FACULTY OF SCIENCE AND TECHNOLOGY
LANCASTER ENVIRONMENT CENTRE

Assessment of plant hormone quantification as a new physiological trait for crop breeding using a new classification method for crop performance under drought.

Arnauld Thiry

B.S. Agricultural Engineer, Gembloux Agro-Bio Tech – University of Liège (GxABT - ULG), Belgium

MSc. Bio-Engineering – Agricultural Science, GxABT - ULG, Belgium

Thesis submitted for the degree of Ph.D. at Lancaster University

August 2017

Declaration

I declare that the contents in this thesis are my original research work and it has not previously been submitted, in part or in full, for the award of a higher degree at any other institution. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement.

Additionally, it is needed to comment that part of Chapter 2 has been published in September 2016 at the Journal of Experimental Botany.

Arnauld A. A. Thiry

Lancaster University

August 2017

To my son ”Alexandre”

For you I have intended to make it better.

Acknowledgment

When somebody changes the course of your life and makes it without personal interest something of great value is pushing your life and your projects ahead.

For giving me the possibility to intend to develop new resources for the human being I'm grateful to the person who did stimulate this idea.

Dra Perla Chavez Dulanto is this person and was the necessary link to introduce me to two of the most famous scientists I have been in touch with.

First Dr Matthew Reynolds (CIMMYT – Mexico) who left me responsibilities in his department of physiology

A few months later, Prof. Bill Davies (Lancaster university - UK) who accepted to be the base of this beautiful and revolutionary concept. He gave me all his faith and did stimulate all my energy correcting me the best he could.

I have tried to respect this feeling of honesty, humility and I have done my best to advance in this project.

No doubt that after all these years as said the famous philosopher Socrates reported by Plato: "*I know one thing; that I know nothing*".

Thank you, Perla, Matthew, Bill and of course friends, parents and all the ones who helped me in the achievement of this exciting work.

You are in my heart for ever and in my future projects.

Project like this one would not have been possible without the financial support of the International Wheat Yield Partnership (IWYP), CGIAR Research Program on Wheat and SAGARPA trough MasAgro-Trigo. Thank you.

Abstract

The need to accelerate the selection of crop genotypes that are both resistant to and productive under abiotic stress is enhanced by global warming and the increase in demand for food by a growing world population. In this thesis, a new method is proposed for evaluation of wheat genotypes in terms of their resilience to stress and their production capacity. The method quantifies the components of the new index related with yield and yield components under abiotic stress. The index method, based on a scoring scale, offers a simple and easy visualization and identification of resilient, productive genotypes, according to their yield and yield components. This new selection method could help breeders and researchers by defining clear and strong criteria to identify genotypes with high resilience and high productivity and to reveal where genotypes express their susceptibility to a stress environment, providing a quantitative classification of contrasts in terms of yield and yield components.

This index method has allowed 1) the identification of contrasting genotypes from a small population (CIMCOG-ROOT, 10 genotypes), and 2) the quantification of their contrasts, in terms of yield and yield components (grain number per spike), both constituting a key opportunity to test whether a stress hormone and/or hormone balance (ABA, ethylene and/or ABA/ethylene) could be used as a physiological trait for breeding for abiotic stress resilience. Due to the complexity of spatial and temporal variation of hormone accumulation (ABA and ethylene) and their different effects in plant development in response to stress environments, it is necessary to investigate how factors other than environment can influence the production of hormones. The factors considered in this work were: day time, water management (irrigation), tissue specificity (leaf and spike) and wheat phenological development (phenological stages).

The present study of hormone (ABA and ethylene) quantification in wheat has shown that the genotypic variation in hormone signalling cannot be identified at every developmental stage of the plant. In fact, only two stages were identified for differences in ethylene emission (late-booting and heading), and two for ABA accumulation (late-booting and half-emergence), both on leaf tissue. However, the ratio ABA/ethylene (ABA/ETH) emerges as a better method to study genotypic variation in response to stress environments, in terms of hormone accumulation. In fact, both tissues, leaf and spike at all stages during the pre-anthesis stage (from booting to heading), have shown significant genotypic variation in terms of ABA/ETH balance.

The resilience index of grain number per spike and this study of hormone (ABA and ethylene) quantification under drought stress (on leaf and spike), have shown that at late-booting stage, a lower leaf hormone ratio ABA/ETH and higher leaf ethylene emission is associated with a higher resilience of spike fertility (grain per spike resilience). However, under controlled conditions, a possible optimum threshold is observed in terms of leaf hormone ratio ABA/ETH. These results have been obtained with statistical significance in eight genotypes under field conditions and four genotypes under controlled environments.

It is suggested in this work that a possible method for early selection of genotypes for high spike fertility resilience under drought stress could be developed by quantifying hormone signalling (leaf ethylene and the leaf ratio ABA/ETH at late-booting stage). However, some improvements in the process of hormone quantification need to be made before recommending this method to breeders.

Table of Contents

List of Figures

Chapter 2

Chapter 4

Chapter 5

Appendix 2

Appendix 3

Appendix 4

Appendix 5

Appendix 6

List of Tables

Chapter 2

Chapter 6

Appendix 2

Abbreviation list

[3^{H}]-ABA	Isotope 3H-labelled ABA
[ABA]	Abscisic acid concentration
[ETH]	Ethylene concentration
ABA	Abscisic acid
ABA-GE	ABA-glucose ester
ACC	1-aminocycopopane-1-carboxylic acid
ACO	ACC-oxidase
ACS	ACC-synthase
At	Anthesis
AtBG1	Arabidopsis thaliana β-glucosidase 1
BGF	Beginning of grain filling
Boot	Booting
CE	Controlled environments
CIMCOG	CIMMYT core germplasm
CK	Cytokinin
CT	Canopy temperature
D	Drought stress
DAE	Day after emergence
DR	Double ridge
Em	Emergence
ETH	Ethylene
FI	Floret initiation
Fw	Fresh weight
FY	Farm yield
G x E	Genotype–environment interaction
G#/m^2	Grain number per square meter
G#/spk	Grain number per spike
G#/spklt	Grain number per spikelet
GN	Grain number
GS1	Growth stage 1
GS2	Growth stage 2
GS3	Growth stage 3
GW	Grain weight
H	Heat
Hdg	Heading
IPM	Integrated pest management
nl	Nanoliters
PBS	Phosphate-buffered saline
PC	Production capacity

PCI	Production capacity index
PGR	Plant growth regulators
PSY	Phytoene synthase
PY	Potential yield
RC	Resilience capacity
RCB	Randomized complete block
RCI	Resilience capacity index
SD	Semi-drought
SF	Spike fertility
SI	Stress intensity
Spk#/m^2	Spike number per square meter
Spklt#/spk	Spikelet number per spike
SSI	Stress susceptibility index
STI	Stress tolerant index
TGW	Thousand grain weight
T-PCI	Trait production capacity index
T-RCI	Trait resilience capacity index
TS	Terminal spikelet
T-SSI	Trait score stress index
WAMI	Wheat Association Mapping Initiative
WS	Water stress
WW	Well-watered
Yp	Yield potential
YPSI	Yield potential score index
Ys	Yield under stress conditions
YSSI	Yield stress score index

Chapter 1. General Introduction

1.1. Climate change

The Fifth Assessment report from the Intergovernmental Panel on Climate Change (IPCC) has projected that by 2100 the increase in global mean temperature will be between 1.8 and 4.0 °C, depending on the level of the greenhouse gas emission. If, for example, the atmospheric CO_2 concentrations stabilise around 450 ppm then the global mean temperature is predicted to rise by around 2-3 °C (Pachauri *et al.*, 2015). In the worst case, if the global economy and human population continue growing at their current rates the increase would be bigger (maybe up to 6.4°C) (Pachauri *et al.*, 2015).

Based on different models and adopting a probabilistic approach, global climate changes, are expected to cause significant restrictions on production of the three main cereal crops (rice, maize and wheat), associated with increases in temperature and drought stress (Lobell and Field, 2007; Tebaldi and Lobell, 2008). Plant growth and development are both adversely affected by increased temperatures and soil water deficiency (Barnabás *et al.*, 2008).

1.2. Food supply and demand

Bread wheat (*Triticum aestivum* L.) is one of the world's most important staple crops, with more than 218 million hectares of land harvested annually and more than 700 tonnes produced in 2013 (FAO, 2015). Today, the global food security brought by wheat in terms of food supply for a growing population is threatened by a changing climate, what is one of the most important challenges for the society in the 21st century (Lal *et al.*, 2005). In order to satisfy the global food demand, it has been estimated that if the population continues to show current food consumption preferences and without any substantial reduction in food waste, global cereal production should increase by a 50% by 2050 (Rosegrant and Cline, 2003; Porter *et al.*, 2014).

In the mid-1960s, the introduction of new semi-dwarf wheat varieties resulted in a drastic yield increase of this crop. This sudden yield increase is now widely known as the "green revolution" which averted the development of widespread hunger and starvation in Asia. Nevertheless, during the last decade, the progress (gain) in wheat yield potential has been limited (Fischer and Edmeades, 2010). Since the green revolution, the genetic gain in yield potential (Yp) has been around an average of 1% per year with the introduction of modern wheat varieties (Reynolds *et al.*, 1996; Sayre *et al.*, 1997; Abbate *et al.*, 1998; Araus *et al.*, 2004), which is actually lower than the predicted annual increase in global food demand (Reynolds *et al.*, 2009). A more recent analysis showed that genetic gains in Yp, during the last 30 years, may have fallen to 0.3% and 0.6% per year, in the UK and Mexico (CIMMYT) respectively (Fischer and Edmeades, 2010).

Rising temperatures and water scarcity due to global climate change, have a negative impact on wheat yield and it is expected to be worse in the near future (Tubiello *et al.*, 2000; Ortiz *et al.*, 2008; Macková *et al.*, 2013; US EPA, 2015), as it is projected that the risk of experiencing high temperatures and decreases of rainfall at critical crop developmental stages is increasing (Tebaldi and Lobell, 2008; Battisti and Naylor, 2009; Tardieu, 2012; Porter *et al.*, 2014). Drought stress is considered as the most important environmental stress in agriculture worldwide, and therefore, improving crop yields under drought stress is a major goal for plant breeders (Cattivelli *et al.*, 2008). Wardlaw *et al.*, (1989) have predicted that wheat production may decline by 3 to 4% for each increase of 1ºC in temperature, which would correspond to 4-5 million tons, and recently, Asseng *et al.* (2015) estimated, using a crop model tool, that wheat production could fall by 6% for each 1ºC increase in global mean temperature. These prognoses turn more critical considering that researchers have estimated that by 2050 the global mean temperature will increase from 1,4 to 3 ºC (Rowlands *et al.*, 2012).

1.3. Drought stress

Drought is a normal, recurring characteristic of climate; it occurs in virtually all climatic zones including high and low rainfall areas (Wilhite and Glantz, 1985; Wilhite, 2010). Wilhite (2010) has pointed out that there is a difference between drought and aridity. Aridity is a permanent characteristic of climate and is restricted to low rainfall areas while drought is considered as a temporary aberration of climate and is mostly related to the reduction in the amount of precipitation received over an extended period of time, such as a season or a year (Mishra and Singh, 2010; Wilhite, 2010). However, other factors play a significant role in the occurrence of droughts such as low relative humidity, high temperatures, strong winds and the distribution, intensity and duration of rain throughout the cropping season (Mishra and Singh, 2010).

A unique definition of drought does not exist and it is generally classified into four categories depending on the perspective of the different disciplines: meteorological, agricultural, hydrological and socio-economic (Wilhite and Glantz, 1985). Agricultural drought is usually linked to the reduction of soil moisture caused by a decrease of the frequency and intensity of rain and abnormally high evapotranspiration which result in reducing plant growth and production (Wilhite and Glantz, 1985; Mishra and Singh, 2010).

Soil moisture content is habitually used to characterise agricultural drought (Wilhite, 2010). However, not all the soil water content (% of w/w or v/v) is available for plants and soil moisture content hardly describes the extent of the drought stress challenge (i.e. drought severity). Therefore, to evaluate this degree, other indicators are used such as temperature and precipitation (intensity and duration) (Brouwer *et al.*, 1985; Mishra and Singh, 2010; Wilhite, 2010). The soil water availability is a constant variable for a given soil but it varies widely between different soil types (texture and structure) (Brouwer *et al.*, 1985). The plant water

availability is calculated by the difference between the field capacity (when the soil after saturation of water has drained away below the root zone) and the permanent wilting point which is the minimal point of soil moisture the plant requires not to wilt (Brouwer *et al.*, 1985).

In the literature, different terms can be found to describe a drought stress, no stress (well-watered plant), mild or moderate stress and severe stress. However, there is no specific scale which identifies the range of these classes of drought stress. The degree of plant water deficit generated by a drought is associated with its water demand which fundamentally depends on weather conditions, the plant's biological characteristics, the specific growth stage, and the physical and biological properties of the soil (Wilhite and Glantz, 1985; Mishra and Singh, 2010). A proper definition of agricultural drought should take into account the plant's water status and the variable susceptibility of crops during different phenological stages, from emergence to maturity (Wilhite and Glantz, 1985).

In the soil-plant system, the water uptake from soil by the roots is mainly driven by the water potential gradient (soil to plant (root)) and the water always moves from high water potential to a low water potential (Kramer and Boyer, 1985; Kirkham, 2005). Water potential is a negative scale, the highest value is zero (pure water) and for the permanent wilting point, the soil water potential is around -1.5 MPa for most plants (Kramer and Boyer, 1985; Kirkham, 2005).

Plant water stress is generally quantified by leaf water potential (Siddique *et al.*, 2000) and is usually measured using a Scholander pressure chamber (Scholander *et al.*, 1965). However, this method is time consuming and requires numerous repetitions to characterise the field (Jackson, 1992). Sinclair and Ludlow (1985) suggested that leaf relative water content (RWC, [(Fresh weight – Dry weight) / (Turgid weight – Dry weight)] × 100%) is a better indicator of the plant

water status than the plant water potential. However, the estimation of RWC shows some sources of error: (i) changes in dry weight (mainly due to respiratory losses), (ii) increases in water content in excess of full turgidity, and (iii) water accumulation in intercellular spaces (Barrs and Weatherley, 1962; Pask *et al.*, 2012). Also, plant growth, development and functioning is influenced by plant water potential which can vary substantially while water content does not always change so significantly. Ehrler *et al.* (1978) stated that the canopy temperature, in wheat, is often a good indicator of the plant water potential (temperature difference between plant canopy and air).

1.4. How does drought stress affect yield during the pre-anthesis period?

The potential grain number on a wheat plant is determined during the early stages of plant development, a critical period which is highly sensitive to abiotic stress (e.g. heat and/or drought). Drought, in a wheat crop, during the development of the young microspore stage of the pollen (Dolferus *et al.*, 2011) (which starts at booting stage) is a particular problem (Acevedo *et al.*, 2002). During the pre-anthesis period, it has been reported that an increase in the setting of grains (i.e., grain number) can be obtained by extending the duration of stem elongation and the spike growth periods, a change that improves the floret survival (Miralles *et al.*, 2000; González *et al.*, 2011). It has been suggested that a better understanding of the physiological and molecular processes leading to pollen abortion induced by the stress may be key elements to allow a good setting of grains and development of a high grain number under stress conditions (Dolferus *et al.*, 2011).

Drought stress is known to adversely affect pollen development in wheat and rice (Dorion *et al.*, 1996; Sheoran and Saini, 1996; Ji *et al.*, 2010), and heat stress in cereals affects both pollen dehiscence and grain-filling (Wardlaw and Wrigley, 1994; Jagadish *et al.*, 2007). Ji *et al.*,

(2010) suggested that pollen sterility may be the main cause of grain loss in wheat during drought conditions, especially when the abiotic stress coincides with meiosis. Therefore, the identification of contrasting germplasm, tolerant and susceptible, for reproductive stage stress responses should enable us to identify the molecular basis of the resilience mechanism (Dolferus *et al.*, 2011).

1.5. Selection of drought-adapted genotypes

Addressing the negative impact of climate change on crop yield and also the increasing food demand, the main target of wheat yield breeding and pre-breeding programmes is to improve grain yield by identifying and creating new varieties. However, it is important to keep in mind that breeding and genetic gains in the last decade, have been responsible for only a 30% to 50 % of the yield improvements achieved, while the other 50% has come from improvement in field management and agronomic practices (Araus *et al.*, 2004; Richards, 2006).

Yield breeding and pre-breeding programmes for abiotic stress adaptation mostly focus on improving yield potential (yield without any biotic or abiotic stresses) and this method has been a major contributor to improved crop yields (Slafer and Araus, 2007; Reynolds *et al.*, 2009; Fischer and Edmeades, 2010). The reason for this is that high yielding genotypes under yield potential conditions usually also perform better under a wide range of environments including drought (but importantly, not under severe drought, high temperatures, and salinity) (Ceccarelli and Grando, 1991; Slafer and Araus, 2007; Reynolds *et al.*, 2009; Fischer and Edmeades, 2010). Additionally, it has been recognised that the genetic gain in yield improvement seems to be higher in more favourable environments (Blum, 1996) and the selection under yield potential conditions is also considered more effective due to relative ease of repeatability of procedures under that environment compared to a selection under a stress environment (Richards, 1996).

However, Ceccarelli and Grando (1991) suggested that it may be better to select genotypes under the target environments, in order to increase the selection efficiency and therefore to more rapidly discover new genotypes adapted to severe stress. Additionally, Blum (1996) explained that often a negative relationship is expressed between high yielding genotypes under non-stress conditions (yield potential) and their drought resistance when the stress is sufficiently severe but some rare exceptions to this generality do exist. At the present time, there is no really effective means of screening for these exceptional genotypes which show combined high yield and tolerance to stress environments.

Slafer and Araus (2007) point out that the identification of physiological traits indicating simultaneously high yield potential and tolerance to stress would be critical to yield improvement. Up to now, significant progress in the understanding of the physiology of the wheat plant shows a high potential to boost wheat productivity. Three main targets which may help to increase yield potential have been highlighted: 1) an increased efficiency of carbon gain, 2) the increasing spike fertility and the partitioning of assimilates to the growing spike and grains, and 3) an improvement of lodging resistance of these genotypes with bigger spikes (Reynolds *et al.*, 2009; Parry *et al.*, 2011; Foulkes *et al.*, 2011).

At the present time, the conceptual model of a breeding programme for drought adaptation usually considers yield under drought to be a function of: (1) yield potential; (2) flowering date (which indicates whether the crop will avoid drought stress); and (3) secondary traits that provide drought resistance (Monneveux *et al.*, 2012). Physiological secondary traits can also be used for the selection of parents to be included in the crossing block (Monneveux *et al.*, 2012).

However, in work of this kind, the drought resistant ideotype is not always well defined and traits which might deliver high productivity under drought are not always clear (Blum, 2005). In fact, the concepts of drought tolerance as set out in the literature can differ significantly. The ecological definition of drought resistance is the ability to stay alive during periods of water scarcity (Turner, 1979). Alternatively, for crop species, drought tolerance is defined as the ability of plants to grow and reproduce satisfactorily while producing harvestable yield with limited water supply or under periodic water deficit (Turner, 1979; Fleury *et al.*, 2010). It has also been suggested that rather than grain yield under stress, yield stability is a better indicator of genotypic drought-resistance (Blum *et al.*, 1989). In terms of physiological mechanisms, the drought resistance is often considered as a compromise between 'dehydration avoidance' and 'dehydration tolerance' both of which can have variable impacts on yield (Fischer and Maurer, 1978; Turner, 1979, 1986; Levitt, 1980). Therefore, to identify new key mechanisms involved in delivering a combination of high crop productivity and stress resilience, there is a need to define and properly characterise what it is meant by the term 'stress tolerant genotype'.

Reliable screening methods (e.g. field phenotyping) are a major bottleneck for improvement of stress tolerance during the reproductive stage (Dolferus *et al.*, 2011; Araus and Cairns, 2014) and despite extensive research by physiologists to identify drought-adaptive traits such as large seed size, tiller survival, leaf waxiness and/or leaf rolling, these physiological adaptive traits have shown little impact in traditional breeding programs as only few breeders incorporate them into their selection programmes (Richards, 1996; Monneveux *et al.*, 2012). There are several reasons for this lack of success. Some of the main reasons are that breeders are not convinced of the proposed criteria and the evaluation of the traits proposed by plant physiologists is usually time-consuming or expensive and therefore could not be applied to trials of hundreds of genotypes (Richards, 1996; Monneveux *et al.*, 2012). According to Richards (2006) and

Monneveux *et al.* (2012) an ideal secondary physiological trait should be: (1) genetically associated with grain yield under drought; (2) genetically variable; (3) highly heritable; (4) easy, inexpensive and quick to be measured or observed; (5) non-destructive; (6) stable over the measurement period; and (7) not associated with yield penalties under favourable conditions. This last condition may be a problem as many authors now report opposing effects of traits depending upon the severity of stress (see e.g. Tardieu, 2012). Traits showing a positive impact on yield under severe stress often show a negative effect under milder stress or *vice versa* (Tardieu, 2012). Therefore, it is commonly proposed that selected traits should be associated with the likely drought scenario for the crop or the target environment.

Recent advances in data processing has increased the applications in remote sensing technologies, under field and control environment, that allows the development of rapid and non-destructive method for high-throughput phenotyping (Leinonen and Jones, 2004; Jones *et al.*, 2007; White *et al.*, 2012; Araus and Cairns, 2014; Tattaris *et al.*, 2016). For example, canopy temperature which is an interesting secondary physiological trait for selection as the measurement is quick, simple, inexpensive (Cossani *et al.*, 2012). Canopy temperature can be measured remotely by the use of hand-held infrared thermometer (IRT) without interfering with the crop (Pask *et al.*, 2012). However, there is still an issue in the sensitivity of the measurement to the environment. Largely windless and cloud-free days are required to obtain reliable data (Cossani *et al.*, 2012).

More recently, the estimation of canopy temperature through thermal images (taken by unnamed aerial vehicle (UAV)) was identified to be better associated to performance traits such as yield than proximal data collected with an IRT (Reynolds *et al.*, 2015; Tattaris *et al.*, 2016). Remote sensing phenotyping methods allow us to screen a wide range of plant genetic resources

at the same time (Araus and Cairns, 2014; Reynolds *et al.*, 2015; Tattaris *et al.*, 2016). Additionally, remote sensing technologies offer more opportunities for detecting different stresses affecting crops even before the appearance of visual symptoms (Chavez *et al.*, 2009; Chávez *et al.*, 2010).

Yield is a complex trait as it is formed continuously from sowing to harvest and almost all a plant genes are involved in a direct or indirect way to produce the grain yield (Slafer, 2003). Therefore, the final yield of a crop will be the result of a balance of complex processes leading to growth and development. Most of these will be highly influenced by an interaction with different environmental factors (GxE) (Slafer, 2005). Instead of using yield as a trait itself, to study the GxE interaction, Slafer (2003) recommends a focus on more simple traits which compose yield. The most popular approach, used by crop scientists, agronomists and breeders, is to divide yield into yield components, where the two major components are the weight of an individual grain (sometimes expressed as thousand grain weight (TGW)) and the number of grains per square meter (G#/m^2) (Slafer, 2003, 2005).

In wheat, grain yield improvement has been highly associated with the increased grain number per unit area (Sayre *et al.*, 1997; Peltonen-Sainio *et al.*, 2007). Wheat yield of modern cultivars under yield potential conditions has shown evidence of sink limitation rather than source limitation during the grain filling period (e.g. Borrás *et al.* (2004)). Therefore, to improve yield, it is firstly necessary to improve the number of grains per unit area and therefore to improve the spike fertility (SF) which is defined as the number of grains per spike chaff dry weight (see e.g. Abbate *et al.* (2012)). In this field of research, consideration of variation in the production and/or interaction of plant growth regulators have been considered as a possible way to explain the relation of spike fertility with environment and its genetic basis (Zeng and King, 1986; Yang

et al., 2006*a*; Hays *et al.*, 2007; Wilkinson *et al.*, 2012; Davies *et al.*, 2013). For example, in rice a gene coding for cytokinin oxidase has been identified to increase panicle fertility. In fact, the cytokinin oxidase leads to an accumulation of cytokinin in inflorescence meristems and increases the number of reproductive organs (Ashikari et al., 2005).

1.6. Plant growth regulators

In the recent past, plant hormones have received a great attention from physiologists and developmental biologists owing to their multifunctional roles as effectors of plant functioning, growth and development. In addition, hormones can play a key role in mediating the plants' responses to abiotic stress (Davies, 2004*a*; Santner and Estelle, 2009; Colebrook *et al.*, 2014; Kumar *et al.*, 2016). By regulating the production, distribution or signal transduction of hormones, plants are able to regulate and coordinate both growth and/or stress tolerance to promote stress survival or an escape strategy (Colebrook *et al.*, 2014). There are currently five major classes of plant hormones: auxins, gibberellins (GAs), cytokinins, abscisic acid (ABA) and ethylene (Davies, 2004*b*; Hopkins and Hüner, 2008), each of which is characterized as being effective at low concentrations to regulate many aspects of plant growth and development (Davies, 2004*b*; Hopkins and Hüner, 2008).

Under drought stress, ABA and ethylene are generally considered to be the two major hormones linked to the plant responses to water stress (Wright, 1980; Bradford and Hsiao, 1982; Wilkinson and Davies, 2002, 2010; Davies, 2004*b*; Salazar *et al.*, 2015). Initially, these plant growth regulators have been studied individually to understand their action on plant development and several reviews have been written on the individual hormone signal transduction pathways (e.g. Himmelbach *et al.*, 2003 for ABA and Guo and Ecker 2004 for ethylene).

1.6.1. Abscisic acid

Abscisic acid (ABA) was identified and characterized by Frederick Addicott and his associates in the 1960s (Ohkuma *et al.*, 1963). They thought initially that ABA has an effect on the abscission of cotton bolls and called it "abscisin II", while, at the same time, the group of Philip Wareing identified a role for the hormone in bud dormancy and called it "dormin"(Doorenbos, 1953). When the chemical structures of dormin and abscisin II were compared, the hormone was renamed ABA and the name was retained in spite of discovering that ethylene was the hormone with a controlling influence on abscission of different plant parts (Wasilewska *et al.*, 2008; Vankova, 2012).

Many aspects of plant growth and development are now known to be affected by changes in ABA concentration (such as seed dormancy, induction of protein storage in seed, stomatal regulation, primary root elongation). Its regulatory effect varies in response to G x E interaction and is pronounced under stress environments such as drought (Tardieu and Davies, 1992; Wilkinson and Davies, 2002; Sharp and LeNoble, 2002), salinity (Finkelstein *et al.*, 2002; Finkelstein, 2013), or cold (Davies, 2004*a*; Finkelstein, 2013). The substantial increase in ABA production, under drought stress compared with well-watered conditions, suggests that ABA is likely to be a key hormone in the control of the stomatal aperture (Tardieu and Davies, 1992), root growth (Sharp and LeNoble, 2002) and plant water status (Tardieu and Davies, 1992; Lee and Luan, 2012). Via the promotion of stomatal closure and promotion of primary root growth, ABA confers to the plant some avoidance or tolerance of drought stress (Sharp and LeNoble, 2002; Claeys and Inzé, 2013). This may be, for example, via partial stomatal closure in response to increased ABA accumulation, increasing water use efficiency (Davies *et al.*, 2002) while allowing the entry of sufficient CO_2 for a rate of photosynthesis that may be optimal for the amount of water available to the plant (Acharya and Assmann, 2009). Low concentrations of

ABA may also promote primary root elongation allowing plants to access water which is deeper in the soil profile (Sharp and LeNoble, 2002). It was initially thought that the plant water status was solely responsible for the control of the plant response (stomatal behaviour, reducing leaf expansion) to water stress scarcity, but it is now well accepted that the plant response to soil drying can be observed without any changes in the water status and that chemical signalling can be a key under many circumstances, i.e., ABA signalling (Wilkinson and Davies, 2002).

Under reduced water availability ABA concentration is enhanced in all parts of the plant such as roots, xylem sap and leaves (Davies and Zhang, 1991; Puértolas *et al.*, 2013). It has been proposed that ABA is a long distance signal in the plant, moving from root to shoot (Davies *et al.*, 2002, 2005). The large quantities of ABA synthesised in the leaf are undoubtedly sufficient to induce stomatal closure (Finkelstein, 2013; Hu *et al.*, 2016) and this local production can be the main contributor to stomatal closure under drought (Manzi *et al.*, 2015; McAdam *et al.*, 2016) but is still possible that the circulation of ABA in the stele provides the shoot with a 'measure' of the edaphic conditions around the root and the root physiological status (Hartung *et al.*, 2002; Hu *et al.*, 2016). The long distance signalling concept considers that ABA is produced in or recirculated from the roots and then transported through the xylem to reach the target cells, where it is firstly accumulated into the symplasm and finally reaches the apoplast, where ABA promotes stomatal closure at low concentration (Wilkinson and Davies, 2002; Davies *et al.*, 2002). This concentration can be lower than the ABA concentration ([ABA]) found into the xylem sap and the symplasm (Wilkinson and Davies, 2002; Davies *et al.*, 2002).

ABA is usually considered to be a plant growth inhibitor, a conclusion based on research where exogenous hormone (ABA) is applied to plants under non-stress conditions (Sharp *et al.*, 1994; Sharp and LeNoble, 2002). However, other research has shown that at low water potential, a

reduced level of endogenous ABA generated by application of fluridone - a carotenoid synthesis inhibitor which reduces ABA production - was associated with the inhibition of root elongation and promotion of shoot elongation in maize (Saab *et al.*, 1990). Additionally, under drought stress, shoot growth is usually more restricted than root growth, which can benefit the plant in maintaining an adjusted water supply from the soil to the shoot (Sharp, 2002). Saab *et al* (1990) concluded that at low water potentials (vermiculite - substrate), ABA accumulation plays an important role in both the maintenance of primary root elongation and the inhibition of shoot elongation, although Guo *et al.* (2009) found that exogenous application of ABA decreases the number of lateral roots. Under severe drought stress, ABA tends to maintain primary root growth and inhibit root branching, resulting in a deeper but less dense root system (Tardieu et al., 2010). In contrast, at high water potential (vermiculite - substrate), ABA-deficient mutants and fluridone applications have shown little effect on the elongation of root and shoot (Saab *et al.*, 1990).

The accumulation of ABA in anthers has been associated with an increased pollen sterility in wheat under drought conditions (Ji *et al.*, 2011), and in rice under cold conditions (Oliver *et al.*, 2007), both during meiosis. Ji *et al.* (2011) have observed that drought-tolerant wheat genotypes tend to accumulate lower ABA levels in the spike and additionally are more sensitive to applied ABA compared with responses of more drought-sensitive genotypes. In the same research, drought-tolerant genotypes show greater reductions of yield when anthers are exposed to a higher ABA levels. In contrast, Dembinska *et al.* (1992) concluded that the induction of spikelet sterility in wheat under drought stress during meiosis is not primarily regulated by an increase of ABA content in spikelet (plants under partial root drying increase ABA content in spikelet but do not reduce the grain set). In contrast, exogenous application of ABA into the leaf sheath has shown that ABA inhibited floret development, and decreased the number of fertile florets

and grain set at almost all development stages (floret initiation, terminal spikelet formation, meiosis, and floret degeneration) of wheat (Wang *et al.*, 2001).

Thus, ABA has different roles in the vegetative (source tissues) and reproductive (sink tissues) plant parts (Dolferus *et al.*, 2011) and accumulation of high [ABA] in these tissues can show a positive or negative correlation with drought stress tolerance. These correlations also depend on the phenological stage of the plant.

1.6.2. Ethylene

In the 19th century, the Russian plant physiologist Neljubov (1879–1926) observed that etiolated pea seedlings grew horizontally in his laboratory but not outside and he firstly associated this growth habit to the presence of high concentrations of contaminating illuminating gas in his laboratory. Later, he showed that the active component of the illuminating gas was ethylene (ETH) (Abeles *et al.*, 2012).

Ethylene is a gaseous plant hormone which is produced by almost all parts and organs of higher plants, and it is involved in a range of developmental processes, such as promoting senescence of plant organs, leaf abscission, fruit ripening, stem thickening, root hair development and adventitious root formation, but also retarding the stem elongation and floral development (Yang and Hoffman, 1984; Davies, 2004*b*; Sisler *et al.*, 2006; Acharya and Assmann, 2009; Abeles *et al.*, 2012). Ethylene synthesis is promoted by almost all biotic and abiotic stress conditions, such as flooding, changes in day length and light intensity, extremes of temperature, drought, pathogens and herbivore attack (Davies, 2004*b*; Cristescu *et al.*, 2013).

Ethylene is often synthesized within the same tissue where it induces changes in development and functioning (Davies, 2004*b*; Abeles *et al.*, 2012) but ethylene is also considered to be a long-distance signal under drought stress conditions. In fact, it has been argued that after

flooding irrigation the immediate precursor of ethylene, 1-aminocycloprane-1-carboxylic acid (ACC), is accumulated in the roots as it cannot be transformed into ethylene due to the lack of oxygen which impedes the activity of ACC-oxidase (ACO) (Yang and Hoffman, 1984; Kende, 1993; Tholen *et al.*, 2006; Van de Poel and Van Der Straeten, 2014). The ACC is therefore transported through the xylem sap to the shoot (Bradford and Yang, 1980) where it is converted into ethylene by ACO, which is already present in the leaves (English *et al.*, 1995).

Ethylene is usually considered as a growth inhibitor mainly associated with the triple response in seedling plants, which is probably the most well-known effect of ethylene on plant growth: 1) inhibition of stem elongation, 2) thickening of the stem, and 3) formation of an apical hook (Guzmán and Ecker, 1990; Pierik *et al.*, 2007; Abeles *et al.*, 2012). Elucidating the impact of ethylene role on plant growth is now an active research area (Pierik *et al.*, 2006, 2007). It has been demonstrated that when plants are under abiotic stress, ethylene, at low concentration, can be a promotor of growth. For example, when plants compete for light in a plant community, ethylene-insensitive plants show a reduced shoot growth rate, compared with the wild type which offers a shade avoidance response (Pierik *et al.*, 2003, 2004, 2006). Fine tuning of plant growth rates and impacts on survival of plants, may depend on an interaction between hormones (such as ethylene and ABA e.g. Sharp and LeNoble, 2002 or ethylene and gibberellin e.g. Pierik *et al.*, 2004) and environmental stress. A low concentration of ethylene in wheat has shown some promoting effect on shoot growth, while a high concentration has an inhibitory effect on shoot (Pierik *et al.*, 2006) and primary root growth (Sharp and LeNoble, 2002; Muday *et al.*, 2012). The biphasic effect of ethylene, on shoot growth, shows the existence of an 'optimum concentration' of the hormone (ethylene) for a particular response which depends on plants species, environmental factors and other potential signals (such as levels of other hormones) (Sharp and LeNoble, 2002; Pierik *et al.*, 2003, 2006, 2007; Abeles *et al.*, 2012).

Ethylene has a negative effect on pollen fertility and anther development. In fact, anther dehiscence can be inhibited by an increase in ethylene concentration ([ETH]) (Campbell *et al.*, 2001). Under heat stress conditions, ethylene synthesis often increases and Klassen and Bugbee (2004) suggested that the cause could be increased activity of the enzyme 1-aminocycloprane-1-carboxylic acid oxidase (ACO) which is a key enzyme in the synthesis of ethylene. Furthermore, Hays *et al.* (2007) showed that increased ethylene levels under heat stress lead to kernel abortion and Yang *et al.* (2007) showed that under drought stress, the application of ABA or amino-ethoxyvinylglycine (AVG - an inhibitor of ethylene synthesis), to the rice panicles during meiosis, significantly reduced spikelet sterility. Yang *et al.* (2006*a*, 2007) found that the grain filling rate after anthesis is enhanced in the basal and apical grains, in wheat and rice, when the ratio ABA/ethylene or ABA/ACC is increased.

Spollen *et al.* (2000) suggested that under drought stress, increased levels of endogenous ABA in roots are required to prevent an excess of ethylene production, which has a negative effect on root elongation, but ABA itself was not identified to have a root promoting effect. On the other hand, ethylene and its primary precursor ACC were observed to promote root hair initiation and elongation in synergy with auxin (Muday *et al.*, 2012).

It has been suggested that ethylene could have a role in the regulation of stomatal behaviour (Wilkinson *et al.*, 2012). In the absence of ABA, it has been observed that ethylene can induce a reduction in stomatal conductance (Vysotskaya *et al.*, 2011). In contrast, under drought conditions, ethylene can antagonize ABA-induced stomatal closure (Tanaka *et al.*, 2005; Wilkinson and Davies, 2009). It has been suggested that the contrasting effects of ethylene on stomata as well as the negative effect of stress ethylene on grain filling rate, grain abortion and

leaf senescence, increase the difficulty in identifying a specific role for ethylene in influencing drought tolerance and yield development (Wilkinson *et al.*, 2012).

Recent research has shown that several plant drought stress responses can be explained most effectively as functions of the actions and interaction of two hormones, abscisic acid (ABA) and ethylene (Peleg and Blumwald, 2011; Davies *et al.*, 2013). Plant hormone balance may control various plant growth and survival mechanism which are associated to a specific organ or tissue and can have an impact on yield crop performance (Wilkinson *et al.*, 2012). Hormone balance may be a function of environmental factors and the crop's developmental stage (Wilkinson *et al.*, 2012). It has recently been suggested that ABA-ethylene concentration ratios can represent novel targets for plant selection and breeding programs for adaptation or for high yielding under drought stress (Yang *et al.*, 2007; Wilkinson *et al.*, 2012; Davies *et al.*, 2013)

1.7. Research objectives and thesis structure

The aim of the work described in this thesis is to investigate whether plant hormone status (ethylene and ABA) has utility as a new secondary physiological trait for breeding selection during the pre-anthesis period when wheat plants are most sensitive to stress environments (from booting to heading stage). The extension of this work would be to develop an effective field-based high throughput method for selection of genotypes adapted to drought stress, to improve the efficiency of plant breeding programmes by reducing cost and time and improve agronomic practices.

The work in Chapter 2 sought to define and characterize (in a systematic fashion) what constitutes a stress resilient genotype in terms of yield under stress environments (heat and drought).

The work in Chapter 3 was designed to better understand the complexity of yield responses to abiotic stress (heat and drought) and the interaction between genome and environments in terms of variation in the magnitude of yield components to define new selection criteria related to yield and spike fertility resilience under stress environment. This work allowed the identification of contrasting genotypes for further mechanistic research.

Chapter 4 describes new methodology to quantify plant hormone status (ethylene and ABA) under field conditions. Ethylene and ABA are quantified at different times in relation to the irrigation process.

In Chapter 5, the genetic variation in hormone accumulation and hormone balance was quantified under field conditions, to discover key phenological stage (s) and tissue (s) in the expression of resilience to drought stress during pre-anthesis.

In Chapter 6, hormone quantification and hormone balance evaluated under controlled environment conditions were related to field assessments of spike fertility at the same phenological stage and tissue as reported in Chapter 5.

Chapter 7 is a general conclusion and discussion of all the work reported in this thesis.

Chapter 2. A new crop screening method based on productivity and resilience to abiotic stress

2.1. Introduction

In agriculture, drought is by far the most important environmental stress that constrains crop yield (Blum, 2011). More than 40 % of the world is classified as dry land, of which 8% is dry sub-humid area and 16% is semiarid area (Pretty *et al.*, 2005; Middleton *et al.*, 2011). In addition, increasing temperature is an important component of climate change and its negative impact on yield is expected to increase in the future. Indeed, it has been demonstrated that growing wheat crops under heat stress (30/25 Cº) can lead to a 30-35% reduction in yield grain weight, when compared with controls (18/13 Cº) (Wardlaw *et al.*, 1989), and the importance of incorporating a heat tolerance trait into wheat germplasm has been highlighted (Sareen *et al.*, 2012). Breeders need to develop genotypes with the capacity to yield significantly under heat stressed environments (Sareen *et al.*, 2012). Therefore, understanding more about the mechanisms involved in plant tolerance/resilience to high temperature and drought stress becomes key for future improved crop production under stress as the climate in many food producing regions becomes hotter and drier (Blum, 2011; Macková *et al.*, 2013).

Many efforts have been made to ameliorate crop productivity under water-limiting conditions. While breeding activity has directed selection towards increasing the economic yield of cultivated species, natural selection has favoured mechanisms of adaptation and survival (Cattivelli *et al.*, 2008). More than 80 years of breeding activities have focused on the increase of yield under drought environments for different crop plants. Meanwhile, significant gains in the understanding of the physiological and molecular responses of plants to water deficits have been provided by fundamental research (Cattivelli *et al.*, 2008). However, it is not always clear

which traits might identify genotypes with higher productivity under drought stress and the drought resilient ideotype is not always properly defined (Blum, 2005).

The term 'stress tolerant genotype' needs to be defined and properly characterised. The definitions of a drought tolerant genotype found in the literature vary substantially as a function of the field of research at issue (Ecological, Crop breeding and/or Crop physiology) and other considerations such as yield production (Turner, 1979; Fleury *et al.*, 2010) or yield stability (Blum *et al.*, 1989). In terms of physiological mechanism, it is also important to take into account that some genotypes, which can show a better yield performance under stress conditions, show an "escape strategy" by accelerating their phenology in order to avoid the intense stress during critical phenological stages. Genotypes with this kind of response should not be considered as drought resilient (Fischer and Maurer, 1978; Turner, 1979, 1986; Levitt, 1980).

Plant breeding programmes mainly focus on selecting genotypes which have high yield firstly under yield potential conditions (non-stress) and secondly under stress conditions (Monneveux *et al.*, 2012). To reach this aim, the classical postulate, widely accepted by breeders for selection, is that a genotype with high yield potential will perform well under most environments (Blum, 2005). However, this selection method does not include the concept of yield stability neither does it consider adaptation to a stress environment. Such shortcomings can be a cause of slow progress in breeding (Ceccarelli and Grando, 1991; Blum, 1996).

Several stress indices, described in more detail in Appendix 2A, have been proposed to allow screening for drought stress adaptation. Fisher & Maurer (1978) developed a stress susceptibility index (SSI), Rosielle & Hamblin (1981) defined the tolerance index (TOL) and the mean productivity index (MP), and Fernandez (1982) analysed the latter and created two

new indices, the geometric mean productivity index (GMP) and the stress tolerance index (STI) in an attempt to improve the MP index so that it would identify highly productive genotypes under both – stress and non-stress - environments. These various indices consider the relationships between traits, under non-stress (yield potential, irrigated conditions) and stress (drought mainly) environments. According to Rosielle and Hamblin (1981); Fernandez (1992) and Sareen *et al.*, (2012), these indices can be grouped into 2 classes. The first class represents the susceptibility indices (SSI and TOL) which tend to distinguish between the stress-tolerant and the stress-susceptible genotypes, showing a negative relationship with yield. The second class represents the tolerance indices (MP, GMP and particularly STI) which tend to identify genotypes with stress-tolerance and high average yield, showing a positive relationship with yield. However, tolerance and susceptibility indices are not ideal to characterise genotypes with high yield performance and high stress tolerance under both environments. Genotype yield performance under stress and non-stress conditions has been categorised by Fernandez (1992) into four groups: A) genotypes express uniform superiority in both stress and no-stress conditions, B) genotypes express good performance only in yield potential but not under stress conditions, C) genotypes present a relatively higher yield only under stress, and D) poor yield performance in both environments. Additionally, Fernandez (1992) evidenced some failures of the defined indices to distinguish between certain of these groups and suggesting that stress tolerance index (STI) is generally able to better distinguish genotypes in group A from those in groups B and C (Table 2.1).

Table 2.1: Summary of the interpretation of tolerances according to the previously developed indices (stress susceptibility index (SSI), tolerance index (TOL), mean productivity index (MP), geometric mean productivity index (GMP) and stress tolerance index (STI)) and their failures to distinguish the different response groups of plants defined by Fernandez (1992). Group A genotypes express uniform superiority in both stress and non-stress conditions; group B genotypes express good performance only in yield potential but not under stress conditions; group C genotypes present a relatively higher yield only under stress; group D genotypes have poor yield performance in both environments. Classes 1 and 2 correspond to susceptibility indices and tolerance indices, respectively, where class 1 tends to distinguish between the stress- tolerant and the stress-susceptible genotypes and class 2 tends to identify genotypes with stress tolerance and high average yield.

Index	Index value	Tolerance	Fails	Class
SSI	High	Low	Fails to distinguish A and C	1
TOL	High	Low	Fails to distinguish A and C	1
MP	High	High	Fails to distinguish A and B	2
GMP	High	High	Same failure as MP in distinguishing better A compared to MP	2
STI	High	High	Same failure as MP in distinguishing better A compared to MP and GMP	2

There is a clear need to develop an accurate tool able to identify the yield performance and resilience capacity of genotypes under stress conditions, since previous research has focused only on yield performance without taking resilience or stability into account. Currently, STI, GMP and MP are the most recommended indices to identify genotypes with high yield under both non-stress and stress environments (heat and drought) (Khodarahmpour *et al.*, 2011; Mohammadi *et al.*, 2011; Sareen *et al.*, 2012). In contrast, Khayatnezhad *et al.*(2010) stated that none of these indices could clearly identify cultivars with high yield under both environments (stress and non-stress).

Importantly, there is not yet a precise screening index which can be recommended in breeding programmes to select genotypes for abiotic stress adaptation and high yield under both stress and non-stress environments. However, it has been suggested that a combination of stress indices (tolerance and suceptibility indices) might provide a more useful criterion for improving drought stress tolerance selection in common bean and heat stress tolerance selection in maize (Ramirez-Vallejo and Kelly, 1998; Khodarahmpour *et al.*, 2011). Nevertheless, it is not yet clear how to combine stress indices appropriately.

Therefore, a major objective of the present work was to develop a new simple tool based on the complementarities of two classes of indices (class 1: susceptibility indices, and class 2:

tolerance indices, in Table 2.1) to express crop yield, to elucidate the characteristics of the best performing and adapted genotypes under stress. To achieve this goal, a methodology was developed to allow a combining of indices. Finally, suggestions are made on how this tool can be used in crop breeding programmes and how the new indices can be used to provide a focus for mechanistic research aimed at understanding the basis of the sensitivity of crop yield to environmental stresses.

2.2. Materials and methods

2.2.1. Site of experiments

Field trials were conducted at the Mexican Phenotyping Platform (MEXPLAT), located in the highly productive irrigated spring wheat growing environment in the Yaqui Valley, near Obregon City, NW Mexico (27° 22′ 6.9′′ N, 109° 55′ 21.6′′ W, 38 meters above sea level). This site is a temperate high radiation environment, and with adequate irrigation, average yield of the best lines is approximately 8 t/ha (Sayre et al., 1997).

2.2.2. Experimental material and stress treatments

Ten lines selected from the CIMCOG trial (acronym of CIMMYT Core Germplasm), were used in this study. The selection was made initially for other research on partitioning and related traits and in particular to allow study partitioning of resources to roots and shoots growth. The ten selected lines were sown in an independent trial named CIMCOG-ROOT. Table 2.2 illustrates the cross name of these ten lines.

Table 2.2: Name of the wheat genotypes or cross name of the CIMCOG-ROOT trial.

Entries	CROSS NAME
1	BACANORA T 88 (Hist 4)
2	BRBT1*2/KIRITATI (HIST 14)
3	PBW343*2/KUKUNA*2//FRTL/PIFED
4	SIETE CERROS T66 (Hist 1)
5	TACUPETO F2001/BRAMBLING*2/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
6	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
7	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
8	WBLL1*2/ KIRITATI CGSS01B00063T-099Y-099M-099M-099Y-099M-27Y-0B
9	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
10	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343

These wheat lines were evaluated during two cropping seasons, 2012-13 and 2013-14, in three different environments: irrigated conditions (yield potential (Yp)) for the two cropping seasons (23 November 2012 to early May 2013 and 27 November 2013 to early May 2014), and under drought and irrigated heat stress during the later cropping season, i.e., from December 4th 2013 to late May 2014 and from February 26th to June 2013, respectively. The sowing date for the heat stress was chosen at that period in Mexico (Yaqui Valley) as it is representative of the crop sowing conditions in the autumn of hot areas such as India (e.g. Delhi), Brazil or Sudan (Ortiz et al., 2008; Braun and Payne, 2012). All trials were conducted with optimal crop management following a preventive biotic stress control strategy to reduce other stresses and with conventional nutrients supplied practiced in CIMMYT.

Wheat plants were sown with a density of 5g/m^2 on raised beds which were 80 cm wide with a 40 cm planting surface with four rows and a 10 cm spacing between the rows (Figure 2.1). Plots size was 10 meters long for yield potential and 5 meters long for stress environment (heat and drought).

For all of the experiments, the testing area was surrounded with one raised bed with four rows of durum wheat (*Triticum durum*) that acted as a windbreak to reduce edge effects. The

experimental design was a totally randomized block design with three replications for the yield potential (Yp) and drought (D) trials, and two replications for the heat (H) trial. Gravity-fed flood irrigation was applied for all experiments. For the drought stress trial, the last irrigation was at 50% of seedling emergence, and in the case of the Yp and irrigated heat (H) trial, 6 additional irrigations were applied, after 50 % of emergence, every three weeks until 15 days before maturity.

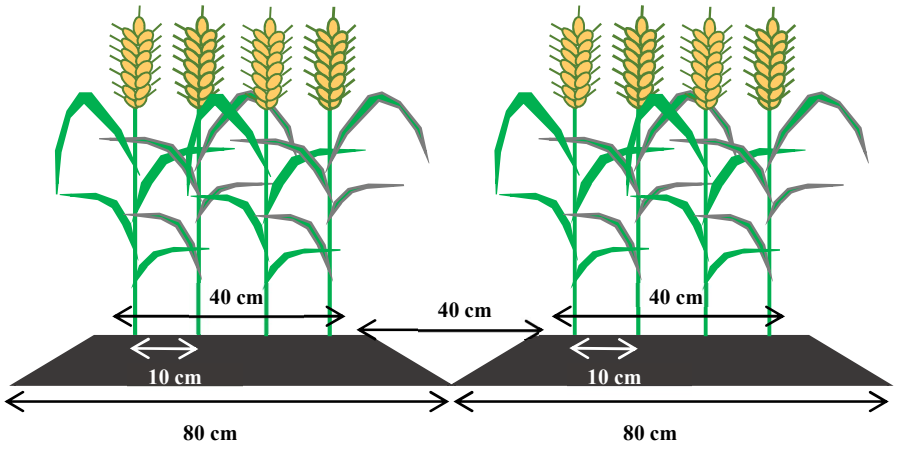


Figure 2.1: Diagram showing the experimental planting on raised beds which were 80 cm wide with a 40 cm planting surface with four-row and 10 cm spacing between the rows. Planting density was 5g per m^2.

Additionally, data from a set of 294 elite genotypes - the WAMI trial (acronym of Wheat Association Mapping Initiative) - grown under yield potential conditions during the 2011-12 cycle (November to May), and under heat conditions from February to June 2012, have been used to test the robustness of the new indices.

2.2.3. Selection of stress adapted genotypes within a population under field conditions

The selection of crop genotypes under field conditions presents additional difficulties due to the variability, intensity, timing and duration of the abiotic stress (heat and/or drought), as well as the development of several stresses at the same time (e.g. pest invasion and nutrient stress). Therefore, it is important to compare genotypes within the population response in order to

identify genotypes more or less susceptible and/or tolerant to the stress in question. Screening for stress-adapted genotypes under field conditions results in screening for susceptibility to cumulative stress and the interaction between responses to these stresses (Fleury *et al.*, 2010; Dolferus *et al.*, 2011). Consequently, to study a specific mechanistic response to environmental stress by plants under field conditions it is highly important to control as much as possible the other collateral stresses that can appear during a growing season (biotic stress, nutrient stress, *etc*.) in order to reduce their effects on the crops (Chapin *et al.*, 1987; Herms and Mattson, 1992; Dolferus *et al.*, 2011). However, the control will never completely reduce the pressure of the other stresses, and we therefore assume that a population of genotypes grown during the same season would have suffered an equivalent pressure of cumulative stress (abiotic and biotic stress). Therefore, each genotype response to stress environment should be compared to the response of the whole population, for each cropping season, to better understand the stress adaptation.

2.2.4. Basis of the development of the new stress indices and their uses

As mentioned above, different approaches are used to identify tolerant genotypes from class 1 and 2 (Table 2.1). Class 1 tends to discriminate the tolerant from the susceptible, and class 2 tends to distinguish the tolerant with high mean yield. However, the failures of the indices in identifying the best yielding genotypes under stress (Table 2.1) show that a high yield under non-stress conditions does not automatically indicate a good performance under stress, and similarly, a high yield under stress does not automatically indicate high resilience. The outcome of a stress challenge will depend on the severity of the stress and obviously on the characteristics of the genotype (genotypic background).

Nevertheless, both classes of indices (class 1 and 2, susceptibility and tolerance indices, respectively) (Table 2.1) explain a part of the behaviour of the genotypes under stress. Therefore, based on the previous concept developed by Fernandez (1992), Fisher & Maurer (1978) and Rosielle & Hamblin (1981), we propose here two new indices which are compiled through the combination of the score indices that show a high correlation with yield under stress and non-stress environments. The score indices have been classified within two new scales called resilience and production capacity, based on classes 1 and 2 of the existing stress indices (Table 2.1), respectively.

The new resilience and production capacity indices are defined as follows:

Resilience Capacity Index (RCI) expresses the yield decrease of the genotypes under stress (Ys) within a population, compared to yield potential conditions (Yp);

Production Capacity Index (PCI) expresses the mean production of the genotypes under both stressed (Ys) and non-stressed (Yp) environments within a population.

These indices RCI and PCI constitute an attempt to improve the use of the five previous indices (SSI, TOL, MP, GMP, STI), as both new indices (RCI and PCI) are required if we are to understand the basis of any yield limitations under stress.

2.2.5. Why combine the indices?

It is important to analyse the different groups of yield responses (from A to D). Groups A and D represent the extremes – in terms of grain yield - as the best and worst genotypes. However, extreme responses are rare and genotypes in these two groups would tend towards groups B or C, like e.g. as AB or AC and DB or DC. Nevertheless, this could explain why both classes of indices have a relatively good relationship with both yields (non-stress and stress), as shown by Fernandez (1992), as they both fail to correctly identify the middle index values of the linear

regression with yield (non-stress and stress). In turn, the middle values can have two tendencies, a medium-high or a medium-low value, for both environments. For example, group A with a value close to the boundary line value, which discriminates group A from C under non–stress and from group B under stress conditions (Figure 2.2). Indeed, to distinguish these values which are more A than C under non-stress and vice versa, it will be used the terms medium-high and medium-low, respectively. Considering this, medium values in the linear regression obtained with the indices, must be readjusted in order to express better the yield trait under non-stress and stress environments. This can be achieved by combining the indices.

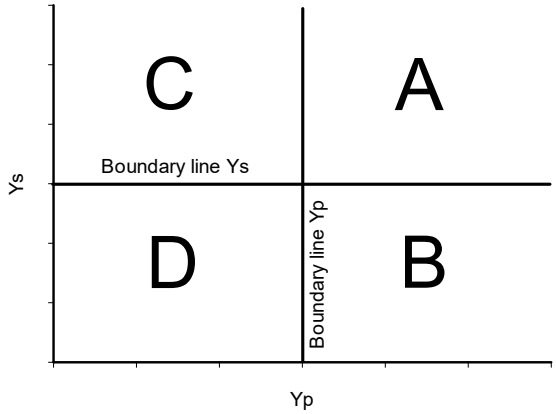


Figure 2.2: Representation of the different response groups (A, B, C, and D), defined by, according to their grain yield under abiotic stress conditions (Ys) and grain yield under yield potential conditions (Yp). Group A genotypes express uniform superiority in both stress and non-stress condition; group B genotypes express good performance only in yield potential and not under stress conditions; group C genotypes present a relatively higher yield only under stress; and group D genotypes express poor yield performance in both environments. The boundary lines (bold line) create the limit between one group and the others. The boundary line value corresponds to the yield value from a check under yield potential conditions (boundary line Yp) and yield under drought stress (boundary line Ys).

2.2.6. How can the indices be combined, as their values are totally different?

In order to classify the trait (e.g. tolerance) from the highest to the lowest, the indices (SSI; TOL; MP; GMP; STI) are each given their own numerical value, as individual index values can only be interpreted inside each index itself, because the scale or reference of the different indices is not the same. Additionally, indices of class 1 have a reverse scale to that of class 2, where low values mean high tolerance. Therefore, to enable comparison of the different indices, a scale has been created on an equal reference for all indices by scoring the results from 1 to

10, where a high value means always a good response in terms of resilience or production capacity. Afterwards, the five indices show a value for each genotype which is comparable between the different indices. The idea of scoring is to have an easy visualization of the information given by the indices for the population under study, and to be able to compare one index with the others. A simple number, on a 1 to 10 scale, provides an easier interpretation than decimal values allocated to the original equations. Additionally, it opens new insights by permitting arithmetic operations between the indices in a simple way.

2.2.7. How to create the scoring scale?

The scoring scale for each index is calculated on the global response within the overall population under study. Thus, the scale is adjusted with the minimum and maximum value obtained with the original equation of the index. The difference of these two values gives the range of the scale for each index. This range is divided into 10 parts and each part has a score from 1 to 10. Therefore, each part represents the 10%, 20%,..., or 100% of the range value.

Additionally, we have inverted the value of TOL and SSI, so a high value obtained with the original equation will receive a lower score. It allows the two classes of indices to have the same scale, where a high score will always mean a 'good' genotype. For example, score value 2 is obtained in the different indices for all the values within the 10% and 20% of the range for MP, GMP, STI and 80% and 90% of the range for TOL and SSI.

Once the scores have been obtained, they can be easily combined and tested versus yield under stress and non-stress conditions. The outcomes should indicate whether genotypes are better adapted to express yield under stress and/or non-stress by identifying or more effectively distinguishing groups B and C from group A based on the Resilient Capacity Index (RCI) and Production Capacity Index (PCI). These are terms which indicate much more specifically what the indices are showing.

2.2.8. Statistical analysis

Analysis of variance (ANOVA: one-way or two-way), correlations and regression were performed using Statgraphics Centurion XVII (StatgraphicsNet, Spain), SPSS 21.0 (IBM, USA) was and MS Excel (Microsoft Corporation, USA) softwares.

2.3. Results

2.3.1. Testing the methodology and the score indices

Firstly, the score indices have been tested against their original value from each index. Table 2.3 shows the Pearson correlation coefficient between the Score Stress Susceptibility index and the Score Tolerance Index values (SSIs and TOLs, respectively) and their original index values (SSI and TOL), calculated on yield data from the WAMI trial (294 genotypes). These correlations are highly negative (ranging from -0.78 to -0.98), as the score scale has been inverted in order to create a scale showing resilience instead of susceptibility. The Pearson correlation coefficient between the original value (MP, GMP and STI), and the score indices (MPs, GMPs, STIs), is highly significant.

Table 2.3: Pearson correlation coefficient between the score indices defined in this paper (SSIs, TOLs, MPs, GMPs, STIs) and their original indices (SSI, TOL, MP, GMP, STI) defined by the original authors, calculated on yield data from the WAMI trial (294 genotypes) under heat stress and non-stress environments. SSI and TOL show a negative correlation with SSIs and TOLs, respectively, as the score scale has been inverted. *P<0.05.

	SSI	**TOL**	**MP**	**GMP**	**STI**
Class 1					
SSIs	-0,98*	-0,80*	0,07	0,38	0,38
TOLs	-0,78*	-0,97*	-0,51	-0,21	-0,21
Class 2					
MPs	-0,09	0,49	0,98*	0,93*	0,93*
GMPs	-0,40	0,19	0,93*	0,98*	0,98*
STIs	-0,39	0,19	0,93*	0,98*	0,99*

2.3.2. How to combine the score indices? What is the best combination to define new indices?

Figure 2.3 shows the linear regression and the coefficient of determination of the different score indices versus yield under non-stress and heat stress environments, calculated on 294 genotypes from the WAMI trial. In each class of index (susceptibility and tolerance), SSI and STI show the highest relationship with yield under heat stress. In contrast, TOL and MP show a high relationship with yield potential. These responses would suggest that the combination of the score indices from each class would improve the relationship between the indices *per se* and grain yield.

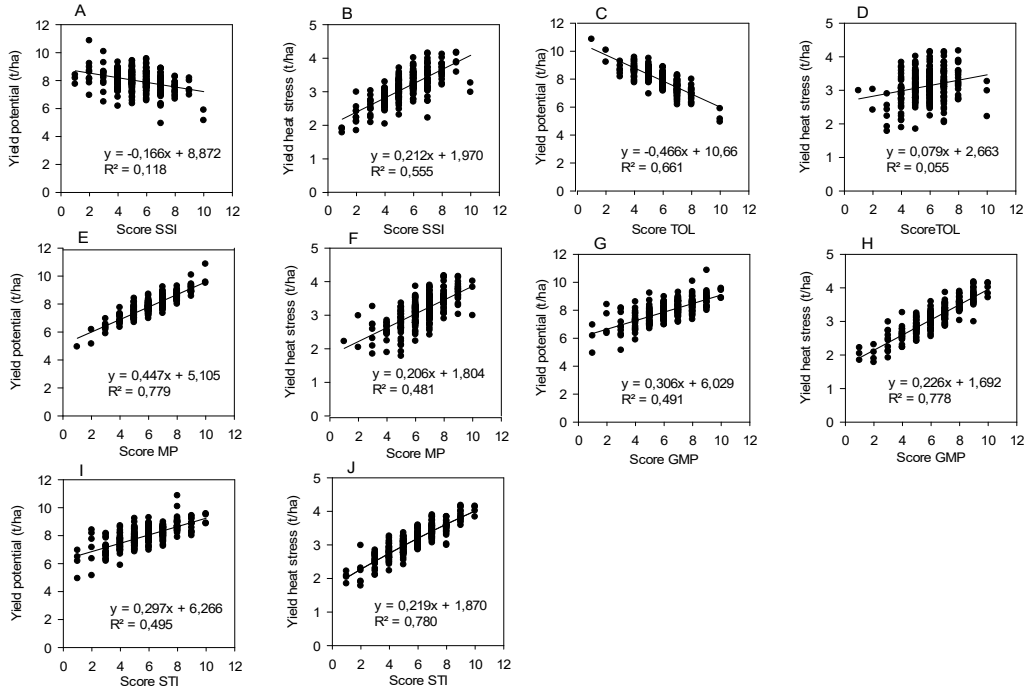


Figure 2.3: Linear regression and the coefficient of determination of the different score indices *versus* grain yield under non-stressed and heat stressed environments. Calculations use yield data from WAMI trial (294 genotypes) under heat stress and non-stress-conditions during the cropping season 2011-12: (A) Yield potential *versus* Score SSI; (B) Yield under heat stress *versus* Score SSI; (C) Yield potential *versus* Score TOL; (D) Yield under heat stress *versus* Score TOL; (E) Yield potential *versus* Score MP; (F) Yield under heat stress *versus* Score MP; (G) Yield potential *versus* Score GMP; (H) Yield under heat stress *versus* Score GMP; (I) Yield potential *versus* Score STI; (J) Yield under heat stress *versus* Score STI.

The new indices defined here are based on the combination of score indices. To easily illustrate the values of score indices and the contrast within the whole population, data from a smaller trial of ten genotypes from the CIMCOG-ROOT trial has been used to make the visualization

easier (compared with a table with 294 genotypes). Nevertheless, the method to interpret and use the score is the same for ten, 294 or even more genotypes.

Table 2.4 is an example of a score index table using grain yield data of ten genotypes from the CIMCOG-ROOT trial. The Yp (irrigated trial) data used in this table are a mean of the grain yield under Yp conditions for each genotype from two cropping seasons (2012-13 and 2013-14), in order to provide more consistent information on the yield potential of the genotype, considering that the Yp represents the maximum grain yield that a genotype is able to produce.

Table 2.4: Example of a Score index table based on grain yield data from the CIMCOG-ROOT trial (ten genotypes) under yield potential and heat stress environments, for the Stress Susceptibility Index (SSI), Tolerance Index (TOL), Mean Productivity Index (MP), Geometric Mean Productivity Index (GMP), and Stress Tolerance Index (STI) during the 2012-13 cropping season (Y12-13). The score indices show slight score differences but keep the same magnitude into each class, where Class 1 tends to distinguish between the stress-tolerant and the stress-susceptible genotypes and Class 2 tends to identify genotypes with stress-tolerance and high average yield.

Entries	Class 1		Class 2		
	Score SSI	Score TOL	Score MP	Score GMP	Score STI
1	1	3	3	1	1
2	4	6	4	3	3
3	2	3	6	4	4
4	6	10	1	1	1
5	4	2	10	9	9
6	10	10	9	10	10
7	5	7	6	5	5
8	5	5	8	7	7
9	2	1	10	7	7
10	4	3	9	7	7

At this point, the next question will be, which combination of these score indices could be considered as the best indices to express yield under stress and non-stress conditions? Several combinations have been studied to generate a new index with the two components (RC and PC). The methods used and the different combinations and formulae are shown in Appendix 2 Table 1. The combinations considered, for each case, were achieved by pairs or groups of four score indices (combinations of two by two score indices from class 1 and 2), adding or subtracting components. Each combination was correlated with grain yield values under stress (Ys) and non-stress conditions (Yp) by calculating the Pearson correlation coefficient (Appendix 2 Table 2 to Appendix 2 Table 4). Some combinations show a better correlation with

Ys and Yp than others, and two of them are outstanding. Indeed, Equation 1 presents the highest Pearson correlation coefficient with Ys (YSSI), and Equation 2 with Yp (YPSI), for the three trials (CIMCOG-root under drought and irrigated heat, and WAMI). In both equations, the first and second components correspond to PCI and RCI, respectively. The relationship between Ys and Yp and Equations 1 and 2 is illustrated in Figure 2.4 and Figure 2.5, respectively. At this point, Equation 1 will be called Yield Stress Score Index (YSSI) and Equation 2 will be called Yield Potential Score Index (YPSI).

$$\mathbf{YSSI} = \frac{(\mathbf{STIs+SSIs})}{2} \quad \textbf{Equation 1}$$

$$\mathbf{YPSI} = \left(\frac{(\mathbf{MPs+STIs})}{2} - \frac{(\mathbf{SSIs+TOLs})}{2}\right) \quad \textbf{Equation 2}$$

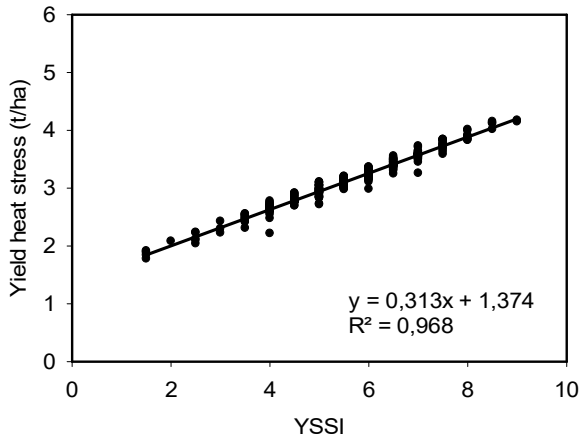


Figure 2.4: Linear regression and the coefficient of determination of grain yield under heat stress (Ys) and the Yield Stress Score Index (YSSI). Calculations use yield data from WAMI trial (294 genotypes) under heat stress and non-stress conditions during the cropping season 2011-12.

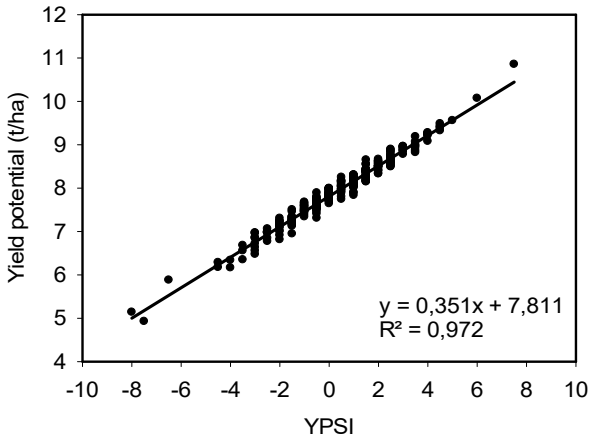


Figure 2.5: Linear regression and the coefficient of determination of grain yield under a yield potential environment (Yp) and the Yield Potential Score Index (YPSI). Calculations use yield data from WAMI trial (294 genotypes) under heat stress and non-stress conditions during the cropping season 2011-12.

2.4. Discussion

The test of the scoring indices method and the high Pearson correlation coefficient values, made on a huge panel (294 genotypes), have demonstrated that the score index method can be used as a surrogate of the original index value (SSI, TOL, MP, GMP; STI). (Table 2.3)

The study of the relationship of the score indices *versus* yield under stress and non-stress shows that no index, used individually, could clearly identify the high yielding genotypes,

independently of the environment. This result confirms the conclusion of Khayatnezhad *et al.*(2010), made from a study of 22 genotypes of durum wheat. However, it is important to recognize that the tolerance indices (MP, GMP, STI) usually show a better correlation between both yield (stress and non-stress) compared with the susceptibility indices (SSI and TOL) as observed by Khodarahmpour *et al.* (2011); Mohammadi *et al.* (2011) and Sareen *et al.* (2012). The score indices of the ten genotypes provide an illustration of small differences between SSI and TOL (Table 2.4). On the other hand, GMP and STI are very similar, but both are slightly different from MP (Table 2.4). It is important to observe that in both cases the values are generally of the same magnitude within the two classes (Table 2.4). Additionally, Table 2.3 indicates a relationship between the indices within each class of indices in the 294 genotypes trial. Thus, these score values (Table 2.4) and the Pearson correlation coefficient (Table 2.3) confirm that SSI and TOL, as well as MP, GMP and STI, can be associated to class 1 and 2, respectively (Table 2.1), demonstrating that these classes address on two different characteristics, resilience capacity (RC) and production capacity (PC), respectively.

These correlations (Figure 2.4; 2.5) demonstrate that yield, either under stress or non-stress, can be expressed by two components, resilience (RCI) and production (PCI). Moreover, the combination of score indices has improved the utility of the original indices and their relationship with yield. It is true that the validation of YSSI and YPSI is based on the yield values of the same year (Figure 2.4; 2.5), as was the validation of the original indices (SSI, TOL, MP, GMP, STI) by Fernandez (1992) but with a much higher correlation than the original indices (Figure 2.3 - 2.5). This demonstrates that YSSI is an improved surrogate of yield under stress which offers the option of selecting genotypes with high yield and high resilience separately by using RCI and PCI (the components of YSSI). Additionally, to demonstrate the robustness of this index, it has also been calculated using data from multiyear trials with a range

of populations (WAMI, Seri/Babax, CIMCOG-ROOT) previously studied in CIMMYT under different abiotic stresses (Pinto *et al.*, 2010; Lopes *et al.*, 2015; Sukumaran *et al.*, 2015). Table 2.5 shows the Pearson correlation coefficient and coefficient of determination (R^2) of yield under stress versus YSSI. The consistency of the correlations between yield under stress and the index demonstrates the reliability of the index.

Table 2.5: Summary table showing the Pearson correlation coefficient and coefficient of determination (R^2) of Yield Stress Score Index (YSSI) versus yield under stress (Ys) from multiyear populations (WAMI, Seri/Babax, CIMCOG-ROOT) previously studied in CIMMYT under different abiotic stresses (heat, drought, drought under drip; semi-drought: drought applied at booting stage). The consistency of correlations demonstrates the reliability of the index.

Trials	Entries	Environments	Years	Correlations	R^2
WAMI	294	Heat	2009-2010	0.988	0.976
WAMI	294	Heat	2010-2011	0.983	0.967
WAMI	294	Heat	2011-2012	0.987	0.975
WAMI	294	Drought	2009-2010	0.987	0.973
Seri/Babax	169	Heat	2004-2005	0.993	0.986
Seri/Babax	169	Heat	2005-2006	0.992	0.984
Seri/Babax	169	Heat	2009-2010	0.955	0.912
Seri/Babax	169	Drought	2005-2006	0.994	0.988
Seri/Babax	169	Drought (Drip)	2007-2008	0.989	0.978
Seri/Babax	169	Drought	2008-2009	0.991	0.982
Seri/Babax	169	Drought (Drip)	2009-2010	0.974	0.948
CIMCOG ROOT	10	Semi-drought	2012-2013	0.996	0.992
CIMCOG ROOT	10	Heat	2012-2013	0.975	0.95
CIMCOG ROOT	10	Drought	2013-2014	0.981	0.962

In Appendix 2B, an explanation is provided of why PCI and RCI are complementary and why these combinations of these indices (PCI and RCI) better identify the group A genotypes compared to an analysis using the previous indices (SSI, TOL, MP, GMP, STI).

A further combination of the five score indices, named Mean Score Index (MSI) (Equation 3), is slightly better correlated with Ys than the correlation between Ys and YSSI (Figure 2.6). Nevertheless, this formula contains a disproportion between its productivity and resilience components, giving more weight to the first one with 3 indices, while containing only 2 indices

for resilience, such that productivity has a greater impact on the output. This could be associated to a better correlation observed between the productive indices *versus* both yield (under stress and non-stress) than the correlation observed with the resilience indices (Figure 2.3). On the other hand, a possible explanation of why Equation 3 and Equation 1 work similarly with or without a disproportion between the resilience and productivity, is probably because the conditions of the experiment are under severe heat stress and it is possible that under a mild stress environment, productivity might contribute more than the resilience. This hypothesis is supported by the fact that breeding under yield potential is usually performed under a wide range of environment but this is not the case under severe stress and the selection is improved when it is made under the target environment (severe stress). However, this difference in the weight of the indices does not modify the use of the components of the indices to select genotypes for high yield (PCI) and high resilience (RCI). As the aim is to identify an easy method to distinguish resilience and productivity, the MSI has not been addressed in this Chapter.

$$\boldsymbol{Mean\ Score\ Index} = \frac{\mathbf{SSIs+TOLs+MPs+GMPs+STIs}}{\mathbf{5}} \quad \textbf{Equation 3}$$

Therefore, to improve the selection and identification of contrasting genotypes in terms of PC (production capacity) and RC (resilience capacity) under stress conditions, YSSI and its components PCI and RCI, which are the Scored STI and the Scored SSI indices, respectively, are the focus indices for the remaining work described in this thesis.

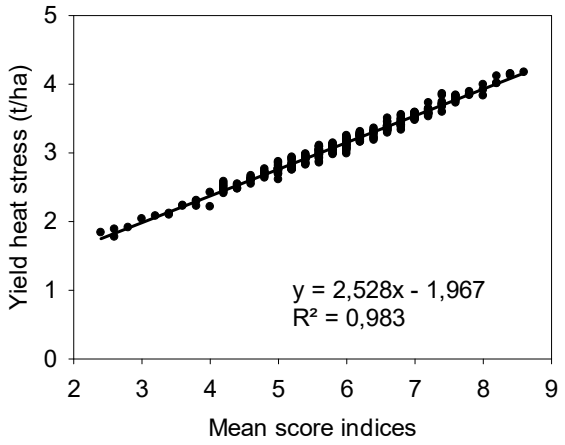


Figure 2.6: Linear regression and the coefficient of determination of grain yield under heat stress (Ys) and the mean score index. Calculations of the score indices use yield data from WAMI trial (294 genotypes) under heat stress and non-stress conditions during the cropping season 2011-12.

2.4.1. How can these indices be used to identify resilient and productive genotypes?

The score indices provide two main advantages. Firstly, the interpretation of yield data collected across large populations of genotypes is much easier, than using yield per se, as everything is on a similar scale, allowing the visualization of the score, (being 1 to 10) to detect the lowest, medium or highest response. Secondly, the score indices enable us to better understand genotype behaviour under stress, indicating if a high yield under stress is due to tolerance (resilience) or due to a high production capacity (mean yield performance), or both. This can be achieved by analysing the components of YSSI, where high resilient/tolerant and high productive genotypes should have a high value in both indices (RCI and PCI, respectively).

Table 2.6 presents an extended summary of the use of the indices (RCI and PCI, respectively) and their combined value as a function of the values obtained under Yp and Ys.

Table 2.6: Summary of the values expected for the different index scales within a population and the impact of their combination as YSSI and YPSI. A unique combination of the Resilience Capacity Index (RCI) and the Production Capacity Index (PCI) values differentiates the four response groups of plants (A, B, C and D) defined by Fernandez (1992), according to Yield Stress Score Index (YSSI) and Yield Potential Score Index (YPSI) values. Group A genotypes express uniform superiority under both stress and no-stress conditions; group B genotypes express good performance only under yield potential but not under stress conditions; group C genotypes present a relatively higher yield only under stress and group D poor yield performance under both environments. Yp: grain yield under yield potential conditions, Ys: grain yield under stress conditions

Groups	Yp	Ys	RCI	PCI	Combination's range of values	
					YSSI	YPSI
A	Med-high to High	Med-high to High	Med-high	High	High to Med-high	High to Med-high
B	Med-high To Med	Med-low to Low	Med	Med	Med	Med
			Low	Low	Low	Med.
				Med	Med-low	Med-high
				High	Med-high	High
C	Med To Med-low	Med-high to high	High	Med	High to Med-high	Med to Med-Low
D	Med-low to Low	Med-low to Low	High	Low	Med-high	Low
			Med		Med-Low	Med-low
			Low		Low	Med-Low

The groups are delimitated by a boundary line (Figure 2.2) which represents the minimum or maximum for each group. The boundary line could be represented by the average grain yield within the population under the corresponding environments or by using the yield of a local check (both could be used depending on the aim of the research). Consequently, depending on the range of values of Yp and Ys inside the groups, a range of values for RCI and PCI is expected to correspond to the variation of Yp and Ys.

As shown in Table 2.6, a unique combination of RCI and PCI values identifies and differentiates perfectly the four groups defined by Fernandez (1992), the only case where the combination is not unique is for the low yield under stress which could be obtained from group B or D, both with low PCI and RCI value. These responses could be differentiated using the Yp value, which is higher for genotypes B than genotypes D. In general, the unique combination and distinction of the different groups is illustrated by an example in Figure 2.7, where the resultant value, in this case YSSI, can be similar for genotypes included into groups A and C, however RCI and PCI will be different between these groups. In this particular case, genotypes C show a better

resilience (RCI) than genotypes A, and genotype A shows a better yield performance under non-stress.

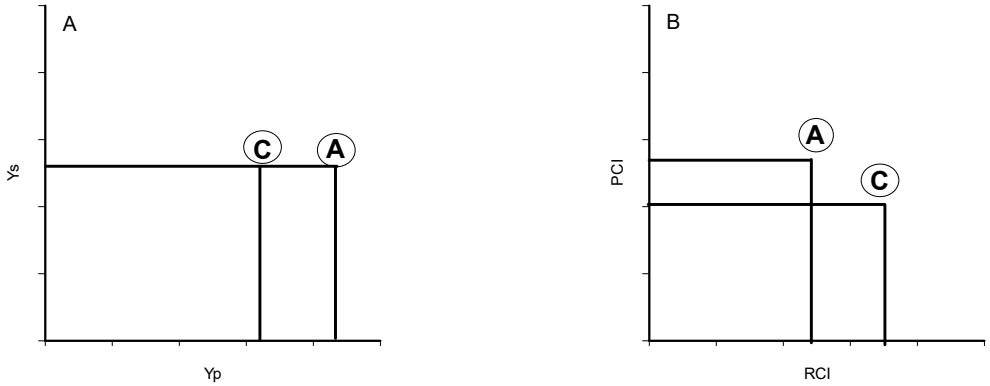


Figure 2.7: Schematic illustration of a particular case of two genotypes according to grain yield performance defined by Fernandez (1992): A) Schematic illustration of two genotypes from groups A and C with a similar grain yield value under stress (Ys) and different grain yield value under yield potential conditions (Yp), where: group A represents genotypes expressing uniform superiority in both stress and no-stress conditions, and group C represents genotypes expressing a relatively higher yield only under stress. B) Schematic representation of the distribution of values of the Production Capacity Index (PCI) and the Resilience Capacity Index (RCI) where genotypes A show a higher PCI compared with genotypes C, and *vice versa*, in terms of RCI.

These differentiations can be very useful for a crop breeding program focussed on discovering highly resilient and productive genotypes or only highly resilient ones for crossing with highly productive genotypes. For mechanistic research, contrasting genotypes in terms of resilience or productivity could provide an understanding of the impact of specific trait expression such as stomatal conductance, waxiness, hormone production, etc... For example, high yield production under stress can be derived from a genotype which is tolerant or has a good yield performance under non-stress, or both. Indeed, some genotypes from groups A and C can have a similar yield value under stress conditions, but genotypes from group C will present a lower yield under a non-stress condition (compared with genotypes from group A) but they will not reduce much their yield under stress and consequently, will have a better resilience to the stress which can be identified by a higher RCI value. Therefore, the score indices offer the possibility of easily visualizing the plasticity of genotypes in response to a particular stress by looking at the RCI and PCI values.

Table 2.7 shows a simple example for a small trial of ten genotypes under heat stress during the 2012-13 cropping season. One example of a contrasting genotype selection for fundamental research, based on yield, can be taken from these data: Genotypes 6 and 1 have a similar YPSI (Yp) but the YSSI (Ys) values are totally opposed, being the highest and the lowest, respectively. Additionally, genotype 6 has the highest PCI and RCI, and genotype 1 shows the lowest index values within the whole population.

Table 2.7: Values of the Resilience Capacity Index (RCI), the Production Capacity Index (PCI), and the result of their combination as the Yield Stress Score Index (YSSI) and the Yield Potential Score Index (YPSI). Values are calculated using the grain yield data from the CIMCOG-ROOT trial (ten genotypes) under yield potential conditions and heat stress, for the 2012-13 cropping season (Y12-13).

	Yield Y12-13			
Genotypes	RCI	PCI	YSSI	YPSI
1	1	1	1.00	0.00
2	4	3	3.50	-1.50
3	2	4	3.00	2.50
4	6	1	3.50	-7.00
5	4	9	6.50	6.50
6	10	10	10.00	-0.50
7	5	5	5.00	-0.50
8	5	7	6.00	2.50
9	2	7	4.50	7.00
10	4	7	5.50	4.50

At this point, an important question has to be raised. Does phenology influence the index selection method? It is widely known that the ability of plants to recover from abiotic stress (drought or heat) principally depends on the developmental stage at which the stress is experienced by the plant (Jäger *et al.*, 2008). In wheat, meiosis is a stage that is very sensitive to abiotic stress and stress at this point in development results in reduced pollen fertility and consequently a reduction in final grain number yield (Saini *et al.*, 1984; Acevedo *et al.*, 2002; Jäger *et al.*, 2008). Additionally, Tewolde *et al* (2006) stated that early-heading genotypes under heat stress had a longer grain filling period and completed a greater fraction of the grain filling earlier in the season when air temperatures were lower and generally more favourable compared to the later-heading cultivars. However, early-heading could be considered as an escape strategy

instead of having a tolerance and/or resilient adaptation. Consequently, in order to improve genotype selection for mechanistic research to discover new traits for stress resilience and to avoid selecting genotypes which may have an escape strategy, phenology should be taken into account. Effectively, as the index compares genotypes within the whole population, early genotypes showing a "good" RCI and/or PCI due to an escape strategy would modify the general range of resilience and/or production capacity with the risk to discriminate some genotypes with late phenology. In fact, these late genotypes could show better adaptive/tolerant traits to endure the stress although reducing more their yield and consequently showing a lower RCI and/or PCI compared with early genotypes. Considering this, in order to improve a contrasting selection by integrating the phenology into the index selection method, two approaches can be recommended:

The first approach, used for small trials of perhaps a ten of genotypes, analyses the whole population and identifies separately the early, mid and late genotypes and selects genotypes into groups of similar phenology. As an example of the first approach, in a small trial like CIMCOG-ROOT used for fundamental research, it was observed that genotypes 2 and 4 show an early phenology, reaching booting stage 5 and 7 days before the late genotypes (9 and 10), respectively, when the mean population reaches this stage 3 days before genotypes 9 and 10. Additionally, when genotypes 9 and 10 started meiosis, genotypes 2 and 4 were at the middle in progress of that phase. Two observations can be made on the performance of the early genotypes (genotype 2 and genotype 4). First observation, genotype 4 is the earliest genotype which starts meiosis 4 days before to the mean population, currently behaving as resilient (RCI = 6). So, it could show an escape strategy, and therefore becoming susceptible, if it would receive the stress at the same phenological stages with the same intensity as, for example, genotypes 7 and 8 (RCI=5). Second observation, genotypes 2 and 10 show similar susceptibility

(RCI=4), although genotype 10 received a higher stress during the susceptible phenological stages compared with genotype 2. Consequently, genotype 10 could be considered more resilient to heat stress than genotype 2 if phenology is taken into consideration. Therefore, in this specific case of the CIMCOG-ROOT trial, genotype 6 seems to be the most successful genotype, in terms of adaptation to stress and harvestable yield under stress. However, this genotype will be classified into the group C instead of group A, because its Yp (irrigated conditions) is lower than the mean yield of the population. It seems appropriate to recommend, for selection for abiotic stress tolerance and suitable yield performance, to consider genotypes included either into groups A and C showing high PCI and RCI value.

The second approach which will integrate phenology into the use of the index is recommended for interrogation of performance of a huge panel of hundreds of genotypes. The principle is to analyse the whole population and/or analyse separately the early, mid and late genotypes. For a breeding program, the problem of different phenology in a huge panel is similar either using conventional selection on yield or the use of the selection method based on the proposed indices (RCI and PCI), with the only difference being that the index method allows us to create “new populations” or “sub-populations” by grouping them in function of their phenology, as for example early, medium and late genotypes, and compare each group/sub-population with the check line or the mean of the “sub-population. The more uniform the population is, in terms of phenology, the better the index will perform in identifying contrasting genotypes on RCI and PCI, as one of the bases of the score index method is to compare the response of genotypes within the whole population. For example, the WAMI trial has been used to compare the conventional selection method - based on yield under both environments (stress and no stress) – with the two selections index method suggested here: 1) the index selection method without the integration of phenology, and 2) the index selection by grouping the genotypes according

to phenology (in this case, heading). For all these cases, the selection is done using a check line (Sokoll) as the reference. The yield method selects genotypes with higher or equal yield under both environments, compared with Sokoll. For the index selection method, the selected genotypes have similar score or higher compared with the check line (Sokoll). The first observation is that the index selection, compared to the conventional yield method, reduces the number of selected genotypes by 33%, and the index and phenology method reduces the number of genotypes selected by 48%. The second observation is that with the index method, 64% of genotypes match those of the yield method, being those genotypes classified into group A, while 36% are genotypes that would never have been selected with the conventional method, coming from group C. The third observation comparing the index method and the index integrating the phenology is that late genotypes are generally discarded using the index method.

2.5. Conclusions

The analysis and discussion in this Chapter show that score indices offer an easy-to-use new method to classify and visualize quickly which are the best or the worst crop genotypes within a population, in terms of resilience to stress and production. Additionally, score indices allow arithmetic operations to create a new index, YSSI, which expresses yield under stress into a simple score scale value. This expression of yield has demonstrated that yield under stress can usefully be perceived as a function of two major crop characteristics, the resilience capacity (RC) and the production capacity (PC).

This analysis opens new insights for selection of genotypes in crop breeding programmes, helping breeders and researchers to understand better the genotypic responses under stress. However, it has been observed that high productive and high resilient genotypes (Group A) are rare in nature and Blum (1996) has noted that an apparently negative association between yield

potential and drought resistance has been found in different researches, where genotypes with a superior adaptation to drought stress may have a lower yield under yield potential environments although some exceptions do exist. Blum (1996) suggested that the identification of factors involved in the negative relationship will be important to enable researchers to design a more efficient approach to be used in breeding for high yield and yield stability.

This work suggests that a wheat improvement program could beneficially use the score indices, RCI and PCI, simultaneously, in order to identify those rare genotypes which do not show these negative relationships. Such a course of action could reduce considerably the number of selected genotypes, focusing on resilience and productivity, allowing breeders to reduce costs and save time.

Finally, the use of a uniform criterion in fundamental research like RCI and PCI, would ensure more valid and useful comparisons between research results obtained across a selection panel where there is only a hazy understanding of potential selection criteria.

The next Chapter (Chapter 3) focuses upon testing the indices (RCI and PCI) on the different yield components in order to try to better understand the basis of yield under stress and identify where, in term of yield components, genotypes expressed their susceptibility to stress. This will allow easier identification of contrasting genotypes for mechanistic research.

Chapter 3. New selection criteria to enhance understanding of the basis of physiological and developmental resilience to abiotic stress

3.1. Introduction

A main target in most wheat crop breeding and pre-breeding programmes is to increase grain yield mainly by identifying high yielding wheat genotypes under favourable environmental conditions (yield potential conditions) (Araus *et al.*, 2004; Blum, 2005; Reynolds *et al.*, 2009; Foulkes *et al.*, 2011). Alternatively, under target stress environments (heat, drought, etc.), progress in increasing yield in future environments can be made through increased understanding of the mechanisms involved in plant tolerance/resistance to abiotic stress (Blum, 2011; Macková *et al.*, 2013).

It is expected that a better understanding of crop yield physiology will increase the efficiency of breeding to increase yield (Araus *et al.*, 2004). However, several proposed selection criteria have delivered little or no impact on crop yield under heat or drought and in most breeding programmes there is almost no direct selection for physiological traits (Richards, 1996, 2006). In order to be considered by breeders, selection criteria need to be easy and quick to measure and inexpensive but also it is crucial to be able to convince breeders about the usefulness of the proposed selection criteria (Richards, 1996; Monneveux *et al.*, 2012).

Grain yield is the main trait used by breeders during the last 50 years to make their decisions on selection (Araus *et al.*, 2004, 2008). However, yield is a complex trait and it has been suggested that yield should be studied as the function of the action and interaction of different simpler traits, and not used as a trait itself (Slafer, 2003). Consequently, in order to boost the genetic gains of yield potential, some have argued that we need to identify new selection tools by an improved understanding of crop yield physiology (Slafer, 2003; Araus *et al.*, 2004, 2008;

Fischer, 2007; Pask *et al.*, 2012; Reynolds *et al.*, 2012*a*). Such an approach might improve the effectiveness of breeding programmes. Calculation of yield components is a good approach to increase understanding of the complex make up of yield, where the two major components are the thousand grain weight (TGW) and the number of grains per square meter (G#/m^2) (Slafer, 2003, 2005). Nevertheless, G#/m^2 and TGW are variables that are almost as complex as yield itself. Effectively, these two components can be impacted by environment along the various stages of phenological development. It is usually considered that grain number per m^2 can be affected from emergence to anthesis and TGW is usually considered to be affected from a few days before anthesis until maturity (Slafer, 2005). Their variation depends on several G x E interactions (where G is genotype and E is environment) during organ differentiation along the phenological stages of development. These phenological stages are germination, emergence (Em), tillering, floral initiation (FI), double ridge (DR), terminal spikelet (TS), jointing or onset of stem elongation, booting (Boot), heading or spike emergence (Hd), anthesis (At), and physiological maturity (Acevedo *et al.*, 2002). These stages have been classified into 4 groups: emergence (Em) from germination to emergence; growth stage 1 (GS1) from emergence to double ridge; growth stage 2 (GS2) from double ridge to anthesis; and growth stage 3 (GS3), which includes the grain filling period, from anthesis to maturity (Hanft and Wych, 1982) (Figure 3.1).

The determination of the grain number per square metre (G#/m^2) is more complex than determination of thousand grain weight (TGW) as it is the result of the expression of many sub-components which can be influenced for long periods during plant development, from sowing to the beginning of the grain filling (Slafer and Rawson, 1994). The different sub-components of grain number per square meter (G#/m^2) are spikes per square meter (Spk#/m^2) and grain number per spike (G#/spk) which can be sub-divided into number of grains per spikelet

(G#/spklt) and number of spikelets per spike (Spklt#/spk). Spikelets per spike is usually affected during the early reproductive phase (from floret Initiation to terminal spikelet (TS), Figure 3.1) and grains per spikelet (G#/spklt) during the late reproductive phase (from terminal spikelet (TS) to anthesis (At), Figure 3.1).

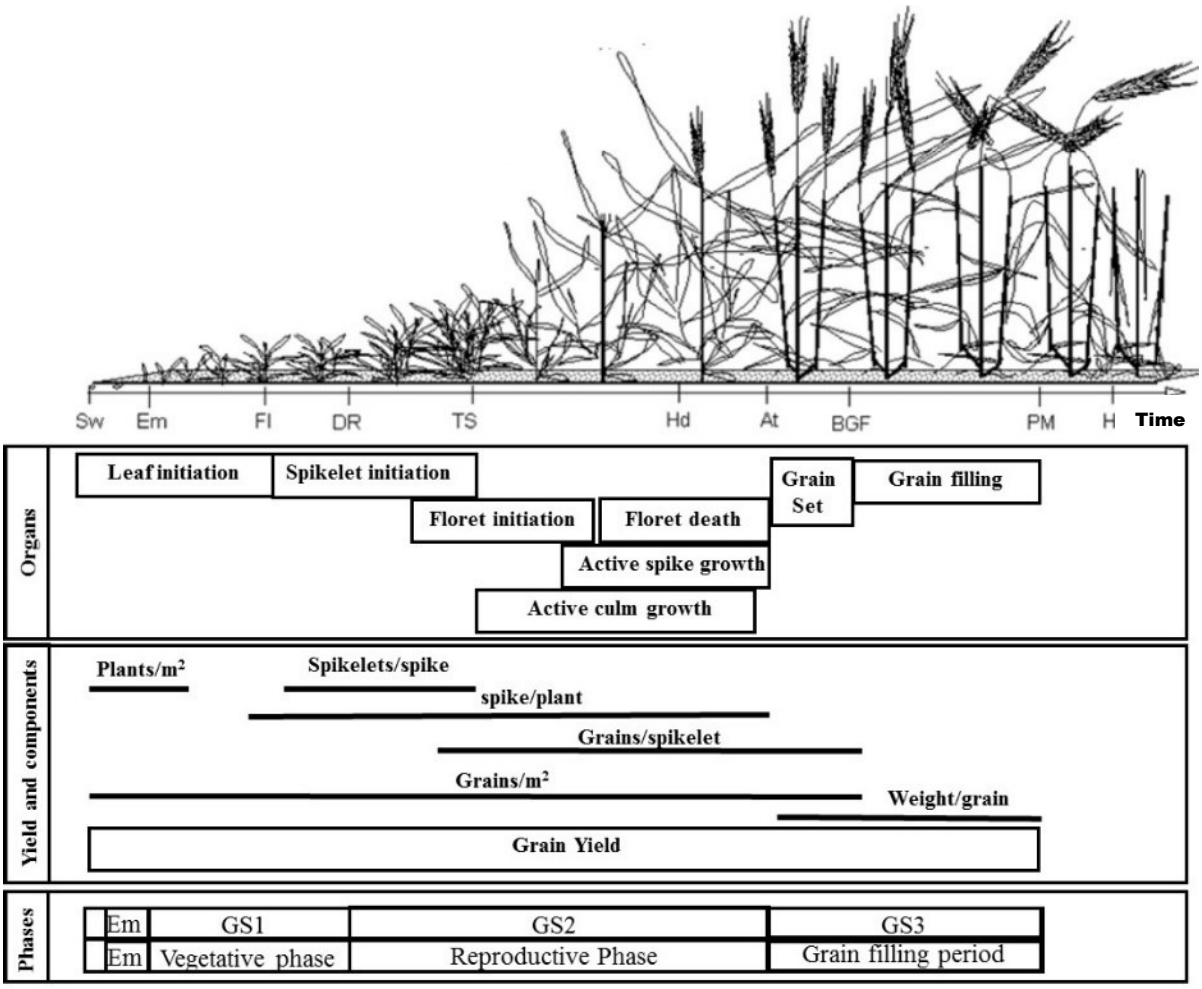


Figure 3.1: Schematic diagram of wheat growth and development stages, periods of initiation or growth of specific organs and periods of different components of Grains yield. Sw: sowing; Em: emergence; FI: Floral initiation; DR: double ridge appearance; TS: terminal spikelet initiation; Hd: heading; At: anthesis; BGF: beginning of Grains filling period; PM: physiological maturity; Hv: harvest; GS: growth stage. Source from Slafer and Rawson, 1994 and (Slafer, 2012)

The number of spikes/m^2, a strong function of tillering, tiller mortality and sowing density, is not associated to a specific developmental stage (from sowing to anthesis (AT), Figure 3.1) and can be influenced by environmental factors (such as: plant density, abiotic stress, nutrient

availability (nitrogen availability, etc.) and genetic factors (e.g. potential number of tillers) (Longnecker *et al.*, 1993; Acevedo *et al.*, 2002)

The manipulation of a single sub-component of yield seems a logical strategy to increase yield. However, a fundamental problem with this strategy is a frequently found negative correlation between sub-components, which means that an improvement in one sub-component will usually result in a negative impact on another(s) (Fischer, 1984; Slafer, 2003). This is partly associated with a competition for carbon, between vegetative and reproductive tissues, during the early stage of plant development (Acevedo *et al.*, 2002) but is also a function of the amount of resource capture, efficiency of use and allocation. It is imperative to better understand these negative relationships in order to improve yield (Slafer *et al.*, 1996). Recently, Slafer *et al.* (2014) concluded that agronomic practice will be able to enhance yield, mainly through an increase in grains number per m^2 ($G\#/m^2$) with negligible trade-offs in grain size, which means that a large change in $G\#/m^2$ may have a little impact on grain weight.

There is a high level of complexity of the response of yield to the environment due to the huge genotypic variation in the responses. This makes it difficult to guess the response of a genotype to the main environmental variables from the responses of other genotypes. This is partly due to the fact that genotype can show different sensitivity to main environmental variables (e.g. vernalisation, photoperiod, temperature, etc.), and the genotype response (sensitivity) to a single environmental factor can differ when this genotype is facing more than one factor (such as photoperiod and vernalisation), generating sometimes a cumulative response on plant development. This cumulative and/or primary responses can also be different between developmental phases (Slafer and Rawson, 1994).

An investigation of the determination of individual yield components and then the identification of the genetic basis of this variation would be an effective way to investigate the genetic basis of yield (Slafer, 2003). More recently Slafer *et al.* (2014) concluded that the regulation of spike number per m^2 is mainly driven by the environmental factors while grain number per spike is mainly driven by genotype and both yield components significantly influence the regulation of yield.

Plants will respond to stress with different susceptibility along their phenological development and this will affect the final yield production. Heat and drought have a direct or indirect impact on nearly all aspects of crop growth and development (Blum, 2011; Ferrise *et al.*, 2011; Zingaretti *et al.*, 2013), such as reduction in photosynthesis, increase in respiration, dry matter partitioning and inhibition of starch synthesis in the growing kernel (Rezaei *et al.*, 2015), morphological and anatomical modifications (Zingaretti *et al.*, 2013), plant development, accelerated senescence and root growth (Shpiler and Blum, 1990).

A better understanding of these effects of abiotic stress on reproductive development will drive the development of more reliable screening methods (Dolferus *et al.*, 2011) focused on the variation of grain number per m^2 ($G\#/m^2$), which has been identified as the best component to explain wheat yield variations under stress (Fischer, 1985), and on spike fertility (SF) (a major component of $G\#/m^2$) (Fischer, 1984; Abbate *et al.*, 1998; Martino *et al.*, 2015). However, a major complication of these traits, $G\#/m^2$ and SF, is the negative relation with grain weight (Martino *et al.*, 2015).

It is expected that future increases in understanding of plant stress resilient mechanisms will be driven by the recent progresses of fast and efficient screening methods for tolerant genotype as well as by the identification of highly susceptible germplasm and germplasm that shows

contrasts in resilience (Dolferus *et al.*, 2011). The effectiveness of a selection criterion, depends principally upon the relative importance of genetic and environmental factors in the expression of phenotypic differences between genotypes in a population, which correspond to the heritability of the selection criterion (Martino *et al.*, 2015).

In the previous Chapter, it has been shown that a better selection criterion for grain yield under stress should incorporate a consideration of stress resilience and not focus only on the productive capacity of a cultivar.

Therefore, the objectives of this Chapter are to better understand the complexity of yield responses under abiotic stress (heat and drought) and the interaction between genome and environments in terms of yield components, by first testing whether yield components under stress environment can be expressed by the inclusion of resilience and productivity factors in the stress score index. Further, it is important to know whether the expression of resilience and productivity is constant in different genotypes along the year and in response to different stresses. A discussion is also conducted to identify which yield components, in term of resilience or productivity, are mostly related to yield under stress and to identify where, in term of yield components, a particular genotype expresses its sensitivity/susceptibility to drought stress. This information will contribute to identification of contrasting genotypes for further evaluation of physiological responses to stress.

3.2. Materials and methods

Location and crop season of field experiments are described in Chapter 2 (Section 2.2.1).

The same panels used in Chapter 2 have been used in this work (namely, CIMCOG-ROOT and WAMI). These lines were evaluated under the same conditions that were described in Chapter 2 (Section 2.2.2, Table 2.2) with an additional environment included from the CIMCOG-ROOT

trial (namely, semi-drought stress). CIMCOG-ROOT lines were evaluated under semi-drought stress during the cropping seasons, 2012-13 from December to late May. The experimental design was the same as that described for drought stress in Chapter 2.

To generate a semi-drought stress, irrigation was applied at 50 % of emergence and 3 additional irrigations were also applied. These applications took place every 3 weeks, with the last irrigation at 65 days after emergence (DAE) when the earliest genotypes reached heading stage. The latest developing genotypes reached the heading stage 3-4 days later. With this kind of stress treatment applied in field conditions, the latest developing genotype (i.e. genotypes which reached a phenological stage (such as booting or heading) at a later point in the growing season) are likely to be more affected by the stress, as they received the stress at an earlier developmental stage.

3.2.1. Phenology

There are relatively few different scales for the identification of the developmental stages of the wheat crop. A non-destructive and relatively easy and fast method of scoring is the Zadoks scale, based on ten major developmental stages (germination, seedling development, tillering, stem elongation, booting, heading, anthesis, kernel and milk development, dough development, ripening) with each stage divided into 10 sub-stages for a total of 100 growth stages (Zadoks *et al.*, 1974; Pask *et al.*, 2012) (Full scale shown in Appendix 3 Table 1).

In our experiment, the evaluation of phenology of the 10 genotypes under field conditions was very precise as it was evaluated every day in the field at the same time between 8:30 am and 10:30 am. It was determined that a field plot has reached a particular developmental stage when 50% of the plants in the plot reached that stage. Seven phenological stages were evaluated:

initiation of booting (GS 41), booting (GS45), late booting (GS47), 1st spikelet (GS51), half-emergence (GS55), heading (GS 59) and anthesis (GS65) (Zadoks *et al.*, 1974).

3.2.2. Determination of yield components and harvest processes

At physiological maturity, yield components (thousand grain weight (TGW), grain number per spike and spike number per square meter) were determined by adapting the method described in Pask *et al.* (2012) as described into the following sections.

3.2.2.1. Biomass, harvest and yield

The crops were harvested by hand, cutting the stems at ground level in an area of one square meter (1.25 x 0.8m) per plot. To prevent plant material losses, all harvested biomass was laid on a large plastic bag spread on the ground at the time of cutting. All harvested biomass was inserted into a cloth bag by first inserting the spikes and then folding the stems, emptying all the residues lying over the plastic bag into the cloth bag. Cloth bags were tied to prevent loss of material during transport. At postharvest, the plot area harvested was re-measured to precisely quantify the harvested area. The pre-tare full cloth bags were weighed to determine the total above ground dry biomass, and the content was then threshed for grain yield weight.

3.2.2.2. Thousand grain weight

To determine the thousand grain weight (TGW), a sub-sample of 200 grains was counted from a random sample of whole grains harvested for each plot. Broken and aborted grains were discarded but not the small grains. The weight of these 200 grains was multiplied by 5 to obtain the TGW expressed in grams (g).

3.2.2.3. Grain number per spike

Outside the marked square meter plot (used for biomass, described above), 50 spikes from each plot under heat (H), semi-drought (SD) and drought (D) stress environments, and 100 spikes

from each plot under yield potential (Yp) conditions, were cut at the ground level randomly. In the field, spikes were separated from the stem at the node level, into two different pre-labelled paper bags. The full bags were weighed with a pre-tare balance before drying them in the oven (75° C) for 48 hours, including a bag sample in order to have a dry tare paper bag after the drying process. The dry paper bags were weighed. Before threshing the 50 spikes, the number of spikes in each bag was verified, and if necessary calculations were adjusted. Finally, the grains harvested were weighed. The number of grains per spike is estimated by dividing the weight of grains from the harvest of the 50 spikes by the weight of a single grain (TGW/1000) and the number of spikes collected.

3.2.2.4. Spikes per square meter

Before harvesting, at physiological maturity, in the selected meter square of the plot, the number of stems was counted 3 times for each row. The result was compared with the estimation of spikes/m^2 using the relation between the harvest from 50 spikes and the full yield.

3.2.3. Adaptation of the stress indices based on yield components

In the previous Chapter, it was proposed that yield under stress is determined by two components, the resilience and the production capacity index. These indices offer an easy avenue to compare and study the yield responses to stress environments within a whole population from two different aspects, the resilience and the production capacity. Genotypes under stress will show different response and susceptibility to stress at different stages of phenological development. The analysis of yield components will help to identify where and when (in terms of phenological development) these genotypes are relatively more susceptible. However, the values of the different yield components have different units and it is difficult to compare them, and therefore, also difficult to identify whether the yield has been mostly affected by one or more specific susceptibility factors.

In the previous Chapter, it was shown that yield production under stress can be expressed by the yield stress score index (YSSI), which is a combination of the resilience capacity index (RCI) and production capacity index (PCI) (Equation 1). This method offers an easy visualization by the use of a scoring scale of different traits expressed in different units. Additionally, the methodology offers an avenue to study the yield resilience to stress environments within a whole population integrating the effect of cumulative stress endured by plants during development.

$$\mathbf{YSSI = RCI + PCI} \quad \textbf{Equation 1}$$

Equation 1 has been adapted for yield components by changing the yield trait values (grain yield value under stress and non-stress environment) used in the calculation of RCI and PCI (shown in Chapter 2) to the trait values of individual yield components (yield components values under stress and non-stress environment such as TGW or G#/m^2) (Equation 2 and Equation 3) in order to determine whether the value of each yield component under stress condition could be also expressed by a combination of resilience and production capacity.

The RCI and PCI (Equation 1) correspond to the score value of previous indices, the Stress Susceptibility Index (SSI) (Fischer and Maurer, 1978) and the Stress Tolerant Index (STI) (Fernandez, 1992), respectively. The score value follows a scoring scale from 1 to 10, where low SSI value and high STI value are awarded a high score following the scoring method (described in the previous Chapter in the methods Section 2.2.6 “How to create a scoring scale?”)

$$\textbf{Adapted Resilience Capacity index} = [1-(ts/tp)]/[1-Ts/Tp)]_{score} \quad \textbf{Equation 2}$$

$$\textbf{Adapted Production Capacity Index} = [(ts \times tp)/(tp)^2]_{score} \quad \textbf{Equation 3}$$

In these equations, "ts" is the trait mean value (yield components, such as, TGW, G#/m^2, G#/spike, Spike/m^2, etc.) of the genotype under stress, "tp" is the trait mean value of the genotype under potential conditions. As "tp" is a reference value which represents the maximum value of each trait that each genotype can reach, the individual genotype value used for "tp" was the average of the two crop seasons under yield potential (irrigated condition). Ts and Tp are mean values of ts and tp of all the population under stress and non-stress (potential) conditions, respectively.

The calculation of the Trait Stress Score Index (T-SSI) is shown in Equation 4

$$\mathbf{T-SSI} = [\mathbf{1-(ts/tp)}]/[\mathbf{1-Ts/Tp})]_{\mathbf{Score}} + [(\mathbf{ts \times tp})/(\mathbf{tp})^{\mathbf{2}}]_{\mathbf{Score}} \quad \textbf{Equation 4}$$

3.2.4. Statistical Analysis

Statistical Analysis was performed as described in Chapter 2 Section 2.2.8.

3.3. Results

3.3.1. Can yield components be expressed through score stress indices?

Table 3.1, Table 3.2 and Table 3.3 show that all trait stress score indices (T-SSI, Equation 4) showed a positive and significant correlation with their respective original values of the various yield components under stress conditions (heat (H), drought (D) and semi-drought (SD), respectively). These values of the Pearson correlation coefficient are usually over 0.9 and very close to 1.

Table 3.1: Pearson correlation coefficient between the yield components score indices (Yield-SSI, TGW-SSI, G#/m2-SSI, G#/spk-SSI, Spk#/m^2-SSI, G#/spklt-SSI, Spklt#/spk-SSI) and their original value (yield, TGW, G#/m2, G#/spk, Spk#/m^2, G#/spklt, Spklt#/spk) obtained in the field, calculated on yield and yield components data from the CIMCOG-ROOT trial under heat stress during the crop season 2012-2013 (10 genotypes). Heat stress was irrigated along the crop cycle. (TGW: thousand grain weight, G#/m^2: number of grains per square meter, G#/spk: number of grains per spike, Spk#/m^2: number of spikes per square meter, G#/spklt: number of grains per spikelet, Spklt#/spk: number of spikelets per spike) * $P < 0.05$; ** $P < 0.01$

Heat Y12-13							
	Yield	TGW	G#/m^2	G#/spk	Spk#/m^2	G#/spklt	Spklt/spk
Yield-SSI	0.997**	0.576*	0.037	0.198	-0.151	0.303	0.020
TGW-SSI	0.424	0.925**	-0.845**	-0.492	-0.271	-0.353	-0.150
Gs#/m^2-SSI	0.215	-0.631*	0.964**	0.425	0.445	0.397	0.045
Gs#/spk-SSI	0.163	-0.250	0.475	0.996**	-0.522	0.800**	0.428
Spks#/m^2-SSI	-0.172	-0.463	0.408	-0.643*	0.993**	-0.488	-0.386
Gs#/spklt-SSI	0.154	-0.200	0.406	0.728*	-0.322	0.976**	-0.303
Spklt/spk-SSI	0.064	0.074	-0.047	0.422	-0.421	-0.157	0.995**

Table 3.2: Pearson correlation coefficient between the yield components score indices (Yield-SSI, TGW-SSI, G#/m2-SSI, G#/spk-SSI, Spk#/m^2-SSI, G#/spklt-SSI, Spklt#/spk-SSI) and their original value (yield, TGW, G#/m2, G#/spk, Spk#/m^2, G#/spklt, Spklt#/spk) obtained in the field, calculated on yield and yield components data from the CIMCOG-ROOT trial under semi-drought stress during the crop season 2012-2013 (10 genotypes). Semi-drought stress means that water supply was stopped at 65 days after emergence. (TGW: thousand grain weight, G#/m^2: number of grains per square meter, G#/spk: number of grains per spike, Spk#/m^2: number of spikes per square meter, G#/spklt: number of grains per spikelet, Spklt#/spk: number of spikelets per spike). * $P < 0.05$; ** $P < 0.01$

Semi-Drought Y12-13							
	Yield	TGW	G#/m^2	G#/spk	Spk#/m^2	G#/spklt	Spklt#/spk
Yield-SSI	0.997**	0.547	0.408	-0.290	0.741*	-0.460	-0.066
TGW-SSI	0.553*	0.873**	-0.398	-0.852**	0.166	-0.788**	-0.097
G#/m^2-SSI	0.660*	-0.230	0.917**	0.430	0.806**	0.197	-0.225
G#/spike-SSI	-0.198	-0.756*	0.644*	0.990**	0.045	0.842**	-0.165
Spk#/m^2-SSI	0.841**	0.213	0.583*	-0.232	0.907**	-0.442	0.097
G#/spklt-SSI	-0.499	-0.691*	0.247	0.814**	-0.317	0.962**	-0.464
Spklt#/spk-SSI	0.244	0.155	0.057	-0.212	0.273	-0.582*	0.885**

Table 3.3: Pearson correlation coefficient between the yield components score indices (Yield-SSI, TGW-SSI, G#/m2-SSI, G#/spk-SSI, Spk#/m^2-SSI, G#/spklt-SSI, Spklt#/spk-SSI) and their original value (yield, TGW, G#/m2, G#/spk, Spk#/m^2, G#/spklt, Spklt#/spk) obtained in the field, calculated on yield and yield component data from the CIMCOG-ROOT trial under drought stress (10 genotypes) during the crop season 2013-2014. Drought stress means that the last water supply was applied when 50% of the plot have reached emergence. (TGW: thousand grain weight, G#/m^2: number of grains per square meter, G#/spk: number of grains per spike, Spk#/m^2: number of spikes per square meter, G#/spklt: number of grains per spikelet, Spklt#/spk: number of spikelets per spike). * $P < 0.05$; ** $P < 0.01$

Drought Y13-14							
	Yield	TGW	G#/m^2	G#/spk	Spk#/m^2	G#/spklt	Spklt/spk
Yield-SSI	0.996**	0.542	0.150	-0.154	0.420	-0.028	-0.180
TGW-SSI	0.522	0.822**	-0.750*	-0.622*	-0.097	-0.448	-0.252
Gs#/m2-SSI	0.255	-0.620*	0.975**	0.553*	0.494	0.651*	-0.314
Gs#/spike-SSI	-0.093	-0.738*	0.814**	0.972**	-0.261	0.890**	0.120
Spks#/m2-SSI	0.393	0.020	0.323	-0.451	0.994**	-0.212	-0.597*
Gs#/spklt-SSI	0.033	-0.645*	0.802**	0.856**	-0.099	0.984**	-0.294
Spklt/spk-SSI	-0.152	-0.038	-0.071	0.045	-0.186	-0.383	0.875*

Under semi-drought and drought stress, the thousand grains weight under the different abiotic stresses (TGWs, where "s" corresponds to the respective stress) showed a slightly lower coefficient of correlation (r = 0.873 and r = 0.822, respectively) with the Trait Stress Score Index (T-SSI = (T-PCI + T-RCI)/2), where the trait "T" in this case is TGW) compared with

the other correlations between the original value of yield components and the respective T-SSI. Table 3.1, Table 3.2 and Table 3.3 show additional information about the negative relationship between the different yield components, such as thousand grain weight and grain number per square meter.

3.3.2. Comparison of yield and yield components between season and year

Table 3.4 shows that the score values and the mean score values of the different yield components are constant between the different stress environments (heat, drought and semi-drought) for each genotype when they are compared within the same population. Additionally, the method identifies which yield components of each genotype show more susceptibility. Four responses could be distinguished, in this population of 10 genotypes by focussing upon the Trait Stress Score Index (T-SSI) of the 2 major components TGW and G#/m^2 of yield of the different genotypes: 1) genotypes with a medium high TGW and medium high G#/m^2 (genotype 6), 2) genotypes with high TGW and low G#/m^2 (genotypes 5 and 9), 3) genotypes with low TGW and medium high G#/m^2 (genotypes 1), and finally, 4) genotypes with medium low TGW and medium high G#/m^2 (genotypes 2, 3, 4, 7, 8 and 10). For the determination of G#/m^2, similar constancy can be observed along the different stresses in terms of Spk#/m^2 and G#/spk (Table 3.4).

Table 3.4: Comparison of the score value on yield components under three different stresses, drought stress (D), heat (H) and semi-drought (SD). The colour scale is made into each environment and green shows the highest observed value, yellow is a medium value and the red value, the lowest. (Ys: yield under stress, YSSI: yield stress score index, SSI: stress score index, TGW: thousand grain weight, G#/m^2: number of grains per square meter, G#/spk: number of grains per spike, Spk#/m^2: number of spikes per square meter)

Entries	Ys (T/ha)	YSSI	TGW-SSI	G#/m^2-SSI	Spk/m^2-SSI	G#/spk-SSI
1 -H	2.11	1.0	1.7	6.0	5.0	6.5
1 -SD	4.01	5.5	1.7	8.5	5.5	9.5
1 -D	2.21	1.0	1.0	6.5	5.5	8.5
Mean			1.4	7.0	5.3	8.2
2 -H	2.34	3.0	5.7	3.0	3.5	5.0
2 -SD	4.01	5.5	4.3	5.5	5.0	7.0
2 -D	2.75	8.0	5.7	5.5	4.5	7.5
Mean			5.2	4.7	4.3	6.5
3 -H	2.33	4.5	2.7	6.5	4.5	8.5
3 -SD	3.86	5.5	4.7	6.0	6.0	7.0
3 -D	2.67	3.0	4.3	4.5	5.5	4.0
Mean			3.9	5.7	5.3	6.5
4 -H	2.34	3.5	4.7	5.0	10.0	1.0
4 -SD	3.46	2.5	4.7	3.0	3.0	4.5
4 -D	2.57	5.5	4.7	6.0	6.0	7.0
Mean			4.7	4.7	6.3	4.2
5 -H	2.60	6.0	7.7	3.0	4.0	4.0
5 -SD	3.86	4.5	5.7	2.5	5.5	2.5
5 -D	2.44	3.5	7.7	1.5	3.5	2.5
Mean			7.0	2.3	4.3	3.0
6 -H	3.00	10.0	8.0	6.5	5.0	5.5
6 -SD	4.57	9.0	8.7	5.5	4.5	4.5
6 -D	2.86	9.5	7.0	5.5	9.5	2.5
Mean			7.9	5.8	6.3	4.2
7 -H	2.52	5.00	5.00	5.50	4.00	6.50
7 -SD	4.13	6.50	4.67	7.00	7.00	5.00
7 -D	2.72	8.00	5.00	6.00	5.50	7,50
Mean			4.9	6.2	5.5	6.3
8 -H	2.59	6.0	3.7	7.0	4.5	7.0
8 -SD	4.86	10.0	7.3	7.0	9.0	3.0
8 -D	2.92	10.0	5.0	7.0	6.5	8.0
Mean			5.3	7.0	6.7	6.0
9 -H	2.46	4.5	10.0	1.0	1.5	3.5
9 -SD	3.26	1.0	5.0	1.0	1.0	4.5
9 -D	2.70	7.0	6.7	4.0	3.5	6.0
Mean			7.2	2.0	2.0	4.7
10 -H	2.53	5.5	4.0	5.5	2.0	9.5
10 -SD	3.62	3.0	3.3	4.5	3.5	7.0
10 -D	2.60	6.0	4.3	5.0	3.0	8.0
Mean			3.9	5.0	2.8	8.2

3.4. Discussion

3.4.1. T-SSI a useful tool to understand yield behaviour?

All yield components have shown a positive correlation with their respective trait stress score index (T-SSI, Equation 4) (Tables 3.1-3.3) which demonstrate that T-SSI can be used as a

surrogate of the yield components. However, TGW shows a slightly lower coefficient of correlation under semi-drought and drought (Table 3.2 and Table 3.3, respectively) *versus* the Trait Stress Score Index (T-SSI = (T-PCI + T-RCI)/2), where the trait "T" in this case is TGW) compared with the other correlations between the yield components and the respective T-SSI. Analysing the relationship between TGW-RCI and TGW-PCI *versus* TGWs, TGWs shows a higher correlation with the production capacity index (TGW-PCI) than when compared with T-SSI (in this case: TGW-PCI + TGW-RCI) (Table 3.5). This suggests that the reduction of TGW under stress conditions (TGW-RCI) does not have the same influence on the regulation of TGWs compared with TGW-PCI. However, the relationship can be improved by modifying the calculation of TGW-SSI by doubling the PCI value in the equation (Equation 5, Table 3.5, and Appendix 3A). This could be justified by the fact that grain size is usually more stable than the other yield components such as grain number (e.g. Dolferus *et al.*, 2011).

$$\boldsymbol{TGW - SSI = (TGW - RCI + 2 * (TGW - PCI))/3} \quad \textbf{Equation 5}$$

Table 3.5: Pearson coefficient correlation (r) and coefficient of determination (r^2) of the thousand grain weight (TGW) *versus* 1) the TGW resilience capacity index (TGW-RCI), 2) the TGW production capacity index (TGW-PCI), 3) the trait stress score index (TGW-PCI + TGW-RCI)/2 and 4) the TGW- stress score index (TGW-RCI+2*TGW-PCI)/3. ** $P < 0.01$

	TGWs					
	Drought (D)		Heat (H)		Semi-drought (SD)	
	r	r^2	r	r^2	r	r2
TGW-RCI	0.022	0.000	0.501	0.251	0.346	0.120
TGW-PCI	0.936**	0.876	0.952**	0.906	0.957**	0.916
(TGW-PCI + TGW-RCI)/2 (Equation 4)	0.822**	0.676	0.925**	0.856	0.873**	0.762
(TGW-RCI+2*TGW-PCI)/3 (Equation 5)	0.982**	0.964	0.985**	0.970	0.977**	0.955

TGW-PCI explains 87.6%, 90.6% and 91.6% of the variability in TGWs (where "s" corresponds to the respective stress) of this population, under drought, heat and semi-drought stress, respectively which is higher than the Trait Stress Score Index (T-SSI, Equation 4) (67.6%, 85.6% and 76.2%, respectively) (Table 3.5). However, the adapted index for TGW (Equation 5) improves the explanation of the variability of TGWs to 96.4%, 97.0% and 95.5% in this population under drought, heat and semi-drought, respectively.

The calculation of Equation 5 suggests 1) thousand grain weight (TGW) can be expressed by the two components (resilience and production capacity) 2) the regulation of TGW under stress (TGWs) is coarsely regulated by production capacity index of TGW (2/3 of TGW) and finely regulated by the resilience capacity index of TGW (1/3 of TGW). This could be explained by the fact that TGW under stress (TGWs) and TGW under non-stress (TGWp) are correlated and therefore more stable, which is not the case for the other yield components (Table 3.6).

Table 3.6: Pearson coefficient correlation (r) and coefficient of determination (r^2) on the different yield components under yield potential (Yp) *versus* each stress environment (H, D, SD). The letter "p" and "s" at the end of each yield component correspond to the value obtained under yield potential and the value obtained under the respective stress (D, H, SD), respectively. (TGW: thousand grain weight, G#/m^2: number of grains per square meter, G#/spk: number of grains per spike, Spk#/$m^{2;}$ number of spikes per square meter, G#/spklt: number of grains per spikelet, Spklt#/spk: number of spikelets per spike). *P< 0.05

	Drought (D)		Heat (H)		Semi-drought (SD)	
	r	r^2	r	r^2	r	r^2
Yp *vs* Ys	-0.149	0.022	0.146	0.021	-0.044	0.002
TGWp *vs* TGWs	0.880*	0.774	0.846*	0.716	0.855*	0.731
G#/m^2p *vs* G#/m^2s	0.310	0.096	0.346	0.120	0.567	0.322
Spk#/m^2p *vs* Spk/m^2s	0.339	0.115	0.568	0.322	0.713	0.509
G#/spk p *vs* G#/spk s	0.405	0.164	-0.003	0.000	0.126	0.016
G#/ Spklt p *vs* G#/spklt s	0.414	0.172	-0.357	0.128	0.165	0.027
Spklt /spike p *vs* Spklt/spk s	0.718*	0.516	0.245	0.060	0.408	0.167

This correlation between TGWs and TGWp suggest that TGW is more influenced by the genetic background and less influenced by the environmental factors. In this regard, Sareen *et al.*, (2012) have recommended the stress tolerance index (STI) as the best index to identify genotypes with high TGW under both stresses. However, data presented in their research on twenty-eight synthetic wheat lines under heat stress suggest that TGWs and TGWp are also highly correlated. (Appendix 3A shows the same proposal tested on a bigger panel of 294 genotypes termed WAMI.)

Under the three stress environments (heat – Table 3.1, drought – Table 3.2 and semi-drought – Table 3.3), the two major components, TGW *versus* G#/m^2, show a negative and significant relationship, as well as the sub-components of G#/m^2 (G#/spike *versus* G#/spklt), under drought and semi-drought stress. However, the correlation becomes non-significant under heat stress

(TGW *versus* G#/spike or G#/spklt). Initially it was thought that the negative relationship between the two major components was largely independent of carbon competition, as their determination occurs during different growth stages (Miralles and Slafer, 1995). Effectively, the G#/m^2 is mainly determined during the reproductive phase (Growth Stage 2 (GS2), Figure 3.1) and the grain weight is mainly determined during the grain filling period (Growth Stage 3 (GS3), Figure 3.1). However, grain size depends on carpel size and its development overlaps the determination of grain number during the spike growth period (Calderini *et al.*, 1999; González *et al.*, 2011) and this could partially explain the negative relationship. On the other hand, it seems that large improvement in grain number will have more impact on improving the final yield compared with large improvement on TGW (Slafer *et al.*, 2014). Consequently, it is necessary to achieve a better understanding of the basis and the dynamics of the negative relationships between 1) G#/m^2 and TGW, and 2) between the grains per spike (G#/spike) and number of spikes per square meter (Spk#/m^2) in order to manipulate them properly to increase yield (Slafer *et al.*, 1996, 2014). The T-SSI index might be a useful tool to understand the basis and the dynamics of these relationships between the yield components and for this it is needed to identify the consistency of the traits.

3.4.2. Comparison of yield and yield components between season and year

Yield components (traits) are expressed on different scales and therefore, they cannot be compared between themselves or between years (as the environment between years and locations always shows some variation). It has been shown that the use of a scoring scale makes the visualization easier to compare different values expressed on different scales (described in the previous Chapter).

In order to understand the dynamics of the plasticity of the different yield components, it is important to identify contrasting genotypes on the basis of reliable traits. It will be useful to determine whether or not wheat genotypes follow a similar pattern in their response to different abiotic stresses and if they show a particular susceptibility on particular yield components when they are confronted by stress.

The use of the index allows the investigation of the basis of the response to stress shown by different genotypes and shows whether or not these responses are consistent under different abiotic stresses. Due to the complexity of the relationship between yield components and sub-components, this analysis is first focussed on the two major components (TGW and G#/m^2) and secondly on the first two sub-components of grain number per square meter (G#/m^2) which are grain number per spike (G#/spk) and spike number per square meter (spk#/m^2).

The use of the score index allows an easy visualization of the plasticity in terms of yield components of a population under different environments (Table 3.4). Actually, the original trait values show high variability between years and stresses (heat (H), drought (D), semi-drought (SD)), and this is why it is difficult to compare them and to understand how wheat plants respond to the stress in terms of yield components. The proposal is that the score index method is an ideal tool to put all the variables on a similar reference scale (1-10 score values, as explained in Chapter 2 for yield), making the comparison easier between population, yield components, years and stresses (Table 3.4).

In terms of yield production (expressed by the YSSI) there are more variations, but if we compare heat and drought environments, genotype 1 always yields less and genotypes 6 and 8 yield more, compared to the whole population. This is a first approach which demonstrates that

these 10 genotypes tend to modify their yield components with a similar susceptibility under different stresses when the response is compared to the whole population.

In order to visualize better the constancy of the indices under the different stress, Table 3.7 shows the correlation of the indices (T-SSI) between the different stresses on a pairwise basis (drought *versus* heat, drought *versus* semi-drought, heat *versus* semi-drought). Table 3.7 shows that the trait stress score indices (T-SSI) of the two major components (thousand grain weight (TGW) and grain number per square meter (G#/m^2)) are significantly correlated between the different stress environments (heat, drought and semi-drought) with the only exception for TGW between heat and semi-drought. This suggests some genotypes in this population respond similarly to the different stresses in terms of TGW and G#/m^2, but it is not in the case for the sub-components of G#/m^2, except for the grain number per spike between drought and semi-drought.

Table 3.7: Table Pearson coefficient of correlation (r) and coefficient of determination (r^2) between the two major yield components, thousand grain weight (TGW) and grains per square meter (G#/m2), and the first two sub-components of G#/m^2, number of spikes per square meter (spk#/m^2) and number of grains per spike (G#/spk). TGW and G#/m^2 show significant correlation across the different environments with the exception of TGW under heat and semi-drought. ** $P<0.01$, * $P<0.05$.

	TGW-SSI		G#/m^2-SSI		Spk#/m^2-SSI		G#/spk-SSI	
	r	r^2	r	r^2	r	r^2	r	r^2
D vs H	0.875**	0.766	0.637*	0.406	0.469	0.220	0.267	0.071
D vs SD	0.732*	0.536	0.723*	0.523	0.313	0.098	0.620*	0.385
H vs SD	0.537	0.289	0.728*	0.531	0.066	0.004	0.243	0.059

Appendix 3 Table 3 shows the full table of the resilience capacity index and productive capacity index of all the genotypes calculated on each of the yield components and demonstrates that there is constancy in their expression under different stress environments when their variations are compared within the same population between year and stress. Since these traits show a certain constancy between year and stress, in order to identify a good selection criterion, it is necessary to identify which yield components in terms of resilience or productivity are best related to yield.

3.4.3. Can T-RCI and T-PCI increase understanding of the genetic basis of yield under stress?

Table 3.8 shows that yield production under heat and drought stress (Y-H, Y-D, respectively) and yield production capacity index (Y-PCI) is a compromise between the mean productivity, in terms of grain weight (TGW-PCI), and the number of grains per unit area (G#/m^2-PCI). Effectively, TGW-PCI and G#/m^2-PCI show a negative and significant correlation under D, H and SD environments. Additionally, TGW-PCI shows a significant and positive correlation with yield under D and H, while G#/m^2-PCI shows a significant negative correlation with yield under D and H, but this is not significant under SD conditions. On the other hand, G#/spike-PCI shows a negative correlation with yield under SD conditions. These results confirm the conclusion of Avci *et al.*, (2005) where number of grains per spike has been identified to have a negative contribution to yield under drought conditions.

Table 3.8: Pearson coefficient of correlation (r) and coefficient of determination (r^2) between yield under three different stress environments, drought (Y-D, table A), heat (Y-H, table B) and semi-drought (Y-SD, table C) *versus* the production capacity index of (1) yield (Y-PCI), (2) thousand grain weight (TGW-PCI), (3) grain number per square meter (G#/m^2-PCI), (4) spike number per square meter (Spk#/m^2-PCI) and (5) grain number per spike (G#/spk-PCI). **P* < 0.05; ** *P*< 0.01.

A	Y-D		Y-PCI		TGW-PCI		G#/m^2-PCI		Spk#/m^2-PCI		G#/spk-PCI	
	r	r2	R	r2	R	r2	r	r2	R	r2	R	r2
Y-D	1.00	1.00										
YPCI	0.92**	0.84	1	1								
TGW-PCI	0.64*	0.41	0.82**	0.68	1.00	1						
G#/m^2-PCI	-0.56*	0.32	-0.72*	0.52	-0.97*	0.94	1.00	1				
Spk/m^2-PCI	-0.12	0.01	-0.21	0.05	-0.37	0.14	0.50	0.25	1.00	1		
G#/spk-PCI	-0.30	0.09	-0.39	0.15	-0.59	0.35	0.53	0.28	-0.40	0.16	1.00	1
B	Y-H		Y-PCI		TGW-PCI		G#/m^2-PCI		Spk/m^2-PCI		G#/spk-PCI	
	r	r2	R	r2	R	r2	r	r2	R	r2	R	r2
Y-H	1.00	1.00										
YPCI	0.93**	0.86	1.00	1.00								
TGW-PCI	0.73*	0.53	0.78**	0.60	1.00	1.00						
G#/m^2-PCI	-0.56*	0.31	-0.61*	0.37	-0.93**	0.87	1.00	1.00				
Spk/m^2-PCI	-0.33	0.11	-0.44	0.20	-0.67*	0.45	0.82**	0.67	1.00	1.00		
G#/spk-PCI	-0.16	0.02	-0.01	0.00	-0.20	0.04	0.10	0.01	-0.47	0.22	1.00	1.00
C	Y-SD		Y-PCI		TGW-PCI		G#/m^2-PCI		Spk/m^2-PCI		G#/spk-PCI	
	r	r2	R	r2	R	r2	r	r2	R	r2	R	r2
Y-SD	1.00	1.00										
YPCI	0.98**	0.97	1.00	1.00								
TGW-PCI	0.45	0.20	0.49	0.24	1.00	1.00						
G#/m^2-PCI	-0.01	0.00	-0.04	0.00	-0.85**	0.72	1.00	1.00				
Spk/m^2-PCI	0.49	0.24	0.46	0.21	-0.42	0.17	0.79**	0.62	1.00	1.00		
G#/spk-PCI	-0.67*	0.45	-0.66	0.43	-0.73	0.53	0.47	0.22	-0.14	0.02	1.00	1.00

Table 3.9 shows that yield production under stress (Y-D, Y-H, Y-SD) and yield resilience capacity index (Y-RCI) are highly associated with the resilience in terms of number of grains per unit area (G#/m^2-RCI) and resilience on TGW (TGW-RCI) seems to have a much reduced or no influence on the determination of yield under the respective stresses. Effectively, G#/m^2-RCI shows a positive and significant correlation with yield under the three different stresses (r = 0.869, for drought, 0.756 for heat and 0.850 for semi-drought stress) while TGW-RCI show no relation (r = -0.268, for drought, -0.083 for heat and 0.364 for semi drought stress).

Table 3.9: Pearson coefficient of correlation (r) and coefficient of determination (r^2) between yield under three different stress environments, drought (Y-D, table A), heat (Y-H, table B) and semi-drought (Y-SD, table C) *versus* the resilience capacity index of (1) yield (Y-RCI), (2) thousand grain weight (TGW-RCI), (3) grain number per square meter (G#/m^2-RCI), (4) spike number per square meter (Spk#/m^2-RCI) and (5) grain number per spike (G#/spk-RCI). *$P < 0.05$; ** $P < 0.01$.

A	Y-D		Y-RCI		TGW-RCI		G#/m^2-RCI		Spk#/m^2-RCI		G#/spk-RCI	
	r	r2	R	r2	r	r2	r	r2	r	r2	R	r2
Y-D	1.00	1.00										
Y-RCI	0.91**	0.82	1.00	1.00								
TGW-RCI	-0.27	0.07	-0.13	0.02	1.00	1.00						
G#/m^2-RCI	0.87**	0.76	0.88**	0.77	-0.57*	0.32	1.00	1.00				
Spk/m^2-RCI	0.77**	0.59	0.72*	0.52	-0.15	0.02	0.74*	0.54	1.00	1.00		
G#/spk-RCI	0.12	0.01	0.31	0.10	-0.35	0.12	0.35	0.12	-0.30	0.09	1.00	1.00

B	Y-H		Y-RCI		TGW-RCI		G#/m^2-RCI		Spk/m^2-RCI		G#/spk-RCI	
	r	r2	R	r2	r	r2	r	r2	r	r2	r	r2
Y-H	1.00	1.00										
Y-RCI	0.90**	0.82	1.00	1.00								
TGW-RCI	-0.083	0.01	-0.12	0.01	1.00*	1.00						
G#/m^2-RCI	0.76*	0.57	0.82**	0.67	-0.65*	0.42	1.00*	1.00				
Spk/m^2-RCI	0.067	0.01	0.32	0.10	0.44	0.19	-0.03	0.00	1.000	1.00		
G#/spk-RCI	0.38	0.14	0.37	0.14	-0.92	0.85	0.80*	0.63	-0.53	0.28	1.00	1.00

C	Y-SD		Y-RCI		TGW-RCI		G#/m^2-RCI		Spk/m^2-RCI		G#/spk-RCI	
	r	r2	R	r2	r	r2	r	r2	r	r2	r	r2
Y-SD	1.00	1.000										
YRCI	0.96**	0.91	1.00	1.00								
TGW-RCI	0.36	0.13	0.45	0.20	1.00	1.00						
G#/m^2-RCI	0.85**	0.72	0.88**	0.77	0.02	0.00	1.00	1.00				
Spk/m^2-RCI	0.72*	0.52	0.65*	0.42	0.20	0.04	0.64*	0.41	1.00	1.00		
G#/spk-RCI	0.33	0.11	0.40	0.16	-0.25	0.06	0.59	0.35	-0.15	0.02	1.00	1.00

In order to generalise a conclusion about which trait is more related to yield between the two major yield components (thousand grain weight (TGW) and grain number per m^2 (G#/m^2)) and their indices (RCI and PCI), this was investigated with a bigger panel (WAMI 294 genotypes under heat stress).

Table 3.10 shows the Pearson coefficient correlation and the coefficient of determination between yield (on WAMI under heat 294 genotypes) *versus* TGW and G#/m^2 and their respective components in terms of resilience and production capacity index (RCI and PCI, respectively), under heat stress. Yield under heat stress, in this case (294 genotypes) is highly correlated with the resilience and productive components of yield (Y-RCI, Y-PCI, respectively) and grain number per square meter (G#/m^2-SSI) and its resilience components (G#/m^2-RCI). Additionally, Y-RCI shows a positively and significant correlation with G#/m^2 and in particular with G#/m^2-RCI, similarly to the results shown in Table 3.9 with 10 genotypes (CIMCOG-ROOT). However, with 294 genotypes (WAMI), yield is neither related with the stress score index of thousand grain weight (TGW) nor its components (RCI, PCI), in opposition with the results obtained with the CIMCOG-ROOT trial under heat stress (Table 3.8 and Table 3.9). The relationship found between TGW and grain yield in the small panel (Table 3.8 and Table 3.9) could be due to the contrast existing among these genotypes, as they were previously selected mainly to study partitioning of carbohydrates to roots and this could have resulted in reduced genetic variability and/or diversity in this small panel.

Table 3.10: Pearson coefficient of correlation (r) and coefficient of determination (r^2) between yield under stress (Ys), thousand grain weight (TGW) and number of grains per square meter (G#/m^2) and their respective resilience and production capacity index (RCI and PCI, respectively). Data from WAMI trial, 294 genotypes, under heat stress during the cropping season 2012-2013. * $P < 0.05$

	Ys		Y-RCI		Y-PCI		G#/m^2		G#/m^2-RCI		G#/m^2-PCI		TGW		TGW-RCI		TGW-PCI	
	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2
Ys	1.00	1.00																
Y-RCI	0.78*	0.61	1.00	1.00														
Y-PCI	0.89*	0.79	0.17	0.03	1.00	1.00												
G#/m^2	0.80*	0.64	0.66*	0.44	0.69*	0.47	1.00	1.00										
G#/m^2-RCI	0.78*	0.61	0.77*	0.59	0.59	0.35	0.81*	0.66	1.00	1.00								
G#/m^2-PCI	0.61	0.37	0.42	0.18	0.59	0.34	0.89*	0.79	0.48	0.23	1.00	1.00						
TGW	-0.06	0.00	-0.11	0.01	0.01	0.00	-0.60	0.37	-0.33	0.11	-0.65*	0.42	1.00	1.00				
TGW-RCI	-0.06	0.00	-0.16	0.03	-0.22	0.05	-0.52	0.28	-0.47	0.22	-0.42	0.18	0.58*	0.33	1.00	1.00		
TGW-PCI	-0.23	0.05	-0.05	0.00	0.10	0.01	-0.52	0.27	-0.22	0.05	-0.61*	0.38	0.95*	0.90	0.33	0.11	1.00	1.00

Additionally, Table 3.10 shows that the negative relationship between G#/m^2 and TGW seems to be more associated with G#/m^2-PCI (r = -0,65), rather than with G#/m^2-RCI (r = -0,33) and similarly between TGW-PCI versus G#/m^2-PCI (r = -0,61). Consequently, this differentiation

could be an opportunity to increase yield by increasing the resilience of the wheat plant in terms of grain number per m^2 without reducing that much the TGW under stress environment. This conclusion supports the conclusion of Slafer *et al.* (2014) that with the above knowledge there is a possibility that a crop breeding programme, added to the improvement of agronomic practices, could increase grain number per m^2 with negligible trade-offs in grain size.

This possibility offers new perspectives for breeding selection where the resilience capacity of grain number per square meter (G#/m^2-RCI) might be a good candidate to identify new physiological traits associated with higher yield and/or to link new gene expression directly to the resilience of grain number per square meter. As suggested by Slafer (2003), yield should be considered as the result of the action and interaction of different traits (yield components) determined by particular genes instead of a trait itself and he recommends a focus on two principal genetic factors influencing yield which are 1) genes conferring adaptation to stress environment, and, 2) genes more related with the potential productivity. The resilience capacity index (RCI) should show how sensitive the different genotypes are to the environment and the production capacity index (PCI) should give more information about the mean production capacity or potential productivity in terms of yield and yield components between stress and non-stress environments. It could be proposed that these two indices (PCI and RCI) could help to identify new genes delivering on the two principal genetic factors highlighted by Slafer (2003) (adaptation and potential productivity) based on straightforward traits (yield components). However, more experiments on a mapping population should be done to test this hypothesis.

Appendix 3B shows that the variation of the production capacity index (PCI), between genotypes under stress environments, seems to be more related to the genetic background and

less influenced by the environment. This conclusion is based on high correlations, close to 1, of the different traits' production capacity index (T-PCI) (the different traits are: thousand grain weight, grain number per m^2, grain number per spike, spike per m^2) when they are compared under different stress environments (drought *versus* heat, drought *versus* semi-drought and heat *versus* semi drought) (Appendix 3 Table 4 in Appendix 3B). This suggests that the production capacity index is more related to the potential yield of each genotypes than the effect of the environment. In contrast, the resilience capacity index (RCI) of the same traits (thousand grain weight, grain number per m^2, grain number per spike, spike per m^2) shows more variability when compared under different stress environments (drought *versus* heat, drought *versus* semi-drought and heat *versus* semi drought) (Appendix 3 Table 5 in Appendix 3B). The sub-components of the resilience component of grain number per m^2 (G#/m^2-RCI = spike number per m^2-RCI + grain number per spike -RCI) show in particular more variability in response to the different stresses (drought, heat, semi-drought). This suggests that these sub-components of G#/m^2-RCI (spike number per m^2-RCI and grain number per spike -RCI) could be interesting traits to identify the susceptibility of genotypes under a specific stress environment.

In the next section, contrasting genotypes are identified based on the resilience component of grain number per m^2 to start further investigations on physiological responses.

3.4.4. Identifying contrasting genotypes for drought stress resilience using T-RCI.

In much fundamental research, the selection of contrasting genotypes to compare the response of susceptible and tolerant genotypes is the basis of many investigations, in order to understand the mechanisms involved in the plant's physiological and developmental responses to a particular stress to identify genotypic variation in specific traits. Therefore, tolerant and susceptible genotypes need to be carefully distinguished to improve the understanding of stress

resilient mechanism as concluded by Dolferus *et al.* (2011). The reproductive stage (GS2) is the most sensitive developmental stage to stress, but a reliable screening method which will help at improvement of abiotic stress resilience at this stage does not yet exist (Dolferus *et al.*, 2011). This could be provided by the T-RCI.

Table 3.11 shows the different RCI and PCI values calculated on yield and yield components under drought stress. Genotypes have been grouped by pairs of contrasting responses on yield production, when it was possible, or by similar production under stress. By analysing these groups, it is possible to find some apparent stress resilience and a range of susceptibility in terms of yield components.

Table 3.11 (RCI) shows that the resilience or the susceptibility of the genotypes, in response to drought stress, can be expressed differently, in term of yield components. For example, the high yielding genotypes (genotypes 6 (Y-PCI=9) and 8 (Y-PCI =10), Table 3.11 (PCI)), show higher resilience in terms of grain per m^2 (G#/ m^2). For genotype 6 this resilience (G#/ m^2) was mainly driven by a high resilience in terms of spike per m^2 and a medium resilience in terms of grain per spike while for genotype 8 the overall resilience appears to be driven by the resilience in terms of grain per spike and medium resilience in terms of spike per m^2, when the responses are compared with the whole population. Similarly, poor yielding genotypes (genotypes 1 (Y-PCI =1) and 5 (Y-PCI=5), Table 3.11 (PCI))) show their susceptibility on different sub-components of the grain number per m^2. Effectively, genotype 1 was more susceptible in terms of spikes number per m^2 and genotype 5 is more susceptible in terms of grains per spike. Additionally, it is important to note that genotype 1 shows a high resilience in terms of grains per spike while genotype 5 shows a medium score value in term of spikes per m^2 and a high

resilience in terms of thousand grain weight. The other genotypes do not arouse special interest for contrasting selection, as they show similar responses in terms of Y-RCI.

Table 3.11: Summary table of the components of the trait stress score index, the trait resilience capacity index (T-RCI) (left table) and the trait production capacity index (T-PCI) (right table) under drought stress, during the cropping season 2013-14. Genotypes are grouped by pairs of contrast on yield resilience for the first four genotypes and the other four are grouped by their resilience on yield and/or grain number per m^2 (Data from CIMCOG-ROOT - 10 genotypes). (Ys: yield under stress, TGW: thousand grain weight, G#/m^2: number of grains per square meter, Spk#/m^2: number of spikes per square meter and G#/spk: number of grains per spike)

RCI						
Entries.	Ys (T/ha)	Y	TGW	G#/m^2	Spk#/m^2	G#/spk
1	2.21	1	1	3	1	9
6	2.86	10	1	10	10	4
5	2.44	2	7	1	4	1
8	2.92	10	1	9	5	10
3	2.67	8	4	6	4	10
7	2.72	10	3	8	6	8
2	2.75	9	3	8	8	7
4	2.57	8	10	4	5	6
9	2.70	6	2	6	6	4
10	2.60	6	3	5	4	6

PCI						
Entries.	Ys (T/ha)	Y	TGW	G#/m^2	Spk#/m^2	G#/spk
1	2.21	1	1	10	10	8
6	2.86	9	10	1	9	1
5	2.44	5	8	2	3	4
8	2.92	10	7	5	8	6
3	2.67	6	4	7	6	7
7	2.72	6	6	4	5	7
2	2.75	7	7	3	1	8
4	2.57	3	2	8	7	8
9	2.70	8	9	2	1	8
10	2.60	6	5	5	2	10

The use of the resilience index on yield components (T-RCI) and the previous analysis on both high and poor yielding genotypes allow identification of where there is weakness or strength of the genotypes, in response to the environment, in terms of yield components. This is a new insight, at least for mechanistic research, as this technique allows selection of better contrasting genotypes not only by their resilience or susceptibility on yield but also by their susceptibility on yield components which can be associated to a phenological stage or developmental period (Figure 3.1). Additionally, T-RCI and T-PCI provide better understanding of the existing negative relationship between yield components as it is necessary to visualize clearly where the genotypes are compensating yield components in response to stress. For example, the relationship between thousand grain weight and grain number per square meter can be explained by the variation in grain number per spike or by the variation in spike per square meter or both (Table 3.11). As the indices show the data on a same scale it is easier to identify the weakness.

Into this population of ten genotypes (CIMCOG-ROOT trial), in order to study the hormone response to drought stress and relate it with a mechanism of resilience, only 4 genotypes (1, 5, 6, 8) were identified showing an appropriate contrast. Genotypes 6 and 8 can be considered as tolerant genotypes in terms of grain yield and grain per m^2 and genotypes 1 and 5 can be considered as sensitive. But these contrasts change when the focus is on the number of grains per spike, one of the sub-components of grain number m^2, where genotype 1 shows a better resilience in term of grain per spike compared to genotype 6.

This change of tolerance classification may be associated with the sowing method (described in the methods section of Chapter 2) practiced by CIMMYT (Physiology group) in all of their field trials (including the experiments described here). Although this is a standard trials procedure, we should recognise that sowing all genotypes at $5g/m^2$ could induce an error in quantification of yield components. By sowing a fixed weight of seed when grain weight shows differences between genotype may result in a different seedling density. An increase in seedling density can increase grain yield and ear weight of genotypes with low tillering potential (Valério *et al.*, 2013). Seedling density may affect the number of spikes per square meter (see e.g. Appendix 3 Table 7) which shows that genotype 1, a low yielding genotype, with a lower TGW compared to the population, was sown with a higher density (136 seeds per m^2) while genotype 6, a high yielding genotype, was sown with a lower density (95 seeds per m^2). It was shown by Valério *et al.* (2013) that a higher density increases yield until an optimal threshold (350 seed per m^2) is reached and the difference of seeding could have an effect on selection. Similarly, genotypes 5 and 8 which also contrast on yield have shown similar seeding density and do not change their tolerance classification in terms of grain per metre square and grain per spike.

Finally, to show the usefulness of the score indices, Appendix 3D shows the analysis of the relationship between the resilience and production components of trait stress score index (T-RCI and T-PCI) and the canopy temperature which is the temperature of the vegetative cover and recommended by CIMMYT's pre-breeding programme for selection of tolerant genotypes under severe stress (heat and drought) (Blum et al., 1989; Reynolds et al., 2012b). In this population of ten genotypes, CT shows a negative relationship with yield resilience, due to its relationship with the resilience capacity on grain number per square meter (G#/m2-RCI) (Appendix 3 Table 8 and Appendix 3 Figure 1). This suggests some mechanistic basis for using CT as a screening method but for resilience only and not for productivity as is usually thought to be the case. However, this apparent linkage needs further investigation on a bigger data set.

3.5. Conclusion

To conclude, the trait resilience capacity index (T-RCI) is a good approach to identify adaptation to stress and the score indices on yield components are also a good tool to identify contrasting genotypes (in terms of yield components) for mechanistic research. The analysis has shown that both resilience and susceptibility can show different bases in response to stress. As suggested by Slafer (2003) an effective way to investigate the genetic basis of yield will be to investigate the determination of individual yield components and then identify the genetic basis of this variation. In order to identify new physiological responses to abiotic stress resilience, such as hormone signals, it could be feasible to select contrasting genotypes which contrast in two yield components while the other variables are fixed.

Therefore, the recommendation is to select contrast genotypes with the same weakness or strength in the different yield components in order to avoid or reduce any error in the interpretation of the response. However, this kind of selection requires a huge panel of

genotypes. In this work, the panel under study was too small and in order to study resilience mechanisms in terms of G#/spike and in terms of G#/m2 during the reproductive stage (GS2) under drought stress, genotypes 1, 5, 6 and 8 have been identified, from the population of 10 genotypes (CIMCOG-ROOT) as the most appropriate for the focus of the work.

This analysis has been focused only on drought stress as the further experiments (field and controlled environments) described in this thesis focus upon hormone quantification in droughted plants. However, Appendix 3C presents the results of an equivalent analysis of heat effects showing where these 10 genotypes expressed their susceptibility to this stress.

Chapter 4. Preliminary experiments in controlled environments to define the field methodology for hormone sampling

4.1. Introduction

In the previous Chapters, a new method has been devised to quantify the mechanistic basis of responses of wheat genotypes to environmental stress. This method, based on two score indices (production and resilience capacity) will be used to test the hypotheses that hormones balance could be an effective physiological trait, for deployment in a plant improvement programme. It is important to keep in mind that a new trait for breeding selection needs to be easy, fast and stable over the measurement period otherwise the criteria will not be used by breeders (Richards, 1996, 2006; Monneveux et al., 2012). Therefore, a simple and fast method to quantify hormones is required to evaluate a potentially huge number of genotypes under field conditions but also to set a robust methodology to compare results between years and environments.

Attribution of the accumulation of a hormone in a field-grown plant to a single stress factor can be difficult, since synthesis of ethylene, for example, may be affected by pest damage, disease, chemical applications, heat, drought, mechanical wounding (Abeles *et al.*, 2012). Other factors such as circadian regulation of plant growth regulator synthesis (PGR) must be taken into account (Mcclung, 2000). The diurnal periodicity of ethylene production has been quantified in barley, wheat and rice (Ievinsh and Kreicbergs, 1992; Kobayashi and Saka, 2000) and also in other crops such as cotton (Jasoni *et al.*, 2002) or *Vicia faba* (faba bean) (El-Beltagy and Hall, 1974). Most of those researches have observed a peak of ethylene at mid-photoperiod (Thain *et al.*, 2004) and this has been associated with a rhythm in mRNA abundance for the enzyme ACC oxidase (ACO) (which transforms ACC to ethylene), the ACC oxidase activity,

in sorghum (Finlayson *et al.*, 1999) and to the expression of multiple ACC synthase (ACS) enzyme ACC oxidase (ACO) (which transforms ACC to ethylene), the ACC oxidase activity, in sorghum (Finlayson *et al.*, 1999) and to the expression of multiple ACC synthase (ACS) genes, in *Arabidopsis thaliana* (Thain *et al.*, 2004). Some evidence has shown that the regulation of Abscisic Acid (ABA) synthesis is also under circadian control. In *Arabidopsis thaliana* grown under controlled environment conditions, foliar ABA concentration peaked at mid-day (Lee *et al.*, 2006). In maize, genes encoding enzymes involved in the biosynthesis of carotenoids such as phytoene synthase (PSY, a precursor in ABA biosynthesis) were under circadian control, with a peak expression during in the early morning (Khan et al., 2010). In Arabidopsis, the diurnal fluctuation of ABA is associated with the fluctuation of the enzyme b-glucosidase (AtBG1) which transforms, by hydrolysis of the biologically inactive ABA (ABA-glucose ester (ABA-GE)) to ABA (Lee *et al.*, 2006). In maize, ABA accumulation is linked with the rhythm of the abundance of mRNA encoding for phytoene synthase, the enzyme governing the first committed stage in carotenoid synthesis (Khan *et al.*, 2010). This kind of variation may affect the stability and/or reliability of the quantification of ethylene and/or ABA which is needed to identify genetic variations in potential hormone traits.

Much of the field work for this thesis was undertaken at CIMMYT's CENEB station (Campo Experimental Norman E. Borlaug), near Ciudad Obregón, in the state of Sonora, northern Mexico. Here, flood irrigation is practiced, which could induce extra ethylene production based on studies of wheat plants grown in controlled environments. Beltrano *et al.* (1997) showed that when plants (in pots) were re-watered after drought stress, ethylene emission from plants was increased. The second experiment of this Chapter were conducted to investigate the possibility that flood irrigation will significantly impact ethylene accumulation under field conditions so that this can be taken into account when constructing screening protocols.

It is also important to note that under drought stress, some inconsistency appears in the literature in terms of ethylene production (Morgan and Drew, 1997). It has been suggested that rapid induction of plant water stress will promote ethylene production, and slow induction of water stress should inhibit its production. It might therefore be expected that under field conditions where plant water stress might develop slowly, ethylene accumulation might be reduced compared with hormone levels in irrigated trials. In contrast under laboratory conditions using restricted volume and highly porous media for root development, ethylene might be increased compared with the well-watered (WW) conditions (Morgan and Drew, 1997). Others researchers argue that the inconsistency of results is due to the method of measuring ethylene (Morgan *et al.*, 1990; Narayana *et al.*, 1991). Indeed, the most commonly used method is the headspace method which allows estimation of the production rates of ethylene (or ethylene accumulation) of a detached plant organ in a sealed container with a saturated air in water (Abeles *et al.*, 2012; Chen *et al.*, 2013). It has been argued that studying ethylene by using detached leaves, plants under water stress tend to increases ethylene production, while studies carried out in intact plants under the same conditions reduce ethylene synthesis compared with control plants (Morgan *et al.*, 1990; Narayana *et al.*, 1991). Additionally, it has been shown that ethylene production can vary according to the tissue, plant species and phenological stage (Wheeler *et al.*, 1996, 2004). Consequently, to quantify properly ethylene and ABA on a large scale, it is necessary to define a protocol which will be followed for all experiments (controlled environment conditions and field) allowing consistent and reliable quantification of hormones. For this purpose, two experiments are described in this Chapter A) to identify the best time of the day to quantify the ethylene and ABA production/accumulation and B) to test the effect of re-watering plants on ethylene production following a transient drought stress.

4.2. Materials and methods

All of the experiments described in this Chapter have been performed under controlled environment conditions (in the greenhouses or in the controlled environment (CE) rooms at the Lancaster Environment Centre).

4.2.1. ABA sampling and radioimmunoassay (RIA)

Leaf samples (the first or the second youngest fully expanded leaf) were placed into pre-labelled Eppendorf tubes (2 ml) and immediately frozen in liquid nitrogen contained in a cool box. The samples were kept in a -80ºC ultra-freezer for three days. After this time, the samples were stored at -20ºC in a freezer before being freeze-dried during 48 hours. Afterwards, dry leaves tissues (enclosed in the 2 ml Eppendorf) were ground using a ball mill (MM400 Retsch GmbH, Germany) during 4 minutes for each cycle. A maximum of 20 samples can be ground per cycle.

The ABA analysis was carried out at Lancaster LEC laboratories using an adapted radioimmunoassay (RIA) method described by Quarrie *et al*. (1988). For extracting the ABA from the ground tissue, +/- 20 mg of ground material was mixed with deionized water at the ratio 1:40 (mg:µl) in a 1.5 ml Eppendorf tube and shaken at 4°C during 13 hours (overnight). Afterwards, the Eppendorfs were centrifuged at maximum speed (1500 RPM) during 4 minutes and 50 µl of supernatant was added to each tube containing 200 µl of phosphate-buffered saline (PBS). Then, 100 µl diluted [3H]-ABA was added to each tube and finally 100 µl diluted antibody MAC 252. To homogenize the mixture, the tubes (previously sealed) were centrifuged (1500 RPM) for one minute and then kept in the fridge at 4-6 degrees for 45 minutes. Then 500 µl of saturated ammonium sulphate was added to each tube and mixed by hand. The mixture was left at room temperature for 30 minutes in the dark. After this, the tubes were centrifuged (1500 RPM) for 4 min to precipitate the ABA-antibody complex at the bottom of the tube. The

supernatant was then removed. To eliminate the possible excess of unbound radioactivity, a washing process consisting of re-suspending the pellet in 1.0 ml of 50% saturated ammonium sulphate and then centrifuging (1500 RPM) for 5 minutes to form a new pellet. The new supernatant was then removed. Finally, 100 µl deionized water was added to each tube and the pellet was re-suspended and after 1.5 ml of Econsint H was added to each tube. The radioactivity was measured with a liquid scintillation counter (Packard TriCARB 1600TR liquid scintillation analyser, Canberra, CT, USA). In order to interpret the result and calculate the concentration of hormone, a standard curve is necessary. During the process, 8 ABA standards were used to determine the optimum for maximum and minimum binding, Bmax and Bmin respectively (0 (Bmax), 62.5, 125, 250, 500, 1000, 2000 and 2×106 pg 50 µl-1 (+)-ABA (Bmin)). These ABA standards were prepared from (±)-ABA (A1049, Sigma-Aldrich). The quantification of ABA in the standards followed the process described above. The tubes (standards and samples) were placed on a foam rack with 50 free spaces, from which 8 spaces were used for standards with a maximum of 42 used for samples. The analysis of each experiment for each tissue and environment was conducted in the same foam rack. In general, two foam racks were run simultaneously (time necessary for one person to run two foam racks is around 3 hours 20 minutes to 4 hours and 6 minutes are required to analyze each sample by the liquid scintillation counter and the analysis was run overnight). This method allows the analysis of a maximum of 240 samples per day (by running three foam racks simultaneously) and radioactivity of all samples was counted using a liquid scintillation counter.

4.2.2. Ethylene sampling and quantification

The choice of the method to quantify hormones in this study has had to bear in mind the laboratory facilities and the logistics of the field work. For example, ethylene emission can be quantified under dark conditions as described in Beltrano *et al.* (1994) but this method presents

problems for field work as it involves putting plant tissue between two sheets of moist filter paper for an hour in the dark at 27°C, and secondly incubating the material rubber-sealed tube for an hour in the dark and at constant temperature (27°C) before finally extracting the gas from the tubes. This methodology was not reproducible under Mexican field conditions and therefore the method used to quantify ethylene emission rates was adapted from the method described in Wilkinson and Davies, (2009), Iqbal *et al.* (2011) and Chen *et al.* (2013) for field and laboratory conditions.

The fresh tissues (the youngest fully expanded leaf) were cleanly cut (leaf at the ligule level and spike at the node level) and then quickly and gently inserted into 25 ml glass test tubes, containing a fully moistened (deionized water) filter paper. Tubes were then sealed with rubber stoppers (Suba-Seal, SLS, Nottingham, UK), for incubation under the same light environmental conditions where the plants were sampled. For the CE-room studies, these were 500 µmol photons. m^{-2} s^{-1} and for greenhouse studies, 550 µmol photons m^{-2} s^{-1}, for 1 hour and 20 minutes. After incubation, 1ml of gas was extracted from each tube with a 1 ml syringe and immediately injected into a pre-labelled hermetically sealed 6 ml vial with a rubber lid previously crimped with an aluminium seal.

Before and after the sampling gas extraction process, the vials were kept out of direct light in a cool box. In the same cool box after incubation, incubation tubes were kept. The fresh weight was determined by the weighing tube method, which consisted of an estimate of the fresh weight of the plant sample (the difference between weight of a pre-prepared tube with the fully humidified filter paper and the weight of the same tube containing the fresh material) (Appendix 4A). This method was developed to allow accurate fresh weight measurement at the field level.

The gas contained in the individual vials (6 ml) was measured through a laser-based ethylene detector system ETD-300 (Sensor Sense B.V., Nijmegen, The Netherlands). These vials were connected to inlet and outlet cuvettes of the VC-6 system (which allowed measurement of six vials per cycle of 30 minutes). The ETD-300 was set up in sampling mode with air flow at 4.5 l/h, so that sensor switched automatically after 5 minutes to each succeeding sample. To remove any traces of external ethylene or other hydrocarbons, the airflow was passed through a platinum-based catalyser before entering the vials. A scrubber with KOH and $CaCl_2$ was placed in line in front of the ethylene detector to reduce the CO_2 and water content of the gas sample. The EDT measures the volume of ethylene contained in the samples (nl). To determine ethylene emission rate all the data were corrected, for incubation time, tube volume, tissue fresh weight (Fw), and expressed in nL g Fw^{-1} h^{-1} or nmol gFw^{-1} h^{-1}. The ETD-300 allows analysis of around 100 samples per day.

4.2.3. Diurnal variation of hormone accumulation under well-watered conditions.

Four contrasting genotypes of wheat from the CIMCOG trial (60 genotypes) were used in this experiment. Two tolerant (T1 and T2) and two sensitive (S1 and S2) to heat stress. This selection was initially based on the yield reduction between yield potential (irrigated trial) and heat stress using yield data of a combined analysis of two years (2010-11 and 2011-12). Within the population, the genotypes which showed bigger differences in yield between these two environments were considered as sensitive and *vice versa* as tolerant.

Seeds were germinated in Petri dishes on saturated filter paper (deionized water) and covered with absorbent paper saturated with water (deionized) under dark conditions. When coleoptiles reached 3 cm in length (around 1 week), the germinated seeds were placed in pots (volume = 3.73 L, height = 21 cm, diameters: top = 17 cm and bottom = 13 cm), initially filled with John

Innes nº2 compost, three seeds per pot and six pots per genotype. Then, plants were placed under well-watered conditions, in a controlled environment room (CE Room), under a 12 hours photoperiod (from 8:00 am to 8:00 pm, 500 µmol photons m^{-2} s^{-1}) and day/night temperatures of 25ºC and 18ºC, respectively. The last watering was performed two days before sampling. The pot watering was applied to soil level. Leaf tissues were sampled at tillering stage (when second tillers started to become visible) at three different times of day under non-stress conditions, 1) at 8:30 am which corresponds to 30 minutes after the light was turned on, 2) at 12:00 pm corresponding to mid-photoperiod (4 hours after light was turned on) 3) and finally at 05:30 pm which is the latest time to measure ethylene emissions rate in the CE, 2 hours and 30 minutes before the light was turned off. The youngest mature leaf was sampled for ethylene emission (as described above), and the second youngest leaf was sampled for ABA (as described above). The experiment was a randomized complete block design (RCB) with 6 replications.

4.2.4. Ethylene production by plants re-watered after drought stress.

Four contrasting genotypes of wheat from a sub-set of the CIMCOG trial (30 genotypes) were used in this experiment. Two tolerant genotypes (T3 and T4) and two genotypes that were more sensitive (S3 and S4) to heat stress. This selection was initially based on the yield reduction between yield potential (irrigated trial) and heat stress (described in the previous Section 4.2.3) using the yield data of a combined analysis of two years (2010-11 and 2011-12) under heat stress and yield potential of a sub-set of the CIMCOG trial (30 genotypes).

The protocols for seed germination and seedling growth are described in the previous Section 4.2.3. Four seeds per pot and 8 pots per genotype were used for this experiment. The plants were placed in the glasshouse under a 12 hours photoperiod (550 µmol photons m^{-2} s^{-1}). The

day/night temperatures were respectively 25ºC/18ºC, and the mean humidity 50% during the night period and 40% during the day period.

At tillering stage (2-3 tillers), around 20 to 24 days after planting, the pots were moved to a controlled environment room (CE room) (500 µmol photons m^{-2} s^{-1}) to allow better control on the photoperiod compared with the glass house condition. The sampling was done at mid photoperiod 4 hours after the light was turned on.

During the first ten days of acclimation to the new environment (from glasshouse to CE room) and before starting the ethylene quantification, at tillering stage, the plants were kept under well-watered conditions. The plants were set out on a tray for easy re-watering and to avoid any additional stress due to the re-watering process, by refilling the tray every two days with the corresponding volume (for each tray, of 200 ml/pot).

The design of the experiments was a completely randomized with four replications and divided into four steps. Step 1: corresponds to initial ethylene status with no stress (day 1) and the last watering was performed 2 days before; Step 2 corresponds to the ethylene quantification with drought stress (day 4); Step 3 corresponds to the ethylene quantification three hours after re-watering (day 5); Step 4 corresponds to the ethylene quantification one day after the re-watering (day 6). This procedure was followed in order to see how the plants recovered their ethylene production status and to better understand the effect of the re-watering process on the ethylene emission. Figure 4.1 shows the mean soil moisture measured at the first 6 centimetres (measured with the Delta-T model HH2, UK) at the four different steps to quantify ethylene emission before, during and after a drought stress.

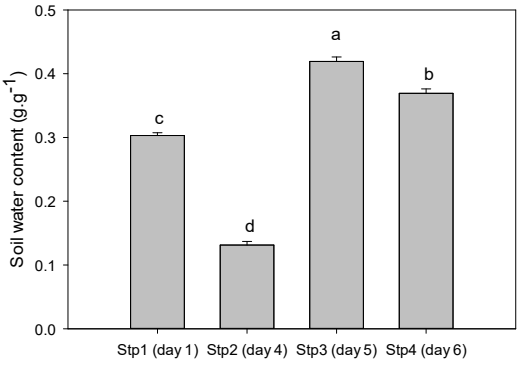


Figure 4.1: Mean soil moisture measured at the four different steps to quantify ethylene emission before, during and after a drought stress treatment (CE room). (Stp1): corresponds to initial ethylene status with no stress (day 1) and the last watering was performed 2 days before, (Stp2) corresponds to the ethylene quantification under drought stress (day 4), (Stp3) corresponds to the ethylene quantification three hours after re-watering (day 5), (Stp4) corresponds to the ethylene quantification one day and three hours after the re-watering (day 6). The days are counted from the first day of sampling. Measurement performed at the first 6 centimetres (measured with the Delta-T model HH2,UK). Bars represent means ± SE of 8 replicates, with different letters indicating significant ($P<0.05$) differences.

For both experiments, a deeper study of the contrasting response of the four genotypes to heat stress on yield was made using the index score based on yield (explained in Chapter 2) to compare the initial selection made before the development of the index and the contrast obtained with the index method (Appendix 4B experiment 1 and 2). The index method shows the contrast of these genotypes is mostly in yield resilience under heat stress (Y-RCI) and not in the yield production capacity index (Y-PCI) for both experiments. However, these experiments are an attempt to identify whether genetic variability does exist in the diurnal variation of hormone accumulation and/or in the effect of the re-watering process and whether any variation should be considered when defining a protocol for hormone quantification (ethylene and ABA). Therefore, the lack of contrast on productivity does not represent a problem here.

4.2.5. Statistical Analysis

Statistical Analysis was performed as described in Chapter 2 Section 2.2.8.

4.3. Results

4.3.1. Diurnal variation of hormone accumulation under well-watered conditions.

Figure 4.2 (A) shows a significant peak of ethylene production 4 hours after the light was turned on (12:00 pm) when compared with the ethylene production 30 mins after the light was turned on (08:30 am), for four contrasting genotypes under well-watered (WW) conditions at tillering stage. Additionally, significant differences, in term of ethylene accumulation, are observed between T2 and S2 at 12:00 pm. However, it is important to notice that genotypes which are more sensitive to heat stress (S1 and S2) have showed a significant reduction of leaf ethylene emission rate at 05:30 pm compared with the peak production at 12:00 pm, while tolerant genotypes (T1, T2) did not.

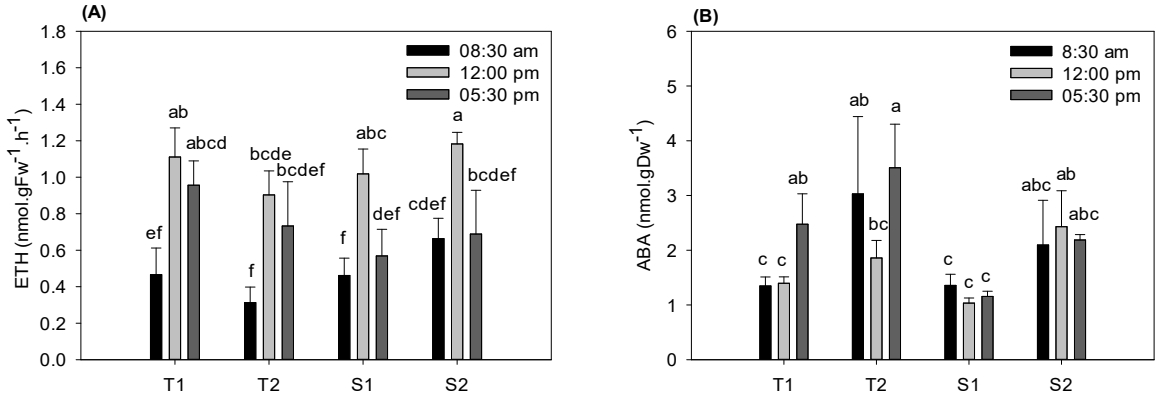


Figure 4.2: Wheat leaf hormone diurnal variation at three different times (8:30 am, 12:00 pm, 05:30 pm) of A) Ethylene emission and B) ABA accumulation under well-watered at tillering stage of four contrasting genotypes. T1 and T2 are the tolerant genotypes and S1 and S2 are the sensitive, in term of yield resilience under heat stress. Bars are means ± standard error of 6 replicates, with different letters indicating significant ($P < 0.05$) differences among the genotypes and times

ABA accumulation in leaves was not significantly different throughout the day for all genotypes, with the exception of one tolerant genotype (T1) which showed a significant peak of ABA accumulation at 05:30 pm (Figure 4.2 B).

4.3.2. Effect on the re-watering after a drought period

Figure 4.3 shows that the ethylene emission rate varies along the four steps, before, during and after a drought stress, and following re-watering the pot. During the drought stress (Stp2), the

ethylene concentrations is reduced significantly, for most of the genotypes (T3, T4, S4 ($p<0.05$)), compared with the initial ethylene emission rate (Stp1, Figure 4.3). Three hours after the re-watering (Stp3) three of four genotypes (T3, T4, S3) showed a significant increase ($P < 0,05$) of ethylene emission rate compared with the ethylene emission rate under severe drought stress (Stp2) but this was not the case for S4. Finally, it is observed that the ethylene emission rate at Stp4 (ethylene emission one day after re-watering) is significantly lower than the emission at Stp1 for T3, S3 and S4.

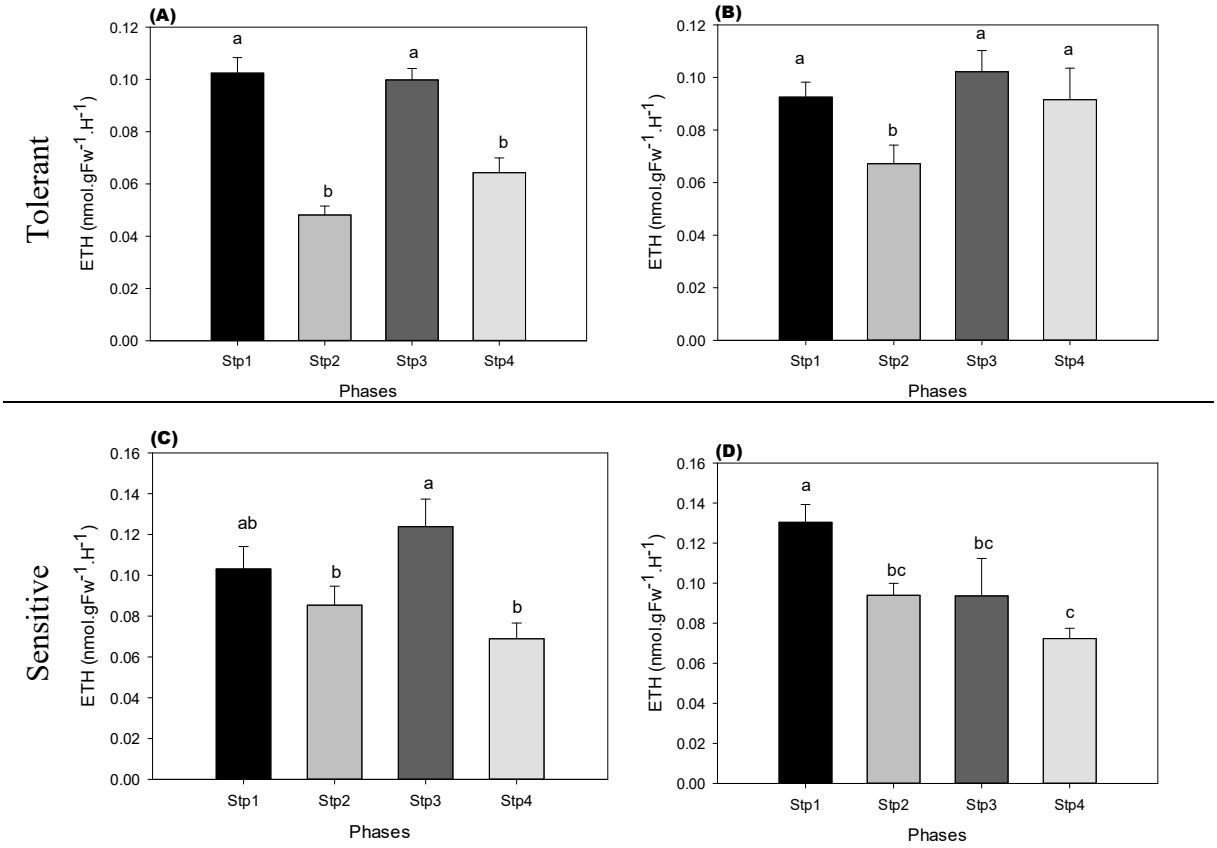


Figure 4.3: Variation of Ethylene emission on leaf, before and after a drought, of four contrasting genotypes to heat stress, two tolerant (T3 and T4 – (A), (B), respectively) and two sensitive (S3 and S4 - (C) and (D), respectively). The ethylene emission is studied at four steps: (Stp1) corresponds to initial ethylene status with no stress (day 1), (Stp2) corresponds to the ethylene quantification with drought stress (day 4), (Stp3) corresponds to the ethylene quantification three hours after re-watering (day 5), (Stp4) corresponds to the ethylene quantification one day and three hours after the re-watering (day 6). The days are counted from the first day of sampling. Columns and bars are means ± standard error of 4 replicates with different letters indicating significant ($P < 0.05$) difference among the steps.

4.4. Discussion

4.4.1. Diurnal variation

This experiment shows that ethylene emission is influenced by the diurnal variation and the genetic background. The peak of ethylene emission observed at 12:00 pm of well-watered wheat at tillering stage (Figure 4.2 (A)) conforms with the results obtained by Thain *et al.* (2004) in *Arabidopsis* and by Finlayson *et al*. (1999) in sorghum. Additionally, the ethylene rhythm was already observed in wheat seedlings by Ievinsh and Kreicbergs (1992) with a peak of ethylene accumulation that tended to coincide with periods of minimum growth during the day.

Foliar ABA concentration does not significantly differ during the day which is in contrast with the research of Lee *et al.* (2006) in *Arabidopsis thaliana* who observed a peak of ABA accumulation at midday. However, genetic differences could be observed between T1 and S1 which accumulate less ABA compared to T2 and S2. Thus, these differences could not be associated with a trait of tolerance, in terms of yield.

On the other hand, to confirm whether these differences between tolerant and sensitive genotypes on ethylene emission and ABA production at two different time of the day (12:00 pm and 5:30 pm) could be associated to an exploitable trait, it would be necessary to repeat this experiment under field conditions on a bigger panel but not before having identified the key tissue(s) and phenological stage(s) for measurement. However, it is clear that the time of the day is an important factor which has to be set when aiming to quantify hormones such as ethylene and ABA.

4.4.2. Effect of re-watering after drought

The ethylene emission rate response to the re-watering is genotypically dependent. These results (Figure 4.3) confirm the results of Beltrano *et al.* (1997) on wheat which observed a peak of ethylene emission rate after re-watering as observed here for most of the genotypes (T3, T4, S3). However, an exception is observed with one genotype, which did not show any peak ethylene emission after re-watering (Figure 4.3 D). The peak of ethylene emission, observed after re-watering, could be associated with a long distance response mediated by the transport 1-aminocyclopropane-1-carboxylic acid (ACC) from the root to the shoot during a flooding irrigation or root hypoxia (Van de Poel and Van Der Straeten, 2014). It is suggested that the lack of oxygen in the root system will induce the expression of ACC-synthase (ACS) resulting in an accumulation of ACC which cannot be converted to ethylene due to the lack of oxygen (plants exposed to hypoxic to anoxic conditions under flooded soil) and therefore the absence of ACC-oxidase (ACO) (Van de Poel and Van Der Straeten, 2014). When the plant is re-watered, ACC accumulated in the root is transported to the shoots (leaves) through the xylem sap (Bradford and Yang, 1980) where it is converted into ethylene due to the presence of ACC-oxidase (ACO) (English *et al.*, 1995).

The day after re-watering (Stp4), the soil water potential was higher than the soil water status at Stp1 (Figure 4.3) but the ethylene emission rate was lower at Stp4 for most of the genotypes (T3, S3 and S4) compared to Stp1 (Figure 4.3). Ievinsh and Kreicbergs (1992) observed a certain asynchronism in the oscillations of ethylene diurnal variation of the individual seedlings wheat, and their hypotheses to explain this variation was related with a difference in the physiological or phenological stages. In our experiment, Stp1 and Stp4 are separated by six days and the variation of ethylene emission could be associated with the different phenological stages of the individual plants between these two steps (Figure 4.3).

4.5. Conclusion

The inconsistency of the ethylene response to drought stress found in the literature (Narayana *et al.*, 1991) could be in part due to the methodology used for quantifying the hormone. In addition to this, there is clear evidence from the literature, supported by this study that there is consistent diurnal variation in ethylene emission from leaves. Therefore, this thesis needs to establish a protocol for hormone quantification and analysis to ensure that all procedures are standardised with respect to stage of plant development, time of day, method and tissue for sampling, methods and time of storage and consistency of analytical chemistry. Consequently, the choice of the method to quantify hormones has considered the laboratory facilities and the logistics of the field work. On the other hand, measuring ethylene and ABA production of ten genotypes at two times per day (12:00 pm and 05:30 pm) under field conditions, in order to study hormone responses to a stress environment, was not possible due to the complexity of the challenge under field conditions (such as several phenological stages, incubation time, material availability). Instead, it was decided to measure plant tissues at mid-day (01:00 pm), when the peak of ethylene accumulation was observed. The hypothesis was that whether these hormone(s) have an effect, positive or negative effect on plant resilience mechanisms during their phenological development, it could be most significant at peak concentration which was observed with ethylene at mid-photoperiod (Figure 4.2 A), a phenomenon that additionally coincides with the maximal temperature under field conditions, and should therefore represent the maximum stress experienced by the plant. Appendix 4 Figure 2 shows the mean temperature and the relative air humidity observed during the cropping season 2013-2014 for the months of February and March 2014 and indicates that the variables (temperature and humidity) appear to be relatively constant during the ethylene sampling (from 01:20 pm to maximum 03:40 pm).

Yang *et al.* (2007) took a similar decision on sample time but their choice was based on the leaf water potential, which was at its lowest value at that time.

Finally, the experiment on re-watering highlights the fact that differences can appear in ethylene production between genotypes after re-watering. The peak of ethylene after the re-watering process is an indicator of the sensitivity of ethylene production to be modified by field management such as irrigation and the genotypic variation observed could be an important trait. However, for the moment this variation could not be linked to a resilience mechanism. Therefore, it was decided to not measure ethylene emission just after an irrigation (field conditions) or re-watering (controlled conditions) to avoid a possible induced bias into the analysis of the data.

The next Chapter will focus on determining when (in terms of phenological stage) and where (in terms of tissue) hormone should be measured in order to study hormone and hormone interaction in response to stress. One aim in this thesis is to develop a high-throughput screening method for drought tolerance based on hormone quantification so the sampling of tissue needs to be fast. Consequently, as ethylene and ABA are produced by almost all the parts of the plant, it is necessary to define in which tissue, when and how hormone concentrations have to be quantified.

Chapter 5. Using plant hormone balance (ABA-ETH) as a physiological trait for drought stress resilience. Defining key tissue(s) and phenological stage(s)

5.1. Introduction

Plant hormones can play an important role in regulating the growth and development of individual plant tissues and exert a controlling influence on reproductive processes (Davies, 2004*b*). Regulation of hormone concentration in different tissues and intracellular compartments depends on several external factors (Chang and Bleecker, 2004), such as heat and drought stress, which are the main environmental constraints of crop production (Araus et al., 2002; Gurmani et al., 2013). It is generally believed that abscisic acid (ABA) and ethylene are strongly linked to a suite of plant stress responses (Wilkinson and Davies, 2002, 2010; Davies, 2004*b*). Both are usually considered as plant growth inhibitors, but recent studies show a biphasic response of different plant tissues to these hormones, such as the promotion or inhibition of root and leaf growth depending on the concentrations accumulating, the status of other hormones and impact of other environmental factors (Sharp and LeNoble, 2002; Pierik *et al.*, 2006; Wilkinson *et al.*, 2012).

Ethylene is produced by almost all parts of the plant and is involved in an extensive range of effects on developmental processes along the plant cycle, such as breaking of dormancy, regulation of stem swelling, root hair development and adventitious root formation but also retarding stem elongation, kernel abortion in wheat, ripening of fruit, senescence, and abscission (Yang and Hoffman, 1984; Chang and Bleecker, 2004; Davies, 2004*b*; Sisler *et al.*, 2006; Hays *et al.*, 2007; Acharya and Assmann, 2009; Abeles *et al.*, 2012). Due to the huge range of effects on plant development, its production is a tightly regulated process controlled

by the developmental signal and the response to different environmental factors, both biotic and abiotic (Davies, 2004*b*; Cristescu *et al.*, 2013). Its production can vary quickly and induce effective biological responses at very low concentration (nanomolar) and the lag time for ethylene responses can vary from minutes (10-15 minutes, case of seedling inhibition) to days (promoting leaf senescence) (Bleecker and Kende, 2000; Davies, 2004*b*; Guo and Ecker, 2004; Abeles *et al.*, 2012). Moreover, ethylene emissions from a plant tissue can vary depending on plant species, tissues (e.g. root, leaf and flower) and developmental stage of plant (Cristescu *et al.*, 2013). Additionally, Beltrano *et al.* (1994) has shown that ethylene concentration of wheat spike during the grain filling period from early milk to dormant stage shows significant variations with a peak at the hard dough stage. Similarly, Yang *et al.* (2006*a*) have shown that ethylene and ABA accumulations in the grain vary along the grain filling period from anthesis to maturity. These findings (Beltrano *et al.* (1994) and Yang *et al.* (2006*a*)) suggest that ethylene and ABA regulation changes along the phenological stages and emphasise the importance of identifying properly the phenological stage at the time of quantifying hormones in different tissues, in order to interpret properly different effects on plant growth and/or survival.

ABA concentration ([ABA]) in tissue is determined by the balance between its production and its degradation (Cutler and Krochko, 1999). Biosynthesis and degradation are influenced by the plant developmental stage, environmental factors and also other growth regulators (Cutler and Krochko, 1999; Finkelstein, 2013). Investigation of the effect of environmental factors such as reduced water availability on ABA concentration, has shown a reproducible enhancement of ABA concentrations in roots, xylem sap and leaves (Davies and Zhang, 1991; Puértolas *et al.*, 2013; Hu *et al.*, 2016). On the other hand, the importance of the tissue used to quantify the hormone concentration, has been highlighted by the research of Hu *et al.* (2016), showing that

ABA biosynthesis can be first promoted either in the leaf or in the root depending on which tissue is first stressed and therefore the ABA concentration is enhanced in that tissue.

More recently, several studies have shown the importance of studying hormone interactions. Both ethylene and ABA can play an important and interrelated role in determining stomatal responses to drought where ethylene acts as an antagonist of ABA and reduces the sensitivity of stomatal closure in response to drought stress, in wheat (Wilkinson and Davies, 2009, 2010; Chen *et al.*, 2013), and in *Arabidopsis* sp. (Tanaka *et al.*, 2005). Additionally, the ABA/ethylene balance affects plant developmental processes such as: 1) root growth, where higher concentration of ABA apparently is required to prevent excess ethylene production from root tissues under drought stress in order to maintain the growth of the primary roots in maize (Sharp and LeNoble, 2002) or 2) the grain-filling rate and seed-setting, where low concentration of ABA and high ethylene will slow-down the filling rate in the inferior and superior grains while reducing seed set in wheat and rice (Yang *et al.*, 2006*a*,*b*, 2007).

Additionally, Hays *et al.* (2007) have shown that under heat stress, ethylene (ETH) in the developing kernels and embryos is involved in signalling that leads to kernel abortion during the post-anthesis period. Also the accumulation of ABA in the floral organs leads to an increase in sterility in wheat under drought and heat stress (Saini and Aspinall, 1982; Saini *et al.*, 1984; Westgate *et al.*, 1996) and in rice under cold conditions during meiosis (Oliver *et al.*, 2007). However, hormones can act antagonistically and it has been shown that high ABA/ETH ratios, in superior and inferior spikelet during the post-anthesis period, can be key in establishing high spikelet fertility in rice (Yang *et al.*, 2007), and high grain-filling rate in wheat (Yang *et al.*, 2006*b*). Also, ABA accumulation can restrict the ethylene production in order to maintain root growth at low soil water potential (Spollen *et al.*, 2000).

In consequence, it has been suggested that hormones, and in particular hormone interaction (ABA/ETH balance), could be an innovative trait for breeders to identify adapted genotypes to stress environments such as drought stress. It is suggested that this approach could help breeders in their research to increase yield under present and future challenging stress environments (Wilkinson *et al.*, 2012).

Grain number per m^2 has been identified as the most relevant trait related to final grain yield under yield potential conditions (Sayre *et al.*, 1997; Shearman *et al.*, 2005; Peltonen-Sainio *et al.*, 2007; Dolferus *et al.*, 2011) and in Chapter 3 the resilience component of grain number per m^2 (G#/m^2-RCI) was shown to be mostly correlated with yield under drought stress. G#/m^2 can be considered as the product of spike dry weight (SDW) per m^2 and the number of grains per unit of SDW, that is an indicator of spike fertility (Fischer, 1984; Abbate *et al.*, 1998; Martino *et al.*, 2015). Under drought stress, grain number per m^2 is significantly reduced when the stress occurs during the spike growth period (Hochman, 1982) from booting stage to anthesis. At booting stage in wheat, the meiosis process, which originates the embryo sac in the carpel and the pollen in the anthers, starts firstly in the middle of the spike, continuing with the basal and apical parts (Zadoks *et al.*, 1974; Acevedo *et al.*, 2002). Meiosis is a period that is very sensitive to drought stress that results in yield reduction. During this period, floret abortion coincides with the maximum growth rate of the spike and stem (Siddique *et al.*, 1989) and it is associated with carbon competition between spike and stem elongation (Kirby, 1988) and also to a reduction of nitrogen availability (Acevedo *et al.*, 2002). Also, the number of spikelets per spike of fertile tillers can be reduced during the spike-growing stage (Hochman, 1982). Under drought stress, plant hormones and mainly ABA and ethylene, have been shown to play important roles in the regulation of the growth rate during plant development but also in the control of the reproductive processes (Wilkinson and Davies, 2002, 2010; Davies, 2004*b*).

Due to the complexity of spatial and temporal variation in individual hormone accumulation/production and their different effects on plant development, a lot of questions are raised about when (in terms of phenological stage and/or time of day) and where (in terms of tissue such as leaf, spike) hormone quantification should take place. This is a key question to identify how genetic sensitivity to environmental cues could be identified using a high-throughput method quantifying hormone accumulation.

Several researches have worked on the understanding of a possible endogenous hormone interaction in different tissues, for example on vegetative tissues such as leaf (Wilkinson and Davies, 2010) or root (Sharp and LeNoble, 2002) and on reproductive tissues in rice and wheat such as the spikelet (Yang *et al.*, 2006*a*, 2008) and anthers (Oliver *et al.*, 2007). In spite of the improved understanding of hormone interactions in these tissues, the methodology will be hard to reproduce under field conditions for a high throughput method, for example, the manipulation to detach spikelets (Yang *et al.*, 2006*a*) or the sampling at the anther level (Oliver *et al.*, 2007). Therefore, in order to have a simple and fast sampling protocol, the selections of tissues to study the ethylene emission rate and ABA accumulation were based on two hypotheses about how these hormones could influence the yield under drought stress during the pre-anthesis period: 1) the reduction of the grain number per spike during meiosis could be due to a competition for carbon, driven by the leaf (source tissue) hormone concentration and the photosynthesis rate 2) the reduction in the fertility of pollen and spikelets could be associated with the hormone concentration in spike (sink tissue). Additionally, as meiosis is a critical period which is very sensitive to abiotic stress (Acevedo *et al.*, 2002) it was decided to study the ethylene emission rate and the ABA accumulation in these two tissues (leaf and the full spike) from booting to heading (phenological stages).

Thus, the aims of this Chapter is 1) to determine how production of ABA and ethylene varies between leaf and spike tissues through phenological development under drought stress and irrigated conditions, 2) to study the genotypic variation of hormone accumulation and hormone ratio (ABA/ethylene) in different tissues (leaf and spike) as the plant develops through different phenological stages under drought stress and irrigated conditions 3) to determine key stage(s) and tissue(s) linking genotype stress resilience and hormone production (ABA and ethylene).

5.2. Materials and methods

Location and timing of field experiments are described in Chapter 2 (Section 2.2.1).

The CIMCOG-ROOT panel described in Chapter 2 (Section 2.2.2) has been used for the work described in this Chapter. These ten wheat lines were evaluated, from booting to heading, at the CENEB station in Mexico, during the cropping season 2013-14 under irrigated conditions (yield potential, Yp) from late-November to early-May and under drought conditions from early-December to mid-May. However, only eight genotypes were analysed for hormones, due to some mixture of genotypes observed in the field plots at heading stage (at the end of the sampling period). As a result, six plots were excluded from the hormone analysis of this Chapter.

Within the population under study (CIMCOG-ROOT - 8 genotypes), four genotypes have shown some contrasts (2 resilients and 2 sensitives) in terms of grain yield and in yield components, as described in Chapter 2 and Chapter 3. The contrasts on yield and yield components resilience capacity index (RCI) are summarised in Table 5.1, which shows that genotypes 1 and 5 are identified as yield susceptible genotypes, while genotypes 6 and 8 are identified as yield resilient. Others contrasts can be observed with other yield components, such as grains number per spike (G#/spk) which is related to spike fertility, where 1 and 8 are

identified as resilient genotypes, 5 and 6 behave as more susceptible genotypes. Genotype 6 shows, in terms of G#/spk-RCI, a lower susceptibility (G#/spk-RCI = 4) compared with genotype 5 (G#/spk-RCI = 1) (Chapter 3 - Section 3.3.4. Table 3.11). As the resilience component of grain number per m^2 was identified to be highly related to yield but knowing that spike number per m^2 might be influenced by the sowing density, in particular for genotype 1 and 6 (as explained in Chapter 3 - Section 3.3.4 and Appendix 3 Table 7), in this Chapter, the contrast will be considered in function of grain number per spike and in terms of this variable, genotypes 1 and 8 will be identified as resilient and 6 and 5 as sensitive.

Table 5.1: Summary table of the trait resilience capacity index (T-RCI) under drought stress calculated on yield (Y), thousand grain weight (TGW), grain number per square meter (G#/m^2), spike number per square meter (Spk#/m^2) and grain number per spike (G#/spk), during the cropping season 2013-14. (Data from CIMCOG-ROOT - 8 genotypes).

Genotypes	Y-RCI	TGW-RCI	G#/m2-RCI	Spk#/m2-RCI	G#/spk-RCI
1	1	1	3	1	9
2	9	3	8	8	7
4	8	10	4	5	6
5	2	7	1	4	1
6	10	1	10	10	4
8	10	1	9	5	10
9	6	2	6	6	4
10	6	3	5	4	6

The experimental design and environmental conditions (irrigation, protection, etc.) are as described in Chapter 2 (Section 2.2.2).

The evaluation of phenology is as described in Chapter 3 Section 3.2.1. The phenological stages which were the focus of this Chapter were booting (GS45), late booting (GS47), half-emergence (GS55), heading (GS59), and only for the yield potential conditions trial, anthesis (GS61) as well. These phenological stages were chosen to elucidate whether a relationship between spike fertility and hormone balance does exist during the pre-anthesis period.

5.2.1. Sampling processes and hormone analysis under field conditions

For ABA analysis, two flag leaves and two spikes per plot were sampled, from two individual stems, at four phenological stages (described above). The leaf and spike samples were placed into pre-labelled glassine envelopes, stapled and immediately frozen in liquid nitrogen contained in a cool box. In the laboratory, the samples were kept in -80ºC for around one week before being freeze-dried during 48 hours and finally kept in a self-sealing bag (Ziplock) with silica gel to keep them dry. Afterwards, dry leaves and spikes tissues were subdivided into Eppendorfs (2 ml capacity) respectively, to be ground using a ball mill MM400 (Retsch GmbH, Germany) during 4 minutes for each cycle. When freeze-dry spike was too big to fit into only one Eppendorf (2 ml), the tissue was divided into two or three Eppendorfs depending on the size of the material, then, when the dry material was ground, all ground material from the same spike tissue returned into only one Eppendorf. To homogenise the samples, the Eppendorfs containing all the ground material were run again in the ball mill MM400 (Retsch GmbH, Germany) for 20 seconds.

The ABA analysis was carried out in a laboratory in the Lancaster Environment Centre using an adapted radioimmunoassay (RIA) method described by Quarrie *et al*., (1988). This method was described in detail in Chapter 4 (Section 4.2.1) of this thesis. The samples for analysis for each phenological stage, environment and tissue, were placed into the same foam rack. Usually, only 30 spaces (10 genotypes, 1 tissue, 1 phenological stage, 1 environment) were used per rack for samples and 16 spaces for standards (two sets of 8 standards). In general, two foam racks were analysed simultaneously, one containing leaf tissue and the other one spike tissue, both sampled at the same phenological stage and under the same environment (irrigated (Yp) or drought).

The method for measuring ethylene emission rate is described in Chapter 4 Section (4.2.2). At each phenological stage, two flag leaves (cut at the ligule level) and two spikes (cut at the node level) were sampled from each plot and incubated separately in individual tubes, with 3 replications (3 plots per genotype). Therefore, a total of six replications per tissue for each phenological stage and genotype were sampled, representing a total of 960 samples for both environments. The tissue sampling and the incubation time started at 01:00 pm, when ethylene shows a peak of production during the day, according to the results described in Chapter 4 (Section 4.3.1).

In the field, to incubate the tubes (25 ml) containing the fresh material (leaf or spike) under the same environmental conditions (sunlight), a platform was placed between two plots at one and half meter height (Figure 5.1) – these platforms were placed and removed every day. The incubation time for ethylene emission rate analysis was of 80 minutes which was the maximum needed time to finish sampling the material for ABA, enclose the sample tissues in the tubes for incubation under sunlight (ethylene emission) of all the plots of the trial (30 plots) and return to the first plot to start the gas sampling. Precise times of incubation were respected. Before sampling, the tubes were kept out of the direct light into a cardboard box. At the moment of sampling, the tubes were opened and closed as fast as possible to avoid changing of the "environmental" conditions inside them.

After the incubation time, 1ml of gas was extracted with a 1 ml syringe and immediately injected into a pre-labelled hermetically sealed 6 ml vial with a rubber lid crimped with an aluminium seal. Once the gas had been taken from the incubation tube, vials and tubes were saved in a cool box. After the field work, the vials were stored in the fridge in the laboratory in Mexico until they were sent via mail to Lancaster University where air samples were analysed

in their laboratories as described in Chapter 4 Section 4.2.2. On the day of field sampling, 1 hour after the field work, the fresh weight of tissues, was determined by the weighing tube method (described in Chapter 4 Appendix 4A) in the laboratory in Mexico.

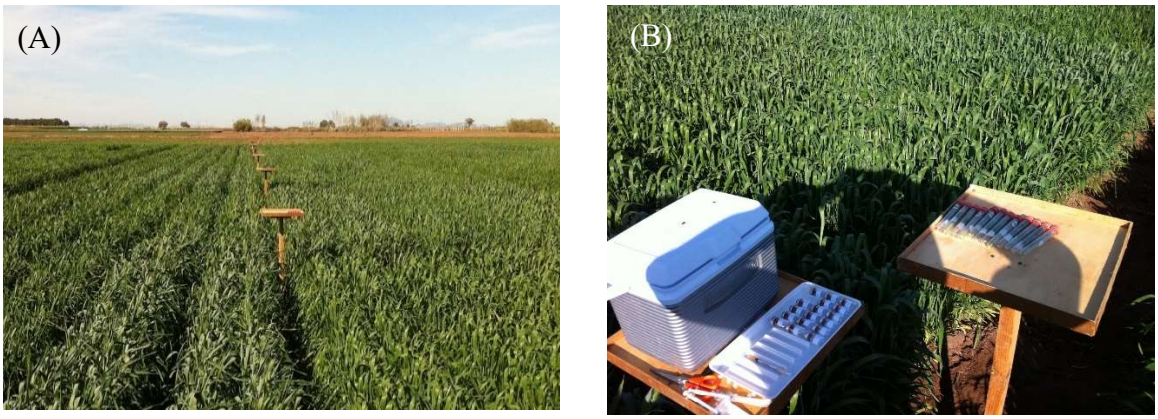


Figure 5.1: Illustration of the field material used to measure ethylene: (A) field distribution of the working incubation surfaces, (B) material used for ethylene extraction: a cool box containing the samples, syringes, vials, incubation tubes, scissors.

It is also important to specify that during the early stages of plant development (before half-emergence), the spike is still enclosed inside the leaf sheath and sometimes it is not visible. Thus, to identify where the spike is, it is necessary to identify the first node formed and cut just above it. At these stages (before half-emergence) spike and stem were incubated together.

Appendix 5A describes the method used in this work to calculate the hormone ratio (ABA/ethylene) to run a proper statistical analysis with enough repetitions when samples have been taken from different stems and/or plants for analysing ethylene emission and ABA accumulation.

5.2.2. Statistical analysis

Statistical Analysis was performed as described in Chapter 2 Section 2.2.8. In all cases when significant differences between genotypes, tissues and/or environment were observed by the ANOVA, a post-hoc test was run to discriminate them (Tukey HSD$p<0.05$).

5.3. Results

5.3.1. General analysis of hormones variation (ethylene and ABA)

Figure 5.2 (A and B) shows ethylene and ABA accumulation by leaf and spike along the different phenological stages under irrigated condition (yield potential (Yp)) and during drought stress (D). Results are means of the data for 8 genotypes from CIMCOG-ROOT. In both figures, it is clearly shown that under both environments (Yp and D) the leaf tissues (source tissue) produce more ethylene and ABA than the spike tissues (reproductive sink tissue) (Figure 5.2 A and B, respectively). An exception is observed under irrigated conditions with the ABA concentration ([ABA]) at half-emergence where leaves and spikes show similar accumulation. This is mainly due to an important reduction of leaf [ABA] and a slight increase of spike [ABA] compared with the previous stage, the late booting (Figure 5.2 B).

Figure 5.2 A shows that leaf ethylene emission rate, under drought stress (D), at booting and heading stages is slightly superior to that under irrigated conditions (Yp) but the difference is not statistically significant ($P = 0.13$ and $P = 0.053$, respectively).Under both environments (D and Yp), from booting to heading, leaf ethylene emission rate increases significantly ($P<0.001$, for both environments) (Figure 5.2 A).

In contrast, spike ethylene emission, under drought stress, always shows lower values compared with irrigated conditions (yield potential (Yp)) (Figure 5.2 A). Under both environments, spike ethylene emission rate increases significantly from half-emergence to heading stages ($P<0.001$ (Yp) and $P< 0.05$ (D)) and, only under irrigated condition (Yp), reduces significantly between booting and late booting stage ($P<0.05$) (Figure 5.2A).

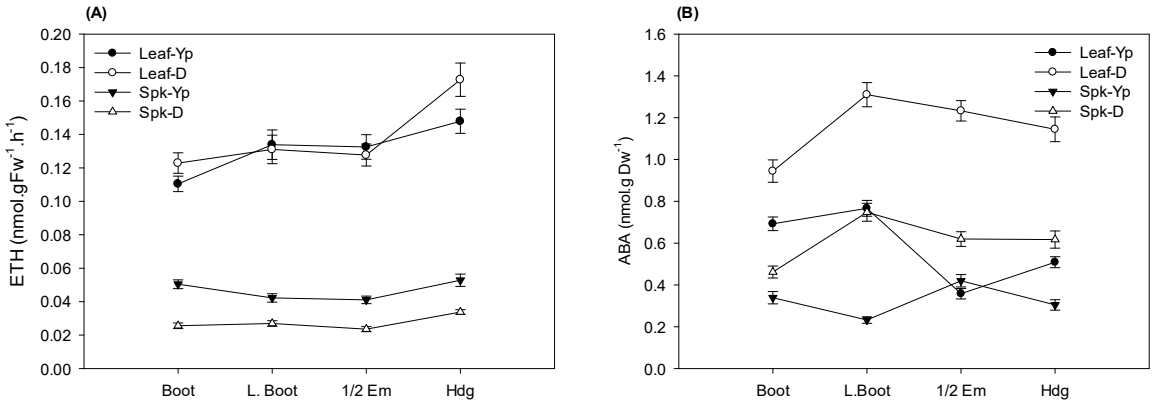


Figure 5.2: Evolution of the production of A) Ethylene (ETH, nmol.gFw^{-1}.h^{-1}), and B) ABA (nmol.g Dw^{-1}), in leaf (circle) and spike (triangle, spk) tissues under yield potential (closed symbols, Yp) and drought (open symbols, D) conditions along the phenological stages booting (Boot), late booting (L. Boot), half-emergence (1/2 Em) and heading (Hdg). Data were collected on eight genotypes from the CIMCOG-ROOT trial during the crop season 2013-2014 in Mexico, CENEB station. Figures are means ± standard error.

Figure 5.2 B shows that under drought stress (D), both, leaf and spike tissues accumulate significantly more ABA along the phenological stages, compared with wheat plants under irrigated conditions (yield potential (Yp)). Under irrigated conditions (Yp), a significant reduction in leaf [ABA] is observed at half emergence, compared with leaf [ABA] at late-booting stage ($P<0.001$). In contrast, under the same condition, spike [ABA] shows a significant increase at half emergence compared with spike [ABA] at late booting and heading stages ($P< 0.001$). Under drought stress (D), from late-booting to heading, flag leaves and spikes reduced their ABA accumulation ($P<0.05$ between late-booting and heading for both tissues).

5.3.2. Hormone quantification and genotypic variation along the phenological stages

Figure 5.3 and Figure 5.4 show that the production of ethylene and ABA in leaf and spike tissues, respectively do not always show significant genotypic variation for every phenological stage. This variation occurs in leaf ethylene emission only at late booting and heading stages, under irrigated and drought conditions (Figure 5.3 C and G). At late booting stage, under drought stress, genotypes 1 (resilient) and 8 (resilient) show the highest leaf ethylene production while genotypes 5 (sensitive) and 9 produce the lowest ethylene levels (Figure 5.3 C). At

heading stage, under drought stress, genotype 1 (resilient) shows the highest ethylene emission while genotype 2 shows the lowest emission (Figure 5.3 G). Leaf ethylene emission rate is enhanced under drought stress compared with irrigated condition at late booting stage (Figure 5.3 C), for genotypes 2*, 4* where * means $P<0.05$ - and at heading stage (Figure 5.3 G), for genotypes 1* (resilient), 8* (resilient). At late booting stage, the four contrasting genotypes (1, 5, 6, 8) do not show significant variation in ethylene emission but under drought stress compared with irrigated condition, increased for genotypes 1 (resilient), 8 (resilient) and reduced for genotypes 5 (sensitive), 6 (sensitive) (Figure 5.3 C). These differences at these specific stages (late booting and heading) between environments were not observed in the general analysis (Figure 5.2).

Leaf [ABA] shows significant genotypic variation at two developmental stages, late booting and at half-emergence under drought stress (D) (Figure 5.3 D and F) and at half-emergence only under irrigated condition (Yp) (Figure 5.3 F). However, it is important to note that the drought stressed genotype 4 at late booting (Figure 5.3 D) and the irrigated genotype 10 at half-emergence (Figure 5.3 F) show a higher [ABA] compared with the other ones which did not show significant genotypic variation between them in term of leaf [ABA].

In contrast, under drought stress, more genotypic variation is observed in leaf [ABA] at half-emergence, the genotype 4 shows the highest leaf [ABA], followed by genotypes 2, 1 (resilient), 5 (sensitive) and 8 (resilient) (Figure 5.3 F).The lowest leaf [ABA] is observed in genotypes 6 (sensitive) and 10. At all stages, leaf [ABA] was higher under drought stress (D) compared to the irrigated treatment (Yp), with two exceptions at booting stage, where genotypes 2 and 8 (resilient) show a constant leaf [ABA] (Figure 5.3 B).

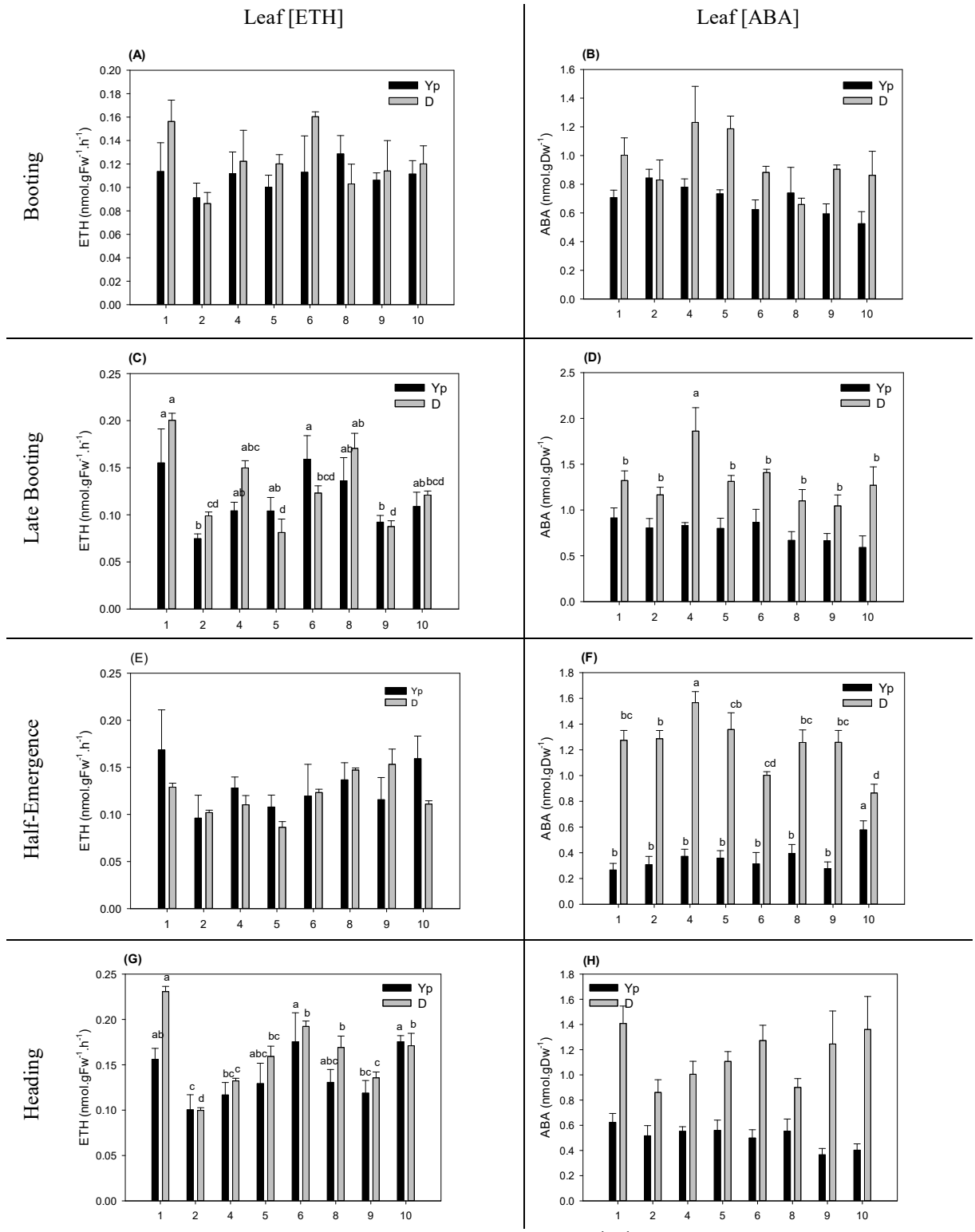


Figure 5.3: Genotypic variation on flag leaf ethylene emission rate (nmol.gFw^{-1}.h^{-1}) (A, C, E, G) and leaf ABA concentrations (nmol.g Dw^{-1}) (B, D, F, H) under different environments (irrigated (Yp, dark bar) and drought stress (D, grey bar)) at different phenological stages: Booting (A and B), Late Booting (C and D), Half-Emergence (E and F) and Heading (G and H). Data were collected on 8 genotypes from CIMCOG-ROOT during the crop season 2013-2014. Columns and bars are means ± standard error of 6 replicates with different letters indicating significant ($P < 0.05$) difference among genotypes within an environment and only when significant differences exist between the genotypes.

Similarly, spike ethylene emission and spike [ABA] (Figure 5.4) under both environments (irrigated (Yp) and drought (D)) do not show genotypic variation at all stages.

Under irrigated conditions (Yp), spike ethylene emission does not show genotypic differences at any stage (Figure 5.4 A, C, E, G) and under drought stress (D) some differences are observed at booting and heading stages (Figure 5.4 A and G, respectively). In fact, at booting stage, genotypes 2 and 6 (sensitive) show the lowest spike ethylene production, while genotype 1 (resilient) shows the highest ethylene level. In contrast, at heading stage, genotype 6 (sensitive) shows the highest spike ethylene emission, followed by genotypes 1 (resilient), 5 (sensitive) and 9, while genotypes 2 and 4 show the lowest ethylene level (Figure 5.4 G). Spike ethylene emission rate for all genotypes, is lower under drought stress compared with irrigated condition but differences are not always significant (Figure 5.4 A, C, E, G).

Under irrigated conditions (Yp), spike [ABA] shows significant differences at half-emergence and at heading stages (Figure 5.4 F and H, respectively). At half-emergence, genotype 2 shows the highest spike [ABA] and the genotype 6 (sensitive) the lowest. At heading stage, genotypes 1 (resilient), 2 and 5 (sensitive) show the highest spike [ABA], and the lowest values are observed with genotypes 8 (resilient), 6 (sensitive) and 9.

Under drought stress (D), spike [ABA] shows significant genotypic variation at late-booting and at half-emergence (Figure 5.4 D and F, respectively). At late-booting, genotypes 1 (resilient), 6 (sensitive) and 9 show the highest spike [ABA] and genotypes 4, 5 (sensitive), 8 (resilient) the lowest (Figure 5.4 D). At half-emergence, genotypes 2 and 8 (resilient) show the highest spike [ABA], followed by genotype 1 (resilient), while the lowest value is observed for genotypes 6 (sensitive) and 5 (sensitive).

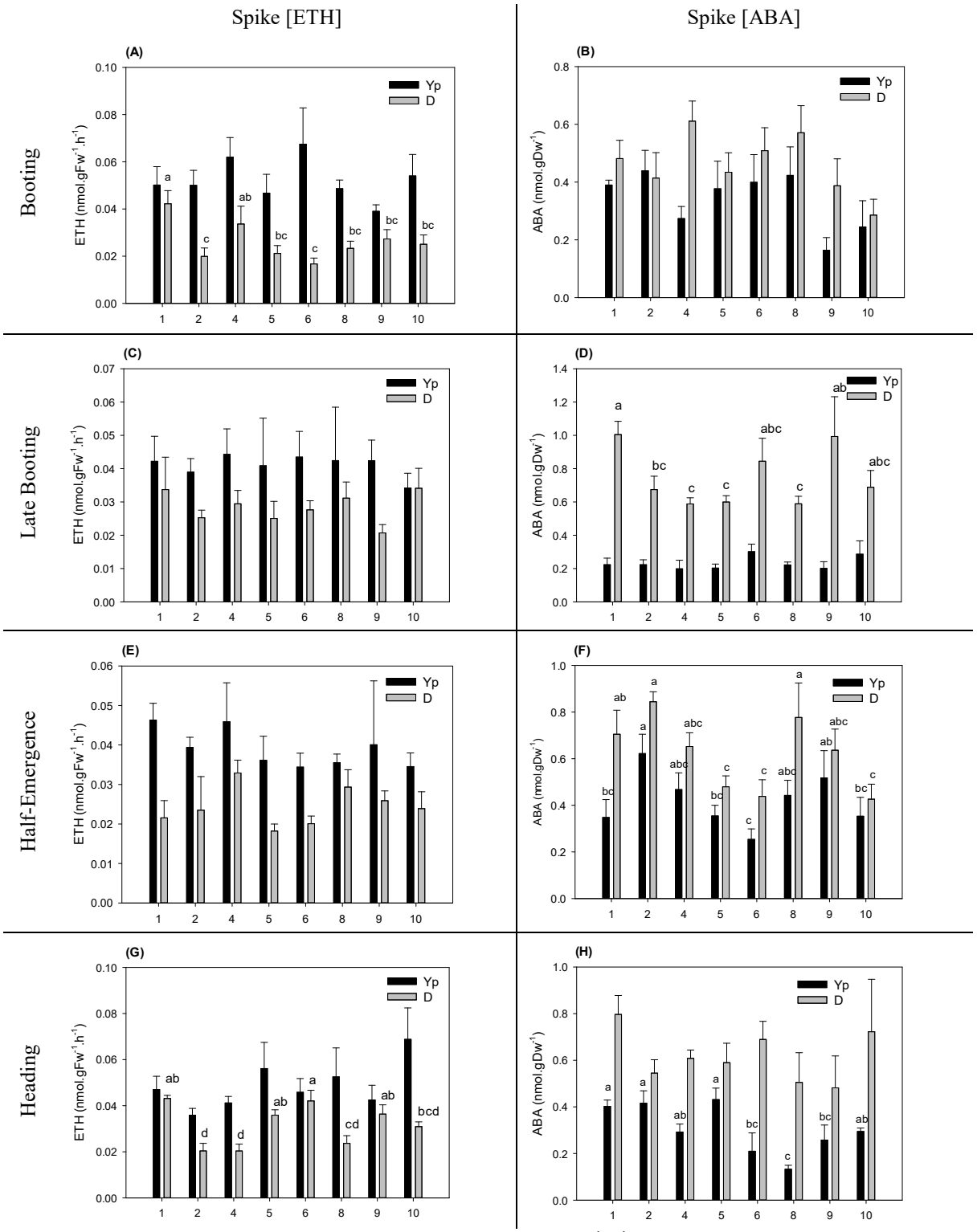


Figure 5.4: Genotypic variation on spike ethylene emission rate (nmol.gFw^{-1}.h^{-1}) (A, C, E, G) and spike ABA concentrations (nmol.g Dw^{-1}) (B, D, F, H) under different environment (irrigated (Yp, dark bar) and drought stress (D, grey bar)) at different phenological stages: Booting (A and B), Late-Booting (C and D), Half-Emergence (E and F) and Heading (G and H). Data were collected on 8 genotypes from CIMCOG-ROOT during the crop season 2013-2014. Columns and bars are means ± standard error of 6 replicates with different letters indicating significant ($P < 0.05$) difference among genotypes within an environment and only when significant differences exist between the genotypes.

The hormone ratio ABA/ETH, (the calculation method is described in Appendix 5A) shows significant genotypic variation at all developmental stages, for all tissues and under both environments (Figure 5.5). Under drought stress, the value of hormone ratio ABA/ETH, either for leaf or spike, is generally superior to the value of ABA/ETH observed under irrigated condition (Yp). Few exceptions are observed in leaf tissue, at booting stage and at late-booting where some genotypes do not show any differences between environments, it is the case of genotypes 1 (resilient), 2, 6 (sensitive) and 8 (resilient) at booting stage (Figure 5.5 A) and genotypes 1 (resilient), 2, 8 (resilient) at late-booting (Figure 5.5 C)

At half-emergence, under drought stress, the leaf hormone ratio ABA/ETH shows two groups of genotypes that are significantly different, with a higher ratio ABA/ETH for genotypes 2, 4, 5 (sensitive) and a lower one for genotypes 1 (resilient), 6 (sensitive), 8 (resilient), 9, 10 (Figure 5.5 E). At half-emergence, under irrigated conditions (Yp) genotype 1 (resilient) shows the lowest leaf ratio ABA/ETH compared with the rest of the population (Figure 5.5 E).

In spike tissue, the biggest variation, in terms of hormone ratio ABA/ETH, between irrigated and drought conditions, is observed at all phenological stages (Figure 5.5 B, D, F, H).

To sum up, it was observed that the genotypic variation in ethylene emission and [ABA] appears 1) in leaves tissues under drought stress, at late-booting and heading stages for ethylene emission and for [ABA] at late-booting and half-emergence 2) in spike tissues under drought stress, at booting and heading stages for ethylene emission, and for [ABA] at late-booting and half-emergence. However, the hormone ratio ABA/ETH apparently effectively expresses genotypic variation in hormone production at all stages. Thus, to see if the hormone production can be related, with a resilience/sensitive response to drought stress, in terms of yield and yield components, the next analysis principally focused on the stages that had shown genotypic variation, in terms of hormone production and ratio, in response to drought stress.

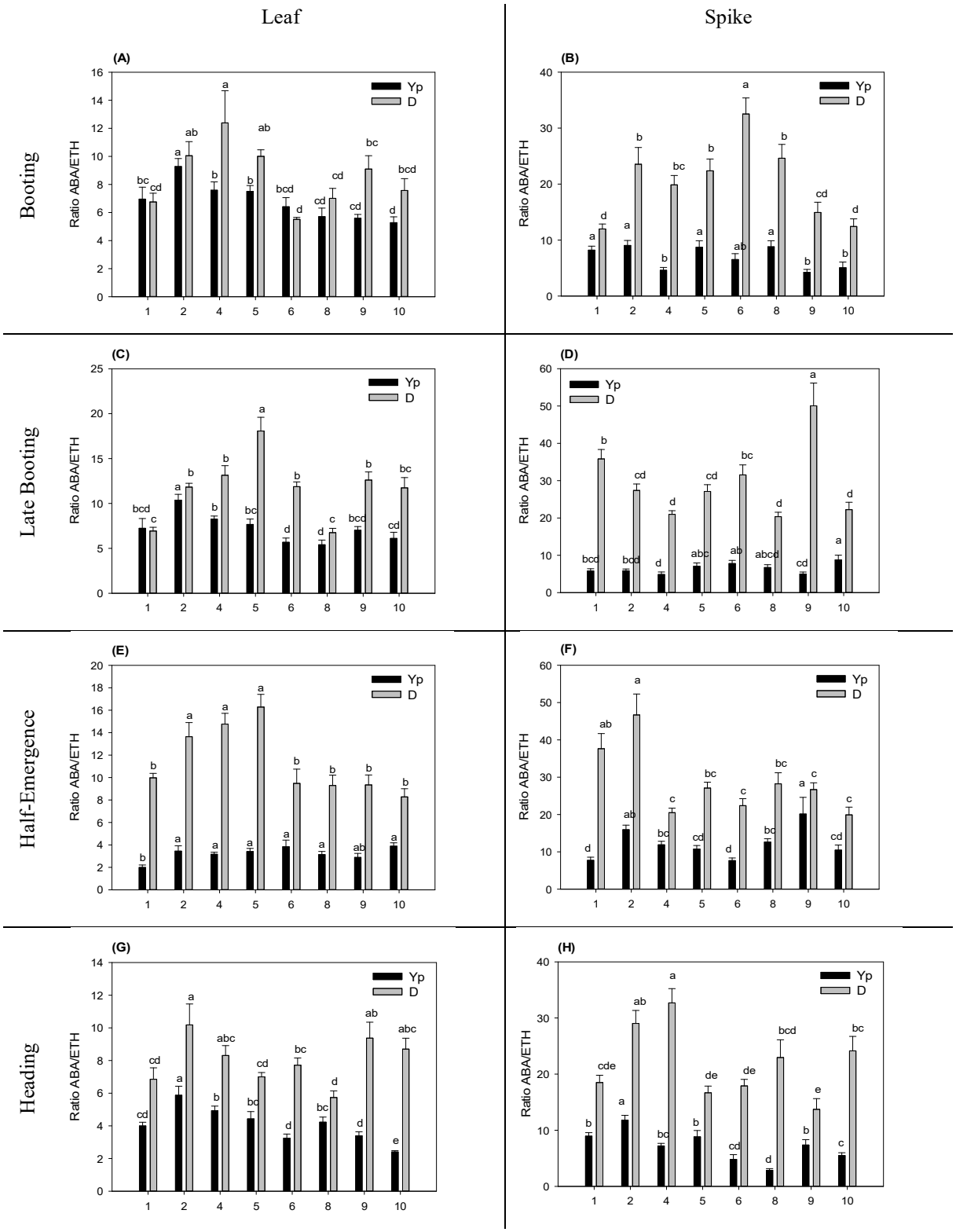


Figure 5.5: Genotypic variation of the ratio ABA/ETH in leaf (A, C, E, G) and spike (B, D, F, H) tissues, and their changes under different treatments yield potential (Yp, dark bar) and drought (D, grey bar) along different phenological stages, Booting (A and B), Late Booting (C and D), Half-Emergence (E and F) and Heading (G and H). Data were collected on 8 genotypes from CIMCOG-ROOT during the crop season 2013-2014. Columns and bars are means ± standard error of 6 replicates, with different letters indicating significant ($P < 0.05$) difference among the genotypes within an environment and only when significant differences exist between the genotypes.

5.3.3. Could hormone and/or hormone balance be a trait for drought stress resilience?

Table 5.2 shows the Pearson coefficient correlation between the accumulation of ABA and ethylene and the hormone ratio (ABA/ETH) on leaf tissue at the different stages *versus* the resilient capacity index (RCI) calculated on yield and yield components.

Leaf ABA concentration ([ABA]) shows a significant positive correlation between the resilient component calculated on Thousand Grain Weight (TGW-RCI) *versus* leaf ABA concentration at late-booting stage (r=0.74, $P<0.05$) (Table 5.2).

Leaf ethylene emission shows a significant and positive relationship with the resilient component of grain number per spike (G#/spk-RCI) at late booting stage (r=0.80, $P < 0.05$) (Table 5.2).

Table 5.2: Table of Pearson coefficient correlation between the Resilient Capacity Index (RCI) calculated on yield and yield components *versus* mean flag leaf ABA concentration, mean ethylene production and the mean ratio ABA/ETH under drought stress. RCI: resilient capacity index, Y: yield, TGW: thousand grain weight, G#/m^2: grain number per m^2, Spk#/m^2 spike number per square meter, G#/spk: grain number per spike, at the different stages (booting, late-booting, half-emergence and heading) where genotypes have shown significant differences with the leaf hormone accumulation (ABA, ethylene) and hormone ratio ABA/ETH. * P<0.05, **P<0.01.

Hormone	Stages	Y-RCI	TGW-RCI	G#/m^2-RCI	Spk#/m^2-RCI	G#/spk-RCI
ABA Flag Leaf	L. Booting	0.02	0.74*	-0.30	-0.05	-0.12
	Half-Emergence	-0.14	0.63	-0.38	-0.22	0.01
ETH Flag Leaf	L. Booting	-0.07	-0.23	0.04	-0.49	0.80*
	Heading	-0.44	-0.44	-0.14	-0.45	0.22
ABA/ETH Flag Leaf	Booting	-0.05	0.87*	-0.46	-0.08	-0.27
	L. Booting	-0.20	0.65	-0.44	0.22	-0.93**
	Half-Emergence	-0.20	0.80*	-0.51	-0.02	-0.44
	Heading	0.19	0.13	0.11	0.41	-0.25

The flag leaf hormone ratio ABA/ETH shows a positive relationship with the resilient component of TWG (TGW-RCI) at booting stage (r= 0.87, $P<0.05$) and at half-emergence (r= 0.80, $P<0.05$). In contrast, leaf hormone ratio ABA/ETH shows a significant negative relationship at late booting stage with the resilient component of grain number per spike (G#/spk-RCI) (r=-0.93, $P<0.01$) which is the best correlation observed (Table 5.2). Figure 5.6

illustrates the linear regression at this stage (late-booting) of G#/spk-RCI plotted *versus* 1) leaf ABA accumulation 2) leaf ethylene emission and 3) leaf hormone ratio (ABA/ETH).

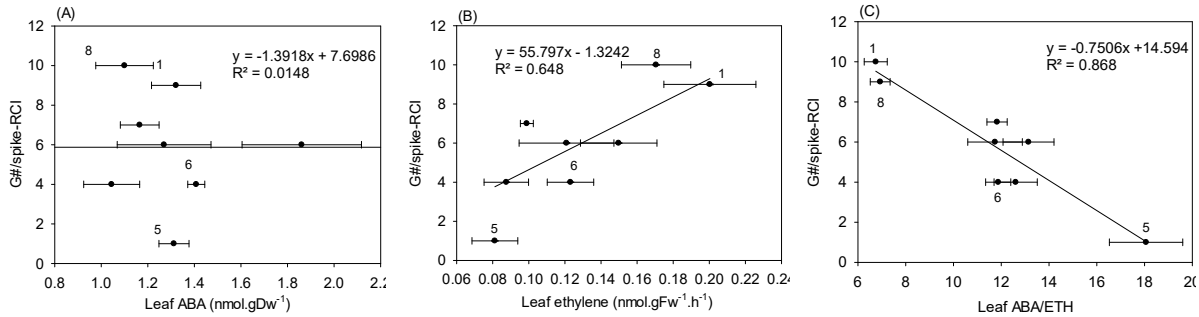


Figure 5.6: Linear regression between the resilient capacity index of grain number per spike (G#/spk-RCI) *vs* (A) mean leaf ABA accumulation (nmol.g Dw^{-1}), (B) mean leaf ethylene emission (nmol.$gFw^{-1}.h^{-1}$) and (C) mean leaf hormone ratio ABA/ETH at late booting stage. Numbers 1, 5, 6 and 8 represent the 4 contrasting genotypes of this population (1 and 8 resilient - 5 and 6 sensitive). Data were collected on CIMCOG-ROOT trial (8 genotypes) during the cropping season 2013-2014.

Table 5.3 shows the Pearson coefficient correlation between the accumulation of ABA and ethylene and the hormone ratio (ABA/ETH) on spike tissues at the different development stages *versus* the resilience capacity index (RCI) calculated on the different yield components. Two significant correlations are observed at booting stage between the resilient component of spike number per m^2 (Spk#/m^2-RCI) and 1) spike ethylene emission (r=-0.74 and P<0.05) and 2) the spike hormone ratio (ABA/ETH) (r=0.77 and P<0.05). However, spike per square meter was discarded, in Chapter 3, as a reliable trait due to the possible influence of the sowing density.

Table 5.3: Table of Pearson coefficient correlation between the Resilient Capacity Index (RCI) calculated on yield and yield components *versus* spike mean ABA concentration, spike mean ethylene production and the spike mean hormone ratio ABA/ETH under drought stress. RCI: resilient capacity index, Y: yield, TGW: thousand grain weight, G#/m2: grain number per spike, Spk#/m2 spike number per square meter and G#/spk: grain number per spike, at the different stages (booting, late-booting, half-emergence and heading) where genotypes have shown significant differences with the spike hormone accumulation (ABA, ethylene) and hormone ratio ABA/ETH. * P<0.05

Hormone	Stage	Y-RCI	TGW-RCI	G#/m^2-RCI	Spk#/m^2-RCI	G#/spk-RCI
ABA spike	Late-Booting	-0.35	-0.58	0.03	-0.09	0.03
	Half-Emergence	0,20	-0,.13	0.22	-0.08	0.67
ETH spike	Booting	-0.62	0.18	-0.59	-0.74*	0.30
	Heading	0.53	0.44	0.18	0.15	0.32
ABA/ETH spike	Booting	0.65	-0.05	0.61	0.77*	-0.17
	L. Booting	-0.31	-0.39	-0.03	0.05	-0.26
	Half-Emergence	-0.12	-0.31	0.08	-0.03	0.36
	Heading	0.44	0.50	0.10	0.08	0.39

5.4. Discussion

The study of plant growth regulators is a challenging undertaking. There is variability in hormone concentrations between the different tissues but also, the responses of different tissues to environmental stress modify the hormonal response. In this work, hormone accumulation varies with phenological stage as does genetic variability (Figure 5.2, Figure 5.3, Figure 5.4). However, due to the variances in temperature and humidity during crop development and therefore during the different phenological stages (described in Appendix 5B) it cannot be concluded with certainty that the phenological stage is the influencing factor on hormone accumulation under field conditions. This may particularly be the case for ethylene when the concentrations change more rapidly with time than do concentrations of ABA (Chapter 4 Section 4.3.1). To address this point, complementary measurements to quantify ethylene under the same environmental conditions were performed by sampling leaf and spike tissues at two close phenological stages on the same day, in the same plot at the same time (described in Appendix 5C). The results indicate that some genotypes show a significant variation in terms of ethylene emission rate between two close stages on leaf and or spike tissues.

Additionally, it has been suggested by Trewavas and Cleland (1983) that the action of hormone in plants depends on a combination of the concentration changes and the tissue sensitivity (Weyers and Paterson, 2001). For example, Ji *et al.* (2011) showed that drought-tolerant rice appeared to be more sensitive to external ABA application which promotes anther sterility but drought-tolerant genotypes accumulate less ABA in the anther under drought stress in contrast with the drought sensitive which increase ABA in the anther and therefore in the spike. In this study, ABA concentration increased in both leaf and spike tissues, but genotypic variation in spike ABA accumulation was only observed at two stages (late booting and half-emergence) (Figure 5.4, D and F). It is difficult to compare properly this experiment with that of Ji *et al.*

(2011) as they refer to the development stage of the anther (young microspore stage) and the present study is focused on the development stages of the plant. In spite of this, at half-emergence, under drought stress, it is observed that sensitive genotypes in terms of grain number per m^2 (5 and 6, Figure 5.4 F) show lower spike ABA accumulation while resilient genotypes (1 and 8, Figure 5.4 F) have higher ABA, in contrast to the results of Ji *et al.* (2011).

ABA variation along phenological stages and possible effect on plant development and fertility.

Several studies show that ABA concentration ([ABA]) is enhanced in leaf, xylem sap and roots under water stress (Zhang and Davies, 1989; Davies and Zhang, 1991; Puértolas *et al.*, 2013). In this thesis, [ABA] is enhanced under drought stress, under field conditions, in both leaf and spike, at all phenological stages (booting, late-booting, half-emergence and heading) and for all the genotypes compared with the [ABA] under irrigated condition (yield potential under field conditions). ABA concentration in the leaf is higher than those concentrations found in the spike, with the only exception at half-emergence, when a reduction of leaf [ABA] is observed under irrigated conditions (yield potential). It is important to note that under yield potential no irrigation was applied during the sampling process from booting to heading and therefore this reduction of leaf ABA concentration could not be associated with an increase of the soil water availability. This reduction of leaf ABA concentration could be associated with the phenology progress, as all genotypes have shown this reduction under irrigated conditions excepted genotype 10 (Figure 5.3 F).

It is relevant to point out that the day before the first sampling at half emergence stage, the fungicide tebuconazol was applied. It has recently been demonstrated that tebuconazol application to maize seedlings can enhance ABA concentration and reduce gibberellin

concentration and that these changes can have a detrimental effect on seedling emergence (Yang *et al.*, 2014). In experimental results reported here, leaf [ABA] under irrigated conditions is significantly reduced at half-emergence (sampling was done 1 to 5 days after the application of tebuconazol) compared with the concentration at late booting, but in contrast, spike [ABA] is increased. This variation was not observed under drought stress. These results suggest that another experiment should be conducted under field conditions without any pesticide application. Such an experiment should show whether the reduction of ABA concentration at half-emergence under irrigated conditions is due to the phenological stage *per se* or to the application of a fungicide or to the impact of an environmental change.

Increased ABA concentration can have a range of effects on growth and development of wheat, which can be beneficial or detrimental under drought conditions (Innes and Quarrie, 1987) affecting both source and sink tissues. Innes and Quarrie (1987) summarized these effects of enhanced leaf ABA concentration on wheat. Most of the suggested effects are related to stomatal closure which can increase water use efficiency but reduce photosynthetic rate (Innes and Quarrie, 1987) and high ABA concentrations in spikelet have also been shown to have a detrimental effect on pollen fertility (Ji *et al.*, 2011) therefore reducing the number of fertile spikelets per ear. In the work reported here, leaf ABA concentration at late booting stage shows a correlation with the resilience component of thousand grains weight (TGW-RCI) (Table 5.2), which is unexpected as TGW is usually considered to be determined during the post-anthesis period (Slafer and Rawson, 1994). It has been shown that carpel size, determined during spike growth, is positively related to thousand grain weight (Calderini *et al.*, 1999; Calderini and Reynolds, 2000) and external application of ABA to flowers of tomato and Arabidopsis increased the number of carpels but concentrations of ABA over 10^{-04} *M* suppressed the growth of anthers in tomato (Chandra Sekhar and Sawhney, 1991; Fitzpatrick *et al.*, 2011). Therefore,

it is possible that high ABA concentration ([ABA]) in leaf could be associated with improved development of the carpel (size or number), perhaps via an increase in water use efficiency (relationship between transpiration and carbon fixation). However, in this work, no correlation was observed between the concentration of ABA in the full spike and the number of grain per spike. This effect of leaf [ABA] or spike [ABA] on carpel (size or number) could be directly tested by quantifying the hormone concentration (leaf or spike) and evaluating carpel size of the basal and apical florets. The experiment could focus initially on florets 3 and 4 (starting counting from the basal spike) as they have been identified to be more affected by environmental stress and it was suggested that the anther and ovary size of these florets (3 and 4) may be better predictors of spike dry weight (Guo *et al.*, 2015). It has been suggested in Chapter 3 of this thesis that the resilience component of thousand grains weight (TGW-RCI) would probably have a fine tuning effect on the regulation of final thousand grains weight under stress conditions (TGW_s) and that the production capacity index (TGW-PCI) is more relevant to the determination of final TGW_s (Chapter 3). Therefore, TGW-RCI may not be the most relevant trait to focus on to improve yield. In contrast, the resilience component of grain number per spike (grain#/spike-RCI) or the resilience component of grain number per m^2 (grain#/m^2-RCI) have been shown to be more related to the determination of yield under stress (Chapter 3, Table3.10 and conclusion).

In this work, leaf and spike ABA concentration could not be related to the resilience component of grain number per spike. Therefore, based on these results, ABA concentration *per se* is not an effective trait for selection for spike fertility under drought stress.

Ethylene variation along phenological stages and possible effect on plant development and fertility.

The effects of ethylene in plants depend upon the developmental stage, the duration of ethylene exposure (when externally applied), its concentration in tissues and the process sensitivity (Høyer, 1996; Abeles *et al.*, 2012; Silva and Finger, 2015). Additionally, Klee and Clark, (2010) suggested that in climacteric fruits, effects of ethylene on developmental processes are regulated both at the level of the synthesis and the perception of the hormone. Furthermore, Klassen and Bugbee (2002) found that the reproductive organs appear to be more sensitive to elevated ethylene concentrations in the atmosphere compared with vegetative organs. Additionally, Wheeler *et al.* (1996, 2004) observed that wheat, grown hydroponically, showed a peak of ethylene emission during rapid vegetative growth (young plant) and close to when the highest rates of photosynthesis, respiration, and nutrient uptake of the stem were occurring. In the same research, they concluded that ethylene could be beneficial somewhere during the early stages of wheat development. However, a development-related peak in synthesis was not observed in the studies reported here with any genotype and tissue either under stress or under irrigated conditions (Figure 5.3, Figure 5.4).

It is commonly reported that ethylene production will increase with environmental stress (Tholen *et al.*, 2006) compared with the emissions under non-stress conditions, but Klassen and Bugbee (2004) report that under drought stress ethylene production will decrease under field conditions but that under drought stress in controlled environments, ethylene emissions may increase as soil dries. This may be due to slow soil drying in the field and more rapid soil drying in small pots in controlled environments.

Differences in the methodology used to measure ethylene could be responsible for differences in ethylene emission in response to stress that are reported in the literature (Morgan *et al.*, 1990; Narayana *et al.*, 1991). The quantification of ethylene using detached tissue enclosed in a tube (method used in this work) often reports an increase of ethylene production under drought stress (e.g. Beltrano *et al.*, 1997 in a controlled environment), while studies on the whole plant, in a controlled environment, suggest a decrease of ethylene synthesis as soil dries, compared with emissions from well-watered plants (Morgan *et al.*, 1990; Narayana *et al.*, 1991). In this study, it has been observed that ethylene emission rates under field conditions can show different responses to drought stress, depending on the genotypes, the phenology and tissues. When leaf tissue is incubated, ethylene production can be enhanced, reduced or be unchanged, as a function of phenology and the plant's genetic background (Figure 5.3 A, C, E, G). In contrast, spike ethylene shows always a reduction of production along the phenological stages under drought stress compared with the irrigated plants (Figure 5.2 A and Figure 5.4 A, C, E, G).

These variations in ethylene emission from leaves and spikes at different phenological stages could be related to positive or negative changes in yield, and the diverse effects of ethylene in regulating plant development. Usually considered as a plant growth inhibitor, some inhibitory effects have been observed e.g. plant height reduction, inhibit primary root growth and reduced spikelet fertility (Morison and Gifford, 1984; Sharp and LeNoble, 2002; Yang *et al.*, 2007; Abeles *et al.*, 2012). However, more recently, it has been suggested that ethylene can also have promoting effects on growth at low concentrations. For example, in Tobacco, a shade-avoidance trait has been associated with a fine regulation of ethylene production at low concentration, expressed as an increase in leaf angles, leaf expansion and stem elongation (Pierik *et al.*, 2003, 2006; Tholen *et al.*, 2006).

Ethylene can also have an inhibitory effect on root growth, when the soil dries, where ABA seems to limit the accumulation of ethylene in roots in order to maintain root growth activity and maybe also sustain shoot growth (Sharp and LeNoble, 2002). Promoting or inhibiting effects of ethylene depends on the concentration of the hormone, where low ethylene concentrations tend to promote growth while high concentrations are inhibitory, although these concentration effects seem to be dependent on the species and environment (Pierik *et al.*, 2006). In rice spikelet, a high concentration of ethylene could be associated with a reduction of fertility under mild drought stress during meiosis (Yang *et al.*, 2007). In the same research, these authors found that ethylene in spikelets is increased under drought stress compared with the well-watered treatment. In this thesis work on wheat, spike ethylene emissions are generally lower under severe drought stress compared with values in spikes from irrigated plants (Figure 5.2 A, Figure 5.4 A, C, E, G). In the research by Yang *et al.* (2007), the application of an inhibitor of ethylene synthesis (amino-ethoxyvinylglycine) to the rice panicles under mild drought stress, at the early meiosis stage, reduced the spikelet sterility. The authors concluded that higher levels of ethylene emission from the rice panicle, has a detrimental effect on spike fertility. In wheat plants which form the basis of this research, it has been found that ethylene emission rate under severe drought stress, at late booting stage was positively correlated with the resilient component of grain number per spike (Figure 5.6 B). Crop-specific differences could be due to the intensity of the stress (Tardieu 2012). This author stresses that a single drought trait can have a positive effect on yield under mild drought stress and a negative effect under severe stress, and/or *vice versa*.

Hormone balance (ABA/ethylene) variation along phenological stages and possible effect on plant development.

Figure 5.3 and Figure 5.4 show that an investigation of the involvement of individual hormones in the plant processes affecting the final yield of a wheat crop should not be undertaken without considering the phenological stage of the plant. However, it has been suggested that higher hormones ratios (e.g. ABA/ETH) could be a new physiological trait for adaptation to mild water stress for maize, wheat or rice (i.e. Wang *et al.*, 2002; Yang *et al.*, 2006*a*, 2007). The results reported here show genetic differences in ABA/ETH in both leaf and spike tissues at all phenological stages (Figure 5.5). Additionally, it was observed, under field conditions, that a higher leaf hormone ratio (ABA/ETH) is negatively related to the resilience index calculated on grain number per spike (G#/spk-RCI) at late-booting stage (Figure 5.6 B and C and Table 5.2). This suggests that at late-booting stage a higher accumulation of leaf ethylene and lower ABA leaf concentration appear to be associated with resilience to severe drought stress which may favour the development of more grains per spike. However, in this work, only one genotype at late-booting stage had a significantly different leaf [ABA] to the others (genotype 4, Figure 5.3 D), but leaf [ABA] was significantly enhanced, for all genotypes, compared with the concentration under irrigated conditions (Figure 5.3 D). In contrast, ethylene showed more variability at this stage (late-booting) (Figure 5.3 C) and a high leaf ethylene production appears to be associated with high resilience in terms of grain number per spike under drought stress (Figure 5.6 B).

The relationship between leaf hormone ratio (ABA/ETH) and the resilient component of grain number per spike (G#/spk-RCI) is negative (Figure 5.6 C) while the relationship between ethylene emission rate *versus* G#/spk-RCI is positive (Figure 5.6 B) , due to the fact that

ethylene emission values are in the denominator of the ratio and as ethylene emission rate values (expressed in $nmol.gFw^{-1}.h^{-1}$) were less than 1 (Figure 5.6 B) and the [ABA] concentrations (expressed in $nmol.gDw^{-1}$) were greater than 1 (Figure 5.6 A).

How can leaf hormones (ABA, ethylene) at late booting stage influence grain number per spike?

Grains number per spike results from a compromise between the pollination at anthesis and the balance of florets production-abortion within each spikelet (Siddique *et al.*, 1989). It is already known that meiosis, which starts at booting stage and finishes at anthesis, is a very stress-susceptible stage of development (Acevedo *et al.*, 2002). In wheat, meiosis starts in the middle of the spike, continuing later above and below this zone (Zadoks *et al.*, 1974). Siddique et al., (1989) have shown that florets death, in wheat, starts at the initiation of booting and these authors did not observe more floret abortion after the ear started to emerge (which might correspond to half-emergence, in this work). In the same research (Siddique *et al.*, 1989), it has been observed that floret death coincides with the period of the highest growth rate of the stem and the peduncle. Kirby (1988) suggest that floret death is a consequence of a carbon competition, between spike and stem, during the maximum growth rate of spike and when translocation may be affected by restriction of the vascular system. It is known that environmental stress speeds up plant development and in contrast, a longer reproductive phase, principally the stem elongation phase, increases the number of fertile florets per spike and finally the number of grains (Miralles *et al.*, 2000).

Consequently, late booting stage could be associated with the timing of meiosis development of apical and basal spikelet. Water stress at meiosis (spike growth stage or boot stage) reduces the availability of Carbon and Nitrogen which is critical during the spike growth stage (Acevedo

et al., 2002) and in turn results in death of florets from the terminal and basal spikelets of the spike (Oosterhuis and Cartwright, 1983).

Therefore, late-booting could correspond to a sensitive stage for the basal and terminal spikelets. As sugar availability seems to be the critical point in the determination of the number of grains per spike, the genotype that has higher sugar availability should reduce less its number of grains per spike. Kobayashi and Saka, (2000) concluded that sucrose content positively regulates the rate of ethylene evolution in the rice seedling leaf, and therefore it could be hypothesised that through this mechanism, higher leaf ethylene production may be then associated with more grains per spike. Additionally, it has been observed that the absence of a functional ethylene receptor on ethylene insensitive Tobacco and *Arabidopsis* sp. leads to a reduction in photosynthetic capacity and rubisco content (Tholen *et al.*, 2007, 2008). Thus, it could be suggested that at this particular stage when the spike reaches the maximum growth rate and competes for carbohydrates, the higher ethylene emission, observed with resilient genotypes in terms of grain number per spike compared with sensitive genotypes, could 1) slow down shoot growth and 2) be associated with plants which have higher sucrose content in the leaf and therefore higher resource availability for reproductive development during the stress period. Both responses can result in an increase of the number of grain and consequently the final grain yield.

5.5. Conclusion

This work demonstrated that it is important to consider the phenological development of the plants and the plant organ tissue in order to study whether plant hormones quantification (ABA and ethylene) could be used as possible traits for drought stress resilience in wheat lines.

Additionally, it has been observed that the hormone ratio (ABA/ETH) may better indicate genotypic variation in stress resilience when compared with data on the production of the individual hormones ABA and ethylene. Leaves at late booting stage (GS 47) were identified to be a key tissue and stage at which ethylene production and the hormone ratio (ABA/ETH) must be determined to indicate the resilience in terms of grain number per spike, under severe drought stress. Following the methods used in this work to quantify ABA and ethylene production, it can be concluded that at late-booting, a lower leaf hormone ratio ABA/ETH is associated with a high resilience term of grain number per spike under severe drought stress where a high ethylene emission rate is a determining factor of this relationship. This genotypic and phenotypic dependence might be useful to develop a new trait for breeding selection for spike fertility resilience under severe drought stress.

Grain per spike resilience under drought stress was observed to be significantly and positively correlated with spike fertility ($r= 0.697$, $n=24$, $P<0.01$, data not shown) which is a major component in the determination of grain number per m^2 and obtained as the quotient between grain number and spike chaff dry weight (Abbate *et al.*, 2013). Therefore, screening for grain per spike resilience (G#/spk-RCI) would help the screening of resilience of spike fertility and yield under drought stress.

It would have been appropriate to conduct another field experiment to confirm these hypotheses, but limited funds did not allow the opportunity for further trialling.

In the next Chapter, an experiment was designed and conducted under controlled environment condition to continue investigating the effects of leaf hormone production (ABA and ethylene) and the relation of hormone ratio with resilience in terms of grain number per spike under contrasting environmental conditions.

Chapter 6. Leaf hormone quantification under controlled environment: a new physiological trait for spike fertility resilience to drought stress?

6.1. Introduction

Phenotyping can be defined as a set of protocols and methodologies used to measure plant growth, architecture, and composition at different biological levels, from organs to canopies (Fiorani and Schurr, 2013), while genotyping is the process to determine the genetic constitution of an individual by analysing DNA sequences. Finding the connection between phenotype and genotype is now one of the major goals of a plant breeding program, in order to speed up selection for high productivity and resilience (Rahaman *et al.*, 2015). Therefore, to establish the link between genes and traits, both, phenotyping and genotyping, are equally important. However, precise phenotyping is the major bottleneck which limits advances in linking genes to phenotypic stress resilience (Cobb *et al.*, 2013; Araus and Cairns, 2014). Phenotyping is generally time-consuming and costly (labour-intensive) and requires destruction of plants at particular phenological stages and/or fixed times (Araus and Cairns, 2014). Nowadays, plant phenotyping programmes have as major goals, 1) to increase the accuracy, precision, and throughput of phenotype interpretation at all levels of biological organisation, such as growth, resistance, architecture, physiology, yield, etc. and 2) at the same time, reduce labour and costs (Rahaman *et al.*, 2015). Indeed, high-throughput phenotyping techniques are developed with the objectives of reducing cost and evaluating more rapidly plants for stress resilience (such as drought, diseases, etc.) by collecting data from plants in a non-destructive manner, such as remote sensing and thermal imaging approaches (Chávez *et al.*, 2009, 2010, 2012; Li *et al.*, 2014). However, precise field phenotyping is a complex issue due to the high heterogeneity of field conditions, with little or no control of a range of environmental factors.

On the other hand, experiments under controlled environments, such as under greenhouse or growth chamber conditions, are often conducted under conditions that are unrepresentative of field situations and the interpretations of results, from controlled environments, can be difficult to extrapolate to the field (Araus and Cairns, 2014). For example, under field conditions, soil - which supplies essential nutrients and water - is heterogeneous, but, under controlled environments, soil tends to be homogeneous, and its volume (in a pot) may be very small compared to the volume of soil available to a field-grown plant (Saint Pierre, 2012). The crop density is another important difference between controlled (generally an isolated plant in a pot) and field conditions (sown in plots with a specific plant density).

Despite these differences between both environments, the choice to phenotype under one or the other environment, depends on the heritability of the trait, the purpose of phenotyping and also the feasibility of collecting the data (Cobb *et al.*, 2013). For example, some experiments are not recommended and/or not authorized to be conducted under field conditions such as those with transgenic plants (Saint Pierre, 2012).

Phenotyping for drought stress resilience of plants in pots under controlled environments is a challenge. In addition to the difference in soil volume between controlled environments and field conditions, laboratory experiments for drought stress resilience present other difficulties such as reproducing the increase of field soil mechanical resistance when potted soil dries (Cairns *et al.*, 2011). Potted plants commonly need to be re-watered several times during the plant cycle under a controlled environment. Therefore, experimental design under controlled conditions studying plant physiology, in particular with pots, needs to be carefully thought through and defined, in order to ensure that physiological responses observed under controlled environments would be likely to be observed in the field (Poorter *et al.*, 2016). A physiological

response found under controlled environments, such as a trait for drought resilience, for example, should be tested in the field before a recommendation is made to breeders.

According to Tyagi *et al.* (2011), many studies on the impacts of terminal drought stress on cereals have been conducted, while there have been fewer investigations on the impacts of drought stress at pre-anthesis developmental stages. Under drought stress, plant hormones and mainly ABA and ethylene, have shown to play important roles in the regulation of the growth rate during plant development and also in controlling a range of reproductive processes (Wilkinson and Davies, 2002, 2010; Sharp and LeNoble, 2002; Davies, 2004*b*; Ji *et al.*, 2011).

In Chapter 5, it was concluded that at late-booting stage under drought stress, high leaf ethylene emission rate and low leaf ABA/ETH were identified as good indicators of the resilience component of grain number per spike, which is in turn directly related to the spike fertility.

Thus, the aim of this Chapter is to test whether the results obtained in Chapter 5 (under field conditions) are also observed under controlled environments, i.e. whether leaf hormone quantification (ethylene, ABA and Ratio ETH/ABA) 1) enables identification of genetic variation at a specific phenological stage and 2) shows a relationship with the genotype resilience in grain number per spike. Finally, consideration is given to whether hormone quantification under controlled condition can be recommended as a useful phenological trait for breeding selection.

All references in this Chapter to field data refer to the data presented in the previous Chapter (Chapter5).

6.2. Materials and methods

For this experiment, four genotypes (1, 5, 6 and 8) were selected from the CIMCOG-ROOT trial. These genotypes showed contrasting responses to drought stress in terms of the resilience component of grain number per spike, grain number per m^2 (according to the index method on yield and yield components described in Chapters 2 and 3 and calculated within a population of 10 genotypes). However, grain number per m^2 cannot be reliably evaluated in small pots due to a difference in the sowing density compared with field conditions. In terms of resilience of grain number per spike, under field conditions, genotypes 1 and 8 were identified as resilient, while genotypes 5 and 6 were identified as more sensitive to drought stress under field conditions (Chapter 3). However, since plants grown under a controlled environment may have altered physiological responses compared to those grown under field conditions, the resilience capacity index (RCI) of the different yield components (thousand grain weight, number of grains per spike, number of grains per spikelet and number of spikelets per spike) was recalculated here with their counterpart results collected under controlled environment conditions (i.e., using the data from well-watered and water-stressed plants) within this population of four genotypes. The assessment of the resilience index of yield components under controlled conditions followed the same methodology described in Chapter 3 Section 3.2.3. The plants were grown in the controlled environment from October to early December (from germination to maturity).

Seed germination procedures, planting and growing conditions were the same as described in Chapter 4 Section 4.2.3. The planting density was four seeds per pot and six pots per genotype, placed under greenhouse conditions with 12 hours photoperiod (from 8:00 am to 8:00 pm, 550 µmol photons. m^{-2} s^{-1}) and day/night temperatures of 25ºC and 18ºC, respectively.

The plants were irrigated adding water at the bottom of the pot to maintain the soil surface dry, to mimic the water uptake from soil by the plants under field conditions through the different phenological stages. Additionally, this approach should result in greater stability of the ABA signal, as Puértolas *et al.*, (2017) demonstrated that soil drying treatments under controlled conditions by high-frequency of deficit-irrigation limited root ABA accumulation and suppressed long-distance ABA signalling in response to stress. Thus, the pots were placed on horizontal trays where the water was added, to ensure the stability of ABA signal through minimum disturbance caused by the irrigation process (adding water).

In terms of water amount per tray, the irrigation was calculated in function of the field capacity of the substrate and the number of pots per tray. The field capacity of the pot was determined using 8 extra pots filled with the same soil substrate (John Innes No2, J. Arthur Bowers, UK) at the same level and bulk density to that of the experimental pots. The substrate was initially wet (0.25 g of water. g^{-1} of soil) at the moment of potting, so these extra pots were left in the greenhouse for 4 days, under the same environmental conditions than the rest of the pots, in order to totally dry the soil. Hence, the pots were weighed to obtain the dry soil weight. Later, the bottom (¾ of each pot) was submerged into a sink full of tap water for 1 hour. Pots were then left to drain for 5 hours before being weighed. The mean difference between the weight of the pot with the dry-soil and the wet-soil was 618 g. The field capacity was rounded to 600 ml of water. Therefore, the irrigation water (added to the tray) for the well-watered treatment consisted of 600 ml (full field capacity) per pot and 45% of the field capacity for the water stress treatment, i.e., 270 ml and it was applied every six days, and was stopped when the plants reached their physiological maturity.

Two sets of 48 pots (6 pots x 4 genotypes x 2 environments) sown with a difference of two weeks to enable sampling of plant material at different phenological stages (late-booting and heading stages where genotypic differences under field conditions were observed (Chapter 5)) at the same day and same time for each treatment (well-watered (WW) and water stressed (WS)) and for each genotype. This method allows fixing any possible environmental variation during the sampling between two distal phenological stages. The experiment was a randomised complete block (RCB) design with same distribution for the two sets.

The flag leaf was sampled from the main stem, for ABA and ethylene quantification with four replications per hormone, treatment and genotype. For the same genotype in the same block of each set, flag leaf was sampled at late-booting (second set sown) and heading stages (first set sown) at the same time (around 5 minutes between the two stages) before sampling the next genotype at both stages. The sampling started 1 month and 20 days after planting the first set. The hormone quantification method used here is described in Chapter 4 Section 4.2.1 (ABA) and 4.2.2 (ethylene).

The determination of phenological stages of development followed the Zadoks scale (Zadoks *et al.*, 1974) (Appendix 3 Table 1).

To determine the yield components, all the spikes, from the six pots which were not used for ABA and ethylene quantification, were harvested at maturity. This ensured a reasonable number of spikes to provide an estimation of the yield components. Usually, under controlled conditions it is not possible to determine all yield components, and only thousand grains weight, grain number per spike, grain number per spikelet and spikelet number per spike can be assessed. Other yield components, such as yield, number of grains per square meter and number of spikes per square meter, cannot be estimated as there is no proper sowing density, compared

with field conditions. The number of tillers per plant was counted for both treatments under controlled conditions in the greenhouse. At harvest, spikes from small tillers that showed total sterility under well-watered conditions, were not considered in the calculation of grain number per spike and its components. Table 6.1 shows the number of spikes collected under well-watered and water stress conditions for the determination of the yield components.

The number of spikelets per spike was firstly determined by counting, then the spike was hand-threshed and grains were counted for each spike, individually. The number of grains per spikelet was determined by the average number of grains per spikelet of individual spike (dividing the total number of grains per spike by the number of spikelets per spike). Thousand grain weight (TGW) was determined by weighing all the grains harvested and dividing this weight by the total number of grains and multiplying by 1000.

Table 6.1: Number of spikes collected from four genotypes (entries) under well-watered and water-stress conditions, in the greenhouse, to estimate the yield components. Spikes from tillers that showed total sterility under well-watered conditions were not included in these calculations.

	Well-watered		Water-stress	
Entries	Main stem	Tillers	Main stem	Tillers
1	8	20	8	3
5	8	13	7	1
6	8	20	8	1
8	8	23	8	0

The assessment of the hormone ratio (ABA/ETH) was carried out following the method described in Appendix 5A.

The statistical analysis was performed as described in Chapter 5 Section 5.2.2.

6.3. Results

6.3.1. Genotypic variation in hormone quantification at two distal phenological stages under controlled conditions

Table 6.2 shows the resilience capacity index (RCI) of thousand grain weight, grain number per spike, grain number per spikelet and spikelet number per spike for each genotype (1, 5, 6, 8). These genotypes will be identified in function of their resilience on grain number per spike (G#/spk-RCI), as follows: genotypes 1 and 8 as resilient (G#/spk-RCI = 10 and 9, respectively), genotype 6 as more resilient than sensitive (G#/spk-RCI = 6), and genotype 5 as sensitive (G#/spk-RCI = 1) (Table 6.2). Under controlled conditions, it was observed that the index of genotype 6 in terms of grain number per spike was slightly different compared with its counterpart under field conditions (G#/spk-RCI = 4, Table 3.11).

Table 6.2: Score table of the resilient capacity index calculated on thousand grain weight (TGW-RCI), grain number per spike (G#/spk-RCI), grain number per spikelet (G#/spklt-RCI) and spikelet number per spike (Spklt#/spk-RCI), calculated with the mean value of each yield components obtained under well-watered (WW) and water-stress (WS) treatments of four contrasting genotypes under controlled environment.

Genotypes	TGW-RCI	G#/spk-RCI	G#/spklt-RCI	Spklt#/spk-RCI
1	5	10	10	10
5	10	1	1	5
6	1	6	9	3
8	8	9	8	1

Figure 6.1 shows the ethylene and ABA concentrations in leaves of the four genotypes, respectively, at late-booting (Figure 6.1 A and B) and heading stages (Figure 6.1 D and E) under water stress and well-watered conditions (greenhouse).

Figure 6.1 A shows that, at late-booting stage under water stress (WS), only genotypes 6 and 8 (both resilient) showed a significant reduction in leaf ethylene emission rate, compared with the well-watered treatment. Under WS, genotype 1 (resilient) showed the highest leaf ethylene production and genotype 5 (sensitive) the lowest, while genotypes 6 and 8 showed an intermediate value not significantly different from 5 and 1. At late-booting stage under well-

watered conditions (WW), genotype 8 (resilient) showed the highest ethylene leaf production, while genotype 5 (sensitive) showed the lowest leaf ethylene emission rate. The other two resilient genotypes (1 and 6) showed an intermediate value not significantly different from 5 and 8 (Figure 6.1 A).

Figure 6.1 B shows that at late-booting stage under water stress (WS), leaf ABA concentration increased significantly compared with the well-watered treatment, except for genotype 6. Genotype 1 (resilient) showed the highest ABA concentration in leaf, while genotypes 6 (resilient), the lowest.

Genotypic variation in terms of leaf ethylene emission rate and leaf ABA accumulation is observed under water stress conditions at late-booting (Figure 6.1 A and B) and only for ethylene emission under well-watered conditions at the same stage (Figure 6.1 A).

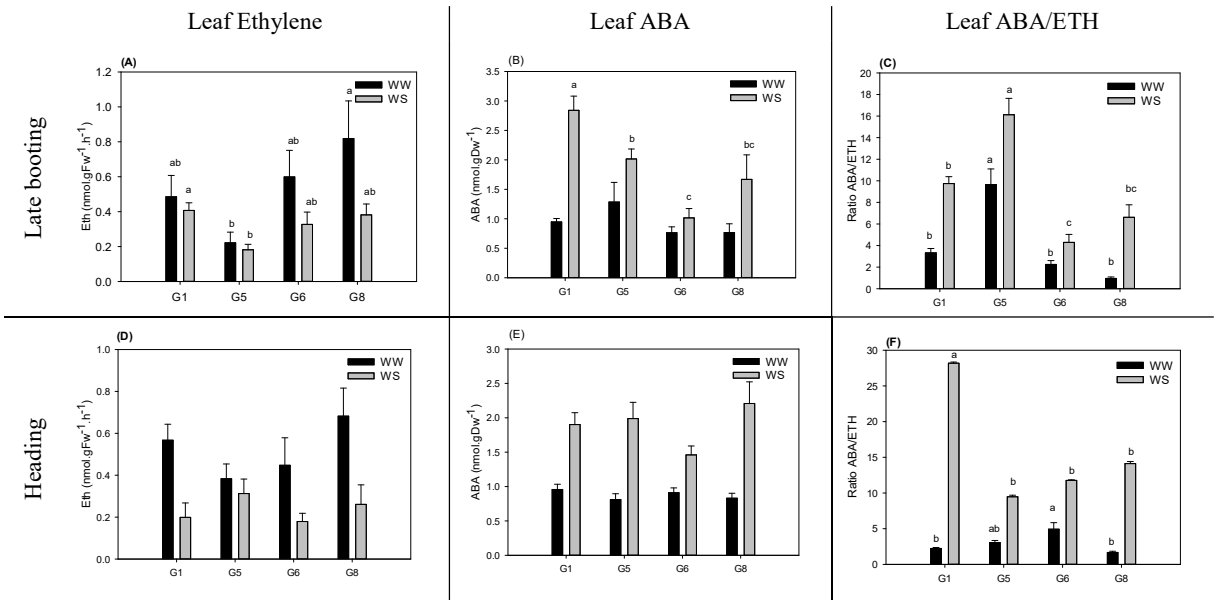


Figure 6.1: Genotypic variation at late booting (A, B and C) and at heading (D, E and F) stages of leaf ethylene emission rate (A and D), leaf ABA concentrations (B and E) and hormone ratio ABA/ETH (C and F) under controlled environment for the well-watered (WW, dark bars) and water-stress (WS, grey bar) treatments. Data were collected on four genotypes from the CIMCOG-ROOT trial (10 genotypes). Columns and bars are means ± standard error of 6 replicates. Different letters indicate significant ($P < 0.05$) difference among genotypes within an environment only when such differences exist between the genotypes.

Figure 6.1 C and F shows that under water stress at both stages (late booting and heading) the leaf hormone ratio is significantly increased compared with well-watered plants, except for genotype 6 at late-booting stage (Figure 6.1 C).

At late booting stage, under water stress, genotype 5 (sensitive) showed the highest leaf hormone ratio value and genotype 6 (resilient) the lowest, while genotypes 8 and 1 (both resilient) showed an intermediate value and under well-watered conditions, genotype 5 (sensitive) showed the highest leaf hormone ratio value while genotypes 1, 6 and 8 (resilient) exhibited the lowest ratios (Figure 6.1 C).

At heading stage, under water stress, genotype 1 (resilient) showed the highest leaf hormone ratio, while genotypes 5, 6 and 8 the lowest and under well-watered conditions, genotype 6 (resilient) showed the highest leaf hormone ratio and genotypes 8 and 1 (both resilient) the lowest (Figure 6.1 F).

Finally, the hormone ratio ABA/ETH showed at both stages, late-booting and heading, significant genotypic variation (Figure 6.1 C and F) under water stress and well-watered conditions.

6.3.2. Could hormone quantification indicate resilience to drought stress when experiments are conducted under controlled environment conditions?

Table 6.3 shows the Pearson coefficient of correlation between leaf hormone production (ABA and ethylene) and the leaf hormone ratio *versus* the resilience index calculated on thousand grain weight (TGW-RCI), grain number per spike (G#/spk-RCI), grain number per spikelet (G#/spklt-RCI) and spikelet number per spike (Spklt#/spk-RCI).

Leaf ABA concentration ([ABA]), at late-booting stage, showed a significant positive correlation with the resilience component of spikelet number per spike (Spklt#/spk-RCI)

(r=0.50, *P*<0.05) (Table 6.3). On the other hand, leaf ethylene emission showed, at late booting stage, a significant and positive correlation with 1) the resilience component of grain number per spike (G#/spk-RCI) (r=0.77, *P*<0.01), and 2) the resilience component of grain number per spikelet (G#/spklt-RCI) (r=0.688, *P*<0.01) (Table 6.3). The leaf hormone ratio ABA/ETH showed at late-booting stage, 1) a positive and significant correlation with the resilience component of thousand grain weight (TGW-RCI) (r=0.510, *P*<0.01), and 2) a negative correlation with the resilience component of grain number per spike (G#/spk-RCI) and grain number per spikelet (G#/spklt-RCI) (r=-0.529 and r=-0.515, respectively, *P*<0.01) (Table 6.3). At heading stage, the leaf hormone ratio ABA/ETH showed positive and significant correlations with the resilient component of grain number per spike (G#/spk-RCI) and grain number per spikelet (G#/spklt-RCI) (r=0.303 and r=0.261, respectively, *P*<0.01) (Table 6.3). The highest correlation is observed, at late booting stage, between the resilience components of grain number per spikelet (G#/spklt-RCI) *versus* leaf ethylene emission rate (positive correlation).

Table 6.3: Pearson coefficient correlation between the Resilience Capacity Index (RCI) calculation based on yield components (TGW: thousand grain weight, G#/spk: grain number per spike, G#/spklt: grain number per spikelet, spklt#/spk: spikelet number per spike *versus* flag leaf ABA concentration, ethylene emission rate and the hormone ratio ABA/ETH at late booting stage and the ratio ABA/ETH at heading under drought stress. The indices have been calculated with the data collected under controlled environment (well-watered and water stress). * indicates *P*<0.05, ** indicates *P*<0.01.

Hormone	Phenological stages	TGW-RCI	G#/spk-RCI	G#/spklt-RCI	Spklt#/spk-RCI
ABA Flag Leaf	Late booting	0.180	-0.003	0.096	0.500**
ETH Flag Leaf	Late booting	-0.303	0.773**	0.688**	0.177
ABA/ETH Flag Leaf	Late booting	0.510**	-0.448**	-0.515**	0.066
	Heading	-0.106	0.303**	0.261**	-0.064

Figure 6.2 illustrates the relationship between the resilience component of number of grains per spike *versus* (A) the mean leaf ethylene emission rate where resilient genotypes (1, 6 and 8) in terms of number of grains per spike show a high ethylene emission rate, while genotype 5 shows the lowest; and (B) the mean leaf hormone ratio (ABA/ETH), where resilient genotypes (1, 6 and 8) in terms of number of grains per spike show a low ABA/ETH, while genotype 5 shows

the highest. However, the distribution is not linear and shows an optimum threshold ABA/ETH ratio of around 10.

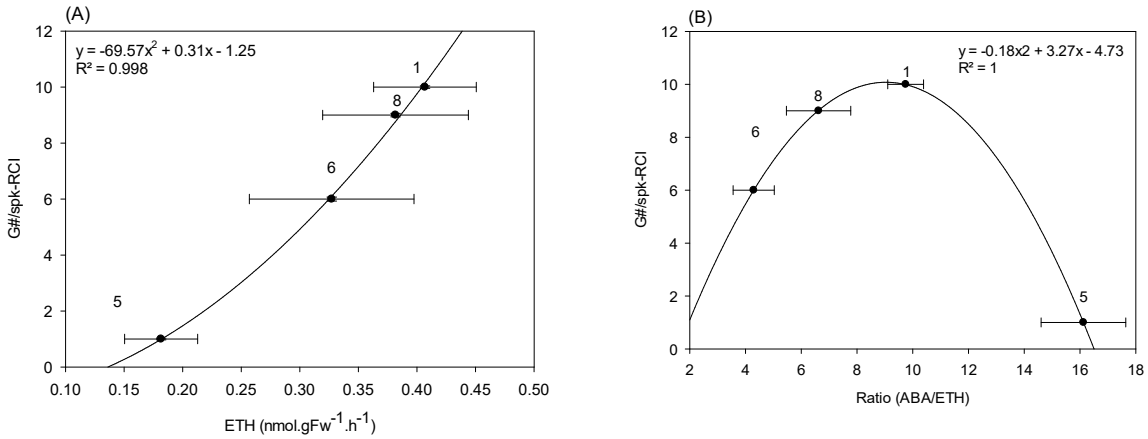


Figure 6.2: Polynomial regression between (A) the mean leaf ethylene emission rate (nmol.gFw^{-1}.h^{-1}), and (B) the mean leaf hormone ratio under water stress (WS) *versus* the resilience capacity index of grain number per spike (G#/spk-RCI) of the four selected genotypes (1, 5, 6, 8) from CIMCOG-ROOT trial.

6.4. Discussion

In this kind of work, the extrapolation of results from controlled environments to field environments is usually difficult due to the variation of factors between these two environments, such as soil temperatures, rates of soil drying, moisture uniformity in pots, depth and volume of soil for root growth, and the availability of nutrients (Saint Pierre, 2012; Araus and Cairns, 2014), among others. Additionally, weaknesses of this experiment were firstly the use of too few genotypes (2 resilient *versus* 2 sensitive) to compare genotype behaviour under field and controlled environments and secondly the application of day and night temperature (25 ºC/18 ºC) could be considered too high with a prolonged photoperiod compared with typical field conditions at these developmental stages, which could introduce some noise into the experimental results. During the drought stress experiment (Chapter 5), the temperature was over 25 ºC only for 6 hours and the night temperature dropped slowly from 19 ºC to 10 ºC (Appendix 4 Figure 2). To improve the experimental design to better mimic field conditions,

night temperature should be fixed at 12 ºC (instead of 18 ºC) and the photoperiod should be reduced to 10 hours. When the photoperiod starts, additional improvement could be made by increasing slowly the temperature during 3 to 4 hours to reach the maximum temperature (25 ºC) then maintaining this temperature for 4 hours and decreasing very slowly to 12 ºC during 16 hours. Despite this, good similarities were observed between the results obtained under field conditions (Chapter 5) and controlled environment (this Chapter).

In fact, the calculation of the indices based on the different yield components shows similarities with their counterpart values obtained with the field data (Appendix 6 Table 1) mainly for the resilience component of grain number per spike (G#/spk-RCI) and the resilience component of grain number per spikelet (G#/spklt-RCI).

It is true that the resilience component of grain number per spike (G#/spk-RCI) for genotype 6 seems to have slightly changed from a score value of four (G#/spk-RCI = 4) under field conditions (Chapter 3, Table 3.11, calculated on a population of 8 genotypes) to a score value of six under controlled environment (G#/spk-RCI = 6, calculated on a population of 4 genotypes) showing a slightly better resilience index in terms of grain number per spike under controlled environment than under field conditions. However, when the resilience capacity index of grain number per spike is calculated within the population of these four genotypes using the field data, the index score value is five for genotype 6 (G#/spk-RCI = 5) (Appendix 6 Table 1), and a difference of one score unit (from 5 to 6) should not be interpreted as a difference. Despite the fact that the genotype 6 showed, under controlled condition, a more resilient behaviour in terms of grain number per spike, in a general vision, for future selection and interpretation, a score value of PCI or RCI (e.g.: G#/spk-RCI) value between 4 and 6 should be considered as a more intermediate behaviour, neither resilient nor sensitive to drought stress.

Therefore, the discussion of this work and conclusion about resilient genotypes should be focus only on genotype 1 and 8 which have shown a constant resilient response between field and controlled environments. This slight variation, between field and controlled environments, stressed the fact that the indices are more precise about the resilience and production capacity when they are calculated on a bigger population. Indeed, for a selection on resilience of contrasting genotypes, it is recommended to calculate the indices with a large population and preferentially over years, if possible, and using a range of score indices. For example, for a selection for high resilience to drought, it would be recommended to select genotypes with highest RCI such as from 10 to 8 or 7. However, in this work, it is observed that the score indices calculated with the field data and the controlled environment data from the same population of four genotypes show similar behaviour in term of resilience capacity of grain number per spike and grain number per spikelet to drought stress (Appendix 6 table 1).

This is a new insight suggesting that the index offers the possibility to identify the resilience capacity of genotypes grown under controlled conditions in terms of grains per spike and per spikelet with a good extrapolation under field conditions. Thus, making a previous selection for these traits under controlled environment, will reduce space for selection (CE such as phenotyping platform) and consequently, reduce time and costs for breeding programme. Similarly, Guo *et al.* (2016) have observed that grain number per spike and grain number per spikelet show a reasonable degree of consistency between greenhouse and field conditions with a relatively high heritability of these traits.

A clear example of G x E interaction was observed between the experiments under field and controlled conditions in terms of hormone production. In the study under discussion here, at late booting stage under water stress (controlled conditions), the ABA accumulation shows

significant differences between genotypes (genotype 1 (resilient) the highest ABA production and genotype 6 (resilient with neutral tendency) the lowest (Figure 6.1 B)) but no differences were observed under field conditions (Chapter 5 Figure 5.3 D). This difference, between field and controlled environments, can be partly associated to the limiting factors of controlled environments mentioned by Saint Pierre (2012), as described in the above paragraph. Additionally, based on the research of Puértolas *et al.* (2017), the high ABA production of genotype 1 under water stress (controlled environment) compared with the other three genotypes, could be associated to a morphological difference in biomass (above and below ground) which modifies the speed at which the soil is drying and varies the intensity of the stress. This means that a genotype which produces more biomass will consume more water and therefore will suffer a higher drought stress intensity than the other genotypes, due to a difference in the evapotranspiration between each pot. Appendix 6 Table 2 shows that genotype 1 has produced, under water stress, more stems (average fertile stem per pot: 4.00) than the other three genotypes (5, 6 and 8, with 3.33, 3.00 and 3.16 fertile stems per pot, respectively) and with a bigger biomass per pot, genotype 1 would probably have suffered a higher stress compared with the others, while genotype 6 with the lowest number of tillers would have suffered a lower water stress under controlled conditions. Therefore, to improve this experiment, it would be recommended to measure the soil moisture to properly evaluate the soil water stress of each pot at three levels (surface, mid-pot and bottom) along the phenological development of the plants. Another option would be maintaining a constant water stress through a high frequency deficit irrigation. However, for this, it will be necessary first to test whether the re-watering at the bottom of the pot limited or not the root ABA accumulation and suppressed long-distance ABA signalling in response to stress, as it was observed by Puértolas *et al.* (2017) when the soil surface was rewatered.

Independently of these differences of hormones production between field and controlled environment, the study of drought stress resilience through hormone quantification has shown similarities.

As it was the case under field conditions (Chapter 5 Section 5.3.2), genotypic variation was more clearly identified in response to drought stress by the leaf hormone ratio ABA/ETH when the variation was observed at both stages (late-booting and heading stage, Figure 6.1 C and F), while the individual leaf hormone quantification (ethylene and ABA) only showed genotypic variation at late booting (Figure 6.1 A and B).

Similarly, with the experiment under field conditions (Chapter 5 Section 5.3.2), it is observed that the analysis of individual hormones (ethylene and ABA) production cannot easily identify a tolerant or sensitive mechanism in response to drought stress. In fact, in this work, the sensitive genotype (genotype 5) showed contrasting responses of individual hormone production (ethylene or ABA) with only one resilient genotype (genotypes 1) (Figure 6.1 A (ETH) and B (ABA)). Under water stress at late booting, genotype 5 shows a lower leaf ethylene emission rate which was significantly different from genotype 1 (resilient) but not significantly different to the other resilient genotype 8 (Figure 6.1 A). At the same stage and treatment, genotype 1 (resilient) shows the highest leaf ABA concentration significantly different from genotype 5 (sensitive) and genotype 8 (resilient) but no significant differences was observed between genotype 5 and 8 (Figure 6.1. B). However, the ratio differentiates significantly sensitive (5) from resilient genotypes (1 and 8), in terms of number of grains per spike at late booting stage (Figure 6.1. C), but not at heading stage (Figure 6.1. F). Thus, as it was also observed under field conditions (Chapter 5) the use of the score index allows a visualization of a relationship between the resilience component of number of grains per spike (G#/spk-RCI)

versus 1) the leaf ethylene emission rate or 2) the hormone balance at late booting stage (Figure 6.1 A and C).

Hormone balance: an indicator of drought stress resilience under controlled environments?

In this work, as was the case under field conditions (Chapter 5 – Section 5.3.3), late-booting and leaf tissue are identified as the key factors to relate hormones quantification (ethylene and ABA) with resilience of spike fertility. In fact, under both environments, a low ethylene emission rate and high hormone ratio (ABA/ETH) indicate a low resilience to drought stress in terms of grain number per spike (case of sensitive genotype 5) and a high ethylene emission rate and low hormone ratio (ABA/ETH) usually indicate a higher resilience to drought stress in terms of grain number per spike (case of genotypes 1 and 8) (Figure 6.2).

Additionally, ethylene emission rate is observed as a determinant factor in the relationship between the leaf hormone ratio (ABA/ETH) and the spike fertility resilience, which is in accordance with the results obtained in Chapter 5. Similar conclusion was made by Valluru *et al.* (2016), who suggested the important role of ethylene in the change of the ratio (ABA/ETH) and the effect on shoot biomass under mild drought at three-leaves stage. However, in Valluru *et al.* (2016) a lower concentration of ethylene and higher ratio was considered to exert a beneficial effect on yield when is this work these levels of ethylene and ratio (ABA/ETH) are associated to a lower resilience (Chapter 5 and 6). These differences, could be explained by the fact that samples were taken at a different phenological stage (three-leaves (Valluru *et al.*, 2016) vs late booting (this work)) and the severity of the stress was lower (mild drought stress (Valluru *et al.*, 2016) vs severe drought stress (this work)) (opposite effects of traits depending upon the severity of stress e.g. Tardieu, 2012). The major importance of ethylene and its direct relation

with spike fertility at late booting stage could be due to the fact that ABA will always increase under drought stress and the fact that the ABA effect on the plant might be regulated by ethylene production (e.g. Sharp and LeNoble, 2002).

However, under controlled environments, genotype 6 shows low ABA production under water stress (Figure 6.1 B), compared with the other genotypes. This influences the value of the ratio and generates the main difference between the results observed under field and controlled conditions. Additionally, genotype 6 and 8 accumulate less ABA than genotypes 1 and 5, while under field conditions no differences were observed between genotypes 1, 5, 6 and 8 at the same stage regarding ABA production. This difference could be associated to the difference of biomass and the variation in the intensity of the development of drought stress between these four genotypes (Puértolas *et al.*, 2017), see discussion and recommendation in the paragraph above. The optimum threshold needs more investigation as the hormone ratio of genotype 6 is the only one who differs from the tendency observed in the field work (Chapter 5). Also, the possibility of an error in the quantification of ABA under water stress under controlled conditions must be considered.

Additionally, in Chapter 5 it was suggested that leaf ethylene emission rate and the leaf hormone ratio might play an important role at late booting stage, in the carbon availability for the development of young microspores of apical and basal spikelet. Ji *et al.* (2010) concluded that maintaining carbohydrate supply to anthers under drought stress during the young microspore development, may be a key element to maintain pollen fertility and consequently spike fertility. However, measurements of the photosynthetic rate at three different stages (booting, late booting and heading, Appendix 6 Figure 1 A, B and C, respectively) do not allow confirmation of such a hypothesis, as at late booting stage only genotype 8 showed a significantly higher

photosynthesis rate compared with the other ones. Consequently, the analyses of sugar content from the floret (basal and apical) at late-booting stage might be a better indicator of the association of leaf ethylene and carbon availability in spike.

6.5. Conclusion

Both hormones, ABA and ethylene, appear as important indicators to identify new traits associated with stress resilience in yield. In addition, leaf ethylene emission rate is observed to be a key factor to identify spike fertility resilience as in both environments (field conditions, Chapter 5, and controlled environments, Chapter 6) it is observed to be positively correlated with spike fertility. The ratio ABA/ETH, as observed in Chapter 5, is a better indicator of genotypic variation, and high hormone ratios are associated with low resilience allowing to discard sensitive genotypes under controlled conditions. Leaf ethylene emission rate and leaf hormone ratio, at late-booting stage, are valuable indicators of stress resilience in terms of number of grains per spike either under field or controlled environments.

These results emphasise the important role of the hormone interaction (ABA, ethylene) and complement other studies that have demonstrated previously the key role of the hormone balance and interaction such as ABA/ETH at grain filling stage during the post-anthesis period in wheat and rice (Yang et al., 2006a, 2007).

This work highlights the possibility to use hormone quantification as a new physiological trait for spike fertility resilience under controlled conditions during the pre-anthesis period. This could be a first step to speed up the selection process by reducing cost and time to breeders allowing to do several experiments per year on a phenotyping platform under controlled environment. However, this possibility needs to be evaluated at late-booting (early stage) on flag leaf tissue following the method of quantification applied in this work.

It would be necessary to repeat this experiment with at least 6 or 8 genotypes showing clear contrast (to avoid the case of genotype 6) in terms of number of grains per spike (resilience

index capacity), from another and bigger population (e.g. WAMI trial, with 294 genotypes) focusing only on leaf hormones (ABA and ethylene) at late-booting stage, to confirm these conclusions.

Chapter 7. General conclusion and discussion

Wheat is one of the world's major food sources and one of the most cultivated cereal crops with more than 220 million ha sown per year (Araus *et al.*, 2008; Shiferaw *et al.*, 2013). The changing climate and a growing population and food demand combine to provide an impetus for more rapid selection of productive and adaptive crop plants for stress environment (Rahaman *et al.*, 2015) such as drought which is one of the major limiting factors of yield worldwide (Blum, 2011). Therefore, a major objective for plant breeding is to maintain or even enhance plant growth and yield production in stress environments (Cattivelli *et al.*, 2008). For this, breeders need quantitative analyses of plant traits to accelerate the selection of crops that are better adapted to resource-limited environments (Fiorani and Schurr, 2013).

The rationale behind this study was to elucidate the role of hormones in bringing about physiological regulation contributing to stress resilience/tolerance. The expectation was that this information would be useful in both the selection of genotypes and improving field management practices. Both advances can enhance stress resilience of field crops.

7.1. What is a stress tolerant genotype?

There is no clear definition of the drought resistant ideotype, traits for high productivity are not always clear (Blum, 2005) and a reliable screening methods are a major bottleneck for improvement of stress tolerance during the reproductive stage (Dolferus *et al.*, 2011; Araus and Cairns, 2014). However, an increase in grain yield is often related with an increase in grain number per m^2 while the grain weight is usually stable or reduced, under yield potential conditions (Sayre *et al.*, 1997; Shearman *et al.*, 2005; Peltonen-Sainio *et al.*, 2007; Dolferus *et al.*, 2011) and under drought stress (Hochman, 1982).

Therefore, in this work, it was firstly necessary to clearly identify where wheat genotypes show a particular sensitivity or resilience to abiotic stress in terms of yield and yield components.

The score index method (described in Chapters 2 and 3) has been shown to be an effective and useful tool to identify genotype resilience to drought stress under both controlled and field conditions. This method helps us to understand better the basis of the genotypic yield response under stress and to identify contrasting genotypes in terms of production and/or resilience to study and identify differences in their physiological and developmental responses. This work has focused on the hormonal regulation of plant growth and yield development.

The use of the index method on yield and yield components, confirmed the positive relationship between grain number and grain yield, where the resilience and production capacity index of grain per m^2 (G#/m^2-RCI and G#/m^2-PCI), were both positively related with the final grain yield under stress environment (Chapter 3, Table 3.9 and Table 3.10). For decades, breeders have selected genotypes based on their production capacity (Araus *et al.*, 2004, 2008) and therefore, a complementary trait (resilience) will be useful for them to allow selection of high yielding genotypes with high resilience. In this work, the index method offers the possibility to identify separately new traits for breeding selection, either for production or resilience for each yield component and discover where (in term of yield components) genotypes are more sensitive or resilient to the environmental stress perceived by a population during the same cropping season.

In this work, the use of the index was the key tool in order to allow research work on hormone quantification. Logistical problems in Mexico meant that field work started with a population (CIMCOG-ROOT, 10 genotypes, Chapter 2 – Section 2.2.2 -Table 2.2) which was not specifically selected for contrasting responses to drought stress. Using the index method,

calculated with yield and yield components data collected under irrigated and drought stress conditions from the CIMCOG-ROOT trial, three genotypes were identified with a contrasting resilience response of grain per m^2 and grain number per spike (2 resilient and 1 sensitive) and one was identified with a more intermediate response. This kind of classification using the score index is novel and gives much more information about the genotype response to the stress environment compared to the usual terminology found in the literature such as tolerant/resilient and sensitive/susceptible genotypes usually without describing in which terms these classifications are made (probably yield). However, the index method presents some limitations, first the score index should be derived from a group of scores (e.g. grouping the three highest and/or the three lowest scores) and the score should ideally be estimated on a large population. The evaluation of a substantial population increases the genotypic diversity and allows the researcher or breeder to avoid the complication of working with genotypes presenting an intermediate response to stress (as it was the case in chapter 6).

The index selection method based on productivity and resilience has shown a particular advantage compared with the selection method based on yield production (widely used by breeders) (Araus *et al.*, 2004) as the number of selected genotypes by the index method compared to the conventional method is reduced by 33 %. In addition, 36% of genotypes discarded by the conventional method are identified, using the index method, as having good potential for resilience and productive yield under stress (Chapter 2 – Section 2.3.4). This index method offers a new perspective for breeding programmes to discoverer new genotype(s) with high adaptation and good productivity under stress environment by analysing quickly previous data base of old trials run under irrigated and stress environments at the field level.

7.2. Hormones: a new physiological trait? Factors to be considered.

Under drought stress, ABA and ethylene are generally considered to be two of the major plant hormones linked to the plant responses to water stress (Wright, 1980; Bradford and Hsiao, 1982; Wilkinson and Davies, 2002, 2010; Davies, 2004*b*; Salazar *et al.*, 2015). Ethylene and ABA can be quantified in all parts of the plant and there are involved in an extensive range of effects on developmental processes along the plant cycle (Chang and Bleecker, 2004; Davies, 2004*b*). Their concentrations are regulated in function of the phenological stage (e.g. Yang *et al.*, 2006*a*,*b*, 2007), tissue (such as leaf or floral organ e.g. Westgate *et al.*, 1996; Ji *et al.*, 2010), the stress environment (such as heat and drought e.g. Davies and Zhang, 1991; Hays *et al.*, 2007; Abeles *et al.*, 2012; Puértolas *et al.*, 2013). During wheat development, meiosis and grain filling stages have been identified as critical stages for wheat yield under stress (Acevedo *et al.*, 2002; Craufurd *et al.*, 2013; Rybka and Nita, 2015). Initially, to understand the action of these plant growth regulators on plant development, the studies were focused upon individual hormones (several literature reviews such as e.g. Himmelbach *et al.*, 2003 for ABA and Guo and Ecker 2004 for ethylene). However, other research suggests that the endogenous hormone production should be a better way to improve the understanding of hormone responses to environment cues (e.g. Yang *et al.*, 2006*a*, 2007; Davies *et al.*, 2013).

Nevertheless, the study of the ABA and ethylene accumulation can vary in function of several factors which increase the complexity of the research which aims to understand the roles of hormones in plant development. In this work, the factors considered were the tissues (leaf or spike), phenological stages, day time and irrigation (frequency and application) (Chapter 4, Chapter 5 and Chapter 6).

7.3. Diurnal variation in hormones: when to measure?

To identify properly the hormone impact on plant development not only tissue and phenology have to be considered but also the time of the day at which to quantify hormone accumulation. In fact, ABA and ethylene accumulation showed diurnal variation which should be integrated into the study of these hormones (Bidinger *et al.*, 1987; Ievinsh and Kreicbergs, 1992; Finlayson *et al.*, 1999; Kobayashi and Saka, 2000; Mcclung, 2000; Thain *et al.*, 2004; Lee *et al.*, 2006; Khan *et al.*, 2010).

It has been shown that sensitive and resilient genotypes show significant differences in terms of leaf ethylene emission between 12:00 pm and 05:30 pm under controlled environmental condition and under well-watered (Chapter 4 – Section 4.3.1.) or drought stress conditions in the field (Thiry *et al.*, 2017). In fact, under well-watered conditions at tillering stage it was observed that resilient genotypes (defined in term of yield under heat stress) did not reduce their ethylene emission between 12:00 pm and 05:30 pm while ethylene emissions of sensitive genotypes did decline significantly (Chapter 4 – Section 4.3.1) and *vice versa* under water stress at late-booting stage (between 01:00 pm and 05:30 pm) (Thiry *et al.*, 2017). These findings need more investigation as the responses between resilient and sensitive genotypes are inconsistent between environments and stages. As ethylene emission has been shown to reduce the sensitivity of the stomata to the ABA signal induced by drought (Wilkinson and Davies, 2009), it could be suggested that these genetic differences, between time of the day and ethylene and ABA production, could be associated with a mechanism of resilience or susceptibility by increasing or reducing the sensitivity of the stomata to ABA under drought stress. Before investigating this hypothesis, it is firstly necessary to identify when (phenological stage) and where (tissues) hormone quantification could be identified as a physiological trait for stress

resilience during the pre-anthesis. That was achieved in this work (Chapter 5 Section 5.3.3 Section and 6 Section 6.3.2).

7.4. Measuring hormones during the crop life cycle: when and where?

In this work, it was suggested that under drought stress, late booting stage and leaf tissue are key elements to relate grains per spike resilience to the quantification of ethylene and ABA accumulation (Chapter 5 and 6). Under field conditions, at late-booting stage, a high flag leaf ethylene emission and a low leaf hormone ratio (ABA/ethylene) indicate a higher resilience in terms of wheat grains per spike under drought stress (Chapter 5 Figure 5.6 B and C and Table 5.2 (field)). However, under controlled condition, at the same stage (late booting) and with the same tissue (Flag leaf), the hormone ratio (ABA/ethylene) differed, where a higher resilience (in terms of grains per spike resilience) was associated with a potential optimum threshold in ABA/ethylene (10) but ethylene was still positively correlated with spike fertility resilience to drought stress (Chapter 6, Figure 6.2 A and B and Table 6.3 (controlled environment)). Therefore, the results of this work show a possible means of identifying genotypes that are resilient to drought stress under controlled condition with a good estimation of resilience under field conditions by using hormone quantification. For this, the ratio and the ethylene emission rate, from leaf at late booting stage, are both needed and a high resilient genotype should show a high ethylene emission rate and a low ratio close to the threshold. More experiments under field and controlled conditions are needed to confirm this conclusion.

Under both environments (field and controlled), at late booting stage, leaf ethylene emission appears as the main driver of the relationship between the leaf hormone ratio (ABA/ethylene) and the spike fertility resilience. Other research already has suggested that ABA does not play a direct role in the regulation of spike fertility. In fact, in the wheat spikelet it was observed that

an increase of ABA concentration without water scarcity does not induce spike sterility (Dembinska *et al.*, 1992) contrasting with results of experiments under low water supply (Westgate *et al.*, 1996). In this work, no relationship between spike hormone quantification and spike fertility resilience could be observed. One possible explanation is that the hormone was quantified from the full spike and the fact that meiosis started in the middle of the spike and continue later on the apical and basal part of the spike (Zadoks *et al.*, 1974). Therefore, the hormone concentration of the different spikelets (which are at different stages) might be different and the hormone quantification of the full spike is not representative of the effect of hormone on sterility. On the other hand, in spite of the improved understanding of hormone interactions in reproductive tissues, the methodology will be hard to reproduce under field conditions for a high throughput method, for example, the manipulation to detached spikelets (Yang *et al.*, 2006*a*) or with sampling at the anther level (Oliver *et al.*, 2007). Therefore, leaf tissue appears to be more appropriate material to develop a high throughput method based on that trait.

7.5. Hormone: a trait for early selection, recommendation and limitation.

The aim of breeders is to rapidly screen a huge range of genotypes for high-yield and high resilience and their selections are mainly based on yield (Araus *et al.*, 2004; Monneveux *et al.*, 2012) but flowering date (which indicates whether the crop will avoid drought stress) and secondary traits providing drought resistance might be incorporated into their criteria if breeders are convinced of their value (Richards, 2006; Monneveux *et al.*, 2012).

The results in this thesis suggest that information on leaf hormone ratio (ABA/ETH) and ethylene emission, at late booting stage, might be useful for breeders, to screen for spike fertility resilience (in terms of maintenance of number of grains per spike), and it could be

recommended as a complementary (secondary) trait. This trait presents two main advantages to speed up genotypic selection for high resilience in terms of spike fertility as it can be assessed at an early stage of the development of the plant, giving an information before plants reach the maturity and it can be quantified either under field or controlled environments (Chapter 5 and 6). This is a new insight which offers to breeders a complementary trait, at early stage, and under controlled environments. This technique (resilience index of numbers of grains per spike and hormone quantification) could be recommended to pre-screen a huge number of genotypes for spike fertility resilience (at an early stage of the plant development), instead of planting all genotypes and evaluating them under field conditions from the first year. The proposed technique, under controlled environments, would reduce cost and time for breeding selection, it will not be dependent of the season and could be repeated several times in a year.

7.5.1. Recommendation for early selection

This approach (hormone quantification) only provides information about the resilience in terms of spike fertility under drought stress and this is only a part of the information breeders need. Therefore, to be more useful in the selection process, this information (about resilience in term of grain per spike) must be associated with information about the production capacity of the genotype.

Several researches have shown that a strong relationship exists between spike dry weight (g/m^2) at flowering time *versus* the grains number per m^2 which in turn is correlated with the number of fertile florets at anthesis and with yield (Sayre *et al.*, 1997; Shearman *et al.*, 2005; Peltonen-Sainio *et al.*, 2007; Fischer, 2011; Dolferus *et al.*, 2011; González *et al.*, 2011). The correlation between spike dry weight at maturity and grain number has also been corroborated during the

experiment described in Chapter 6 under controlled conditions (Figure 7.1, well-watered and water stress).

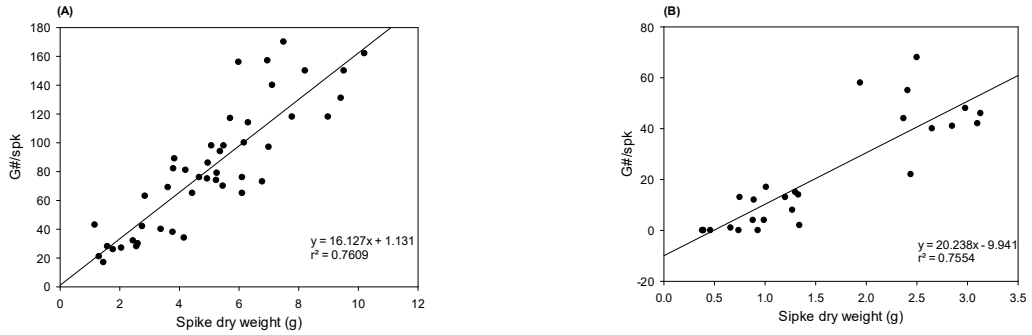


Figure 7.1: Linear regression between spike dry weight (g) and grain number per spike (G#/spk), under controlled conditions: A) well-watered treatment (WW) (n=32), and B) water-stress treatment (WS) (n=30). Spikes from four genotypes selected from the CIMCOG-ROOT trial.

Consequently, a new method could be developed giving a good indication of resilience and production capacity of the genotype, in term of grain per spike, at early developmental stages, by combining the hormone quantification at late booting stage and the spike dry weight at flowering time. The possibility to select plants at early phenological stages, for high resilience and high productivity, will be a huge improvement for a breeding selection program. However, before being recommended as a high-throughput method based on this trait, this method needs to be tested under field condition and after having simplified the hormone quantification.

7.5.2. Limitation of hormone quantification for early selection

To use hormone signalling (ABA and ethylene) as a physiological trait for breeding selection, it is necessary to at least speed up the methodology to quantified ethylene emission rate.

In fact, the ethylene sampling remains as a time-consuming method, even before the sampling process itself (Chapter 4 - Section 4.2.2), by the weighing tubes method (described in Chapter 4 Appendix 4A) which offers the possibility to quantify, quickly and accurately, the fresh weight of the incubated material under field conditions but it consumes time prior the sampling day as it needs the preparation of all the material (tubes, syringes and vials) and the tube

weighing before and after incubation time. Additionally, the incubation time during the sampling, represents a considerable investment of time and also during the sampling, as it is first needed to enclose all material in tubes and then incubate them for one hours and twenty minutes to finally take the gas samples before being able to analyse the samples. Additionally, one person cannot sample more than 30 plots per day in the time defined in this work (from 01:00 pm to 03:40 pm – around two hours and half in total for screening 10 genotypes the same day with three repetitions under field conditions) in comparison with ABA which can be sampled much more quickly (*circa* 50 to 70 tissues samples per person per hour). Therefore, the sampling for ethylene emission is for the moment the bottleneck of developing a high throughput method for a huge population (like 1000 genotypes) using hormone ratio and ethylene emission as an indicator of resilience in terms of spike fertility under field condition.

With the aim of speeding up the ethylene quantification, a small experiment, described in Appendix 7A, was conducted to sample the air inside individual target plots and to compare these measurements with the quantification of ethylene concentration obtained by more conventional methods (describe in Chapter 4 - Section 4.2.2). Figure 7.2 shows a positive relationship between ethylene concentration contained in 1 ml of air sampled from the in-plot *versus* the ethylene concentration contained in 1 ml of gas, sampled from tubes enclosing one leaf which had been incubated for one hour and twenty minutes under sun light (field condition). Therefore, these results indicate that there is a potential method to estimate leaf ethylene production by measuring the in-plot air concentration of ethylene, which could be a faster and easier method to quantify ethylene concentration of individual genotypes. Further experiments need to be carried out to confirm and improve this proposed high-throughput methodology to estimate ethylene emission.

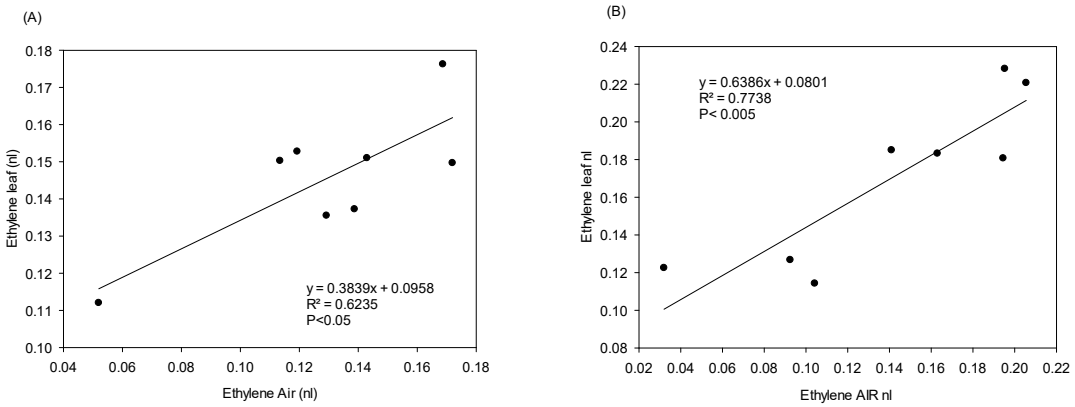


Figure 7.2: Linear correlation between ethylene produced (nl) by the flag-leaf tissue and released into the atmosphere, and the ethylene contained in the plot air (A) under yield potential conditions at late-booting stage, and (B) at booting stage under drought stress conditions.

At the present time, ABA and ethylene analysis through radioimmunoassay and EDT-300 ethylene detector (Sensor Sense B.V., Nijmegen, The Netherlands), respectively, could be, both, considered as high throughput methods. More than hundred samples can be analysed per day (around 100 to 120 samples for ethylene and 180 to 240 for ABA).

Another possibility to improve the method, described in this work, will be to use a multi-analyte physicochemical technique to quantify ABA and ACC (ethylene precursor). However, it will be firstly necessary to identify a link between the ethylene precursor ACC and the ethylene emission rate and secondly to verify if this method of hormone quantification (estimation of ethylene and ABA production) shows also a similar correlation with spike fertility resilience. Knowing that plants synthesize many more than two hormones which can affect the plant response to individual hormones, e.g.: Auxin and cytokinin inhibit ABA-induced stomatal closure by enhancing ethylene production (Tanaka *et al.*, 2006), the multi-analyte physicochemical technique which allows to measure more hormones at the same time from the same sample, could be used to try to understand better hormone interaction and actions in plants. However, if the action of auxin and cytokinin is to enhance the production of ethylene,

it could be hypothesised that ABA and ethylene could be the key hormones to investigate under drought stress.

7.6. Does the cropping design for breeding selection need to be improved?

The different sensitivity of plants to ethylene (Pierik *et al.*, 2006) and the significant differences between genotypes in terms of ethylene concentration in the in-plot air sampled (Figure 7.2), raised more questions about the trialling system used to evaluate wheat performance under field conditions where different genotypes are sown side-by-side, either under yield potential or under stress conditions. It is hypothesised that in this system, the neighbour plots could have some influences on their adjacent plots, for example, if a genotype which produces higher ethylene grows adjacent to a plot with an ethylene-sensitive genotype, this could have a positive or negative effect on yield. However, this needs more investigation to determine whether the randomisation and the space let between each plot used in this kind of experimental design are sufficient to avoid the occurrence of such an issue.

7.7. Impact of agronomic practice and field management on hormone balance and yield

The improvement in agronomic practice and field management, during the last decade, was responsible for the 50% of the yield improvement observed during this period (Araus *et al.*, 2004; Richards, 2006).

This study suggests that around a critical stage, late booting (meiosis), agronomic practice and field management such as application of agro-chemical products (fungicides and/or pesticides) or irrigation might disturb the hormone balance and modify or confound the interpretation of resilience response of the genotype to drought stress during selection trials. For example, application of triazole (fungicide) can alter the hormone balance by inhibiting the gibberellin

synthesis, reducing ethylene evolution and increasing cytokinin (CK) levels (Panneerselvam *et al.*, 2009). These variations of ethylene and cytokinin production could have a beneficial effect on the grain-filling rate (based on the research of Yang *et al.*, 2006*b*; Thomas and Ougham, 2014; Christopher *et al.*, 2016) but it could have a detrimental effect on grain number if it is applied at late booting stage under drought stress (based on this work). Another example, is the peak of ethylene emission observed after a transient drought (Chapter 4 - Section 4.3.1 and Beltrano *et al.*, (1997)) which can be a particular complication, as it could have an unpredictable effect (promoting, inhibiting or no effect) on the final yield and the optimal concentration of ethylene will depend on the developmental stage, the day time, the concentration, the environment and the genotype (Pierik *et al.*, 2006). Other field management effects on plant hormone balance have already been reported, such as tillage conservation promoting yield improvement during the grain filling period under water scarcity by modifying the hormone balance (ABA, ethylene, gibberellins, auxins and cytokinins) (Liu *et al.*, 2013).

It would be logical to recommend avoidance these agronomic practices during these critical stages to improve the selection process (by avoiding confusion in the interpretation of the result). However, in a crop breeding programme, a huge population with thousands of genotypes are evaluated during one cropping season and potentially many of these genotypes will show differences in their phenology at the moment of the field management practices (irrigation, crop protection or others). This means that its effect (on each genotype) will potentially be different due to their genetic background but also due to differences in phenological stage of the plants at the application time. This research suggests some improvements to standard trialling procedure for selection by grouping the genotypes in function of their phenology and select them within sub-groups using the index on resilience and productivity (Chapter 2 and 3). On the other hand, as farmers work with only one genotype (on

crop), they could easily apply the recommendation of avoiding agronomic practices which affect negatively the grain set at critical developmental stages such as late booting stage (as suggested here).

This confirms the potential value of trying to exploit our understanding of crop physiology in field management practices, to raise the yields and to potentially enhance resource use efficiency. Therefore, further research should focus on the interaction between G x E x M (G: genotype, E: environment and M: field management practice). Integrated pest management (IPM) practices, which tend to reduce the use of agrochemicals (Kogan, 1998) may also have an unpredicted effect on plant hormone biology, and therefore, an impact on yield and yield components. Reduced usage of agrochemicals and any beneficial effects on physiology at specific phenological stages, may improve the potential economic impact of a change in management practice which was originally introduced for environmental reasons or to safeguard human health.

Appendix Chapter 2

Appendix 2A - Description of the formulae previously derived as indices of relative performance of populations of crop genotypes under stress

The Stress Susceptibility Index (SSI) by Fisher and Maurer (1978) is expressed by the following relationship:

$$SSI = \frac{1-\frac{Ys}{Yp}}{SI} \quad \textbf{Equation S1}$$

Ys = Yield under stress condition

Yp = Yield under yield potential condition

SI = stress intensity expressed in turn by

$$SI = \left[1 - \frac{\overline{Ys}}{\overline{Yp}}\right] \quad \textbf{Equation S2}$$

Where: $\overline{Ys}$ = mean yields overall population under stress condition;

$\overline{Yp}$= mean yields overall population under yield potential condition.

A greater stress tolerance is shown by smaller values of SSI. However, this index fails to distinguish genotypes in groups A and C (Table 1), owing to the fact that it favours genotypes with medium yield under yield potential and high yield under stress conditions.

Rosielle & Hamblin (1981) created the stress tolerance (TOL) index, which can be explained as the difference between Yp (yield potential) and Ys (yield under stress):

$$TOL = Yp - Ys \quad \textbf{Equation S3}$$

The high value of TOL indicates a low stress tolerance. However, as with SSI, it fails in favouring those genotypes with high yield under stress and low yield under normal conditions. As a result, it cannot discriminate between genotypes in groups A and C.

Rosielle & Hamblin (1981) also proposed the mean production index (MP) by averaging Ys and Yp (Equation S4.), where a high stress tolerance is indicated by a high MP value. However, it fails in favouring genotypes with high yield under yield potential. As a consequence, it cannot differentiate between the group A and group B (Table 1).

$$\boldsymbol{MP = \frac{Ys+Yp}{2}}$$ **Equation S4**

Fernandez (1992) explains that the arithmetic mean used in the calculation of MP presents a bias due to a relative larger difference between Yp and Ys. On the contrary, the geometric mean is less sensitive to large extreme values. Thus, he suggested the geometric mean productivity index (GMP) as a better one to distinguish group A.

$$\boldsymbol{GMP = \sqrt{Ys \,.\, Yp}}$$ **Equation S5**

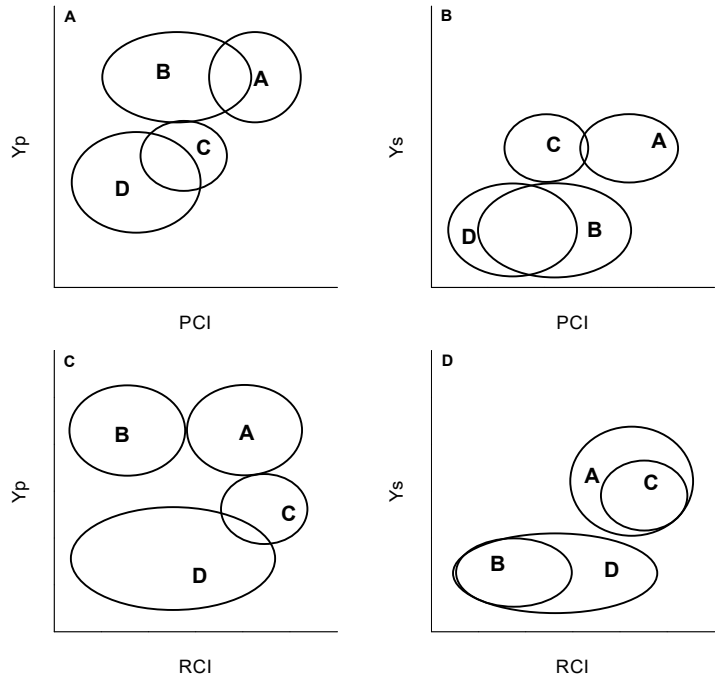
Fernandez (1992) has also defined another stress tolerance index (STI) with the purpose of identifying genotypes that produce high yield under both environments (yield potential and stress conditions) (Group A). It is expressed by the following relationship:

$$\boldsymbol{STI = \frac{Yp}{\overline{Yp}}\frac{Ys}{\overline{Ys}}\frac{\overline{Ys}}{\overline{Yp}} = \frac{Yp \,.\, Ys}{\overline{Yp^2}}}$$ **Equation S6**

The higher value of GMP and STI represents a higher tolerance to stress.

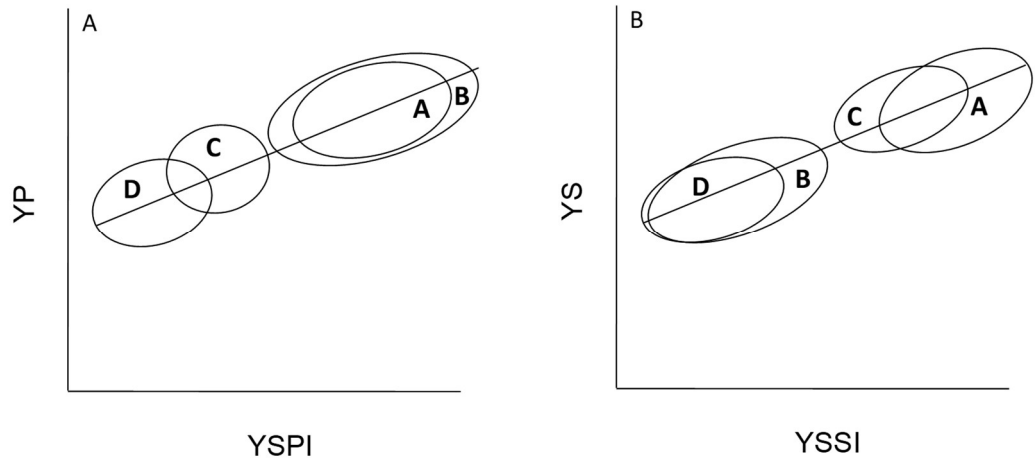
Appendix 2B - Schematic model to explain why the combination of PCI and RCI improves the use of the previous indices

The high correlation of YSSI and YPSI with grain yield (Ys and Yp, respectively) can be explained due to the readjustment of the PCI and RCI values of the different groups defined by Fernandez (1992), where group A) genotypes express uniform superiority in both stress and no-stress condition; group B) genotypes express good performance only in yield potential but not under stress conditions; group C) genotypes present a relatively higher yield only under stress and group D) poor yield performance in both environments. Appendix 2 Figure 1 illustrates the location of the different groups, in the spaces defined by plotting Yp or Ys, against PCI or RCI. The location of groups B and C are the only ones to change when the same index is looking at Yp or Ys. This explains why a good relationship has been observed between the previous indices and both yields (Yp and Ys), since half of the data (groups A and D) keep the same range of values, where group A has the highest value and group D the lowest value.



Appendix 2 Figure 1: Schematic illustration of the position of the different groups defined by Fernandez (1992) as a function of Yp or Ys *versus* PCI or RCI: (A) PCI *versus* Yp; (B) PCI *versus* Ys; (C) RCI *versus* Yp, and (D) RCI *versus* Ys.

The combination of both score indices (RCI and PCI) re-arranges the groups according to grain yield (Yp or Ys) following a linear response (Appendix 2 Figure 2).



Appendix 2 Figure 2: Schematic illustration of the classification of the different groups defined by Fernandez (1992) over the linear regression (A) Yp *versus* YPSI (B) Ys *versus* YSSI.

To understand better YSSI, a particular case, where a similar Ys could be obtained by genotypes producing more or less Yp (groups A and C), is studied. In this case, a genotype from A will have a higher PCI, due to a higher Yp, and a lower RCI, due to a higher reduction of yield compared with a genotype from C. Thus, the combination of a higher PCI and a lower RCI (genotype A) will give a similar value compared with the combination of a lower PCI and a higher RCI (genotype C). Then, for similar Ys, genotype C will be more resilient compared with genotype A.

To understand YPSI, the interpretation is slightly different, a particular case, where two genotypes from groups A and B with a similar high Yp will have different Ys. In terms of PCI and RCI, the genotype from A will always have a higher value than the genotype from B. Thus, the genotype B has a lower PCI value due to its low yield under stress due to its poor resilience. Therefore, if the susceptibility is removed from the mean production (PCI) of genotypes A and B, it is obtained a similar value which correlated with Yp.

Appendix 2 Table 1: List of the different score index combinations assessed to identify the best correlation with grain yield under yield potential and stress conditions.

Addition	Subtraction
By pair	
$\frac{(SSIs+MPs)}{2}$ **Combination 1**	$\frac{(MPs-SSIs)}{2}$ **Combination 7**
$\frac{(SSIs+GMPs)}{2}$ **Combination 2**	$\frac{(GMPs-SSIs)}{2}$ **Combination 8**
$\frac{(SSIs+STIs)}{2}$ **Combination 3**	$\frac{(STIs-SSIs)}{2}$ **Combination 9**
$\frac{(TOLs+MPs)}{2}$ **Combination 4**	$\frac{(MPs-TOLs)}{2}$ **Combination 10**
$\frac{(TOLs+GMPs)}{2}$ **Combination 5**	$\frac{(GMPs-TOLs)}{2}$ **Combination 11**
$\frac{(TOLs+STIs)}{2}$ **Combination 6**	$\frac{(STIs-TOLs)}{2}$ **Combination 12**
By group	
$\left(\frac{(SSIs+TOLs)}{2}+\frac{(MPs+GMPs)}{2}\right)$ **Combination 13**	$\left(\frac{(MPs+GMPs)}{2}-\frac{(SSIs+TOLs)}{2}\right)$ **Combination 16**
$\left(\frac{(SSIs+TOLs)}{2}+\frac{(MPs+STIs)}{2}\right)$ **Combination 14**	$\left(\frac{(MPs+STIs)}{2}-\frac{(SSIs+TOLs)}{2}\right)$ **Combination 17**
$\left(\frac{(SSIs+TOLs)}{2}+\frac{(GMPs+STIs)}{2}\right)$ **Combination 15**	$\left(\frac{(GMPs+STIs)}{2}-\frac{(SSIs+TOLs)}{2}\right)$ **Combination 18**

Appendix 2 Table 2: Pearson coefficient between index combinations (Appendix 2 Table 1) and grain yield under yield potential and heat stress conditions during the cropping season 2012-13 on the CIMCOG-ROOT trial (10 genotypes). * P<0.05

Combinations	Yp	Ys
1	0.411	0.976*
2	0.258	**0.997***
3	0.258	**0.997***
4	0.017	0.976*
5	-0.121	0.937
6	-0.121	0.937
7	0.983*	0.055
8	0.989*	0.233
9	0.989*	0.233
10	0.995*	0.171
11	0.993*	0.293
12	0.993*	0.293
13	-0.146	0.932
14	-0.146	0.932
15	-0.183	0.917
16	**0.997***	0.193
17	**0.997***	0.193
18	0.994*	0.271

Appendix 2 Table 3: Pearson coefficient between index combinations (Appendix 2 Table 1) and grain yield under yield potential and yield under drought stress conditions for the cropping season 2013-14 on the CIMCOG-ROOT trial (10 genotypes). * P<0.05

Combinations	Yp	Ys
1	0.273	0.940
2	0.002	0.994*
3	0.001	**0.996***
4	0.007	0.983*
5	-0.277	0.959*
6	-0.258	0.959*
7	0.980*	-0.223
8	0.992*	-0.018
9	0.987*	0.031
10	0.997*	-0.004
11	0.968*	0.201
12	0.958*	0.239
13	-0.520	0.873
14	-0.510	0.878
15	-0.523	0.870
16	**0.998***	-0.010
17	0.997*	0.011
18	0.983*	0.125

Appendix 2 Table 4: Pearson coefficient between index combinations (Appendix 2 Table 1) and grain yield under yield potential and heat stress conditions during the cropping season 2012-13 on the WAMI trial (294 genotypes). * P<0.05

Combinations	**Yp**	**Ys**
1	0.348	0.980*
2	0.247	0.983*
3	0.258	**0.984***
4	0.173	0.941
5	0.073	0.941
6	0.094	0.949
7	0.896	-0.062
8	0.954*	0.198
9	0.960*	0.222
10	0.985*	0.299
11	0.962*	0.512
12	0.960*	0.522
13	-0.110	0.889
14	-0.103	0.892
15	-0.110	0.892
16	0.985*	0.259
17	**0.986***	0.269
18	0.986*	0.394

Appendix Chapter 3

Appendix 3 Table 1: Zadoks´scale (reproduced from Pask *et al.*, 2012)

GS	Description	GS	Description
	Germination		*Booting*
00	Dry seed	41	Flag leaf sheath extending
01	Water uptake (imbibition) started	43	Boot just visibly swollen
03	Imbibition complete	45	Boot swollen
05	Radicle emerged from seed	47	Flag leaf sheath opening
07	Coleoptile emerged from seed	49	First awns visible
09	Leaf just at coleoptile tip		
			Heading
	Seedling development	51	First spikelet of head visible
10	First leaf emerged	53	¼ of head emerged
11	First leaf unfolded	55	½ of head emerged
12	2 leaves unfolded	57	¾ of head emerged
13	3 leaves unfolded	59	Emergence of head complete
14	4 leaves unfolded		
15	5 leaves unfolded		*Flowering or anthesis*
16	6 leaves unfolded	61	Start of flowering
17	7 leaves unfolded	65	Flowering half complete
18	8 leaves unfolded	69	Flowering complete
19	9 or more leaves unfolded		
			Kernel and milk development
	Tillering	71	Kernel watery ripe (clear liquid)
20	Main shoot only	73	Early milk (liquid off-white)
21	Main shoot and 1 tiller	75	Medium milk (milky liquid)
22	Main shoot and 2 tillers	77	Late milk (more solids in milk)
23	Main shoot and 3 tillers		
24	Main shoot and 4 tillers		*Dough development*
25	Main shoot and 5 tillers	81	Very early dough (slides when crushed)
26	Main shoot and 6 tillers	83	Early dough (elastic, dry and shiny)
27	Main shoot and 7 tillers	85	Soft dough (firm, thumbnail mark not held)
28	Main shoot and 8 tillers	87	Hard dough (thumbnail impression held)
29	Main shoot and 9 or more tillers	89	Late hard dough (difficult to dent)
	Stem elongation or jointing	90	*Ripening*
30	Pseudo stem erection	91	Kernel hard (difficult to divide; 16% water)
31	1st node detectable	92	Kernel hard (not dented by thumbnail)
32	2nd node detectable	93	Kernel loosening in daytime
33	3rd node detectable	94	Overripe, straw dead and collapsing
34	4th node detectable	95	Seed dormant
35	5th node detectable	96	50% of viable seed germinates
36	6th node detectable	97	Seed not dormant
37	Flag leaf just visible	98	Secondary dormancy
39	Flag leaf ligule/collar just visible	99	Secondary dormancy lost

Appendix 3A - Verification of the expression of thousand grain weight by the productive and resilient component.

Due to this modification in the expression of TGWs by the use of RCI and PCI, it was decided to test the adapted index for TGW (equation 5) on a bigger panel (WAMI 294 genotypes) in order to generalise a conclusion on the bigger influence of TGW-PCI on the determination of TGWs. Appendix 3 Table 2 shows that, effectively, TGWs and TGWp are positively and significantly correlated. Additionally, Appendix 3 Table 2 shows that the adapted formula (T-SSI; Equation 4) shows a positive and significant correlation with TGWs (r = 0.93), but this correlation is improved to a higher level by applying Equation 5 (r = 0.98).

Appendix 3 Table 2: Pearson coefficient of the correlation between thousand grain weight under heat stress (TGW_h) *versus* 1) thousand grain weight under yield potential (TGWp), 2) thousand grain weight resilience capacity index (TGW-RCI), 3) thousand grain weight production capacity index (TGW-PCI), 4) trait stress score index calculated on TGW (T-SSI, Equation 4 ((TGW-RCI+TGW-PCI)/2)) and 5) the adapted trait stress score index calculated on TGW (Equation 5 ((TGW-RCI+2*TGW-PCI)/3)) in data from WAMI (294 genotypes) under heat stress for the crop season 2011-12 (Y11-12). ** P<0.01.

WAMI under heat stress		
	r	r2
TGW_h *vs* TGW_p	0.81**	0.66
TGW_h *vs* TGW-RCI	0.58**	0.33
TGW_h *vs* TGW-PCI	0.95**	0.90
TGW_h *vs* Equation 4	0.93**	0.86
TGW_h *vs* Equation 5	0.98**	0.96

It is concluded that TGW can be expressed by the use of the Trait Stress Score Index (T-SSI), which is composed of the resilience and the production capacity index (TGW-RCI and TGW-PCI).

Appendix 3 Table 3: Value of the resilient capacity index of the different yield components (Trait-RCI) and the production capacity index of the different yield components (Trait-PCI) under the different stresses Heat stress (H) (crop season 2012-2013), Drought stress (D) (crop season 2013-2014) and semi-drought stress (SD) (crop season 2012-2013) from the CIMCOG-ROOT trial. This table shows the constancy of the index along the years and between the stresses.

Genotypes	Y-RCI	TGW-RCI	G#/m^2-RCI	G#/spk-RCI	Spk#/m^2-RCI	Genotypes	Y-PCI	TGW-PCI	G#/m^2-PCI	G#/spk-PCI	Spk#/m^2-PCI
1-H	1.00	2.00	2.00	8.00	1.00	1-H	1.00	1.00	10.00	5.00	9.00
1-D	1.00	1.00	3.00	9.00	1.00	1-D	1.00	1.00	10.00	8.00	10.00
1-SD	6.00	3.00	7.00	10.00	1.00	1-SD	5.00	1.00	10.00	9.00	10.00
Mean	**2.67**	**2.00**	**4.00**	**9.00**	**1.00**	**Mean**	**2.33**	**1.00**	**10.00**	**7.33**	**9.67**
2-H	3.00	5.00	3.00	6.00	5.00	2-H	3.00	6.00	3.00	4.00	2.00
2-D	9.00	3.00	8.00	7.00	8.00	2-D	7.00	7.00	3.00	8.00	1.00
2-SD	6.00	3.00	8.00	6.00	8.00	2-SD	5.00	5.00	3.00	8.00	2.00
Mean	**6.00**	**3.67**	**6.33**	**6.33**	**7.00**	**Mean**	**5.00**	**6.00**	**3.00**	**6.67**	**1.67**
3-H	3.00	5.00	3.00	6.00	5.00	3-H	3.00	4.00	6.00	2.00	6.00
3-D	8.00	4.00	6.00	10.00	4.00	3-D	6.00	4.00	7.00	7.00	6.00
3-SD	5.00	2.00	7.00	10.00	3.00	3-SD	4.00	3.00	6.00	7.00	6.00
Mean	**5.33**	**3.67**	**5.33**	**8.67**	**4.00**	**Mean**	**4.33**	**3.67**	**6.33**	**5.33**	**6.00**
4-H	5.00	10.00	2.00	1.00	10.00	4-H	2.00	2.00	8.00	1.00	10.00
4-D	8.00	10.00	4.00	6.00	5.00	4-D	3.00	2.00	8.00	8.00	7.00
4-SD	4.00	10.00	1.00	3.00	1.00	4-SD	1.00	2.00	5.00	6.00	5.00
Mean	**5.67**	**10.00**	**2.33**	**3.33**	**5.33**	**Mean**	**2.00**	**2.00**	**7.00**	**5.00**	**7.33**
5-H	4.00	9.00	2.00	4.00	4.00	5-H	8.00	7.00	4.00	4.00	4.00
5-D	2.00	7.00	1.00	1.00	4.00	5-D	5.00	8.00	2.00	4.00	3.00
5-SD	4.00	5.00	2.00	1.00	6.00	5-SD	5.00	6.00	3.00	4.00	5.00
Mean	**3.33**	**7.00**	**1.67**	**2.00**	**4.67**	**Mean**	**6.00**	**7.00**	**3.00**	**4.00**	**4.00**

Genotypes	Y-RCI	TGW-RCI	G#/m^2-RCI	G#/spk-RCI	Spk#/m^2-RCI	Genotypes	Y-PCI	TGW-PCI	G#/m^2-PCI	G#/spk-PCI	Spk#/m^2-PCI
6-H	10.00	4.00	10.00	10.00	5.00	6-H	10.00	10.00	3.00	1.00	5.00
6-D	10.00	1.00	10.00	4.00	10.00	6-D	9.00	10.00	1.00	1.00	9.00
6-SD	10.00	6.00	10.00	8.00	5.00	6-SD	8.00	10.00	1.00	1.00	4.00
Mean	**10.00**	**3.67**	**10.00**	**7.33**	**6.67**	**Mean**	**9.00**	**10.00**	**1.67**	**1.00**	**6.00**
7-H	6.00	5.00	6.00	8.00	4.00	7-H	4.00	5.00	5.00	5.00	4.00
7-D	10.00	3.00	8.00	8.00	6.00	7-D	6.00	6.00	4.00	7.00	5.00
7-SD	8.00	4.00	10.00	5.00	8.00	7-SD	5.00	5.00	4.00	5.00	6.00
Mean	**8.00**	**4.00**	**8.00**	**7.00**	**6.00**	**Mean**	**5.00**	**5.33**	**4.33**	**5.67**	**5.00**
8-H	5.00	1.00	8.00	10.00	3.00	8-H	7.00	5.00	6.00	4.00	6.00
8-D	10.00	1.00	9.00	10.00	5.00	8-D	10.00	7.00	5.00	6.00	8.00
8-SD	10.00	8.00	10.00	4.00	10.00	8-SD	10.00	7.00	4.00	2.00	8.00
Mean	**8.33**	**3.33**	**9.00**	**8.00**	**6.00**	**Mean**	**9.00**	**6.33**	**5.00**	**4.00**	**7.33**
9-H	3.00	10.00	1.00	3.00	2.00	9-H	6.00	10.00	1.00	4.00	1.00
9-D	6.00	2.00	6.00	4.00	6.00	9-D	8.00	9.00	2.00	8.00	1.00
9-SD	1.00	1.00	1.00	2.00	1.00	9-SD	1.00	7.00	1.00	7.00	1.00
Mean	**3.33**	**4.33**	**2.67**	**3.00**	**3.00**	**Mean**	**5.00**	**8.67**	**1.33**	**6.33**	**1.00**
10-H	5.00	4.00	5.00	9.00	2.00	10-H	6.00	4.00	6.00	10.00	2.00
10-D	6.00	3.00	5.00	6.00	4.00	10-D	6.00	5.00	5.00	10.00	2.00
10-SD	3.00	2.00	5.00	4.00	4.00	10-SD	3.00	4.00	4.00	10.00	3.00
Mean	**4.67**	**3.00**	**5.00**	**6.33**	**3.33**	**Mean**	**5.00**	**4.33**	**5.00**	**10.00**	**2.33**

Appendix 3B: Could T-RCI and T-PCI give information about G x E interaction?

Appendix 3 Table 4 shows that for all traits, PCI is highly significantly correlated across the different environments. Effectively, the linear regression explains more than 80% of the variability of TGW-PCI and G#/m2-PCI between the stress (H, D, SD) (where for TGW-PCI r^2 = 0.920, 0.912 and 0.821 for D *vs* H, D *vs* SD and H *vs* SD, respectively and for G#/m^2-PCI r^2 = 0.863, 0.865 and 0.830 for D *vs* H, D *vs* SD and H *vs* SD, respectively).

Appendix 3 Table 4: Pearson coefficient of correlation (r) and coefficient of determination (r^2) of the production capacity index of thousand grain weight (TGW-PCI), grain number per square meter (G#/m^2-PCI), spike number per square meter (Spk#/m^2-PCI) and grain number per spike (G#/spk-PCI*) versus* the different stress environments Heat (H) Drought (D) and Semi-Drought (SD). ** p<0.01 * p<0.05. Calculated on CIMCOG-ROOT (10 genotypes).

	TGW-PCI		G#/m^2-PCI		Spk#/m^2-PCI		G#/spk-PCI	
	r	r^2	r	r^2	r	r^2	r	r^2
D vs H	0.959**	0.920	0.929**	0.863	0.826**	0.682	0.576*	0.332
D vs SD	0.955**	0.912	0.930**	0.865	0.802**	0.643	0.863**	0.745
H vs SD	0.906**	0.821	0.911**	0.830	0.748*	0.560	0.574*	0.330

The uniformity in PCI between the different stresses could be due to the genetic background codifying for the potential yield, which seems to be independent of the environment when the response is compared with the whole population, as the genotypes are the only parameter that has not changed throughout years and environments.

On the other hand, Appendix 3 Table 5 shows a positive and significant correlation between the resilience of grain number per square meter (G#/m^2-RCI) between the different stresses (D, H, SD). Effectively, the R-square values indicate that the linear regression explains 56.7%, 53.1% and 58.1% of the variability of G#/m^2-RCI between the different stress (D *vs* H, D *vs* SD and H *vs* SD, respectively), suggesting that a 50% of the population responds similarly under different stresses, in terms of G#/m^2 in this population. However, sub-components of G#/m^2-RCI (Spk#/m^2-RCI and G#/spk-RCI) do not show significant correlation between stresses with the exception of G#/spk-RCI between D and SD.

Appendix 3 Table 5: Pearson coefficient of correlation (r) and coefficient of determination (r^2) of the resilience capacity index of thousand grain weight (TGW-RCI), grain number per square meter (G#/m^2-RCI), spike number per square meter (Spk#/m^2-RCI) and grain number per spike (G#/spk-RCI *) versus* the different stress environments Heat (H) Drought (D) and Semi-Drought (SD). * *P*<0.05. Calculated on CIMCOG-ROOT (10 genotypes).

	TGW-RCI		G#/m^2-RCI		Spk#/m^2-RCI		G#/spk-RCI	
	R	r2	r	r2	r	r2	r	r2
D vs H	0.709*	0.503	0.753*	0.567	0.337	0.114	0.392	0.153
D vs SD	0.464	0.215	0.729*	0.531	0.373	0.139	0.608*	0.370
H vs SD	0.033	0.001	0.762*	0.581	-0.077	0.006	0.464	0.215

The results on the resilience of grain number per square meter (G#/m^2) and sub-components suggest that some genotypes could share some pattern of resilience regarding G#/m^2. Indeed, these results confirm that G#/m^2 is a good yield component to explain yield variations under stress environments (Fischer, 1985; Abbate *et al.*, 1995) but the resilience of its sub-components (Spk#/m^2-RCI and G#/spk-RCI) seems to be key traits to increase yield and to understand the basis of stress adaptation. In fact, as more variability is observed in the sub-components of G#/m^2-RCI in response to the different stresses they might be related to an adaptive response to a particular stress. Therefore, the resilience capacity index of the sub-components of G#/m^2-RCI (Spk#/m^2-RCI and G#/spk-RCI) could be recommended for breeding selection.

Appendix 3C: Where the susceptibility is expressed in term of yield components under heat stress

Under heat stress it has been observed that only 2 genotypes show a high resilience in terms of G#/m^2, genotypes 6 and 8. The others show susceptibility in terms of G#/m^2, with the exception of genotype 10, which has a medium score value of G#/m^2-RCI (Appendix 3 Table 6). Three genotypes (4, 5 and 9) show the highest score resilience in terms of TGW compared to the rest of the population, but this does not confer them an especially higher resilience on yield, as observed and concluded in Section 3.3.4.

Appendix 3 Table 6 shows that the same contrasting genotypes, as observed under drought (Section 3.3.4), in terms of resilience of G#/m2 was observed under heat stress (1 and 6, 5 and 8). However, under heat, the genotypes with high resilience in terms of grain number per m2 (G#/m2) seem to be mainly driven by the resilience in terms of grain number per spike (case of genotypes 6 and 8) but genotype 1 shows an exception to that rule and this may be influenced by the sowing process as mentioned in the conclusion of Chapter 3.

Appendix 3 Table 6: Summary table of the components of the trait stress score index, the trait resilience capacity index (T-RCI) (left table) and the trait production capacity index (T-PCI) (right table) under heat stress, during the cropping season 2012-13. Genotypes are grouped by pairs of contrast on yield resilience for the first four genotypes and the other four are group by their resilience on yield and/or grain number per m^2 (Data from CIMCOG-ROOT - 10 genotypes). (Ys: yield under stress, TGW: thousand grain weight, G#/m^2: number of grains per square meter, Spk#/m$^{2;}$ number of spikes per square meter and G#/spk: number of grains per spike)

RCI						
Entries	Ys	Y	TGW	G#/m^2	Spk#/m^2	G#/spk
1	2.11	1	3	2	1	8
6	3.00	10	4	10	5	10
5	2.60	4	9	2	4	4
8	2.59	5	1	8	3	10
3	2.33	3	5	3	5	6
7	2.52	6	5	6	4	8
4	2.34	5	10	2	10	1
10	2.53	5	4	5	2	9
2	2.34	3	5	3	5	6
9	2.46	3	10	1	2	3

PCI						
Entries	Ys	Y	TGW	G#/m^2	Spk#/m^2	G#/spk
1	2.11	1	1	10	9	5
6	3.00	10	10	3	5	1
5	2.60	8	7	4	4	4
8	2.59	7	5	6	6	4
3	2.33	3	4	6	6	2
7	2.52	4	5	5	4	5
4	2.34	2	2	8	10	1
10	2.53	6	4	6	2	10
2	2.34	3	6	3	2	4
9	2.46	6	10	1	1	4

This results confirm previous research made on twenty one wheat spring cultivars where it has been found that grain number per spike (G#/spk) is a good trait for heat stress tolerance (Shpiler and Blum, 1990).

Appendix 3 Table7: Comparison table between sowing 5g of seeds per square meter and a fix number of seeds per square meter (example 100) both showing the difference between seed number and gram. Thousand grain weight under controlled environment is the reference to calculate both variables. Data are from CIMCOG-ROOT during the season 2013-2014.

Genotypes	TGW	5g /m^2 (seeds)	100 seeds/m^2 (g)
1	36.69	136.26	3.67
2	46.90	106.61	4.69
3	42.21	118.46	4.22
4	36.58	136.69	3.66
5	47.26	105.79	4.73
6	53.49	93.48	5.35
7	44.56	112.20	4.46
8	47.60	105.05	4.76
9	51.07	97.90	5.11
10	44.39	112.65	4.44

Appendix 3D: The use of T-RCI and T-PCI to validate actual physiological traits. Case of canopy temperature.

Canopy temperature (CT) has been described as a method that integrates many physiological functions necessary to ensure adaptation to a given environment (Reynolds *et al.*, 2012*b*). It can be incorporated into breeding programmes due to its simple and rapid measurement. Several studies have suggested that variation in CT can be correlated with crop yield (Reynolds *et al.*, 1994, 1998)

Materials and methods

A hand-held infrared thermometer (Sixth Sense LT300 IRT) was used to measure canopy temperature. To avoid the effect of soil temperature, an appropriate angle (45 degrees) and distance (1 meter) from the plot were necessary and the measurements were taken when the IRT viewed a full canopy cover. To reduce the error due to differences of time between the first and the last plots, the temperatures were taken during two successive days between 11 am to 12 pm around anthesis (four measurements per plot), under drought stress and yield potential environments. On the first day, measurements were started from the front to the back of the trial and from the back to the front on the second day. The mean temperature was used in the analysis.

Results

Appendix 3 Table shows that CT at anthesis on the CIMCOG-ROOT trial is negatively and significantly correlated with yield, and the highest relationship is observed between CT and the resilient capacity in terms of G#/m2 (G#/m2-RCI). This negative relationship mean that the

grain number per square meter is usually reduced when genotypes show a higher temperature under drought stress at anthesis.

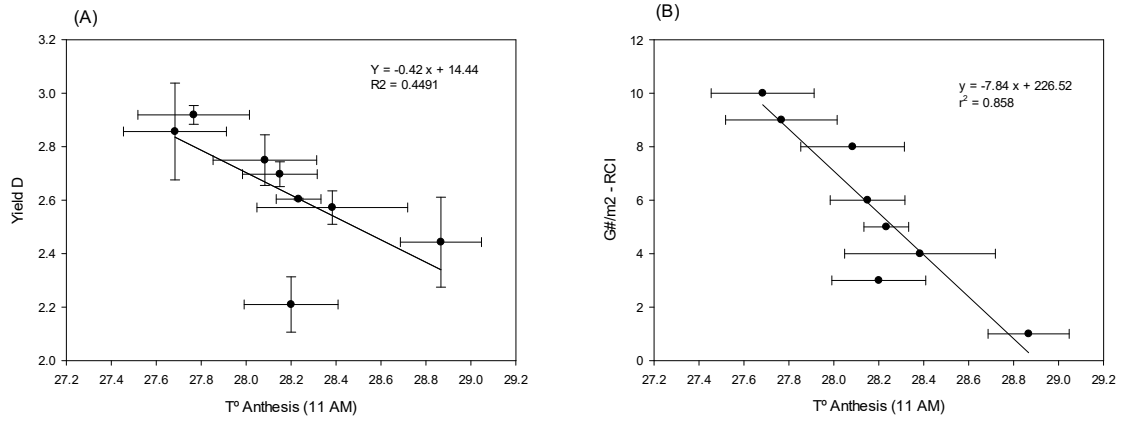
Appendix 3 Table 8: Pearson coefficient correlation between the canopy temperature at anthesis taken at 11 am, 6 days after the earliest genotypes reached anthesis and 2 days before for the latest of CIMCOG-ROOT (10 genotypes) under drought stress *versus* A) trait stress score index (T- SSI), B) trait resilience capacity index (T-RCI) and C) trait production capacity index (T-PCI). (Ys: yield under stress, YSSI: yield stress score index, TGW: thousand grain weight, G#/m^2: number of grains per square meter, Spk#/$m^{2:}$ number of spikes per square meter and G#/spk: number of grains per spike). *P<0.05 **p<0.005

A	Ys		YSSI		TGW-SSI		G#/m^2-SSI		Spk/m2-SSI		G#/spk-SSI	
	r	r2	R	r2	r	r2	r	r2	r	r2	r	r2
T-SSI *vs* Canopy Tº	-0.67*	0.45	-0.72*	0.52	0.40	0.16	-0.74*	0.54	-0.63*	0.40	-0.27	0.07

B	Ys		RCI		TGW-RCI		G#/m2-RCI		Spk/m2-RCI		G#/spk-RCI	
	r	r2	R	r2	r	r2	r	r2	r	r2	r	r2
T-RCI *vs* Canopy Tº.	-0.67*	0.45	-0.71*	0.51	0.71*	0.51	-0.93**	0.86	-0.51	0.26	-0.55	0.30

C	Ys		PCI		TGW-PCI		G#/m2-PCI		Spk/m2-PCI		G#/spk-PCI	
	r	r2	R	r2	r	r2	r	r2	r	r2	r	r2
T-PCI *vs* Canopy Tº.	-0.67*	0.45	-0.60	0.36	-0.26	0.07	0.11	0.01	-0.39	0.15	0.21	0.04

Appendix 3 Figure 1 (A and B) compares the linear regression between temperature and yield *versus* temperature and G#/m^2-RCI. The relationship is clearly improved with the use of G#/m^2-RCI.



Appendix 3 Figure 1: Linear regression between temperature taken between 11 and 12 am on CIMCOG-ROOT at anthesis *versus* a) yield under drought stress b) the resilience of grain number per square meter (G#/m^2-RCI). Temperatures are mean of two following days of measurements.

The use of the components of the trait stress score index (T-RCI and T-PCI) on the different yield components have confirmed that CT may be a good trait to identify yield resilience of wheat genotypes and G#/m^2-RCI is negatively correlated with CT. As mentioned above, G#/m^2 is a good component that explains better yield under drought stress. This analysis suggests that CT is more related with a resilience mechanism then productivity. This is an innovative insight as CT is usually associated with grain yield (productivity) (Reynolds *et al.*, 2012*b*).

The use of the trait stress score index (T-SSI) and its components (T-RCI and T-PCI) is a very interesting tool to identify new physiological traits or to convince breeders of the usefulness of some physiological traits already developed but not necessarily used in breeding programmes (Richards, 1996; Monneveux *et al.*, 2012).

Appendix Chapter 4

Appendix 4A - Protocol used to quantify indirectly sample fresh weight prior to ethylene measurement

The ethylene quantification method used in Wilkinson and Davies (2009) and Chen *et al.* (2013) requires weighing of the fresh materials just before the incubation process and this is not an option under field conditions.

Material and method

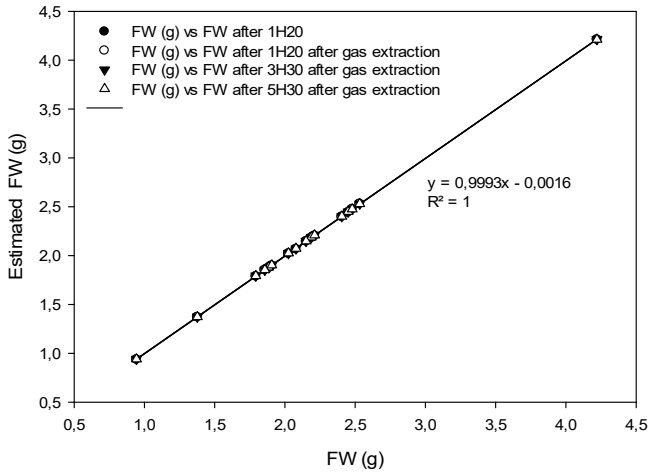
Four plants of each of four genotypes growing under well-watered (WW) conditions in the greenhouse were used to develop this protocol. The protocols for seed germination and seedling growth are described in the previous Section (4.2.3).

One flag leaf, cut at the ligule level, was sampled from each plant at jointing stage. On the day before plant sampling, the pre-labelled incubation glass test tubes (24 ml) were prepared with saturated filter paper (deionized water) and sealed with rubber stoppers (Suba-Seal, SLS, Nottingham, UK) and then weighed. The tubes were kept in a drawer and/or a cupboard at room temperature, approximately 22ºC. On the next day, at the time of leaf tissue sampling, the fresh weight of each sample was determined before the fresh tissues were placed in the tube as described in Wilkinson and Davies (2009). The tubes were incubated in the light in the greenhouse (described above). Each tube , with the fresh samples added, were weighed at four different times: 1) after 1 hour and 20 minutes incubation before the extraction of 1 ml of gas (described above), 2) after 1 hour and 20 minutes incubation after the extraction of 1 ml of gas, 3) after 3 hours and 30 minutes on samples (after incubation and gas extraction the tubes were kept in the dark in a drawer), 4) after 5 hours and 30 minutes (keeping the tube under dark conditions after the previous weighing).

The difference between the weight of tubes enclosing the fresh plant material with the saturated filter paper and the tubes enclosing only the saturated filter paper were compared with the fresh weight before the material was enclosed in the tubes.

Results

Appendix 4 Figure 1 shows a positive correlation between the fresh weight taken just after cutting the leaves and the indirect method using the weight difference between the tubes enclosing the fresh plant material with the saturated filter paper and the tubes enclosing only the saturated filter paper. Additionally, the tube-weighing method showed that the fresh weight does not change after the extraction of 1 ml of gas neither after 5H30 was injected into the tube.



Appendix 4 Figure 1: Correlation between the direct fresh weights measured before incubation following the method described in Wilkinson and Davies (2009) and the weighing tube method which consists to calculate the difference between the weight of tubes enclosing the fresh plant material with the saturated filter paper and the tubes enclosing only the saturated filter paper.

It is concluded that this method can be used under CE and field conditions to determine precisely the fresh weight of plant tissue sampled for hormone analysis. This method offers additional advantages, 1) as the tubes can be stored under dark conditions for more than 5 hours before weighing without any change in the fresh weight of the tissue and 2) saving time during the sampling and incubating process for ethylene quantification, allowing more samples to be taken each day.

Appendix 4B - Comparing the initial contrast selection with the index method

For experiment 1 and experiment 2, a deeper study of the contrasting response of the four genotypes to heat stress on yield was made using the index score based on yield (explained in Chapter 2) and compared it to the initial selection.

Experiment 1: Diurnal variation of hormone accumulation under well-watered conditions.

In this experiment, as for the initial selection, the indices were calculated from data collected from a panel of 60 genotypes of the CIMCOG trial using the data of a combined analysis of two years (seasons 2010-2011 and 2011-2012) under non-stress and heat stress conditions, at the CENED station, Mexico. The index method shows that the contrast of these genotypes is mostly on yield resilience under heat stress (Y-RCI) but not on yield production capacity index (Y-PCI) (similar Y-PCI indices between T1, T2 and S2 excepted S1 with a lower Y-PCI).

Appendix 4 Table 1: Index score table for four previously selected contrast genotypes where T1 and T2 represent the tolerant genotypes and S1 and S2 the sensitive ones based on the yield response to heat stress. Yield data, used to calculate the score indices, came from a combined analysis of two following seasons (season 2010-2011 and 2011-2012) from CIMCOG trial (60 genotypes) under heat stress. The table shows the score value of the resilience and productivity index in terms of yield (Y-RCI and Y-PCI, respectively).

Entries	Y-RCI	Y-PCI
T1	10	8
T2	6	10
S1	1	5
S2	4	8

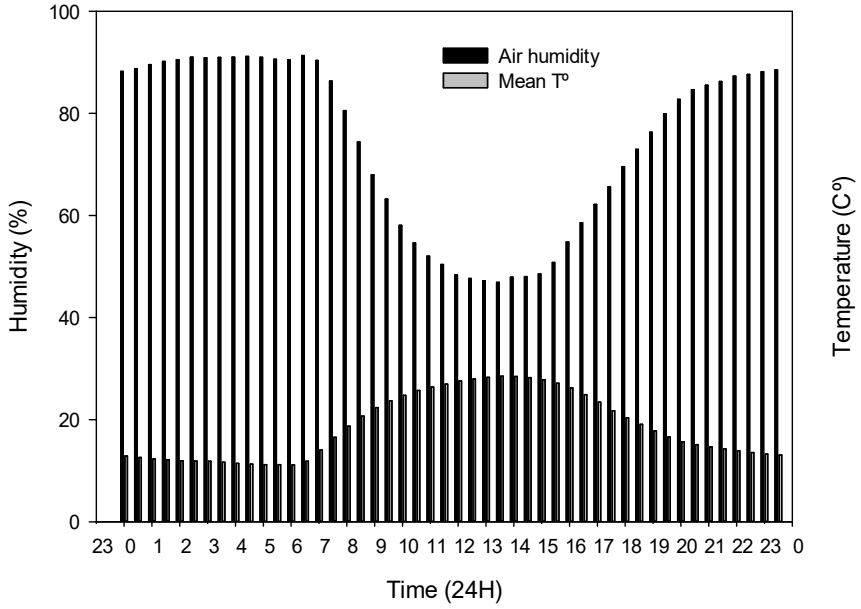
Experiment 2: Ethylene production by plants re-watered after drought stress.

In this experiment, as for the initial selection, the indices (Appendix 4 Table 2) were calculated from data collected from a panel of a sub set of the CIMCOG trial (30 genotypes) using the data of a combined analysis of two years (seasons 2010-2011 and 2011-2012) under non-stress and heat stress conditions, at the CENED station, Mexico. The contrast was confirmed in terms of yield resilience but not on yield productivity for the susceptible genotypes. In fact, genotypes

T3 and T4 showed similar resilience capacity index (RCI = 7 and RCI = 8, respectively) and production capacity index (PCI = 8 and PCI = 10, respectively) on yield. In contrast, genotypes S3 and S4 showed a lower resilience capacity index (RCI = 1 and RCI = 4, respectively) compared with T3 and T4 but only S3 showed a lower production capacity index (PCI = 4), while S4 showed similar production capacity index (PCI = 7) compared with the tolerant T3 and T4 (Table 4.2).

Appendix 4 Table 2: Index score table for four previously selected contrast genotypes where T3 and T4 represent the tolerant genotypes and S3 and S4 the sensitive ones based on the yield response to heat stress. Yield and yield components data, used to calculate the score indices, came from a combined analysis from sub-set of CIMCOG trial (30 genotypes) under heat stress. The table shows the score value of the resilience and production capacity index in terms of yield (Y-RCI and Y-PCI, respectively).

Entries	Y-RCI	Y-PCI
T3	7	8
T4	8	10
S3	1	4
S4	4	7



Appendix 4 Figure 2: Mean temperature and humidity during the period of sampling. Showing that the highest temperature period is observed to start at 01:00 pm and starting a decrease at 03:30 pm, giving therefore a window of three hours for the measurements.

Appendix Chapter 5

Appendix 5A - How to calculate the hormone ratio ABA/ETH

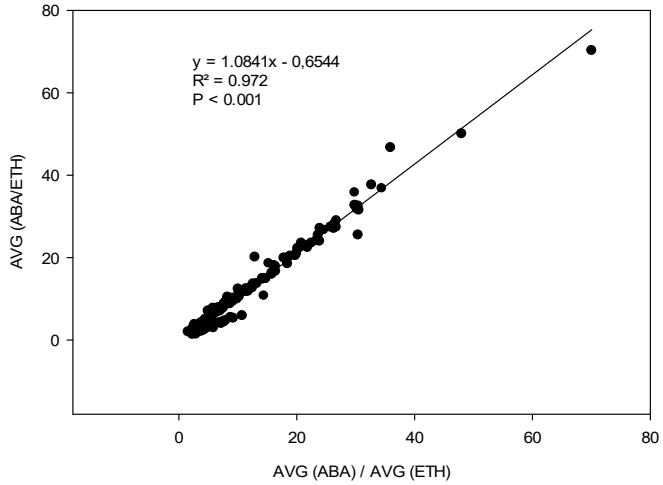
As flag leaf and spike tissues were sampled on one stem from different plants for ABA and for ethylene, the calculation of the ratio ABA/ETH might be undertaken using the mean value of both hormones. However, this calculation does not allow a statistical analysis to study the genotypic variation of this variable (ratio), as only one ratio is obtained per genotype, tissue and environment.

Method

To generate more than one ratio ABA/ETH value per genotype, tissue, phenological stage and environment, each individual ABA concentration was divided by each individual ethylene emission rate. For example, under field conditions, this procedure generated 36 ratios samples (6 ABA values and 6 for ETH) for each tissue, genotype, phenological stage and environment (database of 4608 individual ratios, in total). The mean of these individual ratios was compared with the ratio of the mean value.

Result

Appendix 5 Figure 1 shows a positive relationship between the hormone ratio calculated from the average concentration of ABA and ethylene (AVG (ABA) / AVG (ETH)) per genotype, tissue, phenological stage and environment and the average of the individual ratio between ABA and ethylene calculated by each individual ABA concentration value divided by each individual ethylene emission rate value per genotype, tissue, phenological stage and environment (AVG (ABA / ETH).



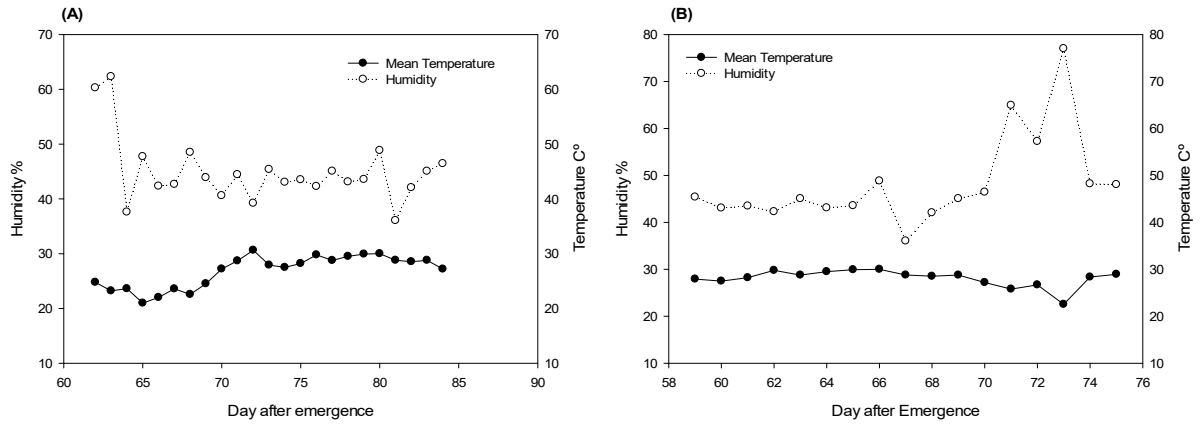
Appendix 5 Figure 1: Linear regression between the hormone ratio calculated from the average concentration of ABA and ethylene (AVG (ABA) / AVG (ETH)) per genotype, tissue, phenological stage and environment and the average of the individual ratio between ABA and ethylene calculated by each individual ABA concentration value divided by each individual ethylene emission rate value per genotype, tissue, phenological stage and environment (AVG (ABA / ETH). Calculated on data from CIMCOG-ROOT (8 genotypes) trial during the cropping season 2013-2014 under stress and irrigated environments.

Conclusion

It is concluded that this method is representative of the hormone ratio ABA / ETH in plants and a statistical analysis such as ANOVA can be undertaken.

Appendix 5B - Field conditions during sampling

The drought stress and irrigated crops grown under field conditions during the cropping season 2013-14, experienced variations of temperature and relative air humidity during plant development and therefore during the sampling for hormones. Appendix 5 Figure 2 shows the mean temperature and relative humidity during the sampling period (from booting to heading) and time (from 01:00 pm to 03:30 pm). During the sampling under drought stress, the temperatures were relatively constant (around 28 ºC) from booting (day after emergence (DAE) 59) to heading (DAE 75) (Appendix 5 Figure 2 B) while the relative humidity showed some variations that coincided with the period where genotypes were developing from half-emergence to heading (Appendix 5 Figure 2 B). For the sampling under irrigated conditions (Yp, sown one month earlier compared with drought stress trial), the temperature showed more variations compared with the drought trial, with the lowest (20◦C) and the highest (30◦C) temperatures observed at DAE 65 and DAE 72, respectively, coinciding with booting to late booting stage for genotypes 5, 9 and 10 and late-booting to half emergence stages for genotypes 1, 2, 4, 6, 8 (Appendix 5 Figure 2 A). Later, the temperature stabilized around the 28◦C from DAE 73 to DAE 85 (Appendix 5 Figure 2 A).



Appendix 5 Figure 2: Mean temperature (dark circle) and relative humidity (open circle), A) for the field trial under yield potential conditions from day 62 after emergence (DAE)- booting stage - to DAE 85 - heading stage, B) for the field trial under drought stress conditions from DAE 58 - booting stage- to DAE 76 - heading stage -. The mean corresponds to the time the measurement was taken from 01:00 pm to 03:30 pm.

Appendix 5C - Complementary measurements under field conditions: Does phenological stage influence ethylene accumulation?

As observed in Chapter 5, ethylene and ABA production shows some variations through phenological development and in different tissues, with some significant differences observed between leaf and spike tissues under Yp and especially under drought conditions (Figure 5.3, Figure 5.4, Figure 5.5). However, as the sampling was done at different dates for the different phenological stages, these differences between phenological stages could be due to the field conditions which imply numerous uncontrollable external factors such as temperature and/or relative humidity (Appendix 5 Figure 2).

The aim of this extra measurement was to determine whether genotypes modified ethylene concentration in response to the environment depending on their phenological stage, or whether the environment is the only key element to promote such variations.

Material and method

Leaf and spike were sampled during the same day in the same plot at two close phenological stages for ethylene quantification. Due to the number of samples taken per day in the field for the main experiment and the relatively short time available to take the ethylene emission (01:00 pm to 03:30 pm, explained in Chapter 4), the study of two close phenological stages, under the same conditions, was undertaken with only a small number of genotypes under yield potential and drought conditions.

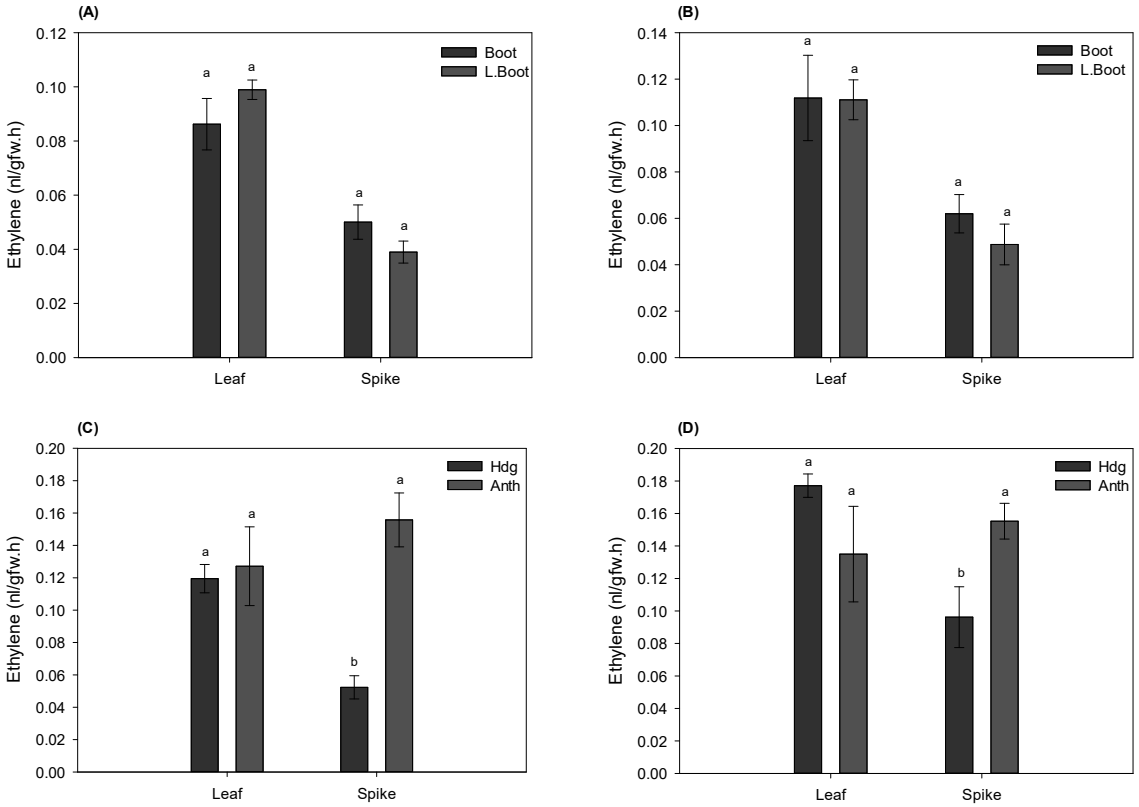
Under yield potential, the genotypes were selected in function of their ability to start their phenological development earlier or later. Therefore, the earliest genotypes (2 and 4) were sampled at booting and late-booting stages and the latest genotypes (9 and 10) at heading and anthesis. Under drought, genotypes 1 and 6 were selected in function of their yield performance

under heat stress (Chapter 3 - Section 3.3.4) being the most sensitive and the most tolerant, respectively, in terms of yield resilience and yield productivity. Genotypes 1 and 6 were sampled at late-booting and half-emergence.

The phenological stages were determined in function of the total amount of samples that it was possible to measure each day.

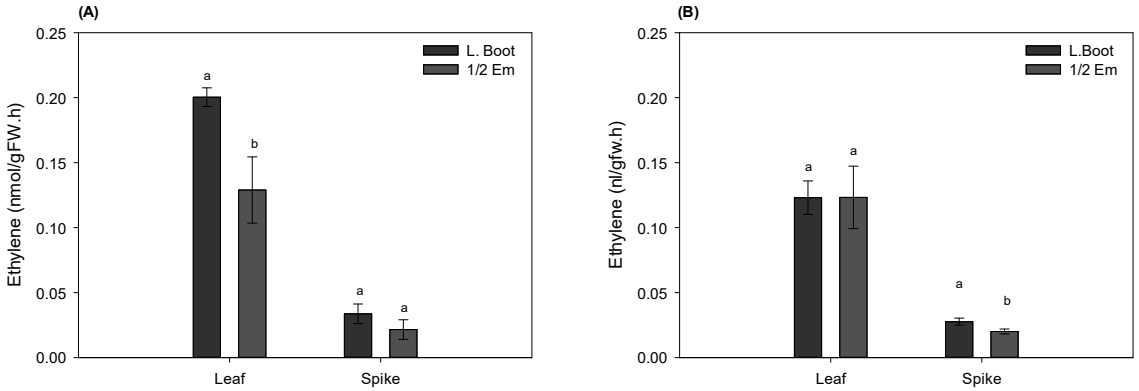
Results

Under irrigated conditions (Yp), leaf and spike ethylene production does not show any significant differences between booting and late-booting for the early genotypes (G2 and G4, Appendix 5 Figure 3: A and B, respectively). In contrast, between heading and anthesis under irrigated conditions, genotypes 9 and 10 show a significantly ($P < 0.05$) higher ethylene emission in spike at anthesis compared with heading stage (Appendix 5 Figure 3: C and D). Leaf ethylene production does not show significant differences under irrigation (Yp) for genotypes 9 and 10 (Appendix 5 Figure 3: C and D).



Appendix 5 Figure 3: Comparison of ethylene production on leaf and spike sampled the same day at the same time in the same plot under yield potential (Yp) at two close phenological stages. Two cases show the ethylene variation on leaf and spike between (1) booting (Boot) and late-booting (L. Boot) stage with genotypes (A) G2 and (B) G4 and (2) between heading (Hdg) and anthesis (Anth) with (C) G9 and (D) G10. Columns and bars are means ± standard error of 6 replicates. Different letters indicate significant difference within tissue ($P < 0.05$).

Under drought stress conditions, leaf ethylene emission showed a significant reduction from late-booting to half-emergence in the case of genotype G1 (P<0.05), but no changes in leaf ethylene production were observed for G6. Spike ethylene production of G6 decreased significantly (p<0.05) between late-booting and half-emergence, but no changes were observed for spike ethylene of G1 between these two phenological stages that were sampled on the same day, at the same time and on the same plot (Appendix 5 Figure 4:).



Appendix 5 Figure 4: Comparison of different phenological stages in terms of ethylene production by leaf and spike tissues under drought stress sampled the same day, at the same time from the same plots. Two cases showing the variation between leaf and spike at late-booting (L. Boot) and half-emergence (1/2 Em) stages: A) G 1, and B) G6. Columns and bars are means ± standard error of 6 replicates. Different letters indicate significant difference within tissue ($P < 0.05$).

Conclusion

It has been observed that under field conditions two close phenological stages show some variation in terms of ethylene and/or ABA production/accumulation. Therefore, these results have shown that phenological stages and tissue are important factors to take into account to quantify hormone production.

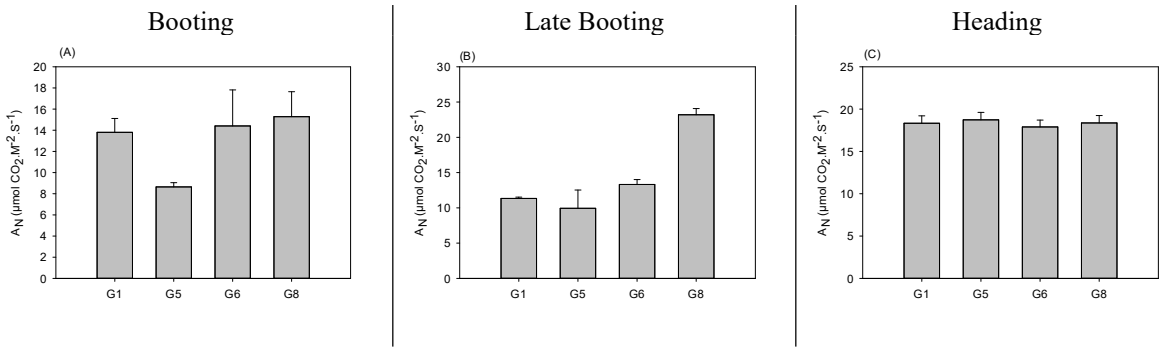
Appendix Chapter 6

Appendix 6 Table 1: Comparison score table of the resilient capacity index (RCI) between different calculations by using 1) the values from well-watered (WW) and water stress (WS) treatments, both under controlled conditions; and, 2) using the values from irrigated trial (Yp) and drought stress (D), both under field conditions for the four genotypes selected from CIMCOG-ROOT trial for controlled environment experiment. RCI calculation was based on grain number per spike (G#/spk-RCI), grain number per spikelet (G#/spklt-RCI) and spikelet number per spike (Spklt#/spk-RCI).

	Genotypes	TGW-RCI	G#/spk-RCI	G#/spklt-RCI	Spklt#/spk-RCI
WW vs WS (Controlled conditions)	1	5	10	10	10
	5	10	1	1	5
	6	1	6	9	3
	8	8	9	8	1
Yp vs D (Field conditions)	1	1	10	10	10
	5	9	1	1	1
	6	10	5	8	5
	8	8	10	10	10

Appendix 6 Table 2: Average of total stems per pot, fertile and sterile stems produced by 12 individual wheat plants, for each genotype, under water stress treatment. Data were collected under a controlled environment from four genotypes from CIMCOG-ROOT trial at physiological maturity.

Genotypes	n	Av fertile stem/pot	Av. sterile stem/pot	Av. Total stem
1	12	4.00	1	5.00
5	12	3.33	0.66	4.00
6	12	3.00	1.33	4.33
8	12	3.16	0.66	3.83



Appendix 6 Figure 1: Measurements of photosynthesis (A_N) taken the same day from all the plants, at A) booting stage, B) late booting stage, and C) heading stage, under water-stress (WS) treatment, by using a portable infrared gas analyser in open gas-exchange system, Li-6400 (Li-Cor Bio Sciences Inc., Nebraska, U.S.A.), under controlled environment (green house). Measurements of A_N were performed on the flag leaves from each plant. All measurements were registered with a CO_2 concentration in the cuvette of 500 µmol CO_2 mol^{-1} air and 1500 µmol photons m^{-2} s^{-1} of light intensity in the growth chamber. Temperature was set at 24°C.

Appendix Chapter 7

Appendix 7A - Speeding up ethylene quantification under field conditions

Ethylene is a gas released into the atmosphere and a preliminary experiment was designed to test the possibility that ethylene accumulation in the air inside the plot could be representative of the ethylene emission quantified by the method describe in Chapter 4 - Section 4.2.2.

The air was sampled, at late booting stage under yield potential conditions and at booting stage under drought stress, from the inner-plot atmosphere (in the middle of the four-sowing row (now called in-plot)), a day with no wind and at the same moment the gas samples was extracted from the tube enclosing fresh material from the same plot (following the method described in Chapter 4 - Section 4.2.2), after incubation time.

To test whether the ethylene in the plot atmosphere could be a useful way to estimate the ethylene released in the atmosphere by the plant, the ethylene concentration produced by the leaf and spike was compared with the concentration contained in the air (in middle of the plot at the flag leaf level). Usually, ethylene emission rate is expressed as a function of the fresh weight of the material and time of incubation (such as in Beltrano *et al.*, 1997 and Chen *et al.*, 2013), but in this case the concentration produced by leaf and spike was not refered to the fresh weight or the incubation time to compare it with the ethylene concentration released in the atmosphere (Figure 7.2).

References

Abbate PE, Andrade FH, Culot JP. 1995. The effects of radiation and nitrogen on number of grains in wheat. The Journal of Agricultural Science **124**, 351–360.

Abbate PE, Andrade FH, Lázaro L, Bariffi JH, Berardocco HG, Inza VH, Marturano F. 1998. Grain yield increase in recent Argentine wheat cultivars. Crop Science **38**, 1203–1209.

Abbate PE, Pontaroli AC, Lázaro L, Gutheim F. 2013. A method of screening for spike fertility in wheat. The Journal of Agricultural Science **151**, 322–330.

Abeles FB, Morgan PW, Saltveit Jr ME. 2012. *Ethylene in Plant Biology*. New York.

Acevedo E, Silva P, Silva H. 2002. Wheat growth and physiology, development. In: Curtis, B.C, Rajaram, S., Macpherson HG, ed. Bread wheat Improvement and Production. Rome, Italy: FAO, Plant Production and Protection Serie, 30.

Acharya BR, Assmann SM. 2009. Hormone interactions in stomatal function. Plant molecular biology **69**, 451–462.

Araus JL, Cairns JE. 2014. Field high-throughput phenotyping: The new crop breeding frontier. Trends in Plant Science **19**, 52–61.

Araus JL, Slafer GA, Reynolds MP, Royo C. 2002. Plant Breeding and Drought in C3 Cereals: What Should We Breed For? Annals of Botany **89**, 925–940.

Araus JL, Slafer GA, Reynolds MP, Royo C. 2004. Physiology of yield and adaptation in wheat and barley breeding. In: Nguyen, Henry T and Blum A, ed. Physiology and biotechnology integration for plant breeding. New York, NY, 1–49.

Araus JL, Slafer GA, Royo C, Serret MD. 2008. Breeding for Yield Potential and Stress Adaptation in Cereals. Critical Reviews in Plant Sciences **27**, 377–412.

Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M. 2005. Cytokinin oxidase regulates rice grain production. Science (New York, N.Y.) **309**, 741–5.

Asseng S, Ewert F, Martre P, *et al.* 2015. Rising temperatures reduce global wheat production. Nature Climate Change **5**, 143–147.

Avci M, Akar T, Meyveci K, Karacam M, Surek D. 2005. Yield performances of cereal varieties in various crop rotations under mediterranean dryland areas. Environments Proceedings of the 7th International Wheat Conference, 27 November - 2 December 2005, Mar del Plata, Argentina (Developments in Plant Breeding).233–242.

Barnabás B, Jäger K, Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell and Environment **31**, 11–38.

Barrs H, Weatherley P. 1962. A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. Australian Journal of Biological Sciences **15**, 413–428.

Battisti DS, Naylor RL. 2009. Historical Warnings of Future Food Insecurity with Unprecedented Seasonal Heat. Science **323**, 240–244.

Beltrano J, Carbone A, Montaldi ER, Guiamet JJ. 1994. Ethylene as promoter of wheat grain maturation and ear senescence. Plant Growth Regulation **15**, 107–112.

Beltrano J, Montaldi E, Bártoli C, Carbone A. 1997. Emission of water stress ethylene in wheat (Triticum aestivum L.) ears: Effects of rewatering. Plant Growth Regulation **21**, 121–126.

Bidinger FR, Mahalakshmi V, Rao GDP. 1987. Assessment of drought resistance in pearl millet (Pennisetum americanum (L.) Leeke). II. Estimation of genotype response to stress. Crop and Pasture Science **38**, 49–59.

Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. Annual review of cell and developmental biology **16**, 1–18.

Blum A. 1996. Yield Potential and Drought Resistance: Are They Mutually Exclusive? Increasing Yield Potential in Wheat: Breaking the Barriers.90–100.

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential - Are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research **56**, 1159–1168.

Blum A. 2011. Drought resistance - is it really a complex trait? Functional Plant Biology **38**, 753–757.

Blum A, Shpiler L, Golan G, Mayer J. 1989. Yield stability and canopy temperature of wheat genotypes under drought-stress. Field Crops Research **22**, 289–296.

Borrás L, Slafer GA, Otegui ME. 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Research **86**, 131–146.

Bradford KJ, Hsiao TC. 1982. Physiological Responses to Moderate Water Stress. In: Lange, 0. L., Nobel, P. S., Osmond, C. B. and Ziegler H, ed. Physiological Plant Ecology, II: Water Relations and Carbon Assimilation. Berlin, Heidelberg: Springer Berlin Heidelberg, 263–324.

Bradford KJ, Yang SF. 1980. Xylem Transport of 1-Aminocyclopropane-1-carboxylic Acid, an Ethylene Precursor, in Waterlogged Tomato Plants. Plant physiology **65**, 322–326.

Braun HJ, Payne T. 2012. Mega-Environment breeding. Physiological breeding I: interdisciplinary approaches to improve crop adaptation.6–17.

Brouwer C, Goffeau A, Heibloem M. 1985. *Irrigation Water Management: Training Manual No. 1-Introduction to Irrigation.* Rome: FAO.

Cairns JE, Impa SM, O'Toole JC, Jagadish SVK, Price AH. 2011. Influence of the soil physical environment on rice (Oryza sativa L.) response to drought stress and its implications for drought research. Field Crops Research **121**, 303–310.

Calderini DF, Abeledo LG, Savin R, Slafer GA. 1999. Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. The Journal of Agricultural Science **132**, 453–459.

Calderini DF, Reynolds MP. 2000. Changes in grain weight as a consequence of de-graining treatmentsat pre- and post-anthesis in synthetic hexaploid lines of wheat. Australian Journal of Agricultural Research **27**, 187–191.

Campbell WF, Salisbury FB, Bugbee B, *et al.* 2001. Comparative floral development of Mir-grown and ethylene-treated, earth-grown Super Dwarf wheat. Journal of plant physiology **158**, 1051–1060.

Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM. 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research **105**, 1–14.

Ceccarelli S, Grando S. 1991. Selection environment and environmental sensitivity in barley. Euphytica **57**, 157–167.

Chandra Sekhar KN, Sawhney VK. 1991. Role of ABA in stamen and pistil development in the normal and solanifolia mutant of tomato (Lycopersicon esculentum). Sexual Plant Reproduction **4**, 279–283.

Chang C, Bleecker AB. 2004. Ethylene biology. More than a gas. Plant physiology **136**, 2895–2899.

Chapin FS, Bloom AJ, Field CB, Waring RH. 1987. Multiple Environmental Factors to Responses Physiological ecology provides tools for studying how environmental interacting. BioScience **37**, 49–57.

Chávez P, Ribas-Carbó M, Medrano H, Mares V, Posadas A, Yarlequé C, Quiroz R, Flexas J. 2009. Early detection of drought stress in potato (Solanum tuberosum L.) and grapevine (Vitis vinifera L.) crops through multifractal analysis applied to remotely sensed data. Tropical roots and tubers in a changing climate: a convenient opportunity for the world. Fifteenth Triennial Symposium of the International Society for Tropical Root Crops, Lima, Peru, 2-6 November 2009. International Society for Tropical Root Crops - Peru Branch, 6–14.

Chávez P, Yarlequé C, Loayza H, *et al.* 2012. Detection of bacterial wilt infection caused by Ralstonia solanacearum in potato (Solanum tuberosum L.) through multifractal analysis applied to remotely sensed data. Precision Agriculture **13**, 236–255.

Chávez P, Yarlequé C, Piro O, Posadas A, Mares V, Loayza H, Chuquillanqui C, Zorogastúa P, Flexas J, Quiroz R. 2010. Applying multifractal analysis to remotely sensed data for assessing PYVV infection in potato (Solanum tuberosum L.) crops. Remote Sensing **2**, 1197–1216.

Chavez P, Zorogastúa P, Chuquillanqui C, Salazar LF, Mares V, Quiroz R. 2009. Assessing Potato Yellow Vein Virus (PYVV) infection using remotely sensed data. International Journal of Pest Management **55**, 251–256.

Chen L, Dodd IC, Davies WJ, Wilkinson S. 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. Plant, cell & environment **36**, 1850–1859.

Christopher JT, Christopher MJ, Borrell AK, Fletcher S, Chenu K. 2016. Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. Journal of Experimental Botany **67**, 5159–5172.

Claeys H, Inzé D. 2013. The agony of choice: How plants balance growth and survival under water-limiting conditions. Plant Physiology **162**, 1768–1779.

Cobb JN, DeClerck G, Greenberg A, Clark R, McCouch S. 2013. Next-generation phenotyping: Requirements and strategies for enhancing our understanding of genotype-phenotype relationships and its relevance to crop improvement. Theoretical and Applied Genetics **126**, 867–887.

Colebrook EH, Thomas SG, Phillips AL, Hedden P. 2014. The role of gibberellin signalling in plant responses to abiotic stress. The Journal of experimental biology **217**, 67–75.

Cossani CM, Pietragalla J, Reynolds MP. 2012. Canopy temperature and plant water relations traits. In: Reynolds, M. P., Pask, A. J. D., Mullan DM, ed. Physiological breeding I: interdisciplinary approaches to improve crop adaptation. CIMMYT, 60–68.

Craufurd PQ, Vadez V, Jagadish SVK, Prasad PVV, Zaman-Allah M. 2013. Crop science experiments designed to inform crop modeling. Agricultural and Forest Meteorology **170**, 8–18.

Cristescu SM, Mandon J, Arslanov D, De Pessemier J, Hermans C, Harren FJ. 2013. Current methods for detecting ethylene in plants. Annals of botany **111**, 347–360.

Cutler AJ, Krochko JE. 1999. Formation and breakdown of ABA. Trends in Plant Science **4**, 472–478.

Davies PJ. 2004*a*. *Plant hormones: biosynthesis, signal transduction, action!* (PJ Davies, Ed.). Dordrecht: Kluwer Academic Publishers.

Davies PJ. 2004*b*. The plant hormones: their nature, occurrence, and functions. In: Davies PJ, ed. Plant hormones: biosynthesis, signal transduction, action! Dordrecht: Kluwer Academic Publishers, 1–15.

Davies WJ, Dodd I, Wilkinson S. 2013. Improving spike fertility by understanding and modifying its sensitivity to environmental cues. Proceedings of the 3rd International workshop of the Wheat Yield Consortium. CIMMYT, 36–38.

Davies WJ, Kudoyarova G, Hartung W. 2005. Long-distance ABA Signaling and Its Relation to Other Signaling Pathways in the Detection of Soil Drying and the Mediation of the Plant's Response to Drought. Journal of Plant Growth Regulation **24**, 285–295.

Davies WJ, Wilkinson S, Loveys B. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytologist **153**, 449–460.

Davies WJ, Zhang J. 1991. Root Signals and the Regulation of Growth and Development of Plants in Drying Soil. Annual Review of Plant Physiology and Plant Molecular Biology **42**, 55–76.

Dembinska O, Lalonde S, Saini HS. 1992. Evidence against the Regulation of Grain Set by Spikelet Abscisic Acid Levels in Water-Stressed Wheat. Plant Physiol **100**, 1599–1602.

Dolferus R, Ji X, Richards RA. 2011. Abiotic stress and control of grain number in cereals. Plant science **181**, 331–341.

Doorenbos J. 1953. Review of the literature on dormancy in buds of woody plants. Mededelingen van de Landbouwhogeschool the Wageningen/Nederland **53**, 1–24.

Dorion S, Lalonde S, Saini HS. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiology **111**, 137–145.

Ehrler WL, Idso SB, Jackson RD, Reginato RJ. 1978. Wheat Canopy Temperature: Relation to Plant Water Potential. Agronomy Journal **70**, 251–256.

El-Beltagy AS, Hall MA. 1974. Effect of Water Stress Upon Endogenous Ethylene Levels in Vicia Faba. New Phytologist **73**, 47–60.

English PJ, Lycett GW, Roberts JA, Jackson MB. 1995. Increased 1-Aminocyclopropane-1-Carboxylic Acid Oxidase Activity in Shoots of Flooded Tomato Plants Raises Ethylene Production to Physiologically Active Levels. Plant Physiology **109**, 1435–1440.

Falconer DS, Mackay TFC. 1996. *Introduction to Quantitative Genetics, 4th edition*. Harlow, Essex, UK: Longmans Green.

FAO. 2015. FAO production statistics, http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor (verified 13/08/15).

Fernandez GC. 1992. Effective selection criteria for assessing plant stress tolerance. Proceedings of the International Symposium on "Adaptation of vegetables and other food crops in temperature and water stress. Tainan. Taiwan: AVRDC Publication, 257–270.

Ferrise R, Moriondo M, Bindi M. 2011. Probabilistic assessments of climate change impacts on durum wheat in the Mediterranean region. Natural Hazards and Earth System Science **11**, 1293–1302.

Finkelstein R. 2013. Abscisic Acid synthesis and response. The Arabidopsis book / American Society of Plant Biologists **11**, e0166.

Finkelstein RR, Gampala SSL, Rock CD. 2002. Abscisic acid signaling in seeds and seedlings. The Plant cell **14 Suppl**, S15–S45.

Finlayson SA, Lee IJ, Mullet JE, Morgan PW. 1999. The mechanism of rhythmic ethylene production in sorghum. The role of phytochrome B and simulated shading. Plant physiology **119**, 1083–1089.

Fiorani F, Schurr U. 2013. Future scenarios for plant phenotyping. Annual review of plant biology **64**, 267–291.

Fischer RA. 1984. Wheat. In: Smith, WH and Banta J, ed. Potential Productivity of Field Crops Under Different Environments.129.

Fischer RA. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. The Journal of Agricultural Science **105**, 447–461.

Fischer RA. 2007. Understanding the physiological basis of yield potential in wheat. The Journal of Agricultural Science **145**, 99–113.

Fischer RA. 2011. Wheat physiology : a review of recent developments. Crop and Pasture Science **62**, 95–114.

Fischer RA, Edmeades GO. 2010. Breeding and cereal yield progress. Crop Science **50**, S-85-S-98.

Fischer RA, Maurer R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Crop and Pasture Science **29**, 897–912.

Fitzpatrick AH, Shrestha N, Bhandari J, Crowell DN. 2011. Roles for farnesol and ABA in Arabidopsis flower development. Plant signaling & behavior **6**, 1189–1191.

Fleury D, Jefferies S, Kuchel H, Langridge P. 2010. Genetic and genomic tools to improve drought tolerance in wheat. Journal of experimental botany **61**, 3211–3222.

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP. 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. Journal of experimental botany **62**, 469–486.

González FG, Miralles DJ, Slafer GA. 2011. Wheat floret survival as related to pre-anthesis spike growth. Journal of experimental botany **62**, 4889–4901.

Guo Z, Chen D, Schnurbusch T. 2015. Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. Journal of experimental botany **66**, 3099–111.

Guo H, Ecker JR. 2004. The ethylene signaling pathway: New insights. Current Opinion in Plant Biology **7**, 40–49.

Guo D, Liang J, Li L. 2009. Abscisic acid (ABA) inhibition of lateral root formation involves endogenous ABA biosynthesis in Arachis hypogaea L. Plant Growth Regulation **58**, 173–179.

Guo Z, Slafer GA, Schnurbusch T. 2016. Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. Journal of Experimental Botany **67**, 4221–4230.

Gurmani AR, Bano A, Najeeb U, Zhang J, Khan SU, Timothy J. 2013. Exogenously applied silicate and abscisic acid ameliorates the growth of salinity stressed wheat (Triticum aestivum L) seedlings through Na + exclusion. **7**, 1123–1130.

Guzmán P, Ecker JR. 1990. Exploiting the triple response of Arabidopsis to identify ethylene-related mutants. The Plant Cell Online **2**, 513–523.

Hanft JM, Wych RD. 1982. Visual Indicators of Physiological Maturity of Hard Red Spring Wheat. Crop Science **22**, 584–588.

Hartung W, Sauter A, Hose E. 2002. Abscisic acid in the xylem: where does it come from, where does it go to? Journal of experimental botany **53**, 27–32.

Hays DB, Do JH, Mason RE, Morgan G, Finlayson S a. 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Science **172**, 1113–1123.

Herms DA, Mattson WJ. 1992. The Dilemma of Plants: To Grow or Defend. The Quarterly Review of Biology **67**, 283–335.

Himmelbach A, Yang Y, Grill E. 2003. Relay and control of abscisic acid signaling. Current Opinion in Plant Biology **6**, 470–479.

Hochman Z. 1982. Effect of water stress with phasic development on yield of wheat grown in a semi-arid environment. Field Crops Research **5**, 55–67.

Hopkins W, Hüner NPA. 2008. *Introduction to Plant Physiology Fourth edition*. John Wiley & Sons, Inc.

Høyer L. 1996. Critical ethylene exposure for *Capsicum annuum* 'Janne' is dependent on an interaction between concentration, duration and developmental stage. Journal of Horticultural Science **71**, 621–628.

Hu B, Cao J, Ge K, Li L. 2016. The site of water stress governs the pattern of ABA synthesis and transport in peanut. Scientific Reports **6**, 32143.

Ievinsh G, Kreicbergs O. 1992. Endogenous rhythmicity of ethylene production in growing intact cereal seedlings. Plant physiology **100**, 1389–1391.

Innes P, Quarrie SA. 1987. Water relations. In: Lupton F, ed. Wheat Breeding: Its Scientific Basis. Chapman and Hall Ltd, 566.

Iqbal N, Nazar R, Syeed S, Masood A, Khan NA. 2011. Exogenously-sourced ethylene increases stomatal conductance, photosynthesis, and growth under optimal and deficient nitrogen fertilization in mustard. Journal of Experimental Botany **62**, 4955–4963.

Jackson RD. 1992. Canopy temperature and crop water stress. In: Daniel Hillel, ed. Advances in irrigation. Volume 1.43–85.

Jagadish SVK, Craufurd PQ, Wheeler TR. 2007. High temperature stress and spikelet fertility in rice (Oryza sativa L.). Journal of experimental botany **58**, 1627–1635.

Jäger K, Fábián A, Barnabás B. 2008. Effect of water deficit and elevated temperature on pollen development of drought sensitive and tolerant winter wheat (Triticum aestivum L .) genotypes. **52**, 67–71.

Jasoni RL, Cothren JT, Morgan PW, Sohan DE. 2002. Circadian ethylene production in cotton. Plant Growth Regulation **36**, 127–133.

Ji X, Dong B, Shiran B, Talbot MJ, Edlington JE, Hughes T, White RG, Gubler F, Dolferus R. 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. Plant physiology **156**, 647–662.

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CLD, Condon AG, Richards R a, Dolferus R. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment **33**, 926–942.

Jones CL, Weckler PR, Maness NO, Jayasekara R, Stone ML, Chrz D. 2007. Remote Sensing to Estimate Chlorophyll Concentration in Spinach Using Multi-Spectral Plant Reflectance. Transactions of the ASABE **50**, 2267–2273.

Kende H. 1993. Ethylene Biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology **44**, 283–307.

Khan S, Rowe SC, Harmon FG. 2010. Coordination of the maize transcriptome by a conserved circadian clock. BMC plant biology **10**, 126.

Khayatnezhad M, Zaeifizadeh M, Gholamin R, Club YR. 2010. Investigation and Selection Index for Drought Stress. Australian Journal of Basic and Applied Sciences **4**, 4815–4822.

Khodarahmpour Z, Choukan R, Bihamta MR, Hervan EM. 2011. Determination of the Best Heat Stress Tolerance Indices in Maize (Zea mays L .) Inbred Lines and Hybrids under Khuzestan Province Conditions. Journal of Agricultural Science and Technology **13**, 111–121.

Kirby EJM. 1988. Analysis of Leaf, Stem and Ear Growth in Wheat from Terminal Spikelet Stage to Anthesis. Field Crops Research Elsevier Science Publishers B.V **18**, 127–140.

Kirkham MB. 2005. *Principles of soil and plant water relations*. Academic press.

Klassen SP, Bugbee B. 2004. Ethylene synthesis and sensitivity in crop plants. HortScience **39**, 1546–1552.

Klee HJ, Clark DG. 2010. Ethylene Signal Transduction in Fruits and Flowers. Plant Hormones. Dordrecht: Springer Netherlands, 377–398.

Kobayashi H, Saka H. 2000. Relationship between Ethylene Evolution and Sucrose Content in Excised Leaf Blades of Rice. Plant Prod. Sci **3**, 398–403.

Kogan M. 1998. Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annual Review of Entomology **43**, 243–270.

Kramer PJ, Boyer JS. 1985. *Water Relations of Plants and Soils*. San Diego, California: Academic press.

Kumar V, Sah SK, Khare T, Shriram V, Wani SH. 2016. Engineering Phytohormones for Abiotic Stress Tolerance in Crop Plants. Plant Hormones under Challenging Environmental Factors. Springer Netherlands, 247–266.

Lal R, Steward BA, Uphoff N, Hansen DO. 2005. Climate Change, Soil Carbon Dynamics, and Global Food Security. Climate Change and Global Food Security.113–143.

Lee SC, Luan S. 2012. ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant, Cell and Environment **35**, 53–60.

Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, Hwang I. 2006. Activation of Glucosidase via Stress-Induced Polymerization Rapidly Increases Active Pools of Abscisic Acid. Cell **126**, 1109–1120.

Leinonen I, Jones HG. 2004. Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. Journal of Experimental Botany **55**, 1423–1431.

Levitt J. 1980. Seccion I: stress concepts. Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses (No. Ed. 2). Academic Press: New York, 1–21.

Levitt J, Sullivan C, Krull E. 1960. Some problems in drought resistance. Bulletin of the Research Council of Israel. Section D. Botany **8**, 173–180.

Li L, Zhang Q, Huang D. 2014. A review of imaging techniques for plant phenotyping. Sensors (Basel, Switzerland) **14**, 20078–20111.

Liu Y, Sui Y, Gu D, Wen X, Chen Y, Li C, Liao Y. 2013. Effects of conservation tillage on grain filling and hormonal changes in wheat under simulated rainfall conditions. Field Crops Research **144**, 43–51.

Lobell DB, Field CB. 2007. Global scale climate–crop yield relationships and the impacts of recent warming. Environmental Research Letters **2**, 14002.

Longnecker N, Kirby EJM, Robson A. 1993. Leaf Emergence, Tiller Growth, and Apical Development of Nitrogen-Dificient Spring Wheat. Crop Science **33**, 154–160.

Lopes MS, S. D, Peña RJ ·, Sukumaran S, Reynolds MP. 2015. Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. Theoretical and Applied Genetics **128**, 453–464.

Macková H, Hronková M, Dobrá J, *et al.* 2013. Enhanced drought and heat stress tolerance of tobacco plants with ectopically enhanced cytokinin oxidase/dehydrogenase gene expression. Journal of experimental botany **64**, 2805–2815.

Manzi M, Lado J, Rodrigo MJ, Zacariás L, Arbona V, Gómez-Cadenas A. 2015. Root ABA Accumulation in Long-Term Water-Stressed Plants is Sustained by Hormone Transport from Aerial Organs. Plant and Cell Physiology **56**, 2457–2466.

Martino DL, Abbate PE, Cendoya MG, Gutheim F, Mirabella NE, Pontaroli AC. 2015. Wheat spike fertility: inheritance and relationship with spike yield components in early generations. Plant Breeding **134**, 264–270.

McAdam SAM, Manzi M, Ross JJ, Brodribb TJ, Gómez-Cadenas A. 2016. Uprooting an abscisic acid paradigm: Shoots are the primary source. Plant Signaling & Behavior **11**, 11–12.

Mcclung CR. 2000. Circadian rhythms in plants : a millennial view. Physiologia Plantarum **109**, 359–371.

Middleton N, Stringer L, Goudle A, Thomas D. 2011. *The Forgotten Billion: MDG Achievement in the Drylands, (UNDP and UNCCD)*. New York.

Miralles DJ, Richards RA, Slafer GA. 2000. Duration of the stem elongation period influences the number of fertile florets in wheat and barley. Australian Journal of Plant Physiology **27**, 931–940.

Miralles DJ, Slafer GA. 1995. Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. Field Crops Research **43**, 55–66.

Mishra AK, Singh VP. 2010. A review of drought concepts. Journal of Hydrology **391**, 202–216.

Mohammadi M, Karimizadeh R, Abdipour M. 2011. Evaluation of drought tolerance in bread wheat genotypes under dryland and supplemental irrigation conditions. Australian Journal of Crop Science **5**, 487–493.

Monneveux P, Jing R, Misra SC. 2012. Phenotyping for drought adaptation in wheat using physiological traits. Frontiers in Physiology **3**.

Morgan PW, Drew MC. 1997. Ethylene and plant responses to stress. Physiologia Plantarum **100**, 620–630.

Morgan PW, He CJ, De Greef JA, De Proft MP. 1990. Does water deficit stress promote ethylene synthesis by intact plants? Plant physiology **94**, 1616–1624.

Morison JL, Gifford RM. 1984. Ethylene Contamination of CO2 Cylinders. Plant Physiol **75**, 275–277.

Muday GK, Rahman A, Binder BM. 2012. Auxin and ethylene: collaborators or competitors? Trends in plant science **17**, 181–195.

Narayana I, Lalonde S, Saini HS. 1991. Water-stress-induced ethylene production in wheat : a fact or artifact? Plant physiology **96**, 406–410.

Ohkuma K, Lyon JL, Addicott FT, Smith OE. 1963. Abscisin II, an Abscission-Accelerating Substance from Young Cotton Fruit. Science (New York, N.Y.) **142**, 1592–1593.

Oliver SN, Dennis ES, Dolferus R. 2007. ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. Plant and Cell Physiology **48**, 1319–1330.

Oosterhuis DM, Cartwright PM. 1983. Spike Differentiation and Floret Survival in Semidwarf Spring Wheat as Affected by Water Stress and Photoperiod. Crop Science **23**, 711–717.

Ortiz R, Sayre KD, Govaerts B, Gupta R, Subbarao GV, Ban T, Hodson D, Dixon JM, Iván Ortiz-Monasterio J, Reynolds M. 2008. Climate change: Can wheat beat the heat? Agriculture, Ecosystems & Environment **126**, 46–58.

Pachauri RK, Meyer L, Plattner GK, Stocker T. 2015. *IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*.

Panneerselvam R, Rajalekshmi KM, Jaleel CA, Azooz MM, Panneerselvam R. 2009. Effect of Triazole Growth Regulators on Growth and Pigment Contents in Plectranthus aromaticus and Plectranthus vettiveroids. Advances in Biological Research **3**, 3–4.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany **62**, 453–467.

Pask A, Pietragalla J, Mullan D, Reynolds MP. 2012. *Physiological Breeding II: A Field Guide to Wheat Phenotyping.* CIMMYT.

Peleg Z, Blumwald E. 2011. Hormone balance and abiotic stress tolerance in crop plants. Current opinion in plant biology **14**, 290–295.

Peltonen-Sainio P, Kangas A, Salo Y, Jauhiainen L. 2007. Grain number dominates grain weight in temperate cereal yield determination: Evidence based on 30 years of multi-location trials. Field Crops Research **100**, 179–188.

Pierik R, Cuppens MLC, Voesenek LACJ, Visser EJW. 2004. Interactions between Ethylene and Gibberellins in Phytochrome-Mediated Shade Avoidance Responses in Tobacco. Plant Physiology **136**, 2928–2936.

Pierik R, Sasidharan R, Voesenek LACJ. 2007. Growth Control by Ethylene: Adjusting Phenotypes to the Environment. Journal of Plant Growth Regulation **26**, 188–200.

Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ. 2006. The Janus face of ethylene: growth inhibition and stimulation. Trends in Plant Science **11**, 176–183.

Pierik R, Visser EJW, De Kroon H, Voesenek LACJ. 2003. Ethylene is required in tobacco to successfully compete with proximate neighbours. Plant, Cell and Environment **26**, 1229–1234.

Saint Pierre C. 2012. Phenotyping in controlled environments vs. field conditions. Physiological breeding I: interdisciplinary approaches to improve crop adaptation. CIMMYT, 124–130.

Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas J-J, Chapman SC. 2010. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics **121**, 1001–21.

Van de Poel B, Van Der Straeten D. 2014. 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene! Frontiers in plant science **5**.

Poorter H, Fiorani F, Pieruschka R, Wojciechowski T, van der Putten WH, Kleyer M, Schurr U, Postma J. 2016. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytologist **212**, 838–855.

Porter JR, Xie L, Challinor AJ, Cochrane K, Howden SM, Iqbal MM, Lobell DB, Travasso MI. 2014. Food Security and Food Production Systems. In: Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea and LLW, ed. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA, 485–533.

Pretty J, Olsson L, Farage P, Warren A, Tschakert P, Ardö J. 2005. *Carbon Sequestration in Dryland Soils*. World Soil Resources Report.

Puértolas J, Alcobendas R, Alarcón juan j., Dodd ian c. 2013. Long-distance abscisic acid signalling under different vertical soil moisture gradients depends on bulk root water potential and average soil water content in the root zone. Plant, Cell and Environment **36**, 1465–1475.

Puértolas J, Larsen EK, Davies WJ, Dodd IC. 2017. Applying 'drought' to potted plants by maintaining suboptimal soil moisture improves plant water relations. Journal of Experimental Botany **68**, 2413–2424.

Quarrie SA, Whitford PN, Appleford NEJ, Wang TL, Cook SK, Henson IE, Loveys BR. 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta **173**, 330–339.

Rahaman MM, Chen D, Gillani Z, Klukas C, Chen M. 2015. Advanced phenotyping and phenotype data analysis for the study of plant growth and development. Frontiers in plant science **6**, 619.

Ramirez-Vallejo P, Kelly JD. 1998. Traits related to drought resistance in common bean. Euphytica **99**, 127–136.

Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Australian Journal of Plant Physiology **21**, 717–730.

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer GA. 2012*a*. Achieving yield gains in wheat. Plant, cell & environment **35**, 1799–1823.

Reynolds MP, Foulkes MJ, Slafer GA, Berry P, Parry M a J, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. Journal of experimental botany **60**, 1899–1918.

Reynolds MP, Molero G, Tattaris M, Cossani CM, Alderman P, Sukumaran S. 2015. Improving crop adaptation to climate change through strategic crossing of stress adaptive traits. Procedia Environmental Sciences. Elsevier B.V., 298–299.

Reynolds MP, Pask AJD, Mullan DM. 2012*b*. *Physiological breeding I: interdisciplinary approaches to improve crop adaptation.* CIMMYT.

Reynolds MP, Rajaram S, McNab A (Eds.). 1996. *Increasing Yield Potential in Wheat : Breaking the Barriers*. Ciudad Obregón, Sonora, Mexico: CIMMYT.

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larqué-Saavedra A, Quick JS. 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. Euphytica **100**, 85–94.

Rezaei EE, Webber H, Gaiser T, Naab J, Ewert F. 2015. Heat stress in cereals: Mechanisms and modelling. European Journal of Agronomy **64**, 98–113.

Richards RA. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regulation **20**, 157–166.

Richards RA. 2006. Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management **80**, 197–211.

Rosegrant MW, Cline SA. 2003. Global Food Security : Challenges and Policies. **302**, 1917–1919.

Rosielle AA, Hamblin J. 1981. Theoretical Aspects of Selection for Yield in Stress and Non-Stress Environments. Crop Science **21**, 943–946.

Rowlands DJ, Frame DJ, Ackerley D, *et al.* 2012. Broad range of 2050 warming from an observationally constrained large climate model ensemble. Nature Geoscience **5**, 256–260.

Rybka K, Nita Z. 2015. Physiological requirements for wheat ideotypes in response to drought threat. Acta Physiologiae Plantarum **37**, 97.

Saab IN, Sharp RE, Pritchard J, Voetberg GS. 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant physiology **93**, 1329–1336.

Saini HS, Aspinall D. 1982. Sterility in Wheat (Triticum aestivum L .) Induced by Water Deficit or High Temperature : Possible Mediation by Abscisic Acid. Functional Plant Biology **9**, 529–537.

Saini H, Sedgley M, Aspinall D. 1984. Development Anatomy in Wheat of Male Sterility Induced by Heat Stress, Water Deficit or Abscisic Acid. Functional Plant Biology **11**, 243–253.

Salazar C, Hernandez C, Pino MT. 2015. Plant water stress: Associations between ethylene and abscisic acid response. Chilean journal of agricultural research **75**, 71–79.

Santner A, Estelle M. 2009. Recent advances and emerging trends in plant hormone signalling. Nature **459**, 1071–1078.

Sareen S, Tyagi BS, Sharma I. 2012. Response Estimation of Wheat Synthetic Lines to Terminal Heat Stress Using Stress Indices. Journal of Agricultural Science **4**, 97–104.

Sayre KD, Rajaram S, Fischer RA. 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Science **7**, 36–42.

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. 1965. Sap Pressure in Vascular Plants. Science **148**, 339–346.

Sharp R. 2002. Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant, Cell & Environment **25**, 211–222.

Sharp RE, LeNoble ME. 2002. ABA, ethylene and the control of shoot and root growth under water stress. Journal of experimental botany **53**, 33–37.

Sharp RE, Wu Y, Voetberg GS, Saab IN, LeNoble ME. 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. Journal of Experimental Botany **45**, 1743–1751.

Shearman VJ, Sylvester-Bradley R, Scott RK, Foulkes MJ. 2005. Physiological processes associated with Wheat Yield Progress in the UK. **45**, 175–185.

Sheoran IS, Saini HS. 1996. Drought-induced male sterility in rice: Changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sexual Plant Reproduction **9**, 161–169.

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds MP, Muricho G. 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security **5**, 291–317.

Shpiler L, Blum A. 1990. Heat tolerance for yield and its components in different wheat cultivars. Euphytica **51**, 257–263.

Siddique MRB, Hamid A, Islam MS. 2000. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica **41**, 35–39.

Siddique KHM, Kirby EJM, Perry MW. 1989. Ear:Stem Ratio in Old and Modern Wheat Varieties; Relationship with Improvement in Number of Grains per Ear and Yield. Field Crops Research **21**, 59–78.

Silva TP, Finger FL. 2015. Ethylene and 1-methylcyclopropene action over senescence of nasturtium flowers. Horticultura Brasileira **33**, 453–458.

Sinclair TR, Ludlow MM. 1985. The unfulfilled potential of plant water potential. Australian journal of plant physiology **33**, 212–217.

Sisler EC, Grichko V, Serek M. 2006. Interaction of ethylene and other compounds with the ethylene receptor : agonists and antagonists. Ethylene action in plants. Springer Berlin Heidelberg, 1–34.

Slafer GA. 2003. Genetic Basis of Yield as Viewed From a Crop Physiologist ’s Perspective. Annals of Applied Biology **142**, 117–128.

Slafer GA. 2005. Physiology of determination of major wheat yield components. In: Buck, H.T., Nisi, J.E., Salomón N, ed. Environments Proceedings of the 7th International Wheat Conference, 27 November - 2 December 2005, Mar del Plata, Argentina (Developments in Plant Breeding). Springer, 557–565.

Slafer GA. 2012. Wheat development: its role Adaptation, phenotyping and improving crop. Physiological breeding I: interdisciplinary approaches to improve crop.109–123.

Slafer GA, Araus JL. 2007. Physiological traits for improving wheat yield under a wide range of conditions. In: Spiertz, J.H.J., Struik, P.C., van Laar HH, ed. Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations. Dordrecht: Springer Netherlands, 147–156.

Slafer GA, Calderini DF, Miralles DJ. 1996. Yield Components and Compensation in Wheat: Opportunities for Further Increasing Yield Potential. In: Reynolds, Matthew P, Rajaram, Sanjaya, McNab A, ed. Increasing yield potential in wheat: breaking the barriers. CIMMYT, 101–133.

Slafer GA, Rawson HM. 1994. Sensitivity of Wheat Phasic Development to Major Environmental Factors: a Re-Examination of Some Assumptions Made by Physiologists and Modellers. Australian Journal of Plant Physiology **21**, 393–426.

Slafer GA, Savin R, Sadras VO. 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Research **157**, 71–83.

Spollen WG, Lenoble ME, Samuels TD, Bernstein N, Sharp RE. 2000. Abscisic Acid Accumulation Maintains Maize Primary Root Elongation at Low Water Potentials by Restricting Ethylene Production. Plant Physiology **122**, 967–976.

Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds MP. 2015. Genome - wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. Theoretical and Applied Genetics **123**, 353–363.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N. 2005. Ethylene Inhibits Abscisic Acid-Induced Stomatal Closure in Arabidopsis 1. **138**, 2337–2343.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. 2006. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis. Journal of experimental botany **57**, 2259–66.

Tardieu F. 2012. Any trait or trait-related allele can confer drought tolerance: Just design the right drought scenario. Journal of Experimental Botany **63**, 25–31.

Tardieu F, Davies WJ. 1992. Stomatal response to abscisic-acid is a function of current plant water status. Plant physiology **98**, 540–545.

Tardieu F, Parent B, Simonneau T. 2010. Control of leaf growth by abscisic acid: Hydraulic or non-hydraulic processes? Plant, Cell and Environment **33**, 636–647.

Tattaris M, Reynolds MP, Chapman SC. 2016. A Direct Comparison of Remote Sensing Approaches for High-Throughput Phenotyping in Plant Breeding. Frontiers in plant science **7:1131**.

Tebaldi C, Lobell DB. 2008. Towards probabilistic projections of climate change impacts on global crop yields. Geophysical Research Letters **35**, 2–7.

Tewolde H, Fernandez CJ, Erickson CA. 2006. Wheat cultivars adapted to post-heading high temperature stress. Journal of Agronomy and Crop Science **192**, 111–120.

Thain SC, Vandenbussche F, Laarhoven LJJ, Dowson-Day MJ, Wang Z-Y, Tobin EM, Harren FJM, Millar AJ, Van Der Straeten D. 2004. Circadian Rhythms of Ethylene Emission in Arabidopsis. Plant Physiology **136**, 3751–3761.

Thiry AA, Reynolds MP, Davies WJ. 2017. ABA and ethylene hormone balance and its diurnal variation as indicators of plant drought stress resilience. Proceedings of the 3rd International TRIGO (Wheat) Yield Potential Workshop. Cd. Obregon, Sonora, Mexico: CIMMYT, 119–128.

Tholen D, Pons TL, Voesenek L a CJ, Poorter H. 2007. Ethylene insensitivity results in down-regulation of rubisco expression and photosynthetic capacity in tobacco. Plant physiology **144**, 1305–1315.

Tholen D, Pons TL, Voesenek LACJ, Poorter H. 2008. The role of ethylene perception in the control of photosynthesis. **3**, 108–109.

Tholen D, Poorter H, Voesenek LACJ. 2006. Ethylene and Plant Growth. In: Khan NA, ed. Ethylene Action in plants. Springer Berlin Heidelberg, 35–49.

Thomas H, Ougham H. 2014. The stay-green trait. Journal of Experimental Botany **65**, 3889–3900.

Trewavas AJ, Cleland RE. 1983. Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? Trends in Biochemical Sciences **8**, 354–357.

Tubiello FN, Rosenzweig C, Goldberg R a, Jagtap S, Jones JW. 2000. U . S . National Assessment Technical Report Part I : Wheat , Potato , Corn , and Citrus. , 1–39.

Turner NC. 1979. Drought resitance and adaptation to water deficits in crop plants. In: Mussell, H., Staples RC, ed. Stress physiology in crop plants. John Wiley and Sons, 343–372.

Turner NC. 1986. Adaptation to Water Deficits: a Changing Perspective. Australian Journal of Plant Physiology **13**, 175–190.

Tyagi K, Park MR, Lee HJ, Lee CA, Rehman S, Steffenson B, Yun SJ. 2011. Fertile crescent region as source of drought tolerance at early stage of plant growth of wild barley (Hordeum vulgare L. ssp. spontaneum). Pakistan Journal of Botany **43**, 475–486.

US EPA CCD. 2015. Agriculture and Food Supply Impacts & Adaptation.

Valério I, Carvalho F, Benin G. 2013. Seeding density in wheat: the more, the merrier? Scientia Agricola **70**, 176–184.

Valluru R, Davies WJ, Reynolds MP, Dodd IC. 2016. Foliar Abscisic Acid-To-Ethylene Accumulation and Response Regulate Shoot Growth Sensitivity to Mild Drought in Wheat. Frontiers in plant science **7:461**.

Vankova R. 2012. Abscisic Acid Signaling in Plants. In: Ahmad, Parvaiz, Prasad MNV, ed. Abiotic stress responses in Plants: Metabolism, Productivity and Sustainability. Springer New York, 359–368.

Vysotskaya L, Wilkinson S, Davies WJ, Arkhipova T, Kudoyarova G. 2011. The effect of competition from neighbours on stomatal conductance in lettuce and tomato plants. Plant, Cell and Environment **34**, 729–737.

Wang Z, Cao W, Dai T, Zhou Q. 2001. Effects of exogenous hormones on floret development and grain set in wheat. Plant Growth Regulation **35**, 225–231.

Wang Z, Mambelli S, Setter TL. 2002. Abscisic Acid Catabolism in Maize Kernels in Response to Water Deficit at Early Endosperm Development. Annals of Botany **90**, 623–630.

Wardlaw IF, Dawson IA, Munibi P. 1989. The Tolerance of Wheat to High Temperatures during Reproductive Growth . 2. Grain Development. Crop and Pasture Science **40**, 15–24.

Wardlaw I, Wrigley C. 1994. Heat Tolerance in Temperate Cereals: an Overview. Australian Journal of Plant Physiology **21**, 695–703.

Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frey NF dit, Leung J. 2008. An Update on Abscisic Acid Signaling in Plants and More …. Molecular Plant **1**, 198–217.

Westgate M, Passioura J, Munns R. 1996. Water Status and ABA Content of Floral Organs in Drought-Stressed Wheat. Australian Journal of Plant Physiology **23**, 763–772.

Weyers JDB, Paterson NW. 2001. Plant hormones and the control of physiological processes. New Phytologist **152**, 375–407.

Wheeler RM, Peterson B V, Sager JC, Knott WM. 1996. Ethylene production by plants in a closed environment. Advances in space research **18**, 193–196.

Wheeler RM, Peterson B V, Stutte GW. 2004. Ethylene production throughout growth and development of plants. HortScience **39**, 1541–1545.

White JW, Andrade-Sanchez P, Gore MA, *et al.* 2012. Field-based phenomics for plant genetics research. Field Crops Research **133**, 101–112.

Wilhite DA. 2010. Quantification of Agricultural Drought for Effective Drought Mitigation and Preparedness: Key Issues and Challenges. In: Sivakumar, M.V. K., Motha, R.P., Wilhite, D.A., Wood DA, ed. Agricultural Drought Indices: Proceedings of Expert Meeting held in Murcia, Spain. Geneva: World Meteorological Organization, 13–21.

Wilhite DA, Glantz MH. 1985. Understanding: the Drought Phenomenon: The Role of Definitions. Water International **10**, 111–120.

Wilkinson S, Davies WJ. 2002. ABA-based chemical signalling : the co-ordination of. Plant, Cell & Environment **25**, 195–210.

Wilkinson S, Davies WJ. 2009. Ozone suppresses soil drying- and abscisic acid (ABA)-induced stomatal closure via an ethylene-dependent mechanism. Plant, cell & environment **32**, 949–959.

Wilkinson S, Davies WJ. 2010. Drought, ozone, ABA and ethylene: new insights from cell to plant to community. Plant, cell & environment **33**, 510–525.

Wilkinson S, Kudoyarova GR, Veselov DS, Arkhipova TN, Davies WJ. 2012. Plant hormone interactions: innovative targets for crop breeding and management. Journal of experimental botany **63**, 3499–3509.

Wright STC. 1980. The effect of plant growth regulator treatments on the levels of ethylene emanating from excised turgid and wilted wheat leaves. Planta **148**, 381–388.

Yang SF, Hoffman NE. 1984. Ethylene Biosynthesis and its Regulation in Higher Plants. Annual Review of Plant Physiology **35**, 155–189.

Yang J-C, Liu K, Zhang S-F, Wang X-M, Wang Z-Q, Liu L-J. 2008. Hormones in Rice Spikelets in Responses to Water Stress During Meiosis. Acta Agronomica Sinica **34**, 111–118.

Yang D, Wang N, Yan X, Shi J, Zhang M, Wang Z, Yuan H. 2014. Microencapsulation of seed-coating tebuconazole and its effects on physiology and biochemistry of maize seedlings. Colloids and Surfaces B: Biointerfaces **114**, 241–246.

Yang J, Zhang J, Liu K, Wang Z, Liu L. 2006*a*. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. The New phytologist **171**, 293–303.

Yang J, Zhang J, Liu K, Wang Z, Liu L. 2007. Abscisic Acid and Ethylene Interact in Rice Spikelets in Response to Water Stress During Meiosis. Journal of Plant Growth Regulation **26**, 318–328.

Yang J, Zhang J, Wang Z, Liu K, Wang P. 2006*b*. Post-anthesis development of inferior and superior spikelets in rice in relation to abscisic acid and ethylene. Journal of experimental botany **57**, 149–160.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research **14**, 415–421.

Zeng Z, King R. 1986. Regulation of Grain Number in Wheat: Changes in Endogenous Levels of Abscisic Acid. Australian Journal of Plant Physiology **13**, 347–352.

Zhang J, Davies WJ. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant, Cell and Environment **12**, 73–81.

Zingaretti SM, Inácio MC, de Matos Pereira L, Antunes Paz T, de Castro França S. 2013. Water Stress and Agriculture. In: Akinci S, ed. INTECH Open Access Publisher. InTech, 151–179.