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Physiological traits and genetic controls associated with heat adaptation of bread wheat

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Statement of originality

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Reyna Suzuky Pinto González

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Thesis abstract

High temperatures affect plant growth and reduce crop productivity. Notwithstanding the extensive research on the effects of heat stress on plant development, to date, the basis of wheat tolerance to high temperatures is unknown. Currently, physiological crop breeding for improved wheat adaptation to heat stress is driven by the utilization of cumulative traits useful under those environments. However many of these traits have yet to be fully exploited.

This study is focused on three key components of heat adaptation: I) the significance of root development for hot irrigated environments in comparison to drought stressed environments and its relation with canopy temperature, II) the contribution and genetic basis of the staygreen attribute to heat adaptation of bread wheat, and III) The relevance of leaf respiration for plant productivity under hot environments.

Chapter 1 presents a literature review focused on providing a general background for the three following publications included in the forthcoming chapters. The relevance of heat stress for the wheat crop is discussed with focus on recent publications that describe the current situation and forecasts for the upcoming years and effect of climate change. The genetic and physiological basis of heat tolerance is also addressed, presenting a generic conceptual model currently applied in wheat breeding for improved heat adaptation.

In Chapter 2, the significance of optimal root development under hot-irrigated environments is addressed and compared with the performance observed in the same germplasm grown under water-limited conditions. This journal publication discusses the association of QTL for canopy temperature with deep and abundant radicular systems. A subset of a Seri/Babax mapping population was evaluated for root distribution patterns and residual soil moisture across the soil profile from surface to 120 cm depth. Results from this study endorse canopy temperature as a valuable tool for assessing expression of plant's water uptake and hence root development under both, hot-irrigated and drought environments.

In Chapter 3, the potential value of the staygreen attribute in breeding for heat adaptation of wheat is addressed. This journal publication estimated a number of parameters associated with the staygreen phenotype including (but not

limited to) the rate of senescence, the percentage of greenness decay and the area under the curve of the normalized difference vegetation index measured over time. The greenness decay of each genotype was fitted to linear or non-linear models to describe their patterns. And finally these staygreen parameters together with other physiological and agronomical parameters were analysed for QTL to aid understanding of the genetic basis of the staygreen attribute in the Seri/Babax population.

Chapter 4 presents a descriptive study on leaf respiration rates of a diverse wheat germplasm set grown under high temperatures during the 2010, 2011, 2012 and 2013 seasons. Agronomic and physiological phenotyping was performed including detailed observations for biomass production, carbohydrates content recorded in a subset during the last year of experiments. The study evaluated the effect of different temperatures and plant stages in leaf respiration rates and also made a comparison between five wheat classes. Results from this study showed genetic diversity for leaf respiration and also genotypic differences between leaf temperature and leaf age (determined by developmental plant stage). Interesting associations were observed between physiological traits, yield and leaf respiration rates.

Finally, Chapter 5 presents general conclusions and future direction of the work. The work described in the thesis suggests that tolerance to high temperature in wheat can be improved by the translation of physiological traits into the breeding process. Three approaches explored in this thesis: canopy temperature, staygreen traits and leaf respiration. The evidence collected support the idea that a strategic, physiological breeding approach can lead to important gains in wheat yield under hot-irrigated conditions and this outcome will be important for the adaptation of wheat to global warming.

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Introduction

Wheat is the most widely cultivated cereal in the world. During the 2014-2015 season about 2.6 billion tonnes of cereals were produced worldwide and wheat accounted for almost 730 million tonnes of this (FAO). Wheat together with rice, maize, barley, pearl millet and sorghum provide 60% of the world's calories consumed. Wheat productivity has been relatively stable during the last decades with slight increments.

World demand for food, including cereals like wheat, is expected to increase every year mainly due to a growing population. In order to meet this demand it is necessary to fully exploit currently cultivated land rather than expand the area under cultivation. The latter cannot be achieved if the characteristics of the available germplasm are unknown and if wheat improvement maintains a focus on raising productivity in high yielding environments. Some authors suggest that wheat yield has reached a plateau in several of the world's high yielding areas (Grassini et al. 2013) and that nearly one-half of world wheat area experiences some type of stress during crop development (Reeves et al. 1999). There is a major opportunity to achieve gains in wheat productivity by breaking the yield barriers in stressed wheat growing areas, including those regions experiencing elevated temperatures. Currently about 50% of the world wheat area is prone to heat stress episodes. Wheat adaptation to high air temperatures is a multidisciplinary challenge given that crop productivity under these conditions is a complex trait controlled by multiple genes and affected by several factors; an integrated approach that shares and combines knowledge across genetics, physiology and molecular disciplines has the potential to generate advances in our understanding of the mechanisms controlling heat tolerance in wheat. The identification of key adaptability traits is also crucial to increase breeding impacts that could increase wheat production for the industry.

One of the least studied wheat features is the plant's root system, probably due to the complexity of performing direct studies on the roots which usually involve destructive processes are both intensive and time consuming. Studies on diverse species have shown that under water limited conditions root development plays an important role in plant adaptation; for example it has been widely documented that in drought adapted germplasm the development of deep roots allow the extraction of moisture from deep soil sections, especially under late-season drought stress where residual soil moisture can be found at

depth (Hund et al. 2012; Manschadi et al. 2006; Reynolds et al. 2007a; Richards and Passioura 1989). Notwithstanding, only a few studies report the utility of this feature under conditions of high air temperatures (herein also referred as 'high temperatures'). Apparently, root development patterns are also a factor determining survival and maintenance of wheat productivity under hot environments with no limitation of water. This is not surprising given that various adaptive strategies are beneficial under both drought and heat stress (Pinto et al. 2010). A strong root system permits the extraction of additional amounts of water, which can help to lower canopy temperatures and increase the production of photosynthetic assimilates. The latter can also be achieved by delaying loss of plant greenness -staygreen- towards the end of grain-filling. Staygreen is a strategy that has proved to provide a significant yield advantage under stressed conditions and especially in drought stressed sorghum, but in wheat results have been inconclusive on its importance for heat adaptation (Kumari et al. 2007; Borrell et al. 2000). Different methods are used to quantify the staygreen attribute but most only consider chlorophyll retention in leaves. Recently, an interesting study using whole plant spectral reflectance (aerial section) showed that the staygreen attribute can be quickly and efficiently determined in spring wheat plants grown under drought and heat stress (Lopes and Reynolds 2012). These kinds of studies provide evidence that cumulative gains in plant adaptation to high temperatures can be accomplished by combining key traits. However, new sources of genes must be identified to increase the possibilities of generating new varieties with superior heat adaptation. In large populations the utilization of high throughtput phenotyping tecniques, such as the recording of the plant spectral reflectance and the canopy temperature, provide powerful tools that allow the collection of large amounts of high quality data in a limited timeframe.

Wheat genetic diversity has been narrowed due to breeding work over the past century. This highlights an imminent danger for global wheat productivity given that much of the worldwide wheat derives from a common genetic base thereby increasing the crop's vulnerability to new diseases and abiotic constrains (Warburton 2006). An advantage in working with the International Maize and Wheat Improvement Center (CIMMYT) is the thousands of wheat genotypes conserved in its collection, which are potentially valuable for wheat improvement research. However, many are still uncharacterized. These wheat collections include landraces, synthetics and wild species that provide a

tremendous opportunity for germplasm screening and for the discovery of useful materials for improving heat adaptive traits. Many wild species and landraces have survived frequent adverse factors suggesting that their utilization in wheat breeding could result in considerable gains for heat adaptation. These materials have been used to increase the allelic diversity of modern wheat cultivars with the objective of improving their drought-adaptation mechanisms (Reynolds et al. 2007b). Some landraces selected for crossing with elite materials have been shown to exhibit favourable expression of biomass and stored stem carbohydrate, traits that have been reported to have a significant effect on the rate of CO_2 consumption or release through variation in photosynthesis and respiration (Covey-Crump et al. 2002; Gifford 2003). The relevance of photosynthesis under stressed and high yielding environments has been extensively studied with results confirming a positive association between elevated photosynthetic activity and crop productivity. Conversely, the role of oxygen consumption through respiration has been poorly studied and is not well understood in the context of plant productivity and heat tolerance. It is known that respiration is necessary for plant growth and maintenance since that many metabolic reactions depend on respiration. The energy generated through the consumption of carbohydrates in respiration is utilized for vital plant activities such as the construction of carbon skeletons, phloem loading or nitrogen fixation (Gifford 2003), but also, it has been observed that the alternative oxidase pathway (AOX) of plant respiration plays a central role in plant adaptation to stress. Stress conditions, such as high temperatures, drives the production of reactive oxygen species (ROS) leading to cell damage (McDonald and Vanlerberghe 2005) but AOX can minimize the negative effects of ROS (Millenaar and Lambers 2003; Yip and Vanlerberghe 2001). Currently, the relevance of respiration for wheat improvement is unknown, especially when breeding for hot environments. Few studies report association between respiration rates and plant performance and even fewer have been focused on its relevance to heat adaptation. For this reason, one of the studies included in this thesis focuses on wheat respiration measured under high air temperatures. The large section on respiration (1.3.5.2.1) in the literature review reflects the fact that respiration was originally intended to be the focus of the thesis. However, other strategies of wheat adaptation to high air temperatures were also investigated. The literature review section on respiration is intended to be submitted as a journal review publication.

The application of a multitrait approach to improve wheat tolerance to high air temperatures is expected to contribute to increase the wheat production required to meet the global goals.

Objectives of the study

This study aims to obtain more information about the physiological and genetic mechanisms involved in the heat tolerance of wheat. The work in this thesis investigated a number of key traits with potential value for increasing plant adaptation to high air temperatures including, root development, delayed loss of greenness during grain-filling and leaf respiration rate.

Exotic and elite germplasm was evaluated for its potential to contribute to heat tolerance breeding. It was anticipated that this work would identify new germplasm with expression of desirable heat adaptive traits suitable for use in breeding programs around the world.

It was also expected that the results would contribute to a deeper understanding of heat stress effects on cereals and heat-adaptive processes.

Hypothesis

General: The heat-adaptive traits studied will show that genetic variability exists in the evaluated germplasm and that their use as selection criteria for wheat improvement can result in yield gains.

Specific hypotheses:

a. An optimal root distribution provides a common physiological base for heat and drought adaptation in spring wheat (Chapter 2).
b. Delayed loss of chlorophyll in aerial parts of the plant, staygreen, is a desirable feature associated with heat adaptation in wheat but the dynamic of the greenness decay from anthesis to maturity is a determining factor affecting plant productivity (Chapter 3).
c. Genetic diversity exists for the staygreen attribute and for traits associated in a population of RILs from the Seri/Babax cross (Chapter 3).

d. QTL for staygreen and associated traits coincide with genomic regions controlling other heat-adaptive traits (Chapter 3).
e. Genetic diversity for leaf respiration rate exists in the evaluated germplasm under heat stressed conditions and its expression is associated with plant performance (Chapter 4).
f. Temperature and plant growth stage affect leaf respiration rate of spring wheat grown under heat stressed conditions (Chapter 4).
g. Genotypic differences for leaf respiration rate exists between different wheat classes grown under heat stressed environments (Chapter 4).

Chapter 1. Literature review

1.1 The Wheat crop: a brief overview

1.1.1 Origins and general characteristics of wheat

It is thought that wheat cultivation started almost 10,000 years ago and that its cultivation set the base for modern civilization. The first domesticated wheat was grown in the Turkey and Iraq regions and the bread wheats resulted from crosses between emmer wheat (*Triticum dicoccum*) and *Aegilopsis tauschii* (Robles 1981). First seed selections and crosses were based on the search for desirable characteristics in the crop, in the same way performed in modern wheat breeding. These features would have included grain productivity, plant resistance to diseases and environmental constraints, as well as selecting by those seeds that facilitate its cultivation and consumption such as spikes that remained intact making harvest easier.

The wheat plant belongs to the *Graminae* botanical family and is composed of a root and a shoots system ending with a spike bearing about 20 spikelets. The wheat root system can reach up to 2m depth but most roots are located at between 0-50 cm. The wheat plant is self-pollinating , which is a reason why its characteristics are very uniform permitting control and maintenance of desirable traits (Bellwood 2005). The stages of the life cycle of the wheat plant can be distinguished based in the descriptive Zadoks scale (Zadoks et al. 1974) but also by three general developmental stages: i) vegetative stage comprising from emergence to tillering, ii) reproductive period starting with tillering and ending with heading, and iii) grain-filling stage from anthesis to maturity.

Optimum growing temperatures for the spring wheat crop range from 20 to 25 °C but breeding strategies have allowed wheat to be grown widely under varied weather conditions (Hopkins and Hüner 2009).

1.1.2 Wheat crop distribution across the world's mega-environments for wheat defined by CIMMYT

Of the world's major cereals, wheat is the widest grown and consumed; in the 2014-2015 season, the world's wheat production of 730 million tonnes represented almost 30% of total cereal production (FAO). Wheat cultivation can be found across the world owing to its relevance in the human diet and its wide

adaptability, but the main producers in the last four years has been the European Union, China, India and the United States of America (FAO 2015).
For breeding purposes the International Maize and Wheat Improvement Center has divided the world wheat growing areas into 12 mega-environments (ME), defined as "*a broad, not necessarily continuous often transcontinental area with similar biotic and abiotic stresses, cropping systems and consumer preferences*" (Braun et al. 1996). The generation of new wheat varieties is focused on the needs of each of these ME which are characterized by: M1-3 and M6 temperate temperatures, ME4-5 hot temperatures, M7-12 cool temperatures (Table 1). While both ME4 and ME5 include spring wheat growing areas experiencing elevated temperatures, in ME4 yearly rainfalls are reduced to less than 500 mm in contrast to ME5 which experiences low/high humidity.

1.2 The wheat crop grown under high temperature environments

Adaptation of spring wheat to global high temperature environments is a main target of wheat breeding programs. While wheat is grown over a wide range of environments, world-wide a large proportion experiences heat stress to some degree (Kumar et al. 2013; Asseng et al. 2011). Average global temperatures are expected to increase during the next years causing yield losses (William 2007; Lobell and Field 2007).
Heat-stressed environments are classified as mega-environment 5 (ME5) (Rajaram and Fischer 1989). ME5 is characterized by temperatures >18°C in the coolest month with humid (ME5A) or dry (ME5B) atmosphere. At least 33 countries are included in this group for which heat stress is a main factor reducing wheat yield, including Australia, The United States, Sudan, Bangladesh, Nepal, Nigeria and Mexico (Paulsen 1994; Rajaram and Fischer 1989). ME5 includes about 7 million hectares experiencing continuous heat stress but almost 50% of the world's wheat growing areas can experience periods of heat stress at some stage (Cossani and Reynolds 2012), and short episodes of extreme heat are becoming more frequent resulting in serious reductions in crop production (Reynolds and Langridge 2016). The global agricultural area experiencing adverse conditions are predicted to increase due to the effect of diverse factors including climate change and many wheat cropping regions in the world will become re-classified as ME5 given the increments expected in temperatures (Table 1). Climate change exacerbates regular fluctuations in environmental conditions and the greatest impact is

expected to take place in spring wheat sowing regions including ME5 (Braun and Payne 2012). Lobell and Field (2007) modeled the impact of climate change in crop production for the period of 1961-2002. These authors estimated that global wheat yield decreased by 2-3% during the 1980's and in the period between 1990 and 2002, which resulted in losses of 19 million tons per year. In the up- coming years, it is expected that major negative effects of climate change on agricultural lands will occur in developing countries where 81% of the wheat consumption depends on local production (Ortiz et al. 2008). Independently of the increased production required for the next decades (Carter et al. 2007), there is a challenge today in breeding for high temperature environments presently affected by this constraint.

Raising productivity to meet the amount of food required for the next years represents a challenge for scientific research. The generation of germplasm tolerant to high temperatures will help meet this challenge. This will involve the identification of traits with cumulative advantageous effects, germplasm selection, and implementation of genetic technologies such as marker-assisted selection of key traits to help breed for better adaptation.

1.2.1 Effect of high air temperatures on plant performance

Temperature is a main determinant of plant growth rate which impacts on crop productivity. The effects of high temperatures on wheat growth depend on the duration of high temperatures, the severity of the heat stress, the developmental stage of the plant, and also the co-occurrence of additional constrains. Different types of heat stress induce different thermo-tolerance responses (Larkindale et al. 2008); the effect of a gradual increase in temperature differs from the effects of sudden heat shock where there is no plant acclimation. Plants at their reproductive stage are more susceptible than those at latter stages (Reynolds et al. 2016). For example, the impact of earlier heat treatments (10 days after anthesis to ripening) on wheat plants is stronger than in plants exposed to high air temperatures at a later stage (20 days after anthesis to ripening) resulting in dramatically reduced yield due to lower kernel number and decreased grain-filling duration. Heat treatment at earlier stage can reduce kernel number by up to 63% which results in yield losses close to 80%. By contrast, kernel number is not reduced when the heat treatment is applied 20 days after anthesis and yield is reduced to a lesser degree (Gibson and Paulsen 1999). Physiological

mechanisms associated with heat induced reduction in kernel number are seed abortion or failed grain set (Hays et al. 2007; Tashiro and Wardlaw 1990). For wheat, heat stress is defined as the occurrence of temperatures > 32 °C at any plant stage which results in yield losses of >10% (Reynolds et al. 2016). In cereal crops the reduction in grain yield is the main concern but this is the result of a number of processes during the whole plant growth cycle with diverse factors influencing it. Wheat grown under hot environments shows dramatic reductions in growth (smaller leaves and spikes and reduced plant height), decreased number of spikes and grains, and the whole plant development period is shortened (Gibson and Paulsen 1999).

Plant gas interchange rates are also affected, with respiration rate being increased by high air temperatures (Bunce 2004; Zha et al. 2001) and photosynthetic rates being decreased, limiting the generation of assimilates. Reductions in photosynthetic rate have negative effects in the grain-filling process producing shapeless and incompletely filled grains as well as grains of poor commercial value, *i.e.* weaker dough properties (Blumenthal et al. 1991). Rijven (1986) suggested that the main limiting factor of grain-filling under heat stress was the heat sensitivity of the soluble starch synthase. Keeling et al. (1993) showed that this enzyme reaches its maximum activity at 25 °C and declines beyond this limit, differing from other enzymes involved in the process that showed a much higher thermo-tolerance, for example the UDP-Glc pyrophosphorylase whose activity increases up to 50 °C.

Bread wheat grown under controlled conditions has shown to be reduced in yield up to 3-5 % per 1°C increased above 15 °C during grain-filling (Gibson and Paulsen 1999). Simulation modelling in Australia estimated that an increase of 2 °C above average temperatures for the growing season could reduce wheat yield by up to 50%, and field studies showed that yield is decreased up to 60% in bread wheat grown under hot-irrigated environments compared to non-stressed conditions (Asseng et al. 2011; Pinto et al. 2010). Lobell and Field (2007) reported that at least 30% of yield variance in the six main cereals (41% in the case of wheat) is related to maximum and minimum temperatures. In 2005 it was estimated that about 9 m ha of wheat worldwide experienced yield reductions due to high temperatures (Lillemo et al. 2005). Heat tolerant wheat cultivars are characterized by maintenance of the photosynthetic rate at high

Table 1. Classification of spring wheat mega-environments (ME) used by the CIMMYT's wheat program using qualitative and geospatial criteria.

ME	Lattitude	Wheat area (m ha)	Criteria	Temperature regime	Major biotic and abiotic stresses	Representative locati ons/regions	Change in ME due to climate change and consequences for germplasm development (adapted from Hodson and White, 2007b)
1	<40°	32	Low rainfall irrigated, coolest quarter (3 consecuti ve months) mean min temp. >3 °C <16 °C	Temperate	Resistance to lodging, SR, LR, YR, KB, *Alternaria* spp.	Yaqui Valley, Mexico; Indus Valley, Pakistan; Gangeti c Valley India; Nile Valley Egypt.	N–Rising temperatures result in large areas evolving to ME5 N–Reduced precipitati on in subtropical regions restricts irrigation; supplementary irrigation results in temporary drought periods requiring germplasm with high yield and tolerance to drought (adapted to ME1 and ME4) P–Reduced irrigation due to impact of elevated CO_2 on water use efficiency N-Increased insect problems
2A	<40°	4	High rainfall in summer; wettest quarter mean min temp. >3 °C <16 °C. wettest quarter (3 consecutive wettest months)	Temperate	As for ME1 + resistance to LR, YR, *Septoria* spp., PM, RDC, BYDV sprouting	Highlands East Africa and Mexico, Andes	N–Rising temperatures result in some areas evolving to ME5 N–Reduced precipitati on results in areas evolving to ME4
2B	<40°	3	High rainfall, winter rain; coolest wuarter mean min temp. >3 °C <16 °C; elevation 1400m	Temperate	As for ME1 + resistance to LR, YR, *Septoria* spp., PM, RDC, BYDV sprouting	Mediterranean Coast, Caspian Sea	U–Changes in precipitati on patt erns in areas will have variable effects N–Frequency of climate extremes over years increase requiring germplasm with high yield potential, wide spectrum of disease resistance and tolerance to drought
3	<40°	1.7	High rainfall acid soil climate as in ME2 and pH <5.2	Temperate	As for ME2 + acid soil tolerance	Passo Fundo, Brazil	N–Rising temperatures result in large areas evolving to ME5 U–Changes in precipitation patterns in areas will have variable effects
4A	<40°	10	Low rainfall, winter rainfall dominant; coolest quarter mean min temp >3 °C <11°C;wettest quarter precipitation >100 mm <400 mm	Temperate	Resistance to drought, *Septoria* Spp., YR, LR, SR, RDC, Hessian fly, Sawfly	Settat, Morocco Aleppo, Syria; Diyarbakir, Turkey	N–Rising temperatures exacerbates water deficits, either further reducing yields or making production uneconomical P–Reduced water deficits through impact of elevated CO_2 on water use efficiency

Continues Table 1…

ME	Lattitude	Wheat area (m ha)	Criteria	Temperature regime	Major biotic and abiotic stresses	Representative locati ons/regions	Change in ME due to climate change and consequences for germplasm development (adapted from Hodson and White, 2007b)
4B	<40°	5.8	Low rainfall summer rainfall dominant; coolest quarter mean min temp >3 °C <11 °C; wettest quarter precipitation >200 mm <500 mm	Temperate	Resistance to, drought Septoria spp., LR, SR, Fusarium spp.	Marcos Juarez, Argenti na.	N–Changes in precipitati on patterns likely to increase drought risk
4C	<40°	5.8	Mostly residual moisture; coolest quarter mean min temp. >3 °C <16 °C; wett est quarter precipitation >100 mm <400 mm.	Hot	Resistance to drought, and heat in seedling stage, SR.	Indore, India	U–Changes in precipitation patterns in areas will have variable effects
5A	<40°	3.9	High rainfall/ irrigated, humid; coolest quarter mean min temp >11 °C <16 ºC	Hot	Tolerance to heat, Helmintho-*sporium* spp., *Fusarium* spp., sprouting; in Brazil Bolivia and Paraguay wheat blast.	Eastern Gangetic Plains in Nepal, India, Bangladesh; Londrina, Brazil.	N-Rising temperatures result in large areas becoming unsuitable for wheat; cropping systems and agronomy practices allowing early sowing of wheat paramount. U-Elevated CO_2 may incerase water use efficiency, but the same mechanism implies increased canopy temperature which likely would exacerbate heat stress.
5B	<40 ºC	3.2	Irrigated, low humidity; coolest querter mean min temp >11ºC <16 ºC	Hot	Resistance to heat and SR, LR.	Gezira, Sudan; Kano, Nigeria.	N-Rising temperatures result in large areas becoming unsuitable for wheat. N-Increasing biotic stress. U-Elevated CO2 may incerase water use efficiency, but the same mechanism implies increased canopy temperature which likely would exacerbate heat stress.
6	>40 ºC	11.0	Moderate rainfall/ summer dominant; high latitude quarter 45 ºN; coolest mean min temp <-13ºC; warmest quarter mean min temp> 9ºC	Temperate	Resistance to drought, SR, LR, Tan spot, Scab, photoperiod sensitivity	Kazakhstan; Siberia; Harbin, China.	P-Rising temperatures allow wheat production in higher latitudes - wheat area espansion likely. P- Lengthen growing season permits marginal areas to become productive. P-Reduced risk of winter-kill allows conversion to more productive winter wheat

N=negative; P=positive; U=unknown; Moisture regime refers to rainfall just before and during the crop cycle. High = >500 mm; Low = <500 mm; Biotic stresses: LR=leaf rust, SR=stem rust, YR=yellow (stripe) rust, PM=powdery mildew, BYDV=barley yellow dwarf virus, KB=Karnal bunt, RDC=root disease complexes. Adapted from Braun & Payne (2012)

temperatures, high number and weight of kernels (Reynolds and Trethowan 2007). Plants with specific morphological and physiological characteristics are expected to show superior tolerance to high temperatures, for example, Blum (1986) suggested that awns may contribute to heat tolerance by heat dissipation. An erect leaf angle has been suggested to contribute to tolerance because of its photo protective function through the avoidance of intercepting excess of radiation (Van Zanten et al. 2010; Mahan et al. 1995). Longer grain-filling periods due to chlorophyll retention and optimal root development also contribute to improved crop yield under hot environments (Kumari et al. 2007; Pinto and Reynolds 2015; Reynolds et al. 2000). More detail is provided in the Chapter 1.3.4.2 from the current study.

1.3 Physiological traits related to wheat adaptation to high air temperatures

Given the complexity of plant response to heat stress, physiological breeding applies an integrative approach which combines key traits specifically useful to improve crop performance in high temperature environments. Strategies for wheat adaptation to heat stress have been reviewed (Cossani and Reynolds 2012) and diverse plant attributes have been reported to give genetic gains in heat tolerance of wheat in earlier studies (Wardlaw et al. 1980; Blum 1986; Al-Khatib and Paulsen 1984; Harding et al. 1990; Reynolds et al. 1994; Blum and Neguyen 1997, Fokar et al. 1998). A conceptual model (Fig 1) proposed by Reynolds and Tuberosa (2008) integrates candidate traits for which genetic value has been documented (Reynolds and Trethowan 2007). The application of this model in wheat breeding has resulted in substantial genetic gains in wheat grown under hot conditions (Reynolds et al. 2001) and derives from a similar model targeted to improve adaptation to drought (Gourdji et al. 2013).Theoretical yield gains in heat stressed environments were estimated as up to 34% with the best expression of key adaptive traits such as final aboveground biomass and harvest index in elite wheats (Reynolds et al. 2007b).

Five main mechanisms are identified as influencing yield under hot conditions: 1) photo-protective traits, 2) radiation use efficiency, 3) partitioning of assimilates, 4) light interception characteristics and 5) water use (Cossani and Reynolds 2012).

It is expected that the strategic combination of these characteristics result in cumulative effects to improve adaptation to high temperatures (Fig 1).

1.3.1 Photo-protective strategies

1.3.1.1 Morphological adjustments in the canopy architecture: leaf rolling and leaf orientation

Specific morphological plant attributes can be advantageous for plant adaptation to high temperatures. Heat stressed environments often experience high irradiances which can promote the accumulation of reactive oxygen species given the increased generation of compounds like $^1O_2$, H_2O_2, O2- and $HO^{\cdot}$ and result in photo-damage to the photosynthetic apparatus of wheat leaves (Suzuki and Mittler 2006; Zhao and Tan 2005). Variation in the canopy architecture relating to leaf orientation, leaf size and leaf angle influences radiation interception and shading and carbon gains. There is wide genetic diversity in wheat for leaf, stem and spike morphological traits but given that a major proportion of total photosynthetic radiation is captured by the leaves, leaf angle is a key component with major effects on the total radiation intercepted (Falster and Westoby 2003). Wheat leaf angle is also affected by environmental factors including temperature and radiation (Ledent 1978; Kimura 1977). An erect leaf angle has been suggested as a valuable trait to increase grain yield in maize (Ku et al. 2010), rice (Wang et al. 2005), wheat (Shibayama 2007) and barley (Tungland et al. 1987), this is because more vertical leaves allows light penetration to lower leaves and avoids self-shading which results in low light interception, reduced carbon gains and the death of the lower leaves (Yanli et al. 2007). In environments of high air temperatures and high irradiances more vertical oriented leaves permit a more balanced light capture across the canopy but also reduce interception of excessive radiation which could increase the leaf temperature and cause photoinhibition (Van Zanten et al. 2010; Mahan et al. 1995). Elevated leaf temperatures can lead to increased transpiration and reduced photosynthesis. Individual leaf angle is a highly sensitive and environment-responsive plant component and extrapolated to whole crop canopies structures result in complex systems. Canopy structure is usually considered a fixed feature, notwithstanding, many plants have the ability to modify their leaf angle via hormone regulation to improve growth efficiency or stress tolerance (Van Zanten et al. 2010).

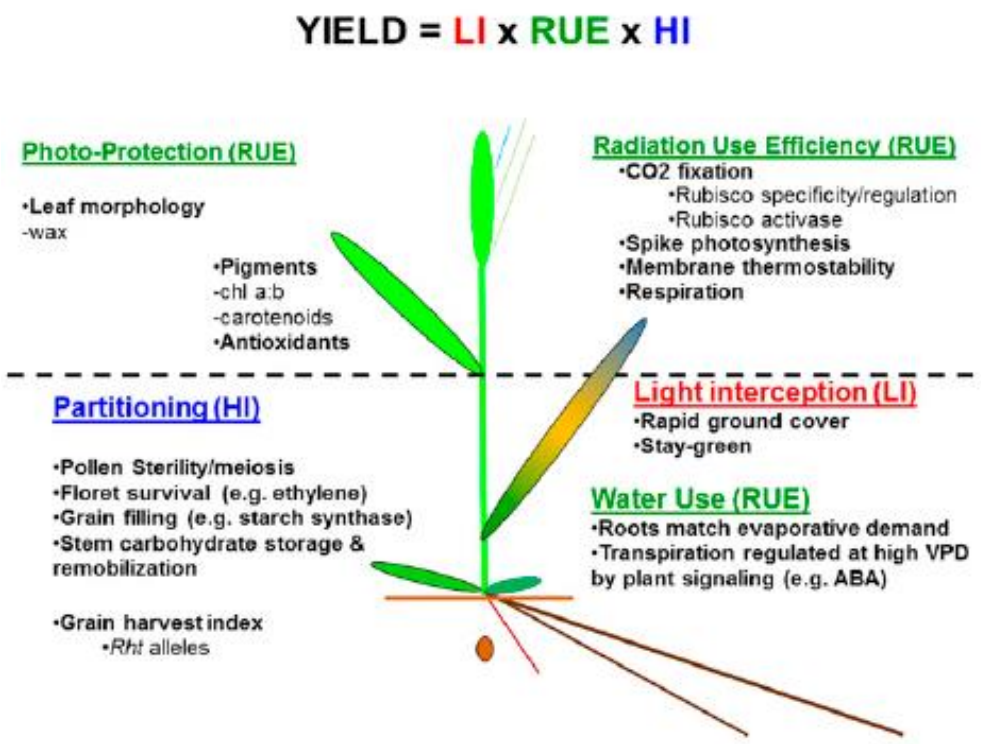

Figure 1. Conceptual model of heat-adaptive traits grouped by three main drivers of yield in the absence of water limitation (adapted from Reynolds et al. 2007b). chl: chlorophyll content; VPD: vapour pressure deficit; ABA: Abscisic acid;. From: Cossani and Reynolds (2012)

1.3.1.2 Production of photo-protective compounds

Fast growing plants like wheat regularly absorb about 50% more radiation energy than can utilize and the excess must be dissipated to avoid photoinhibition. Carotenoids, xanthophylls and chlorophyll absorb a small proportion of the near infrared wavelength from the canopy and reflect some of this radiation (Prasad et al. 2007). The principal carotene in wheat is lutein followed by zeaxanthin and β-carotene (Hidalgo et al. 2006). Carotenes prevent the formation of reactive oxygen species (ROS) which could otherwise cause cell death. Under high irradiance, it is likely that the singlet excited chlorophyll (^{1}Chl) turns to a triple excited state (^{3}Chl) which is highly reactive with the triple ground state of oxygen ($^3O_2$), resulting in a single oxygen state ($^1O_2$) which is a highly toxic ROS. ROS have the ability to oxidize proteins, lipids and DNA and include O_2, H_2O_2, O_{2^-} and HO* (Suzuki and Mittler 2006). ROS are commonly considered damaging, but Larkindale and Vierling (2005) suggested a possible

function as stress signals. Xanthophylls such as lutein, violaxanthin, and zeaxanthin also protect the chloroplasts from photo-oxidation by preventing the formation of the single oxygen state (Hopkins and Hüner 2009). These plant photo-protective pigments can be detected in the field by using the specific pattern in a spectral curve (Sims and Gamon 2002; Gamon et al.1992). Some authors have developed reflectance spectral indices to estimate the leaf concentration of chlorophyll a, chlorophyll b and carotenoids (Chappelle et al. 1992; Blackburn 1998); and the spectral index for carotenoid content has been strongly and positively associated with dry biomass at grain-filling in bread wheat grown under irrigated field environments (Babar et al. 2006) suggesting a potential utility for heat stressed environments. Wax is another plant component with a proto-protective role which can act as a reflective barrier against excessive radiation or to limit water losses; the amount of reflected radiation in the visible and infrared wavelengths is proportional to the amount of wax in the tissue (Shepherd and Griffiths 2006). However, excessive waxiness may have negative impacts on yield, given its association with reduced transpiration efficiency (Merah et al. 2000).

1.3.2 Traits related to water uptake

1.3.2.1 Regulation of transpiration rates and root development

High vapour pressure deficit of heat stressed environments generally results in increased leaf transpiration rates in well irrigated plants allowing evaporative cooling. The latter is expected to result in improved heat stress tolerance given the association of low canopy temperature with yield (Pinto et al. 2010). Reductions in root mass of wheat had been observed when plants were exposed to elevated air temperatures (Batts et al. 1998). Under heat irrigated conditions an adequate root system is necessary to absorb high volumes of water from the soil and at high rates, in order to match the high demand (Amani et al. 1996). Similarly, in drought environments, where adequate root architecture can result in significant differences for plant performance, under hot-irrigated environments an optimum root mass distribution can result in increased efficiency of water extraction (Manschadi et al. 2006; Reynolds et al. 2007a; Lopes and Reynolds 2010). Even though it is well know that roots are

extremely sensitive to high temperatures (Porter and Gawith, 1999) few studies focus in the role that roots play in plant adaptation to heat environments. One of the main reasons is that the study of roots under field conditions is a challenging and time consuming task. However, it is possible to work with surrogate traits which can be indirect indicators of the plant access to soil water and the root development. Recent studies in spring wheat reported that under both heat and drought conditions the best yielding germplasm had cooler canopy temperatures. These results were also supported by co-location of QTL for canopy temperature under heat and drought environments suggesting a common genetic basis for root development on both stresses (Pinto et al. 2008) but this hypothesis has not been tested. For this reason Chapter 2 from the current thesis investigated if the QTL for canopy temperature identified in Pinto et al. (2008) relate to root development traits and, if the root patterns under heat and drought are similar.

1.3.3 Light interception strategies

1.3.3.1 Early ground cover

In contrast to temperate environments, under heat stressed environments total above-ground biomass shows a stronger association with yield than with harvest index indicating that yield is more likely to be limited by photo-assimilation than by partitioning (Reynolds et al. 2001). This suggests high relevance of the canopy light interception traits under heat stress. Rapid early ground cover is linked to high seedling emergence rate which, under high air temperature and high irradiance environments, permits some degree of stress avoidance if heat begins late. Under those conditions early crop establishment can shade the soil and keep it cooler allowing increased tillering (Rawson 1986). Visual scores of plant early vigour have been associated with heat tolerance of durum wheat, with high yielding lines tending to have significantly higher early vigour than the low yielding lines (Nachit and Ketata 1989). More recently Reynolds et al. (2007b) showed that ground cover before anthesis (estimated by spectral reflectance) is associated with grain yield of wheat landraces grown under heat conditions; the highest expression of early ground cover was associated with a 7% yield gain. The potential of this trait for improve heat tolerance in wheat seems promising given the results obtained under

drought stressed environments (Loss and Siddique 1994). However, more studies are needed to evaluate the actual contribution of early ground cover across different types of heat stresses.

1.3.3.2 Potential value of staygreen under hot-irrigated environments

Delayed loss of greenness can be an indicator of higher generation of photosynthetic products in the late stages of the plant development cycle resulting in what is called functional staygreen. Genotypes that have persistent residual greenness after anthesis have shown superior performance under high air temperatures (Kumari et al. 2007; Borrell and Douglas 1996; Borrell et al. 2000). The functional staygreen leads to higher photosynthetic activity due to an extended grainfilling period; the later results in improved plant performance in terms of biomass or yield (Vijayalakshmi et al. 2010). However, when staygreen is not associated with active photosynthesis the plant exhibits a cosmetic staygreen feature that does not contribute to crop productivity (Thomas and Howarth 2000). The dynamics of the staygreen attribute are complex and it is common to find germplasm exhibiting a combination of different types of staygreen. Genetic variability for staygreen has been exploited in several species including cereal crops such as maize, sorghum and wheat, but the complex dynamics of plant greenness losses under high temperatures are not well understood or standarized, complicating its utilization. The quantification of the staygreen trait has be performed through the estimation of leaf chlorophyll content with a SPAD meter, however this technique has the weakness of excluding other photosynthetic organs different than leaves whose contribution to grain yield can be significant under high temperature environments (Maydup et al. 2010). The utilization of a high throughtput technology such as plot spectral reflectance measurement has recently shown to be advantageos in capturing variations in staygreen across different organs of the plant, at the as allowing the collection of a large number of data. In this thesis project the application of this methodology to the Seri/Babax mapping population was expected to result in accurate estimations of the residual plant greenness and extend the current understanding of the staygreen dynamics of wheat grown under high temperatures (Chapter 3).

1.3.4 Partitioning of assimilates

Optimal biomass partitioning can improve performance under heat stress. This is achieved by allocating assimilates generated during early growth stages of the plant, or assimilates that are photosynthetically produced after anthesis, to grain-filling. High biomass partitioning to the root system of the plant has also proven to be a valuable heat adaptive mechanism for wheat grown under hot-irrigated conditions (Pinto and Reynolds 2015). Several studies indicate that the yield component most affected by heat stress is the set grain number per spike (Gibson and Paulsen 1999). However, heat stress after anthesis reduces yield mainly due to reduced grain weight (Wardlaw et al. 1989). Mobilization of carbohydrate reserves stored in the stems (mainly fructans) supports the grain-filling process (Scofield et al. 2009). Estimations indicate that approximately 10-30% of the carbon in wheat grain yield is contributed by the pre-anthesis reserves in pot grown plants exposed to low and high nitrogen treatments

(Gebbing et al. 1999), but given that stress conditions limit the photosynthetic activity the relative contribution of stored and mobilized carbohydrates could be higher in high temperature environments; this will depend on the amount of stored photo-assimilates, the remobilization efficiency and the demand associated with the sink (Asseng and van Herwaarden 2003; Blum 1998). Heat resistant wheat cultivars under heat stress lost three times more non-structural stem carbohydrate than a non-resistant cultivar, further suggesting the capacity to mobilize higher amounts of reserves is associated with heat tolerance (Blum et al. 1994). The accumulation of photo-assimilates in the stems seems to be negatively associated with dwarfing genes. Borrell et al. (1993) showed that the *Rht1* and *Rht2* dwarfing genes reduce stem reserves of wheat by around 40% in high yielding environments due to reduction in stem height. But the mobilization of carbohydrates has not been related to plant height under conditions of limited photosynthesis (Rawson and Evans 1971).

1.3.5 Radiation use efficiency related traits

1.3.5.1 Cell membrane thermostability

High temperatures modify cell membrane structure, plasticity and fluidity. In heat susceptible cultivars, high temperature leads to membrane injury as

evidenced by measuring ion leakage resulting from the cell membrane disruption and protein denaturation. The level of damage to the cells is reflected in the photosynthetic activity and in the overall crop performance at harvest time. Fokar et al. (1998) observed that the intensity of cellular injury is dependent of the developmental stage at which the plant experiences the heat stress, being more susceptible at the reproductive than at the early stages; they showed the cell membrane thermostability in seedlings to be positively and highly correlated with grain yield of wheat grown under a range of hot temperatures. Heat adapted genotypes are expected to show reduced electrolyte leakage after exposure to heat treatments when compared to heat susceptible genotypes. Cellular thermotolerance has been widely used as an indicator of heat tolerance (Gupta el at. 2010; Ibrahim and Quick 2001; Reynolds et al. 1994). For example, a study in wheat cultivars showed that higher membrane thermostability was positively associated with higher yields under warm conditions (Shanahan et al. 1990). Cell membranes also can be damaged by increased accumulation of ROS which results from plant exposure to high temperatures and high irradiance conditions (McDonald and Vanlerberghe 2005; Christiansen 1978).

Further exploration is required in this area given the reduced number of studies showing its utility for wheat improvement for hot environments.

1.3.5.2 Gas exchange processes (photosynthesis and respiration)

Radiation use efficiency is defined as the gross carbon assimilation minus the cost related to cell growth and maintenance (Cossani and Reynolds 2012). Genetic variability in leaf wheat photosynthetic rates has been observed under heat stressed conditions (Wardlaw et al. 1980; Blum 1986) and is negatively associated with premature leaf senescence (Al-Khatib and Paulsen 1984). The high sensitivity of photosynthetic enzymes to elevated temperatures seems to be a major driving factor in photosynthesis; it has been observed that under moderately elevated temperatures (30°C) photosynthesis reductions are linked to deactivation of RuBisCo due to inhibition of rubisco activase (Kurek et al. 2007). Genotypes with improved photosynthesis are suggested to have better adaptation to heat stress. In fact, photosynthetic leaf rate of bread wheat grown under high-temperature field conditions has showed strong and positive association with grain yield when measured at booting, anthesis and grain-filling

stages (Reynolds et al. 1994). During the vegetative stage efficient photosynthesis is expected to promote the development of new organs and therefore positively impact on final grain yield (Asseng et al. 2002). The double functionality of the RuBisCO enzyme as a carboxylase and oxygenase complicates the improvement of photosynthesis. As an oxygenase RuBisCO catalyses the photorespiration and dark respiration process impacting water use efficiency; while as an oxygenase its affinity for CO_2 is reduced with increasing temperatures (Jordan and Ogren 1984). In wheat the introgression of a heat tolerant RuBisCO from *Limonium gibertii* can potentially increase photosynthesis net assimilation by 12% (Parry et al. 2011). Genetic gains in wheat may also be reached by exploiting the genetic diversity of exotic germplasm such as wheat wild relatives or landraces (Lopes et al. 2015; Reynolds et al. 2007a), similar to the process performed for wheat drought adaptation using synthetic wheats (Trethowan et al. 2005). Besides photosynthesis, costs of plant respiratory activity determine the plant's radiation use efficiency; a more efficient use of energy impacts a plant's performance. Under heat stress respiration not only provides the energy required for growth and maintenance processes, but the alternative pathway of respiration is also of high relevance for plant recovery after stress conditions given its association with reduced ROS levels (Wang and Vanlerberghe 2013; Gonzalez-Meler et al. 1999; Millenaar and Lambers 2003). Therefore, selection for wheat genotypes with an optimized balance between captured CO_2 and respired CO_2 can result in improved tolerance to high temperatures.

1.3.5.2.1 Plant respiration

Respiration plays a fundamental role as an energy source for growth and maintenance in plants. The main functions of respiration include the production of C-skeleton intermediates for building more complex molecules and provision of usable energy (ATP) and reducing power [NAD(P)H] for ordinary plant processes such as cell growth and maintenance (Amthor 2000b).

Two main branches of respiratory pathway are distinguished: via cytochrome (*Cyt*), and via the alternative or cyanide resistance pathway (AOP) present in all plants, some protists, fungi, and invertebrate animals (Vanlerberghe and McIntosh 1997; McDonald and Vanlerberghe 2004). Total respiration has been differentiated into growth (R_g) and maintenance respiration (R_m), according to

models presented by McCree and Thornley during the 1970s (McCree 1970; Amthor 2000b; Thornley 2011). Growth respiration comprises the degradation of compounds in processes that result in biomass gains, while maintenance respiration is defined as energy production for processes not resulting in biomass gain, such as maintenance of ion gradients and turnover of proteins and lipids (Chiariello et al. 1989; Amthor et al. 2000a). Under stress conditions (e.g. high irradiance, drought, high temperature), the alternative respiration pathway is activated, therefore, stress protective roles have been hypothesized (Gifford 2003; Sanhueza et al. 2013).

Dry matter accumulation depends on the balance between carbon fixed and carbon respired (Lambers and Van der Werf 1988); some researchers have suggested reducing carbon respiration as a possible strategy for crop improvement, but its essential role would need to be maintained. On average, plant respiration is estimated to consume 50-60% and 30-60% of carbon assimilated from photosynthesis in trees and cereals, respectively (Amthor and Baldocchi 2001).

Photorespiration is not reviewed here, and readers are referred to a recent review from Peterhansel et al. (2010) where the wasteful paradigm is discussed in detail. However, while photorespiration can provide a photo-protective role when CO_2 supply to the Calvin cycle is rate limiting, the oxygenation reaction of RuBisCO is considered wasteful because less 3-Phosphoglycerate is generated and at a higher cost than for RuBP carboxylase activity. Photorespiratory carbon cycling results in approximately 25% of carbon fixed by photosynthesis being re-released as CO_2. Photorespiration also has a cost in terms of ATP and NAD(P)H. Under unfavourable conditions such as at supra-optimal temperatures where oxygenase activity of Rubisco increases, CO_2 losses by photorespiration can reach as much as 40% of the net assimilated carbon in C_3 species, while it is largely avoided in C_4 plants through CO_2 enrichment mechanism in the bundle sheath cells (Zelitch 1992).

While many of the mechanisms regulating plant respiration rates are still unclear, this section reviews current knowledge about factors that can affect respiration rate. Recent findings about respiration pathways and its components are summarized and the relationship between respiration and photosynthesis – and its relevance to breeding – is discussed, including studies where harnessing genetic variation led to improved plant performance. Finally, this

review presents the techniques utilized for measuring respiration, from the traditional Clark electrode to more sophisticated techniques such as isotope fractionation.

1.3.5.2.1.1 Factors affecting plant respiration

The regulation of plant respiration is not sufficiently well-understood for scientists to establish an adequate model (Atkin et al. 2014) similar to the generalized model of photosynthesis proposed by Farquhar et al. (1980), for example. Respiration rate may be affected by variation in temperature, CO_2 level, substrate concentration, light quality/intensity, nutrient availability or stress. The respiration response to any of these factors is not definitive, since different studies have given conflicting results. However, recent multivariate analyses have revealed that growth temperature, leaf nitrogen, and plant functional type account for much of the variation in respiration (Cox 2001; Atkin et al. 2014). The following section reviews a number of studies that analyze the effects of driving factors on plant respiration rates.

1.3.5.2.1.1.1 Temperature

Growth temperature is a principal factor accounting for respiration variation in models, and much recent research is focused on responses to global warming (Prasad et al. 2008). For example, in field grown Mediterranean evergreen species, mean monthly air temperature accounted for 42% of variation ($p<0.01$) in leaf respiration rate when mean yearly air temperature was 16.8 ±6.5 °C (Fig 2; Catoni et al. 2013). Plant respiration rate increases with temperature (Zha et al. 2001; Bunce 2004) but the magnitude of this increment (defined as Q_{10}) is not necessarily constant across species, tissues, and other conditions.

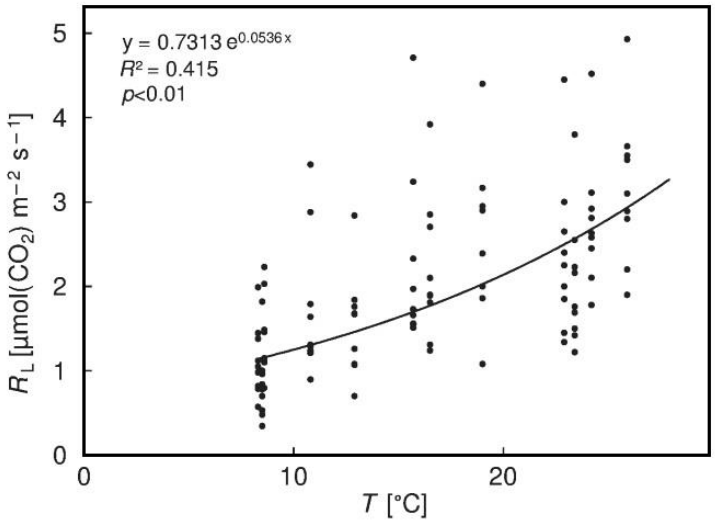

Figure 2. Regression analysis between leaf respiration of Mediterranean evergreen species (*Arbutus unedo*, *Cistus incanus*, *Erica arborea*, *Erica multiflora*, *Phillyrea latifolia*, *Pistacia lentiscus*, *Quercus ilex*, *Rosmarinus officinalis* and *Smilax aspera*) and mean monthly air temperature. Reproduced with permission from Catoni et al. (2013).

Variation in Q_{10}

The temperature response of plant respiration follows a dynamic shape, especially at temperatures that differ greatly from natural conditions (Atkin et al. 2014). This response was originally described to follow a constant Q_{10} of 2, meaning that the rate doubles for each 10 °C rise in temperature. However, Q_{10} does not always equal 2, since the sensitivity of respiration to temperature is reduced with incremental temperatures (Forward 1960; Berry and Raison 1981; Tjoelker et al. 2001; Atkin et al. 2008). The temperature range at which Q_{10} is relatively constant and close to 2 seems to be limited and species dependent (Wager 1941 and references therin; Atkin and Tjoelker 2003).

Results from five boreal trees exposed to high temperature treatments showed that Q_{10} declined with increasing temperature treatment independently of the tree species (Table 2; Tjoelker et al. 2001). The authors compared their results with previous studies which showed that across a large set of experiments the Q_{10} exhibited a negative linear relation with temperature independently of species or temperature range (Fig 3). In crops the same performance has been observed, for example in advanced lines of bread wheat grown under hot irrigated conditions (Tmax = 38 °C), Q_{10} decreased from 1.9 to 1.2 at 20-30 and 27-37 °C, respectively (Pinto et al. submitted).

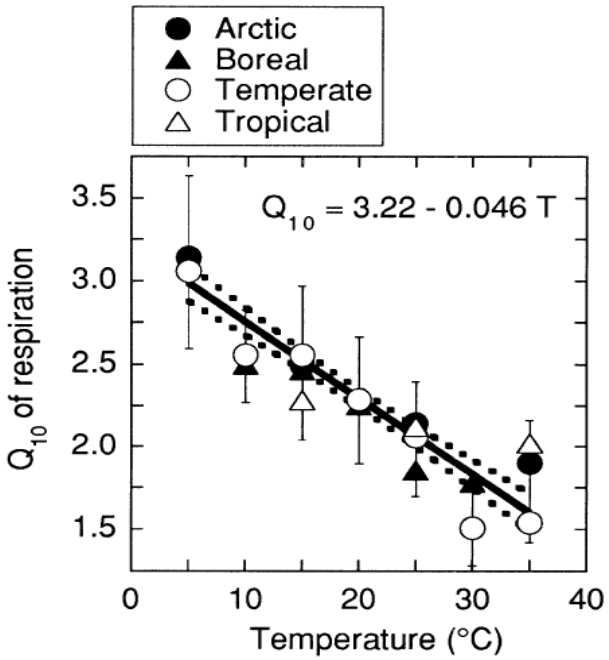

Figure 3. Q_{10} of respiration rates of plants as a function of measurement temperature. Symbols are the mean Q_{10} of species of arctic (16 species), boreal (6 species), temperate (31 species), and tropical (3 species) biomes plotted at the midpoints in 5 °C classes. Error bars indicate ±1 SD of the class means of all observations. Data are of 56 species from 23 studies, including the Tjoelker et al. (2001) experiment with five boreal tree species. Based on covariance analysis, neither slopes (P = 0.50) nor intercepts (P = 0.07) of linear regressions differed among the arctic, boreal, and temperate biome groups, which is evidence for a common relationship across these biomes. The Q_{10} of the tropical species showed no relationship with temperature (P = 0.33). A single linear regression was fit (±95% C.I., dashed lines) to all data points (R^2 = 0.45, n = 238, P < 0.0001). Reproduced with permission from Tjoelker et al. (2001).

The apparently variable response of respiration to temperature may be associated with acclimation mechanisms, since non-acclimated plants can respond more erratically (Amthor 2000b; Zha et al. 2001). Plants able to acclimate show less sensitivity to rising temperatures (translated in lower Q_{10} for respiration rate); that is, the increase in respiration is not as dramatic in acclimated plants as in non-acclimated plants. If thermal acclimation can modify gas exchange, what drives plant acclimation? Night-time temperature seems to be a main factor – as has been shown in studies of gas exchange rate of roots and leaves of *Plantago lanceolata, P. euryphylla* and *Oryza sativa* (Covey-Crump et al. 2002; Mohammed and Tarpley 2009) – possibly because it is during the night that the greatest fraction of respiration occurs. Night and day elevated temperatures promote a higher production of reactive oxygen species (ROS) in many plants (McDonald and Vanlerberghe 2004), which can decrease cell membrane thermostability (Christiansen 1978) due to cell injury. Plants that can cope with the high production of ROS through the activity of enzymes and

antioxidant metabolites may be able reduce cell damage produced by high temperatures. Studies on rice support this idea, showing that at early grain-filling, leaf respiration is increased almost 30% when the plants are grown at high night-time temperatures (32 °C from 20 days after emergence till harvest), compared to ambient temperatures (27 °C). At the same time, cell membrane thermostability decreased by 60% due to high night temperatures (Mohammed and Tarpley 2009).

Substrate availability is another factor modulating Q_{10}. Studies have shown that, at high temperatures, the decline in temperature sensitivity of respiration is a function of substrate availability, while at cool temperatures maximum enzyme activity is the rate determining factor (Dewar et al. 1999; Atkin and Tjoelker 2003). Studies in roots and leaves support this hypothesis, for example, sensitivity of respiration in hydroponically grown roots of *P. lanceolata* was found to be mostly controlled by the glucose supply when exposed to temperatures of 15-30 °C, while at 5-15 °C the substrate supply had no effect, suggesting the enzyme activity to be limiting (Covey-Crump et al. 2002). In needles from Scots pine (*Pinus sylvestris*), Q_{10} declined 15% in trees grown 2-6 °C above the control (Zha et al. 2001), which may be due to reduced substrate availability as high temperatures reduce leaf carbohydrate (starch, glucose, fructose, and sucrose) content.

Experimental Q_{10} values can be found in the range from 1.2 to 5.1 (Wager 1941 and references therin; Azcón-Bieto 1992; Frantz et al. 2004; Catoni et al. 2013) and varying between species (Larigauderie and Körner 1995; Gifford 2003), organs (Covey-Crump et al. 2002), and environments. For example, at 19 °C, the reported Q_{10} was 2.31, 2.22, and 2.42 for temperate, boreal, and artic species, respectively (Tjoelker et al. 2001), while the Q_{10} for Mediterranean evergreen species ranges from 1.5-2 under field conditions (Catoni et al. 2013).

Table 2. Temperature effects on calculated Q_{10} of dark respiration of five common tree species of a boreal forest biome grown at two CO_2 concentrations*.

Species	Growth [CO_2]†	Q_{10} of dark respiration Measurement temperature interval 12–18 °C	18–24 °C	24–30 °C
Populus tremuloides	370	2.26	1.95	1.62
	580	2.23	1.97	1.63
Betula papyrifera	370	2.48	2.27	2.01
	580	2.77	2.37	1.88
Larix laricina	370	2.68	2.34	1.81
	580	2.49	2.44	1.96
Pinus banksiana	370	2.48	2.37	1.88
	580	2.26	2.26	1.91
Picea mariana	370	2.61	2.18	1.93
	580	2.88	2.38	1.95

*Rates of dark net CO_2 efflux of foliage were determined at measurement temperatures of 12, 18, 24 and 30 °C to obtain short-term temperature responses and estimates of Q_{10}, which differed among measurement temperature intervals in each species ($P<0.017$). †Growth CO_2 concentration (μmol mol±1), day/night growth temperature (18/12, 24/18, and 30/24 °C), and the interaction term had no effect on Q_{10} in any species ($P>0.15$). Reproduced with permission from Tjoelker et al. (2001).

Moreover, Q_{10} can be affected by environmental factors such as drought and light stress (Atkin and Tjoelker 2003), which can reduce leaf and plant Q_{10} values. Variation can also be caused by intrinsic factors such as type of tissue; for example, roots and leaves differ in their Q_{10} values, but it is unknown how their Q_{10} changes with temperature. The degree of variability of Q_{10} for individual organs or whole plant respiration still needs to be explored since the acclimation to changes in temperature seems to differ within the same species/organ/tissue under different growing conditions. For this reason, it is illusory to attempt to identify a general range of temperatures at which Q_{10} remains constant. For example, in leaves of *Eucalyptus pauciflora*, Q_{10} of leaves remained constant in the range of 10-22 °C, while in lettuce, tomato, and soybean, Q_{10} was relatively constant and <2 between temperatures of 17-32 °C (Frantz et al. 2004). No acclimation was observed for these fast growing plants that showed a constant 33% increase in respiration for each 10 °C increase in night temperature above 30 °C, over a 15 day period (Figs. 4, 5; Frantz et al. 2004). Kirschbaum and Farquhar (1984) demonstrated that, in leaves of *E. pauciflora*, Q_{10} was close to 2.6 and relatively constant within temperatures of

15-20 °C, but decreased to 1.9 with increased temperatures of 30-35 °C. It can be concluded that, within a given range (which depend of the specie) plant respiration rate increases with temperature. This is logical as enzymatic activity is accelerated at higher temperatures. The degree of response of plant respiration to temperature increments of 10 °C (Q10) follows a non-exponential curve and declines as temperature increases; this performance is attributed to acclimation processes that prevent the plant reaching the carbon compensation point (C fixed = C released) and a negative carbon balance. Plant acclimation will determine the extent of temperature effects on respiration rate, for which the severity and length of the high temperature, as well as the species, organ, and occurrence of stress will play an important role (Table 2, Fig 4). Acclimation seems to be responsible for respiration rates about 20% lower in high-temperature biomes (Atkin et al. 2008).

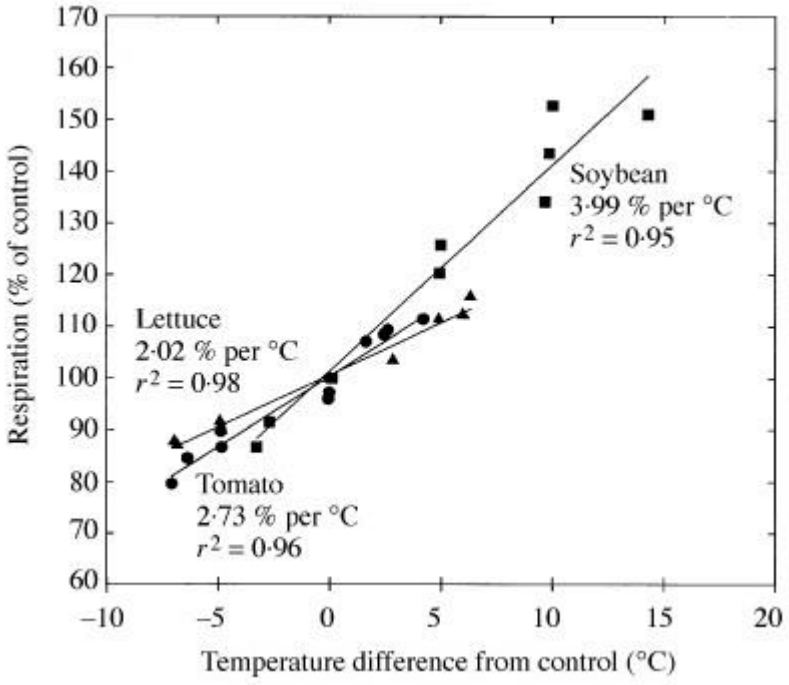

Figure 4. Effect of night temperature on respiration in lettuce, tomato, and soybean relative to the control. Soybean respiration was significantly more temperature-sensitive than tomato ($t = 4.05$, d.f. = 8, $P < 0.005$), and tomato respiration is significantly more sensitive than lettuce ($t = 3.418$, d.f. = 8, $P < 0.025$). Control plants of lettuce and tomato were grown at constant day/night temperature of 25°C, while soybean at constant day/night temperature of 20°C. From Frantz et al. (2004).

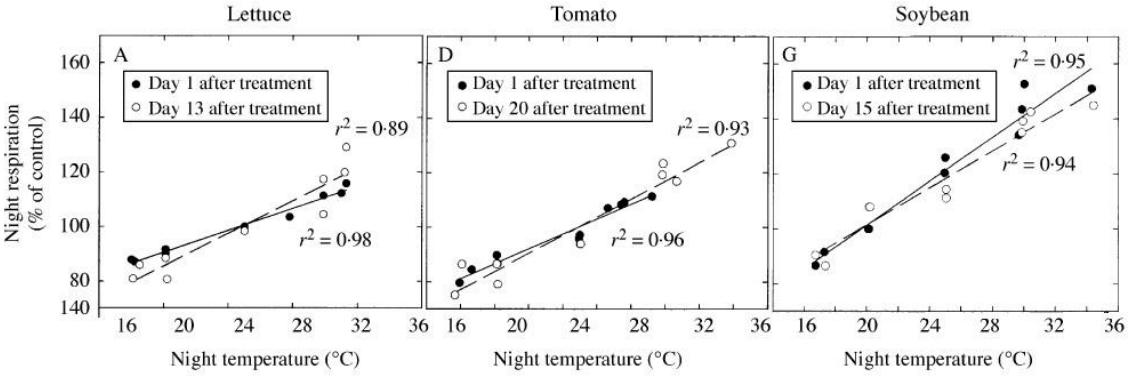

Figure 5. Effect of night temperature on night respiration for lettuce, tomato, and soybean. Data are shown relative to the control for the first and last day of treatment. The night temperatures were changed on day 16 for lettuce and tomato and day 17 for soybean. Control plants of lettuce and tomato were grown at constant day/night temperature of 25°C, while soybean at constant day/night temperature of 20°C. The slopes between the first and last treatment day are not statistically different from one another within a species and parameter (lowest *P* value = 0.29, d.f. = 8), which indicates that there was no acclimation to temperature. From Frantz et al. (2004).

1.3.5.2.1.1.2 CO_2

High atmospheric CO_2 increases the concentration of intercellular CO_2, which eventually leads to increased photosynthesis (Smith and Dukes 2013), assuming no limitation of nutrients (Reich et al. 2006). The literature presents evidence for increased respiration associated with increased photosynthesis at elevated CO_2, for example Free-Air CO_2 Enrichment (FACE) experiments have shown that elevated CO_2 stimulates respiration (expressed on area basis) in soybean. This result was confirmed by transcriptional analyses of carbohydrate status, elevated rates of photosynthesis, growth rate, and respiration (Ainsworth et al. 2006). Transcripts from hundreds of CO_2 responsive genes suggest that high CO_2 promotes the respiratory breakdown of carbohydrates and that long-term exposure to high CO_2 concentrations leads to a metabolic reprogramming that increases respiration, indicated by higher transcription factors encoding enzymes involved in the tricarboxylic acid cycle, glycolysis, and starch and sugar metabolism (Leakey et al. 2009b). Increased respiration at high CO_2 levels provides enough energy to match the high growth rates observed under these conditions. At the molecular level, transcript profiles show that genes encoding respiratory enzymes are up-regulated (Leakeyet al. 2009a). However, changes in transcript levels, enzyme activity, and respiration rates do not always correspond, as observed by Watanabe et al. (2014) who showed that the transcript levels of genes encoding respiratory enzymes increased under

high CO_2 conditions but enzyme activity remained unchanged. These discrepancies were also identified for associated metabolites in the light and dark periods, indicating that high O_2 uptake is not directly linked to metabolite production and enzyme activity, complicating the understanding of these mechanisms.

A plant's response to high CO_2 may be temporary, since it has the ability to acclimate to environmental variations, but other metabolic processes become unbalanced due to the increased gas exchange. Over time, the plant can adjust its growth and maintenance processes by down-regulating photosynthetic activity if the extra carbohydrates cannot be used or stored (Cook et al. 1998; Smith and Dukes 2013 and references therein). This may be the reason for the contradictory results regarding the effect of CO_2 level on respiration reported in the literature. In contrast to the FACE studies, short-term exposure to elevated CO_2 seems to have no effect on respiration rate, for example Ayub et al. (2014) showed that soybean leaf and root respiration is unaffected by CO_2 concentrations of 290, 400, and 700 ppm. In trees, negligible effects of CO_2 have been reported for nocturnal leaf respiration rate of deciduous species exposed to short-term treatments of 800 ppm (Amthor 2000a), and also for *Agrostis capillaris* and *Poa alpina* subjected to a doubling of ambient CO_2 (William et al. 1992). On the other hand, studies also report that high CO_2 can inhibit respiration rate due to reductions in the activity of Cyt *c* oxidase (Azcón-Bieto et al. 1994); a 5% increase in CO_2 concentration can be enough to inhibit the enzymes of the glycolytic pathway and O_2 uptake (Kerbel et al. 1990). Studies have shown that elevated CO_2 concentrations in the range of 700-800 ppm reduced respiration of *Mentha sativa*, *Phragmites australis*, *Quercus ilex*, *Zea mays, Glycine max* and woody plants (Thomas and Griffin 1994; González-Meler et al. 1996; Curtis and Wang 1998; Drake et al. 1999; Pinelli and Loreto 2003), though some researchers have questioned the validity of these results, based on methodological issues (Amthor and Baldocchi 2001; Davey et al. 2004). Notwithstanding, the degree of reduction in respiration rates caused by high CO_2 concentrations varies according to environmental conditions and species. When doubling the ambient CO_2 concentration, Amthor (1997) estimated a reduction of about 15% in the respiration of leaves, roots, and stems of a range of species cited in more than 30 studies; this percentage is close to the 18% reported by Curtis and Wang (1998) in a meta-analysis of the effects of elevated CO_2 (590-790 ppm) in the leaves of woody plants. Leaf respiration rate of soybean was reduced by 25% in plants exposed to a dark

period (8 h, 25 °C) at 1400 ppm CO_2 compared to 370 ppm CO_2 (Bunce 2004). In these experiments, the changes in respiration associated with CO_2 concentration were accompanied by similar variations in nitrate reduction and carbon translocation out of the leaf, suggesting that both processes were co-limited by the reduced energy available from respiration. The reduction in respiration seems to be determined by the plant's ability to acclimate, since the reduction is dramatically less in plants acclimated to high CO_2. For example, leaf respiration rate of soybean was reduced by 30% in plants grown at 352 ppm CO_2, while in plants grown at 703 ppm CO_2 it reduced by only 16% when switched to elevated CO_2 treatment (Thomas and Griffin 1994). Some researchers claim that the discrepancies in the literature may relate to standardization, as results vary when expressed by area, dry mass, or nitrogen content basis (Lambers and Poorter 1992; Curtis and Wang 1998); but others say these factors would not cause contradictions (Azcón-Bieto et al. 1994; Curtis 1996; Ayub et al. 2014).

Under high CO_2 and high air temperature conditions, variation in respiration rate seems to be largely determined by temperature. When studying the combined effect of high CO_2 (+ 350 μmol mol^{-1} for 24 h day^{-1} throughout the year) and high air temperature (+2 and +6 °C) in needles of field grown *Pinus sylvestris* respiration rate increased by as much as 30% compared to the control (ambient conditions), but when the individual effect of these two factors was analyzed, respiration was reduced by high CO_2 and increased by high air temperatures, suggesting that under conditions of high CO_2 and high air temperature, respiration rate is largely driven by temperature controls (Zha et al. 2001). Studies with soybean leaves support these results, showing that if high air temperature (25 °C) is maintained, respiration is increased at least 20% at all CO_2 concentrations from high (1400 ppm), ambient (370 ppm) or low (40 ppm) levels, compared to controls of 20 °C and 370 ppm (Bunce 2004).

The effects of low CO_2 are relatively poorly documented. Given that low CO_2 concentrations promote high nitrogen content in tissues, and given that total respiratory rates are highly influenced by maintenance respiration, higher respiration rates can be expected at low CO_2 levels, though this has not been clarified. Ayub et al. (2014) offered two hypotheses for their findings that low (290 ppm) and variable levels of CO_2 had no effect on soybean leaf and root respiration. In leaves, the authors attributed this result to the fact that measurements were performed on mature leaves where a major proportion of

respiration was maintenance respiration from non-growing tissue. In the case of roots, they proposed that the chemical composition of root tissue and the associated changes in energy demand could be the cause, due to the clear presence of mixed young fast-respiring tissue and mature slow-respiring tissue. In this respect, more studies are needed to better understand the effect of low/high CO_2 on respiration.

In summary, CO_2 effects on respiration rate are highly variable. At high CO_2, the decrease in respiration seems to be associated with inhibition of enzyme activity, while high CO_2 concentration – thus favouring higher photosynthetic rates – result in a higher availability of assimilates able to accelerate respiration (Davey et al. 2004). The latter explanation is stronger and more logical, suggesting that short- and long-term exposure to elevated or reduced CO_2 concentration can result in different plant responses. Nevertheless, there is also strong evidence showing that – irrespective of the CO_2 level – respiration rate remains unaffected and that variations observed are not permanent.

1.3.5.2.1.1.3 Substrate availability

Substrate availability is a major driver of respiration rate in sink tissue (McCree and Troughton 1966; Noguchi and Terashima 1997; Covey-Crump et al. 2002; Gifford 2003). The main substrates of respiration are sucrose and starch, but even though many compounds can be respired, the relative contribution of proteins and lipids to plant respiration is negligible. Several studies show that respiration rate is increased under conditions that promote high availability of carbohydrates (Ayub et al. 2014). By contrast, conditions such as low irradiance environments, temperature variations, and short photoperiods encourage low substrate availability and thus low respiration rates. For example, it has been shown that exposure of *Arabidopsis thaliana* to low irradiances (40-80 µmol m^{-2} s^{-1}) for just one day led to up to 90% less accumulated carbon reserves and about 25-50% reduced plant respiration, when compared to plants exposed 160 µmol m^{-2} s^{-1} (Pilkington et al. 2015).

Rajendrudu et al. (1987) reported that respiration of tropical dicot weeds was highly and positively associated with higher photosynthetic rates and attributed this to the high carbohydrate levels found under those conditions. Previous studies with wheat leaves, tall fescue, and soybean showed high respiratory rates associated with high carbohydrate contents (Fig 6; Coggeshall and

Hodges 1980; Moser et al. 1982; Azcón-Bieto and Osmond 1983; Hrubec et al. 1985). For mature wheat leaves, the rate of CO_2 efflux was higher after a period of photosynthesis than at the end of the night, also suggesting an association with accumulated carbohydrates in the leaf (Table 3; Azcón-Bieto and Osmond 1983).

However, variability has been observed in respiration sensitivity to availability of non-structural carbohydrates in different organs of tall fescue (*Festuca arundinacea*); the organs with higher concentrations of non-structural carbohydrates showed the highest respiration rates (Fig 7; Moser et al. 1982). This study showed that after 20 hours of a dark treatment, leaf terminal meristems decreased their respiration rate if the concentration of non-structural carbohydrates was less than 35%, while collared leaf blades did not show a decline in respiration rate until non-structural carbohydrates dropped to 20%. They concluded that respiration rate is more affected by carbohydrate status in growing tissue i.e. meristems, due to the high growth respiration requirements.

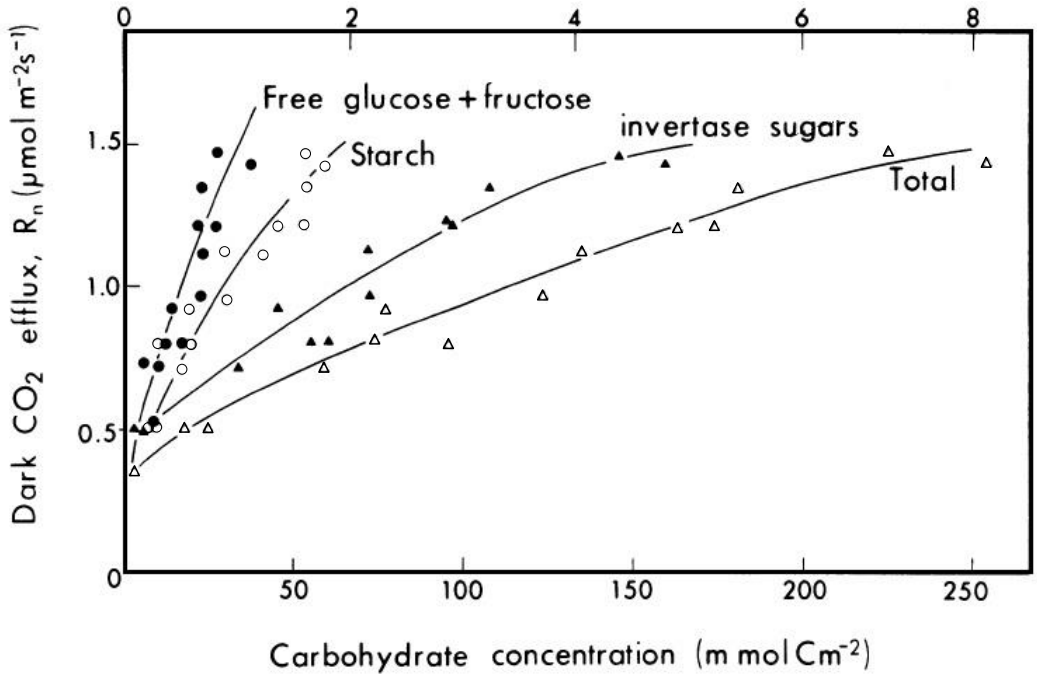

Figure 6. Relationship between dark CO_2 efflux and several carbohydrate fractions in mature wheat leaves. In this study invertase sugars comprised those carbohydrates resulting after hydrolysis with the enzyme invertase (for example hydrolysis of sucrose and small fructosans). Reproduced with permission from Azcón-Bieto and Osmond (1983).

Table 3. R_d of mature leaves of three plant species measured at the end of the night and after a period of photosynthesis of 5 hours. R_d was measured in 21% O2 and leaf temperature of 21 °C in the light and in the dark. Reproduced with permission from Azcón-Bieto and Osmond (1983).

Species	CO_2 during the photosynthetic period (µbar)	Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)	R_d at the end of the night (µmol CO_2 $m^{-2}s^{-1}$)	R_d after 5 hours light (µmol CO_2 $m^{-2}s^{-1}$)
Eucalyptus grandis	340	13	0.82	1.07
	800	17	1.20	1.50
Vicia faba	800	21	0.77	1.05
Lolium perenne	800	22	0.65	0.87

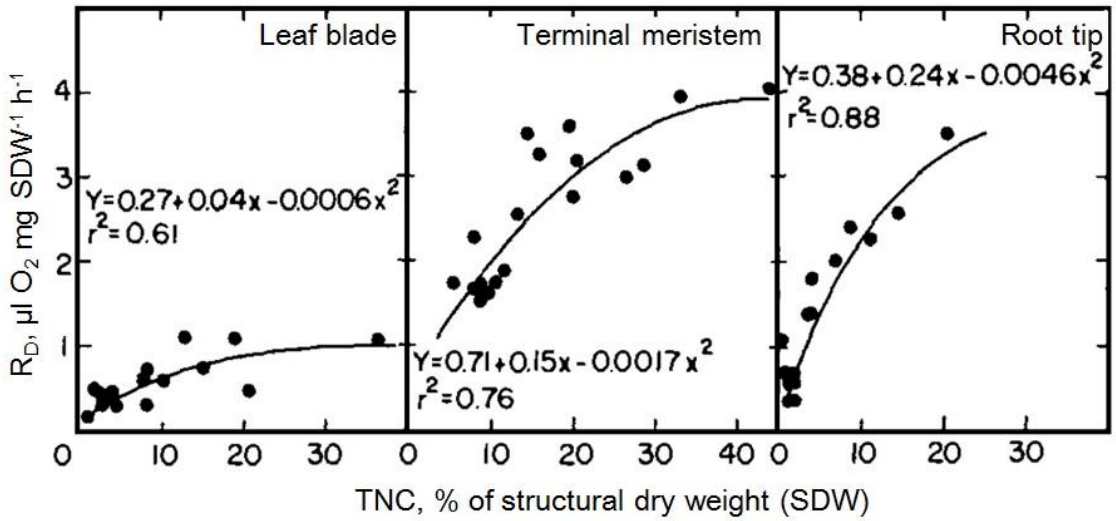

Figure 7. Relationship between dark respiration (R_D) and total non-structural carbohydrate (TNC) concentrations in tall fescue leaf blades, leaf terminal meristems in vegetative tillers (where leaves are initiated), and root tips. From Moser et al. (1982).

According to Azcón-Bieto and Osmond (1983), the photosynthetic process can be inhibited by high carbohydrate levels (Fig 8). Thus it is reasonable to assume that respiration may provide a regulatory function, since sugar accumulation can result in phosphate limitation, reducing the rate of RuBP regeneration (Herold 1980). Earlier studies tried to test this hypothesis, manipulating plant growth by removing the spike and applying DMCU herbicide (King et al. 1967). These studies showed that the accumulation of assimilates inhibited further photosynthesis (Birecka and Dakic-Wlodkowska 1963), though results from defoliation treatments can be confounded by other effects triggered when source and sink are manipulated. Experiments by Azcón-Bieto (1983) tried to exclude these confounding factors by instead increasing/decreasing the CO_2

concentration, or applying a chilling treatment to limit the rate of translocation; wheat leaves were exposed to long periods of illumination and it was showed that CO_2 uptake rate declined when assimilate export rate was limited by chilling the base of the leaf or exposure to low temperatures (Fig 9). The authors indicate that above a critical level of carbohydrate, photosynthesis was inhibited but it recovered when the plant was exposed to a short period of darkness enabling the use of stored carbohydrates; it has been proposed that the accumulation of carbohydrates impairs the production/consumption of energizing molecules in photosynthesis.

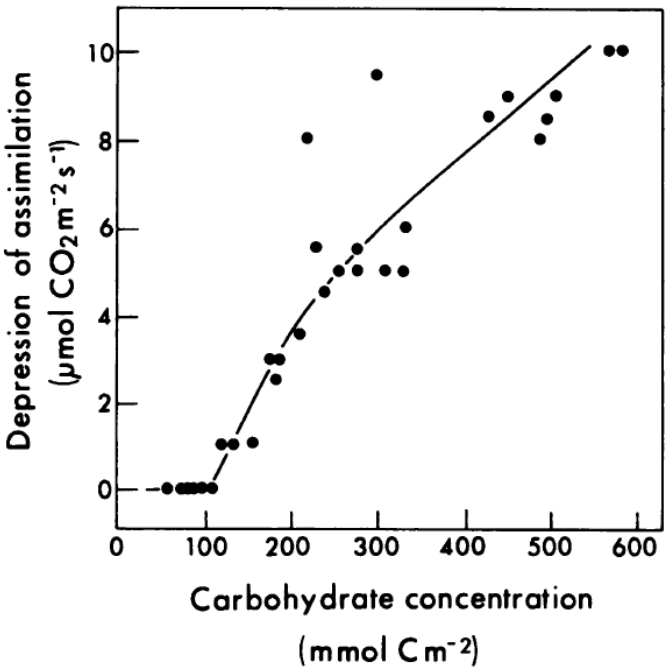

Figure 8. Relationship between net CO_2 assimilation and total carbohydrate concentration in wheat leaves after a period of photosynthesis. This relationship includes data obtained in experiments performed at different temperatures from 20 °C to 30 °C. Reproduced with permission from Azcón-Bieto (1983).

The positive correlation of rates of respiration and photosynthesis has also been tested across species. Villar et al. (1995) exposed *Lepechinia fragans* and *Heteromeles arbutifolia* leaves to conditions that would theoretically encourage high carbohydrate concentration, such as extended light periods (5 hours at photosynthetic photon flux density = 600 μmol m^{-2} s^{-1}) and high CO_2 (500 μl L^{-1} CO_2), and showed that dark respiration was significantly increased compared to respiration rate recorded after 9 hours of dark (Table 4). Similar results were reported by Azcón-Bieto et al. (1983) for wheat and spinach leaves; they observed that the rate of O_2 uptake and dark CO_2 emission decreased during the night when sugar levels were low, but after a period of photosynthesis the respiration rates rose. The authors observed no decrease in respiration rates of

pea leaves, which could be attributed to the more constant level of total carbohydrates observed; further exposure to longer periods of darkening caused a reduction in the level of free sugars and respiratory rates. The common trend observed in these studies provides definitive evidence of the key role of carbohydrate level in regulating plant respiratory rates, though differences between species can be expected.

To summarize, high carbohydrate levels promote high respiratory rates; nevertheless, it is important to clarify if this is a physiological strategy to avoid photosynthesis inhibition, or if it is in fact a matter of substrate concentration dependence of the reaction where the maximum velocity of product generation, in this case CO_2, is reached when there is no limitation of substrate (carbohydrates).

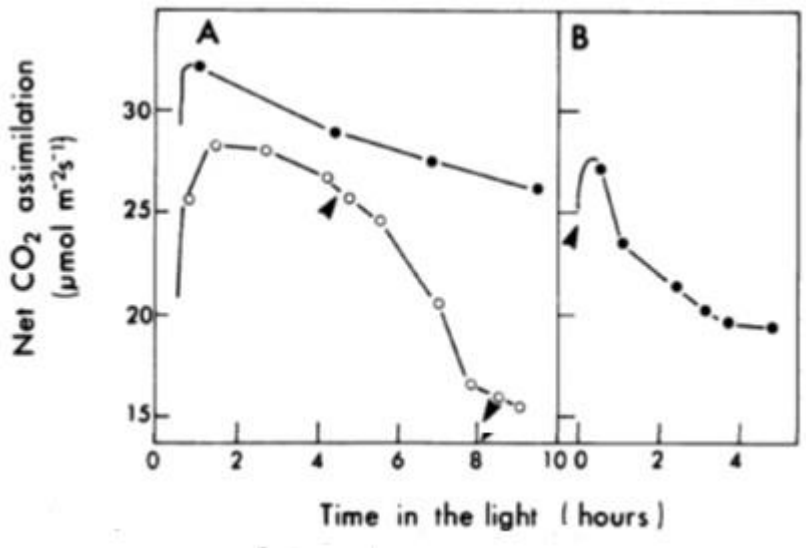

Figure 9. Effect of chilling the base of a wheat leaf on the rate of net CO_2 assimilation. External CO_2 pressures were 700 to 770 *μ*bar (A) and 800 *μ*bar (B). The application of chilling treatments is indicated by arrows. An unchilled control is also shown (●).Reproduced with permission from Azcón-Bieto (1983).

1.3.5.2.1.1.4 Light

This section addresses the effect of light on: a) the rate of non-photorespiratory mitochondrial CO_2 released in the light (Rl), and b) the rate of dark respiration (Rd). Variations in irradiance can affect respiration rate in a *direct* or *indirect* way. The direct effect of light on respiration is studied through the analysis of R_l shifts and seems to result in R_l inhibition; in the literature it is common to find

the % of inhibition of R_l by light in reference to R_d or the R_l:R_d ratio. On the other hand, there is evidence supporting an indirect stimulation of R_d by high irradiance because these conditions are associated with high photosynthetic activity and high availability of respirable substrates.

Table 4. Effect of extended dark and light periods on the values of R_l (dark respiration under light conditions) and R_d (dark respiration) in medium-aged leaves of *H. arbutifolia* and *L. fragans*. Measurements under extended dark and light periods were considered after 9 h of darkness (beginning of the day) and after 5 h at a photosynthetic photon flux density (PPFD) of 600 μmol m^{-2} s^{-1} and an ambient CO_2 concentration of 500 μl^{-1} (prolonged illumination), respectively. Values represent means ± SE of two measurements. From Villar et al. (1995).

Species	Treatment	R_d	R_n	Inhibition
		nml CO_2 g^{-1} s^{-1}	*nml CO_2 g^{-1} s^{-1}*	%
H. arbutifolia	Begining of the day	2.4 ± 0.2	5.6 ± 0.7	57.3 ± 0.7
	Prolongued illumination	3.4 ± 0.1	7.1 ± 0.2	52.4 ± 1.0
L. fragans	Begining of the the day	3.0 ± 0.1	7.8 ± 2.3	57.9 ± 11.9
	Prolongued illumination	4.1 ± 0.2	11.3 ± 0.9	63.4 ± 0.5

The direct effect of light on respiration rate (R_l)

Determining the effect of light on R_l (non-photorespiratory mitochondrial CO_2 release, or dark respiration, in the light) is challenging because, unlike in the dark, other gas exchange processes (photosynthesis, photorespiration, and re-fixation) also occur at the same time, masking the CO_2 and O_2 exchange (for a review see Nunes-Nesi et al. 2007). Even when R_l and R_d are positively associated, R_l tends to be lower than R_d, thus suggesting that light inhibits the former (Villar et al. 1995; Griffin and Turnbull 2013). Under light conditions, oxidative phosphorylation is substituted by photophosphorylation to some degree (Lambers 1997), resulting in reduced release of CO_2. Inhibition of respiration by light has been associated with the regulatory effect of photosynthetic ATP and NADPH on respiratory enzymes (Graham 1980); the ATP produced via photosynthesis increases the cytosolic phosphorylation potential, resulting in inhibition of the oxidative phosphorylation in the light. The

degree of R_l inhibition ranges from 70-80% for wheat and bean leaves (Mangat 1975; Piesker and Apel 1980; Brooks and Farquhar 1985), 55% for *Heteromeles arbutifolia* Ait. and *Lepechinia fragans* Creene (Villar et al. 1994), and 50-60% for evergreen species (Villar et al. 1995).

An interesting study involving variations in temperatures, watering regimes, and CO_2 concentrations was performed by Ayub (2011) to evaluate the degree of inhibition of R_l and R_d of *Eucalyptus saligna* across a range of conditions. The authors showed that light inhibition of respiration is enhanced by water stress (Fig 10); average inhibition across different CO_2 levels and temperatures was 40% when the plants were not subjected to water stress, but this value increased to 60% for plants exposed to drought. However, a study using CO_2 isotopes refuted the inhibitory effect of light over R_l (Loreto et al. 2001). The authors performed an experiment in maize leaves and applied synthetic air with only $^{13}CO_2$ instead of $^{12}CO_2$ (Fig 11), before analyzing the amount of $^{12}CO_2$ exchanged by respiratory processes independently from the $^{13}CO_2$ fixed in photosynthesis. Using this method, they were able to determine that respiration rate R_l is not affected by light, and that 60-90% of total $^{12}CO_2$ generated from respiration under light is re-fixed within the mesophyll by leaves with high rates of photosynthesis. They showed that the amount of re-fixed and emitted $^{12}CO_2$ under light conditions was close to R_d, suggesting that the difference between R_l and R_d was due to the re-fixation process rather than an inhibitory effect of light on R_l, and that R_l may be light-inhibited only under severe water or salt stress when the fraction of re-fixed CO_2 drops. Thus, while the literature seems to show a tendency for light to inhibit R_l, more exploration is required in C3, C4, and CAM plants to clarify the role of CO_2 re-fixation and the underlying mechanisms involved. The degree to which light inhibits R_l seems to be linked to the level of photorespiration, but neither the direction nor mechanism is clear. A number of authors report that when the level of photorespiration is high, R_l decreases, (this is, increased inhibition of R_l) (Wang et al. 2001; Shapiro et al. 2004; Hurry et al. 2005; Zaragoza-Castells et al. 2007), but other studies indicate the opposite. For example, Tcherkez et al. (2008) showed that the degree of inhibition of R_l decreased when leaves of *Xanthium strumarium* were exposed to low CO_2 levels, which promoted an increased demand of TCA substrates associated with the recovery of photorespiratory cycle intermediates in the peroxisome (Ayub et al. 2011; Crous et al. 2011; Griffin and Turnbull 2013).

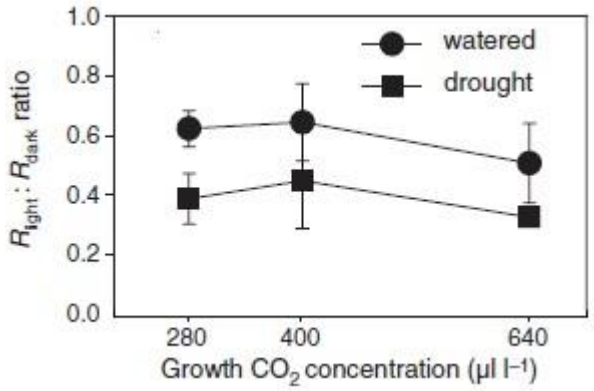

Figure 10. Enhancement of R_l inhibition by sustained drought in *Eucalyptus saligna* plants grown at 26 °C at different atmospheric CO_2 concentrations. Reproduced with permission from Ayub et al. (2011).

Indirect stimulation of high respiration rates (R_d) due to high irradiance conditions

Unlike R_l, respiration rate under dark conditions (R_d) seems to be indirectly stimulated when the tissue/organ/plant is exposed to light during the period preceding the measurement, this phenomenon is known as light enhanced dark respiration. High light conditions increase photosynthetic rates and consequently the amount of substrate available for respiration (Davis 1950; Azcón-Bieto et al. 1983; Bingham and Farrar 1988; Fig 6), which is identified as a main factor controlling respiratory rates (see "*Substrate availability"* section). In *Triticum aestivum, Eucalyptus grandis*, *Vicia faba*, and *Lolium perenne* leaves, R_d recorded after exposure to light (for 6.25 h) increased by about 25-35% compared to R_d measured at the end of the night (Azcón-Bieto and Osmond 1983; Fig 12).

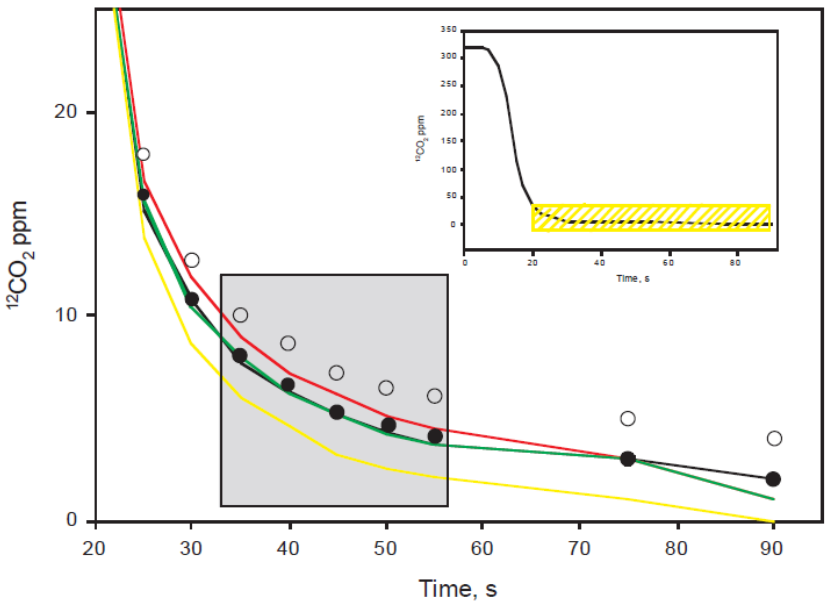

Figure 11. Experiment with maize leaves using CO_2 isotopes. The authors applied synthetic air with only $^{13}CO_2$ instead of $^{12}CO_2$. Time-course of $^{12}CO_2$ wash-out in the gas exchange apparatus. At t = 0 (chosen when photosynthesis was steady) $^{12}CO_2$ at 320 ppm was replaced by an equal concentration of $^{13}CO_2$. The time necessary to wash-out $^{12}CO_2$ in the cuvette without leaf is shown in the inset. The yellow section is magnified in the main figure. Different line colours distinguish the wash-out with the cuvette without leaf (yellow) and those with a *Cichorium intybus* leaf illuminated (1000 μmol photons m^{-2} s^{-1}) and maintained at 21 (red) or 2 (green) $\%O_2$, or exposed for 30 min to darkness (black). Overlapping of lines in some sections of the graph resulted in only one visible line-colour. Symbols represent the wash-out of the empty cuvette when the $^{13}CO_2$ was supplemented with 2 (solid circles) or 4 (open circles) ppm $^{12}CO_2$. Reproduced with permission from Loreto et al. (1999).

Several experiments have shown that variations in light intensity can affect R_d; for example, Pilkington et al. (2015) showed that decreasing light intensity from 160 to 80 and 40 µmol $m^{-2}s^{-1}$ reduced photosynthetic rates of *Arabidopsis thaliana* by about 55-90%, and also reduced R_d by up to 52% in the following night (Fig 13). The authors attributed these large decreases to the estimated reductions in maintenance respiration. Similarly, studies with shade plants showed that respiration rates of mature leaves of *Alocasia odora* were higher when plants were transferred from low (20 μmol m^{-2} s^{-1}) to high (330 μmol m^{-2} s^{-1}) irradiances, suggesting a positive association between exposure to high light intensities and respiration rates (Noguchi et al. 2001). However, for *A. odora,* the authors propose that the change in respiration was associated with the rate of consumption of ATP rather than the availability of substrate, given that they also observed that the addition of sucrose to leaf segments had no effect on the rate of O_2 uptake, unlike the addition of an uncoupler of oxidative

phosphorylation from a respiratory chain activity. It is important to point out that this observation may or may not be valid for non-shade tolerant species, and with this in mind, it would be useful to conduct further exploration to assess if respiration rates are promoted/limited by rates of ATP consumption or by substrate availability. Hill and Bryce (1992) suggested that light enhanced dark respiration is due to malate oxidation given that in their experiments with barley mesophyll protoplasts showed an increase in the accumulation and fast assimilation of malate while the levels of sucrose and glucose did not change.

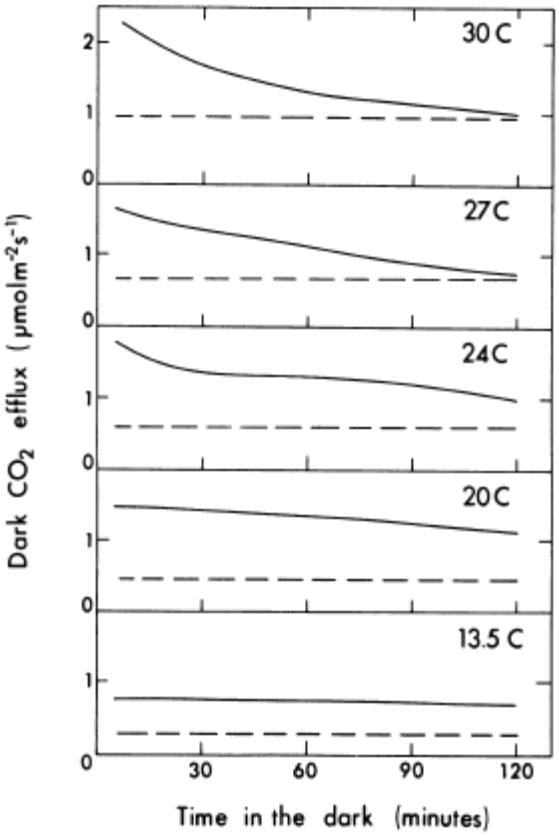

Figure 12. Time course of dark CO_2 efflux of mature wheat leaves after a period of photosynthesis of 6.25 hours at ambient CO_2 and O_2 levels (line) or at the end of the preceding night/dark period (dashed). Reproduced with permission from Azcón-Bieto and Osmond (1983).

Light quality also can modify respiration rate, as shown with shortwave visible radiation. The effect of blue light in chlorophyll-free mutants of *Chlorella* was reviewed by Kowallik (1982), who demonstrated that blue light increased respiration rates, in contrast to red light, which acts as an inhibitor. Several studies have shown that respiration rate is enhanced after a period of illumination with blue light, and that the decay rate until reaching normal respiration levels is slower in blue than red illuminated *Chlorella*. O_2 uptake in cells of *Chlorella* and *Scenedesmus* algae exposed to blue light was reported to

increase 50% compared to respiration in darkness (Kulandaivelu and Sarojini 1980; Miyachi et al. 1980). There is maximum effect between 370 and 460 nm, while no light effects were identified at 550 nm (Kowallik and Gaffron 1966). An enzymatic regulatory effect of blue light on ATP distribution in the cytosol was suggested by the authors. Blue light enhanced the phosphoenol pyruvate carboxylase activity which resulted in increased respiration (Kamiya and Miyachi 1974).

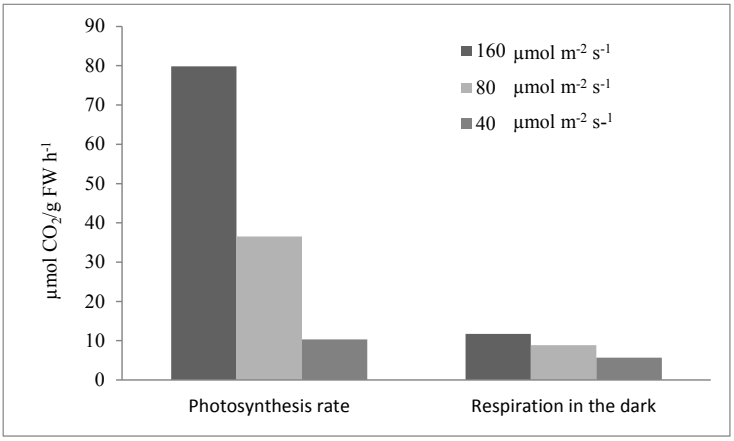

Figure 13. Photosynthesis and R_d rates of *Arabidopsis thaliana* leaves after changing light intensity from 160 to 80 and 40 µmol m^{-2} s^{-1} in the day preceding the measurement. Data from Pilkington et al. (2015) were used to make the figure.

In conclusion, the literature suggests that respiration rate is directly (R_l) or indirectly (R_d) influenced by light intensity and light quality. Generally, R_l seems to be inhibited by light in about 50-80%, but studies using CO_2 isotopes have not supported this idea. There is a link between the degree of R_l inhibition and photorespiration but at this stage it is not possible to clarify the direction. What seems to be consistent across studies is the indirect stimulation of R_d by high light conditions prior to the measurement; nonetheless, the extent of this stimulus would be determined by the combined effect of availability of assimilates and the rate of consumption of respiratory energy (ATP).

1.3.5.2.1.1.5 Drought stress

Negative carbon balances can occur under drought conditions as result of increased respiration and low photosynthetic activity (Reichstein et al. 2002), though – given the limited generation of photo-assimilates under water stress conditions – it is reasonable to expect low respiration rates. In spite of this, low substrate availability is not the only driving mechanism of respiration under

drought stress, the demand for respiratory products and the abundance of mitochondria also play a central role. The literature shows both increases and decreases in respiration rates as a result of drought stress. Respiration response to drought may depend on the severity of the stress; moderate drought can reduce respiratory capacity since lower cellular and enzymatic activity leads to a "*standby"* state, whereas a more severe drought can induce respiration as a result of activation of the drought tolerance mechanisms (Flexas et al. 2005; Atkin and Macherel 2009).

The directionality of the drought effect on respiration can be determined by co-occurring abiotic stresses such as high temperature and high irradiance, i.e. factors that enhance respiratory rates. Contrasts can also be observed under drought stress for whole plant respiration or for individual organs, and the age of the tissue also impacts the effect of drought on respiration. Atkin and Macherel (2009) demonstrates that the respiration of growing tissue (i.e. roots and whole plants) tends to be inhibited by water stress, while in mature tissue (i.e. leaves) the response is more variable, showing reductions or no effect depending on the severity and duration of the drought stress.

Increments of respiration rate by drought stress

Moderately water-stressed young tissue (e.g. bread wheat leaves) has shown large increases (>40%) in respiration under drought stress (leaf relative water content 75%; Bartoli et al. 2005). In this case, the large increase in leaf respiration seemed to be linked to the double O_2 uptake by the alternative pathway (AOP). Recent studies have shown associations between the AOP and changes in mitochondrial respiration under moderated drought. Dahal et al. (2014) showed that – under water stress – AOP knockdown transgenic *Nicotiana tabacum* displayed lower rates of R_l and R_d than the wild type; on average, R_d was reduced by about 18% compared to the wild type. However, the AOP pathway seemed to be more relevant in maintaining R_l under drought, since R_l reductions in the knockdowns lines reached up to 40%; leaf carbohydrate analysis revealed no assimilates limitation during this study. This suggests that the AOP pathway plays a central role in plant recovery after severe water stress, given that it is associated with decreased generation of ROS reducing cell damage (Wang and Vanlerberghe 2013). Gratani et al. (2008) showed that evergreen species grown under drought stress showed increased respiration rates despite a reduction in photo-assimilation, which may

be linked to the remobilization of stored material (recognized as an important drought resistant mechanism; Blum 1998).

Inhibition of respiration rates by drought

In un-watered seedlings (< -0.6 Mpa) of *Fagus sylvatica*, leaf respiration (at 25 °C) was about 20% lower than in normally irrigated plants (Rodriguez-Calcerrada et al. 2010). However, the authors suggested that this difference in leaf respiration was due to lower respiratory capacity rather than differences in carbohydrates, given that they did not find differences in the concentration of soluble carbohydrates between watered and un-watered plants. It seems that stomata closure induced by drought results in lower assimilate levels and reduced respiratory capability (Azcón-Bieto 1983; Gifford 2003; Ciais et al. 2005; Atkin and Macherel, 2009; Rodriguez-Calcerrada et al. 2010; Sanhueza et al. 2013).

In experiments with *Eucalyptus saligna* water stress was induced by stopping irrigations for one_month at five months after sowing, and then limited to maintain daily stomatal conductance between 0.05–0.10 mol H_2O m^{-2} s^{-1}. Similarly to R_d, leaf R_l was shown to be inhibited by sustained water stress (Fig 14); yet R_l exhibited greater sensitivity to water stress, showing 52% inhibition compared to the 17% inhibition reported for R_d measured at ambient temperature (26 °C) and several CO_2 concentrations (average across 280, 400, and 640 *µ*l l^{-1} CO_2; Ayub 2011).

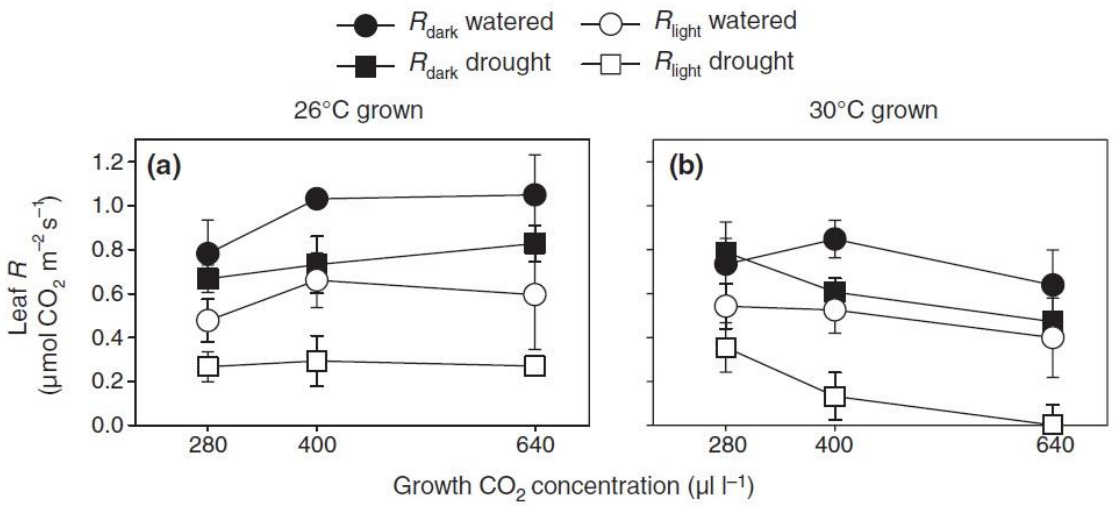

Figure 14. Leaf respiration of *Eucalyptus saligna* in the light (R_l) and the dark (R_d). Effects of growth temperature and sustained drought stress on area based rates of leaf respiration rates. Values are means (n = 4, ±SE). R_l values were calculated assuming infinite internal conductance (i.e. *gi* = ∞), with rates of R_l corrected for irradiance-dependent changes in internal CO_2 concentrations (*ci*). Reproduced with permission from Ayub et al. (2011).

The combined effects of drought and high temperatures seem to decrease respiration on global ecosystem scales (Bowling et al. 2002). Contrary to the expected increase in respiration at high temperatures, an analysis of gross ecosystem primary productivity across Europe showed a decrease of 77 g carbon m^{-2} yr^{-1} in total ecosystem respiration (soil and plant) due to water and heat stress, resulting in reduced gross primary productivity of 195 g carbon m^{-2} yr^{-1} (Ciais et al. 2005).

In summary, drought effects on respiration rates are highly variable with studies showing inhibition, stimulation, or no effect. Growing condition and species effects are observed. Nonetheless, the majority of studies describe a decrease in respiration rates caused by drought. Most studies focus on the impact of drought stress on leaf respiration, and little is known about other tissues. Further research would help explore the effects of drought and co-occurring additional stresses such as high air temperatures and high irradiances, given that real environmental constraints rarely occur in isolation. For a detailed review on the effect of drought stress on respiration see Atkin and Macherel (2009).

1.3.5.2.1.1.6 Chlorosis

Chlorosis is the visual symptom of leaf senescence (Thimann, 1987) but it can be induced by additional factors unrelated to plant phenology, such as nutrient limitation and other stress conditions (Wada et al. 2009). Chlorosis is characterized by low photosynthetic rates that reduce production of assimilates (Proietti 1998); given the triggering effect of carbohydrate availability (see "*Substrate availability"* section), lower respiration rates can be expected in chlorotic tissue, compared to green tissue. This hypothesis is supported by studies using growth regulators to delay senescence (Han 1995; Franco et al. 1997). However, in non-senescent chlorotic leaves, yellowing seems to primarily come from an excessive accumulation of assimilates that down-regulate photosynthesis (Schupp et al. 1992; Sicher 1998; Zhou and Quebedeaux 2003). In this regard, an interesting study performed in apple leaves showed that chlorosis was associated with high respiration rates, as well as higher activity of key enzymes related to glycolysis and the TCA cycle, in comparison to green tissue (Wang et al. 2010). The authors suggested that

increases in respiratory rates were due to the high availability of non-structural carbohydrates, which also inhibited photosynthesis. Non-senescence related leaf chlorosis was studied in chlorotic *Arabidopsis* mutants defective in the mobilization of starch degradation products (Hwang et al. 2013); based on proteomic analyses the authors suggested that chloroplast degradation in this chlorotic mutant was due to proteolytic processes, given the increased expression of a proteasome component named 20S, which suggested up-regulation of proteins involved in degradation of oxidized proteins and increased protein turnover (Coux et al. 1996; Kurepa et al. 2008).

The respiratory response to chlorosis is still poorly studied, compared to the effects on photosynthesis; this can be partly attributed to the challenge of measuring and quantifying plant respiration. The literature shows decreased respiratory rates associated with chlorotic tissue, regardless of the inducing factor. However, the effects of leaf chlorosis on respiration rates needs further exploration in order to determine differences across species and interaction with external factors like the occurrence of stress.

1.3.5.2.1.2 Techniques for measuring respiration

Two approaches are used to determine dark respiration: measurement of CO_2 emission and of O_2 consumption. The rate of CO_2 released is usually recorded using an infrared gas analyser system (IRGA), which detects the signal transmission in the infrared based on the fact that CO_2 absorbs radiation at ˜2000 nm. A controlled flow is applied over the leaf, which is enclosed in an isolated chamber used to detect the change in CO_2 (Fig 15). A gas exchange system in multiple chambers can be used to continually monitor CO_2 evolution (Fig 16); this type of technique is an adaptation of the IRGA system (van Iersel and Bugbee 2000; Frantz et al. 2004). However, the determination of respiratory rates through the measurement of CO_2 flux has the drawbacks that CO_2 is also produced in other metabolic processes (e.g. the oxidative pentose phosphate pathway), and that respiration is not always the main source of evolved CO_2 (Sweetlove et al. 2013).

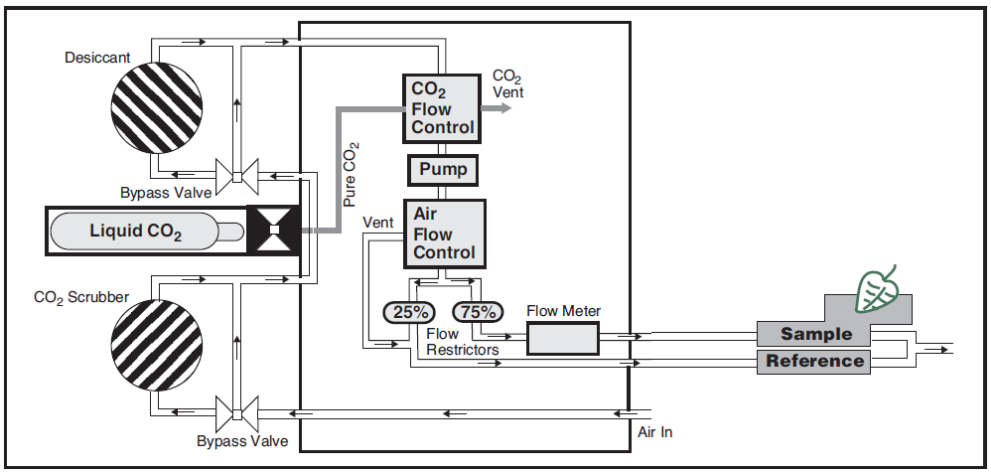

Figure 15. Schematic design of an interchange gas analyser. This figure shows a LI-6400XT flow schematic, with CO_2 mixer by Li-Cor Inc. (Lincoln, NE, USA). Reproduced with permission from Licor Biosciences

O_2 consumption rate is commonly recorded in a Clark type electrode chamber (Clark 1956), which was initially used to measure oxygen in blood (Fig 17). The principle of this instrument relies on the electrochemical reduction of O_2 at a negatively polarized electrode comprised of a silver/silver chloride-anode and a platinum-cathode immersed in an electrolytic solution (KCl). A polarizing voltage of about 0.7 V is applied, resulting in ionization of the electrolyte and its flow throughout the electrode. The magnitude of this flow is proportional to the oxygen dissolved in the electrolyte. The same principle is applied for measurements in gas or aqueous phases. The inconvenience of the Clark electrode is its slow response and high sensitivity to electrical variations. An alternative technique is the fibre optic method, which shows the same response as the Clark electrode but provides more stable signals against electrical disturbances and avoids the large time required for the membrane preparation in the Clark type electrode (Tyystjarvi 1998). This sensor is based on the detection of oxygen fluorescence and phosphorescence quenching when a flourophore is embedded in an oxygen permeable polymer matrix.

Figure 16. A multiple chamber gas-exchange system used for continuous monitoring of respiration and photosynthesis. In the figure five chambers are shown on each side of a walk-in growth chamber. Each chamber has a reflective skirt wrapped around the outside to minimize the side lighting. From Frantz et al. (2004).

Under illuminated conditions, the quantification of non-photorespiratory mitochondrial CO_2 release (dark respiration in the light, R_l) is additionally complicated by the issue of diverse sources releasing or consuming CO_2, i.e. photosynthesis, photorespiration, dark respiration, and CO_2 refixing (Graham 1980; McCashing et al. 1988). Mitochondrial respiration occurs during the 24 hours of the day; CO_2 is released and energy is produced in the TCA in the dark and light. Notwithstanding, as reviewed previously, R_l seems to be inhibited by light, and the degree of inhibition is frequently measured using the Kok and Laisk methods (Villar et al. 1994). The Kok method (Kok 1948) extrapolates the photosynthetic response to low irradiances, assuming a linear response of photosynthesis to light, however care must be taken due to the existence of a breakpoint in the linearity known as the "Kok effect". The Laisk method requires low CO_2 concentrations that limit photorespiration and promote R_l (Tcherkez et al. 2008; Ayub 2011); this response is associated with changes in the cellular energy status. This method utilizes the response curves of photosynthesis to low CO_2 concentrations at several light intensities (Laisk 1977) and assumes no variation of photosynthesis within the irradiances used. Other techniques for the determination of respiration include the use of manometric systems (Warburg

1919), CO_2 isotope fractionation (Fig 11; Loreto 1999), and more sophisticated procedures such as mass spectrometry, electron paramagnetic resonance oximetry, photoacoustic spectrometry, dynamic nuclear polarization-based oximetry, and nuclear magnetic resonance oximetry (Gorkom and Gast 1996; Krishna et al. 2002; Kodibagkar et al. 2006; Presley 2006).

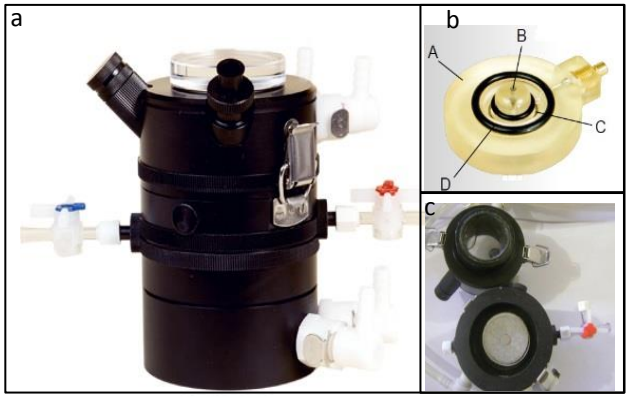

Figure 17. Clark type oxygen electrode chamber. This figure shows a LD2/3 electrode chamber from Hansatech Instruments Ltd. (Norfolk, England). *a*: Outer image of the whole chamber; *b*: electrode disc; *c*: open electrode chamber. In figure *b*, the electrodes are set into an epoxy resin disc (A) with the platinum cathode (B) at the centre of a dome surrounded by a well that contains the silver anode (C). An outer O-ring groove (D) seals the electrodes into the base of the electrode chamber. Reproduced with permission from Hansatech.

1.3.5.2.1.3 Types of respiration

In the literature, plant respiration is classified as either i) respiration from the cytochrome pathway, or ii) respiration from the cyanide resistant or alternative oxidase pathway (AOP). With reference to how the energy provided by respiration is used, at least two basic components are defined: i) growth (R_g) and, ii) maintenance (R_m). However, a number of processes cannot easily be categorized, such as phloem loading for long distance transport of photo-assimilates, nitrogen fixation in the leaves, and nitrate reduction in leaves and roots (Gifford 2003). A third component – "wasteful respiration" – is also sometimes considered. Early studies defined wasteful respiration as that which consumes excess carbohydrates but doesn't contribute to growth or maintenance (Lambers 1979). Some researchers suggest that even the AOP pathway can be considered wasteful respiration, since a reduced amount of energy is produced in comparison to the Cyt pathway (Gifford 2003), however

its protective role against ROS (González-Meler et al. 1999; Millenaar and Lambers 2003) suggests a maintenance function. There is no real consensus about the types of dark respiration, but the two basic components (growth and maintenance respiration) are still frequently referred to in relation to the cytochrome pathway. The classification of respiration into these two components (R_g and R_m) relates mostly to ecological-modelling purposes, though the boundaries between R_g and R_m are not very clear. In biochemical terms, respiration that occurs to maintain the cell is not different from that used in growing processes (van Iersel and Seymour 2000). Growth respiration is respiration that contributes to new growth, while maintenance respiration is respiration that enables the plant to maintain its existing biomass and remain alive. The nature of the components (chemical composition) also determines the specific R_g and R_m values since lipids, carbohydrates, and proteins are associated with inherently different amounts of energy. On the other hand, plant components that are non-biodegradable (e.g. lignin) do not require maintenance (Gifford 2003).

1.3.5.2.1.3.1 Growth respiration (R_g)

Cereal crops respire around 30-50% of assimilated C (Amthor 1989). A fraction of this respired C is used to generate new cellular structures and the rest to maintain the cell. Growth respiration (R_g) includes all processes involved in the production of new biomass, such as the production of ATP and compounds for biosynthesis of molecules, transport processes, and nutrient uptake and reduction (Chiariello et al. 1989). R_g is a function of daily carbon gain (DCG), a measurement of plant growth (van Iersel and Seymour 2000). Estimations of the fraction of total plant respiration that corresponded to R_g showed a linear relation between R_g and daily carbon gain (Fig 18). The efficiency of conversion is calculated as the amount of dry matter produced by each gram of glucose (Gifford 2003). R_g is reported to be unaffected by temperature or CO_2 concentration (McCree 1974; Thomas and Griffin 1994). Experiments comparing the R_g component under normal conditions and R_g under elevated CO_2 and high temperatures found no differences between treatments (Table 5; Zha et al. 2001).

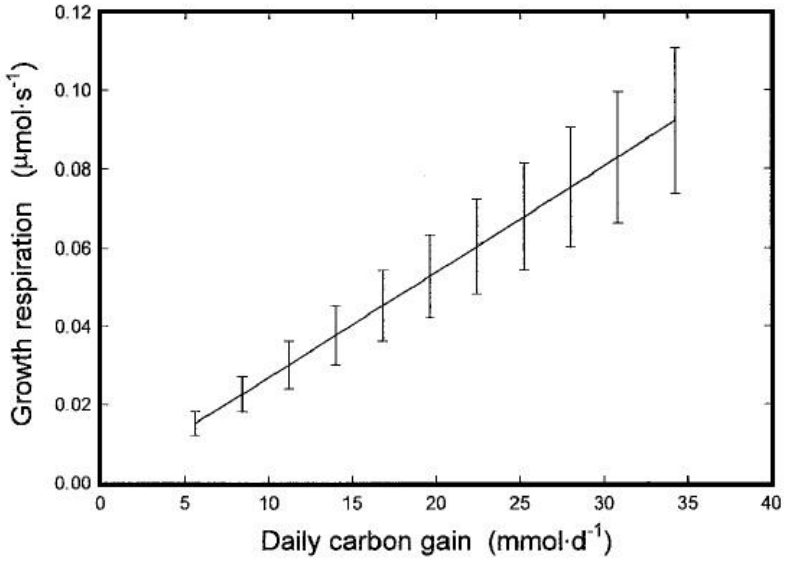

Figure 18. Estimated growth respiration (R_g) as a function of daily carbon gain (a measure of growth rate) of three groups of 28 vinca plants (*Catharanthus roseus*) during a 14-day period (means ±SE). Reproduced with permission from van Iersel and Seymour (2000).

1.3.5.2.1.3.2 Maintenance respiration (R_m)

Maintenance respiration (R_m) is a function of cumulative carbon gain (CCG) and is related to plant size (van Iersel and Seymour 2000). Protein turnover is the main maintenance process, but R_m also includes the maintenance of ion gradients and re-synthesis of other compounds (Chiariello et al. 1989). The coefficient of maintenance is calculated as the amount of glucose that needs to be respired to maintain one gram of dry matter (Gifford 2003). Liu et al. (2011) studied the rate of maintenance respiration in wheat and considered the effects of temperature, drought, and plant age in their mathematical models. The authors used two approaches, the first based on the dry weight biomass (McCree 1974) and a second accounting for nitrogen accumulation, since most of a plant's maintenance costs come from turnover of protein processes (Amthor and Baldocchi 2001). When estimating R_m in early stages, differences were identified between the two approaches that were attributed to the varying nature of the fast growing tissues in the first 45 days after sowing. In later growth stages, both models showed R_m ranging from 0.6-5.5% of dry weight. Previous field studies and theoretical estimations report similar values for R_m (Penning de Vries 1975; McCullough and Hunt 1993). When the effect of water stress was included, both models showed that – as expected – drought increased R_m, but to a minimal degree (Penning de Vries 1975). Water stress

was expected to modify respiration rate since it increases cell ion concentration and enzyme activity, and therefore maintenance requirements.

The R_m component is highly temperature dependent (Zha et al. 2001). In contrast to R_g, R_m increases exponentially then levels off at an optimum temperature level, while further increases in temperature result in reductions of R_m (Stoy 1965; Mitchell et al. 1991; Liu et al. 2011). R_m was shown to increase by 29% when needles of field-grown Scots pine were exposed to a heat treatment (2 and 6 °C above the control conditions) (Zha et al. 2001). This study reported that the R_m coefficient also increased when high temperatures were combined with high CO_2 concentrations, but it decreased 34% as a result of CO_2 alone (Table 5). Similar results were shown by Thomas and Griffin (1994), who reported that the maintenance coefficient of respiration increased by 34% when soybean was exposed to high CO_2 concentrations, whereas the growth coefficient of respiration was not affected. The authors showed that this increase in R_m was a consequence of an increase in the amount of nonstructural carbohydrates in leaves which also require energy for maintenance.

Table 5. Effect of elevated CO_2 (EC), high temperature (ET), and their combination (EC+ET) in the R_g and R_m coefficients of needles of Scots pine trees (*Pinus sylvestris*). *P*= 0.05. From Zha et al. (2001).

Treatment	R_g (mol Kg^{-1})	R_m (mol Kg^{-1} day^{-1})	r^2
CON	23.04 ± 2.53 a	0.26 ± 0.03 b	0.96
EC	24.35 ± 3.25 a	0.17 ± 0.03 c	0.97
ET	25.51 ± 2.66 a	0.37 ± 0.06 a	0.95
EC+ET	26.77 ± 3.27 a	0.33 ± 0.04 a	0.98

1.3.5.2.1.3.3 Alternative Oxidase Pathway (AOP)

During plant respiration, two terminal oxidases – the cytochrome oxidase and the alternative oxidase – compete for electrons from the ubiquinone pool. The alternative oxidase is encoded by the nuclear gene *Aox1* (Vanlerberghe and McIntosh 1997) and is the enzyme that catalyzes the reduction of carbohydrates in the cyanide-resistant, alternative mitochondrial respiratory pathway (AOP), which generally represents about 10-25% of total respiration in all plants (Taiz and Zeiger 1991). It was initially proposed that the AOP

functioned only as an overflow for the Cyt pathway, but it has since been demonstrated that the AOP can be activated by an allosteric effect of pyruvate (Millenaar and Lambers 2003). The initial overflow electrons paradigm indicates that electron flow through the AOP only occurred when the Cyt pathway was saturated, or close to saturation (Bahr and Boner 1973); this led to the utilization of the AOP or Cyt pathway to determine the contribution of each pathway to total respiration. However, later studies offered arguments that challenged this idea; showing, for example, that the Cyt pathway never reaches a saturated rate (leading to underestimation of the AOP activity in the absence of inhibitors) and that the AOP is activated by α-keto acids like pyruvate (Day et al. 1996). The latter may have confounded results that determined the contribution of the AOP using inhibitors of the cytochrome or the alternative pathway (Millenaar and Lambers 2003). The actual contribution of the AOP to plant respiration can be determined by the ^{18}O fractionation technique (Guy et al. 1989).

In higher plants, the non-phosphorylating pathway (via AOP) of the electron transport chain is activated under adverse conditions. The actual physiological value of AOP is unclear; the single confirmed function of AOP is the thermogenic respiration in flowers (*e.g.* arum lily family), where the heat produced volatile aromatic compounds to attract pollinator insects (Meeuse and Raskin 1988). Some authors have suggested a major role in plant survival under stress, but others indicated that it has no obvious use. For example, studies with hybrid peas (*Pisum sativum* L.) showed that AOP might represent a waste of resources, since under high (650 ppm) or normal (350 ppm) CO_2 concentrations, the hybrid lacking the AOP reported higher leaf density and seed production than the hybrid with the AOP (Musgrave et al. 1986). The authors suggested that AOP was energetically wasteful as it was consuming carbohydrates stored in the tissues for no gain in productivity. Azcón-Bieto et al. (1983) showed that, under non-stressed conditions, the rate of leaf respiration of spinach and wheat was increased after a period of illumination, and that much of that rise was due to engagement of the AOP. The increase in total leaf respiration and contribution of AOP was triggered by high levels of sugars, indicating that the concentration of carbohydrates was the determinant for the rate of O_2 uptake and the rise in AOP activity. On the other hand, the AOP of respiration has been linked to protective functions minimizing the effect of ROS when photorespiration is high (Yip and Vanlerberghe 2001; Robson and Vanlerberghe 2002) and was linked to resistance against pathogens in *Nicotiana attenuata* (Zhang et al. 2012). Van Aken et al. (2009) proposed that

the AOP has an important role under stress conditions and plays a programming role in the cell response to stress by suppressing the production of ROS, changing the energy status of the cells, or delaying programmed cell death. Experiments with tobacco (*Nicotiana tabacum*) showed that transgenic cells lacking AOP were more susceptible to programmed death than cells with active AOP (Robson and Vanlerberghe 2002).

Light effects on AOP showed that light promotes electron partitioning and oxygen electron fractionation (Ribas-Carbo et al. 2000). An interesting study with soybean cotyledons revealed an effect of short light flashes on respiration and electron transport through the AOP. In etiolated cotyledon mitochondria of soybean, the authors showed that the AOP cannot compete with the cytochrome path in the absence of light, and they suggested that the respiration AOP does not occur in the absence of inhibitors (Ribas-Carbo et al. 1997; Pastore et al. 2001). They confirmed that the respiration AOP is activated under stress conditions, like those that stimulate high photorespiration rates in order to reduce the effect of the oxidative stress. This was because AOP dissipates energy and controls production of ROS, protecting the cell and improving plant performance under adverse conditions (González-Meler et al. 1999; Gandin et al. 2012). O_2 uptake by the respiration AOP can be confounded by lipoxygenase-mediated O_2 uptake; lipoxygenase is an enzyme that catalyzes the addition of O_2 to linoleic acid that is also insensitive to inhibition by cyanide. For example, experiments with etiolated wheat seedlings have shown that lipoxygenase is the enzyme responsible for the cyanide-insensitive component of O_2 uptake in the tissue (Goldstein et al. 1981). For this reason, the determination of AOP must consider the elimination of any interference in plants that contain lipoxygenase such as lipoxygenase-mediated O_2 uptake.

1.3.5.2.1.4 Root respiration

Roots are major consumers of photosynthetic products. About 50% of the daily photosynthetic assimilates are sent to the roots (Liu et al. 2004) and an average of 30% of the CO_2 fixed in photosynthesis is released back to the environment by root respiration (Lambers et al. 1996). Root respiration provides the energy required for their growth and maintenance and facilitates ion uptake (Atkin et al. 2000). Genetic gains in yield can be assessed through improvement in root

characteristics, and genetic variability between and within species has been shown for root respiration (Atkin et al. 2000). Deep root development enables access to additional water through the extraction of stored residual soil moisture, leading to greater yields under drought stress (Manschadi et al. 2006; Lopes and Reynolds 2010) in the same way a vigorous root system can match high evaporative demands when plants are exposed to heat stress in combination with high vapour pressure deficit, as indicated by cooler canopies (Pinto and Reynolds 2015). Under water limited conditions, reduced root respiration has been associated with enhanced grain yield of spring wheat (Liu and Li 2005b). Root respiration rates are usually higher than those reported for leaves and stems (Atkin et al. 2007) and can be a significant fraction of total plant respiration, depending on the species. For example, in tropical forests, root respiration can contribute 9-15% of total plant respiration; in temperate deciduous forest this contribution is up to 26%; while in grasslands species, roots are expected to account for a lower proportion of respiration (Yoda 1978; Edwards 1981; Amthor and Baldocchi 2001). Similar to other organs, root respiration is sensitive to the effect of external factors like temperature and drought (Table 6). Root respiration increases with temperature up to an optimum (Ryan et al. 1996), but its sensitivity decreases with increasing temperatures, similar to foliage respiration (Tjoelker et al. 2001). In experiments with winter wheat, diurnal fluctuations in wheat root respiration revealed maximum rates at around midday (Ma et al. 2013). Temperature acclimation and Q_{10} of root respiration has been associated with changes in the adenylate control and substrate supply (Atkin et al. 2000). A detailed review of the temperature effects on root respiratory rates is presented by Atkin (2000b).

Similar to leaf respiration, high root respiration rates seem to be associated with high carbohydrate concentration (Lambers et al. 1996). In a study investigating the effects of drought on root respiration of two spring wheat varieties (one drought sensitive and one drought tolerant), the respiration rate of both varieties increased under drought stress; the increase was greater for the drought sensitive variety, which also required more energy for water uptake (Table 6; Liu et al. 2004). Conversely, in *Acer saccharum* and *Pinus radiata*, root respiration has been associated with root nitrogen concentration (Ryan et al. 1996; Pregitzer et al. 1998), a parameter that explained up to 70% of variation in respiration rates (Fig 19, Fig 20, and Fig 21).

The AOP also plays a central role in root respiration. For example, two groups of herbaceous species with contrasting growth rates were studied to investigate their relatively small differences in root respiration rate; the fast growing species reported higher respiration rates than the slow growing species, but lower than those expected given their high growth rates and ion uptake (Scheurwater et al. 1998). The authors showed that the AOP partially explained these small differences between groups, since the fast growing species were more effective in their ATP production and also showed low respiratory costs associated with nutrient uptake and growth and maintenance of root biomass. The chemical compositions of the fast- and slow-growing species suggested that differences in protein and carbohydrate content might have affected the respiratory metabolism of these two groups (Poorter et al. 1991).

Actively growing roots require about 5-10% of respiratory energy (ATP) for maintenance when grown under controlled environments (Scheurwater et al. 1998; Van Der Werf et al. 1988), but as reviewed previously, R_m is expected to increase at higher temperatures (Zha et al. 2001). For fast- and slow-growing European wild species it was estimated that R_m represents only 10% of total root respiration in young tissues grown under high nitrogen conditions, and that R_g accounted for 20-45% of total root respiration, with the largest fraction of energy (50-70%) utilized for nutrient uptake. Root growth respiration increased with increased relative growth rate (Fig 22), where the fast-growing species showed higher costs, possibly associated with protein content (Poorter et al. 1991). Under optimum nutrient supply, a group of species contrasting in their relative growth rate showed that the contribution of the AOP to total root respiration ranged between 14 – 54% within species, but this contribution is not related to the relative growth rate, in contrast to total root respiration (Poorter et al. 1991). However, these results should be interpreted with caution as the experiments were performed using inhibitors, a technique no longer recommended (Millar et al. 1995; Day et al. 1996). Differences in root respiration between species showed higher total root respiration rates, as well as higher relative growth rate and ion uptake, in fast-growing species than in slow-growing species (Poorter et a. 1991).

Table 6. Root respiration rate for different species.

Species	Conditions	Notes	Respiration as cited by the authors	Reference
Triticum aestivum	Watered	DT cultivar	4.2 mg CO_2 h^{-1} g^{-1}	Liu et al. 2004
Triticum aestivum	Watered	DS cultivar	5.9 mg CO_2 h^{-1} g^{-1}	Liu et al. 2004
Triticum aestivum	Drought	DT cultivar	4.4 mg CO2 h^{-1} g^{-1}	Liu et al. 2004
Triticum aestivum	Drought	DS cultivar	9.6 mg CO_2 h^{-1} g^{-1}	Liu et al. 2004
Brassica oleraceavar	Nutrients sufficiency		7.7 mg CO_2 g^{-1} h^{-1}	Singh & Blanke 2000
Brassica oleraceavar	Potassium deficiency		12.2 mg CO_2 g^{-1} h^{-1}	Singh & Blanke 2000
Plantago major	Hydroponic	13 °C	23.0 nmol CO_2 g^{-1} s^{-1}	Atkin et al. 2007
Plantago major	Hydroponic	20 °C	39.2 nmol CO_2 g^{-1} s^{-1}	Atkin et al. 2007
Plantago major	Hydroponic	27 °C	41.7 nmol CO_2 g^{-1} s^{-1}	Atkin et al. 2007
Plantago euryphylla	Hydroponic	13 °C	16.9 nmol CO_2 g^{-1} s^{-1}	Atkin et al. 2007
Plantago euryphylla	Hydroponic	20 °C	41.2 nmol CO_2 g^{-1} s^{-1}	Atkin et al. 2007
Plantago euryphylla	Hydroponic	27 °C	26.4 nmol CO2 g^{-1} s^{-1}	Atkin et al. 2007
Helianthus annuus	this papers	20 °C	4.7 µmol CO2 mol^{-1} N s^{-1}	Szaniawski & Kielkiewicz 1982 (cited by Amthor & Baldocchi 2001)
Solanum tuberosum	Hydroponic	18 °C	3.1 µmol CO2 mol^{-1} N s^{-1}	Bouma et al. 1996 (cited by Amthor & Baldocchi 2001)
Hordeum vulgare L.	Glasshouse	20 °C	˜ 1.8 µmol CO2 g^{-1} s^{-1}	Bloom et al. 1992
Populus tremuloides	25% of full sunlight	23.5 °C	˜ 55 nmol g^{-1} s^{-1}	Walters et al. 1998
Betula papyrifera	25% of full sunlight	23.5 °C	˜ 42 nmol g^{-1} s^{-1}	Walters et al. 1998
Betula allegheniensis	25% of full sunlight	23.5 °C	˜ 47 nmol g^{-1} s^{-1}	Walters et al. 1998
Larix laricina	25% of full sunlight	23.5 °C	˜ 41.5 nmol g^{-1} s^{-1}	Walters et al. 1998
Pinus banksiana	25% of full sunlight	23.5 °C	˜30 nmol g^{-1} s^{-1}	Walters et al. 1998
Picea glauca	25% of full sunlight	23.5 °C	˜ 30 nmol g^{-1} s^{-1}	Walters et al. 1998
Picea mariana	25% of full sunlight	23.5 °C	˜ 31 nmol g^{-1} s^{-1}	Walters et al. 1998
Pinus strobus	25% of full sunlight	23.5 °C	˜ 27 nmol g^{-1} s^{-1}	Walters et al. 1998
Thuja occidentalis	25% of full sunlight	23.5 °C	˜ 22 nmol g^{-1} s^{-1}	Walters et al. 1998
Populus tremuloides	5% of full sunlight	23.5 °C	˜ 39 nmol g^{-1} s^{-1}	Walters et al. 1998
Betula papyrifera	5% of full sunlight	23.5 °C	˜ 40 nmol g^{-1} s^{-1}	Walters et al. 1998
Betula allegheniensis	5% of full sunlight	23.5 °C	˜ 38 nmol g^{-1} s^{-1}	Walters et al. 1998
Pinus banksiana	5% of full sunlight	23.5 °C	˜ 32 nmol g^{-1} s^{-1}	Walters et al. 1998
Pinus strobus	5% of full sunlight	23.5 °C	˜ 22 nmol g^{-1} s^{-1}	Walters et al. 1998

˜ values estimated from figures; All experiments were performed under controlled conditions; DT: drought tolerant; DS: drought susceptible.

Summarizing, similarly to respiration from other organs, root respiration is affected by environmental factors such as temperature and carbohydrate availability. However, given that root respiration rates are usually higher than those found for leaves and stems, root respiration can represent a significant part of total plant respiration depending of species; the latter suggest that modelling of plant CO_2 exchange rates need to be updated to include root contributions for accurate estimations.

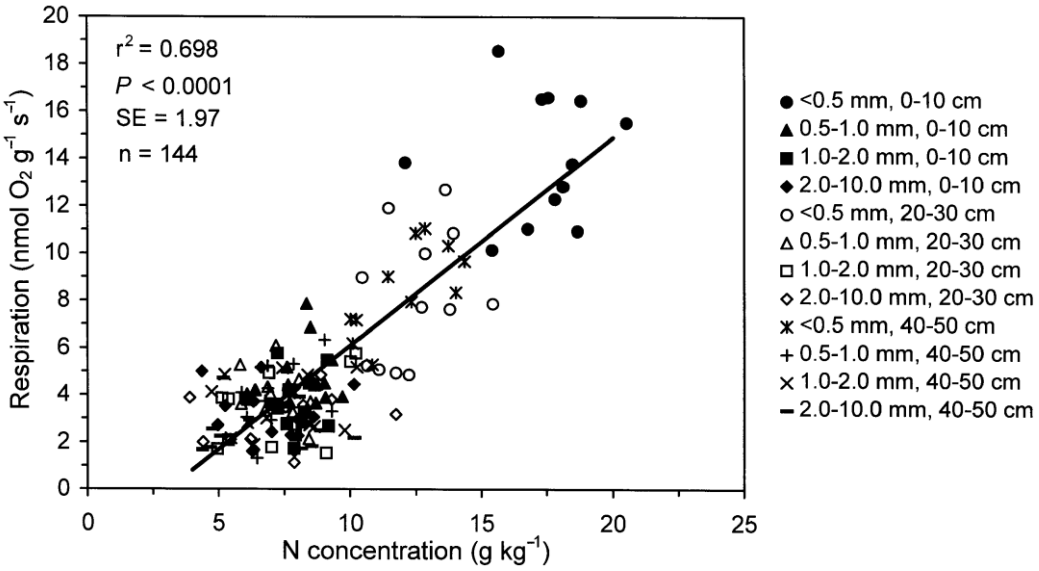

Figure 19. Root respiration versus root tissue N concentration for two Michigan northern hardwood forests. The plotted regression line displays the linear relationship of the data (*n*=144). From Pregitzer et al. (1998).

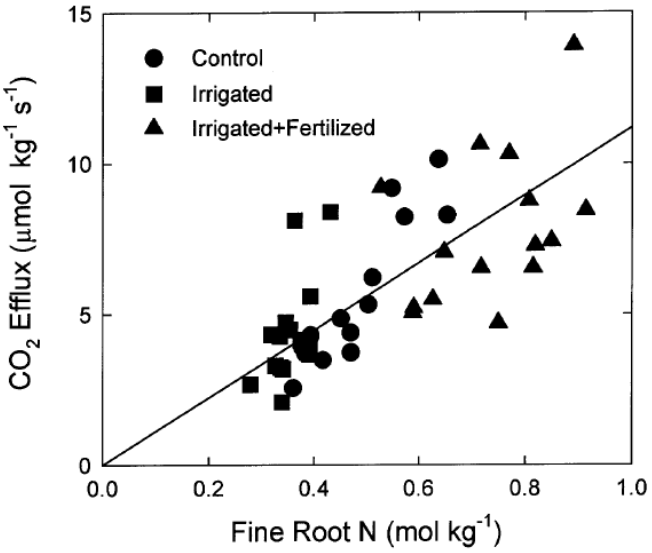

Figure 20**.** Efflux of CO_2 from fine roots (< 2 mm in diameter) corrected to 15 °C versus root N concentration for *Pinus radiata* growing in three treatments. For all treatments combined, dark CO_2 efflux (µmol kg^{-1} s^{-1}) = 11.2N (mol kg^{-1}); R^2 = 0.51. From Ryan et al. (1996).

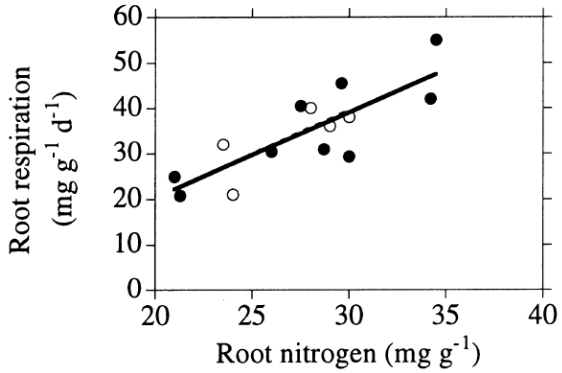

Figure 21. Correlations between root R_d (nmol g^{-1} s^{-1}) and root N concentration, for seedlings of nine boreal species grown at either 5 (empty circles) or 25% (filled circles) of full sunlight .Correlations and *P* values: for 25% sunlight, root R_d = –17.1 + 1.87 × leaf N, P< 0·001, r = 0.83; for 5% of sunlight, root R_d = –18.3 + 1.92 × leaf N, P< 0.001, r = 0.76. Reproduced with permission from Reich et al. (1998)

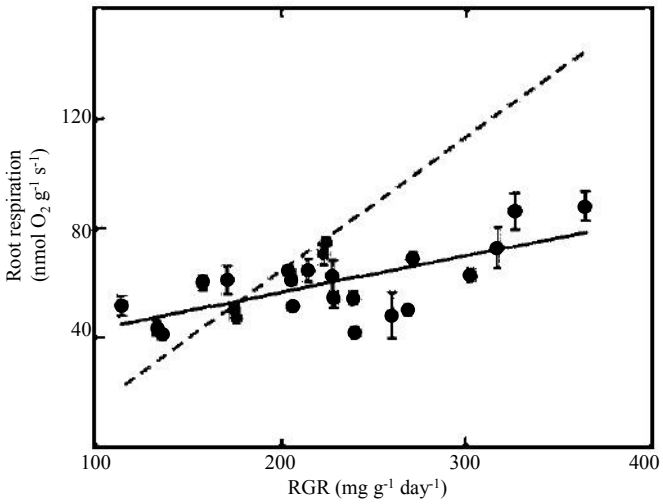

Figure 22. Root respiration rates of 24 wild species differing in growth rate wild species, from a wide range of habitats in western Europe. Mean values ± SE (n = 8). The theoretically expected rate of root respiration (broken line) was determined using actual rates of growth and ion uptake of the 24 species and previously determined specific costs for maintenance, root growth and ion uptake for two slow-growing *Carex* species (Van der Werf et al. 1988). From Poorter et al. (1991).

1.3.5.2.1.5 Relation of respiration to photosynthesis (homeostasis of R/P)

Daily carbon gain is the source of plant growth and crop production and is dependent on the balance between photosynthesis (P) and respiration (R) rates. While the relationship between these two metabolic processes and plant production has been studied, the importance of respiration is commonly underestimated (van Iersel and Seymour 2000). There is a positive association

between R and P since photosynthesis provides the substrate required for respiration (Amthor 1994; Gratani et al. 2008). In fact, at least 30-35% of total carbon assimilated is released as CO_2 via respiration (Amthor and Baldocchi 2001), but depending on species and growth conditions, studies have indicated that respiration can consume up to 70% of all carbohydrates generated by photosynthesis (van der Werf et al. 1992). The positive relationship between respiration and photosynthesis is attributed to the high amount of stored assimilates generated by fast P (Moser et al. 1982; Azon-Bieto 1983c; Rajendrudu et al. 1987), which may be rapidly degraded in order to avoid photoinhibition (Madsen 1974). For example, Gratani et al. (2008) showed that in Mediterranean evergreen species grown under optimal conditions, high respiratory and photosynthetic rates were linked to highly active metabolic processes. The AOP also has been observed to be stimulated by periods of increased photosynthesis (Azcón-Bieto and Osmond 1983). The balance between R and P seems to be relatively constant across moderate temperatures (Atkin et al. 2005); the typical range for R/P is around 0.35-0.70 (Table 7), with cereals like wheat, rice, and sorghum reporting lower values and forest trees reporting the highest (Cock and Yoshida 1973; Gifford 2003). Even when P and R have different sensitivities to increases in temperature, short term temperature changes can lead to acclimation of the P and R processes (Gifford 2003) leading to reach a constant R/P ratio after a period of time (Atkin et al. 2007). For example, R/P was found to be constant at a range 15-30 °C for wheat (Gifford 1995), 20-35 °C for soybean (Ziska and Bunce 1998), and 13-20 °C for *Plantago major* and *Plantago euryphylla* (Atkin et al. 2007). Drought stress is associated with a considerable increase in R/P (Ayub 2011), possibly as the result of an increase in R and a reduced P rate due to limited CO_2 uptake in these environments. Under water–limited conditions, leaf respiration generates almost 50% of the CO_2 consumed by photosynthesis (Gratani et al. 2008); in contrast, CO_2 uptake under non-stressed conditions was 3-5 times greater than the CO_2 released by respiration, resulting in a smaller R/P ratio for optimum environments. The effect of CO_2 concentration was studied by Ziska and Bunce (1998) who observed that the R/P ratio for whole young plants (7-20 days after sowing) decreased in soybean grown at elevated CO_2 (700 μl l^{-1}), compared to ambient CO_2 concentrations at growing temperatures of 20, 25, 30, and 35 °C (Fig 23). This decreased R/P was due to the reduction in respiration rates

observed at high CO_2 levels, irrespective of temperature. These results concurred with previous studies with soybean and other species (see references in the CO_2 chapter 1.3.5.2.1.1.2). As discussed therein, decreased respiration is linked to the reduced activity of the Cyt *c* under conditions of elevated CO_2 (Azcón-Bieto et al. 1994). However, studies with wheat gave opposite results as whole plant R/P was found to be independent of CO_2 level in plants of bread wheat (*Triticum aestivum*) grown at ambient CO_2 or at 710 ppm (Gifford 1995). This study reported R/P ratios of 0.35 and 0.34 for ambient and elevated CO_2 conditions, respectively, and also showed that temperature variations led to small but significant increases in the R/P ratio (Fig 24).
Leaf age can modify the R/P, which decreases as leaves get older since both respiration and photosynthesis are reduced in mature tissue. Older leaves of C3, C4, and C3-C4 tropical weed species (*Amaranthus viridis, Boerhavia diffusa, Alternanthera ficoides,* and *Parthenium argentatum*) were grown under optimum ambient conditions to study the effect of ontogenetic changes in the rate of CO_2 release to that incorporated by photosynthesis. The authors reported that average R/P decreased in older leaves, with R/P values of 0.140, 0.085, 0.075, and 0.070 mg CO_2 dm^{-2} h^{-1} for the first, second, third, and fourth leaf, respectively (Rajendrudu et al. 1987).

In summary, plant performance and crop productivity can be broadly explained by the R/P ratio. The use of a constant R/P value in algorithms that attempt to provide an insight into carbon cycles showed that this approach was effective and practical (Gifford 2003; Cock and Yoshida1973) as there is a clear trend of plant respiration being a relatively constant proportion of photosynthesis, as reviewed above. Nonetheless, care must be taken in studies requiring highest accuracy since variations in tissue age, CO_2 levels, temperature or the occurrence of water stress can modify the R/P ratio.

Table 7. Estimates of the R /P ratio for terrestrial ecosystems.

Ecosystem	*R /P*	Reference
Crop	0.35-0.49	Thomas and Hill (1949)
Alfalfa	~0.30-0.60	Amthor (1989)
Maize, rice, and w heat		
Grassland		
Shortgrass prairie	0.34	Andrew et al., (1974)
	0.51	Detling (1979)
Tall grass prairie		
No grazing	0.61	Risser et al., (1981)
Seasonal grazing	0.65	Risser et al., (1981)
Year-round grazing	0.62	Risser et al., (1981)
Forest		
Tropical moist		
Ivory Coast	0.75	Müller and Nielsen (1965)
Puerto Rico	0.88	Derived from Odum (1970)
Southern Thailand	0.66	Kira (1975)
Temperate		
Warm evergreen	0.72	Kira (1975)
Warm evergreen "oak"	0.66	Kira and Yabuki (1978)
Abies sachalinensis	0.53	Kira (1975)
Castanopis cuspidata	0.58	Kira (1975)
Chamaecyparis obtusa plantation	0.62	Hagihara and Hozumi (1991)
Cryptomeria japonico plantation	0.71	Kira (1975), mean of five estimate
Fagus crenata		
Secondary forest	0.44	Kira (1975)
Plantation	0.56	Kira (1975)
Fagus sylvatica		
8 years old	0.46	Möller et al. (1954)
25 years old	0.39	Möller et al. (1954)
46 years old	0.43	Möller et al. (1954)
85 years old	0.47	Möller et al. (1954)
Fraxinus excelsior plantation	0.37	Kira (1975)
Linodendron tu lipi f era	0.66	Harris et aL (1975)
Picea abies plantation	0.32	Kira (1975)
Pinus densiflora plantation	0.71	Kira (1975)
Pinus ponderosa	0.55	Law et al. (1999)
Pinus taeda plantation	0.58	Kinerson (1975)
Pinus spp.	0.39-0.71[b]	Ryan et al. (1944b)
Pseudotsuga- Tsuga	0.93	Grier and Logan (1977)
Quercus-Acer	0.44-0.55	Amthor (2000)
Quercus-Acer	0.54	M. L. Goulden (personal communication, 1997)
Quercus-Pinus	0.55	Whittaker and Woodw ell (1969)
Quercus spp.	0.61	Satchell (1973), as cited by Edw ards et al. (1981)
Quercus-Carpinus	0.38	Medw ecka-Kornas et al. (1974), as cited by Edw ards et al. (1981)
Subalpine		
Coniferous	0.72	Kitazaw a (1977), as cited by Edw ards et al. (1981)
Abies	0.68	Kira (1975)
Abies veitchii	0. 61	Kira (1975), mean of three estimates
Boreal		
Picea mariana	0.69	M. L. Goulden (personal comm, 1997)
Picea mariana	0.72-0.77	Ryan et aL (1998)
Pinus banksiana	0.68	Baldocchi et al. (1997)
Pinus banksiana	0.69-0.74	Ryan et al. (1998)
Populus tremuloides	0.55[c]	Black et aL (1996)
Populus tremuloides	0.64-0.67	Ryan et aL (1998)
Temperate coastal salt marsh		
Spartina	0.77	Teal (1962)
Spartina-Distichlis	0.69	Woodw ell et al. (1979)
Arctic tundra	0.50	Reichle (1975)

[a] Estimates of R and P are for an entire year, except for crops, where they apply to the growing season. Nonetheless, some of the values in this table represent data from periods less than a full year. These estimates of R/P are based on the assumption that leaf respiration occurs at about the same rate in the light as in the dark, at a given temperature. Data from controlled-environment chambers are included in this summary. [b] Range of values for seven young (16-40 year

old) *Pinus* stands. Ryan *et al.* (1994b) gave daily (24-h) stem, branch, and root respiration rates, but foliage respiration was for nights only. The measure of photosynthesis presented was daytime canopy net CO_2 assimilation. Here, to obtain R the night time foliage respiration amounts. To obtain P the night time foliage respiration amount was added to daytime canopy net CO_2 assimilation. Both our transformations are based on the assumption that daytime foliage respiration was similar to night time foliage respiration. [c] Assuming belowground net primary production (NPP) is 35% of NPP. Reproduced with permission from Amthor and Baldocchi (2001).

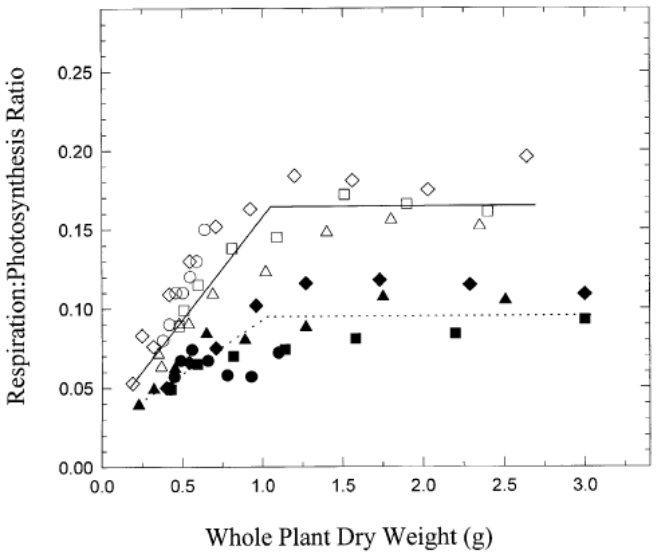

Figure 23. Effect of elevated CO_2 on R/P. The ratio of average night-time respiration rate (R) to average day-time photosynthetic rate (P) for soybean grown at ambient (350 mL L^{-1}, open symbols) and elevated (700 mL L^{-1}, closed symbols) CO_2 concentration at 4 different growth temperatures. Net carbon exchange occurred over 7–9 days. Each point represents the R/P ratio determined on a dry weight basis for a 24-h period for a given CO_2 concentration and growth temperature. Constant day/night growth temperatures were 20 °C (○, ●), 25 °C (□, ■), 30 °C (∆, ▲) and 35 °C (◊, ♦). Lines were hand-drawn. Reproduced with permission from Ziska and Bunce (1998).

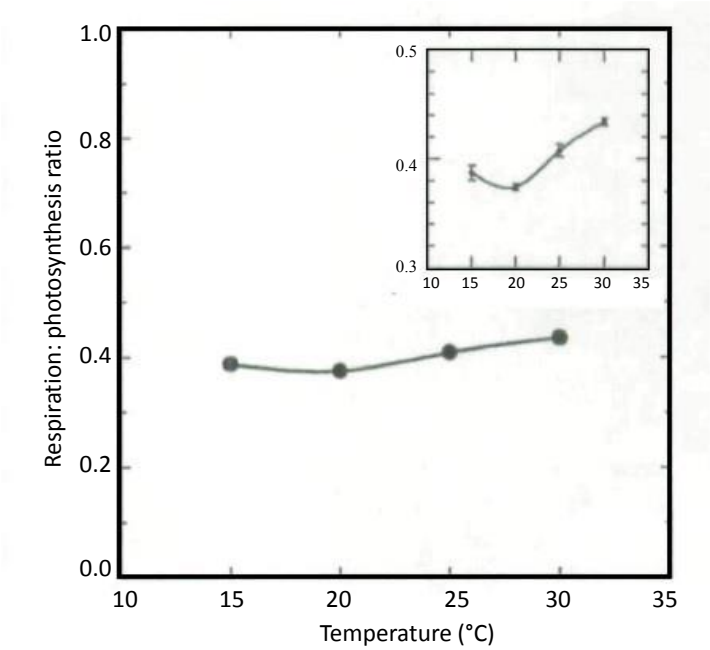

Figure 24. Whole plant respiration: gross photosynthesis ratio (24 h totals) as a function of growth temperature for wheat (*T. aestivum* cv Highbury). The inset expands the scale to show the standard errors of the means. Reproduced with permission from Gifford et al. (1995).

1.3.5.2.1.6 Harnessing genetic variability of respiration for plant selection and crop improvement

Few studies show a clear link between respiration and yield gains (Wilson 1982; Fernandez 1977). One reason is the difficulty of meaningfully measuring whole plant respiration (including roots), while another reason is that a proportion of respired CO_2 is refixed by the photosynthetic structures, e.g, the glumes (Gebbing and Schnyder 2001), which complicates accurate measurement. The perception of R_m as a potentially wasteful component has led to the hypothesis that it can be reduced, and that such reductions would save energy and resources and thus result in biomass gains, but this assumption has not been clearly demonstrated (Earl and Tollenaar 1998). Studies reporting good association of leaf respiration and yield showed genetic variability and high heritability for leaf respiration (Volenec and Nelson 1984; Gent and Kiyomoto 1985; Massacci et al. 1986). One of the few studies comparing the performance of high and low-respiring varieties showed that the productivity, in terms of biomass, of three lines of *Lolium perenne* (two F1 populations and one parent) was associated with their respiration rate. Significantly higher (almost 15% greater) biomass was produced by lines with a low respiration rate in fully grown

leaves, which was attributed to more efficient use of fixed carbon utilized in the production of new tissue, rather than maintenance (Wilson and Jones 1982).
In a comparison of new and old drought-adapted Chinese winter wheats, genetic gains were associated with lower root respiration (Ma et al. 2013). Leaf photosynthesis of the new wheat varieties was equal or even greater than the old varieties, depending on the time of the day. However, respiration rates per unit of root biomass of new varieties were generally about 15% lower than for older low-yielding varieties when measured early in the morning (between 7:00-9:00 h) and late in the evening (19:00-23:00 h). No differences in root respiration were observed during the day when the maximum photosynthetic rates were reported.

In maize, crop growth rates have been associated with differences in leaf respiration. A comparative study between a maize hybrid released in 1959 with a newer hybrid released in 1988 that exhibited 10% greater dry matter accumulation, showed that the R/P ratio was 28% and 14% higher in the older hybrid when grown under non-stressed conditions and under water stress, respectively, suggesting lower maintenance requirements that allowed use of respiratory energy in growth (Nissanka et al. 1997). Later field experiments confirmed this trend with an even larger difference (close to 20%) in respiration rates between hybrids (Earl and Tollenaar 1998). This study showed a strong and negative association (average r = -0.85, p = 0.03) of respiration rate (per unit of dry weight) with dry matter accumulation in maize hybrids grown under high and low nitrogen conditions (200 and 50 Kg N ha^{-1}, respectively) (Fig 25).

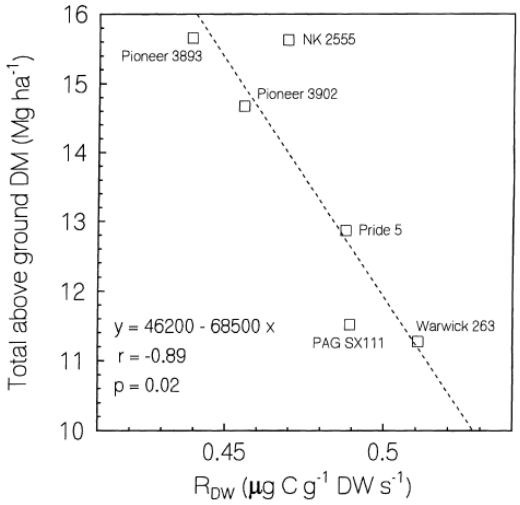

Figure 25. The relationship between total above-ground dry matter accumulation, measured at final harvest, and the mean seasonal leaf dry weight of mature upper leaves for six commercial maize hybrids averaged in two locations under high-N treatment. From Earl and Tollenaar (1998).

Comparative studies of leaf, stem, and canopy respiration rates and dry matter production of rye, wheat, and triticale were conducted under irrigated conditions (McCullough and Hunt 1993). At early stages, the dry matter produced by rye was greater than wheat and triticale, but the dry matter was similar when the ontogenic stages were equalized between crops, though rye had lower canopy respiration rates throughout the whole cycle. The partitioning analysis of respiration into growth and maintenance components showed that the R_m required by rye decreased much earlier than in wheat and triticale, allowing rye to store carbon earlier. Biomass composition analyses revealed no significant differences between crops that could explain the lower R_m in rye.

In conclusion, there are relatively few studies showing an association between plant productivity and respiratory rates; thus it is difficult to draw a general conclusion about the relevance of respiration as an indirect selection criterion. Access to better and higher throughput phenotyping would aid understanding, given that previously described respiration studies frequently compared low numbers of species or varieties. The mechanisms driving respiration are not clear, therefore variations are expected depending of the specific conditions of the measurement.

1.3.5.2.1.7 Conclusions for respiration

Respiration affects plant performance, crop production, plant adaptation, and even whole ecosystem carbon balances. Initially, respiration was considered at least partially a waste of resources, but evidence from recent studies shows that plant respiration is a complex process that provides the energy required for the essential processes for survival, such as cell growth and maintenance, and that alternative pathways have evolved to protect against ROS and pathogens (Millenaar and Lambers 2003; Zhang et al. 2012). Any external factor can modify the specific respiratory rates of vegetative tissues, organs, or whole plants; even the proportion of CO_2 released to that fixed by photosynthesis (R/P) can be affected. Nevertheless, plants tend towards a relatively constant R/P homeostasis under most conditions (Gifford 1995). Genetic variability for respiration (Wilson and Jones 1982; Earl and Tollenaar 1998; Ma et al. 2013) allows the discovery of germplasm and the generation of crosses capable to survive to changing environments, but its effectiveness depends on growing

conditions and the species in question. However, many contradictions exist across studies, complicating the understanding of the physiological basis of respiration. A better consensus is needed to take advantage of the genetic variability of plant respiration, for example to develop standard screening protocols. Studies attempting to exploit the trait have shown it to be challenging, although a few studies have shown that plant performance can be improved when selecting lines with either a better balance between the rate of CO_2 released and CO_2 taken up, or higher efficiency in the generation of energy (triggering of the AOP as a response to stress), or reduced costs of maintenance. In this thesis Chapter 3 explores a physiological approach to asses the association of wheat leaf respiration rates with heat adaptive traits, including grain yield. The study in Chapter 3 also evaluates if genetic differences for leaf respiration exist in a selected set of germplasm. Finally a summary table (Table 8) shows a comparative analysis of the factors affecting respiration, presenting results from several studies for the alternative cyanide resistant respiration (AOP), maintenance (R_m) and growth respiration (R_g).

Table 8. Summary of publications that have studied the effect of a number of factors on the rate of: growth respiration, maintenance respiration, or the alternative respiration (cyanide resistant) pathway, as well as generalities for total plant respiration.

Factors affecting plant respiration	Growth (R_g)	Maintenance (R_m)	Cyanide resistant pathway (AOP)	**Generalities** (Total respiration= R_g+R_m)
	That is required for the synthesis of new molecules for dry matter accumulation	That is required for the maintenance of the cellular structure, gradients, etc. but not associated with dry matter accumulation	Also known as the alternative pathway. Triggered under stress conditions	Respiration: "*Breakdown and oxidation of organic compounds with the transfer of hydrogen (electrons) to molecular oxygen and the release of utilizable energy within the cell"* (Hackett 1959)
Temperature	Unaffected by temperature (McCree 1974; Zha et al. 2001)	Increases exponentially with temperature up to an optimum temperature where the maximum rate is reached with a sigmoidal shape; further increases in temperature result in a rate decay (McCree 1974; Szaniawski and Kielkiewicz 1982; Zha et al. 2001). Proportional to dry weight of plant tissue.	Affected by changes in temperature, e.g. chilling treatment of cucumber increased the proportion of AOP from total respiration (Kiener and Bramlage 1981); low temperature (<19°C) up-regulated AOP in mung bean (*Vigna radiate*; González-Meler et al. 1999), maize (Stewart et al. 1990), and tobacco (Vanlerberghe and McIntosh 1992).	Different responses are seen when comparing long and short term exposure to variations in temperature (Gifford 2003). Up to 25°C, rates have been reported to follow linear and curvilinear patterns for canopy and leaf respiration of wheat, rye, and triticale (McCullough and Hunt 1993). Q_{10} is close to 2 in a reduced range of temperature, but varies between 1.2 and 5.2 across a wider temperature range (Atkin and Tjoelker 2003)

				Temperature sensitivity of respiration is species dependent (Gifford 2003; Frantz et al. 2004) and the environment of origin can have an effect (Criddle et al. 1994).
CO_2	Unaffected by CO_2 (Zha et al. 2001).	Affected by CO_2 (Gifford 1995; Zha et al. 2001). Seems to be inhibited by high CO_2 levels, as reported in shoots and roots of alfalfa (Reuveni and Gale 1985) and leaves of *Xanthium strumarium* L. (Gale 1982).	The absence of this path results in reduced photosynthetic rates (as evidenced by higher total dry matter and specific leaf weight) under elevated CO_2 conditions but similar rates under normal CO_2 (Gandin et al. 2012). AOP is unaffected by variations in CO_2 level (González-Meler et al. 2004). Lesser response to CO_2 enrichment was observed in pea hybrids (*Pisum sativum*) with presence of AOP than in those lacking the pathway (Musgrave et al. 1986).	Usually respiration rates are lower at elevated CO_2 than at ambient conditions (Amthor 1997). Not suppressed by high CO_2 in the short term (Gifford 2003).

Light	Affected by light. High light levels increase R_g due to increases in daily carbon gains. Similarly, its proportion of total respiration is increased (Nemali 2004).	Increased by high light levels due to a higher growth rate but its proportion of total respiration is reduced (Nemali 2004).	Engaged by high light conditions which stimulate elevated rates of photorespiration for example in soybean (Ribas-Carbo et al. 2000; Pastore et al. 2001). The AOP can act, dissipating energy and controlling the production of ROS under elevated irradiance conditions.	Not suppressed by light (Gifford 2003).
Age/Stage	Decreases with age (Stahl and McCree 1988).	Affected by tissue composition of mature and young tissue given that different amount of energy are required to maintain lipids, carbohydrates and proteins. Mature tissue may show lower R_m (Stahl and McCree 1988; van Iersel 2003).	The AOP proportion from total respiration increases with leaf age given that this path is mostly involved with the maintenance component of respiration which is higher in mature tissue (Millar et al. 1998).	Decreases with increases in leaf age (Moser et al. 1982; Villar et al. 1995: Mohammed and Tarpley 2009).
Organ type	Clear organ effects, for example R_g is reported to be higher in roots than in shoots (Hansen and Jensen 1977).	Affected by tissue composition. Depends on organ (Ryan et al. 1996), for example R_m is reported to be higher in roots than in shoots (Hansen and Jensen 1977).	AOP genes show different transcriptional regulation across different organs, (Kearns et al. 1992; Saisho 1997) thus indicating tissue specificity.	Differences between organs are documented, e.g. respiration rates of fully expanded flag leaves of wheat, rye, and triticale are higher than those for the spike and non-elongating basal stem internode (McCullough and Hunt 1993 and references therein; Amthor and Baldocchi 2001).

				Roots usually exhibit higher respiratory rates than leaves and stems (Atkin et al. 2007). Leaf respiration generally accounts for 50% of whole plant respiration (Poorter et al. 1990).
Species/ Cultivars	The growth coefficient (carbon respired per unit of new biomass) for R_g is unaffected by species, according to McCree (1974), but daily carbon gain varies between species (van Iersel and Seymour 2000).	Plant species with significant lignin and woody tissue are likely to have decreased R_m (Amthor and Baldocchi 2001; van Iersel 2003).	In thermogenic plants, the AOP is involved in thermogenesis (Meeuse and Raskin 1988), but in other species AOP is regulated by environmental (such as the occurrence of stress). Differences between species can be attributed to different degrees of regulation of glycolysis and cytochrome pathway (Azcón-Bieto 1983).	Affected, since respiration rates are largely dependent on tissue composition and origin of cultivars (Criddle et al. 1994; Amthor and Baldocchi 2001; van Iersel 2003). Differences between rye, triticale, and wheat are reported (McCullough and Hunt 1993), as well as between eucalyptus, soybean, sorghum, common grape vine, and pea (Gifford 2003), grasses (Scheurwater et al. 1998), wheat cultivars (Ma et al. 2013) and ryegrass (Wilson and Jones 1982).
Interaction with other factors: drought, nutrients	Possibly reduced by drought and nutrient stress since it depends on relative growth rate which is largely reduced under stress conditions (Liu et al. 2011).	Increased by drought (Liu et al. 2011), possibly because use of carbohydrate reserves increase (Wit 1970).	Activated when stress conditions appear (Flores-Sarasa 2007). AOP minimizes the effects of ROS generated under stress conditions such as	Drought effects on respiration are variable; it can be reduced by moderate water stress (Flexas et al. 2005; Atkin and Macherel 2009) but increased by severe

			drought or high temperatures (Maxwell et al. 1999). Activity is increased under nutrient limitations (Theodorou and Plaxton 1993; González-Meler et al. 2001)	drought (Reichstein et al. 2002) Limitation of Pi (Phosphorous) increases respiration (Theodorou and Plaxton 1993), but in a wheat-rice cropping system it was shown that the application of N fertilizer increased respiration of both crops (Sun et al. 2007).
Substrate	Proportional to the substrate level (McCree 1974).	Dependent on the level of non-structural carbohydrates (Thomas and Griffin 1994) but to a lesser degree than R_g (Moser et al. 1982).	May be regulated by water soluble carbohydrate level (WSC), e.g. higher electron flow at higher WSC levels (Azcón-Bieto 1983).	The availability and use of substrate seems to be the main control of respiration. Variability in the sensitivity for substrate availability between tissues have been reported in tall fescue for example (Moser et al. 1982).

General conclusion for the literature review chapter

Improved adaptation of wheat to high air temperatures can be addressed through several approaches as outlined in this chapter. This thesis focuses on a physiological approach based around the idea of strategic trait crossing (Cossani and Reynolds 2012; Reynolds and Langridge 2016), and highlights the utilization of two high throughput phenotyping techniques in the screening of candidate germplasm: the measurement of the canopy temperature (CT) and the determination of residual greenness at the end of the grain-filling phase (staygreen). Both, CT and staygreen can be used as surrogate traits to obtain information about plant access to water and late photosynthetic activity, respectively.

Adaptation to high air temperatures under hot-irrigated conditions could be achieved by improved plant access to water. This would allow the plant to mitigate against heat stress, given that under hot-irrigated conditions the evaporative demand is high and a strong root system permits uptake of soil water and the maintenance of cooler canopies through transpirational cooling (Amani et al. 1996). However, under hot-irrigated conditions the pattern of root development is poorly understood and difficult to measure. Chapter 2 from this thesis examined the relationship of CT with root architecture in the field, and compared it with drought conditions.

The thesis also explores the heat adaptive value of the staygreen trait as a surrogate of extended photosynthetic activity. Elevated air temperatures shorten the whole plant cycle, including the grain-filling phase resulting in premature plant senescence. Reducing the period available to fill the grains is a main factor diminishing final wheat yield, especially under heat stressed conditions. An extended grainfilling period can be observed in staygreen genotypes with the ability of remain green and photosynthetically active. Previous studies have shown that staygreen is a complex character, especially in bread wheat. A further complication derives from the focus on staygreen in wheat leaves through leaf SPAD measurements. This measurement ignores wheat spikes and other photosynthetic organs such as stems, which can be important for grain yield but are not usually quantified in staygreen genotypes. However, recent studies in spring wheat have shown that the use of spectral indexes can improve the staygreen determination and suggest that different parameters can

be assessed to reach a more complete understanding of the staygreen trait in wheat (Lopes and Reynolds et al. 2012). In Chapter 3 from this thesis staygreen and associated parameters were determined using an integrative estimation for the whole wheat plot (including stems, leaves and spikes) and addresses variations in plant phenology.

The biochemical basis for plant responses to heat stress is complex and the exploration of the processes involved can result in interesting discoveries for translation to breeding. The potential value of a direct biochemical approach was explored through measurements of leaf respiration presented on Chapter 4 from this thesis. In general terms, wheat productivity is the result of two basic processes: the balance between the carbon fixed through photosynthesis and the carbon respired. Although a considerable volume of information exists in the literature regarding photosynthesis, relatively little in known on the actual value of respiration, especially in the context of crop productivity. It is interesting that current crop models do not include carbon consumed by respiration, neither the energetic contribution of this process to plant growth nor plant maintenance.

In conclusion, it is clear that several heat adaptive traits can be selected for crop improvement but here, three, potentially additive traits were explored in detail in order to obtain new understanding for translation to wheat breeding.

Chapter 2. Journal paper: Common genetic basis for canopy temperature depression under heat and drought stress associated with optimized root distribution in bread wheat

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Given the complexity of yield, it is highly probable that a multi-trait approach could have significant impact on improvement of wheat's tolerance to heat stress. This chapter focuses on the canopy temperature (CT) as one of three main targets to be explored, given that previous studies in wheat have suggested that CT can be used as a surrogate trait to obtain information about root development and plant access to soil water under drought conditions, and given that recent studies indicate a common genetic and physiological basis between drought and heat tolerance. The study presented in this chapter aimed to investigate if common QTL associated with cooler canopies of spring wheat grown under both heat and drought-stressed conditions relate to root traits; and if root development patterns under heat and drought are similar. Co-location of QTL for canopy temperature under heat and drought has suggested a common genetic basis for tolerance to these stresses (Pinto et al. 2008). The population chosen for this study (Seri/Babax) was previously characterized as relatively homogeneous for phenology and has shown good associations between yield and CT (Olivares-Villegas et al. 2007). The involvement of roots in the improvement of drought tolerance of wheat has been widely reported but little is known about their stress adaptive value under hot-irrigated conditions.

Statement of Authorship

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Contribution to the Paper	Conducted all of the experiments, performed all data analysis and led the write-up.		
Overall percentage (%)	85		
Signature		Date	01/12/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Matthew P. Reynolds**		
Contribution to the Paper	Designed the experiment and participated in all aspects of data analysis and writing of the paper		
Signature		Date	01/12/2015

Common genetic basis for canopy temperature depression under heat and drought stress associated with optimized root distribution in bread wheat

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Key message

QTL related to cooler canopy temperatures are associated with optimal root distribution whereby roots proliferate at depth under drought or near to surface under hot, irrigated conditions

Abstract

Previous research using a bread wheat RIL population of the Seri/Babax cross showed that common QTL were associated with cooler canopies under both drought and heat stressed conditions. A subset of RIL was grown under water-limited and hot-irrigated field environments to test how cooler canopies are related to root development. Eight sisters and the two parents were used in the study with genotypes grouped as COOL or HOT according to their respective QTL for canopy temperature and previous phenotypic data. Root mass production and residual available soil moisture were measured around anthesis at four depth profiles (from 0 to 120 cm depth). When considering different root profiles, there was a clear interaction of QTL with environment. Under water stress, the COOL genotypes showed a deeper root system allowing the extraction of 35% more water from the 30-90 cm soil profile. The strategy under heat was to concentrate more roots at the surface, in the 0-60 cm soil layer where water was more available from surface irrigation. Since COOL genotypes showed better agronomic performance, it can be concluded that their QTL are associated with more optimal root distribution in accordance with water availability under the respective stresses. The study demonstrates the importance of root development under both water limited and hot irrigated environments, and shows a common genetic basis for adaptation to both stresses that appears to be associated with sensitivity of roots to proliferate where water is available in the soil profile.

Keywords: *QTL, soil moisture, water-limited environment, hot, physiology*

INTRODUCTION

While the focus of most research in plants is on the above ground organs, the radicular system represents a high proportion of the total plant's mass and energy requirement. Nonetheless, a comprehensive understanding of root mechanisms involved in, for example, drought and heat response, are imperative to the effort of increasing adaptation of crops to harsher environments under climate change. Roots have a range of functions including anchorage, mechanical support, nutrient and water uptake, and signalling. Roots are also extremely sensitive to water deficit and high temperatures; for example, they show a narrow range of optimum growth temperature compared to other organs (Porter and Gawith, 1999).

Under high temperature field experiments, root growth was observed to be diminished due to a reduction in the carbon partitioned below ground, and the number, length and diameter of roots are especially affected if the heat occurs during the reproductive stage (Batts et al. 1998). Drought has different effects depending on the severity. If a moderate drought occurs root development can be promoted because an increased amount of carbon assimilates is sent to the roots; primary root development is increased while lateral roots are repressed (Smith, 2012). Under drought, high concentrations of ABA can be detected in the roots which have been linked to plant signalling, resulting in stomatal closure and even seed abortion (Prasad et al. 2008). Drought and heat stress symptoms above ground -such as smaller organs and tissue chlorosis- are relatively easy to detect. Nonetheless, relatively few studies have considered the role roots play in stress response mainly because precise, well controlled, field experimental procedures are not straightforward. As a result many researchers opt for studies in controlled environments where rooting volume and temperatures are generally poorly representative of field growing conditions (Anderson 1986). Molecular control of root development has been studied in Arabidopsis (Larkindale et al. 2005) but in cereals relatively little is known. In maize and rice, mutants have been used to study the lateral root development and crown root elongation. The proteomics of the roots of two *Agrostis* grass species exposed to moderated (30°C) and intense (40°C) heat stress were

studied by Xu and Huang (2008) showing more proteins associated with stress response mechanisms were up-regulated in the thermotolerant species.

In the field it has been shown that bread wheat genotypes that invest significant resources in deep root development are capable of extracting residual moisture when drought stress occurs (Reynolds et al. 2007; Lopes and Reynolds, 2010). Under heat stress, well watered plants increase their transpiration rate due to high vapour pressure deficit which permits evaporative canopy cooling. To match evaporative demand requires increased stomatal conductance (Amani et al. 1996) and adequate vascular capacity including in the roots. Some traits can be used as surrogates for the analysis of root development, for example, the measurement of the canopy temperature. Cool canopy temperatures have been associated with increased plant access to water as a result of deeper roots (Lopes and Reynolds, 2010). These authors found that genotypes with cooler canopy temperatures reported 30% more yield associated with an increase of 40% in root dry weight at 60-120 cm. Genomic regions (QTL) associated with canopy temperature have been co-located with regions controlling other drought adaptive traits including kernel number, grain yield and chlorophyll content (Pinto et al. 2010, Diab et al. 2008; Olivares-Villegas et al. 2008). In a previous study, Pinto et al. (2010) identified 15 QTL for canopy temperature (CT) in the Seri/Babax bread wheat population grown under drought, hot irrigated and non-stressed conditions. The authors demonstrated five consistent QTL (1Ba, 2Ba, 3Bb, 4Aa, 7Aa) associated with cooler canopies that were common to both drought and heat environments. Three of the QTL were specific only for drought and heat stress, and the other two were also found under non-stressed conditions. The five QTL for CT explained an average of 7% and 14% of variance under drought and heat, respectively, with maximum of 27.6% under the heat environment in the 4A-a linkage group. On the same linkage group, a QTL explained a maximum of 27.4% and 17.1% of yield variation under drought and heat, respectively. The involvement of roots was inferred since cooler canopies are a result of higher transpiration rates which require adequate access to water. For the current study, lines showing contrast in these five QTL for CT were used for the selection of the sisters together with phenotypic data for CT and yield. These five QTL overlapped with QTL for traits previously associated with drought and heat tolerance including water soluble carbohydrates (WSC), kernel number, yield and plant greenness (Kuchel et al. 2007; Marza et al. 2006; Rebetzke et al. 2008).

The importance of roots in determining yield under stressed environments was highlighted by the recent release of rice varieties with improved performance under drought as a result of deep root development achieved through MAS for QTL associated with root length (Steele et al. 2006). The five QTL used in the current study are regions reported in the literature to be associated with root related traits. A number of QTL for early root length have been mapped in the 1B region of wheat and in its homoeologous region in rice, both crops grown in hydroponic culture (Price and Tomos 1997; Ren et al. 2012). Chromosome 2 was found to be associated with one or more architectural characteristics of seminal roots of durum wheat grown in gel chamber, including length, number and thickness (Sanguineti et al. 2007) and up to 68% of variance for root length of bread wheat was explained by a QTL identified in this region (Ren et al. 2012). Also, in field grown rice a QTL located in the homoeologous chromosome (Chromosome 8, Ahn et al. 1993) to 2B explained more than 30% of variance for root thickness under drought stress (Champoux et al. 1995). It seems that the 2B chromosome might contain genes for evapotranspiration efficiency since wheat experiments in pots under controlled conditions (Ehdaie and Waines 1997) showed several QTL in the long arm of the chromosome 2B of bread wheat associated with biomass production, -including roots, shoots and spikes. Similarly chromosomes 3 and 7 of wheat are associated with deep root development and root thickness and Champoux et al. (1995) report QTL in homoeologous regions of rice controlling these traits under field drought stressed conditions. Up 30% of the root length variance (Price and Tomos1997) was explained by a QTL located in chromosome 11 of rice that was hydroponically grown, which maps with segments of chromosomes 4, 5 and 7 of wheat where QTL for CT, NDVI, yield and grain number were previously mapped in field experiments with the Seri/Babax population (Pinto et al. 2010). The genomic region of chromosome 7 seems to contain several genes associated with drought tolerance in wheat, rice and barley where QTL have been identified for osmotic adjustment and related traits in wheat (Morgan and Tan 1996) and it homoeologues in rice (Zhang et al. 2001; Lilley et al. 1996) and barley (Teulat et al. 1998). Using the subset of sisters grouped according to their phenotype and genotypic data in COOL and HOT canopies, the current study was established with the following objectives: (i) to characterize a subset of contrasting Seri/Babax sisters in their agronomic and physiological performance when grown under drought and heat stresses, (ii) to verify the

potential of the previously identified QTL in marker assisted selection, and (iii) to test the hypothesis that optimal root distribution provides a common physiological response for adaptation under both drought and heat stress.

MATERIALS AND METHODS

Germplasm

The recombinant inbred lines used in this study came from the cross of parents, Seri M82 and Babax (also named *Baviacora M 92* or *Bav 92*), both spring wheat (*Triticum aestivum* L.) semi-dwarf lines with moderate tolerance to drought and heat stress (Olivares-Villegas et al. 2007) and high yield potential. Only Seri M82 carries the T1BL.1RS (rye) translocation from Kavkaz (Villarreal et al. 1998). The population was constructed for mapping of complex traits and therefore shows a relatively narrow range in phenology and height which is useful to avoid the confounding effect of major flowering and *Rht* genes (Pinto et al. 2010). Ten genotypes were included in this study and classified in two groups: COOL and HOT. The list of eight sisters and two parents is presented on Table 1, including their group (COOL/HOT) and the CT QTL used for the classification.

The HOT genotypes generally carried the Seri allele on those regions where any of the five QTL for CT was identified. The allele from Seri accounted for the undesirable expression of high CT and decreased yield (Pinto et al. 2010). In contrast, the COOL genotypes generally carried the Babax allele on the selected QTL for CT, allele responsible for lower canopy temperatures and high yields. For the QTL×E analyses a factorial design was applied using SAS Proc Mixed.

Description of the environments and experiments

Two drought and two heat experiments were established in the Yaqui Valley, NW Mexico during 2008-2009 and 2010-2011 seasons. Climatic conditions for the period of experiments are presented on Table 2. Drought trials received approximately ≤300 mm of water during the whole cycle, including irrigations and precipitation. Genotypes were sown in November and most water was received before the booting stage. Subsequently, there was moderate drought

stress during booting/heading and gradually intensifying stress during grainfilling. To establish the heat experiments, the sowing date was delayed by approximately three months. The air temperature became higher as the cycle progressed, reaching average daily maxima of nearly 37 °C during grainfilling. The heat stressed trials were fully irrigated every two weeks to minimize water limitations that could confound the results. Additionally, the sisters were sown during two years under high yielding conditions with minimal water and temperature limitations as control trials. Pest and diseases were controlled during the season in all the experiments. The four trials were established in a randomized complete block design with four replications. Each experiment consisted of 10 genotypes sown in double raised beds of 3.5 × 0.8 m using a seed density of 13 g/m^2. The soil at the Yaqui Valley is classified as sandy-clay and hyposodic vertisol, smectitic chromic haplotorrert according to the World Reference Base (Verhulst et al. 2009).

Measurements

Agronomic and physiological measurements were performed on all 10 genotypes sown in each environment. For the residual available soil moisture (RASM) and root biomass analyses at heading stage, a subset of four genotypes were selected, two of them COOL and two HOT. Only genotypes number three, four, five and six (Table 1) were included in the root and soil analyses. Traits recorded in the complete pool of genotypes were: yield (g/m^2), aboveground biomass at maturity (g/m^2), stem number at anthesis and maturity (stems/m^2), phenology, water soluble carbohydrates content in the stems at heading ±7 days (%) and canopy temperature during grainfilling (CTg) and vegetative stage (CTv). These measurements were performed using standard protocols cited by Reynolds et al. (2007). Days to heading and days to maturity were determined when 50% of the plot exhibited 50% of the spike (Zadoks 5.5) and when 50% of the plot lost greenness, respectively. For the residual available soil moisture and root biomass analyses an hydraulic soil corer (Giddings Corp. Co., Fort Collins, CO, USA) as cited by Lopes and Reynolds et al. (2010) was used to extract the soil sample from 0 to 120 cm depth. Sampling was done exactly above the row of plants in order to obtain soil and root biomass. Soil sampling was performed at heading time plus 10 days (±2 days). On each plot two and four points were sampled in the 2009 and 2011 seasons, respectively. Soil samples were separated into four depth profiles: 0-30 cm, 30-

60 cm, 60-90 cm and 90-120 cm using plastic bags to avoid soil moisture losses before weighing. In the same plot the two/four subsamples were bulked in a single plastic bag according to the corresponding profile. Samples were kept in the field in a cool box. At the research station the soil was mixed, then, a weighed sample about 100g (fresh weight) was dried in the oven at 75 °C for 24h to determine residual soil moisture. The remaining soil was washed and sieved to obtain root tissue. Roots were dried and weighed to determine root biomass production by soil profile.

A student's T-test was used to compare the two groups of genotypes and determine differences between COOL and HOT genotypes. The statistical analyses were performed using SAS v9.0. Root biomass and RASM data was standardized by the yearly average (SMean). These relative values for root and RASM used to compare between groups were calculated dividing individual data point by the trial mean.

Table 1 List of eight sisters and the two parents (Genotypes 9 and 10) selected from the Seri/Babax bread wheat population for their contrasting phenotypic and genotypic performance under drought and heat stress

Genotype	Cross	Selection History	Group	QTL for which was selected [a]
1	Bav92/Seri	CMSS96Y04084S-0Y-lB-8ITLA-0B-0Y-7IB-0Y-0Y	COOL	1Ba, 2Ba, 3Bb, 7Aa
2	Bav92/Seri	CMSS96Y04084S-0Y-lB-l3ITLA-0B-0Y-lI8B-0Y-0Y	COOL	1Ba, 2Ba, 3Bb, 4Aa, 7Aa
3	Seri/Bav92	CMSS96Y0405lS-0Y-lB-46TLA-0B-0Y-23B-0Y-0Y	COOL	1Ba, 2Ba, 3Bb, 4Aa, 7Aa
4	Seri/Bav92	CMSS96Y0405lS-0Y-lB-46TLA-0B-0Y-24B-0Y-0Y	COOL	1Ba, 2Ba, 3Bb, 7Aa
5	Bav92/Seri	CMSS96Y04084S-0Y-lB-52TLA-0B-0Y-50B-0Y-0Y	HOT	2Ba, 3Bb, 4Aa, 7Aa
6	Bav92/Seri	CMSS96Y04084S-0Y-lB-72TLA-0B-0Y-62B-0Y-0Y	HOT	1Ba, 2Ba, 3Bb, 7Aa
7	Bav92/Seri	CMSS96Y04084S-0Y-lB-93TLA-0B-0Y-l03B-0Y-0Y	HOT	1Ba, 2Ba, 3Bb, 4Aa, 7Aa
8	Bav92/Seri	CMSS96Y04084S-0Y-lB-l3ITLA-0B-0Y-1I7B-0Y-0Y	HOT	1Ba, 3Bb, 4Aa, 7Aa
9	Seri M 82	CM33027-F-l5M-500Y-0M-87B-0Y-0MEX	HOT	1Ba, 3Bb, 7Aa
10	Baviacora M 92	CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y	COOL	1Ba, 3Bb

[a] Indicates the linkage group where the QTL for CT was identified by Pinto et al. (2010)

Table 2 Weather conditions for each drought (Drt) and Heat trial sown during 2008-2009 and 2010-2011 crop seasons in the Yaqui Valley, NW Mexico. Weather data by stage is summarized using the average of daily records for maximum air temperature (Tmax), minimum air temperature (Tmin), sum of precipitation (Rain) and sum of evapotranspiration (Eto), according to data from the Mexican National Water Commission (CNA). The "Irrigation" column indicates the estimated total millimeters of water applied in the whole cycle

Environment	Irrigation (mm)	Year of harvest	Stage	Tmax (°C)	Tmin (°C)	Rain (mm)	Eto (mm)
Drought	≤ 300	2009	emergence to anthesis -15d	26.2	9.5	2	118
			anthesis -15d to anthesis +10d	26.4	7.4	0	66
			anthesis +10d to maturity	29.3	11.0	3	81
		2011	emergence to anthesis -15d	25.9	6.3	2	157
			anthesis -15d to anthesis +10d	27.7	7.5	0	83
			anthesis +10d to maturity	30.7	9.5	0	89
Heat	≥ 700	2009	emergence to anthesis -15d	29.1	10.6	3	125
			anthesis -15d to anthesis +10d	31.2	11.6	0	137
			anthesis +10d to maturity	36.6	15.8	0	153
		2011	emergence to anthesis -15d	30.6	9.6	0	136
			anthesis -15d to anthesis +10d	32.2	12.4	0	153
			anthesis +10d to maturity	36.1	12.3	0	188

d:days

RESULTS

Agronomic and physiological performance of two contrasting groups of sisters

Means for agronomic and physiological traits are presented on Table 3. Differences in CTv and CTg from a previous study (Pinto et al. 2010) were used together with QTL data, to group the lines in two contrasting sets of COOL and HOT genotypes (Table 1). Under drought the two groups reported significant differences of 1.4 and 0.6 °C in CTv and CTg, respectively. In the heat experiments the differences between the two groups were 1.0 and 0.8 °C for CTv and CTg (Table 3). The COOL genotypes yielded 19% and 12% more than the HOT genotypes under drought and heat, respectively, which was in agreement with 20% higher biomass production at maturity under drought and 12% higher biomass under heat. The number of stems was around 20% higher in the group of COOL genotypes (data not shown). While the growing cycles for drought and heat were on average 112 and 83 days, respectively, the

differences in days to heading and maturity between COOL and HOT groups was no more than 3 days in any environment (Table 3). Under drought, the COOL genotypes had 65% more grains per square meter and 15% less WSC in the stems but no differences were found for kernel weight in any environment (data not shown); when grown under heat stress the difference in grain number was 20% more grains produced by the COOL and 40% less WSC. High and significant correlation with yield was found for canopy temperature measured during the grainfilling stage, biomass at maturity, and grain number in the two environments (Table 3). Under non-stressed conditions both groups of genotypes reported statistically equal plant height differing only in four cm (data not shown).

Differences in radicular biomass and residual available soil moisture of the COOL and HOT genotypes

Significant differences were found between COOL and HOT genotypes for root biomass production and residual soil moisture (Table 4), under both drought and heat stresses, with smaller amounts of residual moisture and more extensive roots generally associated with cooler, higher biomass plants (Figure 1 and Figure 2).

Drought. Root mass measured shortly after anthesis and plotted against residual moisture at the same stage (Figure 1) shows that COOL genotypes used more of the available water in deeper soil profiles (Table 4, p= 0.02 for RASM at 30-90 cm and p=0.04 for RAMS at 30-120 cm), and that root mass was also higher in these two regions (p=0.0018 for both, 30-90 and 30-120 cm). It was observed that the moisture at 0-30 cm was close to zero as a result of the larger concentration of roots in this region and soil exposure that allowed evaporative losses. The total residual soil moisture of the COOL genotypes were significantly different (p=0.043) from that of the HOT genotypes, with the COOL genotypes leaving 25% less water in the whole (0-120 cm) soil profile around anthesis.

Heat. Measurements of root development and RASM shortly after anthesis in the heat experiments showed that the HOT genotypes left more residual soil moisture across the whole soil profile (Table 4, p=0.04) down to 120 cm (Figure 2). The strongest contrast was found nearer the surface at profiles 0-30 cm and 30-60 cm (Figure 2) where the COOL genotypes left 60% (p=0.001) and 30%

(*p*=0.032) less moisture than the HOT genotypes, respectively. This result was consistent with the COOL genotypes having relatively more superficial roots than deep roots compared to the HOT genotypes. For example, in the 30-60 cm region the COOL genotypes developed 35% more roots (*p*=0.0003) than the HOT genotypes.

Roots and RASM partitioning under heat and drought. Comparing the total amount of roots (0-120 cm profile), it was found that the COOL genotypes produced only about 10% more root tissue than the HOT genotypes under both heat and drought (Figure 3). However, the analysis of the distribution showed that greater differences were found below 30 cm. Both COOL and HOT genotypes concentrated most of their radicular development (˜ 80%) in the 0-30 cm profile when grown under heat stress, while under drought they tended to be more equally distributed across the 0-30 and 30-120 regions (Figure 3). Under drought 54% of the total root biomass of the COOL genotypes was located in the 30-120 cm profile (Figure 3), while the remaining 46% was superficial (0-30 cm). The HOT genotypes showed a smaller proportion (44%) of roots in the 30-120 cm under drought. The amount of roots found at 0-30 cm under heat, was four times greater than roots from 30-90 and 30-120 cm. Combined analyses across environments and years (QTL × E) showed highly significant interactions between QTL (*i.e.*, COOL v HOT) and the relative distribution of roots across the soil profile which is consistent with the observation that under drought the COOL-QTL favour deeper roots, while under heat stress the COOL-QTL favour more superficial roots (data not shown).

DISCUSSION

Notwithstanding the well documented adaptive value of phenological escape (earliness) from drought and heat stress (Barnabás et al. 2008), the potential confounding effect of phenology was avoided in this study by pre-selecting lines of similar heading time (heading range for two years averaged 7 and 5 days for drought and hot-irrigated environments, respectively), with contrasting agronomic performance and CT.

Drought. Phenotypic differences between the COOL and HOT genotypes were consistent with their agronomic performance and root mass and distribution

profiles. The results showed that under drought, cooler canopy temperatures were associated with genetic gains of 19% in yield, 20% in biomass, and 40% in deep roots, at 30-90 cm and 30-120 cm (Table 3 and Figure 1). Similar results were found by Lopes and Reynolds (2010) who reported that genotypes with lower canopy temperatures developed 40% more root mass at 60-120 cm and 30% higher yields.

Table 3 Means for COOL and HOT genotypes for two years of experiments (2008-2009 and 2010-2011) with Seri/Babax bread wheat grown under drought and heat stress

		Group mean (SE)			
Trait	Env	COOL	HOT	Pr>|t|	r
Canopy temperature during	Drt	23.5 (0.37)	24.9 (0.50)	0.040 **	ns
vegetative stage (°C)	Heat	27.0 (0.16)	28.0 (0.17)	0.000 ***	ns
Canopy temperature during	Drt	26.1 (0.11)	26.7 (0.10)	0.001 ***	-0.70 ***
grainfilling stage (°C)	Heat	31.6 (0.12)	32.4 (0.24)	0.0055 ***	-0.75 ***
Yield	Drt	204 (10.7)	172 (5.7)	0.017 **	-
(g/m^2)	Heat	240 (6.9)	215 (4.9)	0.010 **	-
Biomass at maturity	Drt	525 (24.4)	440 (10.2)	0.007 ***	0.90 ***
(g/m2)	Heat	565 (20.2)	505 (11.6)	0.021 **	0.84 ***
Heading	Drt	79 (0.58)	77 (0.77)	0.035 **	-0.78 ***
(dae)	Heat	51 (0.25)	50 (0.42)	0.003 ***	ns
Maturity	Drt	112 (0.85)	109 (0.56)	0.018 **	-0.65 ***
(dae)	Heat	83 (0.21)	82 (0.48)	0.007 ***	0.58 ***
Number of grains (†)	Drt (‡)	8985 (725)	5438 (605)	0.007 ***	0.93 ***
($grains/m^2$)	Heat	8380 (365)	6885 (349)	0.011 **	0.70 ***
Water soluble carbohydrates (†)	Drt (‡)	32.7 (0.30)	38.6 (1.1)	0.016 **	ns
(%)	Heat	9.0 (0.67)	15.4 (2.0)	0.018 **	ns
Root:shoot at anthesis	Drt	0.32 (0.02)	0.28 (0.02)	0.018 **	nc
	Heat	0.30 (0.04)	0.27 (0.03)	ns	nc

Data for the canopy temperature during vegetative and grainfilling stages was taken from: Pinto et al. (2010) and used for the selection of sister lines included in the current study. Env: environment. Statistically significant values according to Student's t-test at levels *α=0.1, **α=0.05 and ***α=0.01. SE: Standard error of means indicated in brackets. ns: not significant. nc: not calculated. All traits were recorded in the complete set of 10 genotypes except by those indicated by (†) which were measured in the subset selected for root and RASM analyses. (‡) Data for one year under Drt. Phenotypic correlation (Pearson) is shown as *r* for all the traits with yield using raw data for two replications and two years in each environment

Table 4 Significance obtained from the student´s t-test for the standardized means of the COOL and HOT genotypes in two years of experiments (2008-2009 and 2010-2011) in Seri/Babax bread wheat grown under drought and heat stress

	Drought			Heat		
	SMean (SE)		Pr > |t|	SMean (SE)		Pr > |t|
Trait	COOL	HOT	T test	COOL	HOT	T test
	Root development (g/m^2)					
Total Roots (0-120 cm)	1.05 (0.054)	0.927 (0.067)	ns	1.046 (0.046)	0.966 (0.070)	ns
Superficial Roots 0-30 cm	0.98 (0.082)	1.020 (0.104)	ns	1.031 (0.050)	0.968 (0.080)	ns
Roots 30-60 cm	1.12 (0.065)	0.883 (0.081)	0.0409	1.150 (0.029)	0.849 (0.056)	0.0003
Roots 60-90 cm	1.35 (0.112)	0.598 (0.084)	0.0002	1.142 (0.179)	0.858 (0.120)	ns
Roots 90-120 cm	1.22 (0.193)	0.750 (0.181)	ns	0.993 (0.069)	1.005 (0.285)	ns
Roots 0-60 cm	1.03 (0.057)	0.968 (0.059)	ns	1.048 (0.043)	0.952 (0.068)	ns
Roots 0-90 cm	1.05 (0.050)	0.939 (0.055)	ns	1.051 (0.043)	0.949 (0.070)	ns
Roots 30-90 cm	1.16 (0.065)	0.823 (0.052)	0.0018	1.139 (0.049)	0.861 (0.056)	0.0022
Roots 30-120 cm	1.16 (0.070)	0.788 (0.054)	0.0018	1.150 (0.041)	0.888 (0.053)	0.0031
Roots 60-120 cm	1.28 (0.119)	0.626 (0.098)	0.0016	1.154 (0.157)	0.885 (0.105)	ns
	RSM: Residual available soil moisture (mm)					
Total RASM (0-120 cm)	0.798 (0.114)	1.098 (0.068)	0.043	0.806 (0.102)	1.162 (0.106)	0.041
Superficial RASM 0-30 cm	0 (0)	0 (0)	nc	0.609 (0.046)	1.391 (0.145)	0.001
RASM 30-60 cm	0.592 (0.180)	1.551 (0.494)	ns	0.822 (0.061)	1.151 (0.112)	0.032
RASM 60-90 cm	0.704 (0.206)	1.131 (0.082)	0.066	1.22 (0.461)	0.931 (0.272)	ns
RASM 90-120 cm	0.898 (0.093)	0.962 (0.054)	ns	0 (0)	0 (0)	nc
RASM 0-60 cm	0.592 (0.180)	1.551 (0.494)	ns	0.739 (0.045)	1.218 (0.116)	0.006
RASM 0-90 cm	0.660 (0.166)	1.290 (0.169)	0.021	0.806 (0.102)	1.162 (0.106)	0.041
RASM 30-90 cm	0.660 (0.166)	1.290 (0.169)	0.021	0.846 (0.127)	1.132 (0.133)	ns
RASM 30-120 cm	0.798 (0.114)	1.098 (0.068)	0.043	0.846 (0.127)	1.132 (0.133)	ns

SMean: standardized means or relative values of roots and RASM which were obtained dividing each individual data point by the trial mean (year × environment); SE: standard error in brackets; ns: not significant; nc: not calculated

In the current study the differences in the root architecture of the two groups was further supported by the amount of residual soil moisture (RASM) at depth. In the superficial layers both groups of genotypes left similar amounts of moisture. However the analysis of the deeper section from 30-90 and 30-120 cm showed that in these regions the COOL genotypes were able to extract more water leaving 35% and 25% less residual available moisture than the HOT genotypes (Figure 1). The capacity of COOL genotypes to extract extra water in the 60-90 cm profile was also associated with an increase of 115% of root biomass in the same profile under drought (Figure 1). Lopes and Reynolds (2010) reported that genotypes with greater root development in the deep regions had lower amounts of WSC in the stems, perhaps as a result of more WSC being translocated to the roots to support deep root development.

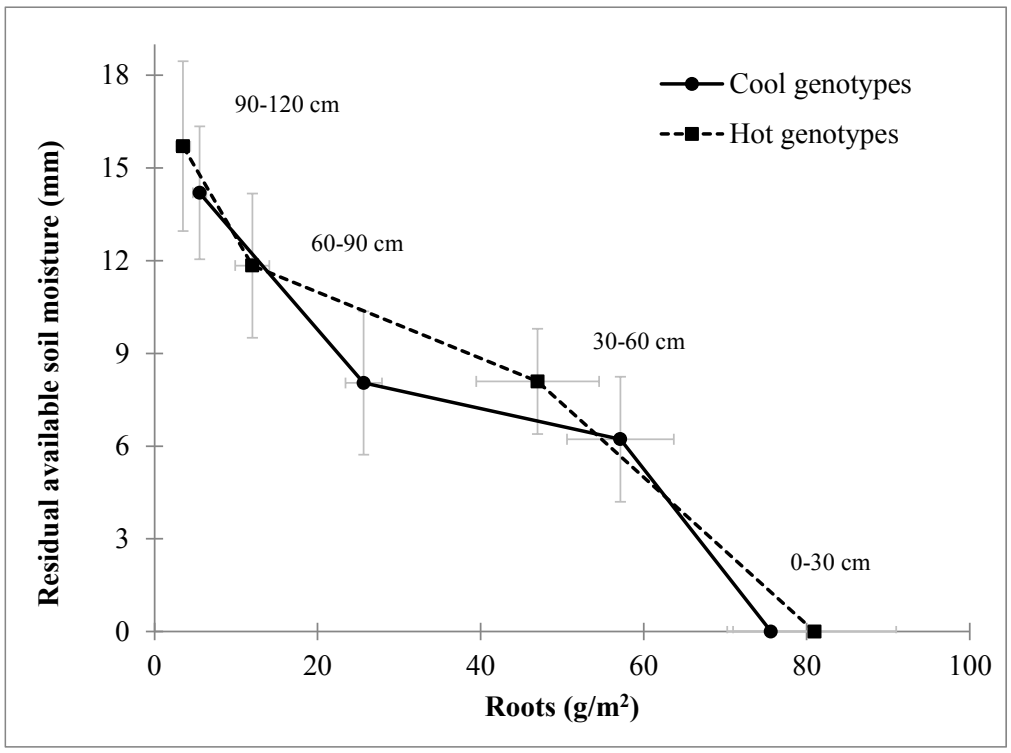

Fig. 1 Average of two years of experiments for root development and residual available soil moisture at heading +10 days in Seri/Babax bread wheat grown under drought conditions NW Mexico during 2008-2009 and 2010-2011 seasons

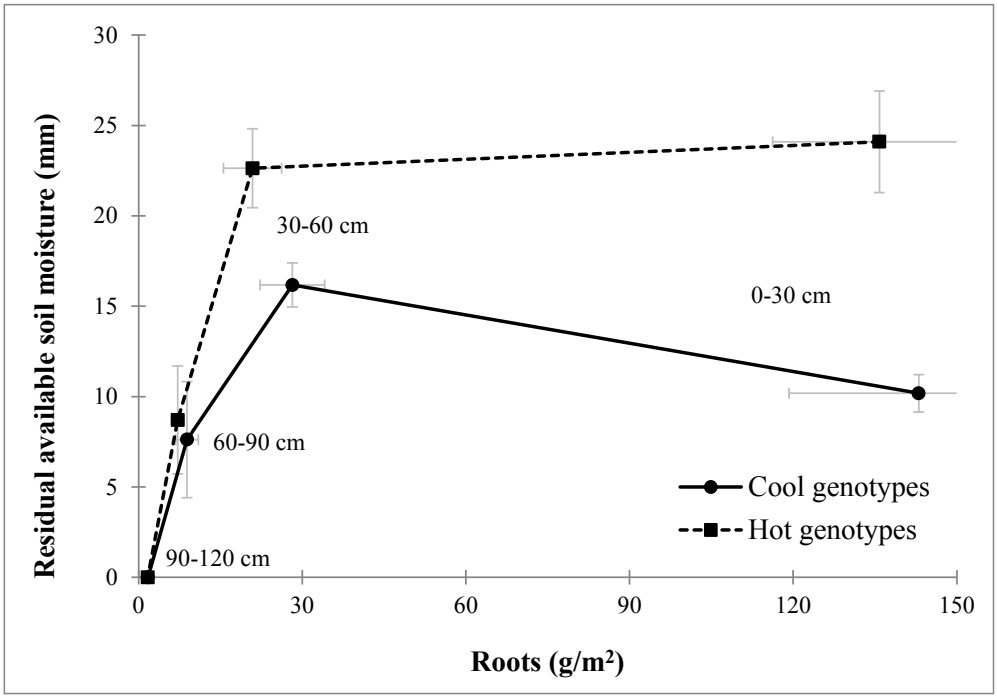

Fig. 2 Average of two years of experiments for root development and residual available soil moisture at heading + 10 days in Seri/Babax bread wheat grown under heat conditions, NW Mexico during the 2008-2009 and 2010-2011 seasons

The current study obtained similar results, showing that the WSC content of the COOL genotypes was 15% lower (Table 3) than the WSC of the HOT genotypes when grown under drought, and 40% lower under heat. The later was supported by phenotypic correlations with the root distribution under drought, which showed that root biomass in the 60-90 (r=-0.54, *p*=0.057), 60-120 (r=-0.68, *p*=0.015) and 90-120 (r=-0.72, *p*=0.006) soil layers was negatively associated with the percentage of WSC (using raw data) in the stems measured at around anthesis.

Mechanisms that may be determined by the "COOL QTL"

Drought stress usually promotes hormone signalling; in particular ABA concentrations are increased in the roots, helping in the maintenance of root growth and water uptake (Prasad et al. 2008). Manschadi et al. (2006) compared two wheat genotypes with different root architecture in root chambers, finding that the root length density below 90 cm in the drought tolerant variety was almost four times greater than in a standard Australian variety. These authors found that the former showed a more compact horizontal root architecture with a narrow angle but greater vertical development. This root pattern allowed superior water extraction capacities of the drought tolerant variety (~25% more water uptake from 60-90 cm). They proved that post anthesis, a drought adapted variety was able to continue development, focusing in the central and deepest soil layers, in contrast with the standard variety which equally extended its roots horizontally and vertically.

Heat. Several studies discuss the relevance of the root development as a key trait for drought tolerance, but scarce information regarding its role under heat stress is available. Deep root development at high temperatures has been associated with higher leaf transpiration rates. Plants with a strong radicular system are able to satisfy the high evaporative demand through elevated transpiration rates under hot irrigated conditions and thus maintain cooler canopies (Amani et al. 1996; Bonos and Murphy, 1999). While studies with tall fescue and ryegrass showed that high temperatures generally decreased root dry weight and photosynthetic rate, tall fescue, considered heat tolerant, exhibited greater root mass at 0-40 cm (Jiang et al. 2001) and a faster depletion of soil water (%).

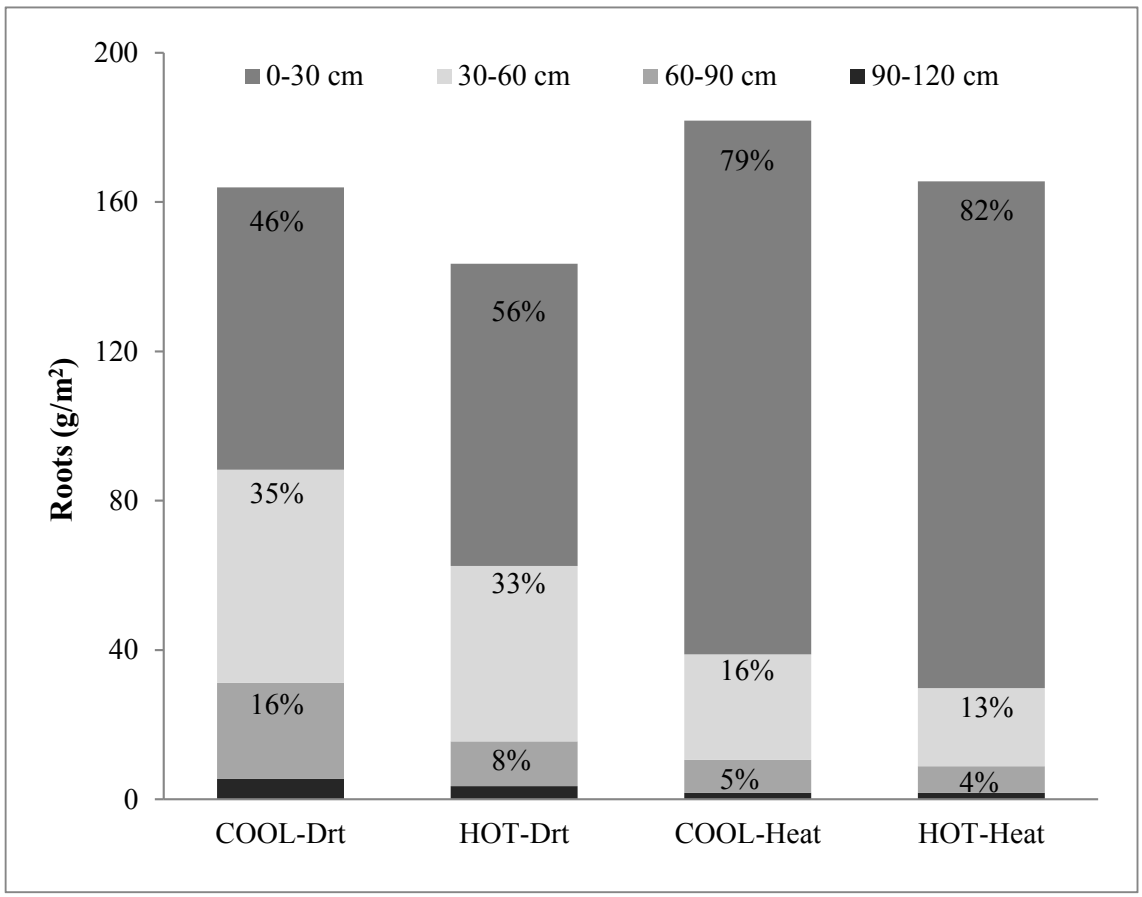

Fig. 3 Root distribution across the whole soil profile (0-120 cm depth) in Seri/Babax bread wheat is presented as the average of two years for each environment. T-tests revealed COOL and HOT genotypes differed significantly for the following profiles: under Drt, 30-60 cm (p=0.04), 60-90 cm (p=0.0002), 30-90 cm (p=0.0013), 30-120 cm (p=0.0018) and 60-120 cm (p=0.0016); under heat, 30-60 (p=0.0003), 30-90 (p=0.0022), 30-120 (p=0.0031). Percentages are the proportion of roots in each profile relative to the total amount of roots produced at 0-120 cm; the proportion in the 90-120 cm profile was <3%for both groups under Drt and Heat

The current study found that the group of COOL genotypes had higher amounts of radicular tissue in all soil profiles down to 120 cm depth, but especially in the 30-60 cm profile. These results showed that under heat stress, cooler canopy temperatures were associated with genetic gains of 12 % in yield, 12% in biomass and 35% in root development in the 30-60 cm layer (Figure 3). When water uptake was studied, the COOL genotypes were more effective in removing soil moisture, resulting in 30% less RASM at 30-60 cm than the HOT genotypes (Figure 2). Studies with Kentucky bluegrass showed that the maintenance of transpiration under heat stress was an important attribute for performance under stress. Comparison between heat tolerant and susceptible cultivars showed that those with canopy temperatures 5°C cooler had 65% more roots at 30-45 cm (Bonos and Murphy, 1999). At the cellular level it has

been observed that the thermal stability of the plasma membrane of wheat roots is affected by high temperatures (Zhao et al. 2011). Structural analyses of proteins located in the root membranes have shown that above 25°C the proportion of α-helix and β-sheet changed due to unfolding and disordering of structures. This change in the structure of plasma membrane proteins resulted in the reduction of H^+-ATPase activity, an enzyme responsible for multiple physiological functions such as nutrient uptake and cell growth, especially under stress conditions (Janicka-Russak, 2011).

Significance of these results to breeding

Genetic confirmation that CT is associated with effective root development. Root growth measurement is challenging and usually involves intensive and destructive techniques in order to obtain root tissue, however, canopy temperature, which is much easier to measure, is associated with the plant's ability to extract deep water (Reynolds et al. 2007; Lopes and Reynolds, 2010) and can be easily measured using infrared technology. Data from a previous study using these lines showed that the genotypes exhibited genetic variation for canopy temperature under heat stressed growing conditions and that the COOL group had lower temperatures; this was supported by the identification of QTL associated with this trait (Pinto et al. 2010). For example, the QTL for CT at chromosome 2B was classified as stress exclusive (drought and heat) and was also reported as the main QTL responsible for root developmental pattern in wheat, namely the maximum root length of lateral and primary roots (Ren et al. 2010; Sanguineti et al. 2007). Studies with rice and barley reinforced the importance of the QTL utilized herein as regions that might contain genes affecting root architectural characteristic and physiological attributes that determine plant performance (Zhang et al. 2001; Teulat et al. 1998; Champoux et al. 1995). Both parents from the experimental population of this study were identified as differing significantly in yield performance under drought while showing high yield potential (Reynolds et al. 2000). Their breeding value -as genetic sources of numerous varieties and cultivars- is recognized by breeding programs elsewhere (Fox et al. 1996; Skovmand et al.1997; IWIS database, CIMMYT Wheat Germplasm Bank). Seri M82 (IWIS CODE: M31 IBWSN S-1 MXI96-97) is a Veery 'S'-derived variety, susceptible to severe drought (moderately tolerant to moisture stress) (S. Rajaram, pers.comm.; CIMMYT 1986); Babax (IWIS CODE: CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y) is

a Baviacora variety sister line, tolerant to severe moisture stress (S. Rajaram, pers.comm.; CIMMYT 1986).

Four of the five CT QTL involved in this study showed favourable expression linked to the presence of the Babax allele. Segregation distortion patterns in the 1B chromosome in the Seri/Babax population result in 75% of the RIL containing the allele from Babax in this region (Mathews et al. 2008). This allele has been reported as responsible for the cool canopies and increased yield in previous studies with the same population (Pinto et al. 2010; Olivares et al. 2007) and other studies have associated the short arm of the 1B chromosome with traits related to transpiration efficiency (Rebetzke et al. 2008). In the subset of RIL included herein, the Seri allele associated with the T1BL.1RS (rye) translocation resulted in negative effects on yield and in warmer canopy temperatures which was in agreement with previous studies involving Seri crosses grown under drought stress and irrigated conditions (Pinto et al. 2008; Mathews et al. 2008; Peake 2003). However, the effect of the T1BL.1RS translocation seems to be environment dependent (Rattey et al. 2009) since it also has been found to be advantageous for drought adaptation in earlier studies (Villareal et al. 1995). In the 4A chromosome, unfavourable effects from the Seri allele were observed on canopy temperature in a previous study; the presence of the Babax allele in the 4A chromosome of the RIL resulted in cooler canopy temperatures which were apparently associated to larger aboveground biomass and yield increments where as much as 27% of genetic variance for these traits have been linked to the Babax parental (Pinto et al. 2010). Results from the current study indicated that the COOL genotypes (which generally possessed the Babax allele in the 4A region) showed significantly higher aboveground biomass production as well as higher radicular development.

Common QTL for heat and drought. Pinto et al (2010) showed for the first time common QTL associated with adaptation of wheat to both drought and hot irrigated conditions in the Seri/Babax population, and inferred the involvement of roots since cooler canopies were associated with better performance in both environments. The current study, by measuring root growth in subsets of iso-QTL lines from the same population has provided definitive evidence for the involvement of roots. However, the response of roots was not simply to grow deeper or more extensively, but rather to adapt to the specific needs of the environment. Namely, under drought, the roots of the cooler lines showed a greater distribution at depth. On the other hand, under heat stress, the roots of

the cooler lines showed a relatively greater proportion of roots at the surface where access to water was more reliable given frequent gravity irrigations in this treatment. This would suggest that the QTL of COOL lines may be exerting their influence at a relatively high level of integration and be involved in determining root distribution pattern in response to environmental cues. This is backed up by work that linked CT to plant growth regulation (Tang et al. 2008; Wardlaw, 1974). This selective root performance observed in bread wheat RIL are supported by results from a recent study with *Arabidopsis* which revealed that water availability determines root development, influencing the position of lateral branches and root hairs. The authors indicate that roots can distinguish between soil areas containing air or humidity and are able to respond according to the environment. This kind of response is known as hydropatterning, a conserved process not exclusive to *Arabidopsis* but present also in cereals like Maize and Rice (Bao et al. 2014). In addition, the specificity of the CT QTL previously reported by Pinto et al. (2010) was supported by an interesting trade off observed between stem water soluble carbohydrates (WSC) content and root growth under both stresses. Under drought, root development in the deep soil layers (60-120 cm) was negatively and significantly associated with WSC, while under heat, the negative association with WSC was found in the upper soil region at 30-60 cm. These results were consistent with the findings from Lopes and Reynolds (2010) regarding the possible contribution of stored stem WSC to the development of deeper roots under drought.

CONCLUSIONS

QTL conferring tolerance to both heat and drought stress provide useful opportunities for adapting wheat to climate change, under which both stresses are expected to increase. If one or more of the QTL can be used to derive close markers, they would be especially useful in molecular breeding since heat and drought are both challenging targets separately, and are expected to increasingly occur together (Sanderson 2011). The result also confirms the value of using CT as a proxy for favourable expression of root traits under both heat and drought stress by putting it on a firmer genetic basis. In addition, the observation that these QTL affect adaptive root response gives a useful lead into understanding the genetic basis of how root growth may be regulated.

Author contribution statement

RSP: Conducted all of the experiments that were based on an earlier study she also published. She performed all data analysis and led the write-up.

MPR: Designed the experiment and participated in all aspects of data analysis and writing of the paper.

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Conflict of interest statement

The authors declare that they have no conflict of interest

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Chapter 3. Journal paper: Modelling and genetic dissection of staygreen under heat stress

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Premature loss of plant greenness is an important factor influencing yield potential under stress (Thomas and Howarth 2000), and under high temperature environments the staygreen phenotype is expected to contribute to longer grain-filling and extended photosynthetic activity (Vijayalakshmi et al. 2010). This strategy can be used to address the issue of heat damage that leads to yield losses and conferring adaptive advantages. In many crops including wheat, the delayed loss of plant greenness has been associated with improved plant performance under stress conditions. However, methods for staygreen determination have not been standardized and large variation is reported across staygreen studies complicating its utilization as a selection tool in wheat improvement. Staygreen has been found to be correlated with wheat yield, but given that greenness loss is heterogeneous across the plant and even within individual organs, it seems that staygreen is difficult to parameterize. The study presented in this chapter applied a novel method for staygreen determination in spring wheat lines grown under hot-irrigated conditions showing that the estimation of derived staygreen parameters can assist in the improvement of heat tolerance of wheat. The study used an integrative technique that measures whole wheat plot spectral reflectance, allowing the determination of the kinetics of canopy greenness during the whole crop cycle. This procedure facilitated the screening of large number of genotypes with a standardized method that accounted for plant phenology variations. The staygreen attribute was analysed in terms of rate of greenness loss (rate of senescence) and was modelled using linear and non-linear models showing that the type of pattern followed was directly associated with crop performance under hot-irrigated conditions. The methods explored in this chapter present new avenues in the discovery of the optimal pattern of greenness loss and provides selection criteria to be used in the improvement of wheat under heat stress.

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	**R. Suzuky Pinto**		
Contribution to the Paper	Conducted field experiments, performed data analysis and led the write-up.		
Overall percentage (%)	60%		
Signature		Date	31/05/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Marta S. Lopes**		
Contribution to the Paper	Conducted field experiments, performed data analysis and participated in the writing of the paper.		
Signature		Date	31/05/2016

Name of Co-Author	**Matthew P. Reynolds**		
Contribution to the Paper	Designed the experiment and participated in all aspects of data analysis and writing of the paper		
Signature		Date	31/05/2016

Name of Co-Author	**Nicholas C. Collins**		
Contribution to the Paper	Provided valuable advice for the improvement of the paper		
Signature		Date	31/05/2016

Modelling and genetic dissection of staygreen under heat stress

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Key message: Staygreen traits are associated with heat tolerance in bread wheat. QTL for staygreen and related traits were identified across the genome co-located with agronomic and physiological traits associated to plant performance under heat stress.

Abstract

Plant chlorophyll retention -staygreen- is considered a valuable trait under heat stress. Five experiments with the Seri/Babax wheat mapping population were sown in Mexico under hot-irrigated environments. Normalized difference vegetation index (NDVI) during plant growth was measured regularly and modelled to capture the dynamics of plant greenness decay, including staygreen (Stg) at physiological maturity which was estimated by regression of NDVI during grainfilling. The rate of senescence, the percentage of plant greenness decay, and the area under the curve were also estimated based on NDVI measurements. While Stg and the best fitted curve were highly environment dependent, both traits showed strong (positive for Stg) correlations with yield, grainfilling rates, and extended grainfilling periods, while associations with kernel number and kernel weight were weak. Stg expression was largely dependent on rate of senescence which was related to the pattern of the greenness decay curve and the initial NDVI. QTL analyses revealed a total of 44 loci across environments linked to Stg and related traits, distributed across the genome, with the strongest and most repeatable effects detected on chromosomes 1B, 2A, 2B, 4A, 4B and 7D. Of these, some were common with regions controlling phenology but independent regions were also identified. The co-location of QTL for Stg and performance traits in this study confirms that the staygreen phenotype is a useful trait for productivity enhancement in hot-irrigated environments.

Keywords: greenness, green biomass, hot environments, QTL, heat tolerance

Introduction

The staygreen attribute, defined as "*heritable delayed foliar senescence*" (Thomas and Stoddart 1975) is considered as a selection criterion for crop improvement to extend grainfilling duration and ensure that grain size is not limited by lack of post-anthesis assimilates. For many years the staygreen character has been empirically included in visual selection of breeding lines (Thomas and Ougham 2014) but its genetic basis is not well understood.

The visible symptom of a staygreen phenotype is the persistence of greenness, which actually represents only one of many processes involved in delayed leaf senescence. The permanence of the pigment can be due to disabled chlorophyll catabolism or modification of the chlorophyll *b* and chlorophyll *a* ratio (Thomas and Howarth 2000). Complex hormonal controls are involved in leaf senescence, where cytokinins are the main inhibitors; plant treatment with cytokinins has resulted in staygreen phenotypes of tobacco and *Arabidopsis* (Gan and Amasino 1995). Five types of staygreen have been distinguished (Thomas and Howarth 2000), which broadly can be grouped as cosmetic staygreen or functional staygreen. As their names indicate, in the first type of staygreen the tissue looks green even when photosynthetic activity has been decreased or stopped in contrast to the functional staygreen (Thomas and Ougham 2014). The latter is obviously the target of plant breeding. Staygreen has been associated with drought and heat tolerance (Kumari et al. 2007); for example in sorghum, grain yield is positively associated with staygreen under water limited conditions (Rosenow et al. 1983; Borrell and Douglas 1996).

Genetic variability for staygreen has been identified and exploited in maize, oat, rice, wheat, fescue, soybean, pea, tomato, pepper, fruits, trees and other species (Barry et al. 2008; Armstead et al. 2006; Duvick et al. 2004; Thomas and Smart 1993; Thomas and Stoddart 1975). A number of studies have modelled the staygreen attribute as an indicator of photosynthetic activity. Deeper understanding of the dynamics and mechanisms affecting staygreen under high temperature environments are required to successfully exploit this attribute and improve plant adaptation to heat stress. Modelling canopy greenness dynamics over the whole crop cycle can help with this, while having obvious application in determining the best time for screening by identifying at what growth stage(s) differences in greenness are best associated with yield and show the best resolution. It is interesting that staygreen is frequently

reported for leaf greenness while other organs that also contribute to total plant photosynthesis such stems and spikes are not always considered. CO_2 absorbed by spikes represents at least 20% of flag leaf CO_2 captured in wheat (Teare et al. 1972) and estimates indicate that the spikes' contribution to grain yield is variable depending of the conditions but can reach up to the 70% in wheat and barley grown under stress (Maydup et al. 2010; Araus et al. 1993; Biscoe et al. 1973; Thorne 1963). Accurate quantification of individual leaf greenness (Harris et al. 2007) can be performed with the SPAD meter, and visual scoring, though more subjective, has been used to estimate greenness for decades (Kumar et al. 2010). The GreenSeeker spectral sensor offers an integrative high throughput approach to precision quantification of staygreen; it measures total canopy variation in green area including leaves, stems and spikes and permits screening of a large number of samples in a relatively short time (Lopes and Reynolds 2012); this enables potential application in large scale phenotyping including for QTL mapping. The current study applies this novel methodology measuring normalized difference vegetative index (NDVI) during the crop cycle so that the pattern of greenness decline could be determined. A number of NDVI-based staygreen related traits can be derived to enhance understanding of the mechanisms affecting plant's greenness persistence; these include the estimation of the velocity of greenness loss (RS), the proportion of plant greenness lost mid grainfilling (Gdecay), and the estimate of total green biomass (StgAUC and TotalAUC), parameters determining light interception (Cossani and Reynolds 2012). The quantification of the staygreen attribute and other related traits in a wheat mapping population allows the identification of genetic loci controlling staygreen which can provide the tools to enable MAS to accelerate and improve efficiency of plant breeding. QTL mapping for staygreen has been performed for several species including *Lolium* (Thorogood et al. 1999), pearl millet (Howarth et al. 1994), wheat (Kumar et al. 2010; Vijayalakshmi et al. 2010), maize (Zheng et al. 2009) and sorghum (Harris et al. 2007; Tao et al. 2000).

It has been estimated that wheat yield is reduced 3-5% per 1°C increased above 15°C during the grainfilling period (Gibson and Paulsen 1999). High temperatures result in accelerated plant growth, reduced plant size and shortened cycle, limiting the amount of light intercepted. In that sense, extending the grainfilling duration through delayed greenness loss seems to be especially advantageous in heat stressed environment. The exact profile of the staygreen attribute as a heat adaptive-trait still needs to be clarified but in the

current study it is proposed that plant greenness during grainfilling is lost following different patterns and that these patterns can be modelled following linear and non-linear regression models. Finally it is anticipated that genotypic differences for the Stg trait and related parameters exist and that this trait can be mapped for QTL to provide new avenues in the understanding of mechanisms controlling plant staygreen and its association with yield and other physiological traits.

The specific objectives of this study were i) to model plant senescence patterns of Seri/Babax RIL grown under heat-stressed, irrigated conditions, ii) to calculate a measure of staygreen (Stg) at physiological maturity using a linear regression model, and iii) to identify QTL linked to this character and additional traits associated with heat tolerance.

Methods

Germplasm and field experiment conditions

The population consisted of 167 RIL derived from crosses between two of CIMMYT´s elite lines: Seri M82 (herein called Seri) derived from a 'Veery' cross (KVZ/BUHO//KAL/BB) and a sister line of the elite variety Baviacora M92 'Babax' (BOW/NAC//VEE/3/BJY/COC). Both parents exhibit drought tolerance and high yield potential (Olivares-Villegas et al. 2007) while the population is characterized by a restricted range of height and phenology and does not segregate for major height, vernalization or photoperiod response genes (Pinto et al. 2010).

Five heat-stressed, irrigated trials were conducted during the seasons 2005, 2006, 2010, 2011 and 2013 in the Yaqui Valley, Northwest México; the site is a high radiation, irrigated environment. In 2005, 2006 and 2010 the trials were sown in February and in 2011 and 2013 the trials were sown in March. Based on the mean temperature at particular developmental stages, the trials were classified as: moderately hot (M), hot (H) or intensely hot (I) and are named with these letters followed by the last two digits of the sowing and harvest year (Table 1). Field experiments consisted of plots of one raised bed of 80 × 100 cm with two rows per bed; all the experiments were sown in two-replicate alpha-lattice designs. Sowing seed density was 15 gm^{-2} in the February and March trials. All trials were fully irrigated when approximately 50% of available soil moisture was depleted in the 0-1 m soil profile.

Phenotyping

Physiological and agronomical traits were recorded in the five trials according to standard procedures detailed elsewhere (Reynolds et al. 2001). These included: repeated measurements during the vegetative (v) and grainfilling stages (g) for the normalized difference vegetation index (NDVI), flag leaf chlorophyll (Chl) and canopy temperature (CT); individual measurements were averaged for these traits and a single value is presented. Also recorded were the number of days to reach heading (heading) and physiological maturity (maturity), plant height (height), grain yield, kernel number (KN), grain weight (TGW) and the grainfilling rate (GFR= yield /[days to maturity – days to heading]). NDVI was measured by canopy reflectance with a GreenSeeker (Optical Sensor Unit, 2002 NTech Industries, Inc., Ukiah, CA, USA). The chlorophyll of the flag leaf was assessed using a portable chlorophyll meter (SPAD-502 Minolta, Spectrum Technologies Inc., Plainfield, IL, USA) and the CT was recorded using an infrared thermometer (Mikron M90 series) 2-3 times per week avoiding cloudy and windy days according to the protocol described in Reynolds et al. (2001).

Estimation of staygreen related traits

Staygreen (Stg) was calculated using linear regression analyses of NDVI readings from heading until shortly after maturity according to Lopes and Reynolds (2012), given that anthesis under heat stress occurs very shortly after heading. The regression equation for each experimental plot was obtained by plotting NDVI during grain filling (NDVIg) against days after heading; Stg was calculated by substituting the maturity day in the equation. Stg is a unitless trait given that it is based on a NDVI ratio. The rate of senescence (RS) for each genotype was calculated from the slope of the NDVIg decline against thermal time (°C) using a linear regression equation (Fig. 1). Greenness decay (Gdecay) was calculated as the percentage of NDVI decline in the first half of the grainfilling stage (in number of days after heading). Staygreen-area (StgAUC) and Total area (TotalAUC) were calculated as the area under the curve with starting points at maximum NDVI (for StgAUC) or at crop establishment (TotalAUC) and using the corresponding thermal time for each case. Stg and staygreen related traits (RS, Gdecay, StgAUC, TotalAUC) were estimated only in three environments: M10, H05 and I13, due to insufficient NDVI data in H11 and I06.

Modelling NDVI along the crop cycle and during the grainfilling period

The modelling of NDVI curves across the crop development period and during staygreen decay in the grainfilling phase were performed in R 3.1.0 (http://www.R-project.org/) applying a sigmoidal function. In the M10 environment NDVIv (NDVI during the vegetative stage) was not recorded before 500 degree-days (dd, °C d) but in order to draw an NDVI trend for the whole cycle, this gap was filled using NDVI from H05 trial, given that comparable values were expected because NDVI for both trials performed similarly after 500 dd. This assumption had no effect on the calculated Stg values or Stg related traits, except on TotalAUC, since only the later included these inferred NDVI values. For this analysis, a non-linear model was developed by combining two sigmoidal functions as given by the following equation:

$$\widehat{NDVI}_{TT} = \frac{NDVI_{max}}{1 + e^{-r_{exp}(TT - i_{exp})}}\left(1 - \frac{1}{1 + e^{-r_{sen}(TT - i_{sen})}}\right)$$

where *TT* is the thermal time (i.e. degree C days), $\widehat{NDVI}_{TT}$ is the simulated NDVI at *TT*, $NDVI_{max}$ is the season maximum NDVI parameter, r_{exp} is a canopy expansion rate parameter, i_{exp} is a canopy expansion inflection point parameter, r_{sen} is a canopy senescence rate parameter, and i_{sen} is the inflection point of canopy senescence. Each genotype was individually modelled for NDVIg after heading following linear and non-linear models using the equations:

Linear model: $NDVI_{TT} = mTT + b$ Curve type 1

Non-linear models: $NDVI_{TT} = -aTT^2 + bTT + c$ Curve type 2

$NDVI_{TT} = aTT^2 + bTT + c$ Curve type 3

The best fitted model was selected based in the Bayesian information criterion (BIC).

Statistical and QTL mapping analyses

Adjusted means were obtained in SAS v9.0 using ANOVA mixed models to obtain the best linear unbiased prediction (BLUPs); spatial adjustment was included in the analysis by adding the effect of row and column according to the location of each plot in the field. Pearson's phenotypic correlations (r_P) were calculated using the formula of Roff (1995) from the adjusted means. The QTL

mapping analyses were performed in GenStat 15th edition in a Composite Interval mapping procedure using a threshold LOD value of 2 to identify all QTL candidates and LOD >3.5 for defining consistent QTL. QTL mapping was performed individually by trial and by trait, and also for each trait combined across environments.

The Seri/Babax population map used here in was previously constructed and consisted of 475 markers: 118 SSR (Single Sequence Repeat), 212 AFLP (Amplified Fragment Length Polymorphism), and 145 DArT (Diversity Array Technology) markers distributed over 20 chromosomes, only the chromosome 3D is missing (McIntyre et al. 2010). Previous QTL mapping studies have been reported using earlier versions of this map (Pinto et al. 2010; Lopes and Reynolds 2012).

Table 1. Average daily temperatures (°C), total evapotranspiration (Eto, mm) and total rain (mm) recorded during the vegetative, reproductive and grainfilling stages for the five Seri/Babax trials grown between 2005-2013 under heat-stressed, irrigated conditions in the Yaqui Valley in Northwest, Mexico.

Environment	Year of sowing and harvest	Month of sowing	Heat stress intensity	Measurements	Days to heading (dae)	GF length (days)	Maximum [a] 3days (°C)	Stage	Daily air temperature (°C)			Rain (mm)	Eto (mm)
									Maximum†	Minimum†	Mean†		
M10	2010	February	Moderate (M)	Stg rel traits	53	31	40.0	Emergence to heading -10 days	28.8	8.8	18.8	0	160
				Agr & Phys traits				Heading ±10 days	30.5	11.7	21.1	0	119
								Heading +10 days to maturity	37.0	12.5	24.7	0	174
H05	2005	February	Hot (H)	Stg rel traits	53	27	37.2	Emergence to heading -10 days	31.8	10.0	20.9	0	231
				Agr & Phys traits				Heading ±10 days	34.7	12.3	23.5	0	141
								Heading +10 days to maturity	35.5	19.0	27.2	0.70	103
H11	2011	March	Hot (H)	Agr & Phys traits	50	26	39.6	Emergence to heading -10 days	33.4	12.6	23.0	0	235
								Heading ±10 days	35.1	12.7	23.9	0	169
								Heading +10 days to maturity	38.4	18.5	28.5	0	119
I06	2006	February	Intense (I)	Agr & Phys traits	55	28	42.4	Emergence to heading -10 days	33.7	10.8	22.3	0	233
								Heading ±10 days	38.4	15.9	27.1	0	154
								Heading +10 days to maturity	39.5	20.8	30.2	0	158
I13	2013	March	Intense (I)	Stg rel traits	49	25	38.9	Emergence to heading -10 days	33.1	12.5	22.8	0	243
				Agr & Phys traits				Heading ±10 days	36.7	15.4	26.1	0	167
								Heading +10 days to maturity	37.0	22.2	29.6	0.3	138

dae: days after emergence; GF: grainfilling; a: maximum average of three days across the whole plant cycle; Eto: evapotranspiration; Agr & Phys: agronomic and physiological; † average of the daily maximum/minimum/mean temperature recorded during the days comprised in the specified period. Non stressed environments are regularly sown during November-December where daily maximum temperatures of the anthesis stage for wheat crop are commonly < 30 °C, season mean is 17.7 °C and ranging between 5.50 to 31.0 °C. Trials are named with letters M (moderately hot), H (hot) or I (intensely hot) followed by the last two digits of the sowing and harvest year.

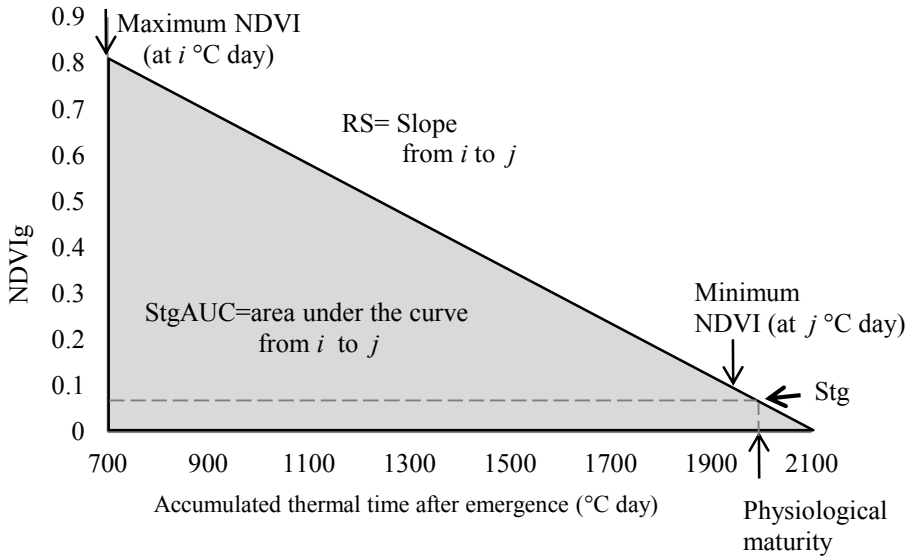

Figure 1. Diagram illustrating calculation of staygreen traits, RS (rate of senescence), StgAUC (area under the curve during the NDVIg decline phase) and Stg (greenness at physiological maturity).

NDVIg: normalized difference vegetative index during grainfilling *i*= thermal time with maximum NDVIg; *j*= Thermal time with minimum recorded NDVIg; RS was calculated as the linear slope from *i* to *j* for all the genotypes; Stg: staygreen, residual greenness remaining at physiological maturity calculated using a linear regression for each genotypes.

Results

Analysis of agronomic and physiological traits

The adjusted means and basic statistics for all traits calculated across the four trials for parents and RILs are presented on Table 2. The two parents showed similar expression for Stg, phenology and other traits while a much wider range was observed in the RIL. The rate of senescence (RS) for both parents averaged across environments indicated that the NDVIg decreased by about 8 SPAD-units each degree day (°C), similarly to the estimated population mean. Gdecay across environments ranged from 18 to 44% and averaged 31.2% for the RILs. Heading time was found to be relatively constant across parents and RILs, with a range of 13 days observed across environments. Pearson's correlations showed that trial associations were positive and significant for yield (Fig. 2). Staygreen (Stg) was found to not well associated (p>0.05) across the three environments (Fig. 3) varying from 0.12-0.38 but Stg showed consistent and positive correlation with kernel number (KN), thousand grain weight (TGW) and yield (Supplementary Figure 1). The correlation between Stg and TGW was the weakest on average (Table 3), although it was still significant (p<0.05). The

distribution of the Stg trait showed that it varied across environments, ranging from 0.2-0.4, 0.05-0.3 and 0.14-0.27 for the M10, H05 and I13 trials, respectively (Supplementary Figure 2). The highest values were observed in M10 which experienced lower heat stress compared with H05 and I13. Unexpectedly, the lowest Stg values were found in H05 and not in I13, but the variability for this trait was reduced under intense heat stress in I13. The rate of senescence for the parents by environment is presented in Supplementary Figure 3.

Modelling NDVI across crop development

Individual measurements of NDVIv and NDVIg were plotted against thermal time and by regression analyses a single curve was fitted for the whole population for each environment. The performance of the NDVI trait across the cycle showed similar patterns in H05 and M10; major differences were observed in the NDVI pattern of the highest stressed environment, I13 (Fig. 4). Maximum NDVI was about 0.80 in M10 and 0.75 in H05, contrasting with I13 where the maximum NDVI was only 0.6. These maximum values were reached at about 750 degree-days in all environments.

During grain filling, Seri showed lower initial NDVIg values than Babax at the same thermal time in the three environments (Supplementary Figure 4). However, the decline in greenness in Seri was slower than the decline in Babax resulting in only marginally lower Stg for Seri. When modelling each mapping line separately, the 169 genotypes were observed to fit one of three types of curves best (Fig. 5). In the I13 environment, higher variation for type of curve was observed, given that the proportion of genotypes that fitted better to a linear curve (55%) was close to the proportion of genotypes that fitted better to a parabolic curve (45%). But when the heat stress was lower the diversity was reduced. In H05, 96% of the population fitted a parabola best (curve type 2 and 3) and only 4% fitted a linear model (curve type 1); while in M10 all the genotypes fitted a parabolic (curve type 2) curve best (data not shown).

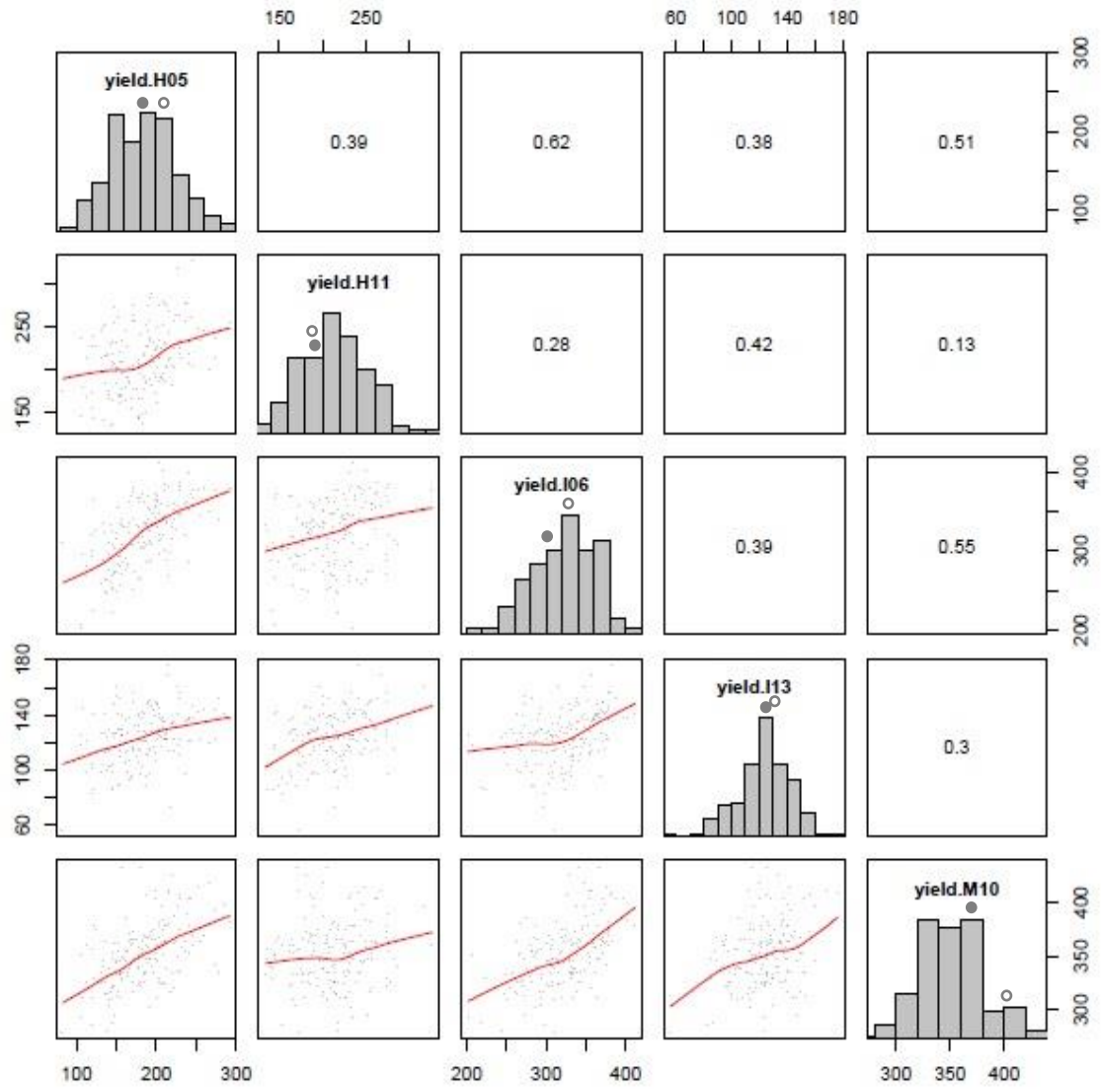

Figure 2. Associations of yield in the Seri/Babax population across five heat-stressed, irrigated environments grown between 2005 and 2013. The diagonal contains the yield histogram for each environment, the lower diagonal a scatter plot and loess smoothing line between all environments, and the upper diagonal shows the phenotypic correlations (r_P)
r_P >0.15 are significant at p=0.05; r_P >0.19 are significant at p=0.01; r_P >0.24 are significant at p=0.001. In the histograms Seri is represented with a filled circle and Babax with an empty circle.

To investigate relation between trait performance and NDVIg curve types, a subset of 53 genotypes with restricted range of phenology (average difference in heading date between groups was restricted to one day) was selected from the I13 environment in order to balance the number of genotypes included on each group. This environment was chosen because it exhibited a larger diversity for type of curve compared to M10 and H05. In an ANOVA, curve type was significantly related to yield (Table 4). Significant differences were found between genotype groups with different curve types, for yield, yield components and physiological traits (Table 4). The curve type with largest StgAUC, curve

type 2, was associated with higher yield, KN, TGW, NDVIg, GFR and GFD. Significant differences were also detected for phenology and plant height, even though differences in heading time between groups were restricted.

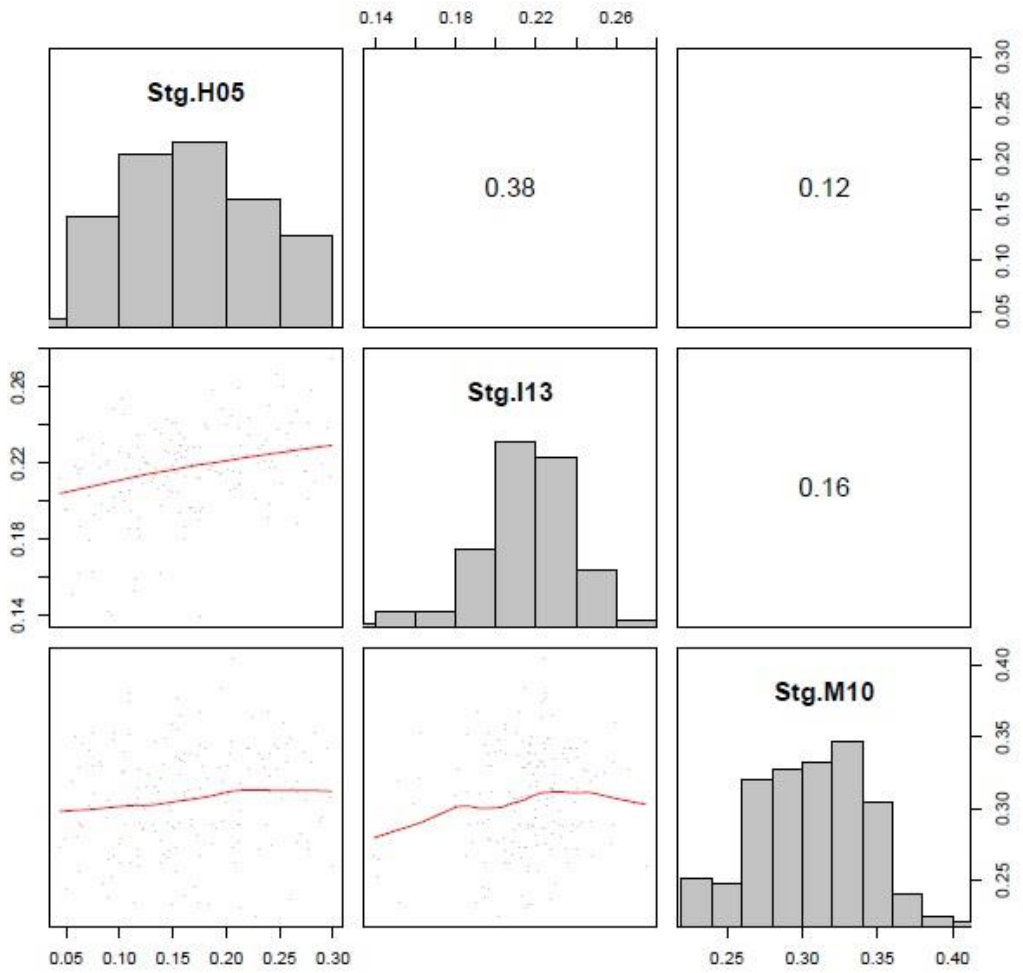

Figure 3. Stg correlations for the Seri/Babax population across three heat-stressed, irrigated environments sown between 2005 and 2013. The diagonal contains the Stg histogram for each environment, the lower diagonal a scatter plot and loess smoothing line between environments, and the upper diagonal shows the phenotypic correlations (r_P)

Table 2. Means and basic statistics for traits measured during the whole development of the Seri/Babax RILs, in five heat-stressed, irrigated environments

Trait	Parents means		RILs				Across environments	
	Seri	Babax	Mean[ł]	Minimum[ł]	Maximum[ł]	$\sigma^{ł}$	h^2	LSD
Staygreen	0.220	0.219	0.230	0.136	0.326	0.042	0.380	0.067
RS (NDVI/ °C day)	7.50×10^{-4}	8.40×10^{-4}	7.90×10^{-4}	5.20×10^{-4}	11.1×10^{-4}	1.10×10^{-4}	0.114	1.60×10^{-4}
Gdecay (%)	31.3	30.5	31.2	18.0	44.0	5.27	0.284	7.94
Yield (g/m^2)	235	258	238	159	317	32.5	0.773	33.0
KN ($grains/m^2$)	8325	7695	8185	4521	11807	1270	0.760	1238
TGW (g)	28.6	33.8	29.4	23.1	36.5	2.32	0.856	1.96
GFR ($g\ m^{-2}/day$)	9.10	10.4	9.62	5.71	13.1	1.39	0.724	1.45
GFD (days)	28.4	27.8	27.5	24.1	31.3	1.23	0.430	1.48
NDVIv	0.516	0.621	0.603	0.514	0.667	0.030	0.740	0.031
NDVIg	0.417	0.435	0.432	0.347	0.533	0.034	0.738	0.029
Chlv (SPAD units)	43.9	43.5	43.4	38.8	47.0	1.57	0.316	2.30
Chlg (SPAD units)	46.0	47.2	46.6	40.6	51.6	2.12	0.453	3.12
CTv (°C)	26.3	26.2	26.5	25.2	27.7	0.483	0.575	0.515
CTg (°C)	31.3	31.2	31.3	30.0	32.9	0.519	0.546	0.665
Heading (dae)	52.7	52.5	52.7	46.9	59.6	2.52	0.938	1.60
Maturity (dae)	79.7	78.7	78.8	73.1	85.5	2.61	0.937	1.66
Height (cm)	61.2	69.1	66.0	56.2	75.9	3.92	0.824	3.54

ł values presented are the averages across each trial's mean/minimum/maximum; Stg: NDVI at physiological maturity; RS: rate of senescence; Gdecay: percentage of greenness lost at mid grainfilling; KN: kernel number; TGW: thousand grain weight; GFR: grainfilling rate; GFD: grainfilling duration. NDVIv: normalized difference vegetative index during vegetative stage; NDVIg: normalized difference vegetative index during grainfilling; Chlv: chlorophyll content at vegetative stage (SPAD); Chlg: chlorophyll content at grainfilling (SPAD); CTv: canopy temperature at vegetative stage; CTg: canopy temperature at grainfilling; dae: days after emergence.

Table 3. Phenotypic correlation (r_P) for Stg and RS with performance traits by individual trial

	H05	M10	I13
Pearson's correlation for the Stg			
Yield	0.275 (0.0003)	0.320 (<0.0001)	0.330 (<0.0001)
GFR	0.430 (<0.0001)	0.440 (<0.0001)	0.430 (<0.0001)
GFD	-0.350 (<0.0001)	-0.580 (<0.0001)	-0.400 (<0.0001)
KN	0.216 (0.0048)	0.130 (0.0906)	0.260 (0.0007)
TGW	0.160 (0.0415)	0.270 (0.0004)	0.056 ns
Pearson's correlation for the RS			
Yield	0.488 (<0.0001)	0.243 (0.0014)	0.110 (0.154)
GFR	0.474 (<0.0001)	0.190 (0.013)	0.138 (0.073)
GFD	0.080 ns	0.005 ns	-0.130 (0.0906)
KN	0.429 (<0.0001)	0.026 ns	0.081 ns
TGW	0.123 (0.113)	0.240 (0.0014)	0.033 ns

The Pearson's correlation for Stg (residual greenness at physiological maturity) and RS (rate of senescence) with yield, grainfilling rate (GFR), kernel number (KN) and kernel weight (TGW) are indicated for each of the three trials. In brackets the *p-values* are shown. ns: not significant. For RS the correlation was calculated using absolute values, *i.e.* positive correlations indicates larger trait values are associated with faster greenness decay.

Table 4. Comparative trait analysis across a subset of Seri/Babax RILs sorted by three different NDVIg curve types in the I13 heat-stressed, irrigated environment. Values in the table represent the average by type of curve best fit in the NDVIg -vs- dae regression analysis

Type of curve	n	StgAUC (NDVI × °C d)	Yield (g/m^2)	Stg	RS (NDVI/°C d)	KN (grains/m^2)	TGW (g)	NDVIg	Heading (days)	Maturity (days)	Height (cm)	GFR (g m^{-2}/day)	GFD (days)
1	23	365 b	129 a	0.235 a	0.00048 a	4965 a	26.2 b	0.332 b	46 b	70 b	60 a	5.4 a	24.1 a
2	15	393 a	130 a	0.228 a	0.00051 a	4553 ab	28.7 a	0.362 a	47 a	72 a	61 a	5.2 ab	24.9 a
3	15	353 c	113 b	0.223 a	0.00043 b	4129 b	27.7 ab	0.313 c	46 b	71 b	59 a	4.7 b	24.4 a
p-value		<.0001	0.023	0.052	<.0001	0.0095	0.0157	<.0001	0.0079	<.0001	0.128	0.0388	0.081

StgAUC: staygreen area under the curve with starting points at maximum NDVI; Stg: NDVI at physiological maturity; KN: kernel number; TGW: thousand grain weight; NDVIg: normalized difference vegetative index during grainfilling; GFR: grainfilling rate; GFD: grainfilling duration

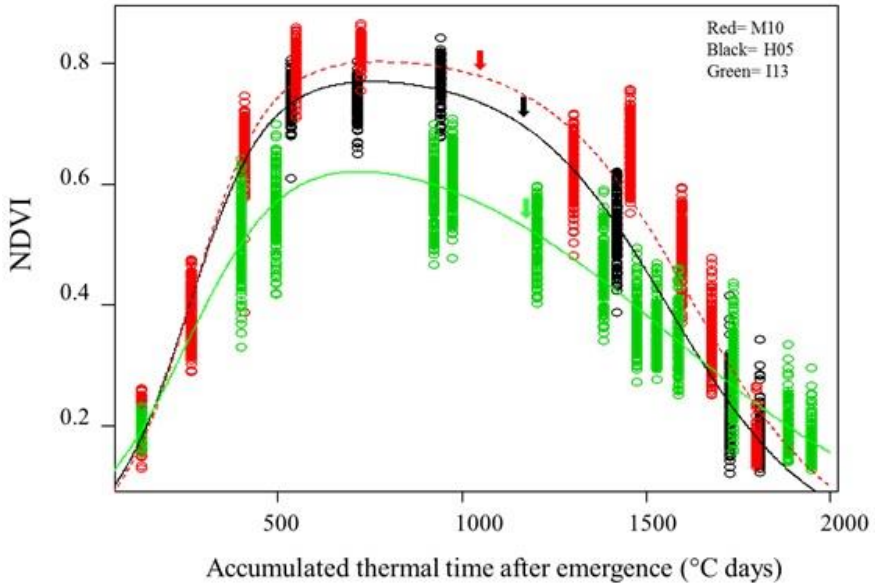

Figure 4. Modelling NDVI across the whole crop cycle. NDVI vs. thermal time (TT) was modelled for each of three trials of the Seri/Babax RILs population. Average days to heading for the environments are indicated by arrows

QTL mapping

The QTL mapping analysis was performed for 19 traits by single and by combined environments resulting in a total of 98 analyses (Trait × Environment combinations). A total of 193 QTL were identified with LOD >2. Of these, 44 QTL were linked to Stg and staygreen associated traits, 37 QTL were associated with yield and yield components and the rest were related to other physiological parameters and phenology. Average LOD scores for all QTL associated with Stg and related traits, yield and yield components and with physiological traits were 3.5, 4.1 and 4.0, respectively. Across all QTL, for all traits and environments, the highest LOD score and the maximum phenotypic variance explained was 18.4 and 36.4% respectively, which was for a QTL on 1B for NDVIv. Additionally, 13 linkage groups contained two QTL located >30 cM apart for the same trait. A summary of results is presented as a matrix in Table 5. More detail about QTL with LOD >3.5 is presented on Table 6; this table shows the related marker(s), maximum variances, size of effects as well as the increasing allele for each QTL. Except for H05, the maximum variances explained in all environments were found for QTL related to traits other than yield.

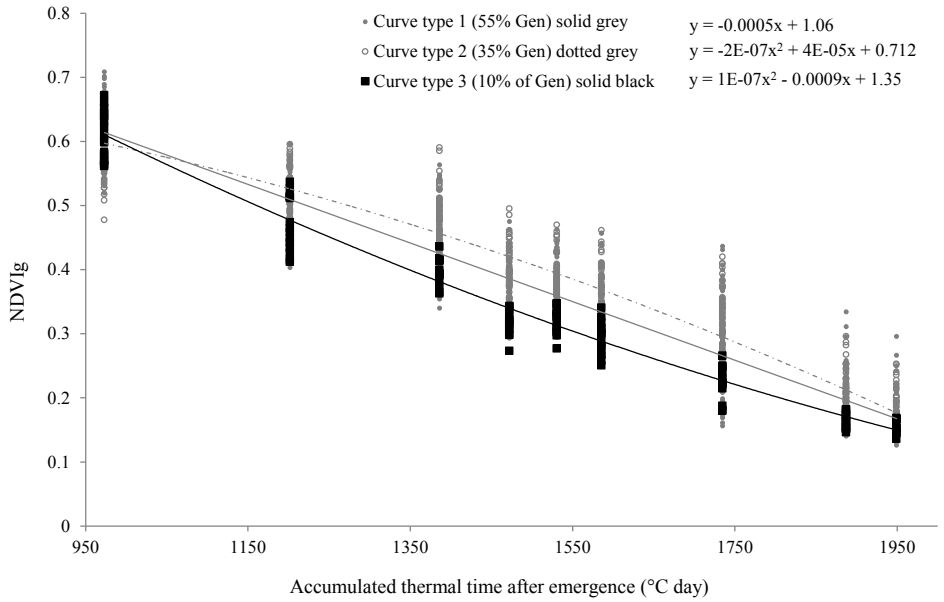

Figure 5. Greenness decay during grainfilling for Seri/Babax RILs in the I13 heat-stressed, irrigated environment. Curves represent the pattern for average NDVIg for RILs individually fitting one of the three different curve types

Gen: genotypes

QTL for staygreen traits

QTL for Stg were located on chromosomes 2A, 4B, 4D, 6A and 7D. The largest phenotypic variance (15%) was for a locus on 7D. This was also the most repeatable Stg QTL detected (two of three environments plus the combined analysis). Stg related traits such as RS, StgAUC, TotalAUC and Gdecay gave 9, 8, 11 and 11 QTL, respectively. The 4B and 7D loci seemed to be the main genomic regions controlling Stg related traits, given that those QTL were identified for multiple environments and traits (Table 5). A QTL on 1B explained around 10% of the phenotypic variance for both RS and Gdecay. On 2B a QTL was detected for RS, TotalAUC, StgAUC and also for Gdecay where the greatest variance explained was about 10% (for Gdecay). Most of the QTL for StgAUC and TotalAUC had LOD values greater than 3.5. QTL on 5B explained 11.3% of the variance for TotalAUC and 7.3% of variance for StgAUC (Table 6). For StgAUC the maximum phenotypic variance, 10.5%, was explained by a QTL on 2A (Table 5). Considering all the environments, alleles from both parents contributed equally to Stg across the genome (Table 6).

QTL for agronomic and physiological traits and co-location with QTL for staygreen traits

A number of QTL associated with agronomic and physiological traits were found co-located (linked to markers <30 cM) with QTL for Stg and staygreen related traits (Table 5). Figure 6 shows a Venn diagram summarizing these genetic overlaps. The 1B, 3B, 4A, 4B and 6B genomic regions appeared to be the most important ones controlling yield and yield components based on repeatability and significance (Table 5). Yield QTL co-located with QTL for Stg and staygreen related traits on 1B, 2A, 2B, 3B, 4A, 4B, 5A, 5B, 6B and 7A, and the QTL on 1B, explained the greatest variances for yield (linked to markers at 61.71-65.36 cM), KN (60.73-66.35 cM) and GFR (61.81-66.35 cM) (Table 6). This yield QTL on 1B appeared in 3 of 5 environments plus in the combined analysis, and was also found at or near QTL for RS, TotalAUC and Gdecay. The strongest effects for yield (16.5 g/m^2) were found on 1B and 4A. For TGW, a QTL on 1A explained close to 12% of variance and had an additive effect of almost 1 g in the M10 and I06 environments. QTL for StgAUC and TotalAUC were also found on chromosome 1A but >30 cM distant from the QTL for TGW. In total, 28 QTL were identified for NDVI, 12 for NDVIv and 16 for NDVIg; most of these QTL showed LOD >3.5. On 1B, a major QTL for early ground cover,

defined by NDVIv, was found in the same region as QTL for RS, TotalAUC and Gdecay; for all the traits the QTL were linked to markers found between 59.7-64.2 cM (Table 6), indicating co-location. This NDVIv QTL on 1B explained more than 36% of phenotypic variance for the trait. On the other hand the maximum variance for NDVIg (12%) was explained by a QTL on 7D (linked to one marker on 2.73 cM) which co-located with QTL for Stg, RS and Gdecay (linked to markers at 2.73-11.1 cM).

On chromosomes 1B, 2B and 3B, there was co-location of chlorophyll content QTL (LOD>3.5), defined by Chlv and Chlg, with Stg QTL related traits; in these three regions the Sgt and Chl QTL were associated with closely linked markers (at ~60, 40 and 113 cM for 1B, 2B and 3B, respectively). Almost 12% of variance for Chlv was explained by a QTL on 6A, while a QTL on 3B explained about 14% of the variance for Chlg. Eight QTL were detected for CTv and eight for CTg. Average LOD scores for all QTL related to canopy temperature was 4.2. For CTg the maximum variance was 15%, explained by a QTL on 7D (at 2.73 cM), which was co-located with a number of QTL for Stg and related traits (at 2.73-11.1 cM). Additionally, the 4A region showed two regions affecting both CTg and yield, the first being located close to 13 cM and the other at around the 111 cM. The maximum variance explained for CTv was for loci on 1B and 4A, each explaining 17% of the variance. Opposite to the 1B QTL, the QTL on 4A was repeatedly detected for CTv and CTg and in all environments, excepted in H11.

Table 5. Co-location of QTL for mapped traits. The number of environments where a QTL was identified is written, followed by +C if the QTL was also detected in the combined QTL analysis across environments

Chromosome	Stg	RS	TotalAUC	StgAUC	Gdecay	Yield	KN	TGW	GFR	GFD	NDVIv	NDVIg	Chlv	Chlg	CTv	CTg	Heading	Maturity	Height
Total Env (n)	3	3	3	3	3	5	5	5	5	5	5	5	3	3	5	4	5	5	5
1A			1+C	1+C			2	5+C		1	1	1		1+C			C	1	3+C
1B		1+C	1+C		2+C	3+C	4+C	C	4+C	1+C	5+C	1		3+C	1+C		1+C	2+C	
1D	1+C		C			1			2	1	1		1		3+C		1+C	1+C	
2A	1	1+C	1+C	1+C		C	1+C	1				3		1					1
2B	C	2	C	1+C	1+C	1	1	3+C	1	2+C	1	2+C	1+C	2	2+C	1	2+C	4+C	2
2D	1+C	1+C			2+C	1	C	2	1	1	2+C		2+C						
3A			1									2				1			3+C
3B		1			1+C	2+C	1+C	5+C	1+C		2+C	1+C	1+C	1+C	2+C		1		2+C
4A		C	1	1	1+C	3+C	3+C	2+C	3+C		2+C	1+C		1	4+C	2+C	1+C	1	
4B	2+C		C	C	1	1	2+C	3+C		1	1+C	1	2					1	3+C
4D	1											1					4	2	
5A	1				1+C	1+C			2+C	1		1			1	2	1+C	2+C	2+C
5B			2+C	2+C		1		1+C	1		2+C	2	1+C						4+C
5D						1				1						2+C	5+C	3+C	1
6A	1		1+C					1		2+C	2+C	1	2+C		1		3+C	2	1
6B		2			1	4+C	3+C	1	2+C	1		C							
6D							3+C	2+C	1	2+C									
7A			1+C	1		1		1+C			3+C	1+C	1	1		1			2
7B																			
7D	3+C			1	C			3+C		2+C	1	4+C				1+C	5+C	5+C	2+C
Max % var explained	15.0	10.4	11.3	10.5	11.1	16.8	18.0	11.9	16.2	9.6	36.4	11.7	11.8	13.5	16.8	14.6	18.1	13.9	7.8
Associated marker	ıcc/cat-1(	act/ctc7	wPt-0103	gwm526	agg/cat-4	acc/cat4	agc/cta-9	aca/cta-2	aag/ctc6	wPt-0298	gwm131	acc/cat-10	gwm617b	barc0164	aac/ctg-3	acc/cat10	acc/cat10	acc/cat10	aca/caa-6
Chromosome	7D	1B	5B	2A	1B	1B	1B	1A	1B	2D	1B	7D	6A	3B	4A	7D	7D	7D	3A

Shaded cells: chromosomes where a consistent QTL (LOD>3.5 in at least one environment) was detected. Stg: staygreen at physiological maturity; RS: rate of senescence; TotalAUC: total area under the curve with starting points at crop establishment; StgAUC: area under the NDVIg curve with starting points at maximum NDVIg; Gdecay: percentage of greenness lost at mid grainfilling; KN: kernel number; TGW: thousand grain weight; GFR: grainfilling rate; GFD: grainfilling duration. NDVIv: normalized difference vegetative index during vegetative stage; NDVIg: normalized difference vegetative index during grainfilling; Chlv: chlorophyll content at vegetative stage (SPAD); Chlg: chlorophyll content at grainfilling (SPAD); CTv: canopy temperature at vegetative stage; CTg: canopy temperature at grainfilling. The number of environments where a given trait was recorded is indicated in the first row below trait names; in the table the number of environments where a QTL was identified is written followed by +C if the QTL was also detected in the combined QTL analysis across environment

The two CTv QTL on 1B and 4A co-located with QTL for yield showing the strongest effects for the trait, but did not co-locate with Stg QTL. The QTL detected for CTv at ~61 cM on 1B also controlled RS. The QTL for CTv and CTg on chromosome 4A co-located with QTL for RS, StgAUC and TotalAUC; only the QTL for RS seems to be different, given the large distances between QTL; The CTv and CTg QTL were found at 13-15 cM while the QTL for RS was located at 72 cM. QTL for Gdecay coincided with QTL for CTv and CTg on 1B, 2B, 3B, 4A and 7D, and in all cases the linked markers were closely located, indicating that it was the same QTL. Plant height was mainly controlled by loci on 3A, 4B and 5B. The strongest QTL for plant height was found on 3A, explained about 8% of phenotypic variance for the trait and had an additive effect of 1.3 cm. This QTL on 3A was not co-located with any QTL for Stg or related traits of LOD>3.5, or for yield or yield components. However the height QTL on 4B and 5B co-located with QTL for Stg, TotalAUC, StgAUC, Gdecay, yield, TGW and KN.

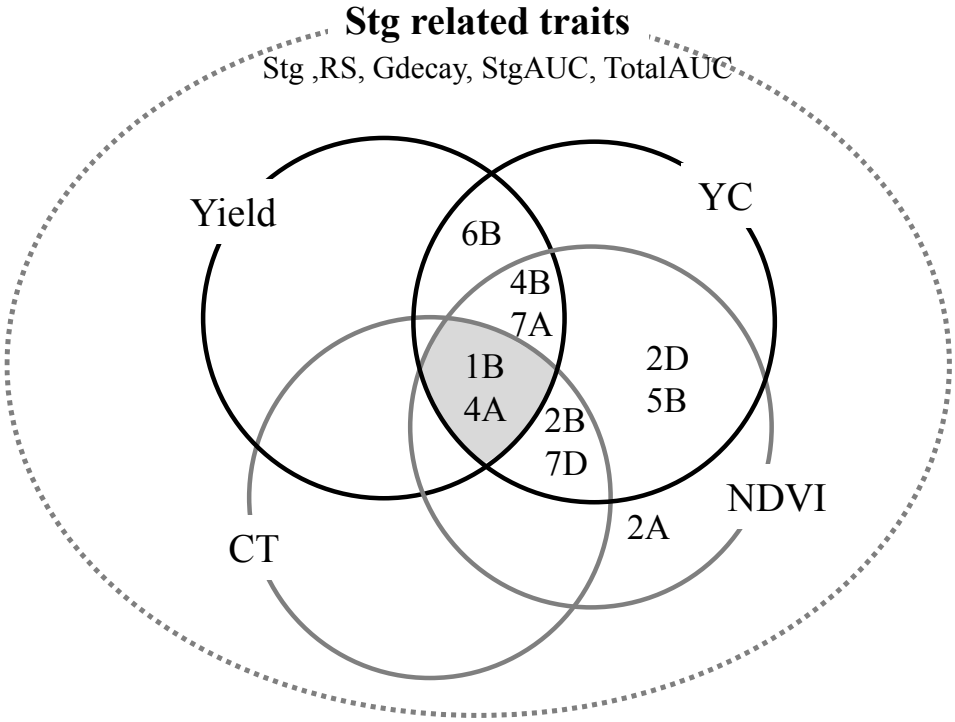

Figure 6. Genetic overlap between QTL loci controlling staygreen and related traits with those controlling other traits. Only consistent QTL (LOD >3.5) are represented, and co-location is defined as positions <30 cM

YC: yield components (kernel number and grain weight); CT: canopy temperature (CTv and CTg); NDVI: normalized difference vegetation index (NDVIv and NDVIg). Staygreen related traits included: Stg, RS, Gdecay, StgAUC and TotalAUC are listed at the top.

QTL for plant phenology

Plant phenology QTL (date of heading and maturity) were positioned across the Seri/Babax genome but with small individual effects (<1.5 days, see Table 6). A QTL on 7D explained the highest variances for both heading and maturity. Based on repeatability and significance it seems that plant phenology was mainly controlled by the 2B, 5D and 7D genomic regions. The consistent QTL (LOD>3.5) on 2B, 4A and 7D co-located with consistent QTL for Stg and all related traits. QTL for all these traits were found linked to markers at 26.8-40.9 cM on 2B, at 12.92-23.65 cM on 4A and on 2.73-11.7 cM on 7D. The phenology QTL on 5D did not co-locate with any QTL for Stg or related traits.

Allele significance for all traits

Considering all the environments, alleles from both parents contributed equally to Stg across the genome (Table 6). QTL for Stg, StgAUC and TotalAUC mostly had Babax contributing the increasing allele *i.e*., these alleles favoured higher areas under the NDVI curve during the whole crop cycle (TotalAUC) and also during the greenness decay phase (StgAUC). Regarding yield, TGW and KN these traits were increased by alleles from both parents across the genome; however, Babax alleles tended to contribute the highest positive effects at loci explaining the maximum variances. Similarly, both parents contributed to increases in NDVI during both the vegetative and the grainfilling stages, depending on the locus. On the other hand, increases in canopy temperature were largely contributed by Seri alleles

Discussion

Understanding the staygreen mechanism in the Seri/Babax population – association with yield and plant performance

The staygreen phenotype has been associated with improved performance of several species under heat stress (Reynolds et al. 2000; Kumari et al. 2013) and in the current study there was a positive and significant association of Stg with yield and yield components (Table 3). However in order to properly exploit the potential of the staygreen trait, a clearer understanding of the underlying mechanisms for the staygreen phenotype in the context of the cumulative effect of traits contributing to yield maintenance in stressed environments is needed.

The current study found Stg to be positively associated with high yield, TGW, GFD, KN, and low CT. While heat stress conditions can reduce the grain number due to seed abortion or reduced grain set (Hays et al. 2007; Tashiro and Wardlaw 1990) crop productivity is also related to longer grainfilling periods and faster grainfilling rates, so it is expected that under heat stress, staygreen traits and green tissue area contribute to heavier grains (Kumari et al. 2013). Canopy temperature depression has also been found to be positively and strongly correlated with staygreen traits suggesting a possible link with root development patterns in bread wheat (Christopher et al. 2008; Kumari et al. 2013), as found in sorghum staygreen genotypes (Borrell et al. 2014a). Herein, the canopy temperature during the vegetative stage (CTv) was also found to be associated with RS and with NDVIv (Supplementary Table 1) further supporting the hypothesis that the RS staygreen attribute in wheat is primarily a consequence of the initial amount of greenness (total biomass and chlorophyll) potentially available for filling the grains This was supported by the fact that genotypes with cooler CTv tended to have higher initial greenness and biomass (NDVIv) and faster rates of senescence during grain filling.

NDVI is an integrative measure of chlorophyll and total plant biomass, confirmed by a significant positive correlation between NDVIg and Chlg and height (Supplementary Table 1). The absolute rate of senescence (RS) was positively correlated with yield in the Seri/Babax population (Table 3), showing that genotypes with higher yields tended to lose chlorophyll faster. Higher absolute RS was also observed in genotypes with higher NDVIv, StgAUC and TotalAUC (Supplementary Table 1) showing that despite higher rates of NDVI decay during grain filling in these genotypes, the total amount of initial NDVIg was higher allowing for higher amounts of photosynthesis per unit degree day to fill grains. Interestingly, higher RS did not result in a faster arrival to maturity (associations of RS with days to maturity were not significant). This suggests that among the Seri/Babax progeny, genotypes with a staygreen phenotype were characterized by a high initial greenness, high StgAUC and TotalAUC and high RS, while attaining maturity within a similar timeframe, compared to non staygreen genotypes.

Table 6. QTL with LOD >3.5 identified for all traits in the Seri/Babax population grown under M10, H05, H11, I06 and I13 heat-stressed, irrigated environments

QTL location	Linked markers with LOD>3.5	Position (cM)	Max % of variance explained R^2	Effect	Allele	Env
Stg						
4B	aac/ctc-9	12.8	10.6	0.021	Babax	H05
7D	acc/cat-10	2.73	15.0	0.025	Seri	H05
RS (NDVI/°C d)						
1B	act/ctc-7	61.1	10.4	0.000	Babax	I13
	aag/ctg-14	61.2				
2A	gwm526	1.34	3.50	0.000	Seri	H05
2B	aag/ctg-12	37.9	8.50	0.000	Babax	M10
	wPt-7750	27.0				
2D	wPt-2644	74.6	6.10	0.000	Seri	H05
TotalAUC (NDVI × °C d)						
1A	wPt-0432	120	6.90	11.6	Seri	M10
1B	aca/cta-9	59.7	5.90	10.4	Babax	H05
2A	gwm526 (1.34)	1.34	6.80	16.9	Babax	I13
5B	wPt-0103 (10.92)	10.9	11.3	21.7	Babax	I13
7A	barc121 (97.45)	97.5	9.20	19.6	Seri	I13
StgAUC (NDVI × °C d)						
1A	wPt-8644	115	6.60	10.0	Seri	M10
2A	gwm526	1.34	10.5	11.9	Babax	I13
4A	act/cag-3	13.2	7.70	9.5	Babax	H05
5B	wPt-0103	10.9	7.30	9.9	Babax	I13
Gdecay (%)						
1B	agg/cat-4	62.2	11.1	1.29	Babax	M10
$2B^I$	wPt-7750	27.0	9.80	1.89	Babax	I13
$2D^I$	gwm102	59.6	6.30	1.51	Seri	I13
4A	act/cag-3	13.2	8.80	1.74	Seri	H05
4B	gwm006a	23.8	7.30	1.63	Babax	I13
6B	aac/ctc-3	83.0	8.20	1.73	Seri	I13
Yield (g/m^2)						
1B	acc/cat-4	61.7	16.8	16.6	Babax	H05
	acg/cta-2	61.5				
	agg/cac-3	65.4				
$3B^I$	gwm301e	44.6	8.90	12.4	Babax	I06
$4A^1$	wmc048d	12.9	16.1	16.7	Babax	I06
	aca/cac-6	103				
4B	wmc048a	9.70	7.00	10.2	Seri	H11
$6B^I$	wPt-2786	36.4	7.30	10.4	Seri	H11
	aac/ctc-3	83.0				
	barc0178	90.3				
7A	aag/cta-3	115	8.50	11.3	Seri	H11
KN ($grains/m^2$)						
$1B^I$	agc/cta-9	66.4	18.0	601	Babax	H11
	gwm301b	61.9				
$3B^I$	barc147	87.7	7.60	449	Seri	I06
$4A^1$	wmc048d	12.9	12.7	582	Babax	I06
4B	gwm375	14.1	7.10	316	Babax	M10
6B	barc0178	90.3	8.50	380	Seri	H05
	agg/cat-8	64.5				
TGW (g)						
1A	aca/cta-2	31.2	11.9	0.897	Babax	I06
	agg/cac-6	42.3				
	aca/cag-13	53.8				
$2B^I$	acc/ctc-2	24.2	6.70	0.531	Babax	M10
2D	gwm102	59.6	5.00	0.457	Seri	M10
$3B^I$	agg/cat-3	89.9	7.40	0.632	Babax	H05
	wPt-2757	86.8				
4A	act/cag-1	75.7	5.40	0.606	Babax	I06
4B	aag/cta-5	11.6	10.8	0.756	Seri	I13
	wPt-1708	9.27				

QTL location	Linked markers with LOD>3.5	Position (cM)	Max % of variance explained R^2	Effect	Allele	Env
GFD (days)						
2D	wPt-0298	70.8	9.65	0.182	Seri	M10
4B	agc/cag-2	12.0	7.70	0.336	Seri	H11
6D	cfd0188	41.4	9.60	0.464	Seri	I06
7D	acc/cat-10	2.73	8.50	0.366	Babax	I13
NDVIv						
1B	gwm131	64.2	36.4	0.024	Babax	H11
	aag/ctc-6	61.8				
	agg/cat-4	62.2				
1D	wPt-1770	9.08	6.70	0.009	Seri	I13
$3B^I$	wPt-8021	40.2	6.60	0.008	Babax	I06
$4A^1$	agg/cta-12	13.6	20.1	0.013	Babax	I06
4B	gwm006a	23.8	6.20	0.009	Babax	I13
5B	aag/ctg-11	6.88	14.1	0.014	Babax	I13
6A	wPt-7599	50.8	6.40	0.009	Seri	I13
$7A^1$	aag/cta-2	98.8	6.90	0.009	Seri	I13
7D	acc/ctc-7	11.7	7.90	0.005	Seri	M10
NDVIg						
2A	gwm526	1.34	10.1	0.013	Babax	I13
2B	wPt-5680	40.9	8.10	0.011	Seri	I13
	aag/ctg-12	37.9				
3A	wPt-7341	2.42	5.20	0.008	Babax	H11
4B	aac/ctc-9	12.8	5.90	0.008	Seri	M10
4D	gdm0129	0.870	7.50	0.009	Babax	H11
7D	acc/cat-10	2.73	11.7	0.011	Babax	I06
Chlv (Spad units)						
2D	wPt-2644	74.6	10.8	0.477	Seri	H05
3B	wPt-1940	112	7.00	0.467	Babax	I06
$4B^I$	aag/cta-5	11.6	9.40	0.445	Babax	H05
5B	wPt-0103	10.9	8.90	0.435	Seri	H05
$6A^1$	gwm617b	28.4	11.8	0.503	Seri	I13
Chlg (Spad units)						
1B	acc/cat-4	61.7	12.6	0.580	Seri	H05
	wPt-7529	61.2				
2B	acg/cta-1	35.7	9.50	0.546	Babax	I06
3B	barc0164	113	13.5	0.601	Babax	H05
CTv (°C)						
1B	acc/cat-4	61.7	16.6	0.157	Seri	H05
$1D^I$	wPt-9380	41.8	11.2	0.154	Babax	H11
2B	acc/ctg-4	25.2	5.90	0.120	Babax	I06
3B	gwm301e	44.6	9.90	0.156	Seri	I06
4A	aac/ctg-3	12.9	16.8	0.202	Seri	I06
	wmc048d	13.8				
CTg (°C)						
$4A^1$	wmc048d	12.9	9.70	0.123	Seri	M10
	agg/cta-12	13.6				
5D	wPt-1400	13.0	7.20	0.220	Seri	H05
7D	acc/cat-10	2.73	14.6	0.314	Seri	H05
Heading (dae)						
2B	wPt-7750	27.0	8.00	0.576	Seri	H11
4D	cfd023	2.25	9.10	0.725	Seri	I13
5D	wPt-5505	12.6	8.60	0.704	Babax	I13
	wPt-1400	13.0				
6A	wPt-0696	34.2	9.80	0.751	Babax	I13
7D	acc/cat-10	2.73	18.1	0.824	Babax	M10
	acc/ctc-7	11.7				

… Continues Table 6

QTL location	Linked markers with LOD>3.5	Position (cM)	Max % of variance explained				QTL location	Linked markers with LOD>3.5	Position (cM)	Max % of variance explained			
			R^2	Effect	Allele	Env				R^2	Effect	Allele	Env
TGW (g)							Maturity (dae)						
5B	gwm371	14.5	6.60	0.592	Babax	I13	2B	gwm388	34.2	9.30	0.742	Seri	H11
6B	barc0178	90.3	10.9	0.767	Babax	H05	5D	wPt-5505	12.6	8.10	0.690	Babax	H11
6D	cfd0188	41.4	8.80	0.684	Babax	I13		wPt-1400	13.0				
	gwm325	37.0					7D	acc/cat-10	2.73	13.9	1.22	Babax	H05
7A	aca/cag-10	78.6	6.20	0.581	Seri	I13	Height (cm)						
7D	cfd0014	41.8	9.00	0.699	Seri	H05	2A	gwm526	1.34	7.00	1.01	Babax	H11
GFR (g m^{-2}/day)							2B	aca/ctg-1	37.5	7.30	0.989	Babax	M10
1B	aag/ctc-6	61.8	16.2	0.763	Babax	H05	3A	aca/caa-6	0.350	7.80	1.33	Seri	I06
	gwm131	64.2					4B	aag/cta-5	11.6	7.50	0.787	Seri	I13
	agc/cta-9	66.4					5B	gwm274	5.51	7.50	1.23	Babax	H05
2B	agg/cac-5	28.6	7.00	0.296	Babax	M10		wPt-9814	0.190				
3B‡	gwm301e	44.6	7.20	0.470	Babax	I06		gwm133	7.47				
	gwm389	92.9	5.50	0.411	Seri	I06	7D	acc/cat-10	2.73	7.00	1.18	Babax	H05
4A‡	wmc048d	12.9	15.1	0.682	Babax	I06							
	gha44	15.2											
6B	wPt-8412	61.2	6.60	0.486	Seri	H05							
	agc/cta-4	79.0											

Stg: staygreen at physiological maturity; RS: rate of senescence; TotalAUC: total area under the curve with starting points at crop establishment; StgAUC: staygreen area under the curve with starting points at maximum NDVI; Gdecay: percentage of greenness lost at mid grainfilling; KN: kernel number; TGW: thousand grain weight; GFR: grainfilling rate; GFD: grainfilling duration. NDVIv: normalized difference vegetative index during vegetative stage; NDVIg: normalized difference vegetative index during grainfilling; Chlv: chlorophyll content at vegetative stage (SPAD); Chlg: chlorophyll content at grainfilling (SPAD); CTv: canopy temperature at vegetative stage; CTg: canopy temperature at grainfilling. For each QTL all linked markers with LOD>3.5 are listed. Only the environment where the maximum variance explained was detected for a given QTL is indicated together with its corresponding effect and allele contributing to increase the trait. For QTL with more than one listed marker the first is the marker related to the maximum R^2. ‡ Chromosomes with two QTL for the same trait since distances between associated markers was >30 cM. dae: days after emergence.

In most species studied so far, a very conservative response has been observed for the staygreen phenotype with low RS and delayed onset of senescence (Thomas and Ougham 2014). However, the wheat Seri/Babax population grown in warm and irrigated environments showed a pattern of staygreen where higher initial greenness is lost at a higher rate without really accelerating time to maturity (Supplementary Table 1, NDVIv and Maturity, r_P= 0.13, p=0.089). Nonetheless, analysis across all environments showed low heritability for Stg especially for RS, similarly to results reported by Lopes and Reynolds (2012) in one staygreen study performed in the same population. Moderate and high heritability was found for physiological and agronomic traits (Table 2).

Differentiating patterns of plant greenness decay

Interpretation of staygreen would be most straightforward when dynamic traits fit a linear model. However during the grainfilling phase plant greenness decay patterns sometimes fitted non-linear models best. Non-linear regression curves have been previously used to describe the percent of greenness retained during grainfilling (Vijayalakshmi et al. 2010). Additionally, a number of genotypes from the Seri/Babax population were found to fit best a parabolic model in the M10, H05 and I13 environments. Parabolic curves were observed in two of the three years in which the Stg attribute was analyzed. Interestingly, the tendency to follow a particular pattern was related to heat stress intensity. Furthermore, the same genotype could fit different curves, depending of the environment, suggesting high G×E for staygreen traits, as reported in previous studies (Bogard et al. 2011; Kumar et al. 2010). The largest genetic diversity for type of curve was observed in the I13 environment which experienced the highest temperatures; in this environment linear and non-linear models applied to an almost equal proportion of genotypes. Lower diversity for the type of curve was observed in the H05 environment in which heat stress was moderate and in which only 4% of the population fitted a linear model (curve type 1). In M10, which was the least heat stressed environment, the whole population fitted a non-linear model best (data not shown).

A curve type 2 (see Fig. 5) during the decay phase resulted in larger area under the greenness curve (StgAUC) which would have allowed more photosynthesis, thus explaining the association of this curve type with higher grain yields (Table 4) (Kumari et al. 2013). By contrast, the lower StgAUC observed for curve type 3 resulted in lower photosynthetic area and genotypes with reduced grain number (KN) (Table 4). The classification of staygreen into four functional types is highly descriptive but in reality it is quite hard to classify a genotype into one or another group because the staygreen phenotype often results from a combination of two or more types (Thomas and Howarth 2000). Additionally, it is important to take into account that the Stg and RS traits by themselves cannot completely describe the staygreen attribute given the high relevance of the initial greenness value, as observed in the current study.

Genetic basis of plant greenness decay – QTL mapping

Heat tolerance is a complex trait influenced by different component traits. Increasing temperatures accelerate plant development and decrease the length and amount of green biomass (through decreased organ size and plant height). The main chromosome regions controlling staygreen related traits in this wheat population were generally co-located with regions controlling agronomic and physiological attributes. Different staygreen traits were calculated and QTL mapped, including the residual greenness at maturity (Stg), the rate of senescence (RS), the green area under the curve (StgAUC) and the percentage of greenness lost at mid grainfilling (Gdecay) – all estimated from NDVI decay curves. The maximum phenotypic variance for any staygreen related QTL was detected on chromosome 7D associated with Stg; this locus has been previously described as associated with permanence of greenness under high temperatures (Vijayalakshmi et al. 2010; Kumar et al. 2010). In the current study, this Stg QTL on 7D co-located with a QTL for NDVIg, CTg (Table 5) and days to heading. Kumari et al. (2013) reported that staygreen in bread wheat was associated with high canopy temperature depression (CTD) such that the warmer plants tended to be non staygreen. There is evidence in sorghum that staygreen genes overlap with root architecture genes (Mace et al. 2012), for example, QTL for root nodal angle have been found to be co-located with Stg QTL including the Stg4 QTL associated with biomass partitioning between root and shoot (Borrell et al. 2014b) . In the present study, the 7D region also controlled Gdecay and StgAUC as well as CTg, with the Seri allele being positive. Gdecay and CTg were positively correlated in the Seri/Babax population indicating that cooler genotypes tended to lose a smaller percentage of greenness in the first half of the grainfilling period. Gdecay and CTg controlled by the QTL on 7D seemed to be affected by plant phenology (Lopes et al. 2013) given the co-location of a main QTL for heading and maturity here (Table 5), but there was no effect of phenology in the 4A region where a consistent QTL was identified for Gdecay and CTg.

The highest phenotypic variability explained for Gdecay (11.1%) and RS (10.6%) was detected on the 1B chromosome. Chromosome 1B has been reported to control a number of performance traits. Yang et al. (2002) found a QTL for grain filling duration on the short arm of chromosomes 1B which co-located with a number of QTL for Stg related traits from this study. Moreover, this QTL on chromosome 1B was co-located with yield, Chlg, NDVIv, CTv,

Gdecay and KN. The 1B region also has been associated with SPAD chlorophyll content (Talukder et al. 2014) and Pinto et al. (2010) reported several QTL on 1B for canopy temperature, yield, and chlorophyll content at the grain filling stage in the Seri/Babax population. Common QTL for Stg related traits, yield, yield components and physiological characters indicate a common genetic basis for these attributes. The strongest QTL for yield detected in the current study was found on chromosome 1B and interestingly, it co-located with a QTL for green leaf duration detected in a previous study of spring wheat grown under heat stress in greenhouse experiments (Naruoka et al. 2012).

In agreement with our results (Table 5), Naruoka et al. (2012) found that the 4A and 3B chromosomes controlled green leaf duration in spring wheat grown under heat and also drought stress; in the Seri/Babax population the 4A and 3B chromosomes seemed to contain genes driving StgAUC, RS and Gdecay. These two genomic regions also showed QTL for yield, yield components, NDVI, GFR, chlorophyll content and canopy temperature which coincided with results from Pinto et al. (2010). During leaf senescence the mechanisms that protect the chlorophyll molecule from photodamage fail and result in leaf yellowing (Thomas and Howarth 2000). In some species, the staygreen phenotype can be conferred by genetic deletions of the locus encoding phaeophorbide *a* oxygenase (PaO), the main regulatory enzyme for chlorophyll catabolism (Vicentini et al. 1995; Roca et al. 2004; Thomas and Howarth 2000). However, the genetic basis of the staygreen phenotype is complex and differs from one species to another. Multiple staygreen genes (SGR) have been identified in several species, but the number of staygreen genes varies between species and homologos genes do not always result in increased greenness persistence. This may be because staygreen genes may also have different functions from one species to another; an example of this is in *Arabidopsis* where over-expression of the SGR2 gene results in a staygreen phenotype whereas over-expression of the SGR1 gene promotes leaf yellowing (Sakuraba et al. 2015). The physiological and biochemical mechanisms by which the staygreen genes affect chlorophyll degradation are unclear but various studies seem to indicate the involvement of a multi-protein complex containing chlorophyll catabolic enzymes (CCEs), the product of the staygreen gene 1 (SGR1) and light-harvesting complex subunits of photosystem II (LHCII). Apparently, this complex channels phototoxic Chl intermediates during chlorophyll catabolism (Sakuraba et al. 2012).

Cosmetic and functional staygreen

Studies have shown that the staygreen phenotype includes a genetic component affected by the phenological clock of the plant and a second component un-related to plant developmental stage. In the current study consistent QTL for staygreen related traits on 2A, 2D, 5B, 6B and 7A were not co-located with phenology QTL; while consistent QTL for staygreen related traits and consistent QTL for heading and maturity co-located on 2B, 4A, 4D and 7D. In general terms, earliness in the Seri/Babax population was associated with longer GFD. Overlapping genomic regions for plant phenology and staygreen attributes suggest common genes controlling these traits. In *Festuca pratensis,* staygreen independent from phenology has been reported as a recessive character generated by changes in a gene regulating the pathway of chlorophyll degradation (Vicentini et al. 1995); *Lolium* and *Festuca* staygreen mutants show expression of the PaO enzyme but with reduced activity (Vicentini et al. 1995; Roca et al. 2004). However, the underlying mechanism associated with the staygreen character seems to vary (Thomas and Howarth 2000). In soybean for example, staygreen can be the result of a cytoplasmic mutation, *CytG,* which makes the chlorophyll *b* structure more stable (Guiamét et al. 1991). The staygreen of these mutants may be classified as Type C or *cosmetic staygreen* (Sakuraba et al. 2015; Thomas and Howarth 2000) which is characterized by the permanence of the greenness, but with unaffected loss of photosynthetic function. Mutant lines have also been used to study staygreen in rice (Cha et al. 2002), wheat (Spano et al. 2003, Thomas et al. 2002, Rampino et al. 2006, Tian et al. 2012), *Arabidopsis* (Grbic and Bleecker 1995) and *Festuca* (Hauck et al. 1997). However, if the genetic lesion resulting in plant greenness persistence is also associated with improved plant performance, the staygreen is classified as *functional staygreen.* An example of functional staygreen is in sorghum where some genotypes remain green and give higher grain weights than the non staygreen genotypes (Duncan et al. 1981; Borrell et al. 2000). In the Seri/Babax population functional staygreen may be controlled by chromosomes where common QTL for Stg, yield and yield components were detected, such as 4B. On the contrary, the staygreen phenotype was unlinked to yield improvement on chromosome 7D suggesting that the locus controlled the cosmetic persistence of greenness.

The staygreen character is a complex trait; its expression is environment dependent suggesting high G×E interaction (Christopher at al. 2008; Bogard et

al. 2011). For example, in sorghum the staygreen attribute is only observed under drought conditions (van Oosterom et al. 1996). In the current study, it was observed that the greenness decay pattern of particular genotypes varied with the growth conditions, resulting in different types of fitted curves (Fig. 5) when grown under moderate, hot or intense heat stress.

Conclusions

Results from this study showed the staygreen attribute to be positively and significantly associated with yield and yield components in bread wheat grown under heat-stressed, irrigated conditions. The NDVI decay trend during grainfilling showed genotypic differences within the Seri/Babax population, and that the type of curve followed during greenness decay was strongly associated with general plant performance parameters. However, the type-curve for greenness decay is highly environment dependent. The association of the Stg character, the rate of senescence and all staygreen related traits with stress tolerance is supported by results showing that the same genomic regions have an effect on yield, grain weight, kernel number, canopy temperature, NDVI and also the length and rate of grainfilling. The staygreen character is clearly complex genetically with environmental influences that require further exploration.

Author contribution statement

R. Suzuky Pinto conducted field experiments, performed data analysis and led the write-up; Marta S. Lopes conducted field experiments, performed data analysis and provided useful advice for data interpretation; Nicholas C. Collins contributed to data interpretation and preparation of the manuscript; Matthew P. Reynolds designed the experiments and participated in all aspects of data analysis, interpretation and writing of the manuscript.

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Conflict of interest: The authors declare that they have no conflict of interest

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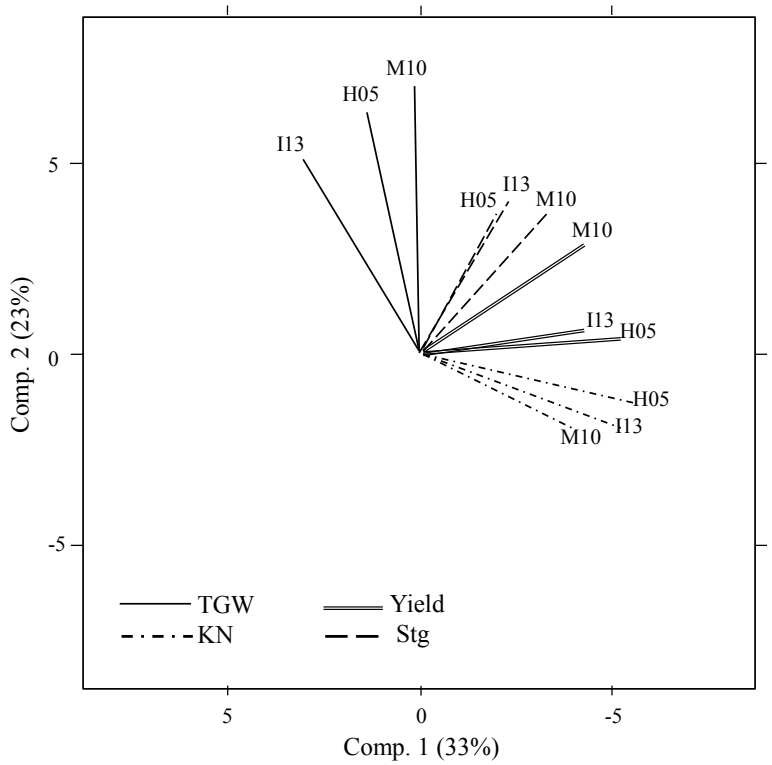
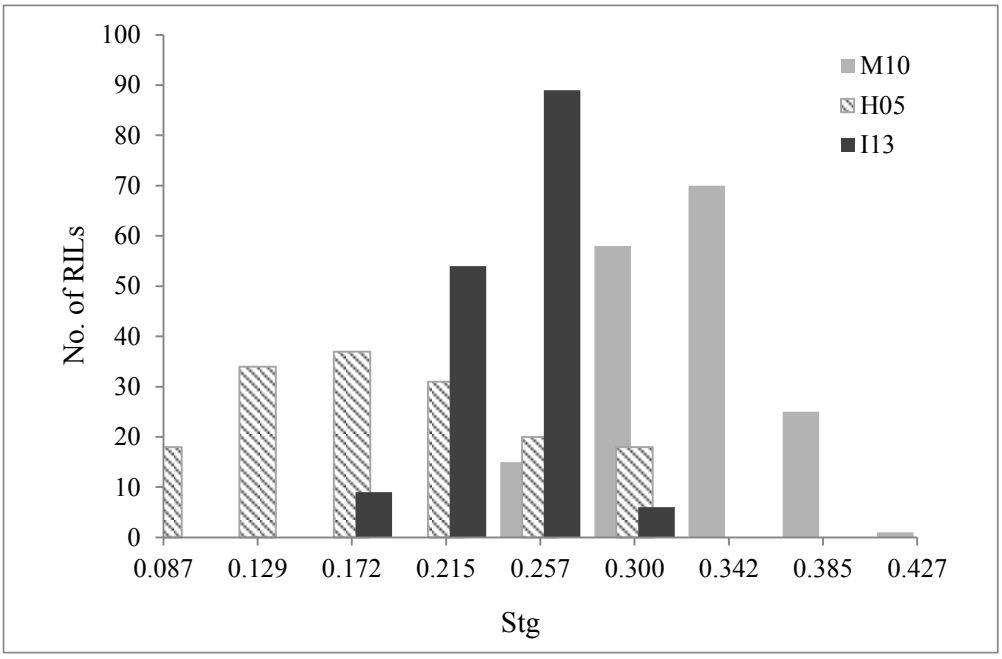
Supplementary material

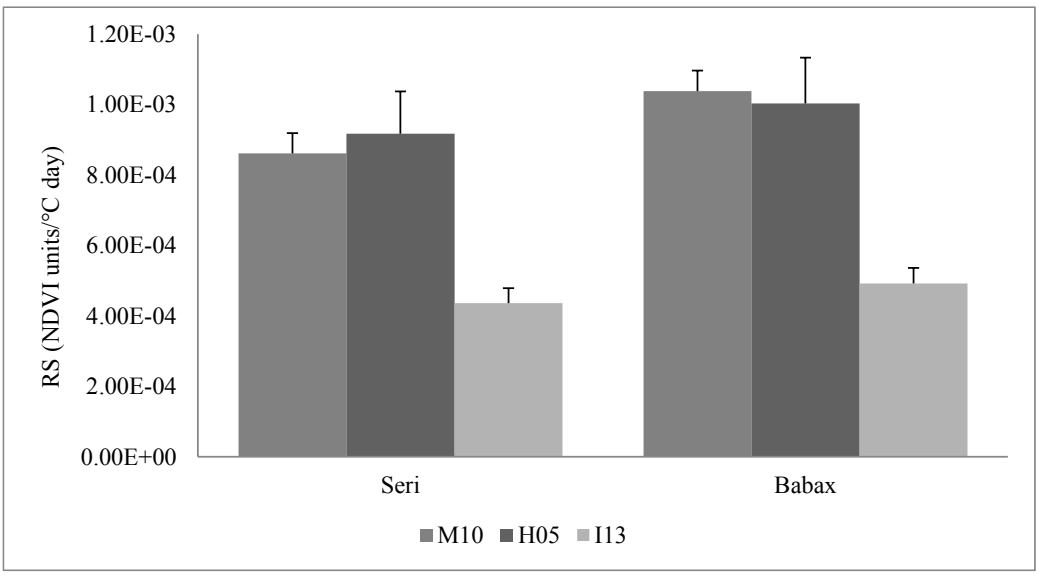


Supplementary Figure 1. PCA for Stg, TGW, KN and yield in the Seri/Babax population grown in M10, H05 and I13 heat-stressed, irrigated environments

TGW: thousand grain weight; KN: kernel number; Stg: residual greenness at physiological maturity; Environments: M10, moderate; H05, hot; I13, intense

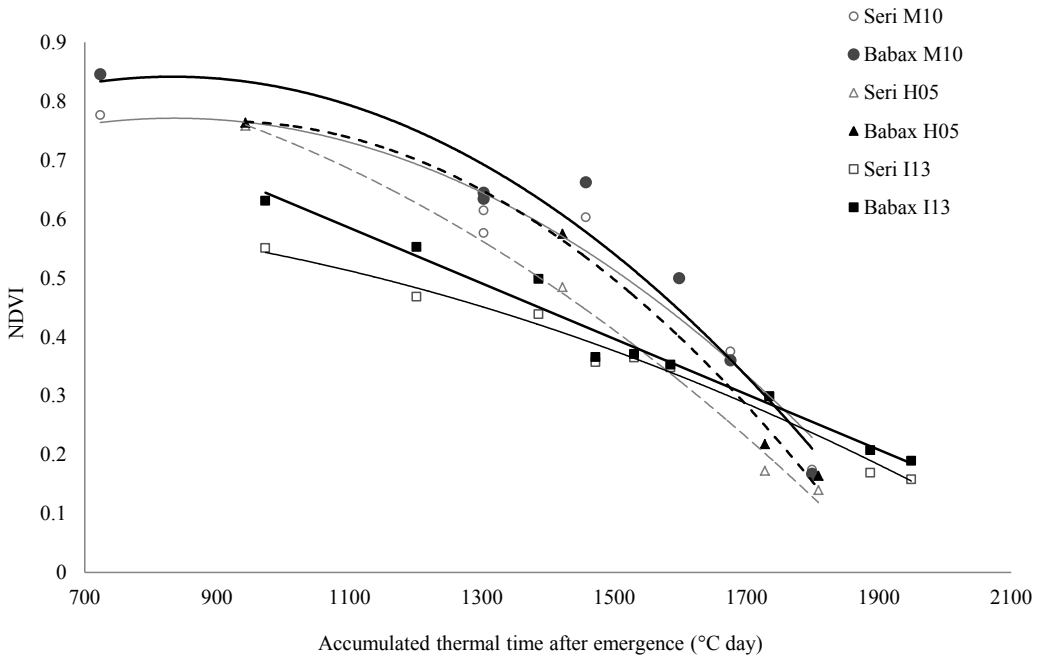


Supplementary Figure 2. Frequency distribution for Stg in the Seri/Babax population grown in M10, H05 and I13 heat-stressed, irrigated environments



Supplementary Figure 3. Absolute rates of senescence (RS) for the two parents Seri and Babax grown under M10, H05 and I13 heat-stressed, irrigated environments

Bars indicate standard error



Supplementary Figure 4. NDVIg patterns resulting after linking the dots of measurements performed on each of the two parents, Seri and Babax, in the M10, H05 and I13 heat-stressed, irrigated environment

Supplementary Table 1. Matrix of phenotypic correlations for all traits averaged across M10, H05, H11, I06 and I13 heat-stressed, irrigated environments

	CTg (°C)	CTv (°C)	Chlg (Spad units)	Chlv (Spad units)	GFD (days)	GFR (gm^{-2}/ day)	GM2 ($grains/m^2$)	Yield (g/m^2)	Gdecay (%)	Heading (dae)	Height (cm)	Maturity (dae)	NDVIg	NDVIv	RS (NDVI/°C d)	Stg	TGW g	StgAUC (NDVI × °C d)	TotalAUC (NDVI × °C d)
CTg	1	0.39	-0.13	0.09	-0.22	-0.07	-0.23	-0.11	0.51	-0.73	-0.40	-0.77	-0.79	-0.28	-0.07	0.52	0.18	-0.67	-0.60
p-value		<.0001	0.094	0.260	0.003	0.357	0.003	0.154	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	0.394	<.0001	0.022	<.0001	<.0001
CTv	0.39	1	-0.03	-0.19	-0.10	-0.57	-0.56	-0.58	0.10	-0.01	-0.04	-0.04	-0.12	-0.29	-0.15	-0.03	0.05	-0.14	-0.17
p-value	<.0001		0.721	0.015	0.200	<.0001	<.0001	<.0001	0.214	0.940	0.575	0.639	0.123	0.000	0.058	0.686	0.554	0.064	0.024
Chlg	-0.13	-0.03	1	0.30	-0.10	0.01	-0.10	-0.03	-0.14	0.14	0.11	0.10	0.21	-0.08	0.06	0.04	0.13	0.19	0.14
p-value	0.094	0.721		<.0001	0.190	0.894	0.207	0.697	0.079	0.074	0.142	0.180	0.006	0.293	0.456	0.633	0.096	0.011	0.059
Chlv	0.09	-0.19	0.30	1	-0.10	0.22	0.13	0.17	0.18	-0.11	-0.04	-0.14	-0.10	0.10	-0.09	0.17	0.03	-0.06	-0.02
p-value	0.260	0.015	<.0001		0.177	0.004	0.098	0.024	0.021	0.138	0.594	0.070	0.191	0.216	0.241	0.028	0.744	0.441	0.803
GFD	-0.22	-0.10	-0.10	-0.10	1	-0.10	0.10	0.16	-0.24	-0.04	0.05	0.26	0.20	-0.15	-0.07	-0.17	0.09	0.13	0.03
p-value	0.003	0.200	0.190	0.177		0.040	0.197	0.043	0.001	0.649	0.553	0.010	0.011	0.059	0.356	0.060	0.242	0.094	0.744
GFR	-0.07	-0.57	0.01	0.22	-0.10	1	0.82	0.96	0.06	-0.37	0.16	-0.39	-0.14	0.43	0.38	0.37	0.07	0.06	0.13
p-value	0.357	<.0001	0.894	0.004	0.040		<.0001	<.0001	0.441	<.0001	0.040	<.0001	0.064	<.0001	<.0001	<.0001	0.394	0.441	0.083
GM2	-0.23	-0.56	-0.10	0.13	0.10	0.82	1	0.84	-0.02	-0.19	0.01	-0.16	-0.04	0.31	0.25	0.11	-0.45	0.07	0.10
p-value	0.003	<.0001	0.207	0.098	0.197	<.0001		<.0001	0.784	0.011	0.904	0.040	0.642	<.0001	0.001	0.145	<.0001	0.356	0.180
Yield	-0.11	-0.58	-0.03	0.17	0.16	0.96	0.84	1	0.01	-0.40	0.15	-0.34	-0.12	0.37	0.37	0.34	0.08	0.07	0.11
p-value	0.154	<.0001	0.697	0.024	0.043	<.0001	<.0001		0.947	<.0001	0.056	<.0001	0.119	<.0001	<.0001	<.0001	0.275	0.356	0.154
Gdecay	0.51	0.10	-0.14	0.18	-0.24	0.06	-0.02	0.01	1	-0.48	-0.33	-0.53	-0.61	-0.07	-0.38	0.31	0.02	-0.58	-0.51
p-value	<.0001	0.214	0.079	0.021	0.001	0.441	0.784	0.947		<.0001	<.0001	<.0001	<.0001	0.390	<.0001	<.0001	0.803	<.0001	<.0001
Heading	-0.73	-0.01	0.14	-0.11	-0.04	-0.37	-0.19	-0.40	-0.48	1	0.26	0.95	0.85	0.17	-0.10	-0.75	-0.26	0.64	0.57
p-value	<.0001	0.940	0.074	0.138	0.649	<.0001	0.011	<.0001	<.0001		0.001	<.0001	<.0001	0.026	0.210	<.0001	0.001	<.0001	<.0001
Height	-0.40	-0.04	0.11	-0.04	0.05	0.16	0.01	0.15	-0.33	0.26	1	0.27	0.46	0.45	0.35	-0.17	0.29	0.58	0.60
p-value	<.0001	0.575	0.142	0.594	0.553	0.040	0.904	0.056	<.0001	0.001		0.001	<.0001	<.0001	<.0001	0.029	0.000	<.0001	<.0001
Maturity	-0.77	-0.04	0.10	-0.14	0.26	-0.39	-0.16	-0.34	-0.53	0.95	0.27	1	0.88	0.13	-0.11	-0.77	-0.22	0.65	0.56
p-value	<.0001	0.639	0.180	0.070	0.010	<.0001	0.040	<.0001	<.0001	<.0001	0.001		<.0001	0.089	0.144	<.0001	0.003	<.0001	<.0001
NDVIg	-0.79	-0.12	0.21	-0.10	0.20	-0.14	-0.04	-0.12	-0.61	0.85	0.46	0.88	1	0.35	0.12	-0.52	-0.08	0.87	0.81
p-value	<.0001	0.123	0.006	0.191	0.011	0.064	0.642	0.119	<.0001	<.0001	<.0001	<.0001		<.0001	0.124	<.0001	0.304	<.0001	<.0001
NDVIv	-0.28	-0.29	-0.08	0.10	-0.15	0.43	0.31	0.37	-0.07	0.17	0.45	0.13	0.35	1	0.27	-0.02	0.09	0.46	0.65
p-value	0.000	0.000	0.293	0.216	0.059	<.0001	<.0001	<.0001	0.390	0.026	<.0001	0.089	<.0001		0.000	0.793	0.265	<.0001	<.0001
RS	-0.07	-0.15	0.06	-0.09	-0.07	0.38	0.25	0.37	-0.38	-0.10	0.35	-0.11	0.12	0.27	1	-0.04	0.13	0.35	0.38
p-value	0.394	0.058	0.456	0.241	0.356	<.0001	0.001	<.0001	<.0001	0.210	<.0001	0.144	0.124	0.000		0.596	0.083	<.0001	<.0001
Stg	0.52	-0.03	0.04	0.17	-0.17	0.37	0.11	0.34	0.31	-0.75	-0.17	-0.77	-0.52	-0.02	-0.04	1	0.32	-0.33	-0.27
p-value	<.0001	0.686	0.633	0.028	0.060	<.0001	0.145	<.0001	<.0001	<.0001	0.029	<.0001	<.0001	0.793	0.596		<.0001	<.0001	0.000
TGW	0.18	0.05	0.13	0.03	0.09	0.07	-0.45	0.08	0.02	-0.26	0.29	-0.22	-0.08	0.09	0.13	0.32	1	0.02	0.04
p-value	0.022	0.554	0.096	0.744	0.242	0.394	<.0001	0.275	0.803	0.001	0.000	0.003	0.304	0.265	0.083	<.0001		0.803	0.649
StgAUC	-0.67	-0.14	0.19	-0.06	0.13	0.06	0.07	0.07	-0.58	0.64	0.58	0.65	0.87	0.46	0.35	-0.33	0.02	1	0.93
p-value	<.0001	0.064	0.011	0.441	0.094	0.441	0.356	0.356	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.803		<.0001
TotalAUC	-0.60	-0.17	0.14	-0.02	0.03	0.13	0.10	0.11	-0.51	0.57	0.60	0.56	0.81	0.65	0.38	-0.27	0.04	0.93	1
p-value	<.0001	0.024	0.059	0.803	0.744	0.083	0.180	0.154	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	0.649	<.0001	

Stg: staygreen at physiological maturity; RS: rate of senescence; TotalAUC: total area under the curve with starting points at crop establishment; StgAUC: staygreen area under the curve with starting points at maximum NDVI; Gdecay: percentage of greenness lost at mid grainfilling; KN: kernel number; TGW: thousand grain weight; GFR: grainfilling rate; GFD: grainfilling duration. NDVIv: normalized difference vegetative index during vegetative stage; NDVIg: normalized difference vegetative index during grainfilling; Chlv: chlorophyll content at vegetative stage (SPAD); Chlg: chlorophyll content at grainfilling (SPAD); CTv: canopy temperature at vegetative stage; CTg: canopy temperature at grainfilling.

Chapter 4. Journal paper: Leaf respiration and other physiological responses to high temperature in genetically diverse wheat lines

Manuscript accepted for publication by Euphytica

Physiological breeding of wheat under heat stressed environments requires combinations of diverse traits representing the main drivers of yield and plant performance at high temperatures (Reynolds and Trethowan 2007). Many of these traits can be evaluated simply, such as CT, while others are more difficult to measure such as those associated with the biochemical and metabolic responses to heat stress. In this study we explore a physiological approach to assess the biochemistry of heat response. Whole plant performance, including crop yield depends on gas exchange, specifically photosynthesis and respiration processes. The relevance of the former is well-known but the role of respiration remains largely a black box. We decided to work with leaf respiration as a key trait given that it consumes up to 60% of carbon accumulated by photosynthesis in cereals (Amthor and Baldocchi 2001), and given that it has been previously associated with plant response to stress conditions. Specifically, the alternative oxidase pathway of plant respiration is suggested to reduce the damage of reactive oxygen species which are generated under stress conditions such as high temperatures and drought (Gifford 2003). Due to the complexity of leaf respiration measurement under field conditions, the study presented in this chapter focused on a selection of germplasm derived from previous screenings. The strategy was to work in a genetically diverse group of wheat lines in order to increase the likelihood of detecting contrasting responses that could lead to the discovery of useful genetic resources for crossing. If successful, this could lead to quite sophisticated and targeted approaches to tackle heat response in wheat and contribute scientific knowledge on the biochemical mechanisms of tolerance to heat stress.

Statement of Authorship

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Name of Principal Author (Candidate)	**R. Suzuky Pinto**		
Contribution to the Paper	Conducted field experiments, performed data analysis and led the write-up.		
Overall percentage (%)	75%		
Signature		Date	31/05/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Gemma Molero**		
Contribution to the Paper	Contributed in field data collection and interpretation, as well as providing valuable advice in writing the manuscript		
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Contribution to the Paper	Designed the experiment and participated in all aspects of data analysis and writing of the paper		
Signature		Date	31/05/2016

Leaf respiration and other physiological responses to high temperature in genetically diverse wheat lines

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Key message: Genetic diversity was found for leaf respiration rate (R_d) of spring wheat grown under hot irrigated conditions. Temperature and plant ontology can influence R_d. Diverse wheat classes are compared in their performance for heat adaptive traits and their association with R_d is discussed.

Abstract

A total of 140 diverse spring wheat genetic resources were characterized for agronomic and physiological traits under hot field conditions, and a subset of thirteen lines were selected for dark leaf respiration studies (R_d). These genetic resources included synthetic-derived lines, landraces, landrace-derived lines, bread wheats, and durum wheats. Initial phenotyping showed that better heat-adapted genotypes (best yielding) exhibited high early biomass (assessed by normalized difference of vegetative index, NDVI), high grainfilling rates, and low canopy temperatures. In accordance with other studies, R_d increased as temperature increased (within the range of 15-40 °C), and decreased with plant age. Correlations between R_d and leaf temperature ranged between 0.50-0.85. R_d was negatively associated with yield when recorded at 30-35 °C during anthesis and grainfilling stages. The comparison between classes of genetic resources showed that synthetic-derived material expressed smaller leaf respiration rates and larger yields than the other classes at warm temperatures.

Keywords: spring wheat, high temperature, heat tolerance, diversity, landrace, synthetic, respiration

Introduction

Predictions of further global warming in the near future (Challinor et al. 2014) are highlighting the importance of improving the heat tolerance of wheat. While genetic gains have been achieved (Gourdji et al. 2013), the complex nature of plant adaptation to abiotic stress means that it requires an integrative approach (Bonnett et al. 2005). Conceptual models for wheat heat tolerance have been proposed (Cossani and Reynolds 2012); the ideal heat tolerant plant is expected to show a combination of two or more of the following characteristics: *i)* rapid aboveground cover during crop establishment, which increases the initial amount of intercepted radiation while also reducing soil temperatures; *ii)* strong radicular system that would absorb high amounts of water to match the evaporative demand and maintain cool canopy temperatures; *iii)* leaves and vegetative structures containing high amounts of carotenoids, chlorophylls, and xanthophylls that act like antioxidants and – together with wax – protect the tissue from high irradiances, reducing damage to the photosynthetic apparatus; *iv)* delayed loss of greenness in the photosynthetic structures, resulting in an extended grainfilling period (staygreen); and *v)* optimized plant gas exchange rates to minimize the negative effects of high temperatures. This latter mechanism is one of the least explored dynamics related to photosynthesis and respiration.

Photosynthesis is one of the most heat-sensitive processes, where inhibition of photosynthetic CO_2 fixation under high temperatures may result in significant reductions in plant growth and yield (Berry and Bjorkman 1980; Seemann et al. 1984; Wardlaw and Wringley 1994; Lafta and Lorenzen 1995; Porter and Gawith 1999). In wheat plants, Rubisco activation is inhibited above 30 °C and irreversibly inhibited above 40 °C, via a direct effect on Rubisco activase (Salvucci and Ogren 1996; Feller et al, 1998). On the other hand, high temperatures increase respiration rates (Kase and Katsky 1984; Kirschbaum and Farquhar 1984). Under optimal growing conditions, plant respiration can release up to about half of the CO_2 fixed by photosynthesis back into the atmosphere (Van Der Werf et al. 1994). Variations in the magnitude of leaf respiration could therefore have an important impact on the carbon economy of a plant in a climate change context. In this sense, a better understanding of plant respiration dynamics is a prerequisite for predicting plant growth and performance resulting in genetic yield gains. However, the effects of increased respiratory rates on growth and yield under high temperatures are not clear and

remain controversial (Prange et al. 1990; Amthor 1994; Mohammed and Tarpley 2009). At this stage, it is difficult to affirm if a heat-adapted wheat genotype is expected to show increased or reduced respiratory rates, compared to heat susceptible germplasm. However, genetic variability for leaf respiration rate has been identified in some species and a number of these studies seem to indicate that reduced respiratory rates are advantageous for general plant performance (Wilson and Jones 1982; Massacci et al. 1986; Nissanka et al. 1997).

Much of the wheat grown worldwide shows a relatively narrow genetic base (Warburton et al. 2006), probably associated with the empirical selection on yield *per se*. Focused research in the study of other traits (not only yield) can contribute to expanding the wheat genepool for crop improvement. Another strategy to increase the presence of novel genes in wheat is to use materials from different sources like landraces (local varieties) and wild wheat relatives to improve current elite varieties. In recent years, greater emphasis has been placed on including these novel and exotic genes in modern wheats (Hede et al. 1999; Skovmand et al. 2001; Dreisigacker et al. 2005), and some physiological traits have been verified in the field (Reynolds et al. 2007a; Cossani and Reynolds 2015). Increased wheat genetic diversity provides a pool of potentially valuable genes for developing new varieties with improved heat tolerance. For years, wheat landraces have survived unfavourable conditions; exploration of this naturally-adapted germplasm could result in the identification of novel genetic resources available for wheat improvement (e.g. Reynolds et al. 2007b). Nevertheless, the physiological mechanisms underlying the heat adaptation of exotic and local cultivars are frequently unexplored and under-utilized. This study aimed to: i) screen a large panel of genetic resources (including landraces, elite, and synthetic lines) for agronomic and physiological performance under heat stress and identify a diverse subset of lines for respiration studies; ii) measure the main effects of temperature and ontology on respiration rates in a subset of 13 diverse genetic resources, and examine interactions between these two factors; and iii) compare respiration rate with performance in the different classes of wheat genetic resources represented by the 13 lines.

Materials and methods

Field experiments

Experiments were conducted over four years (2010, 2011, 2012, and 2013) and were sown under hot irrigated conditions in the Yaqui Valley, northwest Mexico. The study started with an initial collection of 140 diverse wheat lines, from which 70 and then 13 genotypes were selected for more detailed study. Agronomic and physiological data was collected for the first two sets of lines (140 and 70), and for the 13-line subset, leaf respiration rate was also recorded. The 140 diverse wheat lines grown in 2010 were evaluated for grain yield, kernel weight (TGW), kernel number (GM2), grainfilling rate (GFR= Yield/ Maturity − Anthesis), grainfilling duration (GF= Maturity − Anthesis), canopy temperature during the vegetative stage (CTv), canopy temperature during the grainfilling stage (CTg), green biomass production during the vegetative stage assessed through the normalized difference of vegetative index (NDVIv) and during the grainfilling stage (NDVIg), days to heading (Heading), days to maturity (Maturity), and plant height (Height). Grain yield, yield components, phenology, and physiological traits (canopy temperature and NDVI) were measured following standard procedures (Pask et al. 2012). Chlorophyll content in the flag leaf was measured using a SPAD-502 Minolta (Spectrum Technologies Inc., Plainfield, IL, USA). During 2011 and 2012, the first selection of 70 genotypes was evaluated for the same traits, plus the percentage of water soluble carbohydrate (WSC) content in stems at heading ±7 days, leaf chlorophyll content during grainfilling (CHLg), and dry biomass production at maturity (Bmm). These traits were also measured in a selected sample of the 30 best performing genotypes during 2010. WSC in the stems was chemically determined using the anthrone reagent method (Mc Cready et al. 1950).

During the 2013 season, the final set of 13 selected wheat genotypes were sown under hot irrigated conditions and phenotyping was conducted following the same procedures detailed above. Agronomic and physiological traits were recorded as above, as well as leaf respiration rate measurements (R_d).

Germplasm

The initial set consisted of 140 diverse wheat genetic resources selected under heat stressed environments from diversity panels of the World Wheat Collection. This set comprised five different wheat classes: 82 landraces, 21

landrace-derived lines, 5 synthetic-derived lines, 14 durum wheats (*T. turgidum* var. *durum*), and 18 elite bread wheats (*T. aestivum*). The panel was screened in the following years (2011 and 2012) for heat tolerance and traits of interest, and was then reduced to 70 lines and then to 13 lines for detailed respiration studies in 2013. The final set of 13 selected lines was comprised of four synthetic-derived lines (CROC_1/AE.SQUARROSA (213)//PGO/3/BAV92; CROC_1/AE. SQUARROSA (224)//OPATA/3/SOKOLL; CROC_1/AE.SQUARROSA (224)//OPATA/3/ PASTOR; Sokoll), three durum wheats (Atil C2000; T.DICOCCON\PI94628//SOOTY_9/ RASCON_37; SAWALI), three elite bread wheats (SHARP/3/PRL/SARA//TSI/VEE#5/ 5/VEE/LIRA//BOW/3/BCN/4/KAUZ; Weebill1; BABAX/LR42//BABAX/3/ER2000), two landrace-derived lines (WBLL4//OAX93.24.35/WBLL1; WBLL4//OAX93.24.35/WBLL1), and one landrace (MEX94.2.19/PUB94.15.1.12). Atil C2000 was included as a low yielding check in this set. A reference trial was also sown in 2009 to illustrate the association of leaf respiration rate and leaf temperature using a different instrument for measuring respiration. The reference trial was comprised of 24 genotypes including landraces, recombinant inbred line sisters derived from the Seri/Babax cross, and parental lines from mapping populations characterized by good yield under high temperatures and contrasting expression of physiological traits under different environments (for references see Olivares-Villegas et al. 2007; Pinto et al. 2010).

Environment

For the four years of evaluation, germplasm was sown at the end of February and registered emergence dates in March when air temperatures are higher than those regularly observed under normal sowing dates (November-December) (Table 1).

Dark leaf respiration rate measurements

Dark leaf respiration rate (R_d) was recorded in the field using a LI-6400XT portable gas-exchange photosynthesis system (LI-COR, Lincoln, NE, USA) at three plant growth stages (booting, anthesis, and grainfilling) and four temperatures (20, 25, 30, and 35 °C). Leaf respiration measurements were performed at 400 ppm CO_2 concentration, air flux of 400 µmols-1, and during the day or night depending on the required measurement temperature. A minimum of two measurements per plot were recorded for each stage ×

temperature treatment. For daylight measurements, the whole plant was covered with a blanket (black inside and white outside to avoid increasing temperatures above ambient air temperature) and the plant was allowed to adapt to dark conditions for a minimum of 30 minutes before recording leaf respiration rate. In addition to the four main experiments of this study, the reference set of 24 genotypes was sown in 2009 under high temperature conditions, but no agronomic or physiological data was collected, except for leaf respiration rate and leaf temperature, which were measured using a portable analyser (Ciras-1, PP-systems, UK).

Statistical analysis

A complete randomized block design with two replicates was used for all experiments in all years. Statistical analyses were performed in SAS v 9.0 using a Proc Mixed for the ANOVA completed across genotypes and wheat classes.

Results

Agronomic performance of genetic resources

Mean yield of the initial collection of 140 wheat cultivars evaluated in 2010 was 356 gm^{-2}, with a range of 174–475 gm^{-2} (Table 2). TGW had a mean of 34.9 g and a range of 23.0–46.2 g. GM2 mean was 10300 grains m^{-2} with a range of 4300–15017. CTv ranged from 24.3–26.5 °C with a mean of 25.2 °C, while CTg ranged from 30.2–32.6 °C with a mean of 31.1 °C. Green biomass during the vegetative stage assessed through the measurement of the NDVIv showed a mean of 0.580 and range of 0.350–723, but the index mean was reduced to 0.539 during the grainfilling period (NDVIg). Screening this diverse set of wheat genetic resources indicated genetic variability for traits evaluated ($p<0.0001$, Table 2). Yield was positively and well correlated with NDVI measurements averaged across the vegetative and grainfilling stages ($r= 0.46$, $\alpha=0.05$, Fig. 1), and with grain weight and grain number ($r= 0.35$ and $r =0.74$, respectively, $\alpha=0.05$, data not shown), while it was negatively associated with average CT ($r= -0.46$, $\alpha=0.05$, Fig. 2). From the initial panel of 140 lines, 70 wheat cultivars showing high yield, high biomass (NDVI), and low average CT during the whole season, as well as a restricted range of phenology and plant height, were selected for the next season (Figs. 1 and 2). Some genetic resources exhibiting contrasting performance for CT and NDVI were also included in the set of 70

lines to assess different mechanisms of wheat heat tolerance, e.g. Atil C2000, which was included as a low yielding cultivar.

The 70 selected cultivars were grown during the 2011 and 2012 crop seasons; their performance is shown in Table 3. A combined analysis across both years showed a mean yield of 325 gm^{-2}, with a range of 260–380 gm^{-2}. Mean TGW was 37 g, and ranged from 27.2–45.7 g. GM2 ranged from about 7000–11000 grains m^{-2} with a mean close to 9000 grains m^{-2}. The grainfilling period represented about 37% of the whole crop cycle (with a range of 32.1–41.0%) and GFR averaged 10.6 g m^{-2} day^{-1} (with a range of 8.22–12.5 g m^{-2} day^{-1}). CTv averaged 24.1 °C, with a range of 23.3-24.9 °C, while CTg averaged 28.6 °C with a range of 26.8-30.4 °C (Table 3). NDVIv and NDVIg averaged 0.569 and 0.451, with ranges of 0.470–0.630 and from 0.349–0.610, respectively. CHLg ranged from 43.4–51.2 with a mean of 48 (Spad units). Bmm and WSC were measured in a subset of 30 lines. Bmm mean was 620 g/m^2, with a range of 457-730 gm^{-2}, while WSC at heading averaged 17.3% with a range of 13.1–20.3% (Table 3). Compared to the initial collection of 140 lines, the ranges of heading and plant height of the 70-line subset were reduced by 11 days and 12 cm, respectively. Within the 70-line subset, mean yields from 2011 and 2012 were highly correlated with GFR (Fig. 3) and with Bmm (r= 0.85, α=0.05, Fig. 4). From this pool of 70 cultivars, a subset of 13 genotypes was selected for respiration measurements during the 2013 season. This selection attempted to capture lines that performed better under heat stress and could represent candidate parents for breeding and physiological studies. As with previous years, lines were chosen based on high yields (Fig. 5) and good expression of traits associated with heat tolerance such as GFR, CT, and biomass. The final set of 13 diverse wheat lines showed high yield, high Bmm, and low CTv, and was comprised of five types of wheat cultivars (groups), similarly to previous collections, for which the ANOVA showed significant differences between wheat groups in the five experiments grown from 2010-2013 (Table 4).

Landrace-derived lines and synthetics-derived lines showed up to 27% higher yields and 26% higher TGW than the elite bread wheats, durum wheats, and landraces in all experiments (Table 4). This performance was also reflected in Bmm of each group in the pool of 70 and in the subset of 13 lines, where it was observed that the synthetic-derived lines, the landrace-derived lines, and the landraces reported 6-38% more Bmm than the durum wheat lines and the elite

bread wheats. CTg of the landrace-derived lines and synthetic-derived lines were generally lower than CTg reported for other groups. Similar trends were observed for NDVIv and NDVIg, but with some variations across years. The group of synthetic-derived lines and elite bread wheat lines showed the lowest CHLg in the collections of 140 and 70 lines, while the highest CHLg were reported by the landrace-derived lines and the durum wheats (Table 4).

The Rd variance analysis for the 13 genotypes across stages and measurement temperatures showed significant genotypic effects, as well as significant effects of the measurement temperature (T°), plant growth stage (Stage), and significant interactions between Genotype × T°, Genotype × Stage, T° × Stage, and Genotype × T° × Stage (Supplementary table 1). Averaged across stages and temperatures, for the 13 selected genotypes Rd by genotype ranged from 2.1-3.7 µmol CO2 m2s-1. This data was categorized by time of data collection (morning, afternoon, or night) and by plant growth stage (booting, anthesis, or grainfilling) as shown on Fig. 7A. The lowest Rd were observed on measurements performed during the night between 18:00-5:00 hr (Fig. 7B), and in mature tissue during grainfilling while the highest Rd were observed during the afternoon between 12:00-18:00 hr, and in young leaf tissue (Fig. 7C). The variance analysis for the main effect of measurement temperature on R_d across genotypes and plant growth stages showed significant differences between temperatures (Fig. 7B).

Table 1. Weather conditions in the Yaqui Valley, NW-Mexico for the 2010, 2011, 2012, and 2013 seasons.

Sowing	Number of	Date of	Heading	Maturity	T_{max} (°C)			T_{min} (°C)			T_{max} >30 °C (%)			Total rain
& harvest year	genotypes	emergence	(dae)	(dae)	Veg	Ant	GF	Veg	Ant	GF	Veg	Ant	GF	(mm)
2010	140	1-Mar-10	54	86	29.2	30.6	35.1	9.5	11.5	12.1	57	76	91	0
2011	70	5-Mar-11	49	81	30.7	33.2	35.2	10.1	12.5	12.8	65	95	100	0
2012	70	4-Mar-12	54	83	29.4	31.5	35.3	9.7	11.6	12.3	60	76	90	0
2013	13	8-Mar-13	48	77	30.8	33.8	35.9	10.6	12.3	14.6	66	86	100	0

dae: days after emergence; Tmax: average of daily maximum temperatures; Tmin: average of daily minimum temperatures; Tmax >30 °C: percentage of days with daily maximum temperatures >30°C; Veg: vegetative stage; Ant: anthesis ±10 days; GF: grainfilling stage

Table 2. Means for agronomic and physiological traits measured during 2010 ($Tmax^{\dagger}$= 31.6 °C, $Tmin^{\dagger}$=11 °C) for the initial pool of 140 genotypes grown under heat stress in the Yaqui Valley, Mexico.

	Yield	TGW	GM2	GFR	GF	CTv	CTg	NDVIv	NDVIg	Heading	Maturity	Height
	(g/m^2)	(g)	($grains/m^2$)	($g\ m^{-2}/day$)	(%)	(°C)	(°C)			(dae)	(dae)	(Cm)
Mean	356	34.9	10300	11.4	36.6	25.2	31.1	0.580	0.539	54	86	79.3
Minimum	174	23.0	4300	5.65	33.7	24.3	30.2	0.350	0.346	45	77	55.8
Maximum	475	46.2	15017	15.4	41.0	26.5	32.6	0.723	0.653	66	94	109
Stdev	68.5	4.37	2075	2.28	1.47	0.380	0.422	0.062	0.052	2.65	2.83	11.8
p>f Genotype	<.0001	<.0001	<.0001	<.0001	<.0001	0.0037	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Cov heading	ns	0.0047	0.0178	ns	<.0001	ns	0.0006	<.0001	0.0037	-	-	ns
LSD	80.7	3.66	2517	2.66	1.79	0.841	0.646	0.065	0.058	1.73	2.46	5.2
CV	11.4	5.29	12.3	11.8	2.47	1.69	1.05	5.62	5.41	1.61	1.45	3.34

Stdev: standard deviation; Cov: covariate; LSD: least significant differences; CV: coefficient of variability; TGW: thousand grain weight; GFR: grainfilling rate; GF: fraction of the plant growth cycle corresponding to grainfilling; CTv: canopy temperature during the vegetative stage; CTg: canopy temperature during grainfilling; NDVIv: normalized difference of vegetative index recorded during the vegetative stage; NDVIg: normalized difference of vegetative index recorded during grainfilling; dae: days after emergence; ns: not significant; Tmax= daily maximum temperature; Tmin: daily minimum temperature; † average of daily maximum/minimum temperatures observed during the whole crop cycle

Table 3. Means from the combined analysis across years in traits measured in the first subset of 70 genotypes grown under moderated heat stress in the Yaqui Valley, Mexico, during 2011 and 2012 (Tmax[t]= 32.5 °C, Tmin[t]=11.5 °C).

	Yield (g/m^2)	TGW (g)	GM2 ($grains/m^2$)	GFR ($g\ m^{-2}/day$)	GF (%)	CTv (°C)	CTg (°C)	NDVIv	NDVIg	CHLg (Spad units)	Bmm (g/m^2)	WSC (%)	Heading (dae)	Maturity (dae)	Height (Cm)
Mean	325	37.0	8859	10.6	37.1	24.1	28.6	0.569	0.451	48.0	620	17.3	52	82	80.7
Minimum	260	27.2	6986	8.22	32.1	23.3	26.8	0.470	0.349	43.4	457	13.1	47	78	61.5
Maximum	380	45.7	10863	12.5	41.0	24.9	30.4	0.630	0.610	51.2	730	20.3	57	89	103
Stdev	28.8	3.80	910	1.08	1.61	0.411	0.808	0.033	0.063	1.84	56.3	1.94	2.22	2.46	9.4
p>f Genotype	<.0001	<.0001	<.0001	<.0001	<.0001	0.007	<.0001	<.0001	<.0001	<.0001	0.0111	<.0001	<.0001	<.0001	<.0001
p>f Year	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.333	<.0001	<.0001	<.0001
p>f Genotype × Year	<.0001	0.0195	<.0001	<.0001	0.197	0.135	0.0023	<.0001	<.0001	0.796	0.730	0.001	0.0078	0.002	0.0017
LSD	39.9	2.37	1249	1.43	1.74	0.876	0.826	0.048	0.040	3.46	112	3.02	1.39	1.54	5.8
CV	6.2	3.26	7.2	6.8	2.38	1.84	1.47	4.25	4.54	3.65	9.04	8.73	1.36	0.95	3.65

Stdev: standard deviation; Cov: covariate; LSD: least significant differences; CV: coefficient of variability; TGW: thousand grain weight; GFR: grainfilling rate; GF: fraction of the plant growth cycle corresponding to grainfilling; CTv: canopy temperature during the vegetative stage; CTg: canopy temperature during grainfilling; NDVIv: normalized difference of vegetative index recorded during the vegetative stage; NDVIg: normalized difference of vegetative index recorded during grainfilling; CHLg: leaf chlorophyll content measured during grainfilling; Bmm: dry weight of biomass at maturity; WSC: water soluble carbohydrates content; dae: days after emergence; ns: not significant; Tmax= daily maximum temperature; Tmin: daily minimum temperature; [t] average of daily maximum/minimum temperatures observed during the whole crop cycle.

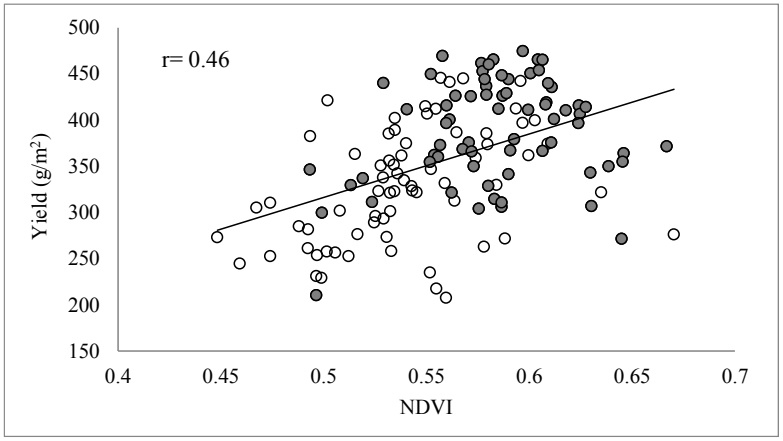

Figure 1. Association of yield with the average normalized difference of vegetative index (NDVI) in the initial pool of 140 wheat lines grown under heat stress during 2010. Filled circles indicate the 70 lines selected for the next season.

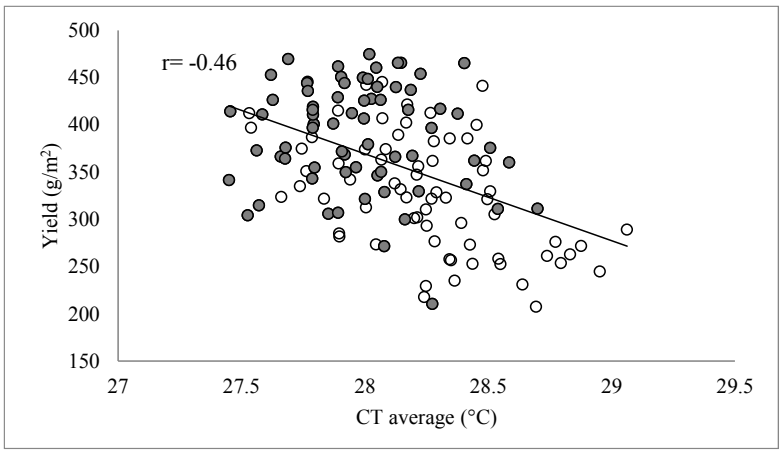

Figure 2. Association of yield with canopy temperature (CT average) in the initial pool of 140 wheat lines grown under heat stress during 2010. Filled circles indicate the 70 lines selected for the next season.

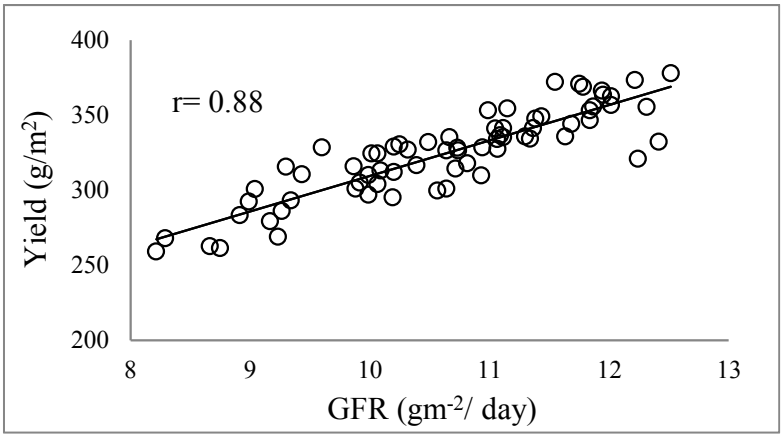

Figure 3. Association of yield with grainfilling rate (GFR) in the first subset of 70 wheat lines grown under heat stress in seasons (combined analysis across two years of experiments in 2011 and 2012).

Table 4. Wheat-type means for agronomic and physiological traits measured in the initial set of 140 wheat lines (2010), in the first subset of 70 lines (2011 and 2012), and in the final 13 lines chosen for respiration measurements (2013) all grown under heat stress in the Yaqui Valley.

Type of wheat	n	Yield (g/m^2)	TGW (g)	GM2 ($grains/m^2$)	GFR ($g\ m^{-2}/day$)	GF (%)	CTv (°C)	CTg (°C)	NDVIv	NDVIg	Heading (dae)	Maturity (dae)	Height (Cm)	CHLg (Spad units)	Bmm (g/m^2)	WSC (%)
Wheat groups in the 140 genotypes (2010)																
Landrace derived	21	423	41.2	10270	13.6	36.1	25.3	30.8	0.619	0.574	55.2	86.4	85.2	nr	nr	nr
Synthetic derived	5	412	35.7	11571	13.4	36.2	25.1	30.9	0.614	0.556	54.5	85.4	81.9	nr	nr	nr
Elite bread wheat	18	349	33.5	10494	11.2	36.6	25.3	31.2	0.583	0.516	53.8	84.9	74.5	nr	nr	nr
Durum wheat	14	340	39.3	8687	11.1	35.2	25.0	30.8	0.495	0.602	56.6	87.3	82.2	nr	nr	nr
Landrace	82	333	35.5	9438	10.1	37.7	25.0	30.8	0.591	0.566	54.7	87.8	92.1	nr	nr	nr
Group p-value		<.0001	<.0001	<.0001	<.0001	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	nr	nr	nr
LSD		36.7	2.05	1052	1.20	1.08	0.254	0.287	0.024	0.031	1.36	1.45	5.34	nr	nr	nr
CV		5.03	2.81	5.30	5.14	1.51	0.513	0.472	2.12	2.84	1.26	0.850	3.26	nr	nr	nr
Wheat groups in the 70 genotypes(combined across 2011 and 2012)																
Landrace derived	2	359	45.1	7959	11.6	37.6	23.8	27.6	0.599	0.430	51.7	82.8	88.2	49.0	685	18.5
Synthetic derived	13	340	37.3	9137	11.1	37.7	23.9	28.0	0.569	0.429	50.7	81.3	81.4	48.2	629	17.8
Durum wheat	2	323	38.0	8445	10.1	37.9	23.9	28.4	0.541	0.464	52.1	83.8	77.4	49.4	587	20.3
Landrace	17	321	37.0	8699	10.4	36.9	24.0	28.3	0.584	0.493	52.9	83.9	86.0	48.6	634	16.1
Elite bread wheat	36	321	36.5	8825	10.6	37.0	24.1	28.5	0.560	0.433	51.4	81.6	77.6	47.7	596	17.1
Group p-value		0.036	<.0001	0.054	0.025	0.501	0.829	0.705	0.005	<.0001	0.003	<.0001	<.0001	0.127	0.041	0.032
LSD		31.1	2.47	771	0.925	1.70	0.889	1.50	0.032	0.038	1.92	1.50	5.52	1.69	68.5	2.36
CV		4.76	3.23	4.55	4.37	2.31	1.89	2.71	2.81	4.32	1.88	0.924	3.42	1.77	5.53	6.63
Wheat groups in the 13 genotypes (across 2010, 2011, 2012 and 2013)																
Landrace derived	2	363	42.2	8644	11.9	36.9	23.9	28.9	0.619	0.465	52.2	82.6	87.0	50.6	695	17.1
Synthetic derived	4	353	34.4	10305	11.5	38.6	24.1	29.6	0.604	0.434	49.0	79.7	78.9	46.9	637	15.8
Landrace	1	340	33.4	10147	11.4	35.7	24.3	30.0	0.614	0.514	54.2	84.2	78.4	48.8	789	23.9
Elite bread wheat	3	332	34.9	9451	11.0	37.3	24.3	30.0	0.578	0.434	50.6	80.8	75.2	46.0	573	15.8
Durum wheat	3	321	35.5	9065	10.3	37.9	23.9	29.5	0.522	0.427	50.9	81.9	68.0	49.5	578	18.6
Group p-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.073	<.0001	0.001
Year p-value		0.036	<.0001	0.002	0.043	<.0001	0.064	0.002	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	0.004	0.040
LSD		30.0	1.73	977	1.07	1.01	0.343	0.567	0.027	0.030	1.05	1.02	3.59	2.49	101	4.22
CV		4.39	2.40	5.13	4.77	1.35	0.711	0.958	2.27	3.28	1.02	0.621	2.32	2.52	7.42	11.1

LSD: least significant difference; CV: coefficient of variability; no recorded

Highest mean R_d across stages and genotypes was observed at 35 °C and 30 °C, with averages of 4.3 and 3.5 µmol $CO_2 m^2 s^{-1}$, respectively, while the lowest R_d were detected at 25 °C and 20 °C. Significant differences were also detected for R_d at different plant growth stages across measurement temperatures and genotypes, with the highest R_d observed during the booting and anthesis periods (Fig. 7C). Genetic variability for R_d was reduced in older leaves.

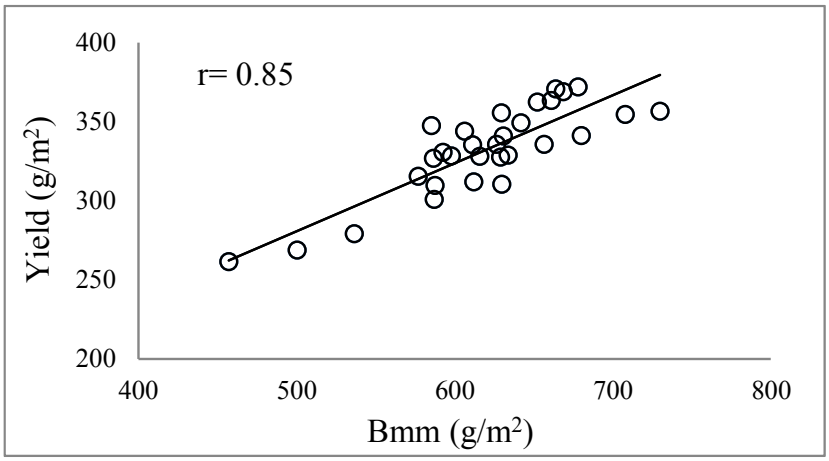

Figure 4. Association of yield with dry weight biomass at maturity (Bmm) in 30 of the 70 wheat lines grown under heat stress in seasons 2011 and 2012.

When averaged across anthesis and grainfilling stages, and measured at 30 °C and 35 °C, R_d was negatively associated with yield traits (α=0.05; Fig. 8). R_d – grouped by wheat type – were highest for durum wheats (averaged across stages and temperatures), followed by wheat landraces, landrace-derived cultivars, and elite bread wheats (Fig. 9). The highest genetic variability inside each group was observed at the earliest plant growth stage at the highest evaluated temperature. When the mean R_d of wheat classes was compared with plant performance traits, it was observed that high yielding wheat resources generally exhibited lower respiration rates (Fig. 9) and that high GFR and NDVI were related to wheat classes with reduced R_d (Fig. 9, Table 4).

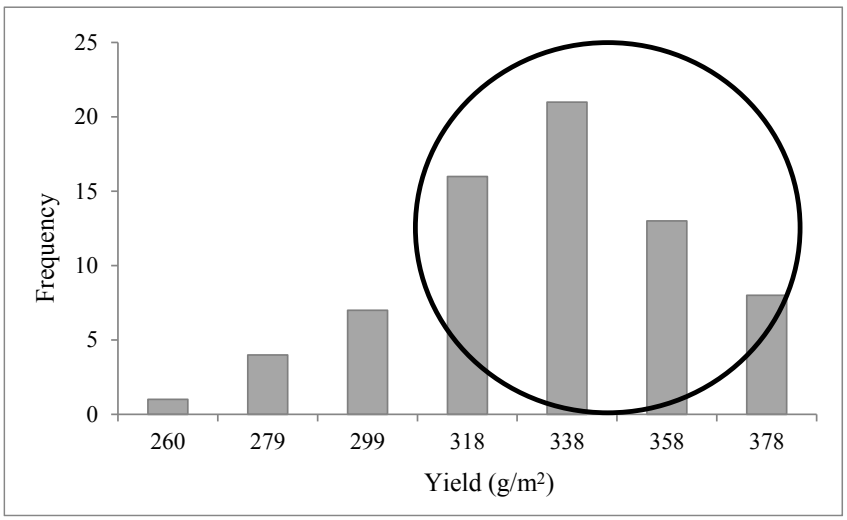

Figure 5. Frequency distribution for yield in the first subset of 70 wheat lines grown under heat stress in seasons 2011 and 2012. The circle contains 12 of the 13 selected lines for leaf respiration studies in the next season (data from the combined analysis across two years of evaluation under heat stress).

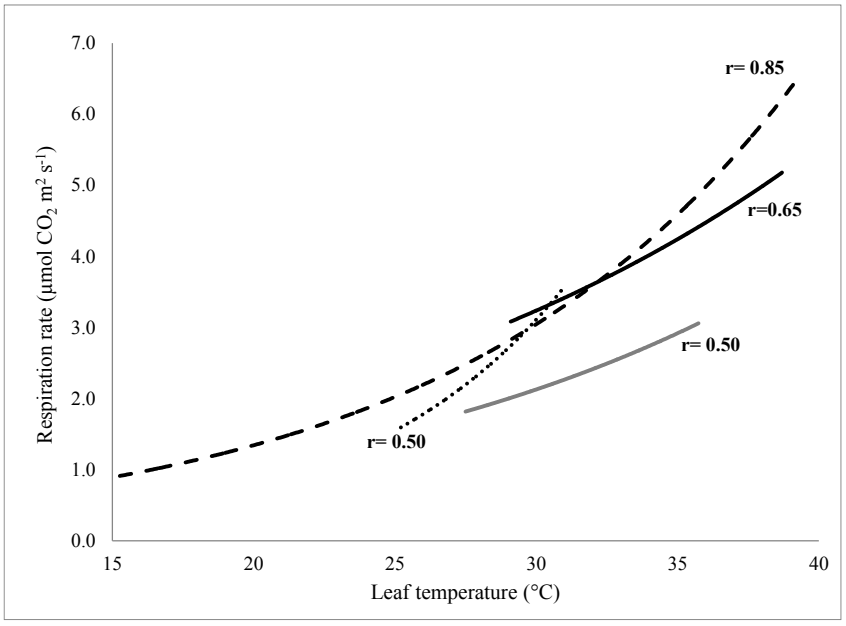

Figure 6. Association of R_d and leaf temperature observed in four experiments sown under heat stress during the 2009 and 2013 crop seasons in the Yaqui Valley, NW-Mexico.

Dashed line: 2013 trial sown in February (n=574 for 13 genotypes); grey line: 2013 trial sown in March (n=75 for 4 genotypes from the 13); black line: 2009 reference trial sown in February (n=52 for 30 genotypes); dotted line: 2009 reference trial sown in March (n=36 for 24 genotypes)

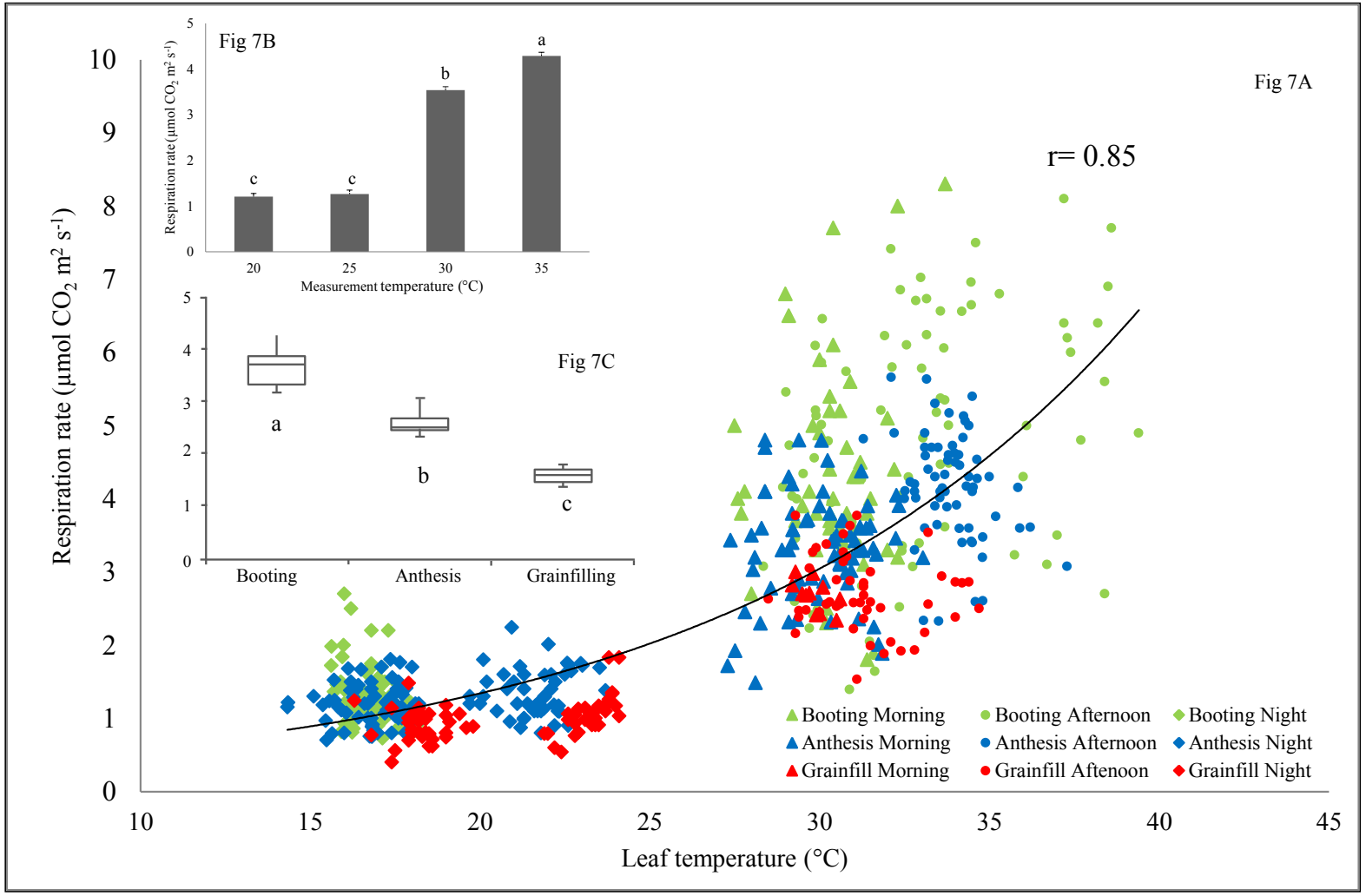

Figure 7. A) Leaf respiration rate recorded in 13 wheat genotypes grown under heat stress during 2013 at six set temperatures between 20-40 °C, three stages (booting, anthesis, and grainfilling), and three data collection times (morning, afternoon, and night); B) main effect of the measurement temperature on leaf respiration rate of 13 wheat genotypes grown under heat stress during 2013; C) main effect of plant developmental stage on leaf respiration rate of 13 wheat genotypes grown under heat stress during 2013.

Discussion

The wheat genetic resources included in this study represent a diverse pool of germplasm selected from CIMMYT's World Wheat Collection grown under heat-stressed environments. High temperatures reduce wheat yield and yield components but also plant performance in general (Al-Khatib and Paulsen 1984; Barnabás et al. 2007). In this study, the mean yield for all wheat trials grown under heat conditions was reduced to almost 50% of the mean reported for the same lines grown under optimum environmental conditions, while TGW and GM2 were reduced by about 25% (data not shown).

Heat adaptive traits identified for germplasm selection

When comparing genotypes under hot irrigated environments, aboveground biomass production is associated with high yield (Reynolds et al. 2007a). Rapid crop establishment results in larger light interception and therefore greater carbon assimilation (López-Castañeda et al. 1995) and has been identified as useful in breeding for both drought and heat environments (Reynolds et al. 2005; Cossani and Reynolds 2012). In this study, NDVI (average of NDVIv and NDVIg) was correlated with yield (Fig. 1), suggesting that genetic gains in heat environments can be achieved selecting for increased biomass across the crop cycle. This hypothesis was confirmed by the high and positive association observed between yield and biomass in a sample of 30 genotypes (Fig. 4).

Superior plant performance under hot environments has also been associated with root development and plant water uptake (Pinto and Reynolds 2015). Assuming no major water limitations, a strong radicular system allows water demand to be met in hot environments where dry air drives high transpiration rates. Given the complexity of measuring root, measurement of CT has been proposed used as a surrogate (Reynolds et al. 2005; Lopes and Reynolds, 2010). In our study, results from the panels of 140 and 70 lines, as well as the final set of 13 genotypes, showed that high yielding genotypes have lower CT than poorly-performing genotypes (Figs. 2 and 5), as has been previously reported (Saint Pierre et al. 2010; Lopes et al. 2012). Efficient partitioning of photosynthetic assimilates to grain has been suggested as a heat adaptive trait (Cossani and Reynolds 2012), and in this study GFR was positively and highly correlated with yield (Fig. 3). Starch synthase, Rubisco, and Calvin cycle enzymes are potential candidates for conferring heat tolerance during grain-

filling since they are normally sensitivity to high temperatures (Keeling et al. 1993; Salvucci and Ogren, 1996).

Main effects of ontology and temperature on leaf respiration rate measured under hot irrigated conditions

Respiration is essential for growth and maintenance of all plant tissue, and plays an important role in carbon and energy metabolism. However, based on empirical estimations, 30-60% of assimilated carbon is lost to respiration (Amthor 1989; Amthor 2000). Selection for a lower respiration rates in the case of ryegrass (*Lolium perenne*) seemed to result in higher yielding lines (Wilson and Jones 1982). The tremendous sensitivity of leaf respiration to high temperatures has been widely reported (Larigauderie and Körner 1995; Atkin et al. 2000; Atkin and Tjoelker 2003; Loveys et al. 2003) and it is estimated that at least 40% of the genetic variability in leaf respiration rates can be explained by mean yearly air temperatures (Catoni et al. 2013). Yet to date, no genetic variation for respiration rate in wheat plants under heat field conditions has been reported in the context of plant breeding.

In this study, genetic variation for R_d was observed among genotypes at specific developmental stages. The differences in R_d in the 13 genotypes studied at various temperatures (Fig. 7A) could be associated with increased activity of respiratory enzymes such as cytochrome oxidase – which exerts up to 50% of the total respiratory control (González-Meler and Siedow 1999) – or by increased carbohydrates due to greater availability of respiratory substrates (Azcón-Bieto and Osmond 1983) and higher energy demand for phloem loading of carbohydrates (Bouma et al. 1995; Amthor 2000). It was notable that the highest respiration rates were observed in the afternoon (12:00-18:00 hr), after a period of up to 13 hours of photosynthetic activity, which may suggest that the assimilate reserves generated during daylight could be triggering an accelerated rate of carbohydrate consumption (Azcón-Bieto and Osmond 1983; Rajendrudu et al. 1987). However, this hypothesis cannot be proved from the current study as no WSC analyses were conducted on the leaves. Instead, this study determined the assimilates stored in the stems, which are an indicator of assimilate remobilization potential. Elevated remobilization of stem reserves under stress conditions favours high yields (Rawson and Evans 1971; Bidinger et al. 1977; Fokar et al. 1998; van Herwaarden et al. 1998), which agrees with our results showing a positive association between stem WSC and yield

(r=0.24). According to the literature, low sink demands also induce assimilate accumulation, which can lead to increased respiration to avoid end-product photosynthesis inhibition (Moser et al. 1982; Azcón-Bieto 1983; Rajendrudu et al. 1987; Foyer and Paul 2001). Results from this study showed a negative association between grain number and R_d at 35 °C (mean across stages, r = -0.39), which partially confirms this hypothesis.

Our results showed that a larger genetic variability for leaf respiration is observed in young tissue (Fig. 7C). Reduced R_d in mature leaves is associated with reductions in maintenance respiration in older tissues (Villar et al. 1995; Van Iersel 2003). Notwithstanding, few studies have harnessed this genetic variability in respiratory rates for crop improvement. This study showed that R_d correlate negatively with yield when measured close to the growing temperature (i.e. 30 °C and 35 °C) during the anthesis and grainfilling stages (Fig. 8). In crop improvement, two contrasting hypotheses can be derived from studies of respiration rate in different species: a) high respiratory rates favour high yields given the positive association between carbohydrate levels and respiration rate (Azcón-Bieto and Osmond 1983), which would indicate that cultivars with high respiration rates also show high photosynthetic rates (Villar et al. 1995), or b) low respiratory rates are linked to higher yields due to more efficient energy use, which allows the best performing genotypes to switch from the traditional cytochrome pathway to the alternative oxidase pathway when exposed to stress conditions such as high temperatures (Wilson and Jones 1982; Van Aken et al. 2009) or due to reduced maintenance respiration (McCullough and Hunt 1993). Our results showed that respiration rates during the early stages (booting) was moderately and positively correlated with yield and yield components, but only when measured at 20 °C and 25 °C. This confirms the complexity of the respiration rate trait, which seems to be highly responsive to external factors such as temperature and stress incidence, but also to intrinsic tissue characteristics like age or composition (Gifford 2003; Flexas et al. 2005; Atkin et al. 2007; Atkin and Macherel 2009; Mohammed et al. 2009).

Respiration rate and performance traits in different wheat classes grown under hot irrigated conditions

The comparison between classes of wheat cultivars showed that the landrace-derived and synthetic-derived lines exhibited the best performance under heat conditions in terms of yield, Bmm, and NDVI (Table 4). Synthetic-derived lines

also reported the lowest respiration rates across wheat classes (Fig. 9). Synthetic- and landrace-derived lines have been used to increase genetic diversity in wheat breeding for drought tolerance (Reynolds et al. 2007b); the synthetics wheat class is characterized by deep root development, which assists plant survival under water limited conditions (Reynolds et al. 2007b; Lopes and Reynolds 2010) and several studies have indicated stress adaptive traits of generic value under both drought and heat environments (Cossani and Reynolds 2012; Pinto and Reynolds 2015). Synthetics also are characterized by their heat tolerance up to 40 °C during the grainfilling phase (Van Ginkel and Ogbonnaya 2007; Cossani and Reynolds, 2015), which concurs with our result that synthetic-derived lines had one of the highest GFR (Table 4). Exotic genetic resources, such as landraces and synthetic-derived lines are a great source of diverse genes and have been proven to possess improved performance under stress conditions (Reynolds et al. 2007a,b). Mexican landraces – such as those included in this study – have been characterized by their ability to tolerate abiotic stresses, resulting not only in plant survival but in high yields (Reynolds et al. 2007a,b). In contrast, the relatively poor performance of elite bread and durum wheats may result from the narrowed genetic base resulting from years of wheat breeding (Warburton et al. 2006). The good performance of the synthetic-derived lines was supported by lower leaf respiration rates compared with other wheat classes (Fig. 9), while the durum wheats reported the highest respiration rates and low yields, suggesting a negative association between these two characteristics. High yielding wheat classes also showed up to 13% and 16% higher GFR and NDVIv, respectively.

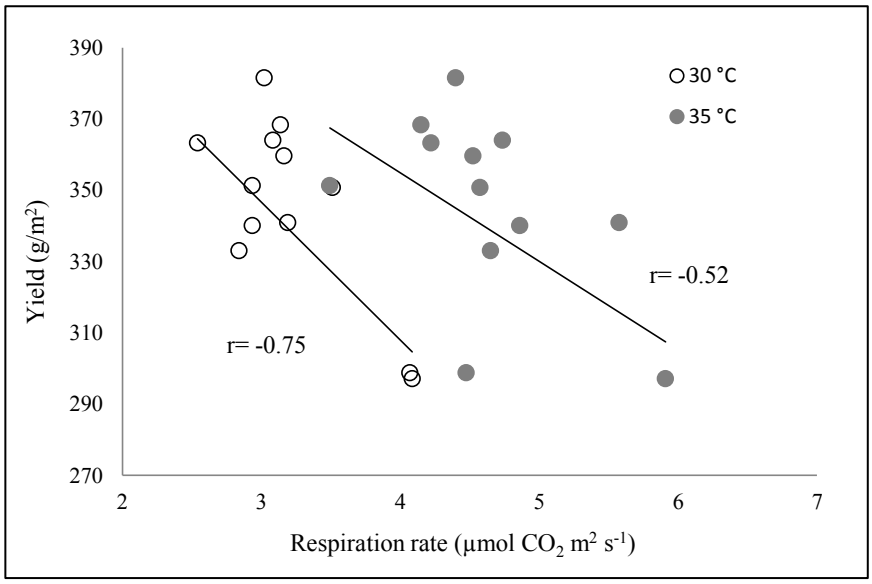

Figure 8. Association of yield with leaf respiration rate measured at 30 °C and 35 °C during anthesis and grainfilling stages in 13 spring wheat lines grown under heat stress during 2013 in the Yaqui Valley, Mexico.

Filled circle: average respiration rate during anthesis and grainfilling stages measured at 35 °C; empty circle: average respiration rate during anthesis and grainfilling stages measured at 30 °C. Only 12 genotypes were used to calculate Pearson's correlations, genotype Atil C2000 was excluded given that – as the susceptible check – it was observed as outlier under all the evaluated environments, disturbing associations with marginal yields under different heat environments and even under high yielding conditions (data not shown).

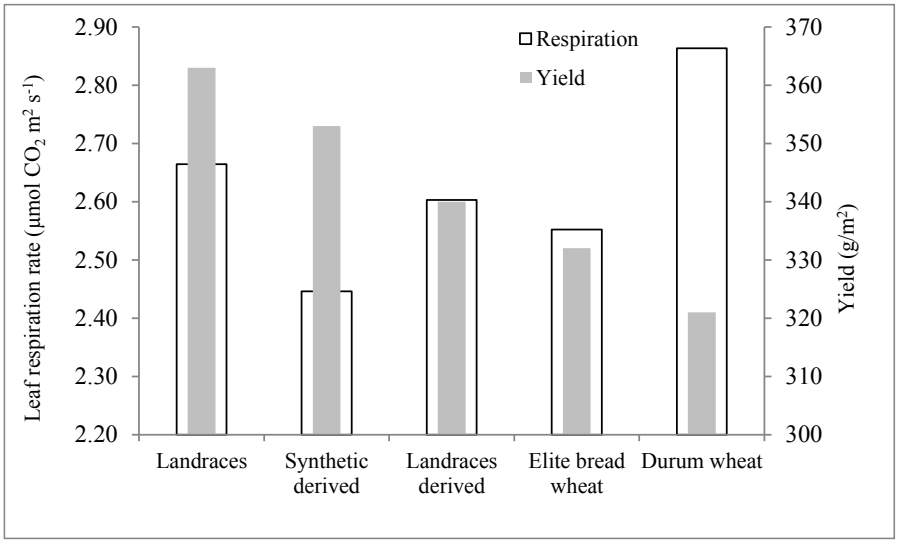

Figure 9. Leaf respiration rate and yield of different classes of wheat genetic resources represented by the final selection of 13 wheat genotypes. (r = -0.524, *p* = 0.365).

Conclusions

Genetic variability for agronomic and physiological traits was detected in the set of genetic resources evaluated in this study across four years, including leaf respiration rates measured in the last experiment. The wheat classes synthetic-derived and landrace-derived lines exhibited the best expression for yield, TGW, GM2 and other heat adaptive physiological traits like biomass production and canopy temperatures. The main effects detected for leaf respiration rate across plant stages and measurement temperatures were in agreement with previous studies; however more studies are required to elucidate the mechanisms controlling these traits under diverse conditions and in genetically diverse cultivars to understand which specific processes were directly affected by temperature and to understand why exotic material had a better respiration rate/yield balance. Results from this study suggest that the best time to capture genetic diversity for leaf respiration rate is at early plant growth stages and at 30-35 °C where the trait was also associated with yield.

Author contribution statement

R. Suzuky Pinto conducted field experiments, performed data analysis, and led the write-up. Gemma Molero contributed in field data collection and interpretation, as well as providing valuable advice in writing the manuscript. Matthew P. Reynolds designed the experiments and participated in all aspects of data analysis, interpretation, and writing.

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Conflict of interest statement

The authors declare that they have no conflict of interest

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Supplementary material

Supplementary Table 1. ANOVA for main effects and interactions of temperature (°T), ontology (stage) and genotype in balanced data 13 spring wheat genotypes grown under heat stress during 2013 in the Yaqui Valley, Mexico

Factor	DF	Pr > F
Genotype	12	<.0001
T°	3	<.0001
Stage	2	<.0001
Genotype × T°	36	<.0001
Genotype × Stage	22	<.0001
T°× Stage	5	<.0001
Genotype ×T°×Stage	48	<.0001

T°: temperature; heading time covariate resulted not significant

Chapter 5. Thesis conclusions

This study provides a background about the current state of knowledge about the mechanisms associated with plant tolerance to high temperatures. Key physiological parameters for wheat adaptation to heat stress were investigated, providing new evidence of their significance and utility in the context of crop improvement. The following findings were achieved during the current PhD project:

- Under water limited conditions and hot-irrigated environments, this study showed that optimal root development was a valuable trait in wheat genotypes adapted to heat and drought conditions as indicated by correlations with yield. Under hot-irrigated environments roots were shown to be concentrated near the surface while under drought roots proliferated at depth.
- QTL related to cooler canopy temperatures were associated with deep roots under drought or near to surface under hot-irrigated conditions in the Seri/Babax population, as well as to better agronomic performance under both heat-irrigated and drought stress.
- Delayed plant greenness could successfully be estimated through high throughput phenotyping of the development of canopy greenness by means of NDVI curves. Additionally, this method offered the possibility of developing associated staygreen parameters (NDVI-based indices) to summarize the progressive decline in NDVI on a genotype basis during the grain-filling phase.
- The staygreen attribute was shown to be positively correlated with yield, grain-filling rates and grain-filling duration in hot-irrigated environments.
- Genetic variability was identified for the NDVI decline-curve type during grain-filling. Linear (curve type 1) and non-linear patterns (curve types 2 and 3) were correlated with contrasting agronomic and physiological plant performance under hot-irrigated environments.
- QTL related to staygreen in the Seri/Babax bread wheat population were co-located with QTL for yield which strongly indicated that the staygreen phenotype is a useful trait for productivity enhancement in hot-irrigated environments.
- Leaf respiration rate (R_d) during anthesis and grain-filling in wheat grown in hot-irrigated environments was negatively associated with yield; and genetic variability was shown for the trait, which was more clearly

detected at 30-35 °C under hot-irrigated conditions than at 20 or 25 °C. Notwithstanding, further studies are required to clarify the underlying mechanisms affecting leaf respiration rates given the high sensitivity of R_d to environmental factors and intrinsic tissue characteristics.

– Genetic differences between wheat classes (synthetic-derived lines, landraces, landrace-derived lines, bread wheats, and durum wheats) were shown for leaf respiration rate, and this variation was associated with agronomic and physiological parameters.

This study provides a valuable basis/platform for the planning and development of further studies to deepen the knowledge of mechanisms of wheat adaptation to heat stress. Especially the respiration study establishes a starting point for the development of protocols (selection of germplasm, and determination of best stages, and time of day), on which larger genetic variability can be detected as well as providing a reference about the yield of the method utilized. Results from this study are the first showing a common basis for root development in heat stressed and in drought stressed environments (Chapter 2); and also the first modelling of different wheat patterns for loss of greenness during grain-filling (Chapter 3), suggesting additional studies to better understand the factors affecting each of these adaptive traits. The study provides evidence of the association between staygreen, leaf respiration rate and plant performance under high temperature environments; and finally another key point is that the study highlights the gaps in knowledge for these topics and identifies the need for more strategic research. Possibilities for future work include: the study of root distribution in other wheat cultivars to confirm the findings detected in the Seri/Babax population; QTL mapping of root characteristics and canopy temperature related traits in the whole Seri/Babax population to compare the genetic basis of root development under both drought and heat stress. The investigation of wheat staygreen patterns provided interesting avenues in the understanding of physiological basis of wheat adaptation to heat stress and future work can use the calculated staygreen parameters as selection criteria in wheat improvement. Finally, it is suggested that future studies on wheat respiration rate will help to respond questions raised from the current project such as: is the temperature or the substrate availability the major factor controlling leaf respiration rate? This question can be addressed with leaf respiration studies performed at different temperatures but same time of

the day after a fix period of photosynthetic activity (to accumulate assimilates). Under heat stress, do stored stem carbohydrates play a key role in leaf respiration, similar to carbohydrates accumulated in the leaf? Which of the two components of respiration R_g or R_m is better correlated with crop productivity?. What is the real contribution of R_d to total carbon balance under heat stressed conditions?. Deeper exploration of these topics is expected to increase the scientific knowledge on wheat adaptation to high temperatures and contribute to the clarification of currently controversial subjects.

In summary, this thesis showed that improved tolerance of wheat to high temperatures can be addressed through the application of the physiological approaches, but an optimal strategy will include a combination of multiple strategies. Here were explored a number of key traits from many additional options suggested in the conceptual model of heat adaptive traits Cossani and Reynolds (2012). Cool wheat canopy temperatures, delayed loss of plant greenness and reduced leaf respiration rates were confirmed as valuable traits associated with enhanced heat tolerance. Depending of the specific characteristics of the heat stress, other approaches that were not explored here, could also be useful. These include optimal expression of traits associated with water use efficiency, light interception, production of photo-protective pigments and antioxidants, optimal partitioning of assimilates and, improved radiation use efficiency. The evidence collected in the current thesis support the idea that a strategic physiological breeding approach of wheat can lead to important gains in wheat yield under hot-irrigated conditions and will be important in the adaptation of wheat to the predicted impact of climate change.

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