0009068	Aspartate family amino acid catabolic process								
GO:0031324	Negative regulation of cellular metabolic process								
GO:0006556	S-adenosylmethionine biosynthetic process								
GO:0009064	Glutamine family amino acid metabolic process								
GO:0006270	DNA replication initiation								
GO:0007018	Microtubule-based movement								
GO:0032259	Methylation								
GO:0005975	Carbohydrate metabolic process								
GO:0032261	Purine nucleotide salvage								
GO:0065003	Macromolecular complex assembly								
GO:0051052	Regulation of DNA metabolic process								
GO:0009066	Aspartate family amino acid metabolic process								
GO:0043173	Nucleotide salvage								
GO:0043101	Purine-containing compound salvage								
GO:0019676	Ammonia assimilation cycle								
GO:0034622	Cellular macromolecular complex assembly								
GO:0009399	Nitrogen fixation								
GO:0009084	Glutamine family amino acid biosynthetic process								
GO:0016051	Carbohydrate biosynthetic process								
GO:0007017	Microtubule-based process								
GO:0005982	Starch metabolic process								
GO:0018205	Peptidyl-lysine modification								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0010389	Regulation of G2/M transition of mitotic cell cycle								
GO:0061640	Cytoskeleton-dependent cytokinesis								
GO:1902749	Regulation of cell cycle G2/M phase transition								
GO:0009744	Response to sucrose								
GO:0034285	Response to disaccharide								
GO:0042126	Nitrate metabolic process								
GO:0042128	Nitrate assimilation								
GO:0000910	Cytokinesis								
GO:0006259	DNA metabolic process								
GO:0000226	Microtubule cytoskeleton organization								
GO:0000086	G2/M transition of mitotic cell cycle								
GO:0000281	Mitotic cytokinesis								
GO:0044839	Cell cycle G2/M phase transition								
GO:0044710	Single-organism metabolic process								
GO:0006520	Cellular amino acid metabolic process								
GO:0006536	Glutamate metabolic process								
GO:1901565	Organonitrogen compound catabolic process								
GO:0006545	Glycine biosynthetic process								
GO:0000278	Mitotic cell cycle								
GO:0000911	Cytokinesis by cell plate formation								
GO:0044711	Single-organism biosynthetic process								
GO:2001057	Reactive nitrogen species metabolic process								
GO:0071941	Nitrogen cycle metabolic process								
GO:0045493	Xylan catabolic process								
GO:1905114	Cell surface receptor signaling pathway involved in cell-cell signaling								
GO:0016055	Wnt signaling pathway								
GO:0198738	Cell-cell signaling by wnt								
GO:0044283	Small molecule biosynthetic process								
GO:0044712	Single-organism catabolic process								
GO:0070482	Response to oxygen levels								
GO:0001666	Response to hypoxia								
GO:0036293	Response to decreased oxygen levels								
GO:0051186	Cofactor metabolic process								
GO:0019685	Photosynthesis, dark reaction								
GO:0009240	Isopentenyl diphosphate biosynthetic process								
GO:0046490	Isopentenyl diphosphate metabolic process								
GO:0010304	PSII associated light-harvesting complex II catabolic process								
GO:0032787	Monocarboxylic acid metabolic process								
GO:0019288	Isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0072330	Monocarboxylic acid biosynthetic process								
GO:0006733	Oxidoreduction coenzyme metabolic process								
GO:0006633	Fatty acid biosynthetic process								
GO:1900409	Positive regulation of cellular response to oxidative stress								
GO:0016053	Organic acid biosynthetic process								
GO:0019682	Glyceraldehyde-3-phosphate metabolic process								
GO:0006631	Fatty acid metabolic process								
GO:1900407	Regulation of cellular response to oxidative stress								
GO:0006972	Hyperosmotic response								
GO:0009658	Chloroplast organization								
GO:0015977	Carbon fixation								
GO:0015994	Chlorophyll metabolic process								
GO:0008610	Lipid biosynthetic process								
GO:0019249	Lactate biosynthetic process								
GO:0046208	Spermine catabolic process								
GO:0046394	Carboxylic acid biosynthetic process								
GO:0008215	Spermine metabolic process								
GO:0042761	Very long-chain fatty acid biosynthetic process								
GO:1904833	Positive regulation of removal of superoxide radicals								
GO:1901671	Positive regulation of superoxide dismutase activity								
GO:1901668	Regulation of superoxide dismutase activity								
GO:1901033	Positive regulation of response to reactive oxygen species								
GO:0010564	Regulation of cell cycle process								
GO:1901990	Regulation of mitotic cell cycle phase transition								
GO:1901987	Regulation of cell cycle phase transition								
GO:0044772	Mitotic cell cycle phase transition								
GO:0044770	Cell cycle phase transition								
GO:1903047	Mitotic cell cycle process								
GO:0022402	Cell cycle process								
GO:0006112	Energy reserve metabolic process								
GO:0005977	Glycogen metabolic process								
GO:0007049	Cell cycle								
GO:0000724	Double-strand break repair via homologous recombination								
GO:0018193	Peptidyl-amino acid modification								
GO:0000725	Recombinational repair								
GO:0016444	Somatic cell DNA recombination								
GO:2000896	Amylopectin metabolic process								
GO:0010021	Amylopectin biosynthetic process								
GO:0007346	Regulation of mitotic cell cycle								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0006302	Double-strand break repair								
GO:0032506	Cytokinetic process								
GO:1902410	Mitotic cytokinetic process								
GO:0010324	Membrane invagination								
GO:0043414	Macromolecule methylation								
GO:0022607	Cellular component assembly								
GO:0051301	Cell division								
GO:0007010	Cytoskeleton organization								
GO:0005984	Disaccharide metabolic process								
GO:0046785	Microtubule polymerization								
GO:0007020	Microtubule nucleation								
GO:0000023	Maltose metabolic process								
GO:0031109	Microtubule polymerization or depolymerization								
GO:1902589	Single-organism organelle organization								
GO:0031047	Gene silencing by RNA								
GO:0006310	DNA recombination								
GO:0006268	DNA unwinding involved in DNA replication								
GO:0006281	DNA repair								
GO:0051258	Protein polymerization								
GO:0009251	Glucan catabolic process								
GO:0000280	Nuclear division								
GO:0048285	Organelle fission								
GO:0009909	Regulation of flower development								
GO:0000272	Polysaccharide catabolic process								
GO:0044275	Cellular carbohydrate catabolic process								
GO:0006656	Phosphatidylcholine biosynthetic process								
GO:0005983	Starch catabolic process								
GO:0043085	Positive regulation of catalytic activity								
GO:0044247	Cellular polysaccharide catabolic process								
GO:0048519	Negative regulation of biological process								
GO:0010556	Regulation of macromolecule biosynthetic process								
GO:0048523	Negative regulation of cellular process								
GO:2000112	Regulation of cellular macromolecule biosynthetic process								
GO:0006974	Cellular response to DNA damage stimulus								
GO:1903046	Meiotic cell cycle process								
GO:0098813	Nuclear chromosome segregation								
GO:0048831	Regulation of shoot system development								
GO:0007126	Meiotic nuclear division								
GO:0009311	Oligosaccharide metabolic process								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0042401	Cellular biogenic amine biosynthetic process								
GO:0009309	Amine biosynthetic process								
GO:0009889	Regulation of biosynthetic process								
GO:0007059	Chromosome segregation								
GO:0060255	Regulation of macromolecule metabolic process								
GO:0050790	Regulation of catalytic activity								
	Molecular function								
GO:0004499	N,N-dimethylaniline monooxygenase activity								
GO:0004553	Hydrolase activity, hydrolyzing O-glycosyl compounds								
GO:0016798	Hydrolase activity, acting on glycosyl bonds								
GO:0031409	Pigment binding								
GO:0016168	Chlorophyll binding								
GO:0002020	Protease binding								
GO:0003735	Structural constituent of ribosome								
GO:0005198	Structural molecule activity								
GO:0103100	UDP-glucose: 6-methylthiohexylhydroximate S-glucosyltransferase activity								
GO:0103103	UDP-glucose: 9-methylthiononylhydroximate S-glucosyltransferase activity								
GO:0103099	UDP-glucose:5-methylthiopentylhydroximate S-glucosyltransferase activity								
GO:0103102	UDP-glucose:8-methylthiooctylhydroximate S-glucosyltransferase activity								
GO:0103101	UDP-glucose:7-methylthioheptylhydroximate S-glucosyltransferase activity								
GO:0030599	Pectinesterase activity								
GO:0003729	Mrna binding								
GO:0044822	Poly(A) RNA binding								
GO:0046910	Pectinesterase inhibitor activity								
GO:0016876	Ligase activity, forming aminoacyl-trna and related compounds								
GO:0016875	Ligase activity, forming carbon-oxygen bonds								
GO:0004812	Aminoacyl-trna ligase activity								
GO:0043531	ADP binding								
GO:0032559	Adenyl ribonucleotide binding								
GO:0030554	Adenyl nucleotide binding								
GO:0036094	Small molecule binding								
GO:0032550	Purine ribonucleoside binding								
GO:0001883	Purine nucleoside binding								
GO:0032549	Ribonucleoside binding								
GO:0001882	Nucleoside binding								
GO:0032555	Purine ribonucleotide binding								
GO:0017076	Purine nucleotide binding								
GO:0000166	Nucleotide binding								
GO:1901265	Nucleoside phosphate binding								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0032553	Ribonucleotide binding								
GO:0097367	Carbohydrate derivative binding								
GO:0005524	ATP binding								
GO:0043621	Protein self-association								
GO:0051082	Unfolded protein binding								
GO:0016887	Atpase activity								
GO:0031625	Ubiquitin protein ligase binding								
GO:0044389	Ubiquitin-like protein ligase binding								
GO:0003723	RNA binding								
GO:0035639	Purine ribonucleoside triphosphate binding								
GO:0031072	Heat shock protein binding								
GO:0051087	Chaperone binding								
GO:0060590	Atpase regulator activity								
GO:0000774	Adenyl-nucleotide exchange factor activity								
GO:0051879	Hsp90 protein binding								
GO:0060589	Nucleoside-triphosphatase regulator activity								
GO:0030544	Hsp70 protein binding								
GO:0080025	Phosphatidylinositol-3,5-bisphosphate binding								
GO:0005507	Copper ion binding								
GO:0044183	Protein binding involved in protein folding								
GO:0005527	Macrolide binding								
GO:0005528	FK506 binding								
GO:0032266	Phosphatidylinositol-3-phosphate binding								
GO:0070678	Preprotein binding								
GO:0001671	Atpase activator activity								
GO:0008144	Drug binding								
GO:0005488	Binding								
GO:0004652	Polynucleotide adenylyltransferase activity								
GO:0016984	Ribulose-bisphosphate carboxylase activity								
GO:0019783	Ubiquitin-like protein-specific protease activity								
GO:0044620	ACP phosphopantetheine attachment site binding								
GO:0051192	Prosthetic group binding								
GO:0050145	Nucleoside phosphate kinase activity								
GO:0000036	ACP phosphopantetheine attachment site binding involved in fatty acid biosynthetic process								
GO:0005515	Protein binding								
GO:0017172	Cysteine dioxygenase activity								
GO:0047800	Cysteamine dioxygenase activity								
GO:0004221	Obsolete ubiquitin thiolesterase activity								
GO:0046975	Histone methyltransferase activity (H3-K36 specific)								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0003755	Peptidyl-prolyl cis-trans isomerase activity								
GO:0031386	Protein tag								
GO:0016531	Copper chaperone activity								
GO:0008135	Translation factor activity, RNA binding								
GO:0016530	Metallochaperone activity								
GO:0016860	Intramolecular oxidoreductase activity								
GO:0046982	Protein heterodimerization activity								
GO:0003677	DNA binding								
GO:0003682	Chromatin binding								
GO:0004356	Glutamate-ammonia ligase activity								
GO:0046983	Protein dimerization activity								
GO:0004478	Methionine adenosyltransferase activity								
GO:0016880	Acid-ammonia (or amide) ligase activity								
GO:0016211	Ammonia ligase activity								
GO:1990939	ATP-dependent microtubule motor activity								
GO:0003676	Nucleic acid binding								
GO:0044877	Macromolecular complex binding								
GO:0005200	Structural constituent of cytoskeleton								
GO:0097159	Organic cyclic compound binding								
GO:1901363	Heterocyclic compound binding								
GO:0030170	Pyridoxal phosphate binding								
GO:0042084	5-methyltetrahydrofolate-dependent methyltransferase activity								
GO:0003871	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity								
GO:0042085	5-methyltetrahydropteroyltri-L-glutamate-dependent methyltransferase activity								
GO:0008705	Methionine synthase activity								
GO:0008732	L-allo-threonine aldolase activity								
GO:0004793	Threonine aldolase activity								
GO:0016781	Phosphotransferase activity, paired acceptors								
GO:0004029	Aldehyde dehydrogenase (NAD) activity								
GO:0008569	ATP-dependent microtubule motor activity, minus-end-directed								
GO:0008017	Microtubule binding								
GO:0015631	Tubulin binding								
GO:0008509	Anion transmembrane transporter activity								
GO:0048037	Cofactor binding								
GO:0003906	DNA-(apurinic or apyrimidinic site) lyase activity								
GO:0016595	Glutamate binding								
GO:0052902	Spermidine:oxygen oxidoreductase (3-aminopropanal-forming) activity								
GO:0052903	N1-acetylspermine:oxygen oxidoreductase (3-acetamidopropanal-forming) activity								
GO:0052904	N1-acetylspermidine:oxygen oxidoreductase (3-acetamidopropanal-forming) activity								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0016859	Cis-trans isomerase activity								
GO:0052894	Norspermine:oxygen oxidoreductase activity								
GO:0052895	N1-acetylspermine:oxygen oxidoreductase (N1-acetylspermidine-forming) activity								
GO:0046592	Polyamine oxidase activity								
GO:0052901	Spermine:oxygen oxidoreductase (spermidine-forming) activity								
GO:0019172	Glyoxalase III activity								
GO:0047517	1,4-beta-D-xylan synthase activity								
GO:0004096	Catalase activity								
GO:0051669	Fructan beta-fructosidase activity								
GO:0009922	Fatty acid elongase activity								
GO:0035250	UDP-galactosyltransferase activity								
GO:0004133	Glycogen debranching enzyme activity								
GO:0008092	Cytoskeletal protein binding								
GO:0019156	Isoamylase activity								
GO:0061731	Ribonucleoside-diphosphate reductase activity								
GO:0004748	Ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor								
GO:0016728	Oxidoreductase activity, acting on CH or CH2 groups, disulfide as acceptor								
GO:0003777	Microtubule motor activity								
GO:0004049	Anthranilate synthase activity								
	Cellular component								
GO:0005782	Peroxisomal matrix								
GO:0031907	Microbody lumen								
GO:0044439	Peroxisomal part								
GO:0044438	Microbody part								
GO:0005777	Peroxisome								
GO:0042579	Microbody								
GO:0005783	Endoplasmic reticulum								
GO:0030076	Light-harvesting complex								
GO:0009522	Photosystem I								
GO:0022625	Cytosolic large ribosomal subunit								
GO:0022626	Cytosolic ribosome								
GO:0010287	Plastoglobule								
GO:0005730	Nucleolus								
GO:0009521	Photosystem								
GO:0009523	Photosystem II								
GO:0044391	Ribosomal subunit								
GO:0015934	Large ribosomal subunit								
GO:0044445	Cytosolic part								
GO:0005829	Cytosol								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0016363	Nuclear matrix								
GO:0009782	Photosystem I antenna complex								
GO:0031981	Nuclear lumen								
GO:0005840	Ribosome								
GO:0031974	Membrane-enclosed lumen								
GO:0070013	Intracellular organelle lumen								
GO:0043233	Organelle lumen								
GO:0030529	Intracellular ribonucleoprotein complex								
GO:0034399	Nuclear periphery								
GO:0048046	Apoplast								
GO:0044428	Nuclear part								
GO:1990904	Ribonucleoprotein complex								
GO:0043202	Lysosomal lumen								
GO:0009533	Chloroplast stromal thylakoid								
GO:0009783	Photosystem II antenna complex								
GO:0000322	Storage vacuole								
GO:0000326	Protein storage vacuole								
GO:0005737	Cytoplasm								
GO:0009517	PSII associated light-harvesting complex II								
GO:0009503	Thylakoid light-harvesting complex								
GO:0005775	Vacuolar lumen								
GO:0022627	Cytosolic small ribosomal subunit								
GO:0009535	Chloroplast thylakoid membrane								
GO:0098796	Membrane protein complex								
GO:0055035	Plastid thylakoid membrane								
GO:0031360	Intrinsic component of thylakoid membrane								
GO:0031361	Integral component of thylakoid membrane								
GO:0044464	Cell part								
GO:0005623	Cell								
GO:0005622	Intracellular								
GO:0042651	Thylakoid membrane								
GO:0044424	Intracellular part								
GO:0009534	Chloroplast thylakoid								
GO:0031976	Plastid thylakoid								
GO:0034357	Photosynthetic membrane								
GO:0098807	Chloroplast thylakoid membrane protein complex								
GO:0046658	Anchored component of plasma membrane								
GO:0044436	Thylakoid part								
GO:0043232	Intracellular non-membrane-bounded organelle								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0044444	Cytoplasmic part								
GO:0009579	Thylakoid								
GO:0005764	Lysosome								
GO:0043228	Non-membrane-bounded organelle								
GO:0015935	Small ribosomal subunit								
GO:0000323	Lytic vacuole								
GO:0044446	Intracellular organelle part								
GO:0044422	Organelle part								
GO:0043227	Membrane-bounded organelle								
GO:0043231	Intracellular membrane-bounded organelle								
GO:0043226	Organelle								
GO:0043229	Intracellular organelle								
GO:0009532	Plastid stroma								
GO:0005788	Endoplasmic reticulum lumen								
GO:0044435	Plastid part								
GO:0031967	Organelle envelope								
GO:0009570	Chloroplast stroma								
GO:0031975	Envelope								
GO:0044434	Chloroplast part								
GO:0044432	Endoplasmic reticulum part								
GO:0009941	Chloroplast envelope								
GO:0009526	Plastid envelope								
GO:0005681	Spliceosomal complex								
GO:0012505	Endomembrane system								
GO:0044429	Mitochondrial part								
GO:0005759	Mitochondrial matrix								
GO:0009507	Chloroplast								
GO:0031090	Organelle membrane								
GO:0044437	Vacuolar part								
GO:0009536	Plastid								
GO:0005774	Vacuolar membrane								
GO:0005789	Endoplasmic reticulum membrane								
GO:0042175	Nuclear outer membrane-endoplasmic reticulum membrane network								
GO:0005634	Nucleus								
GO:0098805	Whole membrane								
GO:0098588	Bounding membrane of organelle								
GO:0005773	Vacuole								
GO:0048492	Ribulose bisphosphate carboxylase complex								
GO:0009573	Chloroplast ribulose bisphosphate carboxylase complex								

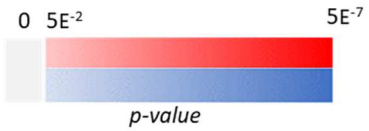
Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0005739	Mitochondrion								
GO:0032991	Macromolecular complex								
GO:0016612	Molybdenum-iron nitrogenase complex								
GO:0016610	Nitrogenase complex								
GO:0001405	Presequence translocase-associated import motor								
GO:0005684	U2-type spliceosomal complex								
GO:0018444	Translation release factor complex								
GO:0005794	Golgi apparatus								
GO:0030532	Small nuclear ribonucleoprotein complex								
GO:0097525	Spliceosomal snrnp complex								
GO:0035061	Interchromatin granule								
GO:0030089	Phycobilisome								
GO:0000124	SAGA complex								
GO:0000786	Nucleosome								
GO:0044815	DNA packaging complex								
GO:0032993	Protein-DNA complex								
GO:0000785	Chromatin								
GO:0044427	Chromosomal part								
GO:0005721	Pericentric heterochromatin								
GO:0005694	Chromosome								
GO:0000792	Heterochromatin								
GO:0000790	Nuclear chromatin								
GO:0000775	Chromosome, centromeric region								
GO:0098687	Chromosomal region								
GO:0044454	Nuclear chromosome part								
GO:0000228	Nuclear chromosome								
GO:0005874	Microtubule								
GO:0099512	Supramolecular fiber								
GO:0099513	Polymeric cytoskeletal fiber								
GO:0015630	Microtubule cytoskeleton								
GO:0044430	Cytoskeletal part								
GO:0043234	Protein complex								
GO:0005871	Kinesin complex								
GO:0045298	Tubulin complex								
GO:0009574	Preprophase band								
GO:0009506	Plasmodesma								
GO:0055044	Symplast								
GO:0005911	Cell-cell junction								
GO:0030054	Cell junction								

Significantly enriched gene ontology terms associated differential expressed genes

Term	Description	Antequera		Bancal		Ardito		Magueija	
		UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
GO:0005576	Extracellular region								
GO:0016020	Membrane								
GO:0031984	Organelle subcompartment								
GO:0071944	Cell periphery								
GO:0010319	Stromule								
GO:0042555	MCM complex								
GO:0005875	Microtubule associated complex								
GO:0005971	Ribonucleoside-diphosphate reductase complex								
GO:0010369	Chromocenter								
GO:0005819	Spindle								
GO:0009501	Amyloplast								
GO:0000347	THO complex								
GO:0009569	Chloroplast starch grain								
GO:0030863	Cortical cytoskeleton								
GO:0010005	Cortical microtubule, transverse to long axis								
GO:0005881	Cytoplasmic microtubule								
GO:0055028	Cortical microtubule								
GO:0030981	Cortical microtubule cytoskeleton								
GO:0043036	Starch grain								

Legend



Supplemental Table 5.3. Heatmap of DEGs involved in heat response ontology in commercial varieties Antequera and Bancal and landraces Ardito and Magueija. Red and blue indicate down and upregulated genes, respectively, and color intensity is related with the log2 fold change value; gray represents unaltered genes.

Supplemental Table 5.3.

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS7A02G451100					(2R)-phospho-3-sulfolactate synthase (ComA) (PF02679)
TraesCS7B02G351000					(2R)-phospho-3-sulfolactate synthase (ComA) (PF02679)
TraesCS7D02G440200					(2R)-phospho-3-sulfolactate synthase (ComA) (PF02679)
TraesCS2D02G013600					AAR2 protein (PF05282)
TraesCS7A02G284700					Activator of Hsp90 ATPase, N-terminal (PF09229)
TraesCS7B02G200400					Activator of Hsp90 ATPase, N-terminal (PF09229)
TraesCS7D02G282500					Activator of Hsp90 ATPase, N-terminal (PF09229)
TraesCS1D02G376300					Alcohol dehydrogenase GroES-like domain (PF08240)
TraesCS4A02G202200					Alcohol dehydrogenase GroES-like domain (PF08240)
TraesCS4A02G202300					Alcohol dehydrogenase GroES-like domain (PF08240)
TraesCS4D02G103300					Alcohol dehydrogenase GroES-like domain (PF08240)
TraesCS7D02G457800					Aminotransferase class-V (PF00266)
TraesCS5A02G201200					AN1-like Zinc finger (PF01428)
TraesCS5B02G200000					AN1-like Zinc finger (PF01428)
TraesCS1B02G013900					Ankyrin repeats (3 copies) (PF12796)
TraesCS5A02G203000					Annexin (PF00191)
TraesCS1A02G221900					Apetala 2 domain (PF00847)
TraesCS1B02G235100					Apetala 2 domain (PF00847)
TraesCS1D02G223600					Apetala 2 domain (PF00847)
TraesCS4D02G298600					Apetala 2 domain (PF00847)
TraesCS5A02G314600					Apetala 2 domain (PF00847)
TraesCS5B02G313000					Apetala 2 domain (PF00847)
TraesCS5B02G315500					Apetala 2 domain (PF00847)
TraesCS6B02G126600					Apetala 2 domain (PF00847)
TraesCS6B02G375400					Apetala 2 domain (PF00847)
TraesCS6D02G084200					Apetala 2 domain (PF00847)
TraesCS7A02G158000					Apetala 2 domain (PF00847)
TraesCS7A02G264100					Apetala 2 domain (PF00847)
TraesCS1D02G310300					Aspartic acid proteinase inhibitor (PF16845)
TraesCS1A02G340100					ATPase family associated with various cellular activities (AAA)(PF00004)
TraesCS1B02G352400					ATPase family associated with various cellular activities (AAA)(PF00004)
TraesCS1D02G342100					ATPase family associated with various cellular activities (AAA)(PF00004)
TraesCS3A02G274400					ATPase family associated with various cellular activities (AAA)(PF00004)
TraesCS3B02G308100					ATPase family associated with various cellular activities (AAA)(PF00004)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS3D02G273600					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS4B02G380800					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS4D02G190600					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS5A02G547300					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS6A02G146400					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS6B02G174500					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS6D02G135600					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS6D02G135600					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS7A02G188300					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS7B02G093400					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS7D02G189400					ATPase family associated with various cellular activities (AAA) (PF00004)
TraesCS3D02G344700					BAG domain (PF02179)
TraesCS4B02G382700					BAG domain (PF02179)
TraesCS4D02G357900					BAG domain (PF02179)
TraesCS5A02G548000					BAG domain (PF02179)
TraesCS7B02G388700					BAG domain (PF02179)
TraesCS1B02G268500					bZIP transcription factor (PF00170)
TraesCS1D02G257400					bZIP transcription factor (PF00170)
TraesCS2B02G167900					bZIP transcription factor (PF00170)
TraesCS2D02G146100					bZIP transcription factor (PF00170)
TraesCS7A02G398400					bZIP transcription factor (PF00170)
TraesCS7B02G299200					bZIP transcription factor (PF00170)
TraesCS5D02G160300					C2 domain (PF00168)
TraesCS3A02G356600					C2H2-type zinc finger (PF13912)
TraesCS5A02G477400					C2H2-type zinc finger (PF13912)
TraesCS5B02G489800					C2H2-type zinc finger (PF13912)
TraesCS5B02G490600					C2H2-type zinc finger (PF13912)
TraesCS5B02G490700					C2H2-type zinc finger (PF13912)
TraesCS5D02G491000					C2H2-type zinc finger (PF13912)
TraesCS7B02G142300					Calcineurin-like phosphoesterase (PF00149)
TraesCS2A02G545600					Calreticulin family (PF00262)
TraesCS2B02G576100					Calreticulin family (PF00262)
TraesCS2D02G546500					Calreticulin family (PF00262)
TraesCS6A02G101800					Calreticulin family (PF00262)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS6A02G101900					Calreticulin family (PF00262)
TraesCS6B02G129800					Calreticulin family (PF00262)
TraesCS6D02G090200					Calreticulin family (PF00262)
TraesCS6D02G090400					Calreticulin family (PF00262)
TraesCS1A02G099300					Chaperone DnaJ domain (PF01556)
TraesCS1A02G395300					Chaperone DnaJ domain (PF00226)
TraesCS1B02G125100					Chaperone DnaJ domain (PF01556)
TraesCS1B02G423600					Chaperone DnaJ domain (PF01556)
TraesCS1D02G107200					Chaperone DnaJ domain (PF01556)
TraesCS1D02G403500					Chaperone DnaJ domain (PF01556)
TraesCS3A02G216800					Chaperone DnaJ domain (PF00226)
TraesCS3B02G247200					Chaperone DnaJ domain (PF00226)
TraesCS3A02G537600					Chaperone DnaJ domain (PF00226)
TraesCS3D02G218800					Chaperone DnaJ domain (PF00226)
TraesCS3B02G603100					Chaperone DnaJ domain (PF00226)
TraesCS4A02G110600					Chaperone DnaJ domain (PF00226)
TraesCS3D02G543100					Chaperone DnaJ domain (PF00226)
TraesCS4B02G193500					Chaperone DnaJ domain (PF00226)
TraesCS4D02G194500					Chaperone DnaJ domain (PF00226)
TraesCS5A02G372900					Chaperone DnaJ domain (PF00684)
TraesCS5A02G426100					Chaperone DnaJ domain (PF00684)
TraesCS5B02G115900					Chaperone DnaJ domain (PF00684)
TraesCS5B02G374900					Chaperone DnaJ domain (PF00684)
TraesCS5B02G428000					Chaperone DnaJ domain (PF00684)
TraesCS5D02G125500					Chaperone DnaJ domain (PF00684)
TraesCS5D02G382400					Chaperone DnaJ domain (PF00684)
TraesCS5D02G434100					Chaperone DnaJ domain (PF00684)
TraesCS6B02G274600					Chaperone DnaJ domain (PF00684)
TraesCS6D02G232600					Chaperone DnaJ domain (PF00684)
TraesCS6D02G356800					Chaperone DnaJ domain (PF00684)
TraesCS7A02G051000					Chaperone DnaJ domain (PF00684)
TraesCS7D02G045900					Chaperone DnaJ domain (PF00684)
TraesCS2A02G146000					Chaperonin 10 Kd subunit (PF00166)
TraesCS2B02G171400					Chaperonin 10 Kd subunit (PF00166)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS2D02G150600					Chaperonin 10 Kd subunit (PF00166)
TraesCS4A02G067500					Chlorophyll A-B binding protein (PF00504)
TraesCS4D02G225400					Chlorophyll A-B binding protein (PF00504)
TraesCS3A02G294500					CS domain (PF04969)
TraesCS3B02G329100					CS domain (PF04969)
TraesCS3D02G227500					CS domain (PF04969)
TraesCS3D02G294300					CS domain (PF04969)
TraesCS7A02G189600					CS domain (PF04969)
TraesCS7B02G094500					CS domain (PF04969)
TraesCS7D02G190700					CS domain (PF04969)
TraesCS1A02G340100					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS1B02G352400					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS1D02G342100					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS3A02G274400					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS3B02G308100					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS3D02G273600					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS4B02G380800					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS5A02G547300					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS6A02G146400					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS6B02G174500					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS6D02G135600					C-terminal, D2-small domain, of ClpB protein (PF10431)
TraesCS3B02G509000					Cytochrome P450 (PF00067)
TraesCS7D02G349400					Cytochrome P451 (PF00067)
TraesCS6A02G350200					Dehydrin (PF00257)
TraesCS6A02G350300					Dehydrin (PF00257)
TraesCS6B02G383200					Dehydrin (PF00257)
TraesCS6D02G332500					Dehydrin (PF00257)
TraesCS2D02G524600					F-box domain (PF08268)
TraesCS3B02G509200					F-box domain (PF08268)
TraesCS5A02G254500					F-box domain (PF00646)
TraesCS5B02G253500					F-box domain (PF08268)
TraesCS5B02G253600					F-box domain (PF08268)
TraesCS5D02G292600					F-box domain (PF00646)
TraesCS7A02G259000					F-box domain (PF00646)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS1A02G290700					Ferric reductase like transmembrane component (PF01794)
TraesCS1D02G289300					Ferric reductase NAD binding domain (PF08030)
TraesCS3A02G182900					Ferric reductase NAD binding domain (PF08030)
TraesCS3B02G212900					Ferric reductase NAD binding domain (PF08030)
TraesCS4D02G270000					Ferric reductase NAD binding domain (PF08030)
TraesCS5B02G099700					Ferric reductase NAD binding domain (PF08030)
TraesCS5B02G212100					Ferric reductase NAD binding domain (PF08030)
TraesCS2A02G050600					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS2A02G277100					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS2B02G063900					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS2B02G294500					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS2D02G050300					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS2D02G276000					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS7A02G257100					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS7B02G153100					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS7D02G257300					FKBP-type peptidyl-prolyl cis-trans isomerase (PF00254)
TraesCS4B02G039300					GAF domain (PF01590)
TraesCS7B02G115300					Gibberellin regulated protein (PF02704)
TraesCS7D02G210500					Gibberellin regulated protein (PF02704)
TraesCS7A02G517000					Glucosidase II beta subunit-like protein (PF07915)
TraesCS1A02G072000					Glutamate-cysteine ligase family 2(GCS2) (PF04107)
TraesCS1A02G168900					Glutaredoxin (PF00462)
TraesCS1B02G188000					Glutaredoxin (PF00462)
TraesCS1D02G166900					Glutaredoxin (PF00462)
TraesCS6A02G213700					Glyceraldehyde 3-phosphate dehydrogenase, NAD binding domain (PF00044)
TraesCS7D02G465500					Glyceraldehyde 3-phosphate dehydrogenase, NAD binding domain (PF00044)
TraesCS2D02G431500					Glycosyl hydrolases family 16 (PF00722)
TraesCS4D02G358700					Glycosyl hydrolases family 17 (PF00722)
TraesCS5A02G548500					Glycosyl hydrolases family 18 (PF00722)
TraesCS7A02G426100					Glycosyl hydrolases family 19 (PF00722)
TraesCS7B02G327200					Glycosyl hydrolases family 20 (PF00722)
TraesCS5A02G429600					Glycosyl hydrolases family 17 (PF00332)
TraesCS5B02G431600					Glycosyl hydrolases family 18 (PF00332)
TraesCS5D02G437700					Glycosyl hydrolases family 19 (PF00332)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS2D02G095800					Glycosyl transferase family 8 (PF01501)
TraesCS4A02G125300					Glycosyl transferase family 9 (PF01501)
TraesCS4B02G179300					Glycosyl transferase family 10 (PF01501)
TraesCS4D02G180800					Glycosyl transferase family 11 (PF01501)
TraesCS5A02G488500					GRAM domain (PF02893)
TraesCS5D02G503400					GRAM domain (PF02893)
TraesCS5A02G176600					GrpE (PF01025)
TraesCS5B02G173300					GrpE (PF01025)
TraesCS5D02G180300					GrpE (PF01025)
TraesCS2A02G064900					Heavy-metal-associated domain (PF00403)
TraesCS6B02G059000					Heavy-metal-associated domain (PF00403)
TraesCS3D02G068900					Helicase conserved C-terminal domain (PF00271)
TraesCS7A02G369500					Helix-turn-helix (PF01381)
TraesCS7B02G259000					Helix-turn-helix (PF01381)
TraesCS7D02G353800					Helix-turn-helix (PF01381)
TraesCS4B02G039300					His Kinase A (phospho-acceptor) domain (PF00512)
TraesCS1A02G375600					HSF-type DNA-binding (PF00447)
TraesCS1B02G396000					HSF-type DNA-binding (PF00447)
TraesCS1D02G382900					HSF-type DNA-binding (PF00447)
TraesCS2A02G401600					HSF-type DNA-binding (PF00447)
TraesCS2B02G419600					HSF-type DNA-binding (PF00447)
TraesCS2D02G151200					HSF-type DNA-binding (PF00447)
TraesCS2D02G211400					HSF-type DNA-binding (PF00447)
TraesCS3A02G289200					HSF-type DNA-binding (PF00447)
TraesCS3B02G323800					HSF-type DNA-binding (PF00447)
TraesCS3D02G289000					HSF-type DNA-binding (PF00447)
TraesCS4A02G062800					HSF-type DNA-binding (PF00447)
TraesCS4B02G278100					HSF-type DNA-binding (PF00447)
TraesCS4D02G276500					HSF-type DNA-binding (PF00447)
TraesCS5A02G314700					HSF-type DNA-binding (PF00447)
TraesCS5A02G383800					HSF-type DNA-binding (PF00447)
TraesCS5B02G315600					HSF-type DNA-binding (PF00447)
TraesCS5B02G556200					HSF-type DNA-binding (PF00447)
TraesCS5D02G244800					HSF-type DNA-binding (PF00447)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS5D02G321000					HSF-type DNA-binding (PF00447)
TraesCS5D02G393200					HSF-type DNA-binding (PF00447)
TraesCS5D02G553300					HSF-type DNA-binding (PF00447)
TraesCS7A02G270100					HSF-type DNA-binding (PF00447)
TraesCS7B02G168300					HSF-type DNA-binding (PF00447)
TraesCS7D02G270600					HSF-type DNA-binding (PF00447)
TraesCS2A02G312900					Hsp20/alpha crystallin family (PF00011)
TraesCS2D02G311400					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G033900					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G034000					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G034500					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G035400					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G112900					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G113000					Hsp20/alpha crystallin family (PF00011)
TraesCS3A02G113100					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G048600					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G049800					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G049900					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G130300					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G130400					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G130500					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G130900					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G131000					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G131100					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G131200					Hsp20/alpha crystallin family (PF00011)
TraesCS3B02G131300					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G044400					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G045500					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G045600					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G045700					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G045800					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G046300					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G046600					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G046700					Hsp20/alpha crystallin family (PF00011)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS3D02G046800					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G114700					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G114800					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G114900					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G115000					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G115100					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G115200					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G115300					Hsp20/alpha crystallin family (PF00011)
TraesCS3D02G115400					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G068200					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G068300					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G092100					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G092600					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G092700					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G092800					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G092900					Hsp20/alpha crystallin family (PF00011)
TraesCS4A02G226700					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G089800					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G211600					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G211700					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G212200					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G212300					Hsp20/alpha crystallin family (PF00011)
TraesCS4B02G225400					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G086200					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G145500					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G145600					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G212200					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G212300					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G212400					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G212500					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G213100					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G213300					Hsp20/alpha crystallin family (PF00011)
TraesCS4D02G226000					Hsp20/alpha crystallin family (PF00011)
TraesCS5B02G245700					Hsp20/alpha crystallin family (PF00011)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS6A02G181700					Hsp20/alpha crystallin family (PF00011)
TraesCS6A02G316200					Hsp20/alpha crystallin family (PF00011)
TraesCS6B02G210600					Hsp20/alpha crystallin family (PF00011)
TraesCS6B02G346700					Hsp20/alpha crystallin family (PF00011)
TraesCS6B02G374100					Hsp20/alpha crystallin family (PF00011)
TraesCS6D02G169100					Hsp20/alpha crystallin family (PF00011)
TraesCS6D02G295500					Hsp20/alpha crystallin family (PF00011)
TraesCS6D02G322300					Hsp20/alpha crystallin family (PF00011)
TraesCS7A02G177600					Hsp20/alpha crystallin family (PF00011)
TraesCS7A02G202200					Hsp20/alpha crystallin family (PF00011)
TraesCS7A02G236700					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G083100					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G083200					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G083400					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G083500					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G088600					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G109100					Hsp20/alpha crystallin family (PF00011)
TraesCS7B02G347100					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179000					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179100					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179200					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179300					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179400					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179500					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G179600					Hsp20/alpha crystallin family (PF00011)
TraesCS7D02G232600					Hsp20/alpha crystallin family (PF00011)
TraesCS1A02G120100					Hsp70 protein (PF00012)
TraesCS1A02G120200					Hsp70 protein (PF00012)
TraesCS1A02G133100					Hsp70 protein (PF00012)
TraesCS1A02G285000					Hsp70 protein (PF00012)
TraesCS1A02G295600					Hsp70 protein (PF00012)
TraesCS1B02G139500					Hsp70 protein (PF00012)
TraesCS1B02G139600					Hsp70 protein (PF00012)
TraesCS1B02G151300					Hsp70 protein (PF00012)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS1B02G294300					Hsp70 protein (PF00012)
TraesCS1D02G121000					Hsp70 protein (PF00012)
TraesCS1D02G121200					Hsp70 protein (PF00012)
TraesCS1D02G131800					Hsp70 protein (PF00012)
TraesCS1D02G284000					Hsp70 protein (PF00012)
TraesCS3D02G352400					Hsp70 protein (PF00012)
TraesCS4A02G066100					Hsp70 protein (PF00012)
TraesCS4A02G097900					Hsp70 protein (PF00012)
TraesCS4A02G098600					Hsp70 protein (PF00012)
TraesCS4B02G205700					Hsp70 protein (PF00012)
TraesCS4B02G206700					Hsp70 protein (PF00012)
TraesCS4B02G243400					Hsp70 protein (PF00012)
TraesCS4B02G397600					Hsp70 protein (PF00012)
TraesCS4D02G140800					Hsp70 protein (PF00012)
TraesCS4D02G206600					Hsp70 protein (PF00012)
TraesCS4D02G207500					Hsp70 protein (PF00012)
TraesCS4D02G243000					Hsp70 protein (PF00012)
TraesCS5A02G268100					Hsp70 protein (PF00012)
TraesCS5A02G479300					Hsp70 protein (PF00012)
TraesCS5B02G087700					Hsp70 protein (PF00012)
TraesCS5B02G111200					Hsp70 protein (PF00012)
TraesCS5B02G267900					Hsp70 protein (PF00012)
TraesCS5B02G492500					Hsp70 protein (PF00012)
TraesCS5D02G093900					Hsp70 protein (PF00012)
TraesCS5D02G276100					Hsp70 protein (PF00012)
TraesCS5D02G492900					Hsp70 protein (PF00012)
TraesCS6A02G042600					Hsp70 protein (PF00012)
TraesCS6A02G276700					Hsp70 protein (PF00012)
TraesCS6A02G337100					Hsp70 protein (PF00012)
TraesCS6B02G058300					Hsp70 protein (PF00012)
TraesCS6B02G304200					Hsp70 protein (PF00012)
TraesCS6D02G049100					Hsp70 protein (PF00012)
TraesCS6D02G257000					Hsp70 protein (PF00012)
TraesCS7A02G457100					Hsp70 protein (PF00012)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS7B02G359100					Hsp70 protein (PF00012)
TraesCS2A02G033700					Hsp90 protein (PF00183)
TraesCS2B02G047400					Hsp90 protein (PF00183)
TraesCS2D02G033200					Hsp90 protein (PF00183)
TraesCS5A02G251000					Hsp90 protein (PF00183)
TraesCS5B02G249000					Hsp90 protein (PF00183)
TraesCS5D02G258900					Hsp90 protein (PF00183)
TraesCS7A02G242200					Hsp90 protein (PF00183)
TraesCS7A02G529900					Hsp90 protein (PF00183)
TraesCS7B02G149200					Hsp90 protein (PF00183)
TraesCS7B02G446900					Hsp90 protein (PF00183)
TraesCS7D02G241100					Hsp90 protein (PF00183)
TraesCS7D02G517800					Hsp90 protein (PF00183)
TraesCS3A02G480500					Inhibitor of apoptosis-promoting Bax1 (PF01027)
TraesCS3B02G525100					Inhibitor of apoptosis-promoting Bax2 (PF01027)
TraesCS3B02G525200					Inhibitor of apoptosis-promoting Bax3 (PF01027)
TraesCS3B02G525800					Inhibitor of apoptosis-promoting Bax4 (PF01027)
TraesCS3D02G475300					Inhibitor of apoptosis-promoting Bax5 (PF01027)
TraesCS5A02G384100					Inhibitor of apoptosis-promoting Bax6 (PF01027)
TraesCS5B02G388900					Inhibitor of apoptosis-promoting Bax7 (PF01027)
TraesCS5B02G440600					Inhibitor of apoptosis-promoting Bax8 (PF01027)
TraesCS5D02G393600					Inhibitor of apoptosis-promoting Bax9 (PF01027)
TraesCS5D02G445000					Inhibitor of apoptosis-promoting Bax10 (PF01027)
TraesCS4B02G382700					IQ calmodulin-binding motif (PF00612)
TraesCS5A02G548000					IQ calmodulin-binding motif (PF00612)
TraesCS5B02G559300					Ku70/Ku80 N-terminal alpha/beta domain (PF03731)
TraesCS5A02G258900					Late embryogenesis abundant protein 18 (PF10714)
TraesCS5B02G257700					Late embryogenesis abundant protein 19 (PF10714)
TraesCS1D02G259400					Leucine Rich repeat (PF13516)
TraesCS2A02G360300					Leucine Rich repeat (PF00560)
TraesCS2A02G397200					Leucine Rich repeat (PF13855)
TraesCS2B02G415500					Leucine Rich repeat (PF13855)
TraesCS2D02G133800					Leucine Rich repeat (PF13855)
TraesCS2D02G395000					Leucine Rich repeat (PF13855)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS3B02G420500					Leucine Rich repeat (PF13855)
TraesCS4B02G040700					Leucine Rich repeat (PF13855)
TraesCS4B02G049800					Leucine Rich repeat (PF13855)
TraesCS4B02G171300					Leucine Rich repeat (PF00560)
TraesCS4D02G106900					Leucine Rich repeat (PF00560)
TraesCS4D02G298800					Leucine Rich repeat (PF13855)
TraesCS5A02G407500					Leucine Rich repeat (PF00560)
TraesCS5D02G417400					Leucine Rich repeat (PF00560)
TraesCS6B02G153100					Leucine Rich repeat (PF00560)
TraesCS6D02G114800					Leucine Rich repeat (PF13855)
TraesCS6D02G271500					Leucine Rich repeat (PF13855)
TraesCS7B02G110700					Leucine Rich repeat (PF00560)
TraesCS7B02G260400					Leucine Rich repeat (PF13516)
TraesCS7B02G261000					Leucine Rich repeat (PF00560)
TraesCS7B02G362800					Leucine Rich repeat (PF13855)
TraesCS7D02G144900					Leucine Rich repeat (PF13855)
TraesCS7D02G357100					Leucine Rich repeat (PF00560)
TraesCS3B02G334400					Leucine rich repeat N-terminal domain (PF08263)
TraesCS3D02G299800					Leucine rich repeat N-terminal domain (PF08263)
TraesCS6B02G250000					Lipocalin-like domain (PF08212)
TraesCS2A02G278400					MatE (PF01554)
TraesCS7A02G497300					MatE (PF01554)
TraesCS7B02G400500					MatE (PF01554)
TraesCS7D02G014200					MatE (PF01554)
TraesCS7D02G484500					MatE (PF01554)
TraesCS7A02G330300					MIR domain (PF02815)
TraesCS7D02G160300					MIR domain (PF02815)
TraesCS7D02G327100					MIR domain (PF02815)
TraesCS1A02G246400					Mitochondrial carrier protein (PF00153)
TraesCS1A02G065700					MIZ/SP-RING zinc finger (PF02891)
TraesCS4B02G135700					MIZ/SP-RING zinc finger (PF02891)
TraesCS5D02G160100					MIZ/SP-RING zinc finger (PF02891)
TraesCS7D02G457800					MOSC domain (PF03473)
TraesCS7A02G369500					Multiprotein bridging factor 1 (PF08523)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS7B02G259000					Multiprotein bridging factor 2 (PF08523)
TraesCS7D02G353800					Multiprotein bridging factor 3 (PF08523)
TraesCS1A02G275800					Myb-like DNA-binding domain (PF00249)
TraesCS1B02G285000					Myb-like DNA-binding domain (PF00249)
TraesCS1D02G275400					Myb-like DNA-binding domain (PF00249)
TraesCS3A02G375500					Myb-like DNA-binding domain (PF00249)
TraesCS3B02G407700					Myb-like DNA-binding domain (PF00249)
TraesCS5A02G225100					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS5A02G269100					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS5A02G517000					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS5D02G168400					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS5D02G276200					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS6A02G147300					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS6B02G420000					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS6D02G136600					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS6D02G365700					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS7A02G396300					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS7A02G398000					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS7D02G391900					NAD dependent epimerase/dehydratase family (PF01370)
TraesCS4B02G381200					Natural resistance-associated macrophage protein (PF01566)
TraesCS4A02G095500					Nucleotide exchange factor Fes1 (PF08609)
TraesCS4B02G208900					Nucleotide exchange factor Fes2 (PF08609)
TraesCS4D02G209700					Nucleotide exchange factor Fes3 (PF08609)
TraesCS5A02G296200					Nucleotide exchange factor Fes4 (PF08609)
TraesCS5B02G295400					Nucleotide exchange factor Fes5 (PF08609)
TraesCS5B02G495500					Nucleotide exchange factor Fes6 (PF08609)
TraesCS5D02G303400					Nucleotide exchange factor Fes7 (PF08609)
TraesCS5D02G496000					Nucleotide exchange factor Fes8 (PF08609)
TraesCS4A02G051400					Nucleotidyl transferase (PF00483)
TraesCS4B02G253000					Nucleotidyl transferase (PF00483)
TraesCS4D02G253100					Nucleotidyl transferase (PF00483)
TraesCS4A02G228900					PDZ domain (PF17820)
TraesCS4B02G087500					PDZ domain (PF17820)
TraesCS4D02G084800					PDZ domain (PF17820)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS7A02G188300					Peptidase family M41 (PF01434)
TraesCS7B02G093400					Peptidase family M42 (PF01434)
TraesCS7D02G189400					Peptidase family M43 (PF01434)
TraesCS2A02G082100					Peroxidase (PF00141)
TraesCS2B02G087400					Peroxidase (PF00141)
TraesCS2B02G096200					Peroxidase (PF00141)
TraesCS2D02G070500					Peroxidase (PF00141)
TraesCS4B02G197900					Peroxidase (PF00141)
TraesCS1A02G065700					PHD-finger (PF00628)
TraesCS4B02G135700					PHD-finger (PF00628)
TraesCS7B02G362800					Phosphatidylinositol-glycan biosynthesis class S protein (PF10510)
TraesCS5D02G160300					Phosphatidylinositol-specific phospholipase C, Y domain (PF00387)
TraesCS6B02G187500					Phosphoglycerate kinase (PF00162)
TraesCS6D02G148700					Phosphoglycerate kinase (PF00162)
TraesCS7B02G310100					P-loop containing NTP hydrolase pore-1 (PF13872)
TraesCS4D02G190600					Proteasomal ATPase OB C-terminal domain (PF16450)
TraesCS2A02G360300					Protein kinase domain (PF00069)
TraesCS2A02G397200					Protein kinase domain (PF00069)
TraesCS2B02G415500					Protein kinase domain (PF00069)
TraesCS2D02G395000					Protein kinase domain (PF00069)
TraesCS3B02G334400					Protein kinase domain (PF00069)
TraesCS3D02G299800					Protein kinase domain (PF00069)
TraesCS4B02G171300					Protein kinase domain (PF00069)
TraesCS4D02G106900					Protein kinase domain (PF00069)
TraesCS6B02G153100					Protein kinase domain (PF00069)
TraesCS6D02G114800					Protein kinase domain (PF00069)
TraesCS7B02G110700					Protein kinase domain (PF00069)
TraesCS7B02G260400					Protein kinase domain (PF00069)
TraesCS7B02G261000					Protein kinase domain (PF00069)
TraesCS7B02G362800					Protein kinase domain (PF00069)
TraesCS7D02G144900					Protein kinase domain (PF00069)
TraesCS6A02G333700					Protein of unknown function (DUF1685) (PF07939)
TraesCS6B02G364100					Protein of unknown function (DUF1685) (PF07939)
TraesCS6D02G312900					Protein of unknown function (DUF1685) (PF07939)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS4B02G230600					Protein of unknown function (DUF775) (PF05603)
TraesCS1D02G259400					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS2D02G133800					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS4B02G049800					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS4D02G298800					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS5A02G407500					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS5D02G417400					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS6D02G271500					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS7D02G357100					Protein tyrosine and serine/threonine kinase (PF07714)
TraesCS1A02G131500					Ras family (PF00071)
TraesCS1B02G152100					Ras family (PF00071)
TraesCS1A02G290700					Respiratory burst NADPH oxidase (PF08414)
TraesCS1D02G289300					Respiratory burst NADPH oxidase (PF08414)
TraesCS3A02G182900					Respiratory burst NADPH oxidase (PF08414)
TraesCS3B02G212900					Respiratory burst NADPH oxidase (PF08414)
TraesCS5B02G099700					Respiratory burst NADPH oxidase (PF08414)
TraesCS5B02G212100					Respiratory burst NADPH oxidase (PF08414)
TraesCS1A02G346800					Reticulon (PF02453)
TraesCS3D02G159100					Reticulon (PF02453)
TraesCS6A02G399600					Ring finger domain (PF13639)
TraesCS6A02G286200					RNA polymerase Rpb4 (PF03874)
TraesCS6B02G315200					RNA polymerase Rpb5 (PF03874)
TraesCS6D02G062500					RNA polymerase Rpb6 (PF03874)
TraesCS1A02G065700					SAP domain (PF02037)
TraesCS5A02G015200					Sec61beta family (PF03911)
TraesCS4A02G089100					Selenoprotein SelK_SelG (PF10961)
TraesCS4B02G215200					Selenoprotein SelK_SelG (PF10961)
TraesCS4D02G215700					Selenoprotein SelK_SelG (PF10961)
TraesCS3D02G227500					SGS domain (PF05002)
TraesCS3A02G294500					Siah interacting protein, N terminal (PF09032)
TraesCS3B02G329100					Siah interacting protein, N terminal (PF09032)
TraesCS3D02G294300					Siah interacting protein, N terminal (PF09032)
TraesCS3D02G068900					SNF2 family N-terminal domain (PF00176)
TraesCS4A02G486300					Squalene/phytoene synthase (PF00494)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS2A02G386800					STI1 domain (PF17830)
TraesCS2B02G404400					STI1 domain (PF17830)
TraesCS2D02G383600					STI1 domain (PF17830)
TraesCS6A02G238600					STI1 domain (PF17830)
TraesCS6B02G285800					STI1 domain (PF17830)
TraesCS6D02G221000					STI1 domain (PF17830)
TraesCS5B02G134400					TAP42-like family (PF04177)
TraesCS1A02G145000					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS1A02G361400					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS1B02G162300					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS1B02G378000					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS1D02G144000					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS1D02G365800					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS4A02G409100					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS4B02G307700					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS4D02G305900					TCP-1/cpn60 chaperonin family (PF00118)
TraesCS2A02G050600					Tetratricopeptide repeat (PF13181)
TraesCS2A02G277100					Tetratricopeptide repeat (PF00515)
TraesCS2A02G386800					Tetratricopeptide repeat (PF13432)
TraesCS2B02G063900					Tetratricopeptide repeat (PF13181)
TraesCS2B02G294500					Tetratricopeptide repeat (PF00515)
TraesCS2B02G404400					Tetratricopeptide repeat (PF13432)
TraesCS2D02G050300					Tetratricopeptide repeat (PF13181)
TraesCS2D02G276000					Tetratricopeptide repeat (PF00515)
TraesCS2D02G383600					Tetratricopeptide repeat (PF13432)
TraesCS3A02G537600					Tetratricopeptide repeat (PF13432)
TraesCS3B02G603100					Tetratricopeptide repeat (PF13432)
TraesCS3D02G227500					Tetratricopeptide repeat (PF13432)
TraesCS3D02G543100					Tetratricopeptide repeat (PF13432)
TraesCS6A02G238600					Tetratricopeptide repeat (PF13181)
TraesCS6B02G285800					Tetratricopeptide repeat (PF13432)
TraesCS6D02G221000					Tetratricopeptide repeat (PF13181)
TraesCS7A02G257100					Tetratricopeptide repeat (PF00515)
TraesCS7B02G153100					Tetratricopeptide repeat (PF00515)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS7D02G257300					Tetratricopeptide repeat (PF00515)
TraesCS1A02G168900					Thioredoxin (PF00085)
TraesCS1B02G188000					Thioredoxin (PF00085)
TraesCS1D02G166900					Thioredoxin (PF00085)
TraesCS5A02G234400					Thioredoxin (PF00085)
TraesCS5B02G232900					Thioredoxin (PF00085)
TraesCS5D02G241200					Thioredoxin (PF00085)
TraesCS6B02G089600					Translation initiation factor eIF3 subunit (PF08597)
TraesCS2B02G392500					Trypsin and protease inhibitor (PF00197)
TraesCS4A02G228900					Trypsin-like peptidase domain (PF13365)
TraesCS4B02G087500					Trypsin-like peptidase domain (PF13365)
TraesCS4D02G084800					Trypsin-like peptidase domain (PF13365)
TraesCS7A02G370000					UAA transporter family (PF08449)
TraesCS7B02G258500					UAA transporter family (PF08449)
TraesCS7D02G353100					UAA transporter family (PF08449)
TraesCS3D02G344700					Ubiquitin family (PF00240)
TraesCS3A02G424000					Ubiquitin-2 like Rad60 SUMO-like (PF11976)
TraesCS3B02G459900					Ubiquitin-2 like Rad60 SUMO-like (PF11976)
TraesCS3B02G460000					Ubiquitin-2 like Rad60 SUMO-like (PF11976)
TraesCS3D02G419200					Ubiquitin-2 like Rad60 SUMO-like (PF11976)
TraesCS3D02G419300					Ubiquitin-2 like Rad60 SUMO-like (PF11976)
TraesCS7A02G318700					U-box domain (PF04564)
TraesCS7B02G219600					U-box domain (PF04564)
TraesCS1D02G335200					X8 domain (PF07983)
TraesCS2B02G180300					X8 domain (PF07983)
TraesCS5B02G431600					X8 domain (PF07983)
TraesCS5D02G437700					X8 domain (PF07983)
TraesCS2D02G431500					Xyloglucan endo-transglycosylase (XET) C-terminus (PF06955)
TraesCS4D02G358700					Xyloglucan endo-transglycosylase (XET) C-terminus (PF06955)
TraesCS5A02G548500					Xyloglucan endo-transglycosylase (XET) C-terminus (PF06955)
TraesCS7A02G426100					Xyloglucan endo-transglycosylase (XET) C-terminus (PF06955)
TraesCS7B02G327200					Xyloglucan endo-transglycosylase (XET) C-terminus (PF06955)
TraesCS1D02G376300					Zinc-binding dehydrogenase (PF00107)
TraesCS4A02G202200					Zinc-binding dehydrogenase (PF00107)

Supplemental Table 5.3. *(continued)*

DEGs and respective encoded products associated with Heat Response Ontology					
Gene stable ID	**Antequera**	**Bancal**	**Ardito**	**Magueija**	**Protein family name (ID)**
TraesCS4A02G202300					Zinc-binding dehydrogenase (PF00107)
TraesCS3A02G424800					#N/D
TraesCS3B02G461100					#N/D
TraesCS3D02G419700					#N/D
TraesCS6A02G112500					#N/D
TraesCS6B02G141700					#N/D
TraesCS6D02G102700					#N/D
TraesCS5A02G107800					#N/D
TraesCS2B02G367100					#N/D
TraesCS2D02G347100					#N/D
TraesCS4B02G251800					#N/D
TraesCS2B02G525800					#N/D
TraesCS1A02G209500					#N/D
TraesCS2D02G497800					#N/D
TraesCS6B02G325000					#N/D
TraesCS4A02G402800					#N/D
TraesCS7A02G104800					#N/D
TraesCS7D02G098600					#N/D
TraesCS3A02G299000					#N/D
TraesCS3B02G337500					#N/D
TraesCS3D02G302700					#N/D
TraesCS4B02G059500					#N/D
TraesCS2A02G396100					#N/D
TraesCS2A02G432300					#N/D
TraesCS2B02G414400					#N/D
TraesCS2D02G393800					#N/D
TraesCS3B02G464600					#N/D
TraesCS6A02G132000					#N/D
TraesCS6A02G295000					#N/D
TraesCS6B02G160300					#N/D
TraesCS6D02G121800					#N/D

Supplemental Table 5.4. KEGG enzyme encoded by DEGs in commercial varieties Antequera and Bancal and landraces Ardito and Magueija. Red and blue indicate down and upregulated genes, respectively.

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Carbohydrate metabolism												
Starch and sucrose metabolism	TraesCS4D02G251000	3.2.1.39	glucan endo-1,3-beta-D-glucosidase				1				1	2
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS7A02G009100	3.2.1.26	beta-fructofuranosidase				1				1	2
	TraesCS1A02G419600	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS7D02G008700	3.2.1.26	beta-fructofuranosidase				1				1	2
	TraesCS1A02G422900	3.2.1.39	glucan endo-1,3-beta-D-glucosidase				1				1	2
	TraesCS5D02G210000	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)			1				1		2
	TraesCS1B02G449700	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS7A02G158900	2.4.1.13	sucrose synthase						1		1	2
	TraesCS1D02G427400	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS3D02G153400	3.2.1.4	cellulase			1				1		2
	TraesCS2A02G310300	2.4.1.18	1,4-alpha-glucan branching enzyme			1				1		2
	TraesCS4B02G142000	3.2.1.39	glucan endo-1,3-beta-D-glucosidase				1				1	2
	TraesCS2A02G468800	2.4.1.21	starch synthase (glycosyl-transferring)				1				1	2
	TraesCS5B02G202200	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)			1				1		2
	TraesCS2B02G145700	2.4.1.25	4-alpha-glucanotransferase			1				1		2
	TraesCS5D02G470400	2.4.1.12	cellulose synthase (UDP-forming)				1				1	2
	TraesCS2D02G293200	3.2.1.26	beta-fructofuranosidase			1				1		2
	TraesCS7A02G009200	3.2.1.26	beta-fructofuranosidase						1		1	2
	TraesCS2D02G468900	2.4.1.21	starch synthase (glycosyl-transferring)				1				1	2
	TraesCS3A02G366400	2.4.1.1	glycogen phosphorylase			1				1		2
	TraesCS3D02G359300	2.4.1.1	glycogen phosphorylase			1				1		2
	TraesCS2A02G373600	2.4.1.21	starch synthase (glycosyl-transferring)			1						1
	TraesCS6D02G305000	3.2.1.39	glucan endo-1,3-beta-D-glucosidase							1		1
	TraesCS1B02G351600	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)							1		1
	TraesCS2D02G220900	3.2.1.2	beta-amylase						1			1
	TraesCS4D02G090300	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS5A02G459400	2.4.1.12	cellulose synthase (UDP-forming)				1					1
	TraesCS2D02G403600	2.4.1.13	sucrose synthase							1		1
	TraesCS6A02G093200	3.2.1.4	cellulase							1		1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS7A02G009600	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS4D02G169800	2.4.1.13	sucrose synthase								1	1
	TraesCS3A02G144600	3.2.1.4	cellulase			1						1
	TraesCS5A02G171900	3.2.1.39	glucan endo-1,3-beta-D-glucosidase				1					1
	TraesCS3A02G366300	2.4.1.1	glycogen phosphorylase							1		1
	TraesCS5B02G431600	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS1D02G275700	3.2.1.39	glucan endo-1,3-beta-D-glucosidase			1						1
	TraesCS2B02G184900	2.4.1.25	4-alpha-glucanotransferase							1		1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
	TraesCS6B02G173000	2.4.1.14	sucrose-phosphate synthase				1					1
	TraesCS3B02G398000	2.4.1.1	glycogen phosphorylase							1		1
	TraesCS2B02G491700	2.4.1.21	starch synthase (glycosyl-transferring)				1					1
	TraesCS1D02G341100	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)							1		1
	TraesCS2D02G126600	2.4.1.25	4-alpha-glucanotransferase			1						1
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS4D02G136800	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS1A02G339300	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)							1		1
	TraesCS4D02G241900	3.2.1.39	glucan endo-1,3-beta-D-glucosidase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS3D02G289100	2.4.1.15	alpha,alpha-trehalose-phosphate synthase (UDP-forming)				1					1
	TraesCS5A02G155600	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS7A02G189000	2.4.1.21	starch synthase (glycosyl-transferring)			1						1
	TraesCS5A02G429600	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS7A02G383900	3.2.1.1	alpha-amylase								1	1
	TraesCS1B02G249000	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS7A02G549100	2.4.1.18	1,4-alpha-glucan branching enzyme			1						1
	TraesCS5B02G469200	2.4.1.12	cellulose synthase (UDP-forming)				1					1
	TraesCS7B02G093800	2.4.1.21	starch synthase (glycosyl-transferring)			1						1
	TraesCS5D02G437700	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS2A02G159300	2.4.1.25	4-alpha-glucanotransferase							1		1
	TraesCS6A02G077800	2.4.1.12	cellulose synthase (UDP-forming)			1						1
	TraesCS7D02G159800	2.4.1.13	sucrose synthase				1					1
	TraesCS6B02G116400	3.2.1.2	beta-amylase							1		1
	TraesCS7D02G314400	3.2.1.4	cellulase			1						1
	TraesCS6B02G468600	2.4.1.34	1,3-beta-glucan synthase							1		1
	TraesCSU02G032700	3.2.1.2	beta-amylase			1						1
	TraesCS2B02G306400	3.2.1.1	alpha-amylase			1						1
	TraesCS4A02G486000	3.2.1.26	beta-fructofuranosidase								1	1
	TraesCS7A02G009500	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS1B02G229000	3.2.1.2	beta-amylase				1					1
	TraesCS7A02G120300	2.4.1.21	starch synthase (glycosyl-transferring)							1		1
	TraesCS4D02G006100	3.2.1.2	beta-amylase				1					1
	TraesCS2D02G166600	2.4.1.25	4-alpha-glucanotransferase							1		1
	TraesCS4D02G088200	2.4.1.14	sucrose-phosphate synthase			1						1
	TraesCS7A02G518200	2.4.1.14	sucrose-phosphate synthase				1					1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS7B02G018600	2.4.1.21	starch synthase (glycosyl-transferring)							1		1
	TraesCS3D02G478000	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS7B02G472500	2.4.1.18	1,4-alpha-glucan branching enzyme							1		1
	TraesCS4A02G131400	3.2.1.4	cellulase								1	1
	TraesCS7D02G117800	2.4.1.21	starch synthase (glycosyl-transferring)							1		1
	TraesCS4A02G175900	3.2.1.39	glucan endo-1,3-beta-D-glucosidase								1	1
	TraesCS7D02G190100	2.4.1.21	starch synthase (glycosyl-transferring)					1				1
	TraesCS4A02G222900	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS7D02G535400	2.4.1.18	1,4-alpha-glucan branching enzyme							1		1
	TraesCS4A02G307900	3.2.1.2	beta-amylase				1					1
	TraesCS1A02G091500	2.4.1.21	starch synthase (glycosyl-transferring)						1			1
	TraesCS4A02G446700	2.4.1.13	sucrose synthase						1			1
	TraesCS4B02G172700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)				1				1	2
	TraesCS5D02G019800	4.2.1.11	phosphopyruvate hydratase				1				1	2
	TraesCS5A02G131100	2.7.1.40	pyruvate kinase				1				1	2
	TraesCS3B02G423200	4.1.2.13	fructose-bisphosphate aldolase				1				1	2
	TraesCS6B02G187500	2.7.2.3	phosphoglycerate kinase				1				1	2
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS6D02G148700	2.7.2.3	phosphoglycerate kinase				1				1	2
	TraesCS4A02G387000	2.7.1.11	6-phosphofructokinase							1		1
	TraesCS5B02G012300	4.2.1.11	phosphopyruvate hydratase								1	1
	TraesCS4D02G174700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)								1	1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS5D02G138800	2.7.1.40	pyruvate kinase								1	1
	TraesCS3A02G359400	5.3.1.1	triose-phosphate isomerase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Glycolysis / Gluconeogenesis	TraesCS1D02G396100	2.7.1.40	pyruvate kinase								1	1
	TraesCS3A02G391100	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS4D02G220500	4.1.1.49	phosphoenolpyruvate carboxykinase (ATP)								1	1
	TraesCS3A02G391500	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS2B02G350500	2.7.1.11	6-phosphofructokinase			1						1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
	TraesCS2D02G075200	1.1.1.27	L-lactate dehydrogenase					1				1
	TraesCS3B02G392000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS4B02G107200	2.7.1.40	pyruvate kinase			1						1
	TraesCS3B02G410400	3.1.3.11	fructose-bisphosphatase			1						1
	TraesCS4D02G107400	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS3B02G422900	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS4D02G213500	4.2.1.11	phosphopyruvate hydratase				1					1
	TraesCS1A02G140900	2.7.2.3	phosphoglycerate kinase							1		1
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS6D02G078300	1.1.1.27	L-lactate dehydrogenase			1						1
	TraesCS5D02G108500	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)	1								1
	TraesCS7B02G067000	5.1.3.3	aldose 1-epimerase						1			1
	TraesCS6A02G213700	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)				1					1
	TraesCS7D02G163100	5.1.3.3	aldose 1-epimerase			1						1
	TraesCS2D02G322200	5.1.3.3	aldose 1-epimerase								1	1
	TraesCS7D02G465500	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)							1		1
	TraesCS3D02G073000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS7B02G104400	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS7D02G200800	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS3D02G370700	3.1.3.11	fructose-bisphosphatase							1		1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
Glyoxylate and dicarboxylate metabolism	TraesCS5A02G165700	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS2D02G065200	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS5D02G169900	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS2A02G066800	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS3B02G186100	4.1.1.39	ribulose-bisphosphate carboxylase						1		1	2
	TraesCS2A02G493700	2.1.2.1	glycine hydroxymethyltransferase			1				1		2
	TraesCS5A02G498000	1.11.1.6	catalase				1				1	2
	TraesCS2B02G079300	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS6D02G163700	3.5.1.9	arylformamidase			1				1		2
	TraesCS2D02G065100	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS4B02G325800	1.11.1.6	catalase				1				1	2
	TraesCS5B02G162800	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS6D02G065600	6.3.1.2	glutamine synthetase							1		1
	TraesCS2B02G528300	6.3.1.2	glutamine synthetase				1					1
	TraesCS2D02G493600	2.1.2.1	glycine hydroxymethyltransferase							1		1
	TraesCS2B02G521700	2.1.2.1	glycine hydroxymethyltransferase			1						1
	TraesCS1B02G158600	6.3.1.2	glutamine synthetase							1		1
	TraesCS5D02G010200	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS4A02G063800	6.3.1.2	glutamine synthetase			1						1
	TraesCS6A02G298100	6.3.1.2	glutamine synthetase			1						1
	TraesCS4A02G266900	6.3.1.2	glutamine synthetase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS6D02G255200	1.17.1.9	formate dehydrogenase								1	1
	TraesCS4B02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS5B02G162600	4.1.1.39	ribulose-bisphosphate carboxylase				1					1
	TraesCS4B02G240900	6.3.1.2	glutamine synthetase			1						1
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS1A02G218700	2.1.2.1	glycine hydroxymethyltransferase								1	1
	TraesCS5D02G169600	4.1.1.39	ribulose-bisphosphate carboxylase				1					1
	TraesCS4D02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS4D02G240700	6.3.1.2	glutamine synthetase			1						1
	TraesCS6B02G330700	1.11.1.6	catalase							1		1
	TraesCS4D02G322700	1.11.1.6	catalase								1	1
	TraesCS1D02G141800	6.3.1.2	glutamine synthetase			1						1
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS2B02G079200	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS5A02G302400	5.1.3.18	GDP-mannose 3,5-epimerase				1				1	2
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS2B02G599300	5.4.2.8	phosphomannomutase				1				1	2
	TraesCS1A02G419600	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS4A02G199200	1.1.1.22	UDP-glucose 6-dehydrogenase				1				1	2
	TraesCS1B02G449700	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS1D02G427400	2.7.7.27	glucose-1-phosphate adenylyltransferase			1				1		2
	TraesCS3A02G044800	5.3.1.8	mannose-6-phosphate isomerase			1				1		2
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS5D02G112600	5.4.2.10	phosphoglucosamine mutase							1		1
	TraesCS4B02G116200	1.1.1.22	UDP-glucose 6-dehydrogenase				1					1
	TraesCS2A02G175400	3.1.1.31	6-phosphogluconolactonase							1		1
	TraesCS1A02G284900	3.2.1.14	chitinase			1						1
	TraesCS2B02G200900	5.4.99.30	UDP-arabinopyranose mutase								1	1
	TraesCS4A02G188300	3.2.1.55	non-reducing end alpha-L-arabinofuranosidase			1						1
	TraesCS2B02G201700	3.1.1.31	6-phosphogluconolactonase			1						1
	TraesCS1A02G203600	3.2.1.14	chitinase			1						1
	TraesCS2B02G526700	5.4.99.30	UDP-arabinopyranose mutase			1						1
	TraesCS5D02G404300	1.1.1.22	UDP-glucose 6-dehydrogenase								1	1
Amino sugar and nucleotide sugar metabolism	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS3D02G036100	5.3.1.8	mannose-6-phosphate isomerase							1		1
	TraesCS2B02G622200	3.2.1.14	chitinase								1	1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS2D02G181700	5.4.99.30	UDP-arabinopyranose mutase								1	1
	TraesCS1D02G283900	3.2.1.14	chitinase			1						1
	TraesCS6B02G141400	2.7.1.6	galactokinase							1		1
	TraesCS5A02G138400	3.2.1.55	non-reducing end alpha-L-arabinofuranosidase			1						1
	TraesCS7A02G270200	3.1.1.31	6-phosphogluconolactonase				1					1
	TraesCS5A02G394900	1.1.1.22	UDP-glucose 6-dehydrogenase			1						1
	TraesCS7B02G471400	3.2.1.14	chitinase			1						1
	TraesCS5D02G309000	5.1.3.18	GDP-mannose 3,5-epimerase								1	1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS1D02G451900	5.1.3.2	UDP-glucose 4-epimerase								1	1
	TraesCS3B02G039000	5.3.1.8	mannose-6-phosphate isomerase							1		1
	TraesCS7A02G548000	3.2.1.14	chitinase			1						1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCSU02G037500	5.4.2.8	phosphomannomutase				1					1
	TraesCS3A02G303200	5.4.99.30	UDP-arabinopyranose mutase							1		1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
	TraesCS4A02G022500	3.2.1.23	beta-galactosidase			1				1		2
	TraesCS7A02G009200	3.2.1.26	beta-fructofuranosidase						1		1	2
	TraesCS7A02G009100	3.2.1.26	beta-fructofuranosidase				1				1	2
	TraesCS2D02G293200	3.2.1.26	beta-fructofuranosidase			1				1		2
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS7D02G008700	3.2.1.26	beta-fructofuranosidase				1				1	2
	TraesCS4D02G090300	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS7A02G009500	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS6A02G042400	3.2.1.22	alpha-galactosidase						1			1
	TraesCS2B02G350500	2.7.1.11	6-phosphofructokinase			1						1
	TraesCS2B02G122900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS1A02G164900	3.2.1.22	alpha-galactosidase				1					1
	TraesCS4D02G279400	3.2.1.23	beta-galactosidase			1						1
	TraesCS2D02G322200	5.1.3.3	aldose 1-epimerase								1	1
	TraesCS1D02G451900	5.1.3.2	UDP-glucose 4-epimerase								1	1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS7B02G067000	5.1.3.3	aldose 1-epimerase						1			1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
Galactose metabolism	TraesCS4B02G280900	3.2.1.23	beta-galactosidase			1						1
	TraesCS1B02G181800	3.2.1.22	alpha-galactosidase				1					1
	TraesCS4D02G220800	3.2.1.23	beta-galactosidase			1						1
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS5B02G011700	3.2.1.22	alpha-galactosidase							1		1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS6B02G141400	2.7.1.6	galactokinase							1		1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS2A02G105900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS7B02G264200	3.2.1.23	beta-galactosidase				1					1
	TraesCS7A02G009600	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS7D02G163100	5.1.3.3	aldose 1-epimerase			1						1
	TraesCS7B02G104400	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS4A02G486000	3.2.1.26	beta-fructofuranosidase								1	1
	TraesCS4A02G083700	3.2.1.23	beta-galactosidase			1						1
	TraesCS7D02G200800	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS4A02G222900	3.2.1.26	beta-fructofuranosidase			1						1
	TraesCS4A02G387000	2.7.1.11	6-phosphofructokinase							1		1
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS3A02G044800	5.3.1.8	mannose-6-phosphate isomerase			1				1		2
	TraesCS2B02G599300	5.4.2.8	phosphomannomutase				1				1	2
	TraesCS3B02G423200	4.1.2.13	fructose-bisphosphate aldolase				1				1	2
	TraesCS4D02G107400	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS3D02G370700	3.1.3.11	fructose-bisphosphatase							1		1
	TraesCS2B02G350500	2.7.1.11	6-phosphofructokinase			1						1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS4A02G387000	2.7.1.11	6-phosphofructokinase							1		1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS7D02G200800	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Fructose and mannose metabolism	TraesCS3D02G073000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS3A02G359400	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS3D02G036100	5.3.1.8	mannose-6-phosphate isomerase							1		1
	TraesCS3A02G391100	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS3A02G391500	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
	TraesCS4D02G051400	2.7.1.28	triokinase			1						1
	TraesCS3B02G039000	5.3.1.8	mannose-6-phosphate isomerase							1		1
	TraesCS7B02G104400	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS3B02G392000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCSU02G037500	5.4.2.8	phosphomannomutase				1					1
	TraesCS3B02G410400	3.1.3.11	fructose-bisphosphatase			1						1
	TraesCS3B02G422900	4.1.2.13	fructose-bisphosphate aldolase				1					1
Pentose phosphate pathway	TraesCS6D02G195700	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)			1				1		2
	TraesCS3B02G423200	4.1.2.13	fructose-bisphosphate aldolase				1				1	2
	TraesCS2B02G201700	3.1.1.31	6-phosphogluconolactonase			1						1
	TraesCS4A02G387000	2.7.1.11	6-phosphofructokinase							1		1
	TraesCS2A02G175400	3.1.1.31	6-phosphogluconolactonase							1		1
	TraesCS2B02G362700	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)						1			1
	TraesCS4D02G107400	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS3A02G391100	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS7D02G030000	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS3A02G391500	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS4A02G126200	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)							1		1
	TraesCS3B02G410400	3.1.3.11	fructose-bisphosphatase			1						1
	TraesCS4A02G455900	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS3B02G422900	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS6A02G211600	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)							1		1
	TraesCS7A02G092300	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS2B02G350500	2.7.1.11	6-phosphofructokinase			1						1
	TraesCS7A02G270200	3.1.1.31	6-phosphogluconolactonase				1					1
	TraesCS7B02G104400	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS3B02G565200	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS7D02G200800	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS3D02G370700	3.1.3.11	fructose-bisphosphatase							1		1
	TraesCS3D02G491400	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
Pyruvate metabolism	TraesCS1D02G252900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS6D02G197700	4.4.1.5	lactoylglutathione lyase				1				1	2
	TraesCS5A02G131100	2.7.1.40	pyruvate kinase				1				1	2
	TraesCS1A02G253400	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS1B02G264900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS1A02G253200	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS5D02G138800	2.7.1.40	pyruvate kinase								1	1
	TraesCS2D02G068100	6.4.1.2	acetyl-CoA carboxylase							1		1
	TraesCS6D02G078300	1.1.1.27	L-lactate dehydrogenase			1						1
	TraesCS2D02G075200	1.1.1.27	L-lactate dehydrogenase					1				1
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS7B02G114000	3.6.1.7	acylphosphatase								1	1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS4D02G220500	4.1.1.49	phosphoenolpyruvate carboxykinase (ATP)								1	1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS1D02G396100	2.7.1.40	pyruvate kinase								1	1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS4B02G107200	2.7.1.40	pyruvate kinase			1						1
Inositol phosphate metabolism	TraesCS5A02G305400	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1				1		2
	TraesCS5D02G312400	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1				1		2
	TraesCS7D02G542800	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS5B02G062000	2.7.1.137	phosphatidylinositol 3-kinase				1					1
	TraesCS4D02G039700	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS2B02G152700	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS2A02G479000	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase			1						1
	TraesCS2B02G525900	2.7.1.158	inositol-pentakisphosphate 2-kinase							1		1
	TraesCS4B02G068300	2.7.1.64	inositol 3-kinase					1				1
	TraesCS2D02G478300	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS1B02G209100	2.7.1.134	inositol-tetrakisphosphate 1-kinase					1				1
	TraesCS3A02G359400	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS5B02G305900	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1						1
	TraesCS5D02G160300	3.1.4.11	phosphoinositide phospholipase C				1					1
	TraesCS7A02G357800	1.13.99.1	inositol oxygenase							1		1
	TraesCS3B02G392000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS7D02G364900	1.13.99.1	inositol oxygenase							1		1
	TraesCS3D02G073000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS1A02G330900	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1						1
	TraesCS4A02G246500	2.7.1.64	inositol 3-kinase				1					1
Pentose and glucuronate interconversions	TraesCS7A02G157300	3.1.1.11	pectinesterase			1				1		2
	TraesCS2D02G409900	3.1.1.11	pectinesterase			1				1		2
	TraesCS2A02G135400	3.1.1.11	pectinesterase					1		1		2
	TraesCS4A02G199200	1.1.1.22	UDP-glucose 6-dehydrogenase				1				1	2
	TraesCS6D02G324600	3.1.1.11	pectinesterase					1				1
	TraesCS5D02G404300	1.1.1.22	UDP-glucose 6-dehydrogenase								1	1
	TraesCS7B02G221200	3.1.1.11	pectinesterase					1				1
	TraesCS3A02G226400	3.1.1.11	pectinesterase						1			1
	TraesCS6A02G343800	3.1.1.11	pectinesterase					1				1
	TraesCS7D02G157800	3.1.1.11	pectinesterase							1		1
	TraesCS2B02G159300	3.1.1.11	pectinesterase							1		1
	TraesCS1D02G264000	3.1.1.11	pectinesterase					1				1
	TraesCS2A02G412600	3.1.1.11	pectinesterase			1						1
	TraesCS5A02G394900	1.1.1.22	UDP-glucose 6-dehydrogenase			1						1
	TraesCS4B02G116200	1.1.1.22	UDP-glucose 6-dehydrogenase				1					1
Ascorbate and aldarate metabolism	TraesCS5A02G302400	5.1.3.18	GDP-mannose 3,5-epimerase				1				1	2
	TraesCS4A02G199200	1.1.1.22	UDP-glucose 6-dehydrogenase				1				1	2
	TraesCS7D02G314200	1.1.3.8	L-gulonolactone oxidase								1	1
	TraesCS7A02G361600	1.10.3.3	L-ascorbate oxidase			1						1
	TraesCS4B02G116200	1.1.1.22	UDP-glucose 6-dehydrogenase				1					1
	TraesCS5A02G394900	1.1.1.22	UDP-glucose 6-dehydrogenase			1						1
	TraesCS7B02G265200	1.10.3.3	L-ascorbate oxidase							1		1
	TraesCS5D02G309000	5.1.3.18	GDP-mannose 3,5-epimerase								1	1
	TraesCS7D02G361300	1.10.3.3	L-ascorbate oxidase			1						1
	TraesCS7D02G364900	1.13.99.1	inositol oxygenase							1		1
	TraesCS7A02G357800	1.13.99.1	inositol oxygenase							1		1
	TraesCS5D02G404300	1.1.1.22	UDP-glucose 6-dehydrogenase								1	1
	TraesCS5A02G522100	2.3.3.10	hydroxymethylglutaryl-CoA synthase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Butanoate metabolism	TraesCS6B02G247900	2.2.1.6	acetolactate synthase							1		1
	TraesCS6A02G218300	2.2.1.6	acetolactate synthase							1		1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS4A02G075600	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS5D02G201100	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS4B02G052300	4.1.1.15	glutamate decarboxylase					1				1
	TraesCS6A02G288000	2.2.1.6	acetolactate synthase							1		1
	TraesCS4D02G232700	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS7D02G269600	2.3.3.10	hydroxymethylglutaryl-CoA synthase			1						1
	TraesCS5A02G189800	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS5A02G411200	1.3.5.1	succinate dehydrogenase				1					1
Propanoate metabolism	TraesCS3B02G221800	6.2.1.5	succinate---CoA ligase (ADP-forming)			1				1		2
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS3A02G193900	6.2.1.5	succinate---CoA ligase (ADP-forming)			1						1
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS3D02G195700	6.2.1.5	succinate---CoA ligase (ADP-forming)							1		1
	TraesCS2D02G068100	6.4.1.2	acetyl-CoA carboxylase							1		1
	TraesCS6D02G078300	1.1.1.27	L-lactate dehydrogenase			1						1
	TraesCS2D02G075200	1.1.1.27	L-lactate dehydrogenase					1				1
	TraesCS2D02G477100	1.2.1.27	methylmalonate-semialdehyde dehydrogenase (CoA-acylating)			1						1
Citrate cycle (TCA cycle)	TraesCS3B02G221800	6.2.1.5	succinate---CoA ligase (ADP-forming)			1				1		2
	TraesCS3A02G193900	6.2.1.5	succinate---CoA ligase (ADP-forming)			1						1
	TraesCS5D02G201100	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS5A02G411200	1.3.5.1	succinate dehydrogenase				1					1
	TraesCS3D02G195700	6.2.1.5	succinate---CoA ligase (ADP-forming)							1		1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS4D02G220500	4.1.1.49	phosphoenolpyruvate carboxykinase (ATP)								1	1
	TraesCS5A02G189800	1.3.5.1	succinate dehydrogenase							1		1
C5Branched dibasic acid metabolism	TraesCS3B02G221800	6.2.1.5	succinate---CoA ligase (ADP-forming)			1				1		2
	TraesCS6A02G288000	2.2.1.6	acetolactate synthase							1		1
	TraesCS6A02G218300	2.2.1.6	acetolactate synthase							1		1
	TraesCS6B02G247900	2.2.1.6	acetolactate synthase							1		1
	TraesCS3A02G193900	6.2.1.5	succinate---CoA ligase (ADP-forming)			1						1
	TraesCS3D02G195700	6.2.1.5	succinate---CoA ligase (ADP-forming)							1		1
Amino acid metabolism												
	TraesCS4A02G398300	2.5.1.16	spermidine synthase			1				1		2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS5D02G177100	2.5.1.16	spermidine synthase				1				1	2
	TraesCS1D02G383100	1.13.11.54	acireductone dioxygenase [iron(II)-requiring]				1				1	2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS2A02G355400	4.1.1.50	adenosylmethionine decarboxylase			1				1		2
	TraesCS5D02G031300	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1				1		2
	TraesCS2B02G260800	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1				1		2
	TraesCS5D02G534600	2.7.2.4	aspartate kinase				1				1	2
	TraesCS2D02G352900	4.1.1.50	adenosylmethionine decarboxylase			1				1		2
	TraesCS6B02G276700	2.5.1.6	methionine adenosyltransferase			1				1		2
	TraesCS3B02G249200	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1				1		2
	TraesCS3D02G233600	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1				1		2

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Cysteine and methionine metabolism	TraesCS5A02G172600	2.5.1.16	spermidine synthase				1				1	2
	TraesCS6B02G217200	2.5.1.47	cysteine synthase					1				1
	TraesCS5D02G487000	2.7.2.4	aspartate kinase				1					1
	TraesCS6D02G364900	2.1.1.37	DNA (cytosine-5-)-methyltransferase							1		1
	TraesCS2D02G493500	3.3.1.1	adenosylhomocysteinase			1						1
	TraesCS6A02G338800	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1						1
	TraesCS3A02G219000	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1						1
	TraesCS6B02G419000	2.1.1.37	DNA (cytosine-5-)-methyltransferase							1		1
	TraesCS3B02G228500	2.5.1.6	methionine adenosyltransferase			1						1
	TraesCS5D02G407800	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS1A02G138500	5.3.1.23	S-methyl-5-thioribose-1-phosphate isomerase							1		1
	TraesCS2B02G372900	4.1.1.50	adenosylmethionine decarboxylase			1						1
	TraesCS1A02G353800	2.3.1.30	serine O-acetyltransferase					1				1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS4A02G032100	2.6.1.52	phosphoserine transaminase					1				1
	TraesCS6B02G276800	2.5.1.6	methionine adenosyltransferase			1						1
	TraesCS4A02G204000	5.3.1.23	S-methyl-5-thioribose-1-phosphate isomerase			1						1
	TraesCS6D02G230000	2.5.1.6	methionine adenosyltransferase			1						1
	TraesCS4A02G298700	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS2A02G489600	3.3.1.1	adenosylhomocysteinase			1						1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS4B02G014700	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS1B02G396200	1.13.11.54	acireductone dioxygenase [iron(II)-requiring]								1	1
	TraesCS4D02G012900	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase							1		1
	TraesCS6A02G247900	2.5.1.6	methionine adenosyltransferase			1						1
	TraesCS5A02G024900	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS6A02G380200	2.1.1.37	DNA (cytosine-5-)-methyltransferase							1		1
	TraesCS7B02G341200	6.3.2.3	glutathione synthase							1		1
	TraesCS6B02G197300	2.5.1.6	methionine adenosyltransferase			1						1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS2B02G521600	3.3.1.1	adenosylhomocysteinase			1						1
	TraesCS5A02G398300	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS6B02G354300	4.4.1.15	D-cysteine desulfhydrase			1						1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS6D02G078300	1.1.1.27	L-lactate dehydrogenase			1						1
	TraesCS5B02G022800	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS6D02G319500	2.1.1.37	DNA (cytosine-5-)-methyltransferase			1						1
	TraesCS5B02G403400	1.2.1.38	N-acetyl-gamma-glutamyl-phosphate reductase				1					1
	TraesCS2D02G075200	1.1.1.27	L-lactate dehydrogenase					1				1
	TraesCS2A02G493600	3.3.1.1	adenosylhomocysteinase			1						1
	TraesCS1D02G211700	2.6.1.42	branched-chain-amino-acid transaminase			1						1
	TraesCS2A02G508900	1.1.1.95	phosphoglycerate dehydrogenase							1		1
	TraesCS1A02G072000	6.3.2.2	glutamate---cysteine ligase				1					1
	TraesCS5A02G221200	4.1.1.50	adenosylmethionine decarboxylase			1						1
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6A02G272200	2.1.3.3	ornithine carbamoyltransferase				1				1	2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS5D02G407800	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS7D02G170500	3.5.1.14	N-acyl-aliphatic-L-amino acid amidohydrolase								1	1
	TraesCS4A02G063800	6.3.1.2	glutamine synthetase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Arginine biosynthesis	TraesCS2B02G528300	6.3.1.2	glutamine synthetase				1					1
	TraesCS4A02G266900	6.3.1.2	glutamine synthetase			1						1
	TraesCS6D02G252300	2.1.3.3	ornithine carbamoyltransferase				1					1
	TraesCS4B02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS4B02G204000	2.3.1.35	glutamate N-acetyltransferase								1	1
	TraesCS1D02G141800	6.3.1.2	glutamine synthetase			1						1
	TraesCS4B02G240900	6.3.1.2	glutamine synthetase			1						1
	TraesCS6A02G298100	6.3.1.2	glutamine synthetase			1						1
	TraesCS4D02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS6D02G065600	6.3.1.2	glutamine synthetase							1		1
	TraesCS7A02G248200	3.5.1.14	N-acyl-aliphatic-L-amino acid amidohydrolase								1	1
	TraesCS4A02G013200	4.3.3.6	pyridoxal 5'-phosphate synthase (glutamine hydrolysing)								1	1
	TraesCS7B02G143300	3.5.1.14	N-acyl-aliphatic-L-amino acid amidohydrolase								1	1
	TraesCS4D02G240700	6.3.1.2	glutamine synthetase			1						1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS5A02G398300	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS7D02G247000	3.5.1.14	N-acyl-aliphatic-L-amino acid amidohydrolase								1	1
	TraesCS5B02G299700	4.3.2.1	argininosuccinate lyase							1		1
	TraesCS1B02G158600	6.3.1.2	glutamine synthetase							1		1
	TraesCS5B02G403400	1.2.1.38	N-acetyl-gamma-glutamyl-phosphate reductase				1					1
Arginine and proline metabolism	TraesCS5A02G172600	2.5.1.16	spermidine synthase				1				1	2
	TraesCS1A02G209100	1.5.5.2	proline dehydrogenase			1				1		2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS1A02G281400	1.2.1.41	glutamate-5-semialdehyde dehydrogenase			1				1		2
	TraesCS4A02G398300	2.5.1.16	spermidine synthase			1				1		2
	TraesCS2A02G355400	4.1.1.50	adenosylmethionine decarboxylase			1				1		2
	TraesCS5D02G177100	2.5.1.16	spermidine synthase				1				1	2
	TraesCS2B02G347800	3.5.3.12	agmatine deiminase			1				1		2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS2D02G328900	3.5.3.12	agmatine deiminase			1				1		2
	TraesCS2D02G352900	4.1.1.50	adenosylmethionine decarboxylase			1				1		2
	TraesCS1B02G223300	1.5.5.2	proline dehydrogenase							1		1
	TraesCS2B02G372900	4.1.1.50	adenosylmethionine decarboxylase			1						1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS5A02G221200	4.1.1.50	adenosylmethionine decarboxylase			1						1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS2A02G334600	3.5.3.12	agmatine deiminase							1		1
	TraesCS3A02G363700	1.2.1.41	glutamate-5-semialdehyde dehydrogenase								1	1
	TraesCS3D02G077300	6.3.5.4	asparagine synthase (glutamine-hydrolysing)						1	1		2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS1D02G141800	6.3.1.2	glutamine synthetase			1						1
	TraesCS4D02G232700	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS1A02G422100	6.3.5.4	asparagine synthase (glutamine-hydrolysing)			1						1
	TraesCS1B02G158600	6.3.1.2	glutamine synthetase							1		1
	TraesCS5B02G152600	6.3.5.4	asparagine synthase (glutamine-hydrolysing)				1					1
	TraesCS4A02G013200	4.3.3.6	pyridoxal 5'-phosphate synthase (glutamine hydrolysing)								1	1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS4A02G063800	6.3.1.2	glutamine synthetase			1						1
	TraesCS4D02G047400	6.3.1.2	glutamine synthetase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Alanine, aspartate and glutamate metabolism	TraesCS4A02G075600	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS4D02G240700	6.3.1.2	glutamine synthetase			1						1
	TraesCS4A02G266900	6.3.1.2	glutamine synthetase			1						1
	TraesCS5B02G299700	4.3.2.1	argininosuccinate lyase							1		1
	TraesCS4B02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS6A02G298100	6.3.1.2	glutamine synthetase			1						1
	TraesCS6D02G065600	6.3.1.2	glutamine synthetase							1		1
	TraesCS2B02G528300	6.3.1.2	glutamine synthetase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS4B02G052300	4.1.1.15	glutamate decarboxylase					1				1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS4B02G180400	4.3.2.2	adenylosuccinate lyase							1		1
	TraesCS4B02G240900	6.3.1.2	glutamine synthetase			1						1
Phenylalanine, tyrosine and tryptophan biosynthesis	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS5D02G173900	4.1.1.48	indole-3-glycerol-phosphate synthase			1				1		2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS3A02G196700	1.1.1.25	shikimate dehydrogenase (NADP+)			1				1		2
	TraesCS4D02G315400	2.4.2.18	anthranilate phosphoribosyltransferase				1			1		2
	TraesCS4A02G089800	4.1.3.27	anthranilate synthase							1		1
	TraesCS4B02G214500	4.1.3.27	anthranilate synthase							1		1
	TraesCS7B02G208700	4.2.1.20	tryptophan synthase			1						1
	TraesCS4B02G318900	2.4.2.18	anthranilate phosphoribosyltransferase							1		1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS7A02G512300	4.1.1.48	indole-3-glycerol-phosphate synthase							1		1
	TraesCS2D02G326400	4.1.3.27	anthranilate synthase							1		1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS3A02G082700	2.7.1.71	shikimate kinase				1					1
	TraesCS3B02G229000	1.1.1.25	shikimate dehydrogenase (NADP+)			1						1
	TraesCS5B02G444300	4.2.1.20	tryptophan synthase							1		1
Glycine, serine and threonine metabolism	TraesCS2A02G493700	2.1.2.1	glycine hydroxymethyltransferase			1				1		2
	TraesCS5D02G534600	2.7.2.4	aspartate kinase				1				1	2
	TraesCS4B02G172700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)				1				1	2
	TraesCS2B02G521700	2.1.2.1	glycine hydroxymethyltransferase			1						1
	TraesCS5D02G407800	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS5B02G444300	4.2.1.20	tryptophan synthase							1		1
	TraesCS2D02G493600	2.1.2.1	glycine hydroxymethyltransferase							1		1
	TraesCS2A02G508900	1.1.1.95	phosphoglycerate dehydrogenase							1		1
	TraesCS3A02G259700	4.2.3.1	threonine synthase							1		1
	TraesCS5B02G403400	1.2.1.38	N-acetyl-gamma-glutamyl-phosphate reductase				1					1
	TraesCS4A02G032100	2.6.1.52	phosphoserine transaminase					1				1
	TraesCS5D02G108500	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)	1								1
	TraesCS4A02G266700	4.3.1.19	threonine ammonia-lyase							1		1
	TraesCS5D02G487000	2.7.2.4	aspartate kinase				1					1
	TraesCS1A02G218700	2.1.2.1	glycine hydroxymethyltransferase								1	1
	TraesCS7B02G208700	4.2.1.20	tryptophan synthase			1						1
	TraesCS4D02G174700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)								1	1
	TraesCS5A02G398300	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Phenylalanine metabolism	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS2A02G381000	4.3.1.24	phenylalanine ammonia-lyase								1	1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS2B02G398200	4.3.1.24	phenylalanine ammonia-lyase			1						1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS1A02G037800	4.3.1.24	phenylalanine ammonia-lyase			1						1
	TraesCS6A02G222800	4.3.1.24	phenylalanine ammonia-lyase								1	1
Tyrosine metabolism	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS2A02G468500	1.10.3.1	catechol oxidase							1		1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS2A02G468200	1.10.3.1	catechol oxidase			1						1
	TraesCS2B02G491100	1.10.3.1	catechol oxidase			1						1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
Tryptophan metabolism	TraesCS6D02G163700	3.5.1.9	arylformamidase			1				1		2
	TraesCS5A02G498000	1.11.1.6	catalase				1				1	2
	TraesCS4B02G325800	1.11.1.6	catalase				1				1	2
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS6B02G330700	1.11.1.6	catalase							1		1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS4D02G322700	1.11.1.6	catalase								1	1
Valine, leucine and isoleucine biosynthesis	TraesCS1A02G411900	1.1.1.86	ketol-acid reductoisomerase (NADP+)								1	1
	TraesCS6B02G108100	4.2.1.33	3-isopropylmalate dehydratase					1				1
	TraesCS6A02G288000	2.2.1.6	acetolactate synthase							1		1
	TraesCS1D02G211700	2.6.1.42	branched-chain-amino-acid transaminase			1						1
	TraesCS6B02G247900	2.2.1.6	acetolactate synthase							1		1
	TraesCS4A02G266700	4.3.1.19	threonine ammonia-lyase							1		1
	TraesCS6A02G218300	2.2.1.6	acetolactate synthase							1		1
Valine, leucine and isoleucine degradation	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS5A02G522100	2.3.3.10	hydroxymethylglutaryl-CoA synthase							1		1
	TraesCS2D02G477100	1.2.1.27	methylmalonate-semialdehyde dehydrogenase (CoA-acylating)			1						1
	TraesCS1D02G067800	1.3.8.4	isovaleryl-CoA dehydrogenase						1			1
	TraesCS7D02G269600	2.3.3.10	hydroxymethylglutaryl-CoA synthase			1						1
	TraesCS1D02G211700	2.6.1.42	branched-chain-amino-acid transaminase			1						1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
Lysine biosynthesis	TraesCS5D02G534600	2.7.2.4	aspartate kinase				1				1	2
	TraesCS5A02G398300	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS5D02G487000	2.7.2.4	aspartate kinase				1					1
	TraesCS5B02G403400	1.2.1.38	N-acetyl-gamma-glutamyl-phosphate reductase				1					1
	TraesCS5D02G407800	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
Lysine degradation	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS4B02G226900	2.1.1.60	calmodulin-lysine N-methyltransferase				1					1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
Histidine metabolism	TraesCS3A02G176300	3.1.3.15	histidinol-phosphatase							1		1
Lipid metabolism												
	TraesCS2D02G364500	1.11.2.3	plant seed peroxygenase				1		1		1	3
	TraesCS2D02G364600	1.11.2.3	plant seed peroxygenase				1		1		1	3
	TraesCS1D02G147900	4.1.99.5	aldehyde oxygenase (deformylating)				1		1		1	3
	TraesCS1D02G029300	2.3.1.20	diacylglycerol O-acyltransferase			1				1		2
	TraesCS7D02G488800	4.1.99.5	aldehyde oxygenase (deformylating)				1				1	2
	TraesCS7B02G408900	4.1.99.5	aldehyde oxygenase (deformylating)				1				1	2

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Cutin, suberine and wax biosynthesis	TraesCS2A02G367700	1.11.2.3	plant seed peroxygenase				1				1	2
	TraesCS1B02G168500	4.1.99.5	aldehyde oxygenase (deformylating)	1			1					2
	TraesCS2D02G382300	1.11.2.3	plant seed peroxygenase			1				1		2
	TraesCS4D02G241500	1.11.2.3	plant seed peroxygenase			1						1
	TraesCS7D02G206200	1.11.2.3	plant seed peroxygenase								1	1
	TraesCS6D02G369400	4.1.99.5	aldehyde oxygenase (deformylating)							1		1
	TraesCS1A02G150900	4.1.99.5	aldehyde oxygenase (deformylating)				1					1
	TraesCS2A02G385600	1.11.2.3	plant seed peroxygenase			1						1
	TraesCS3B02G006700	2.3.1.20	diacylglycerol O-acyltransferase								1	1
	TraesCS6B02G255100	4.1.99.5	aldehyde oxygenase (deformylating)			1						1
	TraesCS3B02G011800	2.3.1.20	diacylglycerol O-acyltransferase								1	1
	TraesCS2B02G385200	1.11.2.3	plant seed peroxygenase								1	1
	TraesCS3B02G339300	2.3.1.20	diacylglycerol O-acyltransferase						1			1
	TraesCS1D02G147800	4.1.99.5	aldehyde oxygenase (deformylating)				1					1
	TraesCS4B02G241900	1.11.2.3	plant seed peroxygenase			1						1
	TraesCS4D02G008300	1.2.1.84	alcohol-forming fatty acyl-CoA reductase							1		1
Fatty acid elongation	TraesCS4D02G242200	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS6D02G166600	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS6B02G207000	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS3A02G075100	4.2.1.134	very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase				1				1	2
	TraesCS4A02G068400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS4B02G297500	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS6A02G177700	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1					1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS6B02G422900	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS4B02G274700	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6A02G073300	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS4A02G007400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS4D02G148300	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6D02G071600	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS3B02G089500	4.2.1.134	very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase								1	1
	TraesCS4A02G164400	2.3.1.199	very-long-chain 3-oxoacyl-CoA synthase								1	1
	TraesCS4D02G296400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
Glycerolipid metabolism	TraesCS3D02G315700	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS5B02G082400	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS1D02G029300	2.3.1.20	diacylglycerol O-acyltransferase			1				1		2
	TraesCS5A02G076300	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS5D02G089700	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS1B02G209900	2.3.1.15	glycerol-3-phosphate 1-O-acyltransferase							1		1
	TraesCS1A02G164900	3.2.1.22	alpha-galactosidase				1					1
	TraesCS1B02G181800	3.2.1.22	alpha-galactosidase				1					1
	TraesCS3B02G339300	2.3.1.20	diacylglycerol O-acyltransferase						1			1
	TraesCS2A02G105900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS4D02G051400	2.7.1.28	triokinase			1						1
	TraesCS2A02G515700	2.7.1.30	glycerol kinase								1	1
	TraesCS5B02G011700	3.2.1.22	alpha-galactosidase							1		1
	TraesCS2B02G122900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS5B02G106000	2.3.1.51	1-acylglycerol-3-phosphate O-acyltransferase						1			1
	TraesCS3A02G315800	2.7.1.107	diacylglycerol kinase (ATP)								1	1
	TraesCS6A02G042400	3.2.1.22	alpha-galactosidase						1			1
	TraesCS3B02G006700	2.3.1.20	diacylglycerol O-acyltransferase								1	1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS3B02G011800	2.3.1.20	diacylglycerol O-acyltransferase								1	1
Fatty acid biosynthesis	TraesCS6D02G166600	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS6B02G207000	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS4D02G242200	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS4A02G068400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS4B02G297500	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1				1	2
	TraesCS4A02G007400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6A02G073300	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS4D02G296400	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6B02G422900	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6A02G177700	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1					1
	TraesCS2D02G068100	6.4.1.2	acetyl-CoA carboxylase							1		1
	TraesCS4B02G274700	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS6D02G071600	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS4D02G148300	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III								1	1
	TraesCS2A02G520200	2.3.1.180	beta-ketoacyl-[acyl-carrier-protein] synthase III				1					1
	TraesCS4D02G191300	2.3.1.39	[acyl-carrier-protein] S-malonyltransferase							1		1
Glycerophospholipid metabolism	TraesCS5A02G540900	4.1.1.65	phosphatidylserine decarboxylase				1				1	2
	TraesCS6D02G203200	2.1.1.71	phosphatidyl-N-methylethanolamine N-methyltransferase			1				1		2
	TraesCS5B02G082400	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS3D02G315700	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS4A02G347800	3.1.4.4	phospholipase D				1				1	2
	TraesCS5D02G089700	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS5B02G106000	2.3.1.51	1-acylglycerol-3-phosphate O-acyltransferase						1			1
	TraesCS3D02G473600	1.1.1.94	glycerol-3-phosphate dehydrogenase [NAD(P)+]					1				1
	TraesCS5D02G379100	3.6.1.13	ADP-ribose diphosphatase								1	1
	TraesCS5B02G372000	3.6.1.13	ADP-ribose diphosphatase								1	1
	TraesCS3A02G315800	2.7.1.107	diacylglycerol kinase (ATP)								1	1
	TraesCS1B02G242500	3.1.4.4	phospholipase D								1	1
	TraesCS5D02G524600	3.1.4.4	phospholipase D								1	1
	TraesCS5A02G076300	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS1B02G209900	2.3.1.15	glycerol-3-phosphate 1-O-acyltransferase							1		1
	TraesCS5A02G337100	3.1.4.4	phospholipase D				1					1
Sphingolipid metabolism	TraesCS4A02G022500	3.2.1.23	beta-galactosidase			1				1		2
	TraesCS4D02G279400	3.2.1.23	beta-galactosidase			1						1
	TraesCS4B02G280900	3.2.1.23	beta-galactosidase			1						1
	TraesCS1B02G181800	3.2.1.22	alpha-galactosidase				1					1
	TraesCS2A02G105900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS4D02G220800	3.2.1.23	beta-galactosidase			1						1
	TraesCS2B02G122900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS5B02G011700	3.2.1.22	alpha-galactosidase							1		1
	TraesCS6A02G042400	3.2.1.22	alpha-galactosidase						1			1
	TraesCS7B02G264200	3.2.1.23	beta-galactosidase				1					1
	TraesCS1A02G164900	3.2.1.22	alpha-galactosidase				1					1
	TraesCS4A02G083700	3.2.1.23	beta-galactosidase			1						1
Steroid biosynthesis	TraesCS5A02G454500	2.5.1.21	squalene synthase				1				1	2
	TraesCS7D02G550700	1.3.1.72	Delta24-sterol reductase				1				1	2
	TraesCS5D02G465000	2.5.1.21	squalene synthase				1				1	2
	TraesCS4D02G236900	1.14.14.17	squalene monooxygenase								1	1
	TraesCS7A02G559400	1.3.1.72	Delta24-sterol reductase								1	1
	TraesCS4A02G059900	1.14.14.17	squalene monooxygenase				1					1
	TraesCS6A02G043400	1.3.1.70	Delta14-sterol reductase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Ether lipid metabolism	TraesCS4A02G347800	3.1.4.4	phospholipase D				1				1	2
	TraesCS1B02G242500	3.1.4.4	phospholipase D								1	1
	TraesCS5D02G524600	3.1.4.4	phospholipase D								1	1
	TraesCS5A02G337100	3.1.4.4	phospholipase D				1					1
Arachidonic acid metabolism	TraesCS2A02G065000	5.3.99.3	prostaglandin-E synthase				1					1
	TraesCS2D02G063100	5.3.99.3	prostaglandin-E synthase				1					1
	TraesCS2B02G077200	5.3.99.3	prostaglandin-E synthase				1					1
alphaLinolenic acid metabolism	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Synthesis and degradation of ketone bodies	TraesCS7D02G269600	2.3.3.10	hydroxymethylglutaryl-CoA synthase			1						1
	TraesCS5A02G522100	2.3.3.10	hydroxymethylglutaryl-CoA synthase							1		1
Fatty acid degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Energy metabolism												
Carbon fixation in photosynthetic organisms	TraesCS6D02G148700	2.7.2.3	phosphoglycerate kinase				1				1	2
	TraesCS5A02G165700	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS3B02G186100	4.1.1.39	ribulose-bisphosphate carboxylase						1		1	2
	TraesCS1A02G253200	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS1A02G253400	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS2D02G065200	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS1B02G264900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS1D02G252900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS5D02G169900	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS2A02G066800	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS6B02G187500	2.7.2.3	phosphoglycerate kinase				1				1	2
	TraesCS2B02G079300	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS2D02G065100	4.1.1.39	ribulose-bisphosphate carboxylase				1				1	2
	TraesCS3B02G423200	4.1.2.13	fructose-bisphosphate aldolase				1				1	2
	TraesCS6A02G213700	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)				1					1
	TraesCS5D02G169600	4.1.1.39	ribulose-bisphosphate carboxylase				1					1
	TraesCS6D02G247400	2.7.1.19	phosphoribulokinase								1	1
	TraesCS3A02G359400	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS3B02G392000	5.3.1.1	triose-phosphate isomerase				1					1
	TraesCS7D02G465500	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)							1		1
	TraesCS5D02G010200	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS4D02G107400	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS3A02G391500	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS4D02G220500	4.1.1.49	phosphoenolpyruvate carboxykinase (ATP)								1	1
	TraesCS2B02G079200	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS3A02G391100	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS3B02G410400	3.1.3.11	fructose-bisphosphatase			1						1
	TraesCS5B02G162600	4.1.1.39	ribulose-bisphosphate carboxylase				1					1
	TraesCS3B02G422900	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS5B02G162800	4.1.1.39	ribulose-bisphosphate carboxylase								1	1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS3D02G073000	5.3.1.1	triose-phosphate isomerase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS1A02G140900	2.7.2.3	phosphoglycerate kinase							1		1
	TraesCS3D02G370700	3.1.3.11	fructose-bisphosphatase							1		1
	TraesCS7D02G440200	4.4.1.19	phosphosulfolactate synthase	1			1				1	3
	TraesCS7A02G451100	4.4.1.19	phosphosulfolactate synthase	1							1	2
	TraesCS5D02G019800	4.2.1.11	phosphopyruvate hydratase				1				1	2
	TraesCS2A02G493700	2.1.2.1	glycine hydroxymethyltransferase			1				1		2
	TraesCS7B02G351000	4.4.1.19	phosphosulfolactate synthase				1				1	2
	TraesCS3B02G423200	4.1.2.13	fructose-bisphosphate aldolase				1				1	2
	TraesCS4B02G172700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)				1				1	2
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS7D02G200800	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS6D02G255200	1.17.1.9	formate dehydrogenase								1	1
	TraesCS3A02G391500	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS3B02G410400	3.1.3.11	fructose-bisphosphatase			1						1
	TraesCS5D02G108500	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)	1								1
	TraesCS3B02G422900	4.1.2.13	fructose-bisphosphate aldolase				1					1
Methane metabolism	TraesCS7B02G104400	2.7.1.11	6-phosphofructokinase								1	1
	TraesCS2A02G508900	1.1.1.95	phosphoglycerate dehydrogenase							1		1
	TraesCS3A02G391100	4.1.2.13	fructose-bisphosphate aldolase				1					1
	TraesCS3D02G370700	3.1.3.11	fructose-bisphosphatase							1		1
	TraesCS5B02G012300	4.2.1.11	phosphopyruvate hydratase								1	1
	TraesCS4A02G032100	2.6.1.52	phosphoserine transaminase					1				1
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS4A02G387000	2.7.1.11	6-phosphofructokinase							1		1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS1A02G218700	2.1.2.1	glycine hydroxymethyltransferase								1	1
	TraesCS2B02G350500	2.7.1.11	6-phosphofructokinase			1						1
	TraesCS4D02G051400	2.7.1.28	triokinase			1						1
	TraesCS2B02G521700	2.1.2.1	glycine hydroxymethyltransferase			1						1
	TraesCS4D02G107400	4.1.2.13	fructose-bisphosphate aldolase								1	1
	TraesCS2D02G493600	2.1.2.1	glycine hydroxymethyltransferase							1		1
	TraesCS4D02G174700	5.4.2.12	phosphoglycerate mutase (2,3-diphosphoglycerate-independent)								1	1
	TraesCS4D02G213500	4.2.1.11	phosphopyruvate hydratase				1					1
	TraesCS1A02G253200	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS1B02G264900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS3B02G221800	6.2.1.5	succinate---CoA ligase (ADP-forming)			1				1		2
	TraesCS1A02G253400	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS1D02G252900	2.7.9.1	pyruvate, phosphate dikinase			1				1		2
	TraesCS5A02G407700	1.1.1.37	malate dehydrogenase					1				1
	TraesCS3D02G195700	6.2.1.5	succinate---CoA ligase (ADP-forming)							1		1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
Carbon fixation pathways in prokaryotes	TraesCS5A02G411200	1.3.5.1	succinate dehydrogenase				1					1
	TraesCS5A02G189800	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS5D02G201100	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS5B02G463700	6.2.1.1	acetate---CoA ligase							1		1
	TraesCS2B02G307300	6.2.1.1	acetate---CoA ligase			1						1
	TraesCS5D02G417600	1.1.1.37	malate dehydrogenase				1					1
	TraesCS2D02G068100	6.4.1.2	acetyl-CoA carboxylase							1		1
	TraesCS3A02G193900	6.2.1.5	succinate---CoA ligase (ADP-forming)			1						1
	TraesCS7D02G523900	4.2.1.1	carbonic anhydrase								1	1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Nitrogen metabolism	TraesCS4D02G296200	4.2.1.104	cyanase					1				1
	TraesCS4D02G047400	6.3.1.2	glutamine synthetase			1						1
	TraesCS1D02G141800	6.3.1.2	glutamine synthetase			1						1
	TraesCS6D02G065600	6.3.1.2	glutamine synthetase							1		1
	TraesCS2B02G528300	6.3.1.2	glutamine synthetase				1					1
	TraesCS4B02G240900	6.3.1.2	glutamine synthetase			1						1
	TraesCS3A02G230100	4.2.1.1	carbonic anhydrase			1						1
	TraesCS4D02G240700	6.3.1.2	glutamine synthetase			1						1
	TraesCS3D02G223200	4.2.1.1	carbonic anhydrase			1						1
	TraesCS6A02G298100	6.3.1.2	glutamine synthetase			1						1
	TraesCS4A02G063800	6.3.1.2	glutamine synthetase			1						1
	TraesCS7D02G523800	4.2.1.1	carbonic anhydrase			1						1
	TraesCS4A02G266900	6.3.1.2	glutamine synthetase			1						1
	TraesCS1B02G158600	6.3.1.2	glutamine synthetase							1		1
	TraesCS4B02G047400	6.3.1.2	glutamine synthetase			1						1
Oxidative phosphorylation	TraesCS2A02G502400	7.1.2.1	P-type H+-exporting transporter								1	1
	TraesCS6A02G076300	1.6.99.3	#N/D				1					1
	TraesCS5A02G411200	1.3.5.1	succinate dehydrogenase				1					1
	TraesCS2B02G530500	7.1.2.1	P-type H+-exporting transporter								1	1
	TraesCS6D02G252400	3.6.1.1	inorganic diphosphatase			1						1
	TraesCS2D02G503000	7.1.2.1	P-type H+-exporting transporter								1	1
	TraesCS5A02G189800	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS3A02G534500	3.6.1.1	inorganic diphosphatase			1						1
	TraesCS5D02G201100	1.3.5.1	succinate dehydrogenase							1		1
	TraesCS3B02G186000	7.1.2.2	H+-transporting two-sector ATPase								1	1
	TraesCS6A02G202900	7.1.1.8	quinol---cytochrome-c reductase			1						1
	TraesCS3B02G280000	7.1.2.2	H+-transporting two-sector ATPase								1	1
	TraesCSU02G073200	7.1.2.1	P-type H+-exporting transporter			1						1
	TraesCS3D02G539900	3.6.1.1	inorganic diphosphatase								1	1
	TraesCS3D02G540100	3.6.1.1	inorganic diphosphatase			1						1
Sulfur metabolism	TraesCS6B02G217200	2.5.1.47	cysteine synthase					1				1
	TraesCS5B02G387300	2.7.7.4	sulfate adenylyltransferase							1		1
	TraesCS1A02G353800	2.3.1.30	serine O-acetyltransferase					1				1
	TraesCS5B02G064500	2.8.1.1	thiosulfate sulfurtransferase				1					1
Photosynthesis	TraesCS3B02G280000	7.1.2.2	H+-transporting two-sector ATPase								1	1
	TraesCS3B02G186000	7.1.2.2	H+-transporting two-sector ATPase								1	1
	TraesCS2A02G252800	1.18.1.2	ferredoxin---NADP+ reductase								1	1
	TraesCS2D02G253400	1.18.1.2	ferredoxin---NADP+ reductase								1	1
Metabolism of cofactors and vitamins												
Thiamine metabolism	TraesCS3B02G155300	2.7.4.3	adenylate kinase		1		1		1		1	4
	TraesCS3A02G137600	2.7.4.3	adenylate kinase				1		1		1	3
	TraesCS3D02G138500	2.7.4.3	adenylate kinase				1				1	2
	TraesCS5D02G186700	3.1.3.2	acid phosphatase				1				1	2
	TraesCS7A02G382500	2.7.4.3	adenylate kinase				1				1	2
	TraesCS4D02G021400	4.1.99.17	phosphomethylpyrimidine synthase				1				1	2
	TraesCS4B02G023800	4.1.99.17	phosphomethylpyrimidine synthase								1	1
	TraesCS5D02G136600	3.1.3.2	acid phosphatase				1					1
	TraesCS7B02G285200	2.7.4.3	adenylate kinase								1	1
	TraesCS7D02G378900	2.7.4.3	adenylate kinase								1	1
	TraesCS4A02G073800	3.1.3.2	acid phosphatase								1	1
	TraesCS7D02G507900	3.1.3.2	acid phosphatase								1	1
	TraesCS7B02G216500	2.7.4.3	adenylate kinase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS5A02G329200	3.1.3.2	acid phosphatase							1		1
	TraesCS4B02G324400	2.7.4.3	adenylate kinase				1					1
	TraesCS5B02G329300	3.1.3.2	acid phosphatase			1						1
	TraesCS2B02G579200	2.7.4.3	adenylate kinase				1					1
	TraesCS5A02G182300	3.1.3.2	acid phosphatase								1	1
	TraesCSU02G129700	4.2.1.96	4a-hydroxytetrahydrobiopterin dehydratase				1				1	2
	TraesCS7B02G315700	4.1.99.12	3,4-dihydroxy-2-butanone-4-phosphate synthase				1				1	2
	TraesCS7A02G415600	4.1.99.12	3,4-dihydroxy-2-butanone-4-phosphate synthase				1				1	2
	TraesCS5A02G545700	4.2.1.96	4a-hydroxytetrahydrobiopterin dehydratase				1				1	2
	TraesCS7D02G457800	2.8.1.9	molybdenum cofactor sulfurtransferase				1					1
	TraesCS1B02G350700	3.4.19.9	folate gamma-glutamyl hydrolase								1	1
Folate biosynthesis	TraesCS3D02G167700	4.1.3.38	aminodeoxychorismate lyase			1						1
	TraesCS4B02G379500	4.2.1.96	4a-hydroxytetrahydrobiopterin dehydratase								1	1
	TraesCS2B02G521000	3.5.4.16	GTP cyclohydrolase I				1					1
	TraesCS1B02G057900	4.1.2.25	dihydroneopterin aldolase								1	1
	TraesCS2B02G547000	1.5.1.3	dihydrofolate reductase			1						1
	TraesCS5D02G070900	4.1.99.22	GTP 3',8-cyclase				1					1
	TraesCS5D02G199800	2.8.1.12	molybdopterin synthase								1	1
	TraesCS2B02G239200	5.3.3.2	isopentenyl-diphosphate Delta-isomerase				1				1	2
	TraesCS7A02G248800	1.1.1.34	hydroxymethylglutaryl-CoA reductase (NADPH)				1					1
	TraesCS7D02G247800	1.1.1.34	hydroxymethylglutaryl-CoA reductase (NADPH)								1	1
	TraesCS2D02G220000	5.3.3.2	isopentenyl-diphosphate Delta-isomerase					1				1
	TraesCS7A02G056000	1.1.1.267	1-deoxy-D-xylulose-5-phosphate reductoisomerase							1		1
Terpenoid backbone biosynthesis	TraesCS3D02G186200	1.3.1.83	geranylgeranyl diphosphate reductase							1		1
	TraesCS7D02G050900	1.1.1.267	1-deoxy-D-xylulose-5-phosphate reductoisomerase							1		1
	TraesCS4A02G442600	1.1.1.267	1-deoxy-D-xylulose-5-phosphate reductoisomerase							1		1
	TraesCS7D02G269600	2.3.3.10	hydroxymethylglutaryl-CoA synthase			1						1
	TraesCS5A02G522100	2.3.3.10	hydroxymethylglutaryl-CoA synthase							1		1
	TraesCS5D02G277500	1.1.1.34	hydroxymethylglutaryl-CoA reductase (NADPH)				1					1
	TraesCS1D02G168700	1.3.1.33	protochlorophyllide reductase				1				1	2
	TraesCS5A02G063800	4.99.1.3	sirohydrochlorin cobaltochelatase			1						1
	TraesCS1B02G191200	1.2.1.70	glutamyl-tRNA reductase				1					1
	TraesCS1A02G171000	1.3.1.33	protochlorophyllide reductase								1	1
	TraesCS2D02G425000	1.3.3.3	coproporphyrinogen oxidase				1					1
Porphyrin and chlorophyll metabolism	TraesCS3A02G191700	1.14.13.81	magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase								1	1
	TraesCS3D02G194300	1.14.13.81	magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase								1	1
	TraesCS2A02G426900	1.3.3.3	coproporphyrinogen oxidase				1					1
	TraesCS6D02G085400	2.5.1.141	heme o synthase						1			1
	TraesCS2B02G447300	1.3.3.3	coproporphyrinogen oxidase				1					1
	TraesCS2B02G593000	1.3.1.33	protochlorophyllide reductase								1	1
	TraesCS5D02G186700	3.1.3.2	acid phosphatase				1				1	2
	TraesCS7B02G315700	4.1.99.12	3,4-dihydroxy-2-butanone-4-phosphate synthase				1				1	2
	TraesCS7A02G415600	4.1.99.12	3,4-dihydroxy-2-butanone-4-phosphate synthase				1				1	2
	TraesCS4A02G073800	3.1.3.2	acid phosphatase								1	1
Riboflavin metabolism	TraesCS5A02G182300	3.1.3.2	acid phosphatase								1	1
	TraesCS5A02G329200	3.1.3.2	acid phosphatase							1		1
	TraesCS7D02G507900	3.1.3.2	acid phosphatase								1	1
	TraesCS5B02G329300	3.1.3.2	acid phosphatase			1						1
	TraesCS5D02G136600	3.1.3.2	acid phosphatase				1					1
	TraesCS4A02G301600	2.1.1.295	2-methyl-6-phytyl-1,4-hydroquinone methyltransferase			1				1		2
	TraesCS6B02G265600	2.1.1.295	2-methyl-6-phytyl-1,4-hydroquinone methyltransferase				1					1
Ubiquinone and other terpenoidquinone biosynthesis	TraesCS5B02G189100	5.4.4.2	isochorismate synthase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS2B02G146000	1.6.5.2	NAD(P)H dehydrogenase (quinone)							1		1
	TraesCS4B02G013100	2.1.1.295	2-methyl-6-phytyl-1,4-hydroquinone methyltransferase			1						1
Vitamin B6 metabolism	TraesCS4D02G353700	1.4.3.5	pyridoxal 5'-phosphate synthase			1						1
	TraesCS5D02G069700	2.7.1.35	pyridoxal kinase								1	1
	TraesCS5A02G529000	1.4.3.5	pyridoxal 5'-phosphate synthase							1		1
	TraesCS4A02G013200	4.3.3.6	pyridoxal 5'-phosphate synthase (glutamine hydrolysing)								1	1
	TraesCS3A02G259700	4.2.3.1	threonine synthase							1		1
	TraesCS4A02G032100	2.6.1.52	phosphoserine transaminase					1				1
Sesquiterpenoid and triterpenoid biosynthesis	TraesCS5D02G465000	2.5.1.21	squalene synthase				1				1	2
	TraesCS5A02G454500	2.5.1.21	squalene synthase				1				1	2
	TraesCS4A02G059900	1.14.14.17	squalene monooxygenase				1					1
	TraesCS4D02G236900	1.14.14.17	squalene monooxygenase								1	1
One carbon pool by folate	TraesCS2A02G493700	2.1.2.1	glycine hydroxymethyltransferase			1				1		2
	TraesCS2D02G493600	2.1.2.1	glycine hydroxymethyltransferase							1		1
	TraesCS2B02G547000	1.5.1.3	dihydrofolate reductase			1						1
	TraesCS1A02G218700	2.1.2.1	glycine hydroxymethyltransferase								1	1
	TraesCS2B02G521700	2.1.2.1	glycine hydroxymethyltransferase			1						1
Pantothenate and CoA biosynthesis	TraesCS6A02G218300	2.2.1.6	acetolactate synthase							1		1
	TraesCS6B02G247900	2.2.1.6	acetolactate synthase							1		1
	TraesCS6A02G288000	2.2.1.6	acetolactate synthase							1		1
	TraesCS1D02G211700	2.6.1.42	branched-chain-amino-acid transaminase			1						1
	TraesCS1A02G411900	1.1.1.86	ketol-acid reductoisomerase (NADP+)								1	1
	TraesCS3A02G169900	2.7.1.24	dephospho-CoA kinase			1						1
Carotenoid biosynthesis	TraesCS2A02G238400	1.3.5.6	9,9'-dicis-zeta-carotene desaturase			1				1		2
	TraesCS2D02G236500	1.3.5.6	9,9'-dicis-zeta-carotene desaturase			1				1		2
	TraesCS7A02G317900	5.2.1.14	beta-carotene isomerase								1	1
Lipoic acid metabolism	TraesCS3A02G297700	2.8.1.8	lipoyl synthase	1								1
	TraesCS4B02G176600	2.3.1.181	lipoyl(octanoyl) transferase								1	1
	TraesCS4A02G128200	2.3.1.181	lipoyl(octanoyl) transferase								1	1
Nicotinate and nicotinamide metabolism	TraesCS3A02G495300	2.7.1.23	NAD+ kinase			1						1
	TraesCS5B02G517900	6.3.4.21	nicotinate phosphoribosyltransferase			1						1
	TraesCS5A02G355300	6.3.4.21	nicotinate phosphoribosyltransferase							1		1
Geraniol degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Limonene and pinene degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Zeatin biosynthesis	TraesCS3A02G311000	1.5.99.12	cytokinin dehydrogenase								1	1
Biotin metabolism	TraesCS6A02G160100	6.3.3.3	dethiobiotin synthase								1	1
Biosynthesis of other secondary metabolites												
	TraesCS3B02G034300	1.11.1.7	peroxidase			1				1		2
	TraesCS2B02G124300	1.11.1.7	peroxidase			1				1		2
	TraesCS4B02G176900	1.11.1.7	peroxidase			1				1		2
	TraesCS2A02G574400	1.11.1.7	peroxidase			1				1		2
	TraesCS2D02G108500	1.11.1.7	peroxidase			1				1		2
	TraesCS7B02G099800	1.11.1.7	peroxidase				1					1
	TraesCS4D02G178600	1.11.1.7	peroxidase							1		1
	TraesCS3D02G516900	1.11.1.7	peroxidase			1						1
	TraesCS2A02G572700	1.11.1.7	peroxidase			1						1
	TraesCS6B02G042600	1.11.1.7	peroxidase						1			1
	TraesCS2A02G573500	1.11.1.7	peroxidase								1	1
	TraesCS2A02G572600	1.11.1.7	peroxidase							1		1
	TraesCS2A02G573700	1.11.1.7	peroxidase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Phenylpropanoid biosynthesis	TraesCS2A02G381000	4.3.1.24	phenylalanine ammonia-lyase								1	1
	TraesCS1B02G095800	1.11.1.7	peroxidase								1	1
	TraesCS6A02G029800	1.11.1.7	peroxidase						1			1
	TraesCS2B02G098400	1.11.1.7	peroxidase							1		1
	TraesCS7A02G339600	1.11.1.7	peroxidase								1	1
	TraesCS1D02G318800	1.11.1.7	peroxidase								1	1
	TraesCS7D02G212900	1.11.1.7	peroxidase				1					1
	TraesCS2B02G124600	1.11.1.7	peroxidase		1							1
	TraesCS3D02G031800	1.11.1.7	peroxidase							1		1
	TraesCS2B02G125800	1.11.1.7	peroxidase			1						1
	TraesCS4A02G389000	1.11.1.7	peroxidase			1						1
	TraesCS2B02G372500	1.11.1.7	peroxidase								1	1
	TraesCS4D02G116600	1.11.1.7	peroxidase			1						1
	TraesCS2B02G398200	4.3.1.24	phenylalanine ammonia-lyase			1						1
	TraesCS4D02G342600	1.11.1.7	peroxidase					1				1
	TraesCS2B02G614100	1.11.1.7	peroxidase								1	1
	TraesCS6A02G222800	4.3.1.24	phenylalanine ammonia-lyase								1	1
	TraesCS2B02G615100	1.11.1.7	peroxidase							1		1
	TraesCS7A02G070900	1.11.1.7	peroxidase			1						1
	TraesCS2D02G107900	1.11.1.7	peroxidase						1			1
	TraesCS7A02G477100	1.11.1.7	peroxidase								1	1
	TraesCS1A02G037800	4.3.1.24	phenylalanine ammonia-lyase			1						1
	TraesCS7D02G084900	1.11.1.7	peroxidase							1		1
	TraesCS2D02G153100	1.11.1.7	peroxidase								1	1
	TraesCS7D02G369900	1.11.1.7	peroxidase			1						1
	TraesCS2A02G084100	1.11.1.7	peroxidase					1				1
	TraesCS3B02G578000	1.11.1.7	peroxidase			1						1
Isoquinoline alkaloid biosynthesis	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS2A02G468500	1.10.3.1	catechol oxidase							1		1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
	TraesCS2A02G468200	1.10.3.1	catechol oxidase			1						1
	TraesCS2B02G491100	1.10.3.1	catechol oxidase			1						1
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
Flavonoid biosynthesis	TraesCS7D02G165800	5.5.1.6	chalcone isomerase								1	1
	TraesCS6A02G337200	5.5.1.6	chalcone isomerase				1					1
	TraesCS5A02G475600	5.5.1.6	chalcone isomerase					1				1
	TraesCS2B02G048400	2.3.1.74	chalcone synthase			1						1
	TraesCS7A02G163700	5.5.1.6	chalcone isomerase				1					1
	TraesCS2B02G558400	2.3.1.74	chalcone synthase			1						1
	TraesCS1A02G036200	5.5.1.6	chalcone isomerase								1	1
	TraesCS2D02G530600	2.3.1.74	chalcone synthase			1						1
Streptomycin biosynthesis	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1
	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
	TraesCS3B02G525400	2.7.1.1	hexokinase				1				1	2
	TraesCS3D02G110300	2.7.1.1	hexokinase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Neomycin, kanamycin and gentamicin biosynthesis	TraesCS1A02G122800	2.7.1.1	hexokinase			1						1
	TraesCS1B02G366400	2.7.1.1	hexokinase							1		1
	TraesCS3D02G475600	2.7.1.1	hexokinase				1					1
	TraesCS3A02G108500	2.7.1.1	hexokinase			1						1
	TraesCS3A02G480900	2.7.1.1	hexokinase				1					1
Tropane, piperidine and pyridine alkaloid biosynthesis	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
Novobiocin biosynthesis	TraesCS6A02G018600	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS7A02G353500	2.6.1.1	aspartate transaminase				1				1	2
	TraesCS6B02G026900	2.6.1.1	aspartate transaminase				1					1
	TraesCS7D02G369300	2.6.1.1	aspartate transaminase				1					1
	TraesCS7B02G274100	2.6.1.1	aspartate transaminase				1					1
Monobactam biosynthesis	TraesCS5D02G534600	2.7.2.4	aspartate kinase				1				1	2
	TraesCS5D02G407800	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS5D02G487000	2.7.2.4	aspartate kinase				1					1
	TraesCS5B02G387300	2.7.7.4	sulfate adenylyltransferase							1		1
	TraesCS5A02G398300	1.2.1.11	aspartate-semialdehyde dehydrogenase				1					1
	TraesCS5B02G403400	1.2.1.38	N-acetyl-gamma-glutamyl-phosphate reductase				1					1
Carbapenem biosynthesis	TraesCS1A02G281400	1.2.1.41	glutamate-5-semialdehyde dehydrogenase			1				1		2
	TraesCS3A02G363700	1.2.1.41	glutamate-5-semialdehyde dehydrogenase								1	1
Phenazine biosynthesis	TraesCS2D02G326400	4.1.3.27	anthranilate synthase							1		1
	TraesCS4B02G214500	4.1.3.27	anthranilate synthase							1		1
	TraesCS4A02G089800	4.1.3.27	anthranilate synthase							1		1
Caffeine metabolism	TraesCS3A02G376400	1.7.3.3	factor-independent urate hydroxylase			1						1
Aflatoxin biosynthesis	TraesCS2D02G068100	6.4.1.2	acetyl-CoA carboxylase							1		1
Glucosinolate biosynthesis	TraesCS1D02G211700	2.6.1.42	branched-chain-amino-acid transaminase			1						1
Biosynthesis of various secondary metabolites	TraesCS1A02G353800	2.3.1.30	serine O-acetyltransferase					1				1
Signal transduction												
mTOR signaling pathway	TraesCS7B02G322900	2.7.11.24	mitogen-activated protein kinase			1				1		2
	TraesCS7A02G410700	2.7.11.24	mitogen-activated protein kinase				1				1	2
	TraesCS7D02G414700	2.7.11.24	mitogen-activated protein kinase			1				1		2
	TraesCS4D02G126200	2.7.11.1	non-specific serine/threonine protein kinase				1				1	2
	TraesCS5B02G146500	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6B02G412400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS6A02G099600	2.7.11.24	mitogen-activated protein kinase							1		1
	TraesCS1B02G431400	2.7.11.24	mitogen-activated protein kinase							1		1
	TraesCS7B02G203800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS2A02G098300	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS5D02G144800	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS2D02G217400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS6B02G127800	2.7.11.24	mitogen-activated protein kinase					1				1
	TraesCS2D02G364200	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS1B02G142300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS3B02G107000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7B02G331800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3B02G544300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS5B02G295300	2.7.11.24	mitogen-activated protein kinase								1	1
	TraesCS3D02G109700	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS6A02G000500	2.7.11.1	non-specific serine/threonine protein kinase				1					1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS3D02G296000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6A02G374700	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3D02G316400	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS6B02G270400	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS4B02G120400	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS6D02G358600	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4B02G178100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7A02G422500	2.7.11.24	mitogen-activated protein kinase							1		1
	TraesCS7D02G231000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1B02G347100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1A02G100400	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS1B02G372400	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1A02G077200	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS7D02G414900	2.7.11.24	mitogen-activated protein kinase			1						1
	TraesCS4D02G179700	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS5B02G004300	2.7.11.1	non-specific serine/threonine protein kinase							1		1
PI3KAkt signaling pathway	TraesCS4D02G126200	2.7.11.1	non-specific serine/threonine protein kinase				1				1	2
	TraesCS6B02G412400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS5D02G144800	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1A02G100400	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS7D02G231000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1B02G142300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS5B02G004300	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS1B02G347100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6A02G374700	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS1B02G372400	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7B02G203800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS2A02G098300	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4B02G178100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS2D02G217400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4D02G179700	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS2D02G364200	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS5B02G146500	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS3B02G107000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6A02G000500	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3B02G544300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS6B02G270400	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS3D02G109700	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS6D02G358600	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3D02G296000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7B02G331800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3D02G316400	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS1A02G077200	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4B02G120400	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS5B02G082400	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS5A02G305400	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1				1		2
	TraesCS5D02G312400	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1				1		2
	TraesCS3D02G315700	2.7.1.107	diacylglycerol kinase (ATP)				1				1	2
	TraesCS2A02G479000	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase			1						1
	TraesCS5D02G089700	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS2B02G152700	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS2D02G478300	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
Phosphatidylinositol signaling system	TraesCS5A02G076300	2.7.1.107	diacylglycerol kinase (ATP)				1					1
	TraesCS3A02G315800	2.7.1.107	diacylglycerol kinase (ATP)								1	1
	TraesCS5B02G062000	2.7.1.137	phosphatidylinositol 3-kinase				1					1
	TraesCS5D02G160300	3.1.4.11	phosphoinositide phospholipase C				1					1
	TraesCS5B02G305900	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1						1
	TraesCS1B02G209100	2.7.1.134	inositol-tetrakisphosphate 1-kinase					1				1
	TraesCS2B02G525900	2.7.1.158	inositol-pentakisphosphate 2-kinase							1		1
	TraesCS1A02G330900	2.7.1.134	inositol-tetrakisphosphate 1-kinase			1						1
	TraesCS7D02G542800	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
	TraesCS4D02G027800	2.7.4.24	diphosphoinositol-pentakisphosphate kinase								1	1
	TraesCS4D02G039700	2.7.1.68	1-phosphatidylinositol-4-phosphate 5-kinase							1		1
Nucleotide metabolism												
Purine metabolism	TraesCS3B02G155300	2.7.4.3	adenylate kinase		1		1		1		1	4
	TraesCS3A02G137600	2.7.4.3	adenylate kinase				1		1		1	3
	TraesCS5A02G131100	2.7.1.40	pyruvate kinase				1				1	2
	TraesCS1B02G238200	3.5.4.6	AMP deaminase			1				1		2
	TraesCS6D02G212000	2.7.1.20	adenosine kinase			1				1		2
	TraesCS1B02G464900	3.6.1.3	#N/D					1			1	2
	TraesCS3D02G138500	2.7.4.3	adenylate kinase				1				1	2
	TraesCS1D02G226400	3.5.4.6	AMP deaminase			1				1		2
	TraesCS5D02G253900	6.3.2.6	phosphoribosylaminoimidazolesuccinocarboxamide synthase			1				1		2
	TraesCS2D02G311500	3.5.3.26	(S)-ureidoglycine aminohydrolase				1				1	2
	TraesCS7A02G204800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS7A02G382500	2.7.4.3	adenylate kinase				1				1	2
	TraesCS7B02G031500	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS1A02G257200	3.6.1.3	#N/D					1			1	2
	TraesCS7D02G207800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS1A02G225000	3.5.4.6	AMP deaminase			1				1		2
	TraesCS1A02G430100	3.6.1.3	#N/D								1	1
	TraesCS2A02G313000	3.5.3.26	(S)-ureidoglycine aminohydrolase								1	1
	TraesCS6B02G251600	2.4.2.7	adenine phosphoribosyltransferase			1						1
	TraesCS1D02G396100	2.7.1.40	pyruvate kinase								1	1
	TraesCS7B02G216500	2.7.4.3	adenylate kinase				1					1
	TraesCS3A02G312300	4.1.1.21	phosphoribosylaminoimidazole carboxylase			1						1
	TraesCS5D02G509100	6.3.3.1	phosphoribosylformylglycinamidine cyclo-ligase					1				1
	TraesCS3A02G376400	1.7.3.3	factor-independent urate hydroxylase			1						1
	TraesCS6D02G204800	2.4.2.7	adenine phosphoribosyltransferase			1						1
	TraesCS1D02G439200	3.6.1.3	#N/D					1				1
	TraesCS2B02G579200	2.7.4.3	adenylate kinase				1					1
	TraesCS1D02G439300	3.6.1.3	#N/D					1				1
	TraesCS7D02G130500	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS4B02G107200	2.7.1.40	pyruvate kinase			1						1
	TraesCS5D02G379100	3.6.1.13	ADP-ribose diphosphatase								1	1
	TraesCS7D02G378900	2.7.4.3	adenylate kinase								1	1
	TraesCS6A02G223400	2.7.1.20	adenosine kinase			1						1
	TraesCS4B02G324400	2.7.4.3	adenylate kinase				1					1
	TraesCS6B02G257900	2.7.1.20	adenosine kinase			1						1
	TraesCS5A02G074200	3.6.1.3	#N/D								1	1
	TraesCS2A02G221500	3.6.1.3	#N/D								1	1
	TraesCS1D02G439400	3.6.1.3	#N/D					1				1
	TraesCS2B02G330100	3.5.3.26	(S)-ureidoglycine aminohydrolase								1	1
	TraesCS5B02G372000	3.6.1.13	ADP-ribose diphosphatase								1	1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS7B02G112000	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS5B02G387300	2.7.7.4	sulfate adenylyltransferase							1		1
	TraesCS7B02G285200	2.7.4.3	adenylate kinase								1	1
	TraesCS5D02G087900	3.6.1.3	#N/D								1	1
	TraesCS2B02G595300	3.5.2.5	allantoinase							1		1
	TraesCS5D02G138800	2.7.1.40	pyruvate kinase								1	1
	TraesCS1D02G439500	3.6.1.3	#N/D					1				1
	TraesCS4B02G180400	4.3.2.2	adenylosuccinate lyase							1		1
	TraesCS3B02G155300	2.7.4.3	adenylate kinase		1		1		1		1	4
	TraesCS3A02G137600	2.7.4.3	adenylate kinase				1		1		1	3
	TraesCS7A02G204800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS7B02G031500	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS7D02G207800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS3D02G138500	2.7.4.3	adenylate kinase				1				1	2
Pyrimidine metabolism	TraesCS3B02G270600	3.6.1.23	dUTP diphosphatase				1					1
	TraesCS7B02G112000	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS2B02G547000	1.5.1.3	dihydrofolate reductase			1						1
	TraesCS3D02G494000	2.4.2.10	orotate phosphoribosyltransferase							1		1
	TraesCS7D02G130500	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS4D02G017900	3.6.1.23	dUTP diphosphatase							1		1
	TraesCS1A02G411600	6.3.4.2	CTP synthase (glutamine hydrolysing)			1						1
	TraesCS1B02G441900	6.3.4.2	CTP synthase (glutamine hydrolysing)			1						1
Metabolism of other amino acids												
	TraesCS7B02G031500	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS6D02G195700	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)			1				1		2
	TraesCS7D02G207800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS2D02G154300	1.11.1.15	#N/D			1				1		2
	TraesCS7A02G204800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS4A02G025200	1.8.1.7	glutathione-disulfide reductase				1				1	2
	TraesCS7B02G065200	1.11.1.15	#N/D				1				1	2
	TraesCS4A02G398300	2.5.1.16	spermidine synthase			1				1		2
	TraesCS5D02G177100	2.5.1.16	spermidine synthase				1				1	2
	TraesCS5A02G172600	2.5.1.16	spermidine synthase				1				1	2
	TraesCS6B02G092200	4.3.2.7	glutathione-specific gamma-glutamylcyclotransferase					1				1
	TraesCS3B02G152200	2.5.1.18	glutathione transferase			1						1
	TraesCS7A02G092300	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS3D02G133100	2.5.1.18	glutathione transferase			1						1
	TraesCS7D02G130500	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS3D02G491400	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS6D02G065900	4.3.2.7	glutathione-specific gamma-glutamylcyclotransferase					1				1
	TraesCS1A02G102700	2.5.1.18	glutathione transferase							1		1
	TraesCS7B02G000300	2.5.1.18	glutathione transferase			1						1
	TraesCS4A02G126200	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)							1		1
Glutathione metabolism	TraesCS7B02G341200	6.3.2.3	glutathione synthase							1		1
	TraesCS1B02G097400	2.5.1.18	glutathione transferase								1	1
	TraesCS7D02G389700	1.11.1.15	#N/D							1		1
	TraesCS4A02G455900	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS6D02G016700	2.5.1.18	glutathione transferase								1	1
	TraesCS4D02G277400	1.8.1.7	glutathione-disulfide reductase								1	1
	TraesCS2B02G362700	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)						1			1
	TraesCS5A02G024100	2.5.1.18	glutathione transferase			1						1
	TraesCS1A02G078800	2.5.1.18	glutathione transferase				1					1

Supplemental Table 5.4. continued)

Differential expressed genes functional anotation through KEGG pathways													
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS7D02G479100	2.5.1.18	glutathione transferase					1				1
	TraesCS3A02G270300	1.11.1.15	#N/D			1						1
	TraesCS1A02G072000	6.3.2.2	glutamate---cysteine ligase				1					1
	TraesCS7B02G112000	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS1B02G113700	2.5.1.18	glutathione transferase							1		1
	TraesCS7D02G030000	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS6A02G068300	4.3.2.7	glutathione-specific gamma-glutamylcyclotransferase					1				1
	TraesCS3B02G152300	2.5.1.18	glutathione transferase			1						1
	TraesCS6A02G211600	1.1.1.49	glucose-6-phosphate dehydrogenase (NADP+)							1		1
	TraesCS3B02G565200	1.1.1.44	phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating)			1						1
	TraesCS6A02G357200	3.4.11.1	leucyl aminopeptidase			1						1
	TraesCS5D02G030000	2.5.1.18	glutathione transferase							1		1
	TraesCS5D02G031300	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1				1		2
	TraesCS7A02G225400	1.8.1.9	thioredoxin-disulfide reductase								1	1
	TraesCS5B02G022800	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS1A02G013800	#N/D	#N/D			1						1
	TraesCS4A02G298700	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
Selenocompound metabolism	TraesCS1B02G017800	2.1.1.12	methionine S-methyltransferase							1		1
	TraesCS5B02G387300	2.7.7.4	sulfate adenylyltransferase							1		1
	TraesCS4B02G014700	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS7B02G301400	6.1.1.10	methionine---tRNA ligase					1				1
	TraesCS4D02G012900	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase							1		1
	TraesCS5A02G024900	2.1.1.14	5-methyltetrahydropteroyltriglutamate---homocysteine S-methyltransferase			1						1
	TraesCS4B02G052300	4.1.1.15	glutamate decarboxylase					1				1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
betaAlanine metabolism	TraesCS4D02G232700	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS4A02G075600	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS2A02G493700	2.1.2.1	glycine hydroxymethyltransferase			1				1		2
Cyanoamino acid metabolism	TraesCS1A02G218700	2.1.2.1	glycine hydroxymethyltransferase								1	1
	TraesCS2D02G493600	2.1.2.1	glycine hydroxymethyltransferase							1		1
	TraesCS2B02G521700	2.1.2.1	glycine hydroxymethyltransferase			1						1
	TraesCS4A02G075600	4.1.1.15	glutamate decarboxylase								1	1
Taurine and hypotaurine metabolism	TraesCS4D02G232700	4.1.1.15	glutamate decarboxylase								1	1
	TraesCS4B02G052300	4.1.1.15	glutamate decarboxylase					1				1
D-Alanine metabolism	TraesCS2A02G092400	6.3.2.4	D-alanine---D-alanine ligase							1		1
	TraesCS1D02G099200	5.1.1.1	alanine racemase				1					1
D-Glutamine and D-glutamate metabolism	TraesCS4A02G013200	4.3.3.6	pyridoxal 5'-phosphate synthase (glutamine hydrolysing)								1	1
Xenobiotics biodegradation and metabolism												
	TraesCS3B02G155300	2.7.4.3	adenylate kinase		1		1		1		1	4
	TraesCS3A02G137600	2.7.4.3	adenylate kinase				1		1		1	3
	TraesCS7A02G204800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS7B02G031500	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS7D02G207800	1.17.4.1	ribonucleoside-diphosphate reductase			1				1		2
	TraesCS3D02G138500	2.7.4.3	adenylate kinase				1				1	2
	TraesCS3B02G152200	2.5.1.18	glutathione transferase			1						1
	TraesCS7B02G000300	2.5.1.18	glutathione transferase			1						1
	TraesCS6D02G016700	2.5.1.18	glutathione transferase								1	1
	TraesCS1B02G097400	2.5.1.18	glutathione transferase								1	1
	TraesCS7B02G112000	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
Drug metabolism other enzymes	TraesCS3B02G270600	3.6.1.23	dUTP diphosphatase				1					1
	TraesCS5D02G030000	2.5.1.18	glutathione transferase							1		1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS3D02G133100	2.5.1.18	glutathione transferase			1						1
	TraesCS1B02G113700	2.5.1.18	glutathione transferase							1		1
	TraesCS7D02G479100	2.5.1.18	glutathione transferase					1				1
	TraesCS1A02G102700	2.5.1.18	glutathione transferase							1		1
	TraesCS1A02G078800	2.5.1.18	glutathione transferase				1					1
	TraesCS7D02G130500	1.17.4.1	ribonucleoside-diphosphate reductase							1		1
	TraesCS4D02G017900	3.6.1.23	dUTP diphosphatase							1		1
	TraesCS3B02G152300	2.5.1.18	glutathione transferase			1						1
	TraesCS5A02G024100	2.5.1.18	glutathione transferase			1						1
	TraesCS3D02G494000	2.4.2.10	orotate phosphoribosyltransferase							1		1
Metabolism of xenobiotics by cytochrome P450	TraesCS5A02G024100	2.5.1.18	glutathione transferase			1						1
	TraesCS7D02G479100	2.5.1.18	glutathione transferase					1				1
	TraesCS6D02G016700	2.5.1.18	glutathione transferase								1	1
	TraesCS1A02G102700	2.5.1.18	glutathione transferase							1		1
	TraesCS3D02G133100	2.5.1.18	glutathione transferase			1						1
	TraesCS1B02G097400	2.5.1.18	glutathione transferase								1	1
	TraesCS5D02G030000	2.5.1.18	glutathione transferase							1		1
	TraesCS1B02G113700	2.5.1.18	glutathione transferase							1		1
	TraesCS7B02G000300	2.5.1.18	glutathione transferase			1						1
	TraesCS3B02G152200	2.5.1.18	glutathione transferase			1						1
	TraesCS1A02G078800	2.5.1.18	glutathione transferase				1					1
	TraesCS3B02G152300	2.5.1.18	glutathione transferase			1						1
Drug metabolism cytochrome P450	TraesCS5A02G024100	2.5.1.18	glutathione transferase			1						1
	TraesCS7D02G479100	2.5.1.18	glutathione transferase					1				1
	TraesCS6D02G016700	2.5.1.18	glutathione transferase								1	1
	TraesCS1A02G102700	2.5.1.18	glutathione transferase							1		1
	TraesCS3D02G133100	2.5.1.18	glutathione transferase			1						1
	TraesCS1B02G097400	2.5.1.18	glutathione transferase								1	1
	TraesCS5D02G030000	2.5.1.18	glutathione transferase							1		1
	TraesCS1B02G113700	2.5.1.18	glutathione transferase							1		1
	TraesCS7B02G000300	2.5.1.18	glutathione transferase			1						1
	TraesCS3B02G152200	2.5.1.18	glutathione transferase			1						1
	TraesCS1A02G078800	2.5.1.18	glutathione transferase				1					1
	TraesCS3B02G152300	2.5.1.18	glutathione transferase			1						1
Aminobenzoate degradation	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS7B02G114000	3.6.1.7	acylphosphatase								1	1
	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
Caprolactam degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Toluene degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Benzoate degradation	TraesCS1D02G231000	4.2.1.17	enoyl-CoA hydratase			1						1
	TraesCS1B02G243300	4.2.1.17	enoyl-CoA hydratase			1						1
Glycan biosynthesis and metabolism												
Other glycan degradation	TraesCS4A02G022500	3.2.1.23	beta-galactosidase			1				1		2
	TraesCS7D02G386900	3.2.1.51	alpha-L-fucosidase						1	1		2
	TraesCS4D02G279400	3.2.1.23	beta-galactosidase			1						1
	TraesCS4A02G083700	3.2.1.23	beta-galactosidase			1						1
	TraesCS7B02G264200	3.2.1.23	beta-galactosidase				1					1
	TraesCS7B02G293100	3.2.1.51	alpha-L-fucosidase			1						1
	TraesCS1A02G087500	3.2.1.24	alpha-mannosidase			1						1
	TraesCS4B02G280900	3.2.1.23	beta-galactosidase			1						1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways												
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS4D02G220800	3.2.1.23	beta-galactosidase			1						1
Glycosaminoglycan degradation	TraesCS4A02G022500	3.2.1.23	beta-galactosidase			1				1		2
	TraesCS4D02G220800	3.2.1.23	beta-galactosidase			1						1
	TraesCS2B02G537300	3.2.1.50	alpha-N-acetylglucosaminidase							1		1
	TraesCS4D02G279400	3.2.1.23	beta-galactosidase			1						1
	TraesCS7B02G264200	3.2.1.23	beta-galactosidase				1					1
	TraesCS4A02G083700	3.2.1.23	beta-galactosidase			1						1
	TraesCS4B02G280900	3.2.1.23	beta-galactosidase			1						1
Glycosphingolipid biosynthesis ganglio series	TraesCS4A02G022500	3.2.1.23	beta-galactosidase			1				1		2
	TraesCS4D02G279400	3.2.1.23	beta-galactosidase			1						1
	TraesCS4D02G220800	3.2.1.23	beta-galactosidase			1						1
	TraesCS4A02G083700	3.2.1.23	beta-galactosidase			1						1
	TraesCS7B02G264200	3.2.1.23	beta-galactosidase				1					1
	TraesCS4B02G280900	3.2.1.23	beta-galactosidase			1						1
Glycosphingolipid biosynthesis globo and isoglobo series	TraesCS2B02G122900	3.2.1.22	alpha-galactosidase								1	1
	TraesCS6A02G042400	3.2.1.22	alpha-galactosidase						1			1
	TraesCS5B02G011700	3.2.1.22	alpha-galactosidase							1		1
	TraesCS1B02G181800	3.2.1.22	alpha-galactosidase				1					1
	TraesCS1A02G164900	3.2.1.22	alpha-galactosidase				1					1
	TraesCS2A02G105900	3.2.1.22	alpha-galactosidase								1	1
Peptidoglycan biosynthesis	TraesCS2A02G092400	6.3.2.4	D-alanine---D-alanine ligase							1		1
	TraesCS2A02G012600	2.4.1.227	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase				1					1
NGlycan biosynthesis	TraesCS6A02G209000	2.4.1.144	beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase								1	1
	TraesCS4A02G131600	2.4.1.267	dolichyl-P-Glc:Man9GlcNAc2-PP-dolichol alpha-1,3-glucosyltransferase			1						1
Lipopolysaccharide biosynthesis	TraesCS2D02G098000	2.5.1.55	3-deoxy-8-phosphooctulonate synthase			1						1
	TraesCS2B02G114700	2.5.1.55	3-deoxy-8-phosphooctulonate synthase							1		1
Other types of Oglycan biosynthesis	TraesCS2D02G274800	2.4.1.255	protein O-GlcNAc transferase							1		1
Translation												
AminoacyltRNA biosynthesis	TraesCS2A02G158300	6.1.1.3	threonine---tRNA ligase				1				1	2
	TraesCS4B02G034500	6.1.1.6	lysine---tRNA ligase			1		1				2
	TraesCS4A02G278500	6.1.1.6	lysine---tRNA ligase			1		1				2
	TraesCS1B02G162400	6.1.1.16	cysteine---tRNA ligase					1			1	2
	TraesCS7B02G107300	6.1.1.15	proline---tRNA ligase				1				1	2
	TraesCS2A02G041300	6.3.5.7	glutaminyl-tRNA synthase (glutamine-hydrolysing)			1				1		2
	TraesCS2D02G165200	6.1.1.3	threonine---tRNA ligase				1				1	2
	TraesCS7A02G516000	6.1.1.5	isoleucine---tRNA ligase				1					1
	TraesCS1D02G144100	6.1.1.16	cysteine---tRNA ligase				1					1
	TraesCS1B02G443700	6.3.5.7	glutaminyl-tRNA synthase (glutamine-hydrolysing)			1						1
	TraesCS1B02G248400	6.1.1.3	threonine---tRNA ligase					1				1
	TraesCS5D02G113200	6.1.1.20	phenylalanine---tRNA ligase			1						1
	TraesCS2A02G562400	6.3.5.7	glutaminyl-tRNA synthase (glutamine-hydrolysing)			1						1
	TraesCS1A02G413700	6.3.5.7	glutaminyl-tRNA synthase (glutamine-hydrolysing)							1		1
	TraesCS2D02G026900	6.3.5.7	glutaminyl-tRNA synthase (glutamine-hydrolysing)						1			1
	TraesCS4B02G033800	6.1.1.6	lysine---tRNA ligase						1			1
	TraesCS7B02G162300	6.1.1.14	glycine---tRNA ligase				1					1
	TraesCS5A02G357400	6.1.1.16	cysteine---tRNA ligase								1	1
	TraesCS7B02G301400	6.1.1.10	methionine---tRNA ligase					1				1
	TraesCS7A02G031700	6.1.1.22	asparagine---tRNA ligase					1				1
	TraesCS7D02G265200	6.1.1.14	glycine---tRNA ligase				1					1
	TraesCS1D02G163400	6.1.1.9	valine---tRNA ligase					1				1
	TraesCS3B02G153500	6.1.1.19	arginine---tRNA ligase				1					1
	TraesCS3B02G309400	6.1.1.2	tryptophan---tRNA ligase						1			1

Supplemental Table 5.4. (continued)

Differential expressed genes functional anotation through KEGG pathways											
Pathways	Gene stable ID	Enzyme ID	Enzyme denominaion	Antequera		Bancal		Ardito		Magueija		
				Down	Up	Down	Up	Down	Up	Down	Up	Total
	TraesCS7D02G028100	6.1.1.22	asparagine---tRNA ligase					1				1
	TraesCS2D02G211400	6.1.1.11	serine---tRNA ligase				1					1
	TraesCS1A02G235200	6.1.1.3	threonine---tRNA ligase					1				1
	TraesCS2D02G286400	6.1.1.14	glycine---tRNA ligase			1						1
Environmental adaptation												
	TraesCS4D02G126200	2.7.11.1	non-specific serine/threonine protein kinase				1				1	2
	TraesCS6B02G412400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS5D02G144800	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1A02G100400	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS7D02G231000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS1B02G142300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS5B02G004300	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS1B02G347100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6A02G374700	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS1B02G372400	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7B02G203800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS2A02G098300	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4B02G178100	2.7.11.1	non-specific serine/threonine protein kinase								1	1
#N/D	TraesCS2D02G217400	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4D02G179700	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS2D02G364200	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS5B02G146500	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS3B02G107000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS6A02G000500	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3B02G544300	2.7.11.1	non-specific serine/threonine protein kinase			1						1
	TraesCS6B02G270400	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS3D02G109700	2.7.11.1	non-specific serine/threonine protein kinase							1		1
	TraesCS6D02G358600	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3D02G296000	2.7.11.1	non-specific serine/threonine protein kinase								1	1
	TraesCS7B02G331800	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS3D02G316400	2.7.11.1	non-specific serine/threonine protein kinase						1			1
	TraesCS1A02G077200	2.7.11.1	non-specific serine/threonine protein kinase				1					1
	TraesCS4B02G120400	2.7.11.1	non-specific serine/threonine protein kinase						1			1

Supplemental Table 5.5. Genbank sequence accession numbers and the correspondent Gene ID in wheat genome annotation used in this work, for the high molecular weight glutenins, granule bound starch synthase (waxy protein) and Puroindolines A and B encoding genes.

Genes		***Genbank***	***Gene ID in wheat genome annotation***
HMW-GS Ax		X61009	TraesCS1A02G317311
HMW-GS Bx		EU287439.1	TraesCS1B02G329711
HMW-GS By		JF736014	TraesCS1B02G329992
HMW-GS Dx		X03346	TraesCS1D02G317211
HMW-GS Dy		X12929	TraesCS1D02G317301
GBSSI	Waxy-A1	KF007194.1	TraesCS7A02G070100
	Waxy-B1	KF007195.1	TraesCS7B02G023400
	Waxy-D1	KF007196.1	TraesCS7D02G064300
Pina-D1		DQ363911.1	TraesCS5D02G004100
Pinb-D1		DQ363913.1	TraesCS5D02G004300

Supplemental Table 5.6. RNA Sequencing data in Sequence Read Archive (SRA) bioproject ID PRJNA750265 - Grain transcriptome dynamics induced by heat in commercial and traditional bread wheat varieties.

Accession	sample_name	title	library_strategy	library_selection	library_layout	platform	instrument_model	design_description	filetype
SAMN20447565	Antequera_Control	RNA-Seq of Bread wheat: Antequera Control	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500	Ten days after anthesis (daa) plants (biological replicates) of each genotype were submitted to two different growth conditions for seven days: control conditions - eight hours of dark at 20 ºC and a 16 hour light period divided in six hours increasing to 25 ºC, four hours at 25 ºC, and six hours decreasing to 20 ºC; or high temperature (HT) regime with a daily plateau of 40 ºC maximum temperature. Immediately after the period of four hours at maximum temperature in the last day of the treatment, immature grains from the middle of each first spike of each plant were collected (17 daa).	fastq
SAMN20447566	Antequera_Heat_Treated	RNA-Seq of Bread wheat: Antequera Heat Treated	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447567	Bancal_Control	RNA-Seq of Bread wheat: Antequera Control	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447568	Bancal_Heat_Treated	RNA-Seq of Bread wheat: Antequera heat treated	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447569	Ardito_Control	RNA-Seq of Bread wheat: Antequera Control	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447570	Ardito_Heat_Treated	RNA-Seq of Bread wheat: Antequera heat treated	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447571	Magueija_Control	RNA-Seq of Bread wheat: Antequera Control	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq
SAMN20447572	Magueija_Heat_Treated	RNA-Seq of Bread wheat: Antequera heat treated	RNA-Seq	PolyA	single	ILLUMINA	NextSeq 500		fastq

Note: All RNA Sequencing data will be available after manuscript publication in https://www.ncbi.nlm.nih.gov/bioproject/750265.

Supplemental Table 5.6. *(continued)*

Accession	filename	filename2	filename3	filename4	filename5	filename6	filename7	filename8	filename9	filename10	filename11	filename12
SAMN20447565	Antequera_Control1_Lane1.fastq.gz	Antequera_Control1_Lane2.fastq.gz	Antequera_Control1_Lane3.fastq.gz	Antequera_Control1_Lane4.fastq.gz	Antequera_Control2_Lane1.fastq.gz	Antequera_Control2_Lane2.fastq.gz	Antequera_Control2_Lane3.fastq.gz	Antequera_Control2_Lane4.fastq.gz	Antequera_Control3_Lane1.fastq.gz	Antequera_Control3_Lane2.fastq.gz	Antequera_Control3_Lane3.fastq.gz	Antequera_Control3_Lane4.fastq.gz
SAMN20447566	Antequera_HT1_Lane1.fastq.gz	Antequera_HT1_Lane2.fastq.gz	Antequera_HT1_Lane3.fastq.gz	Antequera_HT1_Lane4.fastq.gz	Antequera_HT2_Lane1.fastq.gz	Antequera_HT2_Lane2.fastq.gz	Antequera_HT2_Lane3.fastq.gz	Antequera_HT2_Lane4.fastq.gz	Antequera_HT3_Lane1.fastq.gz	Antequera_HT3_Lane2.fastq.gz	Antequera_HT3_Lane3.fastq.gz	Antequera_HT3_Lane4.fastq.gz
SAMN20447567	Bancal_Control1_Lane1.fastq.gz	Bancal_Control1_Lane2.fastq.gz	Bancal_Control1_Lane3.fastq.gz	Bancal_Control1_Lane4.fastq.gz	Bancal_Control2_Lane1.fastq.gz	Bancal_Control2_Lane2.fastq.gz	Bancal_Control2_Lane3.fastq.gz	Bancal_Control2_Lane4.fastq.gz	Bancal_Control3_Lane1.fastq.gz	Bancal_Control3_Lane2.fastq.gz	Bancal_Control3_Lane3.fastq.gz	Bancal_Control3_Lane4.fastq.gz
SAMN20447568	Bancal_HT1_Lane1.fastq.gz	Bancal_HT1_Lane2.fastq.gz	Bancal_HT1_Lane3.fastq.gz	Bancal_HT1_Lane4.fastq.gz	Bancal_HT2_Lane1.fastq.gz	Bancal_HT2_Lane2.fastq.gz	Bancal_HT2_Lane3.fastq.gz	Bancal_HT2_Lane4.fastq.gz	Bancal_HT3_Lane1.fastq.gz	Bancal_HT3_Lane2.fastq.gz	Bancal_HT3_Lane3.fastq.gz	Bancal_HT3_Lane4.fastq.gz
SAMN20447569	Ardito_Control1_Lane1.fastq.gz	Ardito_Control1_Lane2.fastq.gz	Ardito_Control1_Lane3.fastq.gz	Ardito_Control1_Lane4.fastq.gz	Ardito_Control2_Lane1.fastq.gz	Ardito_Control2_Lane2.fastq.gz	Ardito_Control2_Lane3.fastq.gz	Ardito_Control2_Lane4.fastq.gz	Ardito_Control3_Lane1.fastq.gz	Ardito_Control3_Lane2.fastq.gz	Ardito_Control3_Lane3.fastq.gz	Ardito_Control3_Lane4.fastq.gz
SAMN20447570	Ardito_HT1_Lane1.fastq.gz	Ardito_HT1_Lane2.fastq.gz	Ardito_HT1_Lane3.fastq.gz	Ardito_HT1_Lane4.fastq.gz	Ardito_HT2_Lane1.fastq.gz	Ardito_HT2_Lane2.fastq.gz	Ardito_HT2_Lane3.fastq.gz	Ardito_HT2_Lane4.fastq.gz	Ardito_HT3_Lane1.fastq.gz	Ardito_HT3_Lane2.fastq.gz	Ardito_HT3_Lane3.fastq.gz	Ardito_HT3_Lane4.fastq.gz
SAMN20447571	Magueija_Control1_Lane1.fastq.gz	Magueija_Control1_Lane2.fastq.gz	Magueija_Control1_Lane3.fastq.gz	Magueija_Control1_Lane4.fastq.gz	Magueija_Control2_Lane1.fastq.gz	Magueija_Control2_Lane2.fastq.gz	Magueija_Control2_Lane3.fastq.gz	Magueija_Control2_Lane4.fastq.gz	Magueija_Control3_Lane1.fastq.gz	Magueija_Control3_Lane2.fastq.gz	Magueija_Control3_Lane3.fastq.gz	Magueija_Control3_Lane4.fastq.gz
SAMN20447572	Magueija_HT1_Lane1.fastq.gz	Magueija_HT1_Lane2.fastq.gz	Magueija_HT1_Lane3.fastq.gz	Magueija_HT1_Lane4.fastq.gz	Magueija_HT2_Lane1.fastq.gz	Magueija_HT2_Lane2.fastq.gz	Magueija_HT2_Lane3.fastq.gz	Magueija_HT2_Lane4.fastq.gz	Magueija_HT3_Lane1.fastq.gz	Magueija_HT3_Lane2.fastq.gz	Magueija_HT3_Lane3.fastq.gz	Magueija_HT3_Lane4.fastq.gz

5.6.1. Data availability statement

All RNA Sequencing data will be available after manuscript publication in Sequence Read Archive (SRA), with the project ID PRJNA750265 (https://www.ncbi.nlm.nih.gov/bioproject/750265) under the accessions SAMN20447565, SAMN20447566, SAMN20447567, SAMN20447568, SAMN20447569, SAMN20447570, SAMN20447571, SAMN20447572 (Supplemental Table 5.6).

Chapter VI

General Discussion

6 General Discussion

The importance of bread wheat for food and feed is unquestionable, being the third most consumed cereal, after rice and maize (©FAO, 2018). Some projections of population demands in 2050 already anticipate that the annual increase in this crop production will not be sufficient (Ray et al., 2013; Flies et al., 2018). One of the major reasons for these constrains is climate change. In Portugal, climate change has been noted through the increased occurrence of anomalies in air temperature (Nunes et al., 2019). One of these events are heatwaves, defined as five or more consecutive days of heat with daily maximum temperature at least 5ºC higher than the average maximum temperature (WMO, 2015), which are predicted to be particularly frequent and severe in Portugal (Cardoso et al., 2019). To face these limits is essential to search for genotypes with the ability to cope high temperature effects and maintain the desirable nutritional and technological characteristics. Wheat grain yield and quality are based on a combination of many parameters including morphological characteristics, nutritional composition and dough and final products properties, all of which are defined by the genotype, the environment and their interactions (Kaya and Akcura, 2014; Hernández-Espinosa et al., 2018).

The work realized in this thesis aimed to characterize responses of distinct wheat commercial varieties and landraces submitted to thermal stress during grain filling, in order to identify the most tolerant genotypes for the predicted climate conditions. For this we studied seven commercial genotypes recommended to be used in Portugal, Almansor Antequera, Bancal, Estero, Nabão, Pata Negra and Roxo, based on agronomic and technological quality performances for Portuguese edaphoclimatic conditions (ANPOC et al., 2014). Although a recent work showed that commercial genotypes are able to maintain stable breadmaking quality attributes under heat stress conditions (Fleitas et al., 2020), is broadly known that the reduced number of modern varieties used nowadays, resulted in a lack of heterozygosity and caused a genetic erosion. Besides seven commercial varieties we also included in this study four landraces from a collection of traditional genotypes collected in the last century across Portugal (Vasconcellos, 1933). It is well documented the notable successes of landraces in crop improvement (Crossa et al., 2016; reviewed in Dwivedi et al., 2016) as sources of nutritional and technological quality traits (reviewed in Newton et al., 2010; van den Broeck et al., 2010; Migliorini et al., 2016) as well as for the selection of more tolerant genotypes to the predicted climate weather conditions (Trethowan and Mujeeb-Kazi, 2008; Jaradat, 2011, 2013; Lopes et al., 2015). In this context, we selected Ardito and Grécia as sensitive and Magueija and Ruivo as tolerant landraces based on preliminary results assessing the effect of high temperature in grain weight (Scotti-Campos et al., 2011).

With the aim to evaluate the breadmaking quality of the distinct genotypes under study, we used molecular markers to specific alleles of high molecular weight glutenins (HMW), waxy and puroindolines genes. Molecular markers have a great potential as they enable a rapid, cost effective,

simple and early screening of desired characteristics, and several has been published for the main wheat technological characteristics (Nakamura et al., 2002; reviewed in Lafiandra et al., 2007; Ayala et al., 2013; Rasheed et al., 2014). The markers used (Tomás et al., 2020c, Chapter II) are related with major grain quality parameters, as storage protein and starch composition and grain hardness, related with wheat ability to produce bread. High molecular weight glutenins (HMW) assume an essential role for gluten characteristics as their allelic diversity explains about 45-70% of bread-making performance of wheat cultivars (Wang et al., 2018a). Another relevant grain component is starch including amylose, and Granule-bound Starch Synthase I (GBSSI), also called Waxy protein, a key enzyme involved in its biosynthesis. Waxy *loci* allelic composition is related with grain amylose content, which in turn influences flour retention of moist and affect end-use quality, shelf life and nutritional value of wheat products (Guzmán and Alvarez, 2016). Puroindoline genes allelic composition is determinant for kernel textures and *wild-type* (*Pina-D1a/Pinb-D1a*) are necessary for soft wheat, while its absence or any mutation in one of these proteins encoding sequences result in hard texture (Morris, 2002). As expected, small variation was observed in the quality associated markers studied since nowadays commercial varieties are selected accordingly with technological characteristics (Tomás et al., 2020c, Chapter II). A similar genetic screening in traditional genotypes was however not successful due to the absence of sequences amplification with the primer pairs used to characterize commercial genotypes (unpublished results), probably resulting from genetic polymorphisms. The existence of differences in genomic backgrounds is also supported by the lower number of transcripts aligned to exonic regions obtained through RNA sequencing in traditional genotypes (Tomás et al., 2021, Chapter V). Nevertheless two of the traditional genotypes, Ardito and Grécia revealed an interesting Glu-B1al allele ($Bx7^{OE}$), associated to improved dough strength (Butow et al., 2004; Cooper et al., 2016), that was absent in all seven commercial genotypes analyzed (unpublished results). This allele is technologically interesting since it promotes dough strength, compensating the absence or accumulating in the presence of other alleles responsible for this characteristic. This is particularly important in situations like depolymerization of HMW-GS in dough storage under freezing conditions, affecting bread end-use quality (Migliorini et al., 2016). In order to obtain a more complete genomic portrayal of the genotypes in study and also to identify potential molecular markers useful for an early screening of heat tolerant genotypes, namely a single nucleotide polymorphism (SNP) in Heat Shock Protein (HSP) 16.9 (Garg et al., 2012) and several microsatellites associated with grain filling rate (Barakat et al., 2011). However, the results obtained were inconclusive, due to the lack of reproducibility and consistency (unpublished results).

To study the impact of high temperature extreme events on wheat grain yield and quality, the high temperature treatment imposed in this work mimicked a heatwave, an extreme event expected to intensify in the future (Cardoso et al., 2019; Nunes et al., 2019). The treatment period consisted of seven consecutive days with a diary cycle composed by a ramp increase of temperature

until 40ºC, and a plateau phase in this temperature for four hours, followed by a ramp decrease until reaching the night temperature. Control and treated plants of commercial and traditional genotypes, one week after anthesis stage, were maintained in two equal growth chambers and submitted to similar cycles although with distinct temperature regimes. Moreover, to ensure that the temperature treatment was applied in the exact same developing stage, each plant was in a single pot (considered a biological replicate) and only grains from the first spike were analyzed in both immature and mature developmental stages. This is a different approach in comparison with the majority of previous works made in field conditions or with several plants per pot considered as biological replicates (Calderini et al., 1999; Scotti-Campos et al., 2011; Nuttall et al., 2018; Rangan et al., 2019a, 2019b).

Wheat grain yield was appraised thought the evaluation of related agronomic traits in both traditional and commercial genotypes and significant differences between genotypes were detected in almost all yield parameters analyzed in control plants (Tomás et al., 2020a, 2020b, chapters II and IV)). These results consolidate the existence of yield variability of nowadays cultivated (Kaya and Akcura, 2014) and traditional wheat genotypes (Scotti-Campos et al., 2014). Several yield parameters were previously proved to be affected by high temperatures, as vegetative weight and grain number and weight (reviewed in Khan 2021) in a proportion of ~6% for each 1ºC increased (Liu et al., 2019). Significant reduction in the number of grains per spike was observed in two commercial treated varieties, Antequera and Roxo, in opposition with the absence of significant differences in landraces. This was not expected since grain number is strongly affected by high temperatures, during meiosis and fertilization, when occurring between spike initiation and anthesis (Farooq et al., 2011; Liu et al., 2016). In fact, abortions and reduction in grain number resulting from heat before and during anthesis were documented for a few cultivars (Stone and Nicolas, 1995; Hays et al., 2007). On the other hand, in most of the commercial and traditional genotypes assessed, grain weight per spike and ten grain weight significantly decreased after heat stress, except in Bancal and Pata Negra. This is mostly in accordance with previous reports associating reduction in grain mass with high temperature after anthesis, particularly when imposed in early stages of grain filling (Gibson and Paulsen, 1999; Castro et al., 2007; Modarresi et al., 2010; Kaya and Akcura, 2014). Our results showed a reduction in yield parameters variability after high temperature treatment in commercial genotypes, contrasting with the increase induced in traditional ones.

For a qualitative assessment of high temperature influence in grain composition it was unfeasible to use classical chemical analysis methodologies due to the reduced amount of grain obtained in the limited space of growth chambers with precise controlled conditions. Thus, attenuated total reflection Fourier transform infrared (ATR-FTIR) was selected as methodology to assess grain composition and quality. This rapid and non-destructive methodology allows the detection of a range of functional groups and changes in molecular structure. The use of ATR-FTIR constituted a great advantage due to the limited dimension of flour samples even allowing single kernel analysis which

proved to be an accurate predictor of wheat grain composition (Caporaso et al., 2018). Chemical mapping using spectra easily identified peaks that correspond to specific bonds and functional groups and has been successfully applied to a wide range of cereals and food and feed products (Syahariza et al., 2005; Philippe et al., 2006; Wei et al., 2015; Antunes et al., 2016; Sujka et al., 2017; Prates et al., 2018). In wheat, this technique was already used to assess endosperm cell-wall composition, grain infection, and flours quality control (Philippe et al., 2006; Toole et al., 2007; Singh et al., 2017; Sujka et al., 2017). The results obtained in this work unraveled considerable intervarietal divergence in grain composition of commercial genotypes from control conditions. Interestingly, grains from plants of commercial varieties exposed to heatwave-like treatment presented much more similar spectra. However, for each commercial variety, high temperature induced distinct effects, as for example was possible to observe increases or reductions of protein, starch and fat related chemical groups, depending on the genotype. On the other hand, spectra from high temperature-treated plants were overall more intense than control ones in all landraces, indicating a decrease in starch in relation to other grain constituents, which is in accordance with previous works (DuPont et al., 2006; Zhang et al., 2017; Tao et al., 2018). A shift in ATR-FTIR spectra bands mainly assigned to starch suggests also changes in the proportions of different polysaccharides induced by high temperature treatment, in both commercial and landraces genotypes.

Protein is one of the determinant factors to assess wheat quality, and assume an important role in food security since wheat provides 20% of protein for humans (Tilman et al., 2011). Grain protein content depends on a combination of factors like crop genotype and environmental conditions (Triboi et al., 2006). Heat stress generally accelerates the rate of grain-filling and shortens grain-filling duration (Altenbach and Kothari, 2004; Dias and Lidon, 2009; Modarresi et al., 2010), reducing the amount of grain assimilates as starch, and increasing protein content (Hurkman et al., 2009; Altenbach, 2012; Khan et al., 2021). We have generated a model for nitrogen amount prediction for bread wheat grain, which revealed to be very accurate considering the calculated correlation level. Unpredictably, our results showed again that in control conditions commercial genotypes had higher intergenotypic diversity also regarding grain protein content. However, after high temperature treatment, the expected increase in protein content was not observed for all the commercial genotypes, in opposition to the traditional ones, and neither was related with grain weight alterations. Consequently, grain protein content was usually higher in grains from heat treated traditional genotypes but not in grains from commercial ones, as already reported by (Migliorini et al., 2016).

Wheat grain protein can be divided in four fractions accordingly to their solubility (Osborne, 1924), albumins, globulins , glutenins and gliadins, being the last two responsible for dough elasticity and extensibility, respectively, being also determinants for processing quality of several end-products. To evaluate the alterations induced by heat in these specific fractions in small amount of flour, we have adapted the method from Zorb et al (2017) and their quantification was made with

Bradford reagent (Bradford, 1976). Only slight differences were detected after high temperature treatment in protein fractions of grains from most commercial genotypes, although three were significant. The ratio gliadin/glutenin is particularly important since the equilibrium between dough viscosity and elasticity/strength is essential to dough quality (Dhaka and Khatkar, 2015) and an increase in this ratio is associated with poorer dough quality. Only two of the genotypes assessed presented significant alterations in this ratio due to high temperature treatments, namely an enhancement was observed in Almansor while the opposite effect was observed in Antequera.

The comparative study of gene expression in developing wheat grains was further studied to better understand how yield and composition (nutritional and functional qualities) respond to high temperature stress, through the identification of genes or pathways involved in traits determination. For this analysis, wheat grains were collected immediately after the seventh day of four hours exposure at 40ºC. (17 days after anthesis) corresponding to milk stage kernel, when carbohydrates and proteins are actively deposited (Bowden et al., 2007). To perform this assessment, four genotypes were chosen considering their yield and composition characteristics in response to high temperature treatment and agronomic potential. Two commercial varieties with distinct responses to high temperature treatment were selected, namely Antequera presenting a great decrease in protein amount and grain number, and Bancal with only slight reductions don protein content and grain weight (Tomás et al., 2020b, Chapter III). On the other hand, Ardito and Magueija landraces were chosen also due to the contrasting responses to high temperature treatment (Tomás et al., 2020a, Chapter IV). Ardito presents the highest protein content in control conditions and revealed a significant increase after high temperature treatment. Moreover, Ardito is the earlier landrace, with a number of days until flowering similar to the commercial genotypes studied, which can represent a future advantage to avoid heat conditions. Magueija seem to be less affected in terms of yield by heatwave-like treatment showing however a significant increase in spikes and grain number though associated with a detrimental grain quality. It must however be emphasized that although Magueija presented the highest 10-grain weight value, even when compared with commercial varieties, no significant increase was observed in grain protein content after high temperature treatment.

We analyzed the total transcriptome of immature grains of Antequera, Bancal, Ardito and Magueija through total RNA sequencing, and compared control and high temperature treated grains through the analysis of differentially expressed genes (DEG). The results obtained revealed that Bancal was the genotype with less DEGs while both landraces had a considerably higher number in comparison to commercial genotypes. Also, all genotypes except Antequera had more upregulated than downregulated genes. This was also observed for the heat sensitive genotype analyzed by Rangan *et al* (2019b), showing an inferior performance in yield evaluations under heat stress (Rangan et al., 2019a), therefore suggesting that Antequera can also be considered a sensitive genotype (Tomás et al., 2020b, Chapter III). Previous referred results indicating Bancal as the more

tolerant genotype due to the absence of significant changes in yield and quality parameters seem to be reinforced by the reduced number of DEGs identified in this genotype. In fact, the tolerant genotype studied by Nandha *et al* (2019) and Rangan *et al* (2019b) also present a reduced number of DEGs.

The higher number of DEGs detected in landraces as well as the higher percentage of DEGs common to both landraces sustain that these traditional genotypes present a more similar response to high temperature, in comparison with commercial ones, as already observed on yield and grain composition evaluations. DEGs identified in the landraces and also, in lower number, in Bancal were more associated with metabolic pathways, accordingly with previous works (Altenbach and Kothari, 2004; Wan et al., 2008). Additionally, several DEGs identified in these three genotypes were associated with carbohydrates, amino acid and lipid metabolisms, encoding several enzymes involved in synthetic pathways.

Starch fraction in wheat grains comprises around 60-75% and have high importance in wheat nutritional and technological qualities (Shevkani et al., 2017). Granule Bound Starch Synthase I is a determinant gene involved in starch synthesis but no significant differences after high temperature treatment were detected in the expression levels of this gene (Tomás et al., 2020c, Chapter II). In fact, RNA sequencing results showed that the most affected enzymes involved in starch syntheses were starch synthases and starch branching enzymes associated with amylopectin synthesis (unpublished results), showing that this starch fraction is the most depleted by high temperatures. These alterations were observed mostly in landraces indicating once again differences in traditional and commercial genotypes. On the other hand, one gene downregulated in commercial genotypes and upregulated in the traditional ones codes for a transcription factor (*NAC019-A1*), known to be a negative regulator of starch synthesis, kernel weight, and kernel width in wheat developing grains (Liu et al., 2020). This gene differential modulation may be related with the reduction detected in starch amount in mature grains of both commercial varieties and with the increase in some chemical groups associated with polysaccharides in both landraces (Tomás et al., 2020b, 2020a). Genes encoding enzymes involved in lipid metabolism were also altered in Bancal and both landraces, indicating a possible alteration in lipids proportions in response to high temperature as already been referred before (reviewed in Abdelrahman et al., 2020). Although the analysis was done in different developing timepoints, an alteration in fat related chemical groups was also showed in our grain composition analysis (Tomás et al., 2020b, 2020a, Chapters III and IV).

Grain hardness has also an important role in wheat technological quality due to its effects on end-use quality namely milling and baking. Although this phenotype is mainly determined by the allelic composition of *Pina* and *Pinb* genes (Pasha et al., 2010), alterations in their expression levels were also associated with differences in grain hardness (Nirmal et al., 2016). Although the commercial genotypes have an allelic composition corresponding to the hard phenotype, no significant differences were detected in the *Pin* genes expression levels evaluated by RT-qPCR

(Tomás et al., 2020c, Capitulo II) since DEGs analysis only showed *Pinb* downregulation in Ardito (unpublished results).

Almost no significant differences were also observed in any of the HMW-GS encoding genes transcription levels, except the increase of HMW-Dy in Bancal (Tomás et al., 2020c, Chapter II), contrasting with the significant increase in the expression levels of genes encoding for gliadins observed in Antequera an Magueija. On the other hand, cupins that are heat responsive storage proteins (Gábrišová et al., 2016) related with protein synthesis process (Wang et al., 2018b), are downregulation in Antequera reinforcing our previous assumption that protein synthesis is affected in this genotype. Altogether, these results show that gliadins are more affected by high temperature treatment than glutenins, both in wheat commercial varieties and landraces, and reinforce the need to investigate the cupins role in heat stress response.

Overall, our results show that high temperature treatment tend to reduce yield and quality differences observed between commercial varieties in control conditions which are however enhanced in treated landraces. Characteristics like grain weight, protein content and transcription profiles of heat responsive genes on the traditional genotypes studied encourages a deeper analysis of the Vasconcellos collection. Nevertheless, accordingly to all our analysis, the commercial variety Bancal appears as a promising genotype to cope with high temperatures.

Several questions arise from this work confirming that high temperature response results from a complex of physiological, cellular and molecular processes as previously proposed (Jacott and Boden, 2020; Schaarschmidt et al., 2021). Though, several pieces are missing to compose the intricate puzzle of plant response to this abiotic stress (Jagadish et al., 2021; Khan et al., 2021). A deeper exploitation of RNA sequencing data focusing on particular pathways will be needed to unravel any correlations between specific changes in genes expression profiles and phenotype alterations induced by high temperature treatments. In this context, the use of ATR-FTIR will certainly be a good method to pursue molecular phenotypes evaluation after heat treatments as well as to better characterize landraces collection.

6.1 References

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In The End

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Things aren't the way they were before
You wouldn't even recognize me anymore
...

What it meant to me will eventually
Be a memory of a time

I tried so hard and got so far
But in the end it doesn't even matter

In the end, Linkin Park