Transcriptomic, physio-morphological and biochemical analyses of drought stress response in bread wheat *Triticum aestivum L*

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A Thesis Submitted for the Degree of Doctor of Philosophy

School of Natural and Environmental Science Newcastle University

December 2023

Declaration

The research studies detailed within this thesis were carried out in the laboratories of Newcastle University under the supervision of Prof Angharad Gatehouse and Dr Martin Edwards during the period 2018-2023. This thesis submitted to Newcastle University for the requirements of degree of Doctor of Philosophy in Biology. I hereby declare that this thesis is my own work and effort and has not been submitted anywhere for any award. All other sources of information, used in this study, have been acknowledged.

Mohammed Sager Alotaibi

I would like to dedicate this work:

To my father in loving memory

Who has supported me until the last breath of his life and helped me to become what I am today.

To my mother

May she find here the expression of my great affection and eternal gratitude.

I would like to thank them for their constant support, their dedication and their trust,

I owe to them what I am today and what I will be tomorrow.

To my sisters and brothers

For their encouragement over the years and for all our good times.

To all the Alotaibi family.

And finally, to all those who have helped me to become what I am today.

Acknowledgements

I would like to express my deep gratitude to all the people who have kindly guided and supported me throughout this work.

Firstly, I would like to express my sincere gratitude to my supervisors Professor Angharad Gatehouse and Doctor Martin Edwards for helping and supporting me beyond all expectations throughout my thesis. Thank you for your advice, guidance, and encouragement that enabled me to complete this work.

I would also like to extend my warmest thanks to the officials of the Ministry of Higher Education in Saudi Arabia for funding my thesis at Newcastle upon Tyne University, UK., without which this work could have never begun.

I am sincerely grateful for all my colleagues for the good times shared together.

Last but not the least, I would like to thank my family, especially my mother, for supporting me throughout my life.

Abstract

Drought is one of the greatest hazards to the sustainable production of wheat (*Triticum aestivum* L.), one of the world's most vital staple foods. Screening of drought-tolerant wheat cultivars is a major priority for breeding programmes. Ten wheat varieties were subjected, at the maturity and seedling stages, to drought conditions of 50% and 25% water field capacity (WFC), respectively. Selection for drought tolerant cultivars was based on a comparative study of the physio-morphological and biochemical traits of the aerial tissues of the samples under drought and optimal conditions (100-85 WFC%). The results showed that the Atlas and ICARDA32331 cultivars were the most sensitive and tolerant genotypes, respectively. These cultivars were subjected to transcriptomic analysis which revealed that they shared 5068 differentially expressed genes (DEGs) and distinguish ICARDA32331 from Atlas cultivars by 8676 and 10284 DEGs, respectively. Gene ontology terms for ICARDA32331 were mainly related to lipid and cell wall metabolism, results coherent with the significant enrichment of fatty acid elongation, cutin, suberine and wax biosynthesis pathway, unlike the Atlas. The latter cultivar showed an enrichment in down-regulated DEGs, mainly associated with photosynthesis and chloroplast organisation. The cultivars ICARDA32331 and Atlas had an over expression of DEGs associated with dehydrin and proline biosynthesis, indicating that they had undergone osmotic adjustment to adapt to drought. A high number of SNPs and InDels were found in this transcriptomic study, which is a consequence of the genetic diversity of the chosen wheat cultivars. This study highlights the role of lipid metabolism and nitric oxide gene in drought tolerance and provides new molecular factors that can be targeted for the drought tolerance breeding programme.

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List of Abbreviations

Abbreviation	Definition
ABA	Abscisic acid
ABA-WDS	ABA-Water Deficit Stress protein
ABRE	ABA-responsive element
ADC1-2	Arginine decarboxylase
AHC	Agglomerative hierarchical clustering
ALN	Allantoinase
ANOVA	Analysis of variance
AREBs/ABFs	ABA-responsive element-binding proteins/factors
ASR	Abscisic acid Stress Ripening protein
ATP	Adenosine triphosphate
Bp	Base pair
BP	Biological process
bZIP	Basic leucine Zipper
CBF	C-repeat-Binding Factor
CC	Cellular component
cDNA	Copy or complementary DNA
Cm	Centimetre
CO2	Carbon dioxide
DEGs	Differentially expressed genes
Df	Degree of Freedom
DHN	Dehydrin
DNA	Deoxyribonucleic acid
DREB	Dehydration responsive element binding
DTE	Drought tolerance efficiency
DW	Dry weight
ERF	Ethylene response factors / ethylene-responsive element-binding factor
F	Statistic ratio of two variants
FC	Field capacity
FDR	False discovery rate
FPKM	Fragments Per Kilobase of transcript per Million

FW	Fresh weight
g	Gram(s)
G+C	Guanine-cytosine content
GMP	Geometric mean productivity
GO	Gene ontology
GPx	Glutathione peroxidase
H2O2	Hydrogen peroxide
HM	Harmonic mean
HO-	Hydroxyl radicals
HSPs	Heat shock proteins
IAA	Indole-3-acetic acid
Indels	Insertion–deletion mutations
JA	Jasmonic acid
KCS	3-ketoacyl-coa Synthetases
KEGG	Kyoto Encyclopedia of Genes and Genomes
LEA	Late embryogenesis abundant
log2	Logarithm base 2
MAPK	Mitogen-activated protein kinase
MD	Mean difference
MF	Molecular function
MGL	Methionine-gamma-lyase
mM	Millimolar
MP	Mean productivity
mRNA	Messenger RNA
MYB	Myeloblastosis
N	Nitrogen
NAD+	nicotinamide adenine dinucleotide
NL	Number of leaves
NOA1	Nitric-oxide synthase
NS	Number of seeds
NT	Number of tillers
P5CS	Pyrroline-5-carboxylate synthetase
PCA	Principal component analysis

PGAHC	Plant genotypes agglomerative hierarchical clustering
PGD6	6-phosphogluconate dehydrogenase
PH	Plant height
PPT	Podophyllotoxin
RC	Relative change
RIN	RNA integrity number
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSI	Relative stress index
RSN	Reduction of Seeds Number
RSW	Reduction of seed weight
RWC	Relative water content
SA	Salicyclic acid
SD	Standard deviation
SE	Standard error
SeW	Seed weight
SL	Spike lenght
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SSI	Stress susceptibility index
STI	Stress tolerance index
SW	Spike weight
TF	Transcription factor
TOL	Tolerance index
USP	Universal stress protein
VOCs	Volatile organic compounds
WFC	Water field capacity
YI	Yield index
Yp	Yields of relative water content under optimal conditions
Ys	Yields of relative water content under drought stress conditions
YSI	Yield stability index
ZFP	Zinc finger proteins.

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Chapter 1. General introduction

1.1. Importance of wheat for the future food production

Bread wheat, *Triticum aestivum* L, is originally from southwest Asia and is one of world's most commercially important crops after maize. It is cultivated in a wide range of geographical locations, from northern Finland, Norway, and Russia to southern Argentina, in sub-tropical and tropical habitats (Feldman 2001; Levy and Feldman 2022). Asia, mainly China and India, were the top wheat producers in 2020-2021 followed by Europe, the Americas, Oceania and Africa (FAO 2021). The variation in wheat production over the last 5 years has been marked by climatic changes. According to Mäkinen et al. (2018), the increase in temperature, solar radiation and wind speed in the EU have a negative effect on wheat yield.

The production of wheat reached ~ 770 million tonnes in 2020-2021(FAO, 2021); a value in line with its high consumption by a third of the global population. Wheat is the world's leading cereal crop, both in terms of area and production. It has a high nutritional value and is rich in carbohydrates (60–80%), crude fiber (2.2%), lipids (1.5–2%), minerals (1.5–2%), protein (8–17%), Gluten (75–85% of the total protein), vitamins (B-complex and E), and several essential amino acids (Shewry and Hey 2015). It plays an important role in caloric daily intake worldwide (eg. 20% in the UK, 40%-50% Turkey and Egypt) (Shewry and Hey 2015) and is considered as one of the staple foods of humanity. The top five wheat exporting countries are Russia, Australia, the United States, Canada and Ukraine, according to FAO (2021). With the growth of the human population, expected to exceed 9 billion in 2050 (FAO 2021), demand for wheat is set to increase by 60%, requiring an uplift in average annual yield of at least 1.6% rather than the estimated 1% (Lucas 2012). However, wheat production has not increased over the last 10 years due to environmental and climatic constraints.

Wheat production is threatened by drought, which is the most damaging abiotic stress, particularly in rainfed conditions, and which has already affected 50% of the production area in developing countries (Ashraf 2010). In addition, drought stress is expected to intensify in the near future due to climate warming (Yu et al. 2017) and as a consequence, a decline in wheat production is anticipated. This problem calls for greater attention and the search for a better strategy to overcome it. In this context, breeding strategies have been successfully used and applied to develop high drought-tolerant wheat varieties.

1.2. Taxonomic and genomic features of bread wheat, *Triticum aestivum* L

1.2.1. Taxonomic and genomic features of wheat

There are different types of wheat, depending on growing season and geographical location. In South Asia and the middle East, wheat can be harvested in late summer or autumn and is known as spring wheat, while winter wheat is sown between September to November in the northern hemisphere and harvested at the same time as the spring wheat (Curtis 2002). Wheat can be grown and collected at any time of year in regions where the temperature is between 25°C and 32°C (latitudes of 30° and 60° North and 27° and 40° South), while in temperate areas, wheat is ready for harvest between April and September and October-January in the Northern and southern hemispheres, respectively (Nuttonson 1955).

Taxonomically speaking, *Triticum aestivum* L is a hexaploid species belonging to the genus *Triticum* in the grass family *Poaceae* and order *Poales*. This current wheat species is derived from hybridization between diploid wild goatgrass (DD, *Aegilops tauschii*) and tetraploid wheat (AABB, *Triticum dicoccoides*) (Brenchley et al. 2012; Vu et al. 2017). MacKey (1954) classification assigned the following five domesticated subspecies to the *Triticum aestivum* species*:* ae*stivum, spelta, macha*, *compactum*, and *sphaerococcum* ssp. Another semi-wild form, non-domesticated, subspecies, named *tibetanum, has also been* described (Shao et al. 1983; Levy and Feldamn 2022). All these subspecies are referred to as bread wheat and are locally cultivated. *Triticum aestivum* subsp *aestivum* is of global importance and its genome consists of 21 chromosome pairs, genome BBAADD, $2n = 6x = 42$ (Sears 1952) with 16,000 Mbp (IWGS 2018).

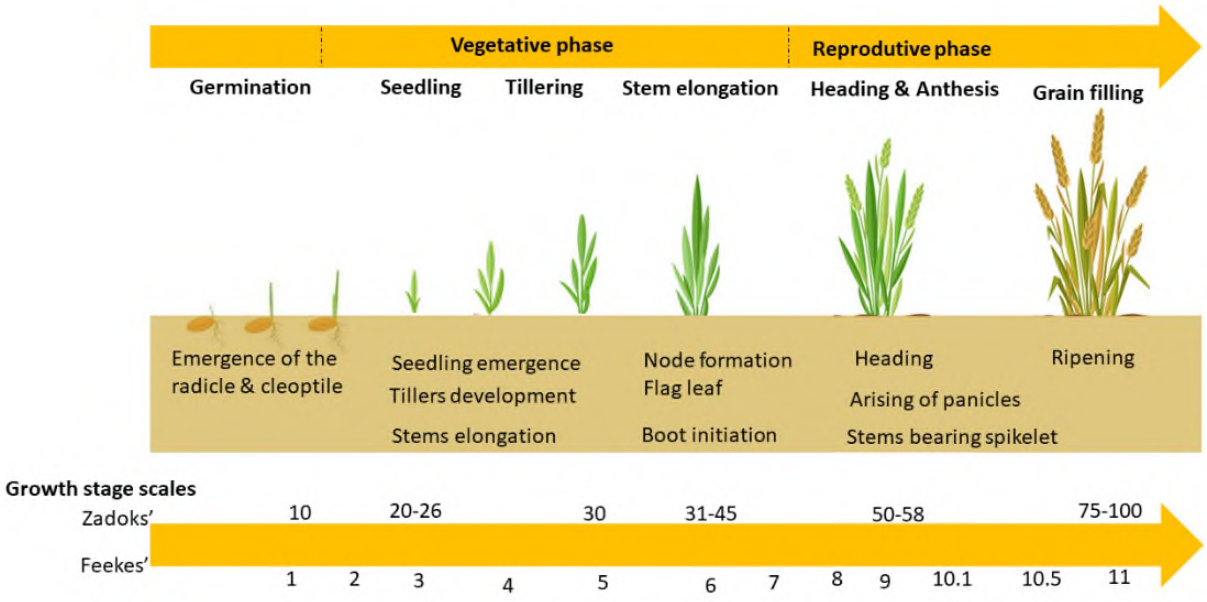
1.2.2. Morphological features and growth stages of wheat

The wheat plant has a height of 2.5-5 feet and its morphology is made up of roots, stems, leaves, inflorescences, and fruits. Wheat has been shown to have two types of roots, primary and secondary, known as seminal and coronal roots, respectively. Primary roots rise from the embryo, while secondary ones grow from the lower nodes. The wheat stem, also known as culm, is formed of nodes and internodes, with a marked variation in length between the lower and upper sides of the shoot. From the underground axillary buds, secondary shoots arise along with the first shoot and then the same growth process is repeated to generate more shoots from the underground buds of the previous shoots. This growth pattern of shoot is named tillering.

The leaves are distributed alternately on the stem and have two auricles called the leaf sheath, which are present on the basal part of the leaf. The wheat leaf is characterised by the presence of leaf lamina and ligule. The ligule is located at the junction of the leaves and features hairy outgrowths. The wheat spike or inflorescence is composed of spikelets that are enclosed in glumes, from which 1 to 5 florets are born, as shown in figure 1.1. The fruit is a single-seeded caryopsis. The kernel (grain) has a tuft of hairs at the tip, a smooth and convex dorsal side and crease ventral side.

To study the impact of drought in wheat, it is crucial to understand its different growth stages, of which there are two classifications known as the Feekes (Large 1945) and Zadoks (Zadoks et al. 1974) scales. Both are based on leaf development and grain filling, but more details on the vegetative phase are provided by Zadoks (Fig 1.1).

(a)



(b)

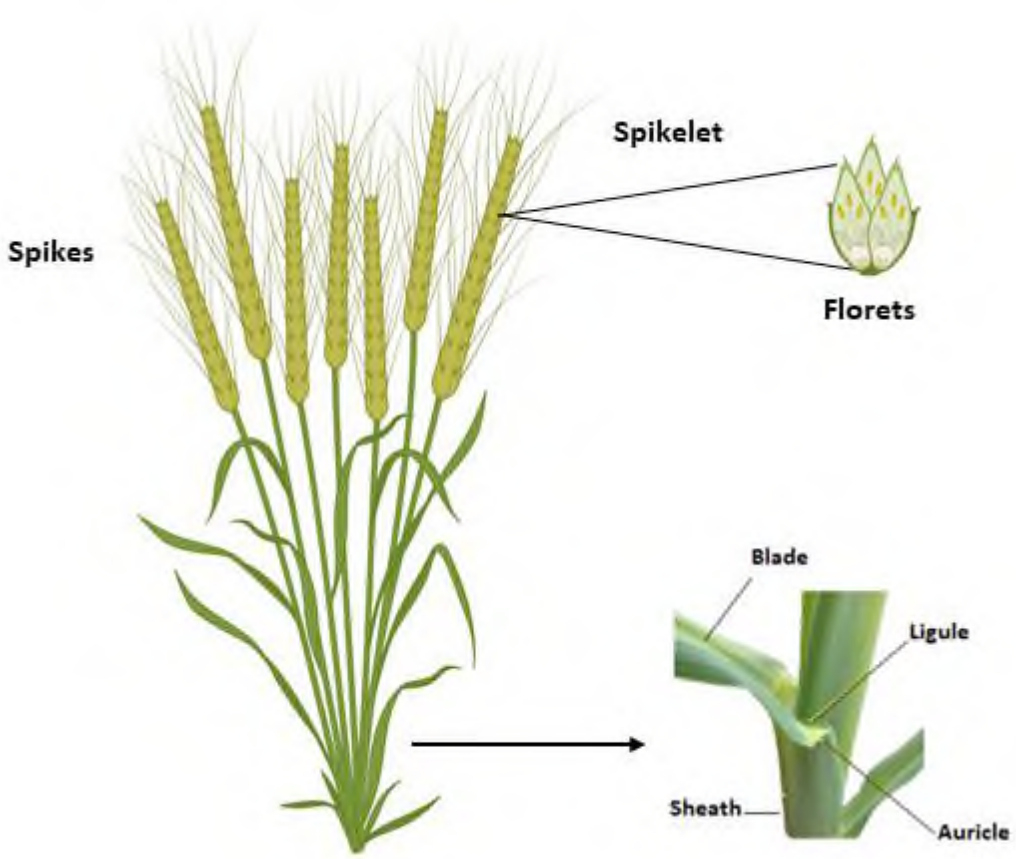


Figure 1.1. (a) Different growth stages of wheat and the Feekes and Zadoks scales (modified version of Large (1954) and Zadoks et al. (1974); **(b)** plant anatomy.

Wheat undergoes five different growth stages: leaf and tiller development, stem elongation or jointing stage, booting stage, heading and flowering, and grain filling and ripening or maturity stage. Germination is marked by the emergence of the radicle and coleoptile, and ends up with the development of the first leaf in the coleoptile. Then, the vegetative phase starts with the emergence of the seedling from the soil surface, accompanied by 4-5 leaves and the development of tillers, and ends with stem-elongation. During this last phase, three visible nodes, the flag leaf and the flag leaf collar develop, along with an initiation of booting. Next, the reproductive phase begins with heading and anthesis, with the arising of panicles from stems bearing spikelet. The head is completely emerged, followed by flowering and pollination, which can be easily noted by the appearance of anthers from the spikelet. The grain-filling stage is marked by milk development and loss of green color as shown in Figure 1.1.

1.3. Effect of drought stress on wheat production

Bread wheat is the second cultivated crop produced under rainfed conditions after maize and the second irrigated crop after rice. A value of 450-650 mm of precipitation is required by wheat during the growth phase (Mehraban et al. 2019), which is well below the annual water precipitation in arid and semi-arid regions of the world.

Global warming and climate change are exacerbating the effects of drought stress on agriculture and, consequently on future food supply. Drought is the shortage of available water over a long period and, unfortunately, it is unpredictable because it depends on rainfall and its distribution, the capacity of the soil to store water and evaporative demand (Wery et al. 1994). Drought stress occurs when the plant's demand for water cannot be met and the level of transpired water required by the plant and that absorbed by the roots are unbalanced. This lack in water is caused by an insufficient precipitation, and a low ground water level, etc. (Lambers et al. 2008). Plant performance in the presence of a long period of drought stress is genotype-dependent and depends on the intensity and duration of stress, growth stage and other factors such as soil fertility (Hazrati et al. 2017; Laxa et al. 2019). For this reason, crop varieties should be selected according to the local climate to better manage drought stress (Daryanto et al. 2017)

1.3.1. Effect of drought stress on morphological features of wheat

The foremost effect of drought stress on wheat has been observed at all growth stages, but mainly at the germination and early seedling growth stage, during which wheat is most sensitive compared with other crops. It has been shown that flowering and seed filling are highly sensitive to environmental conditions (Ma et al. 2017). The flag leaf is considered as the major assimilator of CO_2, since it is involved in up to 50% of wheat assimilation during the grain development stage (Evans 1996). Drought during the early grain development phase reduces grain filling time and thus weight (Saini and Westgate 1999). Water shortage during the grain-filling stage leads to early senescence of the wheat plant and a shortness in the duration of grain filling (Plaut et al. 2004). Drought treatment at the final growth stage leads to a low grain number and consequently negatively affects wheat yield (Dolferus et al. 2011) for most wheat cultivars. However, some drought tolerant cultivars increase the number of seeds under drought, but the seeds are much smaller than under well watered conditions. It has been reported that plants exposed to drought stress before the anthesis stage acquire higher stress tolerance (Wang et al. 2014).

The negative impact of drought on wheat has been manifested by a reduction in yield components, as for example single grain weight, number of spikes and grains, dry matter partitioning, number of plants per unit area (Farooq et al 2009, Hossain et al. 2012, Francia et al 2013). It has been reported that the number of ear heads per square meter, kernel weight and kernel per ear are reduced when drought occurs before the anthesis stage (Guttieri et al. 2001, Farnia and Tork, 2015). Moreover, drought causes a significant decrease in leaf size, plant and shoot/spike length, leaf and stem size, and root extension (Lonbani and Arzani 2011). The root system is one of the plant's sensors for water availability in the soil, which explains the correlation between a well-developed root system and the resistance characteristic of wheat (Wasson et al. 2012). Increased root system growth is accompanied by a decrease in shoot size under water stress conditions. Gregorová et al. (2015) showed that the dry weight and shoot length decreased in wheat under drought stress. However, moderate and high drought stress leads to a reduction in the root system and plant height, but little is known about the metabolic response of wheat to drought stress at cellular and whole plant levels (Fromm and Fichman 2019). Some traits such as the root system and cell membrane stability have been used for evaluating the drought tolerance phenotype of several crops (Ali et al. 2009; Sallam et al. 2019).

1.3.2. Effects of drought stress on physiological features of wheat

Drought stress on the wheat crop has resulted in a 40% yield loss globally, which inhibits CO_2 uptake through stomata and consequently affects respiration and photosynthesis (Daryanto et al. 2016; Zampieri et al. 2017). Photosynthesis is the crucial and fundamental function of plants and a vital process for life on earth. During this process, the plant uses the sunlight energy to transform water and CO_2 into oxygen and sugars. There is a positive correlation between photosynthesis and maximal crop production (Makino 2011; Tankari et al. 2019). Water deficit leads to a significant decrease in cell division and enlargement, alters stomatal oscillations and, consequently, negatively affects photosynthetic activity and crop productivity (Li et al. 2009; Kumawat and Sharma 2018; Mehraban et al. 2019). Photosynthesis is a complex machinery made from different enzymes and proteins and carried out in chloroplasts. Decreasing soil water content or relative leaf water content induces stomal closure, which can also occur in the presence of other parameters such as low humidity or the production of abscisic acid, a phytohormone synthesised following drought stress (Fahad et al. 2017; Camaille et al. 2021). Stomatal restriction causes significant decrease of CO_2 influx and is associated with limiting stomatal (g_s) and /or mesophyll (g_m) conductance to CO_2 (Chaves et al. 2009; Galmés et al

2013; Camaille et al. 2021). Drought stress decreases the Rubisco activities, resulting in reduced photosynthesis (Friso et al. 2004; Gómez-Bellot et al. 2020). Carmo-silca et al. (2010) have shown that these physiological changes, stomatal and non-stomatal limitations, can be induced during photosynthesis. The effects of drought stress on photosynthesis differ from one growth stage of wheat to another. It reduces the carbon assimilation rate at the flag leaf stage and consequently affects wheat height and yield (Frakas et al. 2020; Zhao et al. 2020). Intense and excessive light energy damages photochemical efficiency and leads to photoinhibition, which is enhanced when the amount of absorbed light by the plant is greater than the rate required in the chloroplasts (Giacometti and Morosinotto 2013). Excess light excitation has been shown to be responsible for the overproduction of reactive oxygen species (ROS) (Dat et al. 2000; Reddy and Raghavendra 2006). Normally, the plant converts light energy into electrons to produce the chemical energy, ATP and NADPH, needed to fix CO_2 in the Calvin cycle (Camaille et al. 2021). However, water shortage limits ATP/NADPH use due to reduced CO_2 availability, and leads to chlorophyll photo-oxidation (Farooq et al. 2014; Vurukonda et al. 2016; Keyvan 2010). Consequently, the photosynthesis mechanism is altered (Good and Zaplachinski 1994; Cramer et al. 2011).

Moreover, water shortage in wheat inhibits meiosis in the ovule and, consequently, unsuccessful reproduction during the flowering stage (Saini et al. 1997; Saini and Westgate 1999). Persistent drought perturbs plant's water and mineral nutrition systems and results in a high concentration of abscisic acid, which not only has a negative impact on photosynthesis and chlorophyll content in wheat, particularly at the flag leaf stage, but also leads to wheat pollen sterility (Farooq et al. 2014; Dong et al. 2017). The increased viscosity of plant cellular contents due to drought stress can harm the enzymatic machinery of the plant. Reduced water flux in the xylem correlates with decreased turgor, which negatively affects mitosis, cell division and cell expansion. This results in inhibition of plant growth and reduced yields.

Sheoran et al. (2015) showed that drought stress in winter wheat leads to a significant decrease of nitrogen use efficiency. Consequently, a significant reduction in total protein content in plant tissue has been reported and considered as a physiological indicator of a drought stress sensitive phenotype (Nagy et al, 2013). Several indicators of drought stress tolerant and sensitive genotypes have been reported, including relative leaf water content, proline content and accumulation of soluble sugars in leaves (Qayyum et al. 2011; Soltys-Kalina et al. 2016).

1.3.3. Effects of drought stress on wheat at the molecular level

As mentioned above, drought stress in wheat has a negative effect on photosynthesis at the molecular level. The decrease of intercellular CO_2 concentration causes an interruption in the electron transport chain of photosynthesis due to a reduction in $NADPH^+$ comsumption in the Calvin cycle. The electrons are transferred to oxygen generating ROS (Mehla et al. 2017; Hasanuzzaman et al. 2019; Laxa et al. 2019; Sharma, et al. 2020). This conversion can go up to 50% in wheat under drought stress (Biehler and Fock 1996). ROS manifest as hydrogen peroxide (H_2O_2), hydroxyl radicals (HO-), or superoxide radical ($O2•−$), which can damage cell components, including membrane lipids, proteins and DNA, when over-accumulated (Kasim et al. 2013; Raja et al. 2017), although they are produced as part of normal plant metabolism by chloroplasts, mitochondria, peroxisomes, apoplast, and plasma membranes (Singh et al. 2019).

Osmotic stress generated by drought and salt, stimulates expression of the gene encoding *TaMYB33* mediated by ABA production, conferring some level of resistance in wheat under severe water limited conditions and high salinity (Qin et al. 2012). More genes were found to be expressed in wheat under water –limited and salt stress conditions such as *TaWLIP19* and *TaFBA1* (Baloglu et al. 2014; Zhou et al. 2014, 2015). More metabolites and proteins were found to be highly expressed under drought stress to protect plant cells, such as the cell wall protein, Expansins. The latter is involved in cell wall loosening during the cell elongation stage and is affected by drought stress mainly during leaf development (Zhou et al. 2015). Other genes have been found to be expressed in wheat under drought stress, such as genes associated with abscisic acid (ABA) pathways, and ABA-independant signal transduction pathways (Shinozaki and Yamaguchi-Shinozaki 1997). These genes, their products and the pathways to which they are affiliated are detailed in the regulatory mechanisms section listed below (see section 1.5).

1.3.3.a. Primarily transcriptional responses

Several functional proteins are known to be involved in the plant defence mechanism against drought stress, such as LEA (late embryogenesis-abundant) proteins, chaperones, osmolyte producing enzymes, and water channel proteins, while specific regulatory proteins such as kinases, phosphatases or calmodulin-binding proteins are only produced under drought stress (Kaushal et al. 2016).

The dehydrin (DHN) gene of in wheat is expressed under drought stress and its product contributes to cell dehydration by binding to the partly dehydrated protein in order to protect them from denaturation. As a result, these proteins may have ROS properties. Expression of the DHN gene is often associated with ABA, which is why they are often referred to as RAB proteins (response to ABA). Other specific proteins, such as aquaporin and acetyl-CoA carboxylase, are produced under drought stress conditions with ABA as an inducer (Chaves et al. 2003; Keskin et al. 2010). Heat shock proteins (HSPs) are also involved in response to abiotic stress and are produced under heat and drought stress conditions. The main role of HSPs, which are chaperones, is to maintain protein function and prevent them from misfolding (Kaushal et. 2016). HSPs belong to a large family which contains different types of HSPs as for example Hsp70, which participates in the protein folding, while Hsp100 chaperones are involved in removing denaturated proteins (Wang et al. 2004). However, drought stress in wheat induces Hsp17.8 gene expression up to 3-fold (Kasim et al. 2013).

Volatile organic compounds (VOCs; eg. Isoprenoids, terpenoids, ethylene etc) are produced by plant leaves under several different stress conditions, including drought. They function as volatile signals between plants to reinforce the plant's tolerance to stress via various mechanisms such as chloroplastic thylakoid stabilization. The isoprenoid was found to have several functions, including acting as an antioxidant, maintaining the ROS and lipid peroxidation level, and increasing the adhesive force between the lipid tails of the plant (Possell et al. 2013). However, the release of volatile organic compounds by the plant under stress conditions can interfere with photosynthetic carbon fixation, reducing the growth of the non–stressed neighbouring plant. The production of volatile organic compounds has also been reported in wheat under drought stress and the rate of induced compounds is associated with drought intensity (Timmusk et al. 2014).

1.4. Defence mechanisms against drought stress

To overcome drought stress, plants possess different resistance mechanisms that include morphological, and physiological adaptation (Chaves et al. 2011) with the attempt to respond quickly to avoid cell turgor pressure reduction, which is the pressure inside the cell that presses the plasma membrane into the cell wall (Geilfus 2019) (Figure 1.2). The ability to do this depends on plant genotype/varieties such as a well-developed root system that helps water uptake from a deeper soil level (Du et al. 2020) and/or biochemical ability to synthesise antioxidants, osmolytes, and aquaporins. The latter trait may be associated with the

physiological traits of certain plants for improved water use efficiency and rapid stomatal closure (Farooq et al. 2009). In addition, plants can defend themselves against drought stress due in part to their antioxidant system, which includes several enzymatic (eg. catalase, peroxidase, superoxide dismutase, ascorbate) and non-enzymatic antioxidants (eg. glutathione, flavonoids and alpha-tocopherol). These control and limit the damage caused by high ROS levels (Burke and Mahan 1991).

1.4.1. Escape

This mechanism is known as drought escape, during which the plant attempts to complete its life cycle before water limitation intensifies (Izanloo et al. 2008). This strategy can be adopted by plants with a drought-sensitive phenotype in arid and semi-arid countries and in plants that complete their life cycle at the beginning of March in temperate climates. This escape strategy is also observed in plants that are dormant in late autumn to avoid low winter temperature (Basu et al. 2017).

1.4.2. Avoidance

The stress avoidance strategy is observed when the effect of the stressor is weakened or retarded by the plant. Drought avoidance occurs when plants make some biological adjustments including, reduced leaf expansion and leaf rolling, reduction of stomatal cavity and evapotranspiration (Goufo et al. 2017; Yu et al. 2017).

1.4.3. Tolerance

The drought tolerance mechanism involves osmotic adjustments and an increase in cell wall elasticity (Yi et al. 2016). This mechanism includes a complex network harbouring several transcriptional factors, regulatory proteins, chromatin modifiers, activators and repressors to cope with drought stress (Duma et al. 2022).

1.4.4. Resistance

The resistant genotype is characterised by the plant's ability to either adapt or acclimate through the activation of different coping mechanisms related to stress escape, avoidance and tolerance. Drought resistance involves the activation and augmentation of several antioxidant

metabolic pathways (Reddy et al. 2004). These mechanisms can be initiated by the plants individually or in combination.

Stress avoidance and stress tolerance are known as forms of biological resistance because the plant try to survive environmental stress by adjusting their physiological and molecular characteristics. Agricultural resistance refers to the ability of the crop to cope with abiotic stress due to the activation of defence mechanisms for maintaining stable and good quality yields (Bandurska 2022). More detailed descriptions about the regulatory mechanisms that wheat can adopt to cope with the drought stress are described below (see section 1.6).

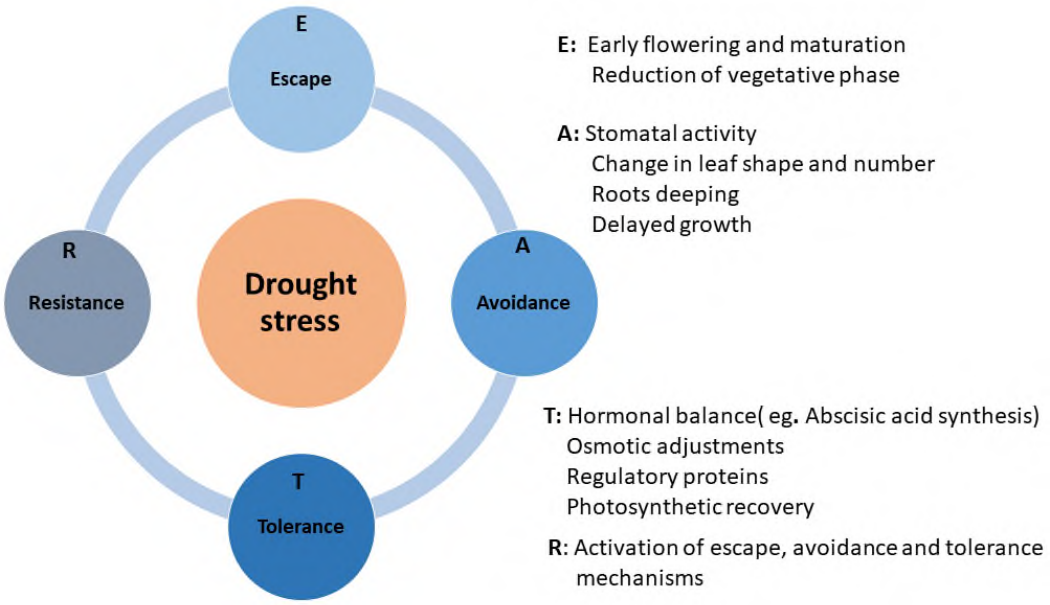


Figure 1.2. Defence mechanisms of wheat against drought stress

1.5. Regulatory mechanisms involved in drought stress

1.5.1. Osmotic balance

Plants are able to adjust and maintain their osmolarity and pH at an adequate level by diminishing their water demands and producing osmolytes such as glycine betaine, proline, polyamines, polyols, and sugars. The latter are soluble molecules that promote water retention and decrease the plant's water potential without affecting the normal metabolism (Farooq et al. 2014). The osmolarity adjustment that is necessary to avoid dehydration involves other elements such amino acids, which are very helpful for stabilising the cell structure of the plant (Lösel 1995).

It has been shown that soluble sugars increase by 80% in wheat after a period of persistent drought lasting 7 days (Gontia-Mishra et al. 2016; Blum 2017). Starch is also considered a stress tolerance enhancing molecule, as its degradation by stress-activated β-amylase1 in the presence of light generates sugar-derived osmolytes (Thalmann et al. 2016). An increase of up to 90% in proline concentration was detected in wheat after 7 days under water deficit conditions (Gontia-Mishra et al. 2016). Vendruscolo et al. (2007) reported that the level of proline in leaf dry weight increases from 1 μmol·g−1 (control group) to 11 μmol·g−1 (samples under drought stress) after 15 days of water stress. The major contribution of the accumulation of proline is towards cellular function and organ protection, while its role in osmolarity stabilisation is minor (Blum 2017). Other amino acids are also increased during drought stress as a result of degradation of proteins, which is an important reaction for osmolarity balance and necessary for *de novo* amino acids biosynthesis (Kaushal and Wani 2016; Blum 2017). Overproduction of glycine betaine has a positive effect on photosynthesis by stabilising cell osmolarity, though its contribution to wheat stress tolerance is more linked to its role in the antioxidant defence mechanism (Wang et al. 2010; Huseynovaet al. 2016).

1.5.2. Oxidative status

Several physiological changes induced by drought stress lead to an increase in ROS production. To overcome this oxidative stress, wheat activates the synthesis of enzymatic (eg. peroxidase, ascorbate peroxidase, catalase, superoxide dismutase (SOD), glutathione reductase and peroxidase) and non-enzymatic antioxidants (eg. ascorbate, carotenoids, glycine betaine, glutathione, flavonoids, and alpha-tocopherol) (Burke and Mahan 1991; Chakraborty et al.

2013; Nadeem et al 2014). The expression of antioxidants in wheat occurs during drought stress and depends on the intensity and duration of the stress as well as the growth stage (Chakraborty et al. 2013). These antioxidants are the key molecules for ROS reduction and enhance drought tolerance by protecting cell components. It has been shown in several species that overproduction of α-tocopherol protects thylakoids, the inner membrane of chloroplasts, against drought stress (Keleş and Önce 2002; Munné-Bosch 2005) (Figure 1.3).

1.5.3. Hormonal effects

Phytohormones play an important role in the growth, development and proliferation of plant tissues (Figure 1.3). They are also involved in the adaptation or regulation of plant growth to a particular condition, such as drought stress. Among the most known phytohormones that improve stress tolerance are abscisic acid, auxin, brassinosteroid, cytokinin, ethylene, jasmonic acid, and salicyclic acid (Peleg and Blumwald 2011). Abscisic acid is one of the first hormones produced by dehydrated wheat roots and is then distributed throughout the whole plant via the xylem, "alarming" water scarcity in the plant (Zhang et al. 2006). A high level of this phytohormone promotes root development and restrains shoot growth (Sharp et al. 2004). In addition, it better controls stomatal closure to prevent further plant dehydration (Farooq et al. 2014). In the presence of light energy, abscisic acid induces starch degradation to help the plant cope with osmotic/drought stress (Thalmann et al. 2016).

Unlike abscisic acid, cytokinins promote wheat growth by boosting photosynthesis and chlorophyll levels at the flag leaf stage; an effect that is inkeeping with its ability to stimulate stomatal opening (Chaves et al. 2003; Farooq et al. 2014). Rivero et al. (2007) and Gujjar and Supaibulwatana (2019) showed that high cytokinin concentrations in transgenic plants delayed plant senescence under drought stress. At the molecular and metabolic level, over production of cytokinin (i) enhances cell division via sugar signalling, which implies a decreased phloem-load and (ii) induces expression of the gene encoding cell wall-associated invertase, an enzyme that breaks down sucrose into glucose and fructore (Rijavec et al. 2009). In addition to the significant role of cytokinin in plant adaptation to drought, via the phosphorelay pathway (Nagar et al. 2015), it also improves nitrogen metabolism in plants (Li et al. 2016). However, cytokinin may also have negative impacts on drought resistance in plants, as an *Arabidospis* mutant with low cytokinin levels has been shown to be more resistant to drought stress than the wild type (Nishiyama et al. 2011).

Brassinosteroids have been found to increase leaf area, relative water content, chlorophyll content and wheat biomass production under moisture stress conditions (Sairam 1994). Moreover, they induce the expression of several stress-related genes that encode for antioxidant enzymes (eg. catalase and peroxidase, superoxide dismutase, etc.). Brassinosteroids activate osmolyte production (eg. proline, soluble sugars, etc.) to maintain photosynthesis so that the plant can cope with drought stress (Li et al. 2008; Peleg and Blumwald 2011).

Ethylene is known for its ability to promote plant growth by enhancing rhizobia nodulation, root proliferation, leaves, flowers and fruit tissue development. Under drought stress, ethylene is overproduced up to a concentration of 25 g/l, above which it becomes toxic to the plant and causes chlorosis, leaf abscission and senescence, following the decline of root and shoot growth (Apelbaum, 1981; Singh, et al. 2015). In wheat, the amount of ethylene is doubled under severe drought stress and leads to a decrease in grain filling rate and final grain weight (Yang et al. 2006). Like other phytohormones, several drought stress related genes are induced by ethylene due to a specific transcriptional factor called ethylene response binding proteins (ERF) (Fujimoto et al 2000). The interaction between ethylene and auxin has been found to promote root system development.

Auxins are known for their rhizogenesis role by increasing cell division and boosting root architecture, which is considered the key target feature for drought tolerance genotype selection (Kohli et al. 2013; Ngumbi and Kloepper 2016; Kulkarni et al. 2017). In this case, auxin modulates root hydraulic traits at the molecular level via the expression of water-saving traits and, consequently, better yield under drought conditions (Sadok and Schoppach 2019). However, auxin levels are reduced by more than 30% under drought stress in wheat due to the > 2-fold upregulation of a transcriptional repressor associated AUX/IAA1 gene (Barnawal, et al. 2017).

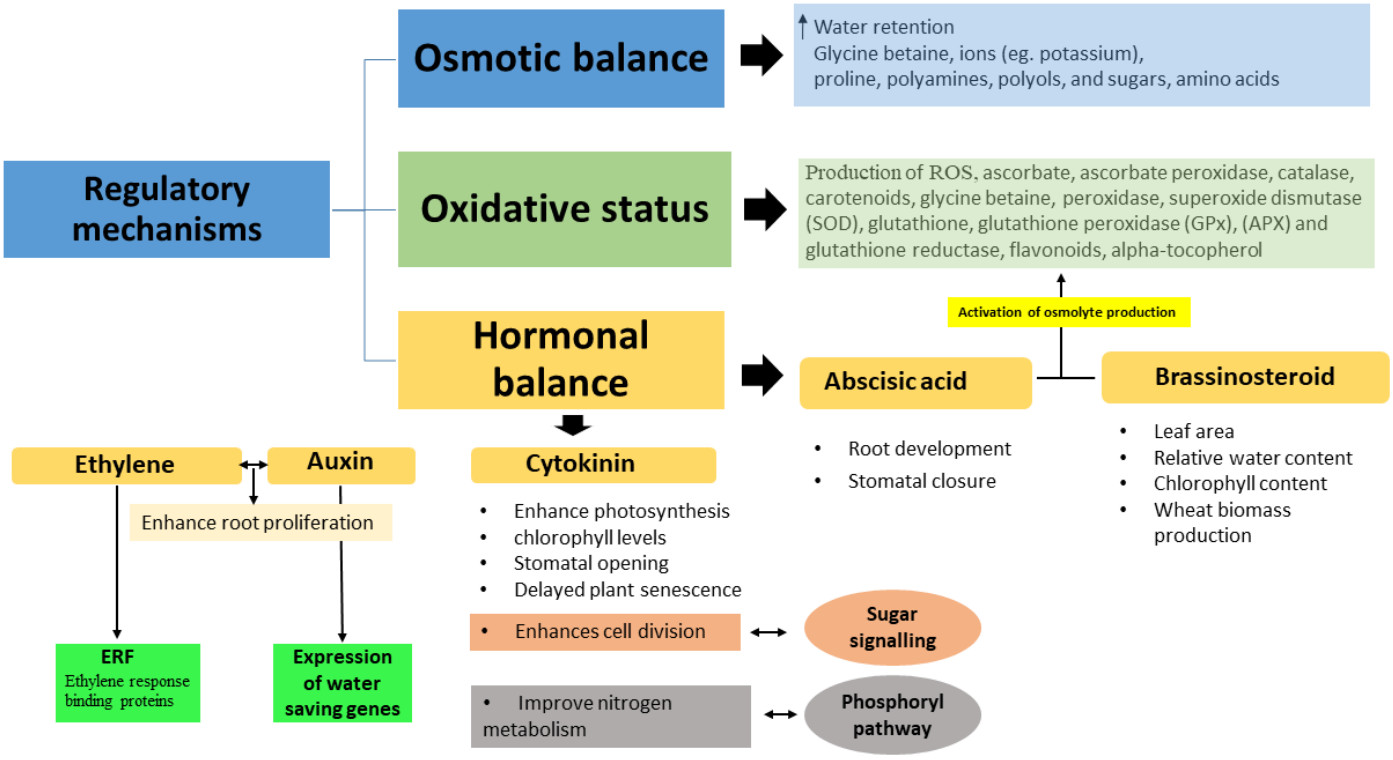


Figure 1.3. Regulatory mechanism of wheat against drought stress

1.6. Transcriptional factors of regulatory mechanisms

Drought stress induces the activation of adaptive mechanisms that include gene expression and regulation of several transcriptional factors associated with morphological and physiological properties (Figure 4). Root architecture and stomatal features are important for water extraction from soil and retention, which explains their morphological and physiological changes to adapt to drought stress. Adaptation to drought stress involves a network of transcription regulators that control and regulate root and transpiration efficiency. Among the well-studied transcription factor families that have been identified as enhancers of drought tolerance in maize, rice, wheat and/or Arabidopsis are: bHLH, bZIP, dehydration responsive element binding (DREB), ethylene response factors (ERF), HD-ZIP, MYB, NAC, WRKY and ZFP (zinc finger proteins). As mentioned above, ABA is the first hormone produced as an indicator for the plant of water-limiting conditions, in addition to its role in modulating stomatal opening. ABA induces the expression of several genes related to drought stress such as oxidative stress related gene, a gene involved in decreasing transpiration, genes encoding enzyme or proteins associated with stress tolerance and other genes involved in carbohydrate metabolism (Urano

et al. 2009; Böhmer and Schroeder 2011). However, expression of several drought stress related genes is not influenced by ABA, signifying that there is another metabolic pathway for drought-inducible genes, independent of ABA, which is also important for drought tolerance in plants. Both ABA dependant and independent pathways are activated by drought stress and lead to the production of transcriptional factors that belong to different protein families. There is a crosstalk between these two pathways for modulating drought-inducible genes.

1.6.1. Abscisic acid dependent pathway

Several studies have demonstrated the production of the transcriptional factors MYC and MYB following the accumulation of ABA under water-restricted condition. It should be noted that the Myc factor is synthesised prior to the induction of the ABA pathway, as it requires binding of Myc to the ABA-responsive element (Zhang et al. 2006). Other gene products detected in response to the ABA signal are a cis regulatory element, ABRE (ABA-responsive element) functioning as an ABA-mediated signal. Overproduction of ABA-responsive element-binding proteins/factors (AREBs/ABFs) in transgenic plants enhanced the sensitivity to ABA and consequently drought tolerance (Yoshida et al. 2015). Moreover, transcriptional factors AREBs/ABFs associated with bZIP (basic leucine Zipper) subfamily have been found to act as up-regulators during drought stress. A molecular study showed that there is an interaction between AREBs/ABFs and DREB2A (dehydration responsive element binding proteins binding protein = DREB) highlighting the existence of crosstalk between the two ABA pathways (Singh and laxmi 2015).

Other genes have also been shown to be expressed in response to ABA and their products WRKY18 and WRKY40 attach to W-box. These transcriptional factors are found in the nuclei and are then exported to the chloroplast where they interact with the H subunit of magnesium-protoporphyrin IX chelatase (Mg-ProtoIX chelatase-H) to trigger the negative impact of drought stress (Shang et al. 2010). A negative regulator of ABA, PP2C, inactivates the protein kinases 2 (SnRK2s) of the subclass III SNF 1, which phosphorylate AREB/ABF and consequently trigger AREB/ABF regulatory gene. This SnRK2s subclass III contributes to stomatal closure and links the ABA dependant and independent pathways by controlling AREB/ABFs and DREB expression (Mustilli et al. 2002). Protein/transcription factors are not the only ones capable of inducing the ABA pathway, which can also be initiated following binding of the transcription factor (bZIP) to the ABRE (ABA response element) in the

promotor domain after activation/modification by an ABA–activated protein (kinase) (Zhang et al. 2006).

1.6.2. Abscisic acid independent pathway

Three hormones are stimulators of this network, gibberellin, jasmonic acid and salicyclic acid. Two cis regulatory elements, DRE and CRT, and the transcriptional factors DREB or CBF are involved in this mechanism. DREB1, CBF and DREB2 are a part of the ethylene-responsive element-binding factor (ERF) family, having the DNA binding motif, AP2/ERF (Liu et al. 2018). Others factors appear to be implicated in the activation or degradation of the DREB2.

The transcriptional factors MYB/MYC and WRKY detected in the ABA dependent network regulate the expression of drought-associated genes via an ABA-independent signalling network (Abe et al. 1997). Moreover, a molecular study showed that NAC transcription factors are essential for both ABA pathways listed above to modulate gene expression under drought stress conditions (Hu et al. 2006). Xu et al. (2013) showed that the ANAC096 gene product interacts with ABF2 and ABF4 to modulate drought related gene expression.

1.6.3. Lipids and cell membrane stability

The cell membrane is the first cellular structure affected by drought stress due to the oxidative reactions of ROS and its impact promoting lipid peroxidation. Cell membrane integrity and stability are important physiological markers for selecting tolerant genotypes (Bajji et al. 2002; Barnabás et al. 2008). Several molecules limit damage to the cell membrane, such as tocopherols, osmolytes, LEA proteins, polyamines (Bouchereau et al. 1999; Reddy et al. 2004). Zang et al. (2017) showed that levels of the plastid outer envelope protein, TaOEP16-2, in wheat was higher under heat and drought stress conditions. TaOEP16-2 was found to increase the rate of survival, improve cell membrane stability and raise sucrose content, thus enhancing heat and drought tolerance in transgenic *Arabidopsis* plants (Zang et al. 2017).

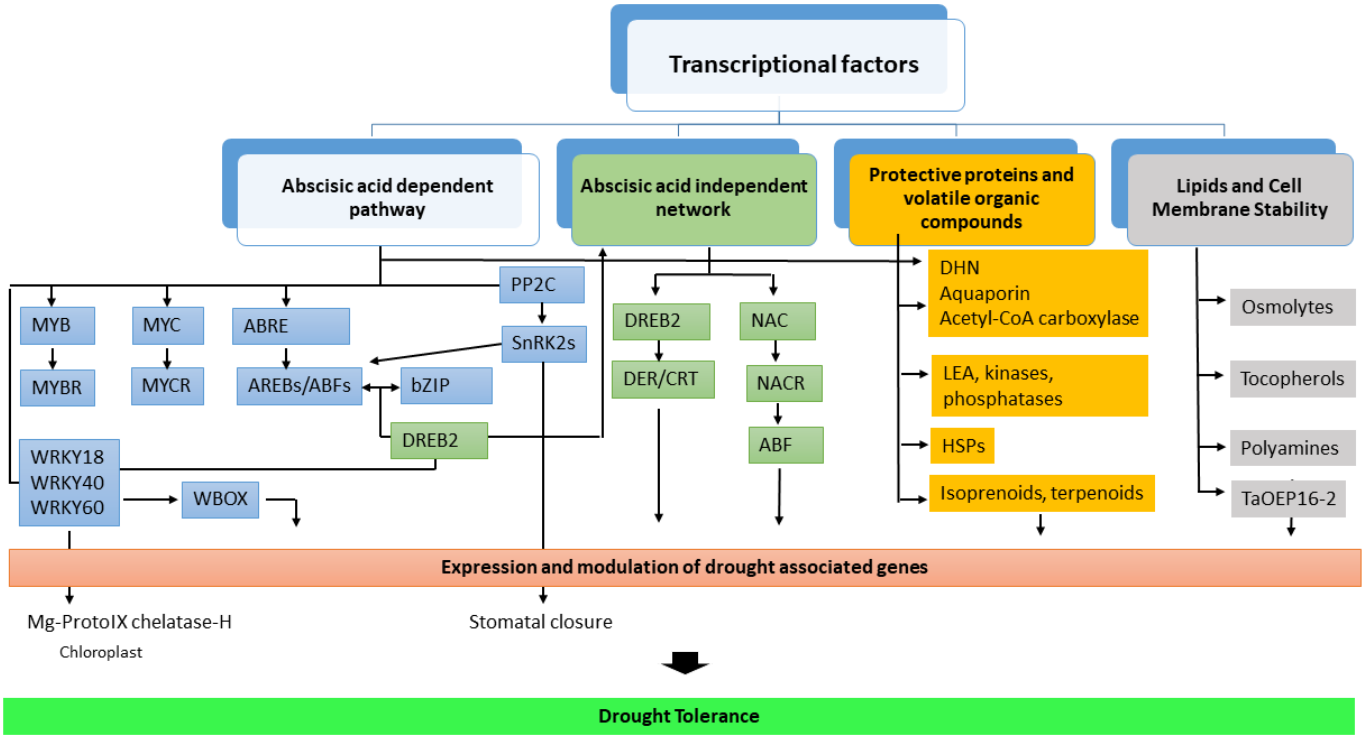


Figure 1.4. Transcriptional factor network for regulatory mechanism of wheat against drought stress.

1.7. Transcriptomic approach to identify drought responsive genes

Drought stress tolerance in wheat is polygenic and involves a complex physiological and molecular network. There is an urgent need to research wheat varieties that are resistant to drought stress due to the increased risk of abiotic stress related to global climate change. Despite the progress made in producing resistant wheat cultivars in different countries, such as Australia, Bangladesh, Europe, and Russia, much remains to be done in this field as several regions of the world with arid and semi-arid climates lack suitable resistant cultivars. Wheat biology is complex and even more complicated when it comes to trying to achieve a better drought response in wheat cultivars due to the enormous genome size (17Gb), which encompasses many repetitive sequences (80-90%) (Šafář et al. 2010).

A transcriptomic approach to identifying drought-associated genes using high throughput next generation sequencing of cDNA (RNA seq) provides a comprehensive qualitative and quantitative study of differentially expressed genes and their functional annotation under drought stress (Kumar et al. 2015, Poersch-Bortolon et al. 2016). This transcriptomic approach

coupled with morpho-physiological and biochemical studies has led to a deeper understanding of the physiological and molecular response of wheat to drought stress, which could pave the way for developing resistant wheat cultivars. Transcriptomic studies of wheat exposed to drought stress have highlighted the expression of more than 300 genes involved in several plant biological process during the reproductive stage (Ma et al 2017). You et al. (2019) showed that highly regulated genes in drought-stressed wheat are associated with photosynthesis, lipid metabolism and amino acid metabolism, endoplasmic reticulum-related protein and plant hormone signalling. Under conditions of water shortage and during the early phases of growth, plant cell organelles such as chloroplast, mitochondria, the nucleus, peroxisomes, and plasma membrane, initiate the production of certain compounds that can prove toxic to the plant when are overproduced under a persistent stress factor, such as ROS. It has been shown that the most common drought response is associated with the expression of cytochrome P450, dehydrins, glutathione transferase, proteinase inhibitors, and transcription factors listed above (sections 1.3.3 and 1.6). Several candidate genes were found to be differentially expressed during drought stress. The gene product TaFER-5B, associated with heat, high iron and oxidation resistance, was found to be overexpressed in wheat under drought stress treatment (Zang et al. 2017). In addition, genes encoding cuticular wax-associated proteins, heat shock proteins, 6-phosphogluconate dehydrogenase (PGD6), and tubulins are also differentially expressed during the drought sensitive period (Ma et al. 2019). Li et al. 2022 showed that differentially expressed genes (DEGs) associated with mTOR and alpha-linolenic acid metabolism are involved in drought resistance during wheat germination. Apart from the compounds associated with the drought stress listed in sections 1.3.3. and 1.6, other compounds and enzymes were found to be expressed in wheat under severe water shortage conditions, such as chitinases and glucanases (Gregorová et al. 2015).

A metabolomic study showed that amino acids were the most abundant of the differentially expressed metabolites in response to drought, followed by phenolic acids (Lv et al. 2022). The secondary metabolites and photosynthesis pathways are the most enriched during drought stress (Lv et al. 2022). Genetic engineering studies on transgenic wheat showed that mtID 1-phosphate mannitol dehydrogenase, and P5CS (delta1-pyrroline-5-carboxylate) gene products are associated with improved drought tolerance in wheat (Abebe et al. 2003; Vendruscolo et al. 2007). The mtID products are necessary for initiating mannitol biosynthesis, while P5CS is a key enzyme involved in proline synthesis (Abebe et al. 2003; Vendruscolo et al. 2007).

By examining the morpho-physiological features of wheat under drought stress conditions and screening for highly regulated genes associated with this abiotic stress, it helps to identify the metabolic pathways and genes involved in drought stress that can be used for breeding for improved tolerance cultivars.

1.8. Breeding approaches for improved drought tolerance

Four types of breeding approaches have been reported. The first two are breeding for high yield under optimal conditions (Blum 1988) or in a target environment (Bidinger and Witcombe 1989). However, these two approaches are limited by the variability of drought stress over time and its influence on plant genotypes. The third approach aims for breeding for high yield stability under stressed and non-stressed conditions (Mitra 2001) through a selection of drought tolerant genotypes based on morphological and physiological traits (Reynolds et al. 2005). The fourth is a polyphasic approach based on the examination of morphological, physiological, genetic and genomic features of crop drought avoidance and tolerance including different agronomic techniques for improved soil moisture conservation in the target environment (Serraj et al. 2005).

1.9. Research rational, hypothesis, and objectives of the research

In this study, screening for drought tolerant wheat genotypes by investigating the morpho-physiological and biochemical changes in wheat at different growth stages was carried out. Tolerant and sensitive genotypes were subjected to comparative transcriptomic study for identification of genes differentially regulated in response to drought as shown in figure 1.5.

Hypothesis

1- Morphological, physiological and biochemical changes in wheat in response to drought stress are regulated via the differential expression of key genes.

Aim

The overall aim of the study was to identify genes that are involved in the response of wheat to drought to inform wheat-breeding programmes for the development of drought-tolerant varieties.

Objectives

1. Screen 10-ICARDA bread wheat genotypes under drought stress to select tolerant and sensitive genotypes based on morpho-physiological parameters. (Chapter 3)
2. Assess the biochemical response of the two most tolerant and two most susceptible ICARDA bread wheat genotypes, identified in objective 1, to drought stress. (Chapter 4)
3. Conduct a transcriptome analysis of tolerant and susceptible genotypes, identified in objectives 1 and 2, to identify genes differentially regulated in response to drought. (Chapter 5)

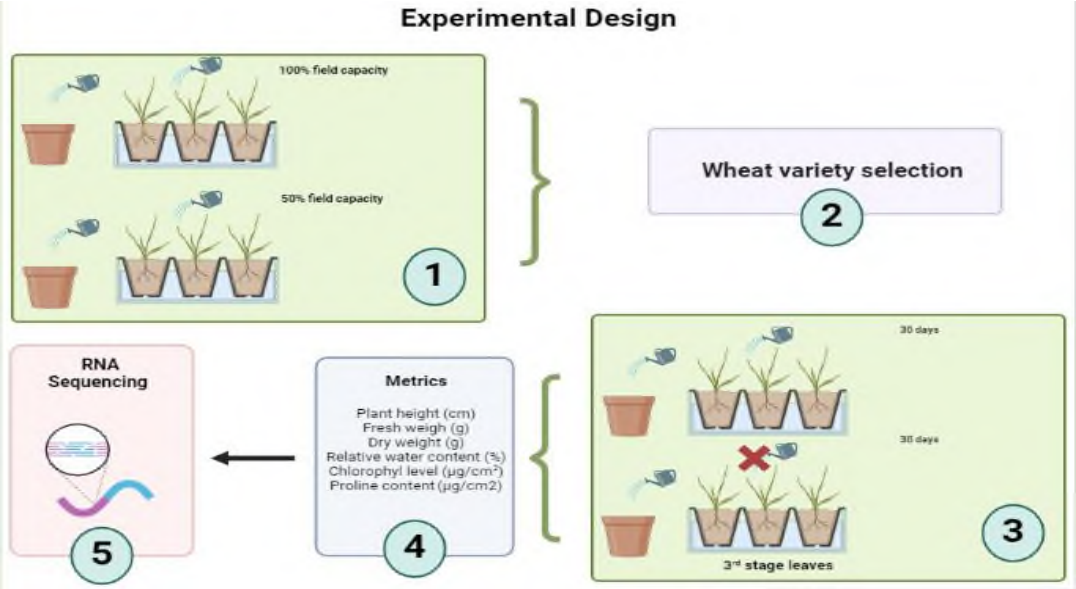


Figure 1.5. Experimental flow for screening for drought tolerant wheat genotypes based on morphological, physiological, biochemical and molecular studies.

Chapter 2. Materials and Methods

For screening drought tolerant in wheat varieties, several experiments have been carried out to highlight the morphological, physiological, biochemical and molecular features of the wheat samples varieties as detailed below in this chapter.

2.1. Plant material, source and maintenance

A total of 10 genotypes of wheat, *Triticum aestivium* subsp *aestivium*, were obtained from Syria ICARDA company (https://www.icarda.org/research/country/syria). Eight bread wheat genotypes (ICARDA) known for their high performance on the rain-fed condition were subjected to drought stress. Two local wheat varieties, Atlas and Terbol, obtained from the same company, were included in this study (Table 1). The reason for selecting this number of cultivars (n =10) is to have a greater chance of obtaining drought-tolerant cultivars after screening. Fifteen seeds for each genotype were ordered and stored in the laboratory at room temperature before the wet lab work. All experiments were carried out in triplicates and performed in the glass house of Ridley building, Newcastle University, UK. The quality of the seeds was carefully checked before proceeding with the pot preparation and only seeds with uniform surface were used.

Table 2.1. The different wheat genotypes used in this present study

Sample names	Name/Pedigree
Atlas	Atlas
Terbol	Terbol
ICARDA 35531	Miskeet-18/sokol/50w90045
ICARDA 33847	Hubara-1/3/Munia/CHTO//Milan/4/GOUMRIA-8/5/AFIF
ICARDA 35621	WEEBILL-1/2*QAFZAH21//KAMB2/PANDION/3/TEG/MIAN YANG 20//CHUM18/5*BCN
ICARDA 35618	QAFZAH-33/FLOKWA-2//Excalibur/3/DOUKKLA/33
ICARDA 32122	CHAM-10/3/TNMU//MILAN/TUI/4/SANDALL-5
ICARDA 35015	GIRWILL-13/2*PASTOR-2//SIDS-1/3/Gladius
ICARDA 32331	GOUMRIA3//MILAN/MUNIA/3/MILAN/KAUZ//HD29/2*WEAVER
ICARDA 31591	WAXWING*2/VIVITSI//SHUHA-8/DUCULA

2.1.1. Experimental design

In a completely randomized design (CRD), five seeds of each wheat genotype were sown at a depth of 1 cm in pots of 1.5 L [(n = 60 (10×3×2)] containing 900 g of John Innes growing media. The planted seeds were supplied with water up to 100% FC (a maximum water content that the soil can retain against gravity) and placed under constant temperature of 20°C-25°C and a regime of light/dark of 16 h/8 h with a light intensity of 350 µmol.m^{-2} s^{-1}. Water in field capacity or the volume of water retained by the soil against gravity was determined by appropriate gravimetric method. In this respect, the soil was dried in an oven of 70°C until a constant weight was obtained (Dw). A determined volume of water (X_0) was used to saturate the dried soil which was hold in a pot with filter paper. The soil was drained for 24h until no more water was draining. The drained water was collected and measured (X1) as well as the saturated wet soil (Sw). The volume of retained water by the soil, which correspond to 100% water field capacity (FC), was calculated as X0-X1 (100% FC). The outcome of this formula was in line with Sw-Dw. Seed germination was observed after 7 days of incubation and only three seedlings of each genotype were used.

2.2. Drought treatment

All seedlings were grown with sufficient watering (100% FC) until three leaves were completely developed and then the drought stress treatment was applied. The wheat samples were supplied with water up to 50% FC. In parallel, two pots containing each 3 wheat samples were used as control samples supplied with water up to 100% field capacity. The control and the drought stress wheat samples were under the same environmental condition of the glass house as mentioned above. All wheat samples were grown until the maturity and ripening stage as shown in Figure 2.1.

2.3. Effect of drought stress on morphological features of wheat at maturity stage

Morphological and physiological features of all wheat samples were evaluated based on the height of plant, length of spike, weight of spike, number of seeds, weight of seeds, number of leaves per plant, leaf area, and fresh and dry weight. The height of all the samples was estimated from the surface of the soil to the top of the main shoot in centimetre (cm). Flag Leaf Area

(LA) was measured for all samples as follow LA= width (middle part) X Length X 0.75. The chlorophyll content of the flag leaf of each sample was determined using a SPAD meter (Opti-Sciences CCM-200). Three random readings were taken at the base, middle, and tip of the leaf. An average value was estimated for each genotype (Pour-Aboughadareh et al 2019).

For plant fresh and dry weight, the shoots of each accession were separately harvested and subjected to shoot fresh weight calculation (SFW). The dried shoot weight (SDW) was estimated after drying the material in an oven at 70°C for 72h.

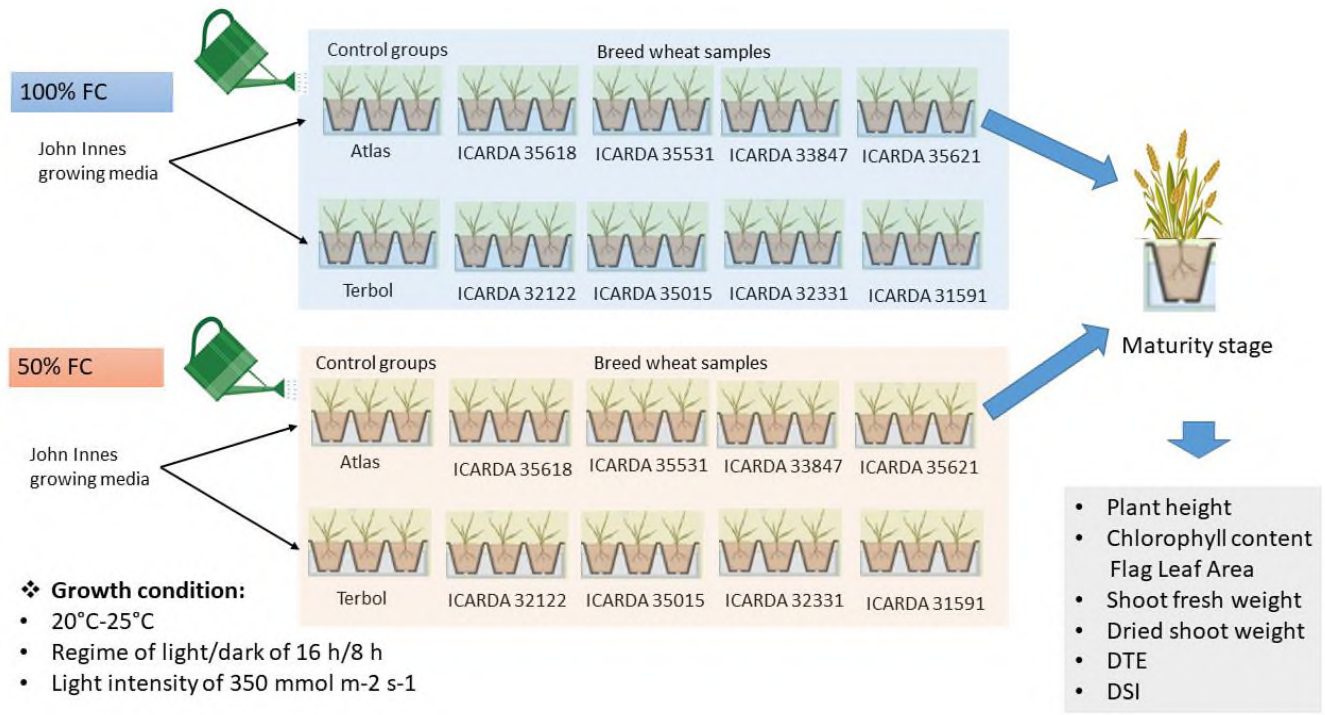


Figure 2.1. Experimental design for effect of drought stress on morphological and physiological features of wheat at maturity stage

2.3.1. Effect of drought stress on wheat yield

Drought tolerance efficiency (DTE) and Drought Susceptibility Index (DSI) of 'total grain yield per plant' have been determined using the following formula:

DTE = Yield under stress condition / yield under control condition X 100.

DSI = (1 – YS/YC) / (1 – XS/XC) [Fischer and Maurer (1978)].

‘YS’ corresponds to ‘yield under stress’ while YC indicates ‘yield under control’. XS is the average yield for all the cultivars under the stress condition, whereas XC is the average yield for all varieties amidst control.

2.4. Effect of drought stress on wheat at seedling stage

Six ICARDA wheat genotypes, Atlas, ICARDA33847, ICARDA 35531, ICARDA 32331, ICARDA 35015, ICARDA 31591, were selected based on their performance in the first experiment. The wheat varieties Atlas, ICARDA33847, ICARDA 35531 were found to be sensitive to drought stress while ICARDA 32331, ICARDA 35015, and ICARDA 31591 were found to be tolerant based on their morphological and physiological features under water shortage condition at maturity stage. These genotypes were subjected to drought stress and their biochemical and molecular traits were evaluated. The seeds were planted in pots of 1.5 L filled with 900g of John Innes growing media and incubated in glass house of Ridley building, Newcastle University. UK, under constant temperature of 20°C-25°C and a regime of light/dark of 16 h/8 h with a light intensity of 350 µmol $m^{-2}s^{-1}$. All experiments were carried out in triplicates and conducted during the seedling stage. The number of pots utilised is as follows: 6 wheat ICARDA varieties × 3 Replications × 2 Treatments = 36 Pots (Figure 2).

A number of 3 wheat seeds were planted within every pot at the depth of 1 centimetre. At the 3-leaf stages, the wheat samples were subjected to 100% FC (well-watered) treatments. Then, the pots were divided into two groups. Group 1 with 100%-85% FC, while group 2 lacked water supply (withholding) for 15 days. The pots were monitored daily and the average water was controlled using an irrometer (W.E.T Sensor kit).

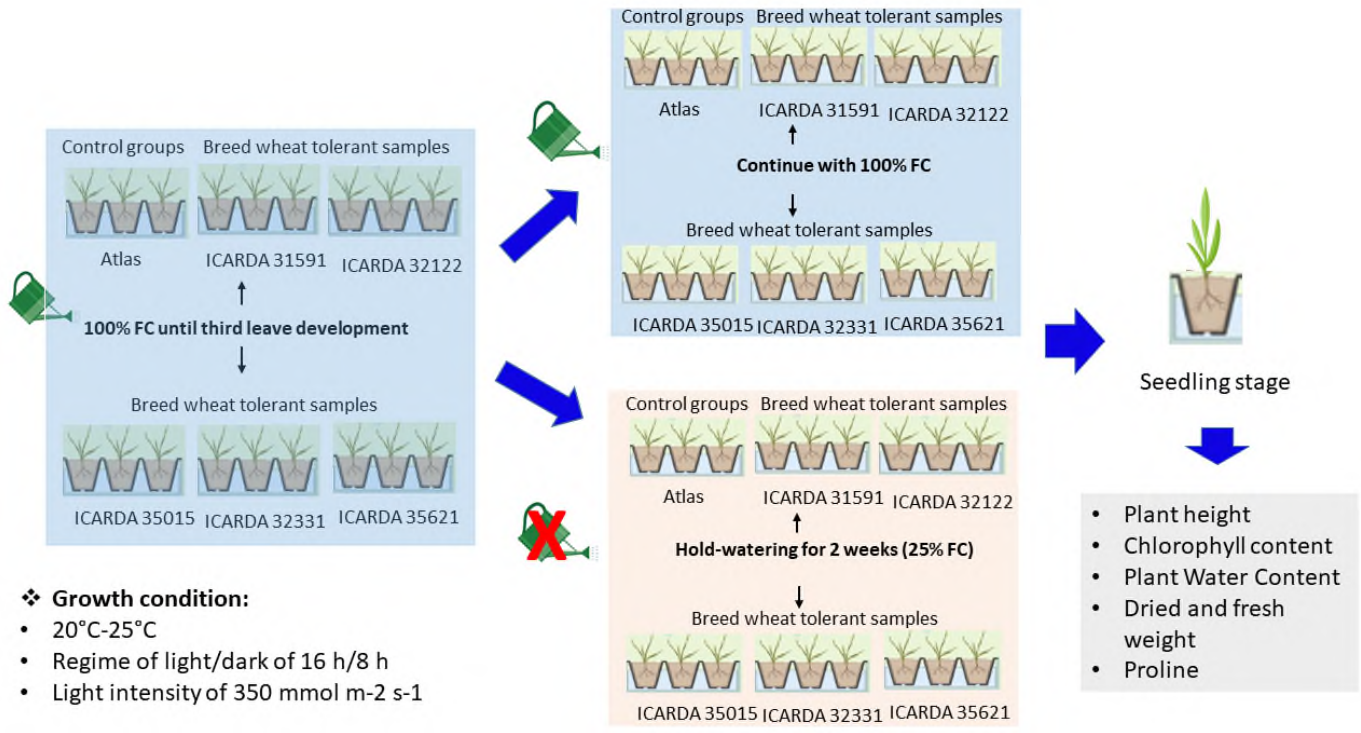


Figure 2.2. Experimental design for effect of drought stress on biochemical and physiological features of wheat at seedling stage.

2.4.1. Data analysis

The effect of the drought stress on the morphological, physiological, and biochemical features of all samples at seedling stage were evaluated. Shoot height, fresh and dry weight, plant water content, chlorophyll and proline content were estimated.

2.4.1.A. Morphological characteristics

The height of the main shoot from the top of the soil to the top of the main shoot was measured (cm) for samples with 100% water irrigation, and those under drought stress.

2.4.1.B. Physiological characteristics

For Plant Water Content (RWC), 1 g of fresh leaf was weighted (Wf) and then dried at 72h at 80°C and measured (Wd). The RWC (%) was calculated as (Wf – Wd) / Wf X 100 (Turner 1986; Barrs and Weatherley 1962).

The chlorophyll content of the 4th and 5th leaves in every plant of each sample was determined using a SPAD meter (Opti-Sciences CCM-200). Three random readings were taken at the base, middle, and tip of the leaf. An average value was estimated for each genotype.

2.4.1.C. Biochemical characteristics

Proline is one of the most important amino acids in plant known for its protecting role against different stresses including drought. Therefore, the wheat samples were subjected to proline content estimation as described by Bates et al. (1973) and modified by Claussen (2005). In brief, 10 ml of a 3% (w/v) aqueous sulfosalicylic acid solution was added to 1g of fresh leaf. After homogenisation, the mixture was centrifuged at 10,000 x g for 10 minutes and the supernatant was collected. 1 ml glacial acetic acid and ninhydrin reagent were added to 1 ml of the homogenate and then incubated in a boiling water bath for 1 h followed by rapid cooling to 21°C for 5 min. A spectrophotometry at 546 nm was used to estimate the absorbance of the red colour developed in samples. A standard curve made using commercial pure L-proline was used to determine the concentration of proline (µmol. g-1 (fW)).

2.5. Statistical analysis

All obtained data on morphological, physiological, and biochemical characters, were statistically analysed with variance (ANOVA) using Tukey test for pairwise mean comparisons of the SPSS software. ANOVA determines whether there are statistically significant differences between cultivars, but does not identify which specific cultivars are different. This limitation is addressed by Tukey's post-hoc analysis, which aims to identify specific cultivars whose means are significantly different from each other (Williams and Abdi 2010). For the first experimental data, we conducted fourteen 2-way Anova tests with 10 varieties of wheat as an independent variable (IV1). Treatment was also considered as an independent variable (IV2) with two levels (control and drought stress). The dependent variables (DV) were plant height measured in cm, spike length measured in cm, spike weight measured in grams, number of seeds per spike, seed weight per spike, number of leaves per spike, number of tillers, leaf length, leaf width, leaf area, fresh weight in grams, dry weigh in grams, chlorophyll, and plant water content. The assumption of normality was checked for all the variables. The linear correlation different traits related to the wheat under control and drought stress conditions was evaluated using Pearson’s correlation coefficient in XLSTAT software (Addisonsoft XLSTAT, Paris, France). The same software was used for agglomerative hierarchical clustering to

determine the relationship between the cultivars based on different physio-morphological parameters.

The same statistical approach was used for evaluating the physiological and biochemical data obtained from the second experiment with 6 varieties of wheat as an IV1. The DV were proline, Plant Water Content, chlorophyll, fresh and dry weight and plant height.

Drought tolerant wheat genotypes were identified based on the stress tolerance index (STI) which was calculated using the iPASTIC toolkit (Belay et al .2021)) and XLSTAT software. Principal component analysis (PCA) was computed for distinguishing between the different wheat varieties associated with drought stress condition using XLSTAT software.

2.6. Transcriptomic studies

Total RNA was extracted from wheat leaves of two genotypes Atlas and ICARDA 32331 (X 4 biological replications x 1 stress treatment X 1 control) using RNA plant kit following the manufacturer's instructions (Novogene, UK). As shown in Figure 2.3, the selection of Altlas (drought sensitive) and ICARDA 32331 (drought tolerant) was based on their different genotypes concluded following the second experiment (section 4). Qualitative and quantitative evaluation of the RNA were performed by 1% agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo fisher scientific Inc, ca USA). The absorbance ratio at 260/280 nm and RNA integrity for all samples were estimated. The RIN is measured using 28S and 18S rRNA ratio. Only RNA sample with ratio between 1.9-2.1 and RIN > 8.0 were considered for further analysis. RNA Seq libraries were prepared by Novogene Ltd, Cambridge, UK (https://www.novogene.com) using Illumina NovaSeq platforms with paired-end 150 bp sequencing method. High quality reads were obtained after measuring the error rate and eliminating low sequence quality (base quality <5 and > 50% of the read) and ambiguous nucleotides (N>10%), removing adapter sequences, and controlling the G+C distribution.

High quality of sequence (error rate of single base < 1%) were aligned with the genome sequence of *Triticum aestivium*, available at NCBI under accession number JAGHKL000000000.1, using HISAT2 algorithm.

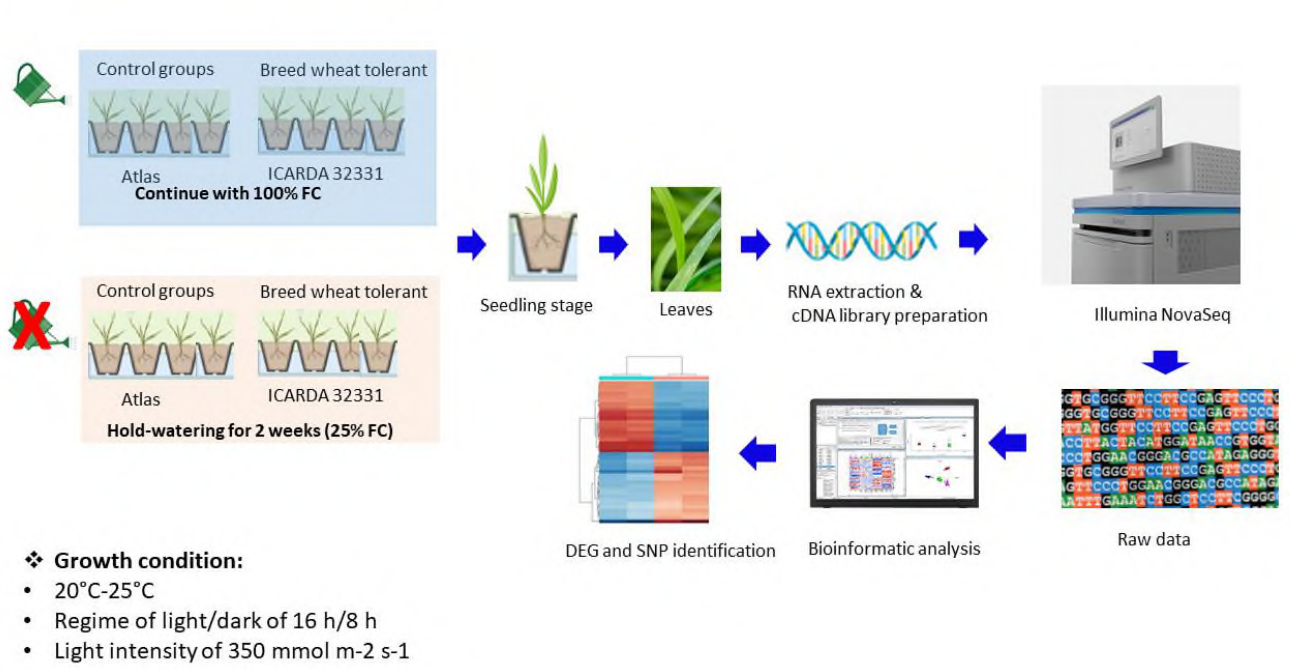


Figure 2.3. Experimental design for transcriptomic studies on the effect of drought stress on molecular traits of wheat at seedling stage.

2.6.1. Differential gene expression (DEGs) analysis

All the below information was kindly provided by novogene company (UK). Statistical analysis was applied to quantify and highlight the significant difference in the gene's expression levels in different conditions. The differential analysis was performed in three steps: (i) the raw readcount is normalized, mainly to correct the sequencing depth; then (ii) the statistical model is used to calculate the hypothesis test's probability (pvalue); and at final, multiple hypothesis test corrections are used to obtain FDR values (false discovery rate) (Anders et al., 2010). The software used for gene expression differential analysis are listed in the table below. The following screening criteria for differential genes were used: |log2(FoldChange)| >= 1 & padj<= 0.05, which are flexibly selected according to the data.

Table 2.2. Software for differential analysis and differential gene screening criteria.

Type	Software	Normalized Method	pvalue Calculation Model	FDR Calculation Method	Differential Gene Screening Threshold
Biological Replicates	DESeq2(Anders et al., 2014)	DESeq	The Negative Binomial Distribution	BH	|log2(FoldChange)| >= 1 & padj <= 0.05
No Biological Replicates	edgeR (Robinson et al., 2010)	TMM	The Negative Binomial Distribution	BH	|log2(FoldChange)| >= 1 & padj <= 0.05

2.6.2. Cluster analysis

Clustering of the two cultivars based on their gene expression pattern under control and drought conditions was performed using the mainstream hierarchical clustering to group the fpkm values of genes, and homogenized the row (Z-score).

2.6.3. Enrichment analysis

All the DEGs were associated to their biological function or pathway using clusterProfiler (Yu 2012) software including GO Enrichment, DO Enrichment, KEGG and Reactome database Enrichment etc, was used for enrichment analysis.

Chapter 3. Effect of drought stress on the physio-morphological traits of bread wheat cultivars: Initial screening of varieties for drought tolerance

3.1. Abstract

Wheat (*Triticum aestivum* L.) is one of the most vital staple food grains worldwide and is in continuous high demand as the global population increases. Drought is one of the greatest hazards for sustainable crop production and has a negative impact on wheat production. Consequently, breeding drought-tolerant wheat cultivars is one of the highest priorities to meet the future needs of the growing world population. In this respect, the aerial tissues of ten wheat cultivars, Atlas, terbol, and ICARDA (33847, 32122, 35531, 35618, 35621, 31591, 35015, 32331, were evaluated under prolonged drought stress conditions (50% water field capacity (WFC)) on the basis of physio-morphological characteristics and in comparison, with control samples (100-85% WFC). The drought stress was applied from the development of the third leaf to the maturity stage.The results showed that 5 (Terbol, ICARDA 35621, ICARDA 31591, ICARDA 35015, and ICARDA 32331) out of 10 cultivars are tolerant to drought with The drought stress was applied (DSI) < 1, high Drought Tolerance Efficiency (DTE) and minimum reduction seeds of weigh (RSW) and reduction of seeds number (RSN) values compared to drought sensitive cultivars (Atlas, ICARDA 33847, ICARDA 32122, ICARDA 35531, ICARDA 35618). Based on these data, the drought tolerant cultivars were ranked in descending order: ICARDA 32331 > ICARDA 35015 > ICARDA 31591> ICARDA 35621>Terbol while the drought sensitive cultivars are as follows: ICARDA 35618> ICARDA 35531> ICARDA 32122> ICARDA 33847> Atlas. It is interesting to mention that Atlas was able under drought stress to reduce the loss related toPH, SL, NL, NT, FW, and DW, except chlorophyll content. Pearson's correlations of the investigated physio-morphological characters highlighted the high correlation value (≥0.7-1) between spike weight, number of seeds, and seed weight, like optimal conditionThe outcome of this study highlighted the relationship between numerous traits that can be very useful for breeding programmes.

3.2. Introduction

Drought stress has been the impetus for several famines in the past and has become one of the greatest hazards for sustainable crop production. Several publications have highlighted the negative impact of drought on wheat yield, estimated at an average reduction of 20.65% and 27.5% (Daryanto et al. 2016; Zhang et al. 2018), observed in several countries, including Australia and Kenya (Rauf et al. 2016; Curtis and Halford, 2014). It has been shown that the 20.6% decrease in wheat yield corresponds to a 40% reduction in global water availability. These studies are consistent with the recent increase in global hunger according to the FAO (FAO 2018). Around half of the annual wheat crop worldwide is frequently altered by drought stress (Pfeiffer et al. 2005). This problem is exacerbated in arid environments, where wheat crop production can be drastically reduced by 50-90% (Olivares-Villegas et al. 2007). It is expected that global climate change, associated with increased aridity, will adversely affect wheat crop production in many regions of the world such as the Middle-East, the North Indian River plain, Southern Australia, Southern Europe, parts of Western Canada and Western China.

The effect of drought on wheat is very complex because it can be influenced by other abiotic factors such as heat or soil type (Nicolas et al. 1984; Eriyagama et al. 2009). Three types of drought have been defined: agricultural, hydrological, and meteorological droughts, which are caused by low precipitation and high evapotranspiration rates, lower water source than the normal average, and higher temperatures and anomalies in the atmosphere, respectively (Dai 2011). Drought intensity depends on the duration and water availability, and can be mild, moderate or severe. There is no clear definition of this classification as it is influenced by other environmental conditions. Some field studies suggest that 55–60% and 35–40% water field capacity (WFC) are moderate and severe drought stress conditions, respectively, while 75–80% WFC is an optimal water condition (Abid et al. 2018). However, in greenhouse experiments, 100% and 50% WFC were considered normal and drought stress conditions, respectively (Ahmed et al. 2019).

Changes in morphological characters and development profile of wheat under drought stress are linked to physiological, biochemical, and molecular adaptations. The development of improved drought-tolerant wheat cultivars has been the main objective of many breeders. A drought tolerant wheat variety should exhibit high grain yield. However, it has been shown that a highly drought

tolerant wheat genotype can have a low grain yield, such as wild wheat (Haque et al. 2021). Therefore, the majority of studies on the search for tolerant cultivars were based on the evaluation of morphological traits associated with grain yield (Hakim et al. 2012; Hossain and Silva, 2012; Rahman et al. 2016). Among the most studied yield-contributing features were plant height, dry and fresh weight, spike length and weight, seed number and weight, tiller number, and leaf number and size (length, width, and area).

Wheat morphology and architecture are influenced by drought stress, manifested by a reduction in plant height, due to poor mitosis and leaf abscission (Elnaggar et al. 2018; Paponov et al. 2020). This negative effect of water stress on the internal plant components is reflected in a reduction in total plant weight and leaf area (Paponov et al. 2020; Kumar et al. 2021). It has been observed in several crops such as maize that shoot length, fresh and dry weight dropped following a drought stress regime (Tumova et al. 2018; Widuri et al. 2018). The leaf was found to be more sensitive to drought stress than the shoot and roots (Kumar et al. 2021). Water-limited conditions alter leaf turgor pressure as a consequence of limited water volume in the upper epidermis, leading to leaf rolling. Decrease in leaf area limits the plant's vital photosynthesis process.

Water deficit can be detrimental to crop yield when it occurs after the anthesis stage and often leads to a reduction in the number of spikes, tillers and grains as well as grain weight. These observations have been recorded in several crops such as barley, *Triticum aestivium* L. *Pennisetum glaucum* L., soybean (Nofouzi et al. 2018; Istanbuli et al. 2020; Malinowska et al. 2020;Kalagare et al. 2021).

3.3. Aims and objectives

The present chapter aimed to evaluate the effect of drought stress (50% WFC) on the aerial tissues of ten wheat cultivars under prolonged drought stress conditions, based on morphological characteristics. The water stress regime was applied from the development of the third leaf to the maturity stage. Biochemical traits related to chlorophyll content were also evaluated.The selected drought-tolerant wheat varieties were subjected to further biochemical, physiological and molecular studies, which are detailed in the following chapters (Chapters 4 and 5) of this thesis.

3.4. Results

In this present study, 10 wheat genotypes were subjected to a long-term drought stress of 50% WFC from the third leaf development stage to the maturity stage. The effect of abiotic stress on the morphological features of the wheat cultivars was evaluated in comparison with control samples that were under 100-85% WFC. The morphological parameters listed in Table 3.1 were estimated for wheat cultivars under both optimal and drought stress conditions.

Table 3.1. Wheat cultivars and their evaluated physio-morphological characters included in this study.

S/No.	Cultivars	Parameters
1	Atlas	Plant height
2	ICARDA 33847	Spike length
3	ICARDA 32122	Spike weight
4	ICARDA 35531	Nunber of seeds
5	ICARDA 35618	Seeds weight
6	Terbol	Nunber of leaves
7	ICARDA 35621	Nunber of tiller
8	ICARDA 31591	Leaf length
9	ICARDA 35015	Leaf width
10	ICARDA 32331	Leaf area
		Fresh weight
		Dry weight
		Chlorophyll

3.4.1. Effects on plant height (cm)

Limited water conditions (50%WFC) had a measurable negative effect ($p < .001$) on the mean plant height of ten wheat varieties (Tables 3.2 and 3.3), which was reduced overall by 41.89%. A variation in plant height, between cultivars in the control group, of 47.25 to 61.97cm with an

average of 52.39 +/ SD (Standard deviation) = 5.00 was obtained. However, under drought conditions, the variation in this parameter was reduced to between 26.60 to 33.91cm, depending on the variety, with a mean value of 30.44 +/- SD = 6.52. Under conditions of 100-85% WFC, the average plant height (cm) of the wheat cultivars was as shown below and in Figure 3.1. However, the average value of this parameter for wheat samples under drought stress conditions was completely different.

Table 3.2. Average plant height (cm) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 35531	61.97	ICARDA 35531	33.91
Terbol	56.13	Atlas	33.04
ICARDA 31591	55.17	ICARDA 32122	32.55
Atlas	52.12	ICARDA 35618	32.38
ICARDA 35618	51.98	Terbol	30.78
ICARDA 32122	51.42	ICARDA 33847	27.87
ICARDA 33847	49.96	ICARDA 35015	27.45
ICARDA 35015	49.11	ICARDA 32331	27.41
ICARDA 35621	48.77	ICARDA 35621	26.60
ICARDA 32331	47.25	ICARDA 31591	23.42

As shown in Table 3.3, variety and treatment had significant effects on plant height, manifested by $F(9,40) = 2.19$, $p < 0.04$. and $F(1, 40) = 233.88$, $p < .001$, respectively. However, it is clear from the Anova results, $F(9,40) = 0.42$, $p = .918$, that plant height was not affected by the interaction between variety and treatment. The mean difference (MD) in plant height between control (MD= 52.39, Standard Erroe (SE) = 1.01) and stressed samples (MD = 30.44, SE = 1.01) was found to be 21.95 cm, $p < .001$. The data clearly show that the control group had the highest plant height value.

Table 3.3. Analysis of variance for plant height under control (100 -85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	2.19	9	**0.044***
Treatment	233.88	1	**<0.001**
Variety / Treatment	0.42	9	0.918
Error	-	40	-

*data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants); df = degree of freedom.

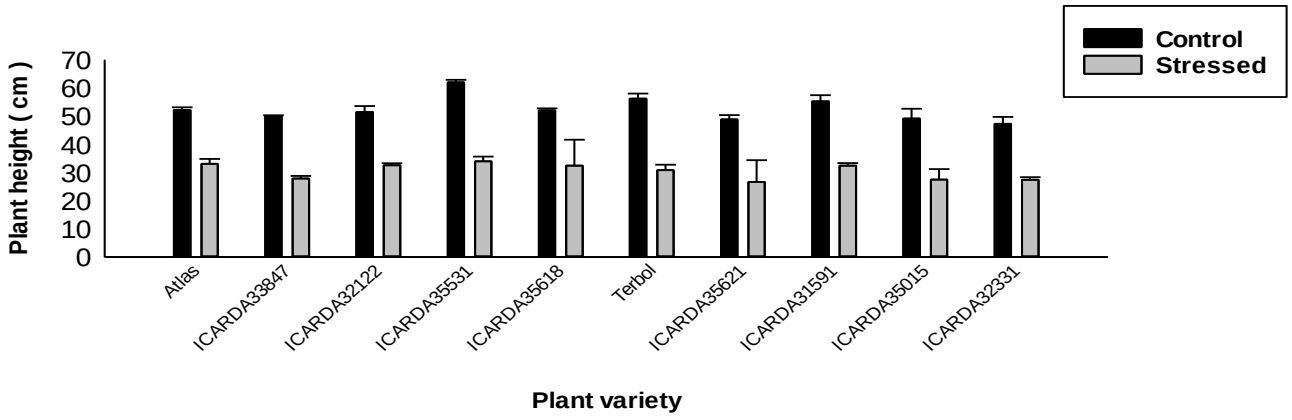


Figure 3.1. Plant height of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = plant height (cm). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 52.39 (cm) and standard deviation = 5. The overall mean under drought stress = 30.44 (cm) and standard divination = 6.52.

Based on the reduction in plant height under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < ICARDA32122 < ICARDA35618 <ICARDA31591< ICARDA32331< ICARDA35015 < ICARDA33847 < Terbol < ICARDA35531< ICARDA35621. Thus, Atlas was the best-performing cultivars under drought conditions.

Post-hoc comparison was conducted to further explore which ICARDA bread wheat varieties had statistically significant plant height under control and drought stress. This comparative analysis revealed that any of the cultivars had significant plant height under drought conditions. A detailed comparison is provided in Table 3.4.

Table 3.4. Post-hoc comparison for plant height (cm) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	2.163±4.54	0.63	-7.00	11.33
		ICARDA 32122	0.70±4.54	0.88	-8.47	9.87
		ICARDA 35531	-9.84±4.54	**0.04***	-19.02	-0.67
		ICARDA 35618	0.14±4.54	0.97	-9.03	9.32
		Terbol	-4.01±4.54	0.38	-13.18	5.16
		ICARDA 35621	3.35±4.54	0.46	-5.82	12.52
		ICARDA 31591	-3.04±4.54	0.51	-12.22	6.13
		ICARDA 35015	3.01±4.54	0.51	-6.16	12.19
		ICARDA 32331	4.87±4.54	0.29	-4.30	14.05
	ICARDA 33847	*ICARDA 32122*	-1.46±4.54	0.75	-10.64	7.71
		ICARDA 35531	-12.01±4.54	**0.01**	-21.18	-2.83
		ICARDA 35618	-2.02±4.54	0.66	-11.19	7.15
		Terbol	-6.17±4.54	0.18	-15.35	3.00
		ICARDA 35621	1.19±4.54	0.80	-7.99	10.36
		ICARDA 31591	-5.21±4.54	0.26	-14.38	3.97

	ICARDA 35015	0.85±4.54	0.85	-8.32	10.02
	ICARDA 32331	2.71±4.54	0.55	-6.46	11.88
ICARDA 32122	*ICARDA 35531*	-10.54±4.54	**0.03**	-19.72	-1.37
	ICARDA 35618	-0.56±4.54	0.90	-9.73	8.62
	Terbol	-4.71±4.54	0.31	-13.88	4.46
	ICARDA 35621	-0.56±4.54	0.90	-9.73	8.62
	ICARDA 31591	-3.74±4.54	0.41	-12.92	5.43
	ICARDA 35015	2.31±4.54	0.61	-6.86	11.49
	ICARDA 32331	4.17±4.54	0.36	-5.00	13.35
ICARDA 35531	*ICARDA 35618*	9.99±4.54	**0.03**	0.81	19.16
	Terbol	5.83±4.54	0.21	-3.34	15.01
	ICARDA 35621	13.19±4.54	**0.01**	4.02	22.37
	ICARDA 31591	6.80±4.54	0.14	-2.37	15.97
	ICARDA 35015	12.86	**0.01**	3.68	22.03
	ICARDA 32331	14.72±4.54	**<0.001**	5.54	23.89
ICARDA 35618	*Terbol*	-4.15±4.54	0.37	-13.33	5.02
	ICARDA 35621	3.21±4.54	0.48	-5.97	12.38
	ICARDA 31591	-6.06±4.54	0.19	-15.23	3.12
	ICARDA 35015	2.87	0.53	-6.30	12.04
	ICARDA 32331	4.73±4.54	0.30	-4.44	13.90
Terbol	*ICARDA 35621*	7.36±4.54	0.11	-1.81	16.53
	ICARDA 31591	0.97±4.54	0.83	-8.21	10.14
	ICARDA 35015	7.02±4.54	0.13	-2.15	16.20
	ICARDA 32331	8.88±4.54	0.06	-0.29	18.06

	ICARDA 35621	*ICARDA 31591*	-6.39±4.54	0.17	-15.57	2.78
		ICARDA 35015	-0.34±4.54	0.94	-9.51	8.84
		ICARDA 32331	1.52±4.54	0.74	-7.65	10.70
	ICARDA 31591	*ICARDA 35015*	6.06±4.54	0.19	-3.12	15.23
		ICARDA 32331	7.92±4.54	0.09	-1.26	17.09
	ICARDA 35015	*ICARDA 32331*	1.86±4.54	0.68	-7.31	11.03
Stressed	*Atlas*	*ICARDA 33847*	5.17±4.54	0.26	-4.00	14.34
		ICARDA 32122	0.49±4.54	0.91	-8.68	9.66
		ICARDA 35531	-0.87±4.54	0.85	-10.05	8.30
		ICARDA 35618	0.65±4.54	0.89	-8.52	9.83
		Terbol	2.26±4.54	0.62	-6.91	11.43
		ICARDA 35621	6.44±4.54	0.16	-2.74	15.61
		ICARDA 31591	0.62±4.54	0.89	-8.56	9.79
		ICARDA 35015	5.58±4.54	0.23	-3.59	14.76
		ICARDA 32331	5.62±4.54	0.22	-3.55	14.80
	ICARDA 33847	*ICARDA 32122*	-4.68±4.54	0.31	-13.85	4.49
		ICARDA 35531	-6.04±4.54	0.19	-15.22	3.13
		ICARDA 35618	-4.52±4.54	0.33	-13.69	4.66
		Terbol	-2.91±4.54	0.53	-12.08	6.26
		ICARDA 35621	1.27±4.54	0.78	-7.91	10.44
		ICARDA 31591	-4.55±4.54	0.32	-13.73	4.62
		ICARDA 35015	0.41±4.54	0.93	-8.76	9.59
		ICARDA 32331	0.45±4.54	0.92	-8.72	9.63
	ICARDA 32122	*ICARDA 35531*	-1.36±4.54	0.77	-10.54	7.81

	ICARDA 35618	0.16±4.54	0.97	-9.01	9.34
	Terbol	1.77±4.54	0.70	-7.40	10.94
	ICARDA 35621	5.95±4.54	0.20	-3.23	15.12
	ICARDA 31591	0.13±4.54	0.98	-9.05	9.30
	ICARDA 35015	5.09±4.54	0.27	-4.08	14.27
	ICARDA 32331	5.13±4.54	0.26	-4.04	14.31
ICARDA 35531	*ICARDA 35618*	1.53±4.54	0.74	-7.65	10.70
	Terbol	3.13±4.54	0.49	-6.04	12.31
	ICARDA 35621	7.31±4.54	0.12	-1.86	16.48
	ICARDA 31591	1.49±4.54	0.74	-7.68	10.66
	ICARDA 35015	6.46±4.54	0.16	-2.72	15.63
	ICARDA 32331	6.50±4.54	0.16	-2.68	15.67
ICARDA 35618	*Terbol*	1.61±4.54	0.73	-7.57	10.78
	ICARDA 35621	5.78±4.54	0.21	-3.39	14.96
	ICARDA 31591	-0.04±4.54	0.99	-9.21	9.14
	ICARDA 35015	4.93±4.54	0.28	-4.24	14.10
	ICARDA 32331	4.97±4.54	0.28	-4.20	14.14
Terbol	*ICARDA 35621*	4.18±4.54	0.36	-5.00	13.35
	ICARDA 31591	-1.64±4.54	0.72	-10.82	7.53
	ICARDA 35015	3.32±4.54	0.47	-5.85	12.50
	ICARDA 32331	3.36±4.54	0.46	-5.81	12.54
ICARDA 35621	*ICARDA 31591*	-5.82±4.54	0.21	-14.99	3.35
	ICARDA 35015	-0.85±4.54	0.85	-10.03	8.32
	ICARDA 32331	-0.81±4.54	0.86	-9.99	8.36

ICARDA 31591	*ICARDA 35015*	4.97±4.54	0.28	-4.21	14.14
	ICARDA 32331	5.01±4.54	0.28	-4.17	14.18
ICARDA 35015	*ICARDA 32331*	0.04±4.54	0.99	-9.13	9.21

*Data in bold represent significant results at 0.05

3.4.2. Effects on spike length (cm)

The mean spike length of ten wheat cultivars subjected to a 50% WFC was reduced by 27.45%; a value consistent with the detrimental effect of drought stress ($p < .001$) on spike length (Tables 3.5 and 3.6). In the control group, a variation of spike length from 05.40 to 09.10 cm, depending upon variety, with an overall mean value of 06.50 +/- SD = 1.02 was obtained. However, a mean value of 04.72 (SD = 0.99) was obtained for the drought-stressed samples, which showed a variation in spike length from 03.61 to 6.20cm. The descending order of the mean spike length for wheat cultivars under 100-85% WFC and for drought-stressed samples was as follows:

Table 3.5. Average spike length (cm) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32122	9.10	ICARDA 32122	6.20
ICARDA 35015	6.78	Atlas	5.20
ICARDA 32331	6.77	ICARDA 35531	5.14
ICARDA 35618	6.63	ICARDA 32331	4.75
ICARDA 33847	6.27	ICARDA 35621	4.70
Terbol	6.10	ICARDA 33847	4.59
ICARDA 35621	6.05	Terbol	4.45
ICARDA 35531	6.02	ICARDA 31591	4.31
Atlas	5.91	ICARDA 35015	4.22
ICARDA 31591	5.40	ICARDA 35618	3.61

Anova statistical results demonstrated a considerable effect of variety, treatment, and interaction between variety and treatment on spike length with $F(9,40) = 7.76$, $p < .001$; $F(1, 40) = 106.75$, $p < .001$; and $F(9,40) = 2.27$, $p = .037$, respectively (Table 3.6). Tukey's test showed a mean difference in spike length of 01.79 cm, $p < .001$ between control (M = 06.50, SE = 0.12) and

drought-stressed samples (M = 04.72, SE = 0.12) with a higher value observed in the control wheat samples (Figure 3.2).

Table 3.6 Two-way ANOVA results for spike length under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	7.763	9	**<0.001***
Treatment	106.757	1	**<0.001**
Variety * Treatment	2.272	9	**0.037**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants); df = degree of freedom.

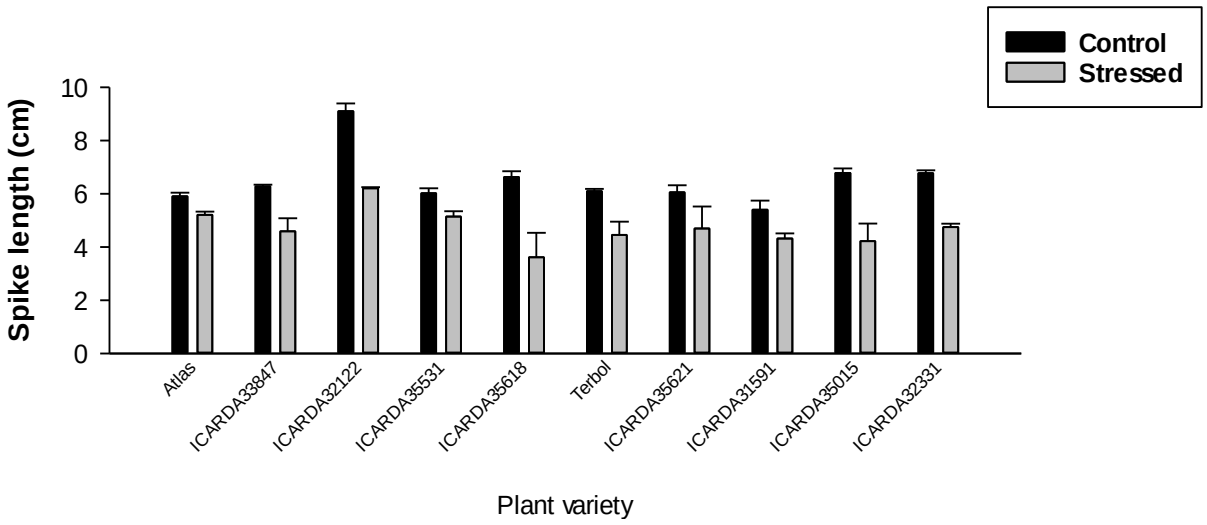


Figure 3.2. Spike length of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = spike length (cm). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 06.50 (cm) and standard deviation = 1.22. The overall mean under drought stress = 04.72 (cm) and standard divination = 0.99.

Based on the reduction in plant height under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < ICARDA35531 < ICARDA31591 < ICARDA35621 <

ICARDA33847 < Terbol < ICARDA32331< ICARDA32122 < ICARDA35015 < ICARDA 35618. Thus, Atlas was the best-performing cultivars under drought conditions based on spike length as well as plant height parameters.

Post-hoc comparative analysis showed that the culivars ICARDA 33847, ICARDA 32122, ICARDA 35618, ICARDA 32331 had a significant spike length under drought conditions. A detailed comparison is provided in Table 3.7.

Table 3.7. Post-hoc comparison for spike length (cm) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-0.37±0.55	0.51	-1.47	0.74
		ICARDA 32122	-3.19±0.55	**<0.001***	-4.29	-2.09
		ICARDA 35531	-0.11±0.55	0.84	-1.22	0.99
		ICARDA 35618	-0.72±0.55	0.19	-1.82	0.38
		Terbol	-0.19±0.55	0.73	-1.29	0.91
		ICARDA 35621	-0.15±0.55	0.79	-1.25	0.96
		ICARDA 31591	0.51±0.55	0.36	-0.59	1.61
		ICARDA 35015	-0.87±0.55	0.12	-1.98	0.23
		ICARDA 32331	-0.87±0.55	0.12	-1.97	0.24
	ICARDA 33847	*ICARDA 32122*	-2.82±0.55	**<0.001**	-3.93	-1.72
		ICARDA 35531	0.25±0.55	0.65	-0.85	1.36
		ICARDA 35618	-0.35±0.55	0.52	-1.46	0.75
		Terbol	0.18±0.55	0.75	-0.93	1.28

	ICARDA 35621	0.22±0.55	0.69	-0.88	1.32
	ICARDA 31591	0.88±0.55	0.12	-0.23	1.98
	ICARDA 35015	-0.51±0.55	0.36	-1.61	0.60
	ICARDA 32331	-0.50±0.55	0.37	-1.60	0.60
ICARDA 32122	*ICARDA 35531*	3.08±0.55	**<0.001**	1.97	4.18
	ICARDA 35618	2.47±0.55	**<0.001**	1.37	3.57
	Terbol	3.00±0.55	**<0.001**	1.90	4.10
	ICARDA 35621	3.04±0.55	**<0.001**	1.94	4.15
	ICARDA 31591	3.70±0.55	**<0.001**	2.60	4.80
	ICARDA 35015	2.32±0.55	**<0.001**	1.21	3.42
	ICARDA 32331	2.32±0.55	**<0.001**	1.22	3.43
ICARDA 35531	*ICARDA 35618*	-0.61±0.55	0.27	-1.71	0.50
	Terbol	-0.08±0.55	0.89	-1.18	1.03
	ICARDA 35621	-0.03±0.55	0.95	-1.14	1.07
	ICARDA 31591	0.62±0.55	0.26	-0.48	1.73
	ICARDA 35015	-0.76±0.55	0.17	-1.86	0.34
	ICARDA 32331	-0.75±0.55	0.18	-1.86	0.35
ICARDA 35618	*Terbol*	0.53±0.55	0.34	-0.57	1.63
	ICARDA 35621	0.57±0.55	0.30	-0.53	1.68
	ICARDA 31591	1.23±0.55	**0.03**	0.13	2.33
	ICARDA 35015	-0.15±0.55	0.78	-1.26	0.95
	ICARDA 32331	-0.15±0.55	0.79	-1.25	0.96
Terbol	*ICARDA 35621*	0.04±0.55	0.94	-1.06	1.15
	ICARDA 31591	0.70±0.55	0.21	-0.40	1.80

		ICARDA 35015	-0.68±0.55	0.22	-1.79	0.42
		ICARDA 32331	-0.68±0.55	0.22	-1.78	0.43
	ICARDA 35621	*ICARDA 31591*	0.66±0.55	0.24	-0.45	1.76
		ICARDA 35015	-0.73±0.55	0.19	-1.83	0.38
		ICARDA 32331	-0.72±0.55	0.19	-1.82	0.38
	ICARDA 31591	*ICARDA 35015*	-1.38±0.55	**0.02**	-2.49	-0.28
		ICARDA 32331	-1.38±0.55	**0.02**	-2.48	-0.27
	ICARDA 35015	*ICARDA 32331*	0.01±0.55	0.99	-1.10	1.11
Stressed	*Atlas*	*ICARDA 33847*	0.61±0.55	0.27	-0.49	1.72
		ICARDA 32122	-1.00±0.55	0.07	-2.10	0.10
		ICARDA 35531	0.06±0.55	0.91	-1.04	1.17
		ICARDA 35618	1.59±0.55	0.01	0.49	2.70
		Terbol	0.75±0.55	0.18	-0.35	1.85
		ICARDA 35621	0.51±0.55	0.36	-0.60	1.61
		ICARDA 31591	0.89±0.55	0.11	-0.21	1.99
		ICARDA 35015	0.98±0.55	0.08	-0.12	2.09
		ICARDA 32331	0.46±0.55	0.41	-0.65	1.56
	ICARDA 33847	*ICARDA 32122*	-1.61±0.55	**0.01**	-2.72	-0.51
		ICARDA 35531	-0.55±0.55	0.32	-1.65	0.55
		ICARDA 35618	0.98±0.55	0.08	-0.12	2.08
		Terbol	0.14±0.55	0.80	-0.97	1.24
		ICARDA 35621	-0.11±0.55	0.85	-1.21	1.00
		ICARDA 31591	0.28±0.55	0.62	-0.83	1.38
		ICARDA 35015	0.37±0.55	0.50	-0.73	1.47

	ICARDA 32331	-0.16±0.55	0.78	-1.26	0.95
ICARDA 32122	*ICARDA 35531*	1.06±0.55	0.06	-0.04	2.17
	ICARDA 35618	2.59±0.55	**0.001**	1.49	3.70
	Terbol	1.75±0.55	**0.001**	0.65	2.85
	ICARDA 35621	1.51±0.55	**0.01**	0.40	2.61
	ICARDA 31591	1.89±0.55	<**0.001**	0.79	2.99
	ICARDA 35015	1.98±0.55	<**0.001**	0.88	3.09
	ICARDA 32331	1.46±0.55	**0.01**	0.35	2.56
ICARDA 35531	*ICARDA 35618*	1.53±0.55	**0.01**	0.43	2.63
	Terbol	0.69±0.55	0.22	-0.42	1.79
	ICARDA 35621	0.44±0.55	0.42	-0.66	1.55
	ICARDA 31591	0.83±0.55	0.14	-0.28	1.93
	ICARDA 35015	0.92±0.55	0.10	-0.18	2.02
	ICARDA 32331	0.39±0.55	0.48	-0.71	1.50
ICARDA 35618	*Terbol*	-0.84±0.55	0.13	-1.95	0.26
	ICARDA 35621	-1.09±0.55	**0.05**	-2.19	0.02
	ICARDA 31591	-0.70±0.55	0.21	-1.81	0.40
	ICARDA 35015	-0.61±0.55	0.27	-1.71	0.49
	ICARDA 32331	-1.14±0.55	**0.04**	-2.24	-0.03
Terbol	*ICARDA 35621*	-0.24±0.55	0.66	-1.35	0.86
	ICARDA 31591	0.14±0.55	0.80	-0.96	1.24
	ICARDA 35015	0.23±0.55	0.67	-0.87	1.34
	ICARDA 32331	-0.29±0.55	0.59	-1.40	0.81
ICARDA 35621	*ICARDA 31591*	0.38±0.55	0.49	-0.72	1.49

		ICARDA 35015	0.48±0.55	0.39	-0.63	1.58
		ICARDA 32331	-0.05±0.55	0.93	-1.15	1.05
	ICARDA 31591	*ICARDA 35015*	0.09±0.55	0.87	-1.01	1.20
		ICARDA 32331	-0.43±0.55	0.43	-1.54	0.67
	ICARDA 35015	*ICARDA 32331*	-0.53±0.55	0.34	-1.63	0.58

*Data in bold represent significant results at 0.05.

3.4.3. Effects on spike weight (g)

As reported for the other morphological features, the spike weight of 10 wheat cultivars was negatively affected by this form of abiotic stress, resulting in reduction of 75.95% (Tables 3.8 and 3.9), with a p value < 0.001.The spike weight of wheat samples varied from 0.55 to 01.16 g and from 0.11 to 0.24 g with mean values of 0.79 +/- SD = 0.28 and 0.19 +/- SD = 0.10, under 85-100% WFC and 50% WFC conditions, respectively. The decreasing order of mean spike weight of wheat varieties under control conditions and drought conditions was (Table 3.8).

Table 3.8. Average spike weight (g) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 35618	1.16	ICARDA 35621	0.24
ICARDA 35621	0.96	ICARDA 32331	0.22
Atlas	0.88	ICARDA 35618	0.22
ICARDA 35531	0.88	ICARDA 31591	0.19
Terbol	0.74	ICARDA 35015	0.19
ICARDA 31591	0.73	ICARDA 32122	0.19
ICARDA 32331	0.72	ICARDA 35531	0.19
ICARDA 33847	0.68	Atlas	0.18
ICARDA 32122	0.64	Terbol	0.18
ICARDA 35015	0.55	ICARDA 33847	0.11

Statistical analysis based on Anova tests showed that variety and the interaction between variety and treatment had no impact on spike weight, with $F(9,40) = 1.30$, $p < .266$; and $F(9,40) = 0.90$, $p = .539$, respectively. In contrast, treatment had a significant effect on this parameter, as

demonstrated by F(1, 40) = 123.16, p < .001 (Table 3.9). Pairwise comparisons of the spike weight of wheat samples under control (M = 0.79, SE = 0.04) and drought stress conditions (M = 0.19, SE = 0.04) showed an average difference of 0.60 g (p < .001), with the higher value for wheat varieties in the control group (Figure 3.3).

Table 3.9. Analysis of variance for spike weight of wheat samples under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	1.30	9	0.266
Treatment	123.16	1	**0.001**
Variety * Treatment	0.895	9	0.539
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants); df = degree of freedom.

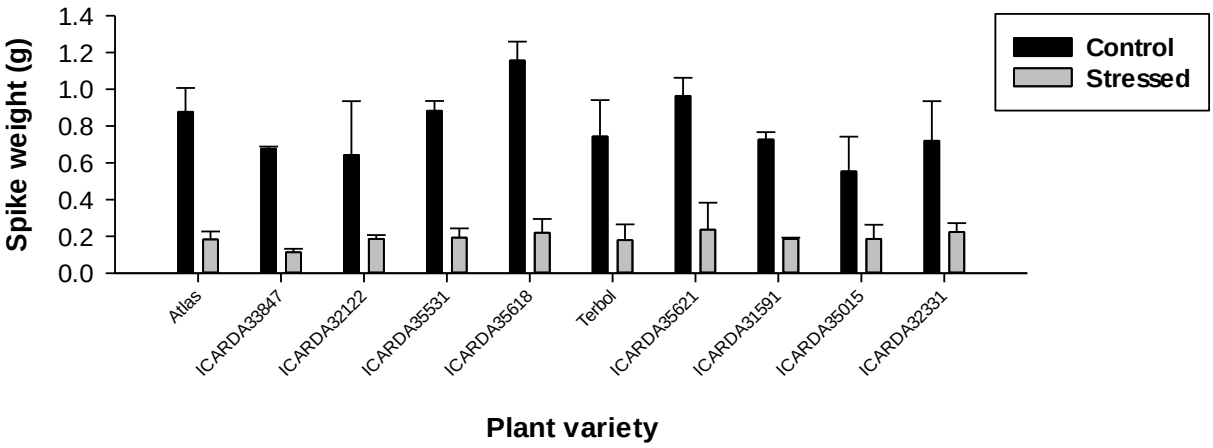


Figure 3.3. Spike weight of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = spike weight (cm). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 0.79 (g) and standard deviation = 0.28. The overall mean under drought stress = 0.19 (g) and standard deviation = 0.10.

Based on the reduction in spike weight under drought conditions, the cultivars were ranked in ascending orders as follows: ICARDA35015 < ICARDA32331 < ICARDA32122 < ICARDA31591 < ICARDA35621 < Terbol < < ICARDA35531 < Atlas < ICARDA35618 < ICARDA33847. Thus, ICARDA35015 and ICARDA32331 topped the list of the best-performing cultivars under drought conditions. However, no cultivar had a significant spike weight under drough conditions based on post-hoc comparative statistical analysis (Table 3.10). As drought tolerance genotype is based on interaction of several several parameters, further statistical analyses were performed in this study (section 3.4.14).

Table 3.10. Post-hoc comparison for spike weight (g) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	0.20±0.17	0.25	-0.15	0.55
		ICARDA 32122	0.23±0.17	0.18	-0.11	0.58
		ICARDA 35531	-0.01±0.17	0.97	-0.35	0.34
		ICARDA 35618	-0.28±0.17	0.11	-0.63	0.07
		Terbol	0.13±0.17	0.44	-0.21	0.48
		ICARDA 35621	-0.09±0.17	0.62	-0.43	0.26
		ICARDA 31591	0.15±0.17	0.39	-0.20	0.50
		ICARDA 35015	0.32±0.17	0.07	-0.02	0.67
		ICARDA 32331	0.16±0.17	0.37	-0.19	0.50
	ICARDA 33847	*ICARDA 32122*	0.03±0.17	0.85	-0.31	0.38
		ICARDA 35531	-0.21±0.17	0.24	-0.55	0.14

	ICARDA 35618	-0.48±0.17	**0.01***	-0.83	-0.13
	Terbol	-0.07±0.17	0.70	-0.41	0.28
	ICARDA 35621	-0.29±0.17	0.10	-0.63	0.06
	ICARDA 31591	-0.05±0.17	0.77	-0.40	0.30
	ICARDA 35015	0.12±0.17	0.48	-0.22	0.47
	ICARDA 32331	-0.04±0.17	0.80	-0.39	0.30
ICARDA 32122	*ICARDA 35531*	-0.24±0.17	0.17	-0.59	0.11
	ICARDA 35618	-0.51±0.17	**<0.001**	-0.86	-0.17
	Terbol	-0.10±0.17	0.56	-0.45	0.25
	ICARDA 35621	-0.32±0.17	0.07	-0.67	0.03
	ICARDA 31591	-0.08±0.17	0.63	-0.43	0.26
	ICARDA 35015	0.09±0.17	0.60	-0.26	0.44
	ICARDA 32331	-0.08±0.17	0.66	-0.42	0.27
ICARDA 35531	*ICARDA 35618*	-0.27±0.17	0.12	-0.62	0.07
	Terbol	0.14±0.17	0.42	-0.21	0.49
	ICARDA 35621	-0.08±0.17	0.64	-0.43	0.27
	ICARDA 31591	0.16±0.17	0.37	-0.19	0.50
	ICARDA 35015	0.33±0.17	0.06	-0.02	0.68
	ICARDA 32331	0.16±0.17	0.35	-0.18	0.51
ICARDA 35618	*Terbol*	0.41±0.17	**0.02**	0.07	0.76
	ICARDA 35621	0.19±0.17	0.27	-0.15	0.54
	ICARDA 31591	0.43±0.17	**0.02**	0.08	0.78
	ICARDA 35015	0.60±0.17	**<0.001**	0.26	0.95
	ICARDA 32331	0.44±0.17	**0.02**	0.09	0.78
Terbol	*ICARDA 35621*	-0.22±0.17	0.21	-0.57	0.13

		ICARDA 31591	0.02±0.17	0.92	-0.33	0.36
		ICARDA 35015	0.19±0.17	0.28	-0.16	0.54
		ICARDA 32331	0.02±0.17	0.89	-0.32	0.37
	ICARDA 35621	*ICARDA 31591*	0.24±0.17	0.18	-0.11	0.58
		ICARDA 35015	0.41±0.17	**0.02**	0.06	0.76
		ICARDA 32331	0.24±0.17	0.16	-0.10	0.59
	ICARDA 31591	*ICARDA 35015*	0.17±0.17	0.32	-0.17	0.52
		ICARDA 32331	0.01±0.17	0.97	-0.34	0.35
	ICARDA 35015	*ICARDA 32331*	-0.17±0.17	0.34	-0.51	0.18
Stressed	*Atlas*	*ICARDA 33847*	0.07±0.17	0.69	-0.28	0.42
		ICARDA 32122	0.00±0.17	0.98	-0.35	0.34
		ICARDA 35531	-0.01±0.17	0.95	-0.36	0.34
		ICARDA 35618	-0.04±0.17	0.83	-0.38	0.31
		Terbol	0.00±0.17	0.98	-0.34	0.35
		ICARDA 35621	-0.05±0.17	0.76	-0.40	0.29
		ICARDA 31591	0.00±0.17	0.98	-0.35	0.34
		ICARDA 35015	0.00±0.17	0.98	-0.35	0.34
		ICARDA 32331	-0.04±0.17	0.82	-0.39	0.31
	ICARDA 33847	*ICARDA 32122*	-0.07±0.17	0.67	-0.42	0.27
		ICARDA 35531	-0.08±0.17	0.64	-0.43	0.27
		ICARDA 35618	-0.11±0.17	0.54	-0.45	0.24
		Terbol	-0.07±0.17	0.70	-0.41	0.28
		ICARDA 35621	-0.12±0.17	0.48	-0.47	0.22
		ICARDA 31591	-0.07±0.17	0.67	-0.42	0.27

	ICARDA 35015	-0.07±0.17	0.67	-0.42	0.27
	ICARDA 32331	-0.11±0.17	0.53	-0.46	0.24
ICARDA 32122	*ICARDA 35531*	-0.01±0.17	0.97	-0.35	0.34
	ICARDA 35618	-0.03±0.17	0.85	-0.38	0.31
	Terbol	0.01±0.17	0.97	-0.34	0.35
	ICARDA 35621	-0.05±0.17	0.77	-0.40	0.30
	ICARDA 31591	0.00±0.17	1.00	-0.35	0.35
	ICARDA 35015	0.00±0.17	1.00	-0.35	0.35
	ICARDA 32331	-0.04±0.17	0.83	-0.38	0.31
ICARDA 35531	*ICARDA 35618*	-0.03±0.17	0.88	-0.37	0.32
	Terbol	0.01±0.17	0.94	-0.33	0.36
	ICARDA 35621	-0.04±0.17	0.80	-0.39	0.30
	ICARDA 31591	0.01±0.17	0.97	-0.34	0.35
	ICARDA 35015	0.01±0.17	0.97	-0.34	0.35
	ICARDA 32331	-0.03±0.17	0.86	-0.38	0.32
ICARDA 35618	*Terbol*	0.04±0.17	0.82	-0.31	0.39
	ICARDA 35621	-0.02±0.17	0.92	-0.36	0.33
	ICARDA 31591	0.03±0.17	0.85	-0.31	0.38
	ICARDA 35015	0.03±0.17	0.85	-0.31	0.38
	ICARDA 32331	0.00±0.17	0.98	-0.35	0.34
Terbol	*ICARDA 35621*	-0.06±0.17	0.74	-0.40	0.29
	ICARDA 31591	-0.01±0.17	0.97	-0.35	0.34
	ICARDA 35015	-0.01±0.17	0.97	-0.35	0.34
	ICARDA 32331	-0.04±0.17	0.80	-0.39	0.30

ICARDA 35621	*ICARDA 31591*	0.05±0.17	0.77	-0.30	0.40
	ICARDA 35015	0.05±0.17	0.77	-0.30	0.40
	ICARDA 32331	0.01±0.17	0.94	-0.33	0.36
ICARDA 31591	*ICARDA 35015*	0.00±0.17	1.00	-0.35	0.35
	ICARDA 32331	-0.04±0.17	0.83	-0.38	0.31
ICARDA 35015	*ICARDA 32331*	-0.04±0.17	0.83	-0.38	0.31

*Data in bold represent significant results at 0.05

3.4.4. Effects on seed number per spike

The ten wheat varieties subjected to drought stress treatment showed an overall decrease in the number of seeds per spike of 79.30%. These findings highlighted the adverse effects of drought on this morphological trait ($p < .001$). As shown in Tables 3.11 and 3.12 a variation of 6.28 to 21.70 in the number of seeds per spike between the 10 genotypes in the control group was recorded. The average number of seeds per spike for all cultivars under these control conditions was 13.52 +/-SD = 6.28. Table 3. 11 illustrates clearly that there is a correlation between the number of seeds per spike and wheat cultivars under stressed and non-stressed conditions. This correlation is revealed by the decreasing number of seeds per spike of wheat, in the control group, from one genotype to another. The same conclusion was drawn for the drought-stressed samples where a significant change in the order of the mean number of seeds per spike per variety was observed as follows (also Figure 3.4):

Table 3.11. Average seed number per spike of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 35618	21.70	ICARDA 35618	4.72
ICARDA 35531	18.42	ICARDA 35621	4.02
ICARDA 35621	17.77	ICARDA 32331	3.77
Atlas	15.97	ICARDA 35531	3.30
ICARDA 31591	12.75	ICARDA 35015	3.14

Terbol	12.42	ICARDA 31591	2.93
ICARDA 33847	12.31	Terbol	2.88
ICARDA 32331	9.19	Atlas	1.42
ICARDA 35015	8.44	ICARDA 33847	0.95
ICARDA 32122	6.28	ICARDA 32122	0.97

These results were consistent with the ANOVA data which confirmed the significant effect of wheat varieties ($F(9, 40) = 2.59$, $p < 0.019$) and treatment ($F(1, 40) = 92.49$, $p < 0.001$) on seed number per spike. However, there was no significant effect of the interaction between varieties and treatment on the number of seeds per spike ($F(9,40) = 1.54$, $p = .169$) as mentioned in Table 3.12.

Table 3.12. Analysis of variance for number of seeds per Spike under Control (100- 85% WFC) and Drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	2.59	9	**<0.019**
Treatment	92.49	1	**<0.001**
Variety * Treatment	1.54	9	<0.169
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

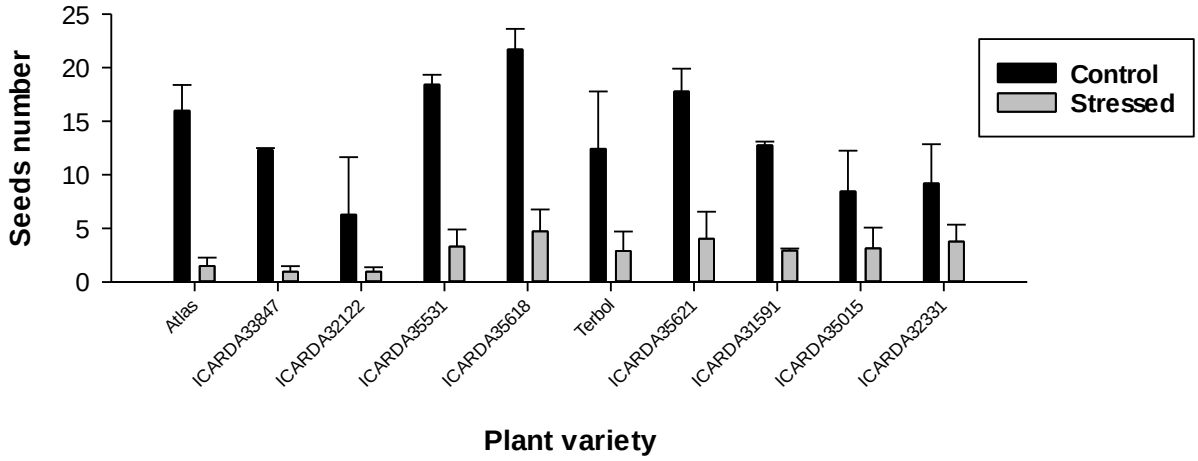


Figure 3.4. Number of seeds per spike in wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = number of seeds per spike. Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 13.52 and standard deviation = 6.28. The overall mean under drought stress = 2.81 and standard deviation = 2.54.

A maximum decrease in the number of seeds per spike of 92.28%, 91.13%, 84.60% was detected for genotypes ICARDA 33847, Atlas, ICARDA 32122, respectively. These results confirmed the sensitivity of these three cultivars to drought stress. However, the cultivars ICARDA 31591 (76.99%), ICARDA 35015 (62.81%) and ICARDA 32331 (59.03%) showed the lowest reduction in the number of seeds per spike, demonstrating that these particular genotypes were more resistant to drought stress. Thus, ICARDA32331 and ICARDA35015 topped the list of the best-performing cultivars under drought conditions; results coherent with those for spike weight. A marked difference in seed number per spike between the control sample (M = 13.52, SE = 0.79) and the drought-stressed samples (M = 2.81, SE = 0.79) was obtained, with an average difference of 10.71 ($p < 0.001$). Like all the other morphological parameters listed above, the control wheat samples showed the highest number of seeds. However, under drought conditions, no statistical significance of seed number per varitety was observed based on post-hoc comparative analysis (Table 3.13). As drought tolerance genotype is based on interaction of several several parameters, further statistical analyses were performed in this study (section 3.4.14).

Table 3.13. Post-hoc comparison for seeds number per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	3.66±3.52	0.30	-3.45	10.78
		ICARDA 32122	9.69±3.52	**0.01**	2.57	16.81
		ICARDA 35531	-2.44±3.52	0.49	-9.56	4.67
		ICARDA 35618	-5.72±3.52	0.11	-12.84	1.39
		Terbol	3.55±3.52	0.31	-3.56	10.67
		ICARDA 35621	-1.79±3.52	0.61	-8.91	5.32
		ICARDA 31591	3.22±3.52	0.36	-3.89	10.34
		ICARDA 35015	7.53*±3.52	**0.03**	0.41	14.65
		ICARDA 32331	6.78±3.52	0.06	-0.34	13.90
	ICARDA 33847	*ICARDA 32122*	6.03±3.52	0.09	-1.08	13.15
		ICARDA 35531	-6.10±3.52	0.09	-13.22	1.01
		ICARDA 35618	-9.39±3.52	**0.01**	-16.51	-2.27
		Terbol	-0.10±3.52	0.97	-7.22	7.01
		ICARDA 35621	-5.45±3.52	0.12	-12.57	1.66
		ICARDA 31591	-o.43±3.52	0.90	-7.55	6.68
		ICARDA 35015	3.86±3.52	0.27	-3.25	10.98
		ICARDA 32331	3.11±3.52	0.38	-4.00	10.23
	ICARDA 32122	*ICARDA 35531*	-12.14±3.52	**<0.001**	-19.26	-5.02

	ICARDA 35618	-15.42±3.52	**<0.001**	-22.54	-8.30
	Terbol	-6.14±3.52	0.08	-13.26	0.98
	ICARDA 35621	-11.49±3.52	**0.01**	-18.61	-4.37
	ICARDA 31591	-6.47±3.52	0.07	-13.59	0.65
	ICARDA 35015	-2.16±3.52	0.54	-9.28	4.95
	ICARDA 32331	-2.91±3.52	0.41	-10.03	4.20
ICARDA 35531	*ICARDA 35618*	-3.28±3.52	0.35	-10.40	3.83
	Terbol	6.00±3.52	0.09	-1.12	13.12
	ICARDA 35621	0.65±3.52	0.85	-6.47	7.77
	ICARDA 31591	5.67±3.52	0.11	-1.45	12.79
	ICARDA 35015	9.97±3.52	**0.01**	2.85	17.09
	ICARDA 32331	9.22±3.52	**0.01**	2.10	16.34
ICARDA 35618	*Terbol*	9.28±3.52	0.01	2.16	16.40
	ICARDA 35621	3.93±3.52	0.27	-3.18	11.05
	ICARDA 31591	8.95±3.52	**0.01**	1.83	16.07
	ICARDA 35015	13.25±3.52	**<0.001**	6.13	20.37
	ICARDA 32331	12.50±3.52	**<0.001**	5.38	19.62
Terbol	*ICARDA 35621*	-5.35±3.52	0.13	-12.47	1.77
	ICARDA 31591	-0.33±3.52	0.92	-7.45	6.79
	ICARDA 35015	3.97±3.52	0.26	-3.14	11.09
	ICARDA 32331	3.22±3.52	0.36	-3.89	10.34
ICARDA 35621	*ICARDA 31591*	5.02±3.52	0.16	-2.10	12.14
	ICARDA 35015	9.32±3.52	**0.01**	2.20	16.44
	ICARDA 32331	8.57±3.52	**0.02**	1.45	15.69

	ICARDA 31591	*ICARDA 35015*	4.30±3.52	0.22	-2.81	11.42
		ICARDA 32331	3.55±3.52	0.31	-3.56	10.67
	ICARDA 35015	*ICARDA 32331*	-0.75±3.52	0.83	-7.87	6.37
Stressed	*Atlas*	*ICARDA 33847*	0.46±3.52	0.89	-6.65	7.58
		ICARDA 32122	0.45±3.52	0.89	-6.67	7.57
		ICARDA 35531	-1.88±3.52	0.59	-9.00	5.23
		ICARDA 35618	-3.30±3.52	0.35	-10.42	3.81
		Terbol	-1.46±3.52	0.67	-8.58	5.65
		ICARDA 35621	-2.60±3.52	0.46	-9.72	4.51
		ICARDA 31591	-1.51±3.52	0.66	-8.637	5.60
		ICARDA 35015	-1.72±3.52	0.62	-8.84	5.39
		ICARDA 32331	-2.35±3.52	0.50	-9.47	4.77
	ICARDA 33847	*ICARDA 32122*	-.017±3.52	0.99	-7.13	7.10
		ICARDA 35531	-2.35±3.52	0.50	-9.47	4.77
		ICARDA 35618	-3.77±3.52	0.29	-10.89	3.34
		Terbol	-1.93±3.52	0.58	-9.05	5.18
		ICARDA 35621	-3.07±3.52	0.38	-10.19	4.04
		ICARDA 31591	-1.98±3.52	0.57	-9.10	5.13
		ICARDA 35015	-2.19±3.52	0.53	-9.31	4.93
		ICARDA 32331	-2.81±3.52	0.42	-9.93	4.30
	ICARDA 32122	*ICARDA 35531*	-2.33±3.52	0.51	-9.45	4.78
		ICARDA 35618	-3.75±3.52	0.29	-10.87	3.36
		Terbol	-1.91±3.52	0.58	-9.03	5.20
		ICARDA 35621	-3.05±3.52	0.39	-10.17	4.06

	ICARDA 31591	-1.96±3.52	0.58	-9.08	5.15
	ICARDA 35015	-2.17±3.52	0.54	-9.29	4.94
	ICARDA 32331	-2.80±3.52	0.43	-9.92	4.32
ICARDA 35531	*ICARDA 35618*	-1.42±3.52	0.68	-8.54	5.69
	Terbol	0.41±3.52	0.90	-6.70	7.53
	ICARDA 35621	-0.72±3.52	0.83	-7.84	6.39
	ICARDA 31591	0.36±3.52	0.91	-6.75	7.48
	ICARDA 35015	0.16±3.52	0.96	-6.96	7.28
	ICARDA 32331	-0.46±3.52	0.89	-7.58	6.65
ICARDA 35618	*Terbol*	1.84±3.52	0.60	-5.28	8.96
	ICARDA 35621	0.70±3.52	0.84	-6.42	7.82
	ICARDA 31591	1.79±3.52	0.61	-5.33	8.91
	ICARDA 35015	1.58±3.52	0.65	-5.53	8.70
	ICARDA 32331	0.95±3.52	0.78	-6.16	8.07
Terbol	*ICARDA 35621*	-1.14±3.52	0.74	-8.26	5.98
	ICARDA 31591	-0.05±3.52	0.98	-7.17	7.07
	ICARDA 35015	-0.25±3.52	0.94	-7.37	6.86
	ICARDA 32331	-0.88±3.52	0.80	-8.00	6.23
ICARDA 35621	*ICARDA 31591*	1.09±3.52	0.75	-6.03	8.21
	ICARDA 35015	0.88±3.52	0.80	-6.23	8.00
	ICARDA 32331	0.25±3.52	0.94	-6.86	7.37
ICARDA 31591	*ICARDA 35015*	-0.20±3.52	0.95	-7.32	6.91
	ICARDA 32331	-0.83±3.52	0.81	-7.95	6.28
ICARDA 35015	*ICARDA 32331*	-0.62±3.52	0.86	-7.74	6.49

*Data in bold represent significant results at 0.05.

3.4.5. Effects on seed weight per spike (g)

Another relevant parameter, seed weight per spike, used for screening resistant genotypes, was considered in this study**.** This parameter appeared to be affected by drought stress for all the studied cultivars, with a p value < .001 (Tables 3.14 and 3.15). An overall decrease in the seed weight per spike of 83.08% was estimated for all the cultivars. This seed parameter varied from 0.28 to 0.91g and from 0.04 to 0.15 g, with a mean of 0.58 +/-SD = 0.27 and 0.10 +/-SD = 0.09 under control and drought stress conditions, respectively.

Given the following order of genotypes in relation to seed weight, there appeared to be some correlation between wheat varieties and seed weight per spike, independent of treatment, particularly for the best performing ICARDA varieties. The average seed weight for samples under control conditions and drought stress were as follows (Figure 3.5):

Table 3. 14. Average seed weight per spike (g) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions in descending order.

Optimal (control) conditions	**Mean**	**Drought conditions**	**Mean**
ICARDA 35618	0.91	ICARDA 35621	0.15
ICARDA 35621	0.75	ICARDA 35618	0.15
Atlas	0.70	ICARDA 32331	0.13
ICARDA 35531	0.69	ICARDA 35531	0.11
ICARDA 31591	0.57	ICARDA 31591	0.11
Terbol	0.51	ICARDA 35015	0.10
ICARDA 33847	0.48	Terbol	0.09
ICARDA 32331	0.48	ICARDA 33847	0.06
ICARDA 35015	0.39	Atlas	0.06
ICARDA 32122	0.28	ICARDA 32122	0.04

However, the Anova data showed no significant effect of variety ($F(9,40) = 2.06$, $p < .057$) on seeds weight, though the order of the cultivars, based on the seed weight values, was affected by the drought stress.The same conclusions were drawn for the interaction between variety and

treatment on seed weight, F(9,40) = 1.221, p = 0.31 (Table 3.15) while it appeared that only treatment (F(1, 40) = 102.908, p < .001) had a significant effect on seed weight.

Table 3.15. Analysis of variance for seeds' weight per spike under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	2.06	9	<0.057
Treatment	102.908	1	**<0.001**
Variety * Treatment	1.221	9	<0.31
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

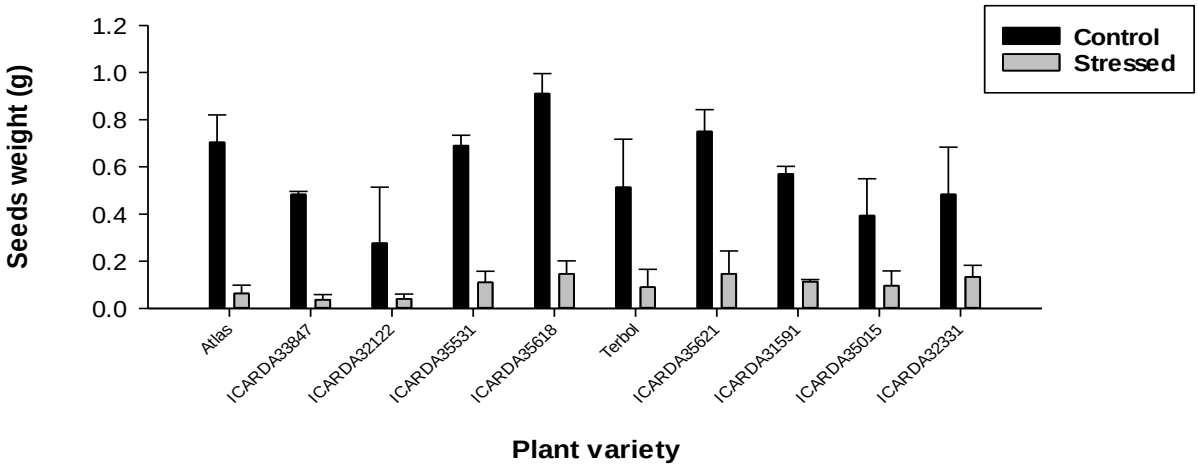


Figure 3.5. Weight of seeds per spike of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = weight of seeds per spike. Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 0.57g and standard deviation = 0.27. The overall mean under drought stress = 0.98g and standard deviation = 0.08.

It was found that the cultivars Atlas, ICARDA 33847, ICARDA 32122 exhibited the greatest decrease in seed weight being 90.96%, 87.76%, 84.66% respectively, highlighting their drought stress sensitive genotype, while varieties ICARDA 31591 (76.99%), ICARDA 35015 (62.81%) and ICARDA 32331(59.03%) had the least reduction in this parameter confirming their resistant genotype to drought stress. Thus, ICARDA32331 and ICARDA35015 topped the list of the best-performing cultivars under drought conditions; results coherent with those for spike weight and number of seeds.

The mean difference in seed weight between the treated and untreated samples was 0.48 g ($p < .001$). These data were consistent with the significant variation in seed weight between control wheat (M = 0.58, SE = 0.03) and drought stress samples (M = 0.10, SE = 0.03). The highest seed weight was from the wheat under control conditions, as were the other parameters mentioned above. However, under drought conditions, no statistical significance of seed weight per varitety was observed based on post-hoc comparative analysis (Table 3.16). These inconclusive data were also obtained for seed number and spike weight. As drought tolerance is based on interaction between different parameters, further statistical analyses were performed in this study (section 3.4.14).

Table 3.16. Post-hoc comparison for seeds weight (g) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	0.22±.15	0.15	-0.08	0.52
		ICARDA 32122	0.43±.15	**0.01**	0.12	0.73
		ICARDA 35531	0.01±.15	0.93	-0.29	0.32
		ICARDA 35618	-0.21±.15	0.17	-0.51	0.09
		Terbol	0.19±.15	0.21	-0.11	0.49

	ICARDA 35621	-0.05±.15	0.76	-0.31	0.26
	ICARDA 31591	0.13±.15	0.38	-0.17	0.44
	ICARDA 35015	0.31±.15	**0.04**	0.01	0.61
	ICARDA 32331	0.22±.15	0.15	-0.08	0.52
ICARDA 33847	*ICARDA 32122*	0.21±.15	0.17	-0.09	0.51
	ICARDA 35531	-0.21±.15	0.17	-0.51	0.09
	ICARDA 35618	-0.43±.15	**0.01**	-0.73	-0.12
	Terbol	-0.03±.15	0.84	-0.33	0.27
	ICARDA 35621	-0.27±.15	0.08	-0.57	0.04
	ICARDA 31591	-0.09±.15	0.56	-0.39	0.27
	ICARDA 35015	0.09±.15	0.55	-0.21	0.39
	ICARDA 32331	-4.16±.15	1.00	-0.30	0.30
ICARDA 32122	*ICARDA 35531*	-0.41±.15	**0.01**	-0.72	-0.11
	ICARDA 35618	-0.63±.15	**<0.001**	-0.93	-0.33
	Terbol	-0.23±.15	0.12	-0.54	0.06
	ICARDA 35621	-0.47±.15	**0.01**	-0.77	-0.17
	ICARDA 31591	-0.29±.15	0.06	-0.59	0.01
	ICARDA 35015	-0.12±.15	0.44	-0.41	0.18
	ICARDA 32331	-0.21±.15	0.17	-0.50	0.09
ICARDA 35531	*ICARDA 35618*	-0.22±.15	0.14	-0.52	0.08
	Terbol	0.17±.15	0.24	-0.12	0.47
	ICARDA 35621	-0.06±.15	0.69	-0.36	0.24
	ICARDA 31591	0.12±.15	0.42	-0.18	0.42
	ICARDA 35015	0.29±.15	**0.05**	-0.01	0.59
	ICARDA 32331	0.20±.15	0.17	-0.09	0.50

	ICARDA 35618	*Terbol*	0.39±.15	**0.01**	0.09	0.69
		ICARDA 35621	0.16±.15	0.29	-0.14	0.46
		ICARDA 31591	0.34±.15	0.02	0.03	0.64
		ICARDA 35015	0.51±.15	**<0.001**	0.21	0.81
		ICARDA 32331	0.42±.15	**0.01**	0.12	0.72
	Terbol	*ICARDA 35621*	-0.23±.15	0.12	-0.53	0.06
		ICARDA 31591	-0.05±.15	0.70	-0.35	0.24
		ICARDA 35015	0.12±.15	0.42	-0.18	0.42
		ICARDA 32331	0.03±.15	0.84	-0.27	0.33
	ICARDA 35621	*ICARDA 31591*	0.18±.15	0.23	-0.12	0.48
		ICARDA 35015	0.35±.15	**0.02**	0.05	0.65
		ICARDA 32331	0.26±.15	0.08	-0.03	0.56
	ICARDA 31591	*ICARDA 35015*	0.17±.15	0.24	-0.12	0.47
		ICARDA 32331	0.08±.15	0.56	-0.21	0.38
	ICARDA 35015	*ICARDA 32331*	-0.09±.15	0.55	-0.39	0.21
Stressed	*Atlas*	*ICARDA 33847*	0.02±.15	0.85	-0.27	0.32
		ICARDA 32122	0.02±.15	0.87	-0.27	0.32
		ICARDA 35531	-0.04±.15	0.75	-0.34	0.25
		ICARDA 35618	-0.08±.15	0.58	-0.38	0.21
		Terbol	-0.02±.15	0.85	-0.32	0.27
		ICARDA 35621	-0.08±.15	0.58	-0.38	0.21
		ICARDA 31591	-0.05±.15	0.74	-0.35	0.25
		ICARDA 35015	-0.03±.15	0.82	-0.33	0.26
		ICARDA 32331	-0.07±.15	0.64	-0.37	0.23

ICARDA 33847	*ICARDA 32122*	-0.00±.15	0.98	-0.30	0.29
	ICARDA 35531	-0.07±.15	0.62	-0.37	0.22
	ICARDA 35618	-0.11±.15	0.46	-0.41	0.19
	Terbol	-0.05±.15	0.72	-0.35	0.24
	ICARDA 35621	-0.11±.15	0.46	-0.41	0.19
	ICARDA 31591	-0.07±.15	0.61	-0.37	0.22
	ICARDA 35015	-0.06±.15	0.69	-0.36	0.24
	ICARDA 32331	-0.09±.15	0.52	-0.39	0.20
ICARDA 32122	*ICARDA 35531*	-0.07±.15	0.64	-0.37	0.23
	ICARDA 35618	-0.10±.15	0.48	-0.40	0.19
	Terbol	-0.05±.15	0.74	-0.35	0.25
	ICARDA 35621	-0.10±.15	0.48	-0.40	0.19
	ICARDA 31591	-0.07±.15	0.62	-0.37	0.22
	ICARDA 35015	-0.05±.15	0.70	-0.35	0.24
	ICARDA 32331	-0.09±.15	0.53	-0.39	0.20
ICARDA 35531	*ICARDA 35618*	-0.03±.15	0.80	-0.33	0.26
	Terbol	0.02±.15	0.89	-0.28	0.32
	ICARDA 35621	-0.03±.15	0.80	-0.33	0.26
	ICARDA 31591	-0.00±.15	0.98	-0.30	0.29
	ICARDA 35015	0.01±.15	0.92	-0.28	0.31
	ICARDA 32331	-0.02±.15	0.87	-0.32	0.27
ICARDA 35618	*Terbol*	0.05±.15	0.70	-0.24	0.35
	ICARDA 35621	1.38±.15	1.00	-0.30	0.30
	ICARDA 31591	0.03±.15	0.82	-0.26	0.33
	ICARDA 35015	0.05±.15	0.74	-0.25	0.35

	ICARDA 32331	0.01±.15	0.92	-0.28	0.31
Terbol	*ICARDA 35621*	-0.05±.15	0.70	-0.35	0.24
	ICARDA 31591	-0.02±.15	0.87	-0.32	0.27
	ICARDA 35015	-0.01±.15	0.96	-0.30	0.29
	ICARDA 32331	-0.04±.15	0.77	-0.34	0.25
ICARDA 35621	*ICARDA 31591*	0.03±.15	0.82	-0.26	0.33
	ICARDA 35015	0.05±.15	0.74	-0.25	0.35
	ICARDA 32331	0.01±.15	0.92	-0.28	0.31
ICARDA 31591	*ICARDA 35015*	0.01±.15	0.91	-0.28	0.31
	ICARDA 32331	-0.02±.15	0.89	-0.32	0.28
ICARDA 35015	*ICARDA 32331*	-0.03±.15	0.80	-0.33	0.26

*Data in bold represent significant results at 0.05

3.4.6. Effects on leaf number per plant:

The number of leaves per plant has been considered in several studies as a good marker to evaluate the effect of drought stress on wheat. The 10 cultivars investigated in the present study, taken together, showed a decrease in leaf number of 24.17% following drought stress treatment. The variation in the number of leaves per wheat sample ranged from 7.23 to 19.13 and from 7.78 to 9.92, with an average of 11.66 +/-SD = 3.76 and 8.84, and +/- SD = 1.42, estimated in wheat samples under control and stress treatment, respectively. These findings confirmed the considerable effect of drought stress on the number of leaves per plant, with the p-value below the cut-off point of 0.05. On the basis of the average number of wheat leaves calculated under both control and drought conditions, the studied cultivars were ranked as follows:

Table 3.17. Average leaf number per plant of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 33847	19.13	ICARDA 32122	9.92
ICARDA 32122	15.86	Terbol	9.50
Terbol	12.47	ICARDA 31591	9.33
ICARDA 32331	12.00	ICARDA 35621	9.13
ICARDA 35531	11.33	ICARDA 33847	9.00
ICARDA 31591	10.47	Atlas	8.72
ICARDA 35015	10.21	ICARDA 35015	8.39
ICARDA 35618	8.98	ICARDA 35531	8.38
ICARDA 35621	8.91	ICARDA 32331	8.26
Atlas	7.23	ICARDA 35618	7.78

As seen for most other parameters investigated in the present study, the order of the wheat varieties under drought stress treatment differed to that under optimal (control) conditions (Figure 3.6, Table 3.17). Based on statistical analyses, variety ($F(9,40) = 7.36$, $p < 0.001$), treatment ($F(1, 40) = 39.37$, $p < .001$), and the association between varieties and treatment ($F(9,40) = 5.42$, $p = 0.001$) showed a significant main effect on leaf number, as shown in Table 3.18. The mean difference of 2.82, ($p < .001$) in leaf number between control ($M = 11.66$, $SE = 0.31$) and drought stressed samples ($M = 8.84$, $SE = 0.31$) was found to be significant. As with other parameters, the control sample had a higher number of leaves (Table 3.18).

Table 3.18. Analysis of variance number of leaves in wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	7.364	9	**<0.001***
Treatment	39.372	1	**<0.001**
Variety * Treatment	5.424	9	**<0.001**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

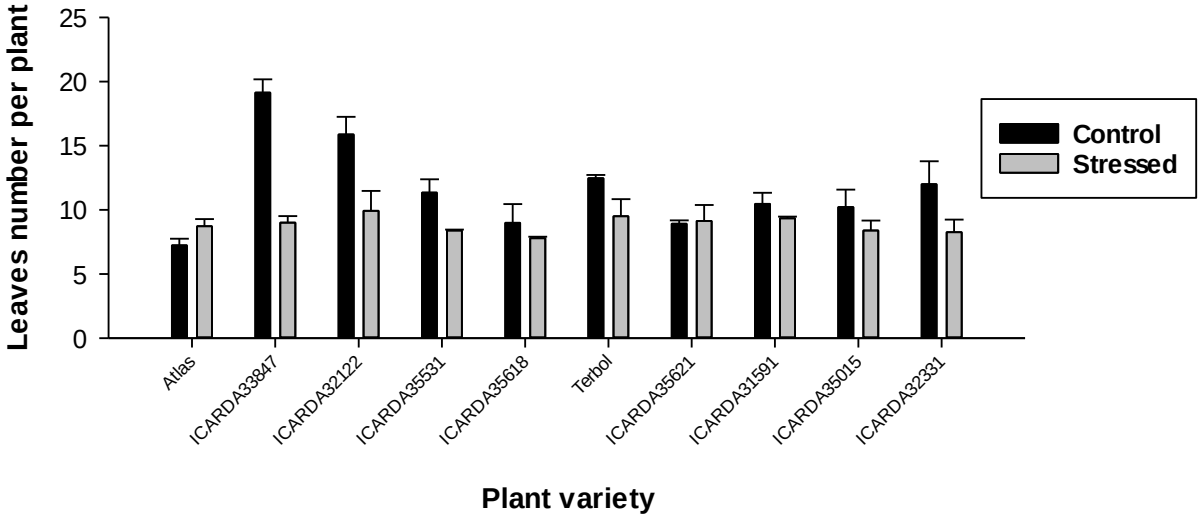


Figure 3.6. Number of leaves per plant for wheat cultivars under control (100-85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = number of leaves per plant. Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 11.66 and standard deviation = 3.76. The overall mean under drought stress = 8.84 and standard deviation = 1.42.

Based on the reduction in number of leaves under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < ICARDA35621 < ICARDA31591 < ICARDA35618 < ICARDA35015 < Terbol < ICARDA35531 < ICARDA32331 < ICARDA32122 < ICARDA33847. Thus, Atlas topped the list of the best-performing cultivars under drought conditions; results coherent with those for plant height, spike length, number of tillers. The low reduction in the number of leaves in the cultivars ranked above (eg. Atlas) under the effect of drought, may be explained by the attempt of the cultivars to complete their life cycle before water limitation intensified. This escape mechanism from drought stress is adopted by plants with a sensitive phenotype in arid and semi-arid countries (Basu et al. 2017). Post-hoc comparison showed that there is no statistical significance of drought on leaf number for the cultivas under drought conditions (Table 3.19). Further statistical tests were performed below (section 3.4.14).

Table 3.19. Post-hoc comparison for number of leaves / plants per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-11.90±1.42	**<0.001***	-14.77	-9.03
		ICARDA 32122	-8.63±1.42	**<0.001**	-11.50	-5.76
		ICARDA 35531	-4.10±1.42	**0.01**	-6.97	-1.23
		ICARDA 35618	-1.75±1.42	0.23	-4.62	1.12
		Terbol	-5.23±1.42	**<0.001**	-8.10	-2.36
		ICARDA 35621	-1.68±1.42	0.24	-4.55	1.19
		ICARDA 31591	-3.23±1.42	**0.03**	-6.10	-0.36
		ICARDA 35015	-2.98±1.42	**0.04**	-5.85	-0.11
		ICARDA 32331	-4.77±1.42	**<0.001**	-7.64	-1.90
	ICARDA 33847	*ICARDA 32122*	3.27±1.42	**0.03**	0.40	6.14
		ICARDA 35531	7.80±1.42	**<0.001**	4.93	10.67
		ICARDA 35618	10.15±1.42	**<0.001**	7.28	13.02
		Terbol	6.67±1.42	**<0.001**	3.80	9.54
		ICARDA 35621	10.22±1.42	**<0.001**	7.35	13.09
		ICARDA 31591	8.67±1.42	**<0.001**	5.80	11.54
		ICARDA 35015	8.92±1.42	**<0.001**	6.05	11.79
		ICARDA 32331	7.13±1.42	**<0.001**	4.26	10.00
	ICARDA 32122	*ICARDA 35531*	4.53±1.42	**<0.001**	1.66	7.40

	ICARDA 35618	6.88±1.42	**<0.001**	4.01	9.75
	Terbol	3.40±1.42	**0.02**	0.53	6.27
	ICARDA 35621	6.95±1.42	**<0.001**	4.08	9.82
	ICARDA 31591	5.40±1.42	**<0.001**	2.53	8.27
	ICARDA 35015	5.65±1.42	**<0.001**	2.78	8.52
	ICARDA 32331	3.86±1.42	**0.01**	0.99	6.73
ICARDA 35531	*ICARDA 35618*	2.35±1.42	0.11	-0.52	5.22
	Terbol	-1.13±1.42	0.43	-4.00	1.74
	ICARDA 35621	2.42±1.42	0.10	-0.45	5.29
	ICARDA 31591	0.87±1.42	0.55	-2.00	3.74
	ICARDA 35015	1.12±1.42	0.43	-1.75	3.99
	ICARDA 32331	-0.67±1.42	0.64	-3.54	2.20
ICARDA 35618	*Terbol*	-3.48±1.42	**0.02**	-6.35	-0.61
	ICARDA 35621	0.07±1.42	0.96	-2.80	2.94
	ICARDA 31591	-1.48±1.42	0.30	-4.35	1.39
	ICARDA 35015	-1.23±1.42	0.39	-4.10	1.64
	ICARDA 32331	-3.02±1.42	**0.04**	-5.89	-0.15
Terbol	*ICARDA 35621*	3.56±1.42	**0.02**	0.69	6.43
	ICARDA 31591	2.00±1.42	0.17	-0.87	4.87
	ICARDA 35015	2.26±1.42	0.12	-0.61	5.13
	ICARDA 32331	0.47±1.42	0.74	-2.40	3.34
ICARDA 35621	*ICARDA 31591*	-1.56±1.42	0.28	-4.43	1.31
	ICARDA 35015	-1.30±1.42	0.37	-4.17	1.57
	ICARDA 32331	-3.09±1.42	**0.04**	-5.96	-0.22

	ICARDA 31591	*ICARDA 35015*	0.26±1.42	0.86	-2.61	3.13
		ICARDA 32331	-1.53±1.42	0.29	-4.40	1.34
	ICARDA 35015	*ICARDA 32331*	-1.79±1.42	0.21	-4.66	1.08
Stressed	*Atlas*	*ICARDA 33847*	-0.28±1.42	0.85	-3.15	2.59
		ICARDA 32122	-1.19±1.42	0.41	-4.06	1.68
		ICARDA 35531	0.34±1.42	0.81	-2.53	3.21
		ICARDA 35618	0.94±1.42	0.51	-1.93	3.81
		Terbol	-0.78±1.42	0.59	-3.65	2.09
		ICARDA 35621	-0.41±1.42	0.77	-3.28	2.46
		ICARDA 31591	-0.61±1.42	0.67	-3.48	2.26
		ICARDA 35015	0.33±1.42	0.82	-2.54	3.20
		ICARDA 32331	0.47±1.42	0.74	-2.40	3.34
	ICARDA 33847	*ICARDA 32122*	-0.92±1.42	0.52	-3.79	1.95
		ICARDA 35531	0.62±1.42	0.67	-2.25	3.49
		ICARDA 35618	1.22±1.42	0.40	-1.65	4.09
		Terbol	-0.50±1.42	0.73	-3.37	2.37
		ICARDA 35621	-0.13±1.42	0.93	-3.00	2.74
		ICARDA 31591	-0.33±1.42	0.82	-3.20	2.54
		ICARDA 35015	0.61±1.42	0.67	-2.26	3.48
		ICARDA 32331	0.74±1.42	0.60	-2.13	3.61
	ICARDA 32122	*ICARDA 35531*	1.53±1.42	0.29	-1.34	4.40
		ICARDA 35618	2.13±1.42	0.14	-0.74	5.00
		Terbol	0.42±1.42	0.77	-2.45	3.29
		ICARDA 35621	0.78±1.42	0.58	-2.09	3.65

	ICARDA 31591	0.58±1.42	0.68	-2.29	3.45
	ICARDA 35015	1.53±1.42	0.29	-1.34	4.40
	ICARDA 32331	1.66±1.42	0.25	-1.21	4.53
ICARDA 35531	*ICARDA 35618*	0.60±1.42	0.67	-2.27	3.47
	Terbol	-1.12±1.42	0.44	-3.99	1.75
	ICARDA 35621	-0.75±1.42	0.60	-3.62	2.12
	ICARDA 31591	-0.95±1.42	0.51	-3.82	1.92
	ICARDA 35015	-0.01±1.42	1.00	-2.88	2.86
	ICARDA 32331	0.13±1.42	0.93	-2.74	3.00
ICARDA 35618	*Terbol*	-1.72±1.42	0.23	-4.59	1.15
	ICARDA 35621	-1.35±1.42	0.35	-4.22	1.52
	ICARDA 31591	-1.55±1.42	0.28	-4.42	1.32
	ICARDA 35015	-0.61±1.42	0.67	-3.48	2.26
	ICARDA 32331	-0.47±1.42	0.74	-3.34	2.40
Terbol	*ICARDA 35621*	0.37±1.42	0.80	-2.50	3.24
	ICARDA 31591	0.17±1.42	0.91	-2.70	3.04
	ICARDA 35015	1.11±1.42	0.44	-1.76	3.98
	ICARDA 32331	1.24±1.42	0.39	-1.63	4.11
ICARDA 35621	*ICARDA 31591*	-0.20±1.42	0.89	-3.07	2.67
	ICARDA 35015	0.74±1.42	0.60	-2.13	3.61
	ICARDA 32331	0.88±1.42	0.54	-1.99	3.75
ICARDA 31591	*ICARDA 35015*	0.94±1.42	0.51	-1.93	3.81
	ICARDA 32331	1.08±1.42	0.45	-1.79	3.95
ICARDA 35015	*ICARDA 32331*	0.13±1.42	0.93	-2.74	3.00

*Data in bold represent significant results at 0.05.

3.4.7. Effects on Number of tillers

The results of our study showed that the 10 wheat cultivars subjected to 50% WFC had a 48.12% drop in the number of tillers ($p < .001$), which highlighted the significant effect of drought stress on number of tillers. For all the wheat cultivars used in this present work, the variation in the number of tillers was from 0.13 to 3.90 and from 0.27 to 1.33 observed in wheat under control (a mean of 1.30 +/-SD = 1.13) and drought stress conditions (a mean of 0.67 +/-SD = 0.57) respectively. The ranking of varieties based on the mean value of the number of tillers in the wheat samples under control conditions differed to the ranking under drought conditions (Table 3.20; Figure 3.7):

Table 3.20. Average number of tillers of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 33847	3.90	ICARDA 31591	1.33
ICARDA 32122	2.08	ICARDA 33847	1.07
ICARDA 32331	1.75	Atlas	0.89
Terbol	1.22	ICARDA 32331	0.70
ICARDA 35531	1.07	Terbol	0.60
ICARDA 31591	1.05	ICARDA 35621	0.60
ICARDA 35015	0.91	ICARDA 35531	0.47
ICARDA 35618	0.57	ICARDA 32122	0.42
ICARDA 35621	0.30	ICARDA 35015	0.39
Atlas	0.13	ICARDA 35618	0.27

Under drought conditions, the number of tillers was significantly increased in the cultivar Atlas, doubled in the cultivar ICARDA 35621 and slightly raised in the cultivar ICARDA 31591, whereas it decreased in the other cultivars. These results were consistent with the number of leaves in Atlas, and ICARDA 35621 where the drought did not reduce the number of leaves. However, these cultivars showed a significant decrease in the seed number and weight under drought conditions (sections 3.4.4 and 3.4.5). It is clear that Atlas, ICARDA 35621, and ICARDA 31591 cultivars tried to adapt and cope with drought stress by allocating their limited resources in nutrient and

water to the development of more leaves and tillers, resulting on less energy for producing seeds (Naseer et al 2024).

Table 3.21. Analysis of variance number of tillers for all the studied wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	7.86	9	**<0.001***
Treatment	20.2	1	**<0.001**
Variety * Treatment	5.68	9	**<0.001**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

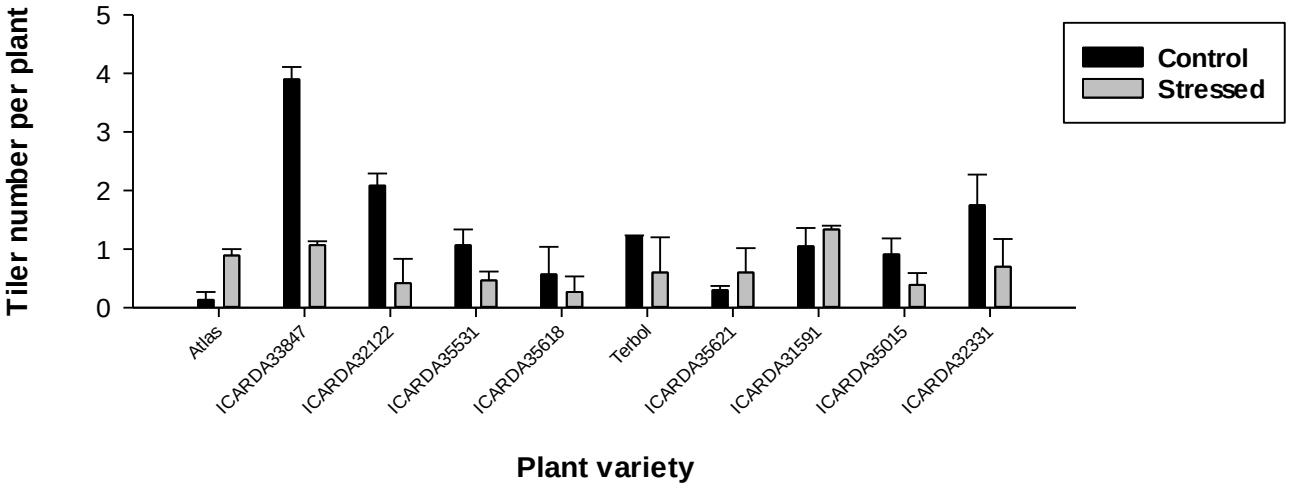


Figure 3.7. Number of tillers per wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = number of tillers. Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 1.29 and standard deviation = 1.13. The overall mean under drought stress = 0.67 and standard deviation = 0.57.

Like seed weight per spike, variety ($F(9,40) = 7.86$, $p < .001$), treatment $F(1, 40) = 20.20$, ($p < .001$), and the interaction of variety and treatment ($F(9,40) = 5.68$, $p = 0.001$) had a significant effect on the number of tiller as shown in Table 3.21. The results of the Tukey tests confirmed the considerable difference in the number of tillers between samples under control ($M = 1.30$, $SE = 0.10$) and under drought stress ($M = 0.67$, $SE = 0.10$) conditions. Pairwise comparison between the two wheat groups showed a mean difference of 0.62 $p < .001$, with the highest number of tillers observed in the control samples.

Based on the reduction in number of tillers under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < ICARDA35621 < ICARDA31591 < Terbol < ICARDA35618 < ICARDA35531 < ICARDA35015 < ICARDA32331 < ICARDA33847 < ICARDA32122. Thus, Atlas topped the list of the best-performing cultivars under drought conditions; results coherent with those for plant height, spike length, number of leaves.

To further explore which ICARDA bread wheat varieties had significant number of tillers under stress regime, Tukey's post-hoc comparison was performed. The results indicated that the cultivars ICARDA 32122, ICARDA 35618, and ICARDA 31591 had significantly greater number of tillers under drought stress. A detailed comparison is provided in Table 3. 22.

Table 3.22. Post-hoc comparison for number of tillers per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-3.77±0.44	**<0.001***	-4.65	-2.88
		ICARDA 32122	-1.95±0.44	**<0.001**	-2.84	-1.06
		ICARDA 35531	-0.93±0.44	**0.04**	-1.82	-0.05
		ICARDA 35618	-0.43±0.44	0.33	-1.32	0.45

	Terbol	-1.08±0.44	**0.02**	-1.97	-0.20
	ICARDA 35621	-0.17±0.44	0.71	-1.05	0.72
	ICARDA 31591	-0.91±0.44	**0.04**	-1.80	-0.03
	ICARDA 35015	-0.78±0.44	0.08	-1.66	0.11
	ICARDA 32331	-1.62±0.44	<**0.001**	-2.50	-0.73
ICARDA 33847	*ICARDA 32122*	1.82±0.44	<**0.001**	0.93	2.70
	ICARDA 35531	2.83±0.44	<**0.001**	1.95	3.72
	ICARDA 35618	3.33±0.44	<**0.001**	2.45	4.22
	Terbol	2.68±0.44	<**0.001**	1.80	3.57
	ICARDA 35621	3.60±0.44	<**0.001**	2.71	4.49
	ICARDA 31591	2.85±0.44	<**0.001**	1.97	3.74
	ICARDA 35015	2.99±0.44	<**0.001**	2.10	3.88
	ICARDA 32331	2.15±0.44	<**0.001**	1.26	3.04
ICARDA 32122	*ICARDA 35531*	1.02±0.44	**0.03**	0.13	1.90
	ICARDA 35618	1.52±0.44	<**0.001**	0.63	2.40
	Terbol	0.87±0.44	0.06	-0.02	1.75
	ICARDA 35621	1.78±0.44	<**0.001**	0.90	2.67
	ICARDA 31591	1.04±0.44	**0.02**	0.15	1.92
	ICARDA 35015	1.17±0.44	**0.01**	0.29	2.06
	ICARDA 32331	0.33±0.44	0.45	-0.55	1.22
ICARDA 35531	*ICARDA 35618*	0.50±0.44	0.26	-0.39	1.39
	Terbol	-0.15±0.44	0.73	-1.04	0.74
	ICARDA 35621	0.77±0.44	0.09	-0.12	1.65
	ICARDA 31591	0.02±0.44	0.96	-0.87	0.91
	ICARDA 35015	0.16±0.44	0.72	-0.73	1.04

		ICARDA 32331	-0.68±0.44	0.13	-1.57	0.20
	ICARDA 35618	*Terbol*	-0.65±0.44	0.15	-1.54	0.24
		ICARDA 35621	0.27±0.44	0.55	-0.62	1.15
		ICARDA 31591	-0.48±0.44	0.28	-1.37	0.41
		ICARDA 35015	-0.34±0.44	0.44	-1.23	0.54
		ICARDA 32331	-1.18±0.44	**0.01**	-2.07	-0.30
	Terbol	*ICARDA 35621*	0.92±0.44	**0.04**	0.03	1.80
		ICARDA 31591	0.17±0.44	0.70	-0.72	1.06
		ICARDA 35015	0.31±0.44	0.49	-0.58	1.19
		ICARDA 32331	-0.53±0.44	0.23	-1.42	0.35
	ICARDA 35621	*ICARDA 31591*	-0.75±0.44	0.10	-1.63	0.14
		ICARDA 35015	-0.61±0.44	0.17	-1.50	0.28
		ICARDA 32331	-1.45±0.44	<**0.001**	-2.34	-0.56
	ICARDA 31591	*ICARDA 35015*	0.14±0.44	0.76	-0.75	1.02
		ICARDA 32331	-0.70±0.44	0.12	-1.59	0.18
	ICARDA 35015	*ICARDA 32331*	-0.84±0.44	0.06	-1.73	0.05
Stressed	*Atlas*	*ICARDA 33847*	-0.18±0.44	0.69	-1.06	0.71
		ICARDA 32122	0.47±0.44	0.29	-0.41	1.36
		ICARDA 35531	0.42±0.44	0.34	-0.46	1.31
		ICARDA 35618	0.62±0.44	0.16	-0.26	1.51
		Terbol	0.29±0.44	0.51	-0.60	1.18
		ICARDA 35621	0.29±0.44	0.51	-0.60	1.18
		ICARDA 31591	-0.44±0.44	0.32	-1.33	0.44
		ICARDA 35015	0.50±0.44	0.26	-0.39	1.39

	ICARDA 32331	0.19±0.44	0.67	-0.70	1.08
ICARDA 33847	*ICARDA 32122*	0.65±0.44	0.15	-0.24	1.54
	ICARDA 35531	0.60±0.44	0.18	-0.29	1.49
	ICARDA 35618	0.80±0.44	0.08	-0.09	1.69
	Terbol	0.47±0.44	0.29	-0.42	1.35
	ICARDA 35621	0.47±0.44	0.29	-0.42	1.35
	ICARDA 31591	-0.27±0.44	0.55	-1.15	0.62
	ICARDA 35015	0.68±0.44	0.13	-0.21	1.56
	ICARDA 32331	0.37±0.44	0.41	-0.52	1.25
ICARDA 32122	*ICARDA 35531*	-0.05±0.44	0.91	-0.94	0.84
	ICARDA 35618	0.15±0.44	0.73	-0.74	1.04
	Terbol	-0.18±0.44	0.68	-1.07	0.70
	ICARDA 35621	-0.18±0.44	0.68	-1.07	0.70
	ICARDA 31591	-0.92±0.44	**0.04**	-1.80	-0.03
	ICARDA 35015	0.03±0.44	0.95	-0.86	0.91
	ICARDA 32331	-0.28±0.44	0.52	-1.17	0.60
ICARDA 35531	*ICARDA 35618*	0.20±0.44	0.65	-0.69	1.09
	Terbol	-0.13±0.44	0.76	-1.02	0.75
	ICARDA 35621	-0.13±0.44	0.76	-1.02	0.75
	ICARDA 31591	-0.87±0.44	0.06	-1.75	0.02
	ICARDA 35015	0.08±0.44	0.86	-0.81	0.96
	ICARDA 32331	-0.23±0.44	0.60	-1.12	0.65
ICARDA 35618	*Terbol*	-0.33±0.44	0.45	-1.22	0.55
	ICARDA 35621	-0.33±0.44	0.45	-1.22	0.55
	ICARDA 31591	-1.07±0.44	**0.02**	-1.95	-0.18

		ICARDA 35015	-0.12±0.44	0.78	-1.01	0.76
		ICARDA 32331	-0.43±0.44	0.33	-1.32	0.45
	Terbol	*ICARDA 35621*	0.00±0.44	1.00	-0.89	0.89
		ICARDA 31591	-0.73±0.44	0.10	-1.62	0.15
		ICARDA 35015	0.21±0.44	0.64	-0.68	1.10
		ICARDA 32331	-0.10±0.44	0.82	-0.99	0.79
	ICARDA 35621	*ICARDA 31591*	-0.73±0.44	0.10	-1.62	0.15
		ICARDA 35015	0.21±0.44	0.64	-0.68	1.10
		ICARDA 32331	-0.10±0.44	0.82	-0.99	0.79
	ICARDA 31591	*ICARDA 35015*	0.94±0.44	**0.04**	0.06	1.83
		ICARDA 32331	0.63±0.44	0.16	-0.25	1.52
	ICARDA 35015	*ICARDA 32331*	-0.31±0.44	0.48	-1.20	0.58

*Data in bold represent significant results at 0.05

3.4.8. Effects on leaf size: Length (cm)

Leaves are the crucial plant organ for photosynthesis and possess all the genetic machinery to overcome the adverse effects of drought stress by adapting their size. In this context, the average leaf length of 10 wheat varieties grown under drought stress conditions showed a significant reduction of 34.41% ($p < 0.001$). All cultivars studied under control and drought stress conditions displayed a range of leaf lengths from 11.06 to 24.20 cm, and from 8.63 to 18.04 cm, respectively (Table 3.23). The average leaf length of samples in the control group was found to be 16.78 +/-SD 4.06, while wheat samples in the presence of 50% WFC was 11.00 +/- SD 3.86.

Based on the mean value of leaf length, the cultivars grown under control and drought conditions were ranked in descending order:

Table 3.23. Average leaf length (cm) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32122	24.20	ICARDA 32122	18.04
ICARDA 32331	18.82	Terbol	13.52
Terbol	18.72	ICARDA 35621	12.5
ICARDA 33847	18.59	Atlas	11.25
Atlas	18.49	ICARDA 35015	9.48
ICARDA 35015	16.56	ICARDA 32331	9.34
ICARDA 35531	15.98	ICARDA 35618	9.23
ICARDA 35618	12.69	ICARDA 35531	9.13
ICARDA 35621	12.67	ICARDA 31591	8.88
ICARDA 31591	11.06	ICARDA 33847	8.63

This classification highlighted the significance effect of the variety ($F(9,40) = 8.82$, $p < .001$) and the treatment ($F(1, 40) = 76.46$, $p < 0.001$) on this morphological parameter. This conclusion was confirmed by statistical analyses as shown in Table 3.24. It was evident that treatment had a substantial effect on leaf length as well as on the other morphological parameters listed above. Pairwise comparisons between the mean leaf length of wheat samples under control ($M = 16.78$, $SE = 0.47$) and those obtained under stress conditions ($M = 11.00$, $SE = 0.47$) showed a significant difference between the two plant conditions with a mean difference value of 5.77 cm, ($p < .001$) and a higher value in the control group. Moreover, the ANOVA results showed that there was a significant effect of the interaction between variety and treatment ($F(9,40) = 2.21$, $p = 0.042$) on leaf length (Table 3.24). These findings were consistent with the results of the Tukey's post-hoc comparison which was performed to determine which of the ICARDA bread wheat varieties had statistically significant leaf length under drought stress. The results demonstrated that Atlas, ICARDA 33847, ICARDA 32122, ICARDA 35618, Terbol cultivars had significant leaf length under drought stress. A detailed comparison is provided in Table 3.25. These cultivars ICARDA 33847, ICARDA 32122, ICARDA 35618 had also a significant spike length. As well as ICARDA 32331.

Table 3.24. Analysis of variance leaf length under control (100- 85% WFC) and drough (50% WFC) conditions.

Model	F	df	*P*-value
Variety	8.83	9	**<0.001***
Treatment	76.46	1	**<0.001**
Variety * Treatment	2.21	9	**0.042**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants df = degree of freedom.

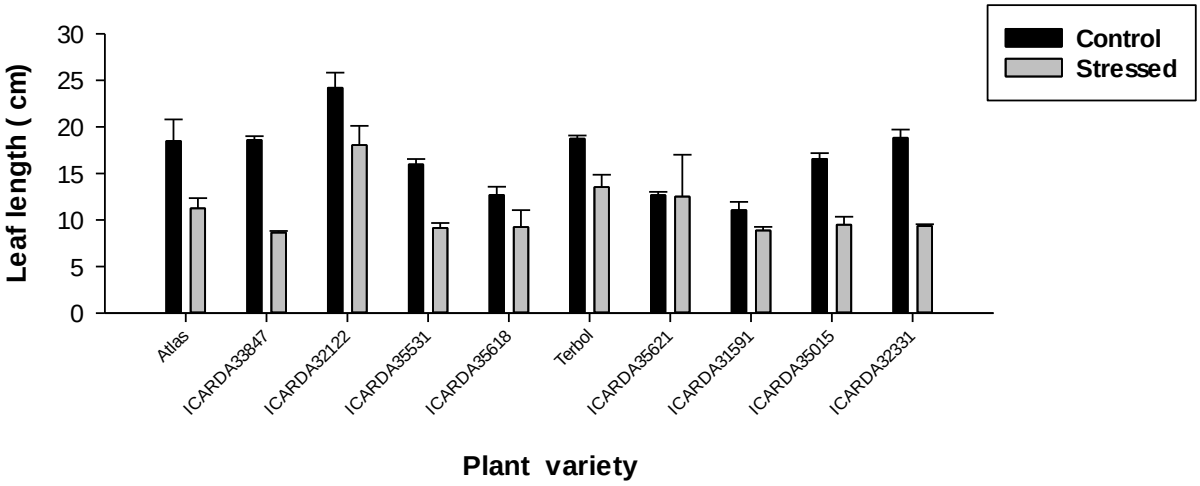


Figure 3.8. Leaf length under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = leaf length (cm). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 16.77(cm) and standard deviation = 4.05. The overall mean under drought stress = 11(cm) and standard deviation = 3.86

Based on the reduction in leaf length under drought conditions, the cultivars were ranked in ascending orders as follows: ICARDA35621 < ICARDA31591 < ICARDA32122 < ICARDA35618< Terbol < Atlas < ICARDA35015 < ICARDA35531 < ICARDA32331 < ICARDA33847. Thus, ICARDA35621 topped the list of the best-performing cultivars under drought conditions.

Plants can adjust morphologically by reducing the number and size of leaves (length, width and surface area) to avoid dehydration and survive in drought conditions. The plant may also assign more resources towards longer leaves when the width and number of leaves are reduced, in order to increase the efficiency of photosynthesis (Yang et al. 2021). In addition, plants may exhibit hormonal regulation, mainly associated with abscisic acid, and osmotic adjustment to maintain cell turgor, which promote leaf elongation (Bertolino et al. 2019; Pamungkas et al. 2022). Leaf length decreased in all cultivars, with the exception of cultivar ICARDA 35621, which maintained its leaf length and number. It appears that cultivar ICARDA 35621 was able to maintain its osmotic balance, which supports cell growth and explains the preservation of leaf length and number, as reported by Bertolino et al. (2019).

Table 3.25. Post-hoc comparison for leaf length (cm) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-0.10±2.09	0.96	-4.32	4.12
		ICARDA 32122	-5.71±2.09	**0.01***	-9.93	-1.49
		ICARDA 35531	2.51±2.09	0.24	-1.71	6.73
		ICARDA 35618	5.80±2.09	**0.01**	1.58	10.02
		Terbol	-0.24±2.09	0.91	-4.46	3.98
		ICARDA 35621	5.82±2.09	**0.01**	1.60	10.04
		ICARDA 31591	7.43±2.09	**<0.001**	3.21	11.65
		ICARDA 35015	1.93±2.09	0.36	-2.29	6.15
		ICARDA 32331	-0.34±2.09	0.87	-4.56	3.88
	ICARDA 33847	*ICARDA 32122*	-5.61±2.09	**0.01**	-9.83	-1.39
		ICARDA 35531	2.61±2.09	0.22	-1.61	6.83

	ICARDA 35618	5.90±2.09	**0.01**	1.68	10.12
	Terbol	-0.13±2.09	0.95	-4.35	4.09
	ICARDA 35621	5.92±2.09	**0.01**	1.70	10.14
	ICARDA 31591	7.53±2.09	**<0.001**	3.31	11.75
	ICARDA 35015	2.03±2.09	0.34	-2.19	6.25
	ICARDA 32331	-0.23±2.09	0.91	-4.45	3.99
ICARDA 32122	*ICARDA 35531*	8.22±2.09	**<0.001**	4.00	12.44
	ICARDA 35618	11.51±2.09	**<0.001**	7.29	15.73
	Terbol	5.48±2.09	**0.01**	1.26	9.70
	ICARDA 35621	11.53±2.09	**<0.001**	7.31	15.75
	ICARDA 31591	13.14±2.09	**<0.001**	8.92	17.36
	ICARDA 35015	7.64±2.09	**<0.001**	3.42	11.86
	ICARDA 32331	5.38±2.09	**0.01**	1.16	9.60
ICARDA 35531	*ICARDA 35618*	3.29±2.09	0.12	-0.93	7.51
	Terbol	-2.75±2.09	0.20	-6.97	1.47
	ICARDA 35621	3.31±2.09	0.12	-0.91	7.53
	ICARDA 31591	4.92±2.09	**0.02**	0.70	9.14
	ICARDA 35015	-0.58±2.09	0.78	-4.80	3.64
	ICARDA 32331	-2.85±2.09	0.18	-7.07	1.37
ICARDA 35618	*Terbol*	-6.04±2.09	**0.01**	-10.26	-1.82
	ICARDA 35621	0.02±2.09	0.99	-4.20	4.24
	ICARDA 31591	1.63±2.09	0.44	-2.59	5.85
	ICARDA 35015	-3.87±2.09	0.07	-8.09	0.35
	ICARDA 32331	-6.14±2.09	**0.01**	-10.36	-1.92
Terbol	*ICARDA 35621*	6.06±2.09	**0.01**	1.84	10.28

		ICARDA 31591	7.67±2.09	**<0.001**	3.45	11.89
		ICARDA 35015	2.17±2.09	0.31	-2.05	6.39
		ICARDA 32331	-0.10±2.09	0.96	-4.32	4.12
	ICARDA 35621	*ICARDA 31591*	1.61±2.09	0.45	-2.61	5.83
		ICARDA 35015	-3.89±2.09	0.07	-8.11	0.33
		ICARDA 32331	-6.16±2.09	**0.01**	-10.38	-1.94
	ICARDA 31591	*ICARDA 35015*	-5.50±2.09	**0.01**	-9.72	-1.28
		ICARDA 32331	-7.77±2.09	**<0.001**	-11.99	-3.55
	ICARDA 35015	*ICARDA 32331*	-2.27±2.09	0.28	-6.49	1.95
Stressed	*Atlas*	*ICARDA 33847*	2.62±2.09	0.22	-1.60	6.84
		ICARDA 32122	-6.79±2.09	**<0.001**	-11.01	-2.57
		ICARDA 35531	2.12±2.09	0.32	-2.10	6.34
		ICARDA 35618	2.02±2.09	0.34	-2.20	6.24
		Terbol	-2.27±2.09	0.28	-6.49	1.95
		ICARDA 35621	-1.26±2.09	0.55	-5.48	2.96
		ICARDA 31591	2.38±2.09	0.26	-1.84	6.60
		ICARDA 35015	1.77±2.09	0.40	-2.45	5.99
		ICARDA 32331	1.91±2.09	0.37	-2.31	6.13
	ICARDA 33847	*ICARDA 32122*	-9.41±2.09	**<0.001**	-13.63	-5.19
		ICARDA 35531	-0.50±2.09	0.81	-4.72	3.72
		ICARDA 35618	-0.60±2.09	0.78	-4.82	3.62
		Terbol	-4.89±2.09	**0.02**	-9.11	-0.67
		ICARDA 35621	-3.88±2.09	0.07	-8.10	0.34
		ICARDA 31591	-0.24±2.09	0.91	-4.46	3.98

	ICARDA 35015	-0.85±2.09	0.69	-5.07	3.37
	ICARDA 32331	-0.71±2.09	0.74	-4.93	3.51
ICARDA 32122	*ICARDA 35531*	8.91±2.09	**<0.001**	4.69	13.13
	ICARDA 35618	8.81±2.09	**<0.001**	4.59	13.03
	Terbol	4.52±2.09	**0.04**	0.30	8.74
	ICARDA 35621	5.53±2.09	**0.01**	1.31	9.75
	ICARDA 31591	9.17±2.09	**<0.001**	4.95	13.39
	ICARDA 35015	8.56±2.09	**<0.001**	4.34	12.78
	ICARDA 32331	8.70±2.09	**<0.001**	4.48	12.92
ICARDA 35531	*ICARDA 35618*	-0.10±2.09	0.96	-4.32	4.12
	Terbol	-4.39±2.09	0.04	-8.61	-0.17
	ICARDA 35621	-3.38±2.09	0.11	-7.60	0.84
	ICARDA 31591	0.26±2.09	0.90	-3.96	4.48
	ICARDA 35015	-0.35±2.09	0.87	-4.57	3.87
	ICARDA 32331	-0.21±2.09	0.92	-4.43	4.01
ICARDA 35618	*Terbol*	-4.29±2.09	**0.05**	-8.51	-0.07
	ICARDA 35621	-3.28±2.09	0.12	-7.50	0.94
	ICARDA 31591	0.36±2.09	0.87	-3.86	4.58
	ICARDA 35015	-0.25±2.09	0.91	-4.47	3.97
	ICARDA 32331	-0.11±2.09	0.96	-4.33	4.11
Terbol	*ICARDA 35621*	1.01±2.09	0.63	-3.21	5.23
	ICARDA 31591	4.65±2.09	**0.03**	0.43	8.87
	ICARDA 35015	4.04±2.09	0.06	-0.18	8.26
	ICARDA 32331	4.18±2.09	**0.05**	-0.04	8.40
ICARDA 35621	*ICARDA 31591*	3.63±2.09	0.09	-0.59	7.85

		ICARDA 35015	3.03±2.09	0.15	-1.19	7.25
		ICARDA 32331	3.17±2.09	0.14	-1.05	7.39
	ICARDA 31591	*ICARDA 35015*	-0.60±2.09	0.77	-4.82	3.62
		ICARDA 32331	-0.47±2.09	0.82	-4.69	3.75
	ICARDA 35015	*ICARDA 32331*	0.14±2.09	0.95	-4.08	4.36

*Data in bold represent significant results at 0.05.

3.4.9. Effects on leaf size: Width (cm)

Leaf width was also assessed for all the wheat varieties under drought stress conditions. This parameter was found to be decreased by 40.27% in wheat under 50% WFC, meaning that drought stress had a significant negative effect not only on leaf number and length but also on leaf width ($p < 0.001$) below the cut-off point of 0.05. Leaf width of cultivars under control and stress conditions varied from 0.98 to 1.48 cm (a mean of 1.27 +/-SD = 0.16) and from 0.62 to 0.91 (a mean of 0.76 cm +/-SD = 0.12), respectively.

The average leaf width of wheat cultivars under control and stress conditions was recorded and the following wheat varieties were ranked in decreasing order as follows:

Table 3.26. Average leaf width (cm) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32331	1.48	ICARDA 35618	0.91
ICARDA 35618	1.36	ICARDA 35015	0.86
ICARDA 32122	1.33	ICARDA 32122	0.83
ICARDA 35531	1.32	ICARDA 31591	0.79
ICARDA 31591	1.31	ICARDA 32331	0.78
ICARDA 35015	1.30	ICARDA 35531	0.74
ICARDA 35621	1.25	Terbol	0.73
Terbol	1.19	ICARDA 35621	0.69
Atlas	1.19	ICARDA 33847	0.63
ICARDA 33847	0.98	Atlas	0.62

It is evident that the highest leaf width was observed in the wheat cultivars under control conditions (Figure 3.9). This observation was confirmed by statistical analysis which showed that the control samples had the highest average leaf width, M = 1.27, SE = 0.02, than drought treated samples, (M = 0.76, SE = 0.02), with a mean difference of 0.51 cm ($p < .001$). These pairwise comparisons were in line with the Anova test for the significant effect of treatment on leaf width with $F(1, 40) = 316.19$, $p < .001$. In addition, there was also a significant effect of wheat variety ($F(9,40) = 5.29$, $p < .001$) on leaf width, but this was not the case for the interaction between these two variables, variety and treatment $F(9,40) = 1.14$, $p = 0.357$), which had no significant contribution to changes in leaf width (Table 3.27).

Table 3.27. Analysis of variance leaf width under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	5.29	9	**<0.001***
Treatment	316.19	1	**<0.001**
Variety * Treatment	1.14	9	0.36
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

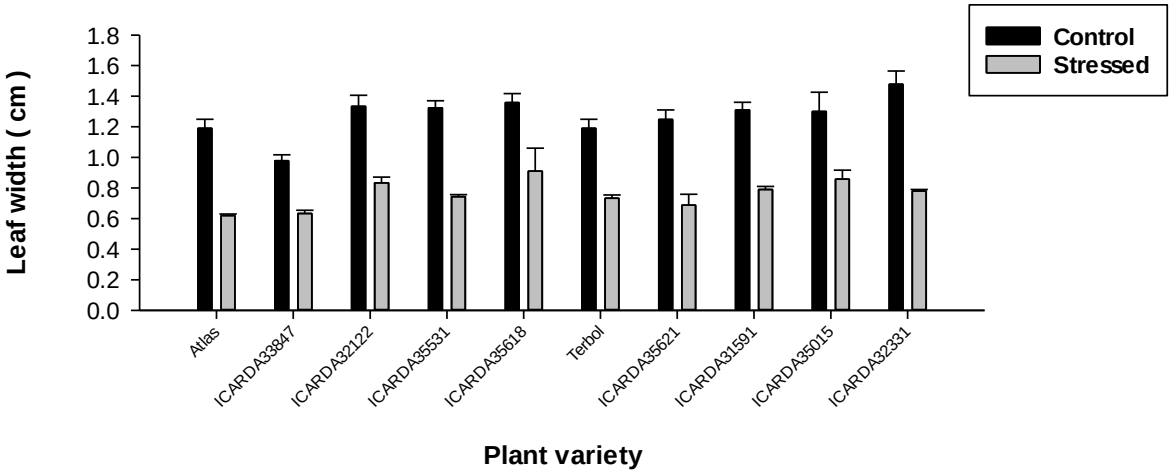


Figure 3.9. Leaf width under control (100-85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = leaf width (cm). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 1.27(cm) and standard deviation = 0.16. The overall mean under drought stress = 0.75(cm) and standard deviation = 0.12.

Based on the reduction in leaf width under drought conditions, the cultivars were ranked in ascending orders as follows: ICARDA35618 < ICARDA35015 < ICARDA33847 < ICARDA32122 < Terbol < ICARDA31591 < ICARDA35531 < ICARDA35621 < ICARDA32331 < Atlas. Thus, ICARDA35618 topped the list of the best-performing cultivars under drought conditions. However, ICARDA35621 was found to be the best performing cultivars based on leaf width parameter. Post-hoc comparison was performed to determine which of the ICARDA bread wheat varieties had statistically significant leaf width under drought stress. The results demonstrated that the cultivars Atlas, ICARDA 33847, ICARDA 35618 had significant leaf width under drought stress, which is coherent with the data of leaf length (except for ICARDA 32122 and Terbol). A detailed comparison is provided in Table 3.28.

Table 3.28. Post-hoc comparison for leaf width (cm) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	0.21±0.09	**0.02***	0.03	0.40
		ICARDA 32122	-0.14±0.09	0.12	-0.33	0.04
		ICARDA 35531	-0.13±0.09	0.15	-0.32	0.05
		ICARDA 35618	-0.17±0.09	0.07	-0.35	0.02
		Terbol	0.00±0.09	1.00	-0.18	0.18
		ICARDA 35621	-0.06±0.09	0.54	-0.24	0.13

	ICARDA 31591	-0.12±0.09	0.19	-0.30	0.06
	ICARDA 35015	-0.11±0.09	0.23	-0.29	0.07
	ICARDA 32331	-0.29±0.09	<**0.001**	-0.47	-0.10
ICARDA 33847	*ICARDA 32122*	-0.36±0.09	<**0.001**	-0.54	-0.17
	ICARDA 35531	-0.35±0.09	<**0.001**	-0.53	-0.16
	ICARDA 35618	-0.38±0.09	<**0.001**	-0.56	-0.20
	Terbol	-0.21±0.09	**0.02**	-0.40	-0.03
	ICARDA 35621	-0.27±0.09	**0.01**	-0.45	-0.09
	ICARDA 31591	-0.33±0.09	<**0.001**	-0.52	-0.15
	ICARDA 35015	-0.32±0.09	<**0.001**	-0.51	-0.14
	ICARDA 32331	-0.50±0.09	<**0.001**	-0.68	-0.32
ICARDA 32122	*ICARDA 35531*	0.01±0.09	0.91	-0.17	0.19
	ICARDA 35618	-0.02±0.09	0.80	-0.21	0.16
	Terbol	0.14±0.09	0.12	-0.04	0.33
	ICARDA 35621	0.09±0.09	0.35	-0.10	0.27
	ICARDA 31591	0.02±0.09	0.80	-0.16	0.21
	ICARDA 35015	0.03±0.09	0.72	-0.15	0.22
	ICARDA 32331	-0.14±0.09	0.12	-0.33	0.04
ICARDA 35531	*ICARDA 35618*	-0.03±0.09	0.72	-0.22	0.15
	Terbol	0.13±0.09	0.15	-0.05	0.32
	ICARDA 35621	0.08±0.09	0.40	-0.11	0.26
	ICARDA 31591	0.01±0.09	0.88	-0.17	0.20
	ICARDA 35015	0.02±0.09	0.80	-0.16	0.21
	ICARDA 32331	-0.15±0.09	0.10	-0.34	0.03
	Terbol	0.17±0.09	0.07	-0.02	0.35

	ICARDA 35618	*ICARDA 35621*	0.11±0.09	0.23	-0.07	0.29
		ICARDA 31591	0.05±0.09	0.61	-0.14	0.23
		ICARDA 35015	0.06±0.09	0.54	-0.13	0.24
		ICARDA 32331	-0.12±0.09	0.19	-0.30	0.06
	Terbol	*ICARDA 35621*	-0.06±0.09	0.54	-0.24	0.13
		ICARDA 31591	-0.12±0.09	0.19	-0.30	0.06
		ICARDA 35015	-0.11±0.09	0.23	-0.29	0.07
		ICARDA 32331	-0.29±0.09	<**0.001**	-0.47	-0.10
	ICARDA 35621	*ICARDA 31591*	-0.06±0.09	0.49	-0.25	0.12
		ICARDA 35015	-0.05±0.09	0.56	-0.24	0.13
		ICARDA 32331	-0.23±0.09	**0.02**	-0.41	-0.05
	ICARDA 31591	*ICARDA 35015*	0.01±0.09	0.91	-0.17	0.19
		ICARDA 32331	-0.17±0.09	0.07	-0.35	0.02
	ICARDA 35015	*ICARDA 32331*	-0.18±0.09	0.06	-0.36	0.01
Stressed	*Atlas*	*ICARDA 33847*	-0.01±0.09	0.88	-0.20	0.17
		ICARDA 32122	-0.21±0.09	**0.02**	-0.40	-0.03
		ICARDA 35531	-0.12±0.09	0.18	-0.31	0.06
		ICARDA 35618	-0.29±0.09	<**0.001**	-0.47	-0.11
		Terbol	-0.11±0.09	0.22	-0.30	0.07
		ICARDA 35621	-0.07±0.09	0.47	-0.25	0.12
		ICARDA 31591	-0.17±0.09	0.07	-0.35	0.01
		ICARDA 35015	-0.24±0.09	**0.01**	-0.42	-0.05
		ICARDA 32331	-0.16±0.09	0.09	-0.34	0.02

ICARDA 33847	*ICARDA 32122*	-0.20±0.09	**0.03**	-0.38	-0.02
	ICARDA 35531	-0.11±0.09	0.23	-0.29	0.07
	ICARDA 35618	-0.28±0.09	**<0.001**	-0.46	-0.09
	Terbol	-0.10±0.09	0.28	-0.28	0.08
	ICARDA 35621	-0.05±0.09	0.56	-0.24	0.13
	ICARDA 31591	-0.16±0.09	0.09	-0.34	0.03
	ICARDA 35015	-0.22±0.09	**0.02**	-0.41	-0.04
	ICARDA 32331	-0.15±0.09	0.11	-0.33	0.04
ICARDA 32122	*ICARDA 35531*	0.09±0.09	0.33	-0.09	0.27
	ICARDA 35618	-0.08±0.09	0.40	-0.26	0.11
	Terbol	0.10±0.09	0.28	-0.08	0.28
	ICARDA 35621	0.15±0.09	0.11	-0.04	0.33
	ICARDA 31591	0.04±0.09	0.64	-0.14	0.23
	ICARDA 35015	-0.02±0.09	0.80	-0.21	0.16
	ICARDA 32331	0.05±0.09	0.56	-0.13	0.24
ICARDA 35531	*ICARDA 35618*	-0.17±0.09	0.07	-0.35	0.02
	Terbol	0.01±0.09	0.91	-0.17	0.19
	ICARDA 35621	0.06±0.09	0.54	-0.13	0.24
	ICARDA 31591	-0.05±0.09	0.61	-0.23	0.14
	ICARDA 35015	-0.11±0.09	0.22	-0.30	0.07
	ICARDA 32331	-0.04±0.09	0.69	-0.22	0.15
ICARDA 35618	*Terbol*	0.18±0.09	0.06	-0.01	0.36
	ICARDA 35621	0.22±0.09	**0.02**	0.04	0.41
	ICARDA 31591	0.12±0.09	0.19	-0.06	0.30
	ICARDA 35015	0.05±0.09	0.56	-0.13	0.24

	ICARDA 32331	0.13±0.09	0.16	-0.05	0.31
Terbol	*ICARDA 35621*	0.05±0.09	0.61	-0.14	0.23
	ICARDA 31591	-0.06±0.09	0.54	-0.24	0.13
	ICARDA 35015	-0.12±0.09	0.18	-0.31	0.06
	ICARDA 32331	-0.05±0.09	0.61	-0.23	0.14
ICARDA 35621	*ICARDA 31591*	-0.10±0.09	0.26	-0.29	0.08
	ICARDA 35015	-0.17±0.09	0.07	-0.35	0.01
	ICARDA 32331	-0.09±0.09	0.31	-0.28	0.09
ICARDA 31591	*ICARDA 35015*	-0.07±0.09	0.47	-0.25	0.12
	ICARDA 32331	0.01±0.09	0.91	-0.17	0.19
ICARDA 35015	*ICARDA 32331*	0.08±0.09	0.40	-0.11	0.26

*Data in bold represent significant results at 0.05

3.4.10. Effects on leaf size: Leaf area

Examination of these 10 wheat cultivars under 50% WFC showed that this form of abiotic stress had a significant negative effect ($p < .001$) on leaf area (Tables 3.29 and 3.30). This detrimental impact was manifested by a 56.44% reduction in average leaf area for all the studied varieties. The leaf area of wheat samples in the control group ranged from 14.68 to 32.54 (mean of 21.39; +/-SD = 6.22), while it varied from 5.55 to 16.10 (mean of 9.32; +/- SD = 5.68) for wheat subjected to drought treatment.

The average leaf area recorded for the wheat samples under control conditions was higher than that for the samples grown under stressed conditions. These findings were consistent with those of Tukey's test and pairwise comparisons between the two treatments, which showed a mean difference in leaf area of 12.07, ($p < .001$). Wheat cultivar samples from plants grown under optimal water conditions (100-85% WFC) showed a mean leaf area value of M = 21.39, SE =0.87, while those in the drought stress condition had M = 9.32 and a SE= 0.87). Based on the average leaf area value, the cultivars under control and drought conditions were ranked as follows:

Table 3.29. Average leaf area of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32122	32.54	ICARDA 31591	16.10
ICARDA 32331	27.97	ICARDA 32122	15.08
Terbol	22.36	Terbol	9.99
Atlas	22.24	ICARDA 35618	8.89
ICARDA 35015	21.66	ICARDA 35621	8.50
ICARDA 35531	21.13	ICARDA 35015	8.01
ICARDA 33847	18.23	ICARDA 32331	7.27
ICARDA 35618	17.27	Atlas	7.04
ICARDA 35621	15.83	ICARDA 35531	6.75
ICARDA 31591	14.68	ICARDA 33847	5.55

This variation in cultivar rankings under control and drought conditions (Figure 3.10) highlights the significant effect not only of treatment ($F(1, 40) = 96.74$, $p < .001$), but also of variety ($F(9,40) = 3.171$, $p < .006$). This conclusion was confirmed by the two way ANOVA test, which also showed the significant effect of the combination of these two parameters, variety and treatment, on leaf area $F(9,40) = 2.50$, $p = 0.022$ (Table 3.30).

Table 3.30. Analysis of variance leaf area under control (100- 85% WFC) and Drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	3.17	9	**<0.006***
Treatment	96.74	1	**<0.001**
Variety * Treatment	2.51	9	**0.022**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

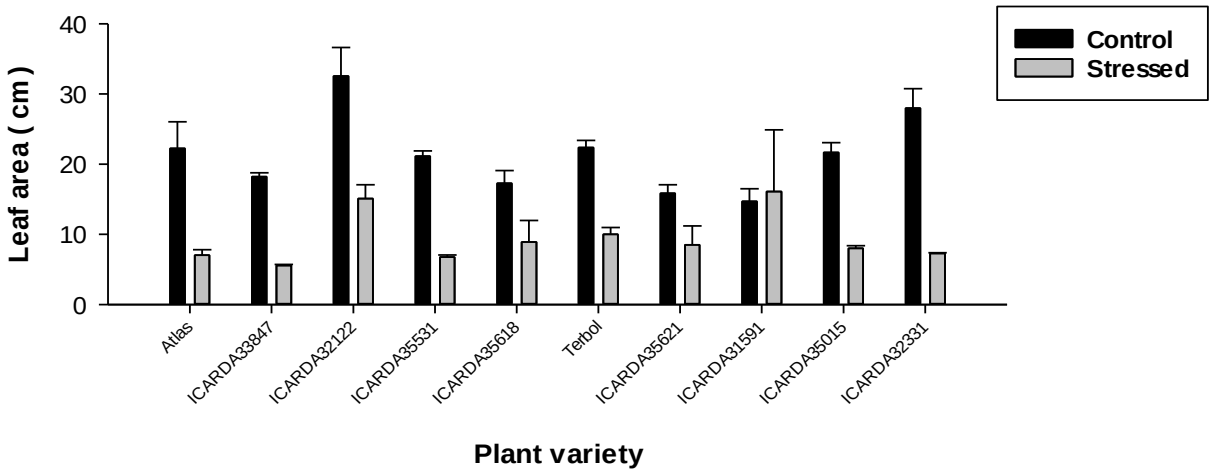


Figure 3.10. Leaf area of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = leaf area. Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 21.39 and standard deviation = 6.21. The overall mean under drought stress = 9.31 and standard deviation = 5.68.

Based on the reduction in leaf area under drought conditions, the cultivars were ranked in ascending orders as follows: ICARDA31591 < ICARDA35621 < ICARDA35618 < ICARDA32122 < Terbol < ICARDA35015 < ICARDA35531 < Atlas < ICARDA33847 < ICARDA32331. Thus, ICARDA31591 topped the list of the best-performing cultivars under drought conditions. This ranking was not coherent with those based on leaf length and width, as ICARDA35621 and ICARDA35618 occupied first place, respectively.

Leaf area is a good indicator for transpiration rate and photosynthesis capacity. The significant decrease in the leaf area of the studied culivars reflects their ability to minimize water loss via transpirsation. These results were in concordance with the reduced leaf length (except ICARDA 35621) and leaf width under drought. Therefore, the cultivars tried to cope with the drought stress by making certain biological adjustments. However, plants can sometime increase the leaf area and reduce the leaf length and width, as reported by (Bertolino et al. 2019; Yang et al. 2021; Pamungkas and Farid 2022). This adaptative mechanism was also detected in the cultivar

ICARDA 31591, which may be explained by its ability to adjust osmotic pressure and maintain cell growth and leaf expansion under drought conditions (Bertolino et al. 2019).

Post-hoc comparison was performed to determine which of the ICARDA bread wheat varieties had statistically significant leaf area under drought stress. The results showed that the cultivars Atlas, ICARDA 33847, ICARDA 32122, ICARDA 35531, ICARDA 31591 had significant leaf area under drought stress. These results were partlly in line with those for leaf length (except Terbol) and leaf width (except ICARDA 35618). A detailed comparison is provided in Table 3.31.

Table 3.31. Post-hoc comparison for leaf area (cm^2) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	4.02± 3.88	0.31	-3.83	11.86
		ICARDA 32122	-10.30± 3.88	**0.01***	-18.14	-2.45
		ICARDA 35531	1.12± 3.88	0.78	-6.73	8.96
		ICARDA 35618	4.98± 3.88	0.21	-2.87	12.82
		Terbol	-0.11± 3.88	0.98	-7.96	7.73
		ICARDA 35621	6.41± 3.88	0.11	-1.43	14.25
		ICARDA 31591	7.56± 3.88	0.06	-0.28	15.40
		ICARDA 35015	0.59± 3.88	0.88	-7.26	8.43
		ICARDA 32331	-5.73± 3.88	0.15	-13.57	2.11
	ICARDA 33847	*ICARDA 32122*	-14.31± 3.88	**<0.001**	-22.16	-6.47
		ICARDA 35531	-2.90± 3.88	0.46	-10.74	4.94

	ICARDA 35618	0.96± 3.88	0.81	-6.88	8.80
	Terbol	-4.13± 3.88	0.29	-11.97	3.71
	ICARDA 35621	2.39± 3.88	0.54	-5.45	10.24
	ICARDA 31591	3.54± 3.88	0.37	-4.30	11.39
	ICARDA 35015	-3.43± 3.88	0.38	-11.27	4.41
	ICARDA 32331	-9.75± 3.88	**0.02**	-17.59	-1.90
ICARDA 32122	*ICARDA 35531*	11.41± 3.88	**0.01**	3.57	19.26
	ICARDA 35618	15.27± 3.88	**0.00**	7.43	23.12
	Terbol	10.18± 3.88	**0.01**	2.34	18.03
	ICARDA 35621	16.70± 3.88	**0.00**	8.86	24.55
	ICARDA 31591	17.86± 3.88	**0.00**	10.01	25.70
	ICARDA 35015	10.88± 3.88	**0.01**	3.04	18.73
	ICARDA 32331	4.57± 3.88	0.25	-3.28	12.41
ICARDA 35531	*ICARDA 35618*	3.86± 3.88	0.33	-3.98	11.70
	Terbol	-1.23± 3.88	0.75	-9.07	6.61
	ICARDA 35621	5.29± 3.88	0.18	-2.55	13.14
	ICARDA 31591	6.44± 3.88	0.10	-1.40	14.29
	ICARDA 35015	-0.53± 3.88	0.89	-8.37	7.31
	ICARDA 32331	-6.85± 3.88	0.09	-14.69	1.00
ICARDA 35618	*Terbol*	-5.09± 3.88	0.20	-12.93	2.75
	ICARDA 35621	1.43± 3.88	0.71	-6.41	9.28
	ICARDA 31591	2.58± 3.88	0.51	-5.26	10.43
	ICARDA 35015	-4.39± 3.88	0.26	-12.23	3.45
	ICARDA 32331	-10.71± 3.88	**0.01**	-18.55	-2.86
Terbol	*ICARDA 35621*	6.52± 3.88	0.10	-1.32	14.37

		ICARDA 31591	7.67± 3.88	**0.05**	-0.17	15.52
		ICARDA 35015	0.70± 3.88	0.86	-7.14	8.54
		ICARDA 32331	-5.62± 3.88	0.16	-13.46	2.23
	ICARDA 35621	*ICARDA 31591*	1.15± 3.88	0.77	-6.69	8.99
		ICARDA 35015	-5.82± 3.88	0.14	-13.67	2.02
		ICARDA 32331	-12.14± 3.88	**<0.001**	-19.98	-4.30
	ICARDA 31591	*ICARDA 35015*	-6.97± 3.88	0.08	-14.82	0.87
		ICARDA 32331	-13.29± 3.88	**<0.001**	-21.13	-5.45
	ICARDA 35015	*ICARDA 32331*	-6.32± 3.88	0.11	-14.16	1.53
Stressed	*Atlas*	*ICARDA 33847*	1.49± 3.88	0.70	-6.35	9.33
		ICARDA 32122	-8.05± 3.88	**0.04**	-15.89	-0.20
		ICARDA 35531	0.28± 3.88	0.94	-7.56	8.13
		ICARDA 35618	-1.86± 3.88	0.64	-9.70	5.99
		Terbol	-2.96± 3.88	0.45	-10.80	4.89
		ICARDA 35621	-1.46± 3.88	0.71	-9.31	6.38
		ICARDA 31591	-9.06± 3.88	**0.02**	-16.90	-1.22
		ICARDA 35015	-0.97± 3.88	0.80	-8.82	6.87
		ICARDA 32331	-0.23± 3.88	0.95	-8.07	7.61
	ICARDA 33847	*ICARDA 32122*	-9.54± 3.88	**0.02**	-17.38	-1.69
		ICARDA 35531	-1.21± 3.88	0.76	-9.05	6.64
		ICARDA 35618	-3.35± 3.88	0.39	-11.19	4.50
		Terbol	-4.45± 3.88	0.26	-12.29	3.40
		ICARDA 35621	-2.95± 3.88	0.45	-10.80	4.89
		ICARDA 31591	-10.55± 3.88	**0.01**	-18.39	-2.71

	ICARDA 35015	-2.46± 3.88	0.53	-10.31	5.38
	ICARDA 32331	-1.72± 3.88	0.66	-9.56	6.12
ICARDA 32122	*ICARDA 35531*	8.33± 3.88	**0.04**	0.49	16.17
	ICARDA 35618	6.19± 3.88	0.12	-1.65	14.03
	Terbol	5.09± 3.88	0.20	-2.75	12.93
	ICARDA 35621	6.58± 3.88	0.10	-1.26	14.43
	ICARDA 31591	-1.01± 3.88	0.80	-8.86	6.83
	ICARDA 35015	7.07± 3.88	0.08	-0.77	14.92
	ICARDA 32331	7.82± 3.88	**0.05**	-0.03	15.66
ICARDA 35531	*ICARDA 35618*	-2.14± 3.88	0.58	-9.98	5.70
	Terbol	-3.24± 3.88	0.41	-11.08	4.60
	ICARDA 35621	-1.75± 3.88	0.66	-9.59	6.10
	ICARDA 31591	-9.34± 3.88	**0.02**	-17.19	-1.50
	ICARDA 35015	-1.26± 3.88	0.75	-9.10	6.59
	ICARDA 32331	-0.51± 3.88	0.90	-8.36	7.33
ICARDA 35618	*Terbol*	-1.10± 3.88	0.78	-8.94	6.74
	ICARDA 35621	0.39± 3.88	0.92	-7.45	8.24
	ICARDA 31591	-7.20± 3.88	0.07	-15.05	0.64
	ICARDA 35015	0.88± 3.88	0.82	-6.96	8.73
	ICARDA 32331	1.63± 3.88	0.68	-6.22	9.47
Terbol	*ICARDA 35621*	1.49± 3.88	0.70	-6.35	9.34
	ICARDA 31591	-6.10± 3.88	0.12	-13.95	1.74
	ICARDA 35015	1.98± 3.88	0.61	-5.86	9.83
	ICARDA 32331	2.73± 3.88	0.49	-5.12	10.57

	ICARDA 35621	*ICARDA 31591*	-7.60± 3.88	0.06	-15.44	0.25
		ICARDA 35015	0.49± 3.88	0.90	-7.35	8.33
		ICARDA 32331	1.23± 3.88	0.75	-6.61	9.08
	ICARDA 31591	*ICARDA 35015*	8.09± 3.88	**0.04**	0.24	15.93
		ICARDA 32331	8.83± 3.88	**0.03**	0.99	16.67
	ICARDA 35015	*ICARDA 32331*	0.74± 3.88	0.85	-7.10	8.59

*Data in bold represent significant results at 0.05

3.4.11. Effects on fresh weight of vegetative tissues (g)

A long period (90 days) of drought stress applied to ten wheat cultivars led to a significant reduction in fresh weight of 71.77% ($p < .001$); a value well below the established ANOVA threshold of 0.05 for statistical significance. The fresh weight of all cultivars under control (4.37 to 13.27g) and drought conditions (1.46 to 3.51 g) showed mean values of 8.92g +/- SD = 2.95 and 2.52g +/-SD = 0.88, respectively. These results were consistent with the outcome of the Tukey test highlighting the significant variation in fresh weight of wheat cultivars under 100-85% WFC ($M = 8.92$, $SE = 0.22$) and those estimated from wheat samples under 50% WFC ($M = 2.52$, $SE = 0.22$) with a mean difference of 6.40 g, ($p < .001$). The pairwise comparison clearly indicated that wheat cultivars grown under control conditions had higher fresh weight values than those subjected to drought stress. Therefore, water stress had a significant effect on fresh weight as shown by $p < .001$, with $F(1, 40) = 423.09$. Moreover, variety had a considerable effect on fresh weight with $p < .001$, $F(9,40) = 10.68$. These findings were in concordance with the variation in descending order of cultivars, based on mean of fresh weight, under control and drought stress conditions:

Table 3.32. Average fresh weight (g) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32122	13.27	ICARDA 32122	3.51
ICARDA 35531	11.62	ICARDA 35015	3.45
Terbol	10.70	Terbol	2.91

ICARDA 33847	10.36	ICARDA 32331	2.59
ICARDA 35015	9.87	ICARDA 35531	2.37
ICARDA 31591	8.41	ICARDA 31591	2.33
ICARDA 32331	8.27	ICARDA 33847	2.21
ICARDA 35618	6.38	Atlas	2.21
ICARDA 35621	5.95	ICARDA 35621	2.14
Atlas	4.37	ICARDA 35618	1.46

It is interesting to note that the cultivar ICARDA 32122 performed the best because it showed the lowest reduction on fresh weight under drought stress unlike the cultivar ICARDA 35621 (Figure 3.11). In addition, the two-way ANOVA demonstrated that there was a considerable effect of both parameters, variety and treatment, on fresh weight, with a p value of 0.001 and F(9,40) of 5.96 (Table 3.33).

Table 3.33. Analysis of variance of fresh weight of vegetative tissues of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	**F**	**df**	***P*-value**
Variety	10.68	9	**<0.001***
Treatment	423.09	1	**<0.001**
Variety * Treatment	5.96	9	**<0.001**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

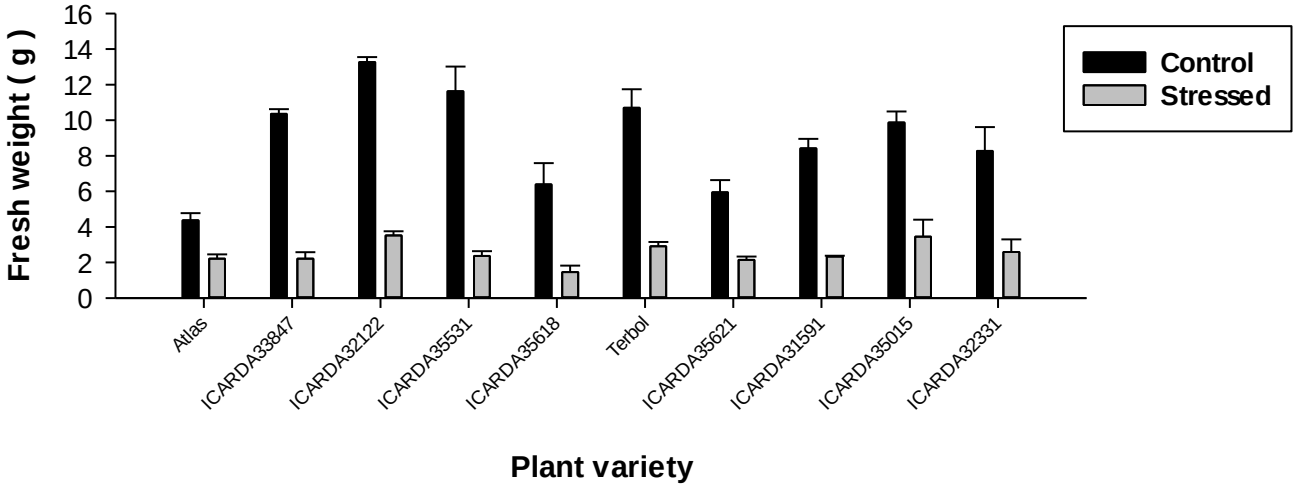


Figure 3.11. Fresh weight of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = fresh weight (g). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 8.91(g) and standard deviation = 2.95. The overall mean under drought stress = 2.51(g) and standard deviation = 0.87.

Based on the reduction in fresh weight under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < ICARDA35621< ICARDA35015< ICARDA32331< ICARDA31591 < Terbol < ICARDA32122 < ICARDA35618 < ICARDA33847 < ICARDA35531. Thus, Atlas was able to be the best-performing cultivars under drought conditions; results coherent with those based on plant height, spike length, number of leaves and tillers.

To further explore which ICARDA bread wheat varieties had significant fresh weight under stress regime, Tukey's post-hoc comparison was performed. The results revealed that ICARDA 32122 and ICARDA 35618 had significantly greater fresh weight under drought stress. A detailed comparison is provided in Table 3.34.

Table 3.34. Post-hoc comparison for fresh weight (g) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-5.99±0.98	**<0.001***	-7.98	-4.00
		ICARDA 32122	-8.90±0.98	**<0.001**	-10.89	-6.91
		ICARDA 35531	-7.25±0.98	**<0.001**	-9.24	-5.26
		ICARDA 35618	-2.01±0.98	**0.05**	-4.00	-0.02
		Terbol	-6.33±0.98	**<0.001**	-8.32	-4.34
		ICARDA 35621	-1.58±0.98	0.12	-3.57	0.41
		ICARDA 31591	-4.04±0.98	**<0.001**	-6.03	-2.05
		ICARDA 35015	-5.50±0.98	**<0.001**	-7.49	-3.51
		ICARDA 32331	-3.90±0.98	**<0.001**	-5.89	-1.91
	ICARDA 33847	*ICARDA 32122*	-2.91±0.98	**0.01**	-4.90	-0.92
		ICARDA 35531	-1.27±0.98	0.21	-3.26	0.72
		ICARDA 35618	3.97±0.98	0.00	1.98	5.96
		Terbol	-0.34±0.98	0.73	-2.33	1.65
		ICARDA 35621	4.41±0.98	**<0.001**	2.42	6.40
		ICARDA 31591	1.94±0.98	0.06	-0.05	3.93
		ICARDA 35015	0.49±0.98	0.62	-1.50	2.48
		ICARDA 32331	2.09±0.98	**0.04**	0.10	4.08
	ICARDA 32122	*ICARDA 35531*	1.64±0.98	0.10	-0.35	3.63

	ICARDA 35618	6.88±0.98	<**0.001**	4.89	8.87
	Terbol	2.57±0.98	**0.01**	0.58	4.56
	ICARDA 35621	7.32±0.98	<**0.001**	5.33	9.31
	ICARDA 31591	4.85±0.98	<**0.001**	2.86	6.84
	ICARDA 35015	3.40±0.98	<**0.001**	1.41	5.39
	ICARDA 32331	5.00±0.98	<**0.001**	3.01	6.99
ICARDA 35531	*ICARDA 35618*	5.24±0.98	<**0.001**	3.25	7.23
	Terbol	0.93±0.98	0.35	-1.06	2.92
	ICARDA 35621	5.67±0.98	<**0.001**	3.68	7.66
	ICARDA 31591	3.21±0.98	<**0.001**	1.22	5.20
	ICARDA 35015	1.76±0.98	0.08	-0.23	3.75
	ICARDA 32331	3.36±0.98	<**0.001**	1.37	5.35
ICARDA 35618	*Terbol*	-4.31±0.98	<**0.001**	-6.30	-2.32
	ICARDA 35621	0.43±0.98	0.66	-1.56	2.42
	ICARDA 31591	-2.03±0.98	**0.05**	-4.02	-0.04
	ICARDA 35015	-3.48±0.98	<**0.001**	-5.47	-1.49
	ICARDA 32331	-1.88±0.98	0.06	-3.87	0.11
Terbol	*ICARDA 35621*	4.75±0.98	<**0.001**	2.76	6.74
	ICARDA 31591	2.28±0.98	**0.03**	0.29	4.27
	ICARDA 35015	0.83±0.98	0.40	-1.16	2.82
	ICARDA 32331	2.43±0.98	**0.02**	0.44	4.42
ICARDA 35621	*ICARDA 31591*	-2.46±0.98	**0.02**	-4.45	-0.47
	ICARDA 35015	-3.92±0.98	<**0.001**	-5.91	-1.93
	ICARDA 32331	-2.32±0.98	**0.02**	-4.31	-0.33

	ICARDA 31591	*ICARDA 35015*	-1.45±0.98	0.15	-3.44	0.54
		ICARDA 32331	0.15±0.98	0.88	-1.84	2.14
	ICARDA 35015	*ICARDA 32331*	1.60±0.98	0.11	-0.39	3.59
Stressed	*Atlas*	*ICARDA 33847*	0.00±0.98	1.00	-1.99	1.99
		ICARDA 32122	-1.30±0.98	0.19	-3.29	0.69
		ICARDA 35531	-0.15±0.98	0.88	-2.14	1.84
		ICARDA 35618	0.75±0.98	0.45	-1.24	2.74
		Terbol	-0.69±0.98	0.49	-2.68	1.30
		ICARDA 35621	0.07±0.98	0.94	-1.92	2.06
		ICARDA 31591	-0.12±0.98	0.91	-2.11	1.87
		ICARDA 35015	-1.24±0.98	0.22	-3.23	0.75
		ICARDA 32331	-0.37±0.98	0.71	-2.36	1.62
	ICARDA 33847	*ICARDA 32122*	-1.30±0.98	0.19	-3.29	0.69
		ICARDA 35531	-0.15±0.98	0.88	-2.14	1.84
		ICARDA 35618	0.75±0.98	0.45	-1.24	2.74
		Terbol	-0.69±0.98	0.49	-2.68	1.30
		ICARDA 35621	0.07±0.98	0.94	-1.92	2.06
		ICARDA 31591	-0.12±0.98	0.91	-2.11	1.87
		ICARDA 35015	-1.24±0.98	0.22	-3.23	0.75
		ICARDA 32331	-0.37±0.98	0.71	-2.36	1.62
	ICARDA 32122	*ICARDA 35531*	1.15±0.98	0.25	-0.84	3.14
		ICARDA 35618	2.05±0.98	**0.04**	0.06	4.04
		Terbol	0.61±0.98	0.54	-1.38	2.60
		ICARDA 35621	1.37±0.98	0.17	-0.62	3.36

	ICARDA 31591	1.18±0.98	0.24	-0.81	3.17
	ICARDA 35015	0.06±0.98	0.95	-1.93	2.05
	ICARDA 32331	0.93±0.98	0.35	-1.06	2.92
ICARDA 35531	*ICARDA 35618*	0.91±0.98	0.36	-1.08	2.90
	Terbol	-0.54±0.98	0.59	-2.53	1.45
	ICARDA 35621	0.22±0.98	0.82	-1.77	2.21
	ICARDA 31591	0.04±0.98	0.97	-1.95	2.03
	ICARDA 35015	-1.08±0.98	0.28	-3.07	0.91
	ICARDA 32331	-0.22±0.98	0.82	-2.21	1.77
ICARDA 35618	*Terbol*	-1.45±0.98	0.15	-3.44	0.54
	ICARDA 35621	-0.68±0.98	0.49	-2.67	1.31
	ICARDA 31591	-0.87±0.98	0.38	-2.86	1.12
	ICARDA 35015	-1.99±0.98	**0.05**	-3.98	0.00
	ICARDA 32331	-1.13±0.98	0.26	-3.12	0.86
Terbol	*ICARDA 35621*	0.76±0.98	0.44	-1.23	2.75
	ICARDA 31591	0.58±0.98	0.56	-1.41	2.57
	ICARDA 35015	-0.54±0.98	0.58	-2.53	1.45
	ICARDA 32331	0.32±0.98	0.75	-1.67	2.31
ICARDA 35621	*ICARDA 31591*	-0.19±0.98	0.85	-2.18	1.80
	ICARDA 35015	-1.31±0.98	0.19	-3.30	0.68
	ICARDA 32331	-0.44±0.98	0.65	-2.43	1.55
ICARDA 31591	*ICARDA 35015*	-1.12±0.98	0.26	-3.11	0.87
	ICARDA 32331	-0.26±0.98	0.80	-2.25	1.73
ICARDA 35015	*ICARDA 32331*	0.86±0.98	0.39	-1.13	2.85

*Data in bold represent significant results at 0.05.

3.4.12. Effects on dry weight of vegetative tissues (g)

The effect of water stress was also estimated on the dry weight of all wheat cultivars and was found to be reduced by 65.16%; a value in line with the significant effect of drought stress on dry weight ($p < 0.001$). Dry weight for all cultivars in the control group ranged from 2.52 to 6.12g with a mean of 4.34 +/-SD = 1.15, while it varied from 1.01 to 2.08g with a mean of 1.51 +/-SD = 0.41, under drought stress. Pairwise comparisons of wheat dry weight under control (M = 4.34, SE = 0.11) and water stress conditions (M = 1.51, SE = 0.11), highlighted the significant negative impact of water stress on this particular parameter. The mean difference between the two treatments was found to be 2.83 g ($p < .001$). Therefore, drought stress had a statistically significant effect on dry weight, evidenced by a $p < .001$, $F(1, 40) = 364.25$.

In addition, the two-way ANOVA showed that there was a significant impact of variety on dry weight $F(9,40) = 7.39$, $p < .001$. These statistical results were in line with the variation in decreasing order of wheat cultivars, based on mean of dry weight, in control and water stress conditions, which were: The order of wheat genotypes under 100-85% WFC conditions was as follows:

Table 3.35. Average dry weight (g) of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	**Mean**	**Drought conditions**	**Mean**
ICARDA 32122	6.12	ICARDA 32122	2.08
ICARDA 35015	5.54	Terbol	1.89
ICARDA 33847	4.64	ICARDA 35015	1.82
ICARDA 35531	4.41	ICARDA 35621	1.50
ICARDA 31591	4.36	ICARDA 32331	1.49
Terbol	4.31	ICARDA 35531	1.45
ICARDA 32331	4.18	ICARDA 31591	1.37
ICARDA 35621	3.89	ICARDA 33847	1.34
ICARDA 35618	3.47	Atlas	1.18
Atlas	2.52	ICARDA 35618	1.01

ICARDA 32122 was the best-performing cultivar,, showing the smallest decrease in dry weight under drought conditions, unlike the cultivars ICARDA 35618 and Atlas. These results were in line with those obtained for fresh weight (Figure 3.12). Statistical analyses were also carried out to evaluate the significance effect of the interaction between variety and treatment on dry weight, which was demonstrated by F(9,40) = 2.66 , p = 0.02 (Table 3.36).

Table 3.36. Analysis of variance of dry weight vegetative tissues of of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	7.4	9	**<0.001***
Treatment	364.26	1	**<0.001**
Variety * Treatment	2.66	9	**0.016**
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants; df = degree of freedom.

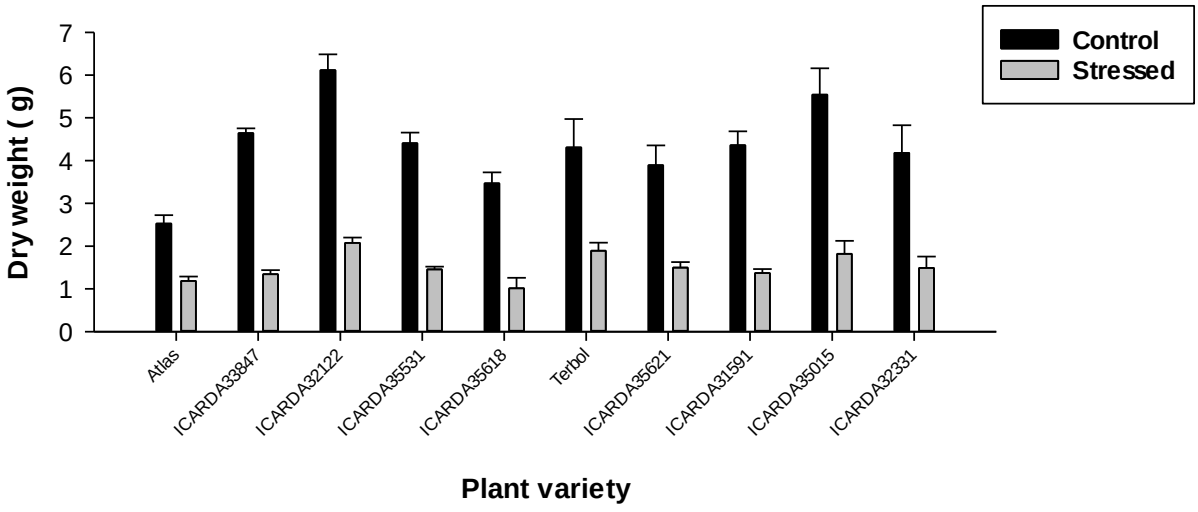


Figure 3.12. Dry weight of wheat cultivars under control (100- 85% WFC) and drought (50% WFC) conditions. X axis = wheat varieties. Y axis = dry weight (g). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean

under control = 4.34(g) and standard deviation = 1.14. The overall mean under drought stress = 1.51(g) and standard deviation = 0.40.

Based on the reduction in dry weight under drought conditions, the cultivars were ranked in ascending orders as follows: Atlas < Terbol< ICARDA35621< ICARDA32331< ICARDA32122 < ICARDA35531< ICARDA35015< ICARDA31591< ICARDA35618< ICARDA33847. Thus, Atlas remained one of the best-performing cultivars under drought conditions in terms of dry and fresh weight, plant height, spike length, number of leaves and tillers.

To further explore which ICARDA bread wheat varieties had significant dry weight under stress regime, post-hoc comparison was performed. The results showed that only ICARDA 32122 had significantly greater dry weight under drought stress. A detailed comparison is provided in Table 3.37.

Table 3.37. Post-hoc comparison for dry weight (g) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	-2.12±0.47	**<0.001***	-3.07	-1.17
		ICARDA 32122	-3.59±0.47	**<0.001**	-4.54	-2.65
		ICARDA 35531	-1.88±0.47	**<0.001**	-2.83	-0.94
		ICARDA 35618	-0.94±0.47	**0.05**	-1.89	0.00
		Terbol	-1.78±0.47	**<0.001**	-2.73	-0.84
		ICARDA 35621	-1.37±0.47	**0.01**	-2.32	-0.42
		ICARDA 31591	-1.84±0.47	**<0.001**	-2.78	-0.89

	ICARDA 35015	-3.02±0.47	<**0.001**	-3.96	-2.07
	ICARDA 32331	-1.66±0.47	<**0.001**	-2.60	-0.71
ICARDA 33847	*ICARDA 32122*	-1.47±0.47	<**0.001**	-2.42	-0.53
	ICARDA 35531	0.24±0.47	0.62	-0.71	1.18
	ICARDA 35618	1.18±0.47	**0.02**	0.23	2.12
	Terbol	0.34±0.47	0.48	-0.61	1.28
	ICARDA 35621	0.75±0.47	0.12	-0.20	1.70
	ICARDA 31591	0.28±0.47	0.55	-0.66	1.23
	ICARDA 35015	-0.90±0.47	0.06	-1.84	0.05
	ICARDA 32331	0.46±0.47	0.33	-0.48	1.41
ICARDA 32122	*ICARDA 35531*	1.71±0.47	<**0.001**	0.76	2.66
	ICARDA 35618	2.65±0.47	<**0.001**	1.70	3.60
	Terbol	1.81±0.47	<**0.001**	0.86	2.76
	ICARDA 35621	2.22±0.47	<**0.001**	1.28	3.17
	ICARDA 31591	1.76±0.47	<**0.001**	0.81	2.70
	ICARDA 35015	0.58±0.47	0.23	-0.37	1.52
	ICARDA 32331	1.94±0.47	<**0.001**	0.99	2.88
ICARDA 35531	*ICARDA 35618*	0.94±0.47	**0.05**	-0.01	1.89
	Terbol	0.10±0.47	0.83	-0.85	1.05
	ICARDA 35621	0.51±0.47	0.28	-0.43	1.46
	ICARDA 31591	0.05±0.47	0.92	-0.90	0.99
	ICARDA 35015	-1.13±0.47	**0.02**	-2.08	-0.19
	ICARDA 32331	0.23±0.47	0.63	-0.72	1.17
ICARDA 35618	*Terbol*	-0.84±0.47	0.08	-1.79	0.11
	ICARDA 35621	-0.43±0.47	0.37	-1.37	0.52

		ICARDA 31591	-0.89±0.47	0.06	-1.84	0.05
		ICARDA 35015	-2.07±0.47	<**0.001**	-3.02	-1.13
		ICARDA 32331	-0.71±0.47	0.14	-1.66	0.23
	Terbol	*ICARDA 35621*	0.41±0.47	0.38	-0.53	1.36
		ICARDA 31591	-0.05±0.47	0.91	-1.00	0.89
		ICARDA 35015	-1.23±0.47	**0.01**	-2.18	-0.29
		ICARDA 32331	0.13±0.47	0.79	-0.82	1.07
	ICARDA 35621	*ICARDA 31591*	-0.47±0.47	0.33	-1.41	0.48
		ICARDA 35015	-1.65±0.47	<**0.001**	-2.59	-0.70
		ICARDA 32331	-0.29±0.47	0.54	-1.23	0.66
	ICARDA 31591	*ICARDA 35015*	-1.18±0.47	0.02	-2.13	-0.23
		ICARDA 32331	0.18±0.47	0.70	-0.77	1.13
	ICARDA 35015	*ICARDA 32331*	1.36±0.47	**0.01**	0.41	2.31
Stressed	*Atlas*	*ICARDA 33847*	-0.16±0.47	0.73	-1.11	0.79
		ICARDA 32122	-0.89±0.47	0.06	-1.84	0.05
		ICARDA 35531	-0.27±0.47	0.57	-1.22	0.68
		ICARDA 35618	0.17±0.47	0.72	-0.78	1.12
		Terbol	-0.71±0.47	0.14	-1.65	0.24
		ICARDA 35621	-0.31±0.47	0.51	-1.26	0.63
		ICARDA 31591	-0.19±0.47	0.69	-1.13	0.76
		ICARDA 35015	-0.64±0.47	0.18	-1.58	0.31
		ICARDA 32331	-0.30±0.47	0.52	-1.25	0.64
	ICARDA 33847	*ICARDA 32122*	-0.73±0.47	0.13	-1.68	0.21

	ICARDA 35531	-0.11±0.47	0.82	-1.06	0.84
	ICARDA 35618	0.33±0.47	0.49	-0.62	1.28
	Terbol	-0.55±0.47	0.25	-1.49	0.40
	ICARDA 35621	-0.15±0.47	0.75	-1.10	0.79
	ICARDA 31591	-0.03±0.47	0.95	-0.97	0.92
	ICARDA 35015	-0.48±0.47	0.32	-1.42	0.47
	ICARDA 32331	-0.14±0.47	0.76	-1.09	0.80
ICARDA 32122	*ICARDA 35531*	0.62±0.47	0.19	-0.32	1.57
	ICARDA 35618	1.06±0.47	**0.03**	0.12	2.01
	Terbol	0.19±0.47	0.69	-0.76	1.13
	ICARDA 35621	0.58±0.47	0.22	-0.37	1.53
	ICARDA 31591	0.71±0.47	0.14	-0.24	1.65
	ICARDA 35015	0.26±0.47	0.59	-0.69	1.20
	ICARDA 32331	0.59±0.47	0.22	-0.36	1.54
ICARDA 35531	*ICARDA 35618*	0.44±0.47	0.35	-0.51	1.39
	Terbol	-0.44±0.47	0.36	-1.38	0.51
	ICARDA 35621	-0.04±0.47	0.93	-0.99	0.90
	ICARDA 31591	0.08±0.47	0.86	-0.86	1.03
	ICARDA 35015	-0.37±0.47	0.44	-1.31	0.58
	ICARDA 32331	-0.03±0.47	0.94	-0.98	0.91
ICARDA 35618	*Terbol*	-0.88±0.47	0.07	-1.82	0.07
	ICARDA 35621	-0.48±0.47	0.31	-1.43	0.46
	ICARDA 31591	-0.36±0.47	0.45	-1.30	0.59
	ICARDA 35015	-0.81±0.47	0.09	-1.75	0.14
	ICARDA 32331	-0.47±0.47	0.32	-1.42	0.47

	Terbol	*ICARDA 35621*	0.39±0.47	0.41	-0.55	1.34
		ICARDA 31591	0.52±0.47	0.27	-0.43	1.47
		ICARDA 35015	0.07±0.47	0.88	-0.88	1.02
		ICARDA 32331	0.40±0.47	0.39	-0.54	1.35
	ICARDA 35621	*ICARDA 31591*	0.13±0.47	0.79	-0.82	1.07
		ICARDA 35015	-0.32±0.47	0.49	-1.27	0.62
		ICARDA 32331	0.01±0.47	0.98	-0.94	0.96
	ICARDA 31591	*ICARDA 35015*	-0.45±0.47	0.34	-1.40	0.50
		ICARDA 32331	-0.12±0.47	0.80	-1.06	0.83
	ICARDA 35015	*ICARDA 32331*	0.33±0.47	0.48	-0.61	1.28

*Data in bold represent significant results at 0.05.

3.4.13. Effects on Chlorophyll content (SPAD value)

Drought stress has been shown to inhibit photosynthesis by decreasing chlorophyll content. This parameter was assessed in all the wheat cultivars under control and water stress conditions. A mean reduction of 70.03% in chlorophyll content was recorded in all wheat samples under 50% WFC. All wheat samples under optimal and stressed-conditions had chlorophyll contents that ranged from 17.00 to 34.40 (a mean of 25.39; +/- SD = 5.68) and from 4.96 to 13.28 (a mean of 7.61; +/- SD = 4.69), respectively (Table 3. 27). The significant effect of drought stress on chlorophyll content was statistically evidenced ($p < .001$) and below the cut-off point of 0.05. It is evident from the data that the highest chlorophyll content was obtained from the wheat samples grown under optimal growth conditions (100-85% WFC) as shown by the mean value, M = 25.39, (SE = 0.77), which is significantly greater to the values (M = 7.61; SE = 0.77) for samples under water stress conditions. The mean difference between the two treatments was 17.78, $p < .001$. These findings were consistent with the Anova data, which confirmed the significant effect of treatment on chlorophyll content with $F(1, 40) = 268.23$, $p < .001$. Moreover, there appeared to be a significant main effect of wheat variety on chlorophyll content, as evidenced by $F(9,40) = 7.36$, $p < .004$.

These data were consistent with the variation in descending order of cultivars, based on chlorophyll content, under control and drought stress conditions, which were:

Table 3.38. Average chlorophyll content of the wheat cultivars, under control (100-85% WFC) and drought (50% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 35618	34.40	ICARDA 35618	13.28
ICARDA 35621	30.46	ICARDA 32122	12.08
Atlas	28.38	ICARDA 32331	7.79
ICARDA 35015	25.39	ICARDA 31591	7.12
ICARDA 35531	25.06	ICARDA 35531	6.90
Terbol	24.60	Terbol	6.79
ICARDA 32331	24.37	ICARDA 35015	6.26
ICARDA 31591	24.01	Atlas	5.63
ICARDA 32122	20.22	ICARDA 35621	5.28
ICARDA 33847	17.00	ICARDA 33847	4.96

Based on the reduction in chlorophyll content under drought conditions, the cultivars were ranked in ascending orders as follows: ICARDA32122 < ICARDA35618 < ICARDA32331 < ICARDA31591 < ICARDA33847 < Terbol < ICARDA35531 < ICARDA35015 < Atlas < ICARDA35621 (Figure 3.13). Thus, cultivar ICARDA35622 and ICARDA35618 proved to be the best-performing cultivars under drought conditions. However, two-way ANOVA showed a p value of 0.055 ($F(9,40) = 2.08$) for the two variables, treatment and variety, confirming that there was no significant interaction between these two parameters on chlorophyll content (Table 3.39).

Table 3.39. Analysis of variance of chlorophyll content for wheat cultivars under control (100-85% WFC) and drought (50% WFC) conditions.

Model	F	df	*P*-value
Variety	3.36	9	**0.004***
Treatment	268.23	1	**<0.001**
Variety * Treatment	2.08	9	0.055
Error	-	40	-

*Data in bold represent results below the threshold of p-value 0.05; F = statistic (ratio of two variants); df = degree of freedom.

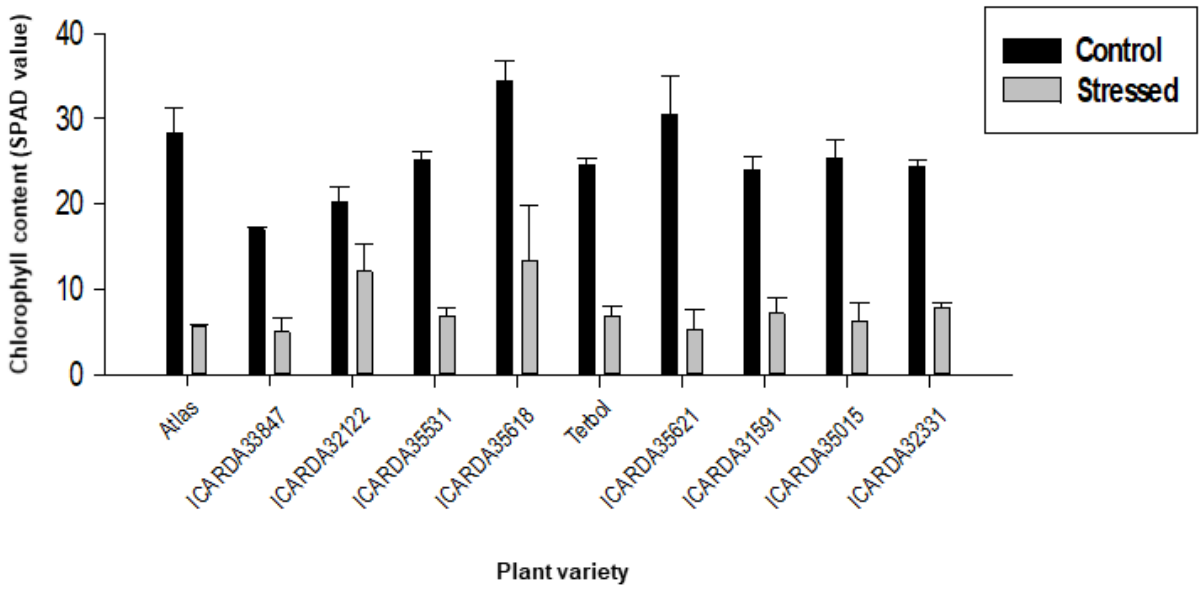


Figure 3.13. Chlorophyll content of wheat cultivars under control (100- 85% WFC) and (50% WFC) conditions. X axis = wheat varieties. Y axis = chlorophyll content (SPAD value). Black bar = wheat varieties under control condition. Grey bar = wheat varieties under drought condition. The overall mean under control = 25.38 and standard deviation = 5.67. The overall mean under drought stress = 7.60 and standard deviation = 4.69.

To further explore which ICARDA bread wheat varieties had significant chlorophyll content under stress regime, Tukey's post-hoc comparison was performed. The results indicated that Atlas, ICARDA 33847, ICARDA 32122, ICARDA 35618 had significantly greater chlorophyll content under drought stress. A detailed comparison is provided in Table 3.40.

Table 3.40. Post-hoc comparison for chlorophyll content (SPAD value) per variety under control (100-85% WFC) and drought (50% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 33847*	11.39±3.43	**<0.001***	4.45	18.32
		ICARDA 32122	8.17±3.43	**0.02**	1.23	15.10
		ICARDA 35531	3.33±3.43	0.34	-3.61	10.26
		ICARDA 35618	-6.01±3.43	0.09	-12.95	0.92
		Terbol	3.79±3.43	0.28	-3.15	10.72
		ICARDA 35621	-2.08±3.43	0.55	-9.01	4.86
		ICARDA 31591	4.37±3.43	0.21	-2.56	11.31
		ICARDA 35015	2.99±3.43	0.39	-3.94	9.93
		ICARDA 32331	4.02±3.43	0.25	-2.92	10.95
	ICARDA 33847	*ICARDA 32122*	-3.22±3.43	0.35	-10.16	3.72
		ICARDA 35531	-8.06±3.43	**0.02**	-15.00	-1.12
		ICARDA 35618	-17.40±3.43	**<0.001**	-24.34	-10.46
		Terbol	-7.60±3.43	**0.03**	-14.54	-0.66
		ICARDA 35621	-13.46±3.43	**<0.001**	-20.40	-6.53
		ICARDA 31591	-7.01±3.43	**0.05**	-13.95	-0.08
		ICARDA 35015	-8.39±3.43	**0.02**	-15.33	-1.46
		ICARDA 32331	-7.37±3.43	**0.04**	-14.31	-0.43
	ICARDA 32122	*ICARDA 35531*	-4.84±3.43	0.16	-11.78	2.10

	ICARDA 35618	-14.18±3.43	**<0.001**	-21.12	-7.24
	Terbol	-4.38±3.43	0.21	-11.32	2.56
	ICARDA 35621	-10.24±3.43	**<0.001**	-17.18	-3.31
	ICARDA 31591	-3.79±3.43	0.28	-10.73	3.14
	ICARDA 35015	-5.17±3.43	0.14	-12.11	1.76
	ICARDA 32331	-4.15±3.43	0.23	-11.09	2.79
ICARDA 35531	*ICARDA 35618*	-9.34±3.43	**0.01**	-16.28	-2.40
	Terbol	0.46±3.43	0.89	-6.48	7.40
	ICARDA 35621	-5.40±3.43	0.12	-12.34	1.53
	ICARDA 31591	1.05±3.43	0.76	-5.89	7.98
	ICARDA 35015	-0.33±3.43	0.92	-7.27	6.60
	ICARDA 32331	0.69±3.43	0.84	-6.25	7.63
ICARDA 35618	*Terbol*	9.80±3.43	**0.01**	2.86	16.74
	ICARDA 35621	3.94±3.43	0.26	-3.00	10.87
	ICARDA 31591	10.39±3.43	**<0.001**	3.45	17.32
	ICARDA 35015	9.01±3.43	**0.01**	2.07	15.94
	ICARDA 32331	10.03±3.43	**0.01**	3.09	16.97
Terbol	*ICARDA 35621*	-5.86±3.43	0.10	-12.80	1.07
	ICARDA 31591	0.59±3.43	0.87	-6.35	7.52
	ICARDA 35015	-0.79±3.43	0.82	-7.73	6.14
	ICARDA 32331	0.23±3.43	0.95	-6.71	7.17
ICARDA 35621	*ICARDA 31591*	6.45±3.43	0.07	-0.49	13.39
	ICARDA 35015	5.07±3.43	0.15	-1.87	12.01
	ICARDA 32331	6.09±3.43	0.08	-0.84	13.03

	ICARDA 31591	*ICARDA 35015*	-1.38±3.43	0.69	-8.32	5.56
		ICARDA 32331	-0.36±3.43	0.92	-7.29	6.58
	ICARDA 35015	*ICARDA 32331*	1.02±3.43	0.77	-5.91	7.96
Stressed	*Atlas*	*ICARDA 33847*	0.67±3.43	0.85	-6.27	7.61
		ICARDA 32122	-6.45±3.43	0.07	-13.39	0.49
		ICARDA 35531	-1.27±3.43	0.71	-8.21	5.67
		ICARDA 35618	-7.65±3.43	**0.03**	-14.59	-0.72
		Terbol	-1.16±3.43	0.74	-8.09	5.78
		ICARDA 35621	0.35±3.43	0.92	-6.59	7.29
		ICARDA 31591	-1.49±3.43	0.67	-8.43	5.45
		ICARDA 35015	-0.63±3.43	0.86	-7.56	6.31
		ICARDA 32331	-2.16±3.43	0.53	-9.10	4.78
	ICARDA 33847	*ICARDA 32122*	-7.12±3.43	**0.04**	-14.06	-0.18
		ICARDA 35531	-1.94±3.43	0.58	-8.88	5.00
		ICARDA 35618	-8.32±3.43	**0.02**	-15.26	-1.39
		Terbol	-1.83±3.43	0.60	-8.76	5.11
		ICARDA 35621	-0.32±3.43	0.93	-7.26	6.62
		ICARDA 31591	-2.16±3.43	0.53	-9.10	4.78
		ICARDA 35015	-1.30±3.43	0.71	-8.23	5.64
		ICARDA 32331	-2.83±3.43	0.41	-9.77	4.11
	ICARDA 32122	*ICARDA 35531*	5.18±3.43	0.14	-1.76	12.12
		ICARDA 35618	-1.20±3.43	0.73	-8.14	5.73
		Terbol	5.29±3.43	0.13	-1.64	12.23
		ICARDA 35621	6.80±3.43	**0.05**	-0.14	13.74

	ICARDA 31591	4.96±3.43	0.16	-1.98	11.90
	ICARDA 35015	5.82±3.43	0.10	-1.11	12.76
	ICARDA 32331	4.29±3.43	0.22	-2.65	11.23
ICARDA 35531	*ICARDA 35618*	-6.38±3.43	0.07	-13.32	0.55
	Terbol	0.11±3.43	0.97	-6.82	7.05
	ICARDA 35621	1.62±3.43	0.64	-5.32	8.56
	ICARDA 31591	-0.22±3.43	0.95	-7.16	6.72
	ICARDA 35015	0.64±3.43	0.85	-6.29	7.58
	ICARDA 32331	-0.89±3.43	0.80	-7.83	6.05
ICARDA 35618	*Terbol*	6.50±3.43	0.07	-0.44	13.43
	ICARDA 35621	8.00±3.43	**0.02**	1.07	14.94
	ICARDA 31591	6.16±3.43	0.08	-0.77	13.10
	ICARDA 35015	7.03±3.43	**0.05**	0.09	13.96
	ICARDA 32331	5.49±3.43	0.12	-1.44	12.43
Terbol	*ICARDA 35621*	1.51±3.43	0.66	-5.43	8.44
	ICARDA 31591	-0.33±3.43	0.92	-7.27	6.60
	ICARDA 35015	0.53±3.43	0.88	-6.41	7.47
	ICARDA 32331	-1.00±3.43	0.77	-7.94	5.93
ICARDA 35621	*ICARDA 31591*	-1.84±3.43	0.59	-8.78	5.10
	ICARDA 35015	-0.98±3.43	0.78	-7.91	5.96
	ICARDA 32331	-2.51±3.43	0.47	-9.45	4.43
ICARDA 31591	*ICARDA 35015*	0.86±3.43	0.80	-6.07	7.80
	ICARDA 32331	-0.67±3.43	0.85	-7.61	6.27
ICARDA 35015	*ICARDA 32331*	-1.53±3.43	0.66	-8.47	5.40

*Data in bold represent significant results at 0.05.

3.4.14. Effect of drought on specific parameters as a measure of drought tolerance in wheat

The Drought Susceptibility Index (DSI) is ‘’a measure of drought, based on loss of yield under drought conditions in comparison to the yield under normal conditions”. This index therefore enables drought tolerance to be measured, indicating performance stability for genotypes under drought stress/optimal conditions. An estimation of drought susceptibility for all 10 genotypes was conducted and expressed as DSI. The decline in the performance of wheat genotypes under drought stress was determined relative to performance under optimal conditions. It must be noted that DSI < 1, points to cultivar tolerance towards drought stress, whilst DSI > 1 shows cultivar susceptibility towards it (Fischer and Maurer, 1978). The aforementioned values were compared for identifying cultivars that are considered as being susceptibille to drought stress. DSI figures for ‘grain weight per plant’ varied between 0.87-1.10 (Table 3.41).

Table 3.41. Impact of drought on specific parameters for drought tolerance of wheat based on grain yield.

Genotype	***DSI***	***DTE***	***RSW***	***RSN***
Atlas	1.10	9.04	90.96	91.13
ICARDA 33847	1.06	12.24	87.76	92.28
ICARDA 32122	1.03	14.46	85.54	84.60
ICARDA 35531	1.01	15.94	84.06	82.08
ICARDA 35618	1.01	16.12	83.88	78.23
Terbol	0.99	17.53	82.47	76.78
ICARDA 35621	0.97	19.56	80.44	77.35
ICARDA 31591	0.96	19.88	80.12	76.99
ICARDA 35015	0.90	25.43	74.57	62.81
ICARDA 32331	0.87	27.58	72.42	59.03

*DTE drought tolerance efficiency, DSI drought susceptibility index, RSW reduction of seed weight, RSN reduction of seeds number.

More specifically, the following genotypes were classified as being resistant to drought (DSI<1): Terbol, ICARDA 35621, ICARDA 31591, ICARDA 35015, and ICARDA 32331. In contrast, the following cultivars were found to be susceptible to drought (DSI > 1): Atlas, ICARDA 33847, ICARDA 32122, ICARDA 35531, and ICARDA 35618.

Moreover, considerable variations were observed in terms of DTE (Drought Tolerance Efficiency) for all cultivars under stress conditions. In this context, DTE for tolerant varieties ranged from 17.53%-27.58% while for susceptible cultivars was between 9.03% and 16.12%. Amongst susceptible cultivars determined based on DSI, the maximum DTE was seen in ICARDA 35618 (16.12%) and the minimum was found in Atlas (9.03%) while among the tolerant varieties, ICARDA 32331 exhibited the maximum DTE of 27.58% with Terbol exhibiting the minimum (17.93%). Terbol, ICARDA 35621, ICARDA 31591, ICARDA 35015, and ICARDA 32331 genotypes were shown to be drought tolerant based not only on the DTE and DSI data but also according to RSW ('Reduction of seeds' weight'), and RSN ('Reduction of seeds' No.'). Among the aforementioned cultivars, ICARDA 32331 had highest tolerance to drought, with ICARDA 35015 coming next, followed by ICARDA 31591. These finding were consistent with the minimum RSW and RSN values obtained for these tolerant genotypes compared to the other cultivars used in the present study (Table 3.41).

Pearson's correlations of the investigated physio-morphological characters relating to the whole plant, leaves, tillers, and grains of the ten cultivars under optimal and drought stress conditions were further analysed. The outcome of this study highlighted the relationship between numerous traits that can be very useful for breeding programmes (Sallam et al. 2019). Under drought stress conditions, a high correlation value (≥0.7-1) between spike weight (SW), number of seeds (NS), and seed weight (SeW) was observed.The results showed that the cultivars had adapted physiologically (eg. proline accumulation, relative water content, etc.) to maintain seed development and weight (Mahdavi et al. 2023). These same correlations were also observed when grown under optimal condition in addition to number of leaves and tillers (Table 3.42).

In addition, leaf number was also found to be strongly correlated with dry and fresh weight. Moreover, under optimal conditions, a high correlation was also obtained between spike length and leaf length, as well as between leaf number (NL), leaf length (LL), and dry weight (DW). It

is interesting to mention that under optimal condition there were a high correlation between chlorophyll content (CC) and SW, NS and SeW in contrast to performance under stress conditions (Table 3.42). These results highlighted the role of photosynthesis in spike and seeds development by producing the necessary energy (Goins et al. 1997; Jobson et al. 2019). Photosynthesis generates several nutrients such as carbon and oxgen that are required for metabolic activities during the grain filling stage (Galili et al. 2014; Rangan et al. 2016; Jobson et al. 2019).

Under control condition, a negative correlation was detected between CC and FW; and CC, NL and NT which could be related to genetic variation, growth stage, nutrient allocation, and physiological factors. Some genotypes may have a high CC and be less efficient in biomass production and others may spend more energy in grain production than leaf growth, as reported by Gao et al. (2024). More nutrients can be allocated to leaves and tillers and less is available for chlorophyll synthesis. The increased number of leaves can lead to the lower leaves being less exposed to light, which impairs chlorophyll production. The high number of leaves and tillers leads to the available nutrients not being sufficient to have optimal chlorophlly synthesis, as they are distributed finely accros the plant (Naseer et al. 2024; Wood et al. 1993; Karele 2001).

More details on the moderate and weak correlations (<0.7) between the different variables are presented in (Table 3.42).

Table 3.42. Pearson's correlations among physio-morphological characteristics of ten bread wheat genotypes under control (100- 85% WFC) and drought-stress (50% WFC) conditions.The upper right-hand side of the Table represents the values for wheat sample under control conditions. The lower left side of the table represents the values for wheat samples under drought stress conditions. The value in bold = highly correlated.

Variables	PH	SL	SW	NS	SeW	NL	NT	LL	LW	LA	FW	DW	CC
PH	**1**	-0.297	0.187	0.366	0.223	-0.075	-0.166	-0.141	-0.022	-0.157	0.338	-0.065	-0.011
SL	0.264	**1**	-0.299	-0.553	-0.570	0.424	0.296	**0.725**	0.272	**0.805**	0.530	**0.659**	-0.286
SW	-0.044	-0.117	**1**	**0.926**	**0.945**	-0.529	-0.513	-0.525	0.139	-0.439	-0.593	**-0.702**	**0.808**
NS	-0.170	**-0.654**	**0.768**	**1**	**0.975**	-0.512	-0.473	**-0.654**	-0.071	**-0.658**	-0.559	**-0.723**	**0.717**
SeW	-0.257	**-0.648**	**0.736**	**0.964**	**1**	-0.626	-0.560	**-0.701**	0.042	**-0.648**	**-0.703**	**-0.803**	**0.812**
NL	0.067	0.570	-0.306	-0.603	-0.573	**1**	**0.968**	0.559	-0.436	0.323	**0.724**	0.613	**-0.866**
NT	-0.020	-0.032	-0.461	-0.389	-0.192	0.333	**1**	0.473	-0.455	0.233	0.586	0.491	**-0.850**
LL	0.161	**0.692**	0.110	-0.388	-0.473	**0.720**	-0.322	**1**	-0.101	**0.893**	0.540	0.435	-0.591
LW	0.140	-0.283	0.416	0.486	0.324	-0.280	-0.550	0.049	**1**	0.357	-0.017	0.095	0.398
LA	0.372	0.226	0.124	-0.121	-0.149	0.618	0.193	0.484	0.424	**1**	0.492	0.454	-0.379
FW	-0.145	0.510	-0.167	-0.412	-0.536	0.501	-0.195	0.541	0.190	0.319	**1**	**0.857**	**-0.712**
DW	-0.162	0.503	-0.078	-0.309	-0.431	**0.636**	-0.280	**0.692**	0.183	0.380	**0.930**	**1**	-0.616
CC	0.434	0.032	0.328	0.204	0.076	-0.121	-0.528	0.340	**0.771**	0.435	-0.040	0.025	**1**

PH =Plant hight, SL= Spike Length, SW= Spike weight, NS=number of seeds, SeW= Seeds weight, NL= Number of leaves, NT= Number of tillers, LL= Leaf length, LW= Leaf width, LA = Leaf area, FW= Fresh weight, DW= Dry weight, CC= Chlorophyl content

The results presented in Table 3.42 agreed with the Agglomerative Hierarchical Clustering (AHC) of the ten cultivars based on the reduction percentage of all morphological features related to grain yield (NS, SW, SL, SeW). As shown in (Figure 3.14), Atlas was found to be closely related to ICARDA 33847, and next to a branch of ICARDA 356189 that was associated to a sub-cluster containing ICARDA 32122, ICARDA 35531, Terbol, ICARDA35621, and ICARDA 31591. However, the most drought-tolerant genotype, ICARDA 32331, together with ICARDA 35015, formed a distinct cluster. The relationship between these genotypes in AHC was consistent with their grain yield data. AHC, based on the reduction percentage of all physio-morphological traits observed under drought stress conditions (Figure 3.15), showed that ICARDA 32331 was closely related to ICARDA 35015 and next to a clade housing ICARDA 35621, ICARDA 31591, ICARDA 35618, ICARDA 32122, ICARDA 33847, Terbol, and ICARDA 35531. However, Atlas was placed in a distinct branch, far from all the other cultivars. The genotypes ICARDA 35531, ICARDA 33847 and Terbol were closely related to each other as they were grouped in the same cluster, while ICARDA 31591 was sub-clustered with ICARDA 35621. This clustering was partially in agreement with that based on percentage of reduction in plant height, dry weight and fresh weight, as Atlas was placed in a separate branch distant from all the others.

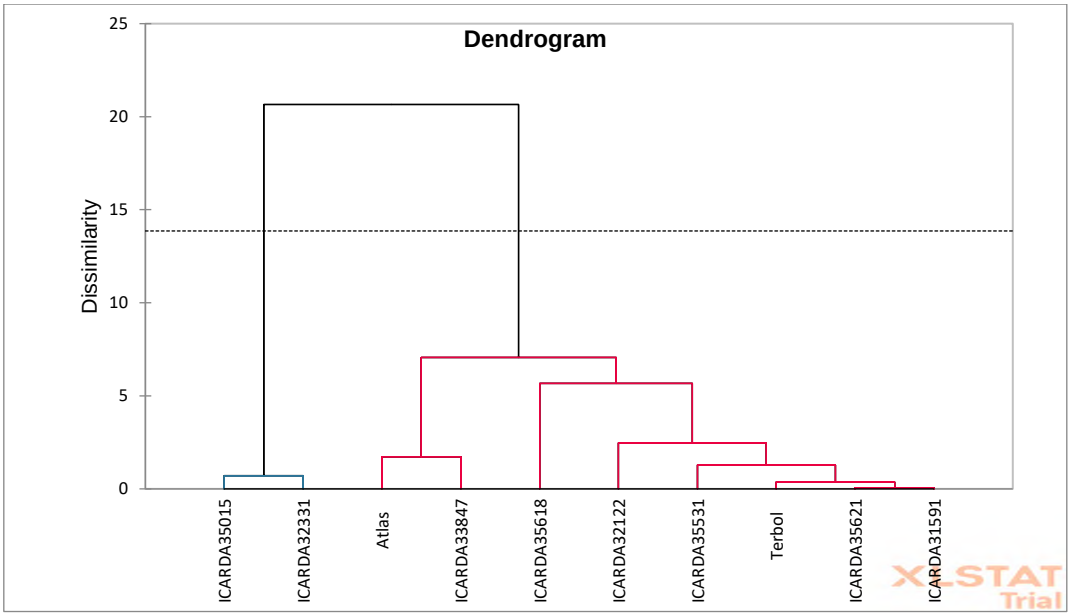


Figure 3.14. Agglomerative hierarchical clustering (AHC) based in the reduction percentage of all the morphological characters relating to NS, SW, SL, SeW of ten wheat cultivars under drought stress conditions. NS = Numer of seeds, SW= Spike weight, SL= Spike length, SeW= Seeds weight.

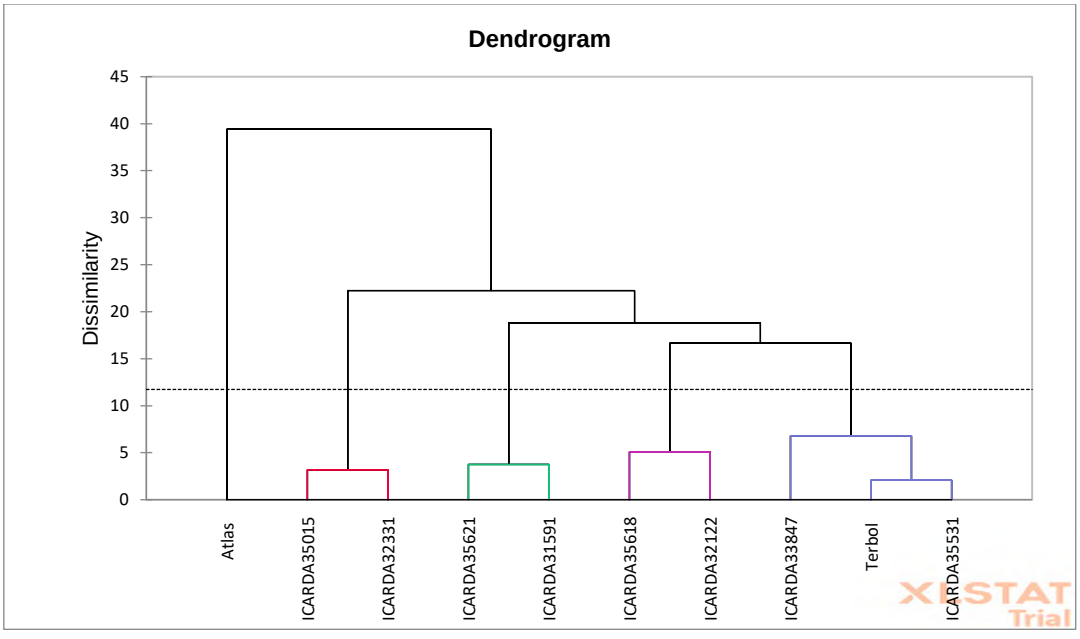


Figure 3.15. Agglomerative hierarchical clustering (AHC) based in the reduction percentage of all the physio-morphological characters of ten wheat cultivars under drought stress conditions.

3.5. Discussion

The impact of drought on grain yield and quality depends on its intensity, frequency, and phenological stage. The morphological traits affected by this abiotic stress, which contribute towards the final grain yield, reflect the significance of drought stress and give an insight into the adaptive mechanisms that the cultivars have to cope with this stress. Studying the physio-morphological features of wheat under drought, independently of grain yield, helps to select for more efficient tolerant cultivars in breeding programmes.

In this study, the physio-morphological characters of wheat cultivars associated with grain yield were affected by the water stress regime. The estimated values for wheat biomass and size, leaf size, seed number and weight per spike, spike and tiller numbers declined significantly when grown under reduced water availability, compared with wheat samples in the control group. The significant effect of drought stress treatment was statistically demonstrated in all physio-morphological characters studied, in contrast to the wheat variety. The reductions measured in the specific morphological traits measured in response to water stress are consistent with previous studies. Kilic and Yagbasanlar (2010) studied the effect of water stress on durum wheat cultivars and demonstrated that plant height, number of spike and grains, and grain weight were reduced under drought conditions.

The dwarf wheat cultivar, with its larger seed head and shorter stem, saved over a billion people from starvation in Mexico, India and Pakistan in 1960. Reducing the height of wheat improves standing ability and devotes more of the plant’s ressources to grain production than to stem (Norman Borlaug Green revolution). Plant height and biomass, including fresh and dry weight, are important parameters for selecting a drought-tolerant genotype. Ten wheat genotypes were screened for their ability to withstand drought stress based on performance of specific characteristics under the two water regimes. These findings were consistent with previous studies focused on drought stress wheat (Faisal et al. 2017; Chowdhury et al. 2021; Hussain et al. 2022). This abiotic stress was found to decrease grain productivity and increase the number of unfilled spike and spikelets (Haque et al. 2021). The genetic background from one wheat cultivar to another is different, meaning that some wheat varieties have the best morphological features and high grain yield and quality under optimal conditions, while they are not able to withstand abiotic stress and show a high percentage of the seed weight and number reduced under water-limited conditions. However, other wheat varieties are genetically less efficient in terms of grain yield under optimal conditions, but can show a lower reduction in seed weight and number under drought, leading to a more stable grain yield under water–limited conditions

(Ahomed 2017; Haque et al. (2020). However, some susceptible cultivars with high yields under optimal conditions may produce higher yields than resistant cultivars with low yields under dought conditions, and others cultivars may have decreased spike and seed numbers but higher seed weight under moderate stress conditions compared with control plants (Khan et al. 2019). Therefore, total grain yield parameters such as DSI, DTE, RSW and RSN are one of the most important criteria for selecting a drought-tolerant genotype. The low RSW and RSN values obtained for 5wheat cultivars, Terbol < ICARDA 35621 < ICARDA 31591< ICARDA 35015 < and ICARDA 32331 (ranked from least tolerant to most tolerant), agreed with the high values of DTE and DSI values < 1. These findings concur with those of Ahomed (2017) and Haque et al. (2020), who recognized that the genotypes Sonalika, Sourav, and BARI gom-28 performed exceptionally well under drought conditions based on their DSI and DTE values, used as selection criteria. The DSI value of the most tolerant genotype in the present study, ICARDA 32331, was similar to that of the previously described tolerant genotypes, Sonalika, Sourav, and BARI gom-28 genotypes (0.74-0.986), although the experimental conditions were not the same.

The Pearson correlation among morphological traits associated with grain yield such as grain weight, number of seeds, and spike weight were found to be highly correlated under drought and control conditions, results consistent with those reported by Moosavi et al. (2020). The same outcome was obtained for the number of leaves and tillers, which is in agreement with a previous study by Pokhrel et al. (2013).

A more in-depth examination of the physio-morphological properties of the present cultivars based on the percentage of reduction of the wheat parameters under drought stress was carried out. Comparative analysis of the physio-morphological parameters of wheat varieties provided insight into the effectiveness and reliability of morpho-physiological characteristics in estimating plant sensitivity to drought stress. In this context, the percentage reduction for all parameters studied was measured and provided a great insight into the key physio-morphological traits that can be used to select for a tolerant genotype. As shown in the results above, the order of cultivars based on the mean value of their physio-morphological characters under optimal and drought stress conditions differed from one characteristic to another. The wheat cultivars showing the lowest reduction in SW, NS and SW were ICARDA 35015 (75.41%; 62.81%; 66.26%), and ICARDA 32331 (72.42%; 59.03%; 68.99%); this is consistent with the data on total grain yield. However, the cultivars Atlas (36.62%) and ICARDA 332122

(36.71%) had the lowest reduction in plant height. Atlas also showed a low reduction in dry and fresh weight under drought stress, despite being found as the most sensitive genotype based on the total grain yield parameter (Table 3.28). In addition, Atlas had a higher number of leaves (NL 8.72) and tillers (NT 0.89) under drought stress than under optimal conditions (7.23; 0.13), as did ICARDA 35621 (9.13; 0.60[NL, NT stress]; 8.91; 0.30 [NL, NT control]). In addition, the ICARDA 31591 genotype showed a greater number of tillers (mean 1.33 vs 1.05) and a better leaf area (mean LA 16.10 vs. 14.68) under the stress regime than under optimal conditions. The lowest reduction in leaf length (LL) was observed in ICARDA 35621 (1.24%), while the lowest reduction in leaf width (LW) was obtained in ICARDA 35618 (32.93%). The lowest reduction in chlorophyll content was estimated for cultivar ICARDA 32122 (40.25%). Based on these results, the genotype Atlas appeared to cope with water stress conditions by reducing the loss of all the following morphological parameters, PH, SL, NL, NT, FW, and DW, except chlorophyll content, unlike the following cultivars ICARDA 33847, ICARDA 32122, ICARDA 35531, ICARDA 35618**,** which proved to have a sensitive genotype based on the total grain yield. These findings are consistent with the DSI data. However, under drought stress, Atlas appeared to perform better than ICARDA 32331 at the morphological level, although it exhibited a low grain yield value. These results would suggest that Atlas to be selected for further physiological, biochemical, and molecular studies, as described in the following chapters of this thesis.

Chapter 4. Effect of drought stress on the physio-morphological and biochemical characters of bread wheat cultivars

4.1. Abstract

Global warming has intensified the deleterious impact of drought stress on wheat production and, consequently, calls for the screening and exploring of drought-tolerant wheat cultivars, which is fundamental to the breeding approach. After an initial screening of the ten wheat cultivars in chapter 3, a total of 6 cultivars were selected for further analysis. The aerial tissues of three tolerant (ICARDA 32331, ICARDA 35015, and ICARDA 31591), and sensitive (ICARDA 33847, Atlas, ICARDA 35531) cultivars at seedling stage were evaluated under drought stress conditions (25% WFC), in comparison with control samples (100-85% WFC). The tolerance of these cultivars was assessed based on physio-morphological and biochemical features, including plant height, fresh weight, dry weight, relative water content (RWC %), chlorophyll content (CHL), and proline content. The following plant stress-related indices were estimated: Yp and Ys which stand for RWC of the genotype under non-stress and stress conditions, tolerance index (TOL), relative stress index (RSI), mean productivity (MP), harmonic mean (HM), yield stability index (YSI), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), and yield index (YI). Statistical analyses showed that the cultivars ICARDA32331, ICARDA35015, and ICARDA33847 merit to be considered as drought-tolerant varieties, while Atlas, ICARDA35531, and ICARDA31591 are drought-sensitive cultivars. These results are consistent with those of the agglomerative hierarchical clustering. Average sum of rank (ASR), calculated using values of drought stress indices for RWC, showed that ICARDA32331 is the best drought-tolerant variety (ASR =14) while Atlas (ASR =60) is the most sensitive cultivar, calling for selection of these cultivars for further comparative transcriptomic analysis to define the key molecular factors underlying drought tolerance. Pearson's correlation coefficient analysis showed that there was a strong positive correlation between plant height, chlorophyll, fresh weight, and relative water content under control conditions. However, under drought stress, a strong positive correlation was detected between the following parameters: chlorophyll and fresh weight (FW), relative water content (RWC) and stress tolerance index (STI); RWC and STI. STI was found to be strongly correlated with RWC > FW > CHL under drought stress. This study highlighted the relationship between several features that can be very useful for breeding programmes and justify the

selection of Atlas and ICARDA32331 cultivars for further RNA-Seq analysis to define the key molecular factors of drought tolerance (chapter 5).

4.2. Introduction

Triticum aestivum L, is the most vital staple food grain for approximately 36% of the global population (Kaur et al. 2020) and is continuously in high demanded, which will need to be increased by 50% by 2050 to meet the needs of the growing global population (FAO 2021; Lucas 2012). The productivity of this annual grass is threatened by abiotic stress such as drought which was behind several crop loss worldwide (Desa 2015). Drought stress induces physio-morphological and biochemical changes in wheat at vegetative and reproductive growth stages such as relative water content, chlorophyll content, and plant biomass. Limited water condition alters metabolic and physiological functions related to osmotic balance, respiration, and photosynthesis. The deleterious impact of drought on plant development and consequently on crop productivity depend on its duration, intensity and magnitude. This abiotic stress is one of the major causes of wheat yield loss globally that can be reduced by more than 50%. Drought can be detrimental at all growth stages but the seedling stage seems to be the most critical and extremely sensitive (Fang and Xiong 2015). Drought stress causes poor germination and seedling growth in several crop including pea (*Pisum sativum* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivium*). Germination is the physiological mechanism that triggers cascade of biochemical activities for seedling establishment (Poudel et al. 2019). The lack of water obstructs the germination process as normally it is initiated with the absorption of water by the dormant dry seed which lead to endosperm rupture and succeeded by the development of plant embryo into primary root (McCormick et al. 2016). Its performance is the result of interaction between physiological mechanism and the various environmental factors such as light, temperature, soil pH, moisture and water availability. Earlier studies showed a significant reduction of seed germination and seedling, under drought stress, that varied from one variety to another (Yassin et al. 2019). These growth phases have been used as indicator to evaluate the tolerance of wheat to drought stress (Rauf et al. 2007; Hubbard et al. 2012). Mérida-García et al. (2019) showed that germination and seedling development are negatively affected in presence of soil with osmotic potential reached 1.5 MPa. Under conditions of limited water, morphological features of wheat such as plant height, and fresh biomass including roots and shoots, decrease significantly as a result of altered cell division and enlargement (Taiz et al. 2015; Paul et al. 2018). Other plant morphological parameters are considered as important

indices for drought tolerant genotypes at seedling stage, such as plant height, dry and fresh weight of seedling, shoot and root length, and root /shoot ratio (RSR), as they were decreased under water stress conditions (Mujtaba et al. 2016; Faisal et al. 2017) except the root length and the ratio RSR which were found to be increased in water limited conditions (Mujtaba et al. 2016; Faisal et al. 2017; Ahmed et al. 2019, 2020; Mohi-Ud-Din et al. 2021). As response to drought stress conditions, the plant root length and RSR are increased for better absorption of water. This change is induced by the level of abscisic acid in roots and shoots (Nezhadahmadi et al. 2013).

Other physiological and biochemical parameters are considered relevant for selecting drought tolerance genotype are relative water content (RWC), chlorophyll, and proline content. RWC is one of the most important parameters to evaluate the water status of the plant. It has been shown that RWC is also reduced under drought stress due to the osmotic stress (Ahmed et al. 2019; 2020) and to the reduction in plant vigor (Kapoor et al. 2020). Chlorophyll is a fundamental component of photosynthesis and is directly influenced by drought stress. The latter damages the thylakoid membrane of the chloroplast and leads to a loss of chlorophyll (Djanaguiraman et al. 2010; Ahmed et al. 2019). The reduction in chlorophyll increases the production of reactive oxygen species (ROS), which promote lipid peroxidation (Khalilzadeh et al. 2016).

At the biochemical level, several parameters were used in plant breeding including proline content which is considered as significant indicator for drought tolerant genotype (Abebe et al. 2013; Bowne et al. 2012; Mwadzingeni et al. 2016). Previous studies showed that the level of proline in the plant was significantly increased for plant under stress condition including drought. This amino acid plays an important protective role such as membrane stabilisation and osmotic adjustment (Hayet et al. 2012; Zadehbagheri et al. 2014). Molecular studies showed that the biosynthesis of proline is related to pyrroline-5-carboxylate synthetase (P5CS) while its degradation is associated with proline dehydrogenase (Kishor et al., 2005; Szabados and Savouré, 2010; Saeedipour (2013). It has been reported that the level of proline is high in drought tolerant genotype of plant under drought stress comparing to the sensitive cultivars (Mwadzingeni et al .2016).

While water stress is detrimental to wheat growth, development, yield and productivity; it has also been shown that drought stress during seedling stage enhance the acclimation or hardening mechanism of the plant so that it can cope with this abiotic stress when it is more intense at

later growth stages (Selote and Khanna-Chopra 2010; Amoah and Seo 2021). This mechanism immunizes the plant against drought by increasing their fitness and performance under water stress conditions. Acclimation is based on physiological and metabolic adjustment of the plant following a limited water supply at seedling stage (Wilson and Franklin 2002). This approach helps the plant to be more resistant to severe drought stress (Thomas 2009) and it has also been applied for other plant and abiotic stress such as tomato and cucumber seedlings under cold stress (Ghanbari and Kordi 2019; Ghanbari and Sayyari 2018). Plants modulate their biological features and remodel their genetic expression to withstand drought stressor. The performance of the plants in the presence of abiotic stress relying strongly on their genetic makeup.

In view of the drastic impact of drought stress on wheat, search and development of drought tolerant or resistant or adaptable genotypes are urgently needed to boost wheat production particularly in semi-arid climatic area (Abhinandan et al. 2018; Amoah and Seo 2021). In this context, initial screening of ten wheat varieties for drought tolerance was performed in chapter 3 and led to 5 drought tolerant cultivars being defined. Selection of the drought tolerant cultivars was based on their physio-morphological features in response to drought stress during the maturity stage. The defined drought tolerant cultivars in chapter 3 was based mainly on their ability to generate high grain yield under drought conditions. As this late stage trait is complex and can be influenced by several factors, the evaluation of wheat cultivars, under water limited conditions, at seedling stage seems to be more targeted to this abiotic stress. Therefore, from the initial screening a total of 6 genotypes (3 drought tolerant [ICARDA 32331, ICARDA 35015, and ICARDA 31591] and 3 drought sensitive cultivars [ICARDA 33847, Atlas, ICARDA 35531]) were selected for further analysis, mainly at the biochemical level.

4.3. Aims and objectives

A total of 6 bread wheat cultivars were selected based on their performance on drought stress condition during the maturity stage. The tolerant and sensitive selected wheat genotypes were based on their morphological and physiological characters. The aim of this present study is to evaluate and identify the tolerance of these selected bread wheat genotypes to the drought stress during the seedling stage based not only on physio-morphological traits but also on their biochemical characters.

4.4. Results

In order to identify the biochemical response of three most tolerant (ICARDA 32331, ICARDA 35015, and ICARDA 31591) and three most sensitive (ICARDA 33847, Atlas, ICARDA 35531) ICARDA bread wheat genotypes to the experimentally induced drought stress, an evaluation of a wide range of plant traits over a seedling stage was performed. The drought stress was exerted for 15 days starting from the development of the third leaf and ended at the appearance of the first tiller. During the seedling stage the water supply was stopped until it reached 25% WFC. The evaluated morphological, biochemical, and physiological traits of the wheat were: Plant height (cm), fresh weight (g), dry weight (g), relative water content (RWC %), chlorophyll content (SPAD value), and proline concentration (µg/l). In general, all these plant traits have been affected by water holding conditions in comparison to those observed in control group.

4.4.1. Effect of drought on plant height (cm)

In general, the impact of drought stress on plant height (cm) was different among ICARDA bread wheat varieties dependent on variety and growth condition denoted as treatment (Figure 4.1, Table 4.1). More specifically, induced drought stress significantly decreased plant height of all six bread wheat genotypes ($p < .001$) by about 40.1% (Figure 4.1). Plant height ranged between 10.45 to 16.26 cm with a mean of 12.95±2.26 under drought stress, while it fluctuated between 13.9 and 27.39 cm with a mean of 21.98±5.47 under control conditions. Table 4.1 showed that the variety ($F(5,24) = 10.73$, $p < 0.001$), treatment ($F(1, 24) = 121.06$, $p < 0.001$), and the interaction between these two variables ($F(5,24) = 4.14$, $p < 0.001$) had a significant main effect on plant height. These results were consistent with those of Tukey pairwise comparisons which reveal that the plant height was significantly different on wheat cultivars in control group ($M = 21.98$, $SE = 0.58$) and those cultivated under drought stress conditions ($M = 12.95$, $SE = 0.58$). The mean difference was 9.03 cm, $p < .001$, with the highest plant height value observed in control group.

Table. 4.1 Two- way ANOVA for plant height (cm) per variety under control (100- 85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	10.73	5	**<0.001**
Treatment	121.06	1	**<0.001**
Variety * Treatment	4.14	5	**0.008**
Error	6.06	24	-

*Data in bold represent significant results at p = 0.05

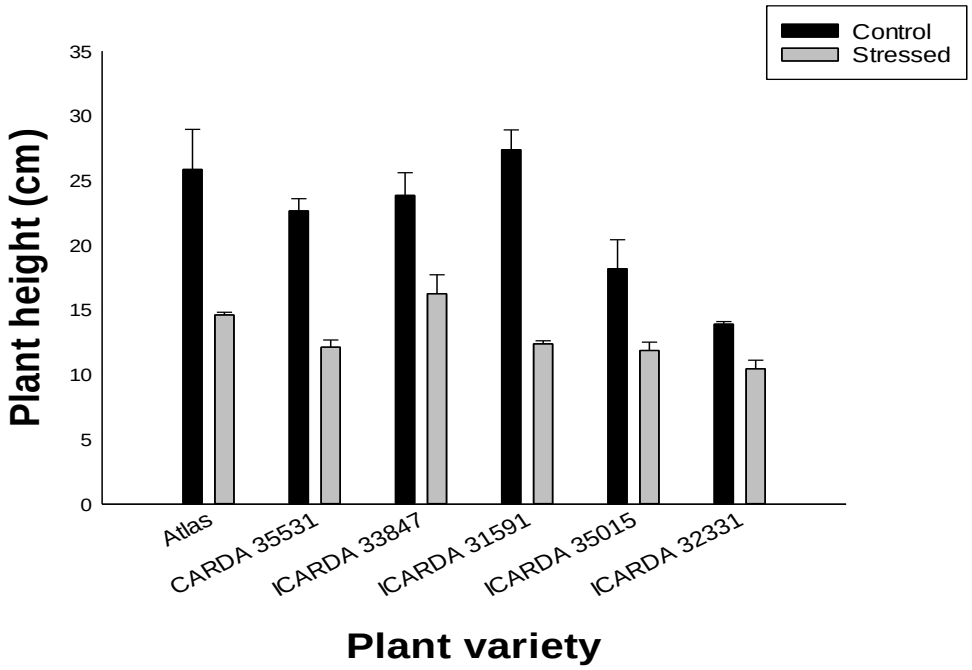


Figure 4.1. Plant height of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = plant height (cm). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 21.98 (cm) and standard deviation = 5.47. The overall mean under drought stress = 12.95 (cm) and standard deviation = 2.26.

As shown in figure 4.1 the mean of plant height (cm) for the six wheat varieties under control and stress conditions. The rank order of plant height in response to the two growing conditions is summarised in Table 4.2 and shows that drought negatively affected plant height but the relative growth rate between cultivars did not differ greatly.

Table 4.2. Average plant height (cm) of the wheat cultivars, under control (100-85% WFC) and drought (25% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 31591	27.39	ICARDA 33847	16.27
Atlas	25.87	Atlas	14.61
ICARDA 33847	23.87	ICARDA 31591	12.38
ICARDA 35531	22.67	ICARDA 35531	12.13
ICARDA 35015	18.20	ICARDA 35015	11.87
ICARDA 32331	13.90	ICARDA 32331	10.45

Interestingly, ICARDA 31591, Atlas, ICARDA 35531 were recognized as the drought-sensitive varieties as they had the greatest reduction in height of 54.81%, 46.5%, 43.51%, respectively. In contrast, plant height reduction was minimal in genotypes ICARDA 32331 (24.80%), ICARDA 33847 (31.84%) and ICARDA 35015 (34.75%) indicating their ability to cope with the drought stress.

Tukey's post-hoc comparison was conducted to further explore which ICARDA bread wheat varieties had statistically significant plant height under control and drought stress. The outcome of this test revealed that only ICARDA 232331 and ICARDA 35015 genotypes had significant plant height under drought and control conditions while ICARDA 31591 had significant plant height under control ($p < 0.001$). A detailed comparison is provided in Table 4. 3.

Table. 4.3. Post-hoc comparison for plant height (cm) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	3.20±2.01	0.46	-5.68	2.62
		ICARDA 33847	2.0±2.01	0.33	-2.15	6.15
		ICARDA 31591	-1.53±2.01	0.46	-5.68	2.62
		ICARDA 35015	7.67±2.01	**<0.001***	3.52	11.82
		ICARDA 32331	11.97±2.01	**<0.001**	7.82	16.12
	ICARDA 35531	*ICARDA 33847*	-1.20±2.01	0.56	-5.35	2.95
		ICARDA 31591	-4.73±2.01	**0.03**	-8.88	-0.58
		ICARDA 35015	4.47±2.01	**0.04**	0.32	8.62
		ICARDA 32331	8.77±2.01	**<0.001**	4.62	12.92
	ICARDA 33847	*ICARDA 31591*	-3.53±2.01	0.09	-7.68	0.62
		ICARDA 35015	5.67±2.01	**0.01**	1.52	9.82
		ICARDA 32331	9.97±2.01	**<0.001**	5.82	14.12

	ICARDA 31591	*ICARDA 35015*	9.19±2.01	**<0.001**	5.04	13.34
		ICARDA 32331	13.49±2.01	**<0.001**	9.34	17.64
	ICARDA 35015	*ICARDA 32331*	4.30±2.01	**0.04**	0.15	8.45
Stressed	*Atlas*	*ICARDA 35531*	2.49±2.01	0.23	-1.66	6.64
		ICARDA 33847	-1.65±2.01	0.42	-5.80	2.50
		ICARDA 31591	2.74±2.01	0.19	-1.41	6.89
		ICARDA 35015	2.74±2.01	0.19	-1.41	6.89
		ICARDA 32331	4.16±2.01	**0.05**	0.01	8.31
	ICARDA 35531	*ICARDA 33847*	-4.14±2.01	**0.05**	-8.29	0.01
		ICARDA 31591	-0.25±2.01	0.90	-4.40	3.90
		ICARDA 35015	0.25±2.01	0.90	-3.90	4.40
		ICARDA 32331	1.67±2.01	0.41	-2.48	5.82
	ICARDA 33847	*ICARDA 31591*	3.89±2.01	0.07	-0.26	8.04
		ICARDA 35015	4.39±2.01	**0.04**	0.24	8.54
		ICARDA 32331	5.81±2.01	**0.01**	1.66	9.96
	ICARDA 31591	*ICARDA 35015*	0.51±2.01	0.80	-3.64	4.66

	ICARDA 32331	1.93±2.01	0.35	-2.22	6.08
ICARDA 35015	*ICARDA 32331*	1.42±2.01	0.49	-2.73	5.57

*Data in bold represent significant results at 0.05

4.4.2. Changes in fresh weight (g) resulting from drought conditions

Plant fresh weight per wheat variety under control and stress conditions was measured (Table 4.4). Data proved that the average fresh weight of six cultivars was significantly affected by the drought stress ($p < .001$) (Table 4.4). Plant fresh weight ranged from 0.28 to 0.7 g with a mean of 0.5±0.2, under drought stress, whereas it varied from 1.34 to 3.48 g with a mean of 2.54±1.02, under control growth conditions. A fresh weight of the six wheat cultivars decreased under stress conditions by 80.11%. As shown in figure 4.2, plant fresh weight average under control condition was always higher than in the stressed group. The trends within the data were very similar and are summarised in the following rank order table.

Table 4.4. Average fresh weight (g) of the wheat cultivars, under control (100-85% WFC) and drought (25%WFC) conditions, in descending order.

Optimal (control) conditions	**Mean**	**Drought conditions**	**Mean**
ICARDA32331	3.48	ICARDA 32331	0.70
ICARDA 35015	3.34	ICARDA 35015	0.66
ICARDA 35531	3.31	ICARDA 33847	0.53
ICARDA 33847	2.35	ICARDA 35531	0.46
Atlas	1.41	ICARDA 31591	0.39
ICARDA 31591	1.34	Atlas	0.28

The variation in the order of the cultivars based on the mean value of their fresh weight under control and drought stress highlighted the significant effect of the genotype and drought on their total fresh biomass. This conclusion was statistically supported by the two-way ANOVA results. Table 4.5 showed that the variety ($F(5,72) = 32.10$, $p < .001$), treatment ($F(1,72) = 631.58$, $p < .001$) and the interaction between these two variables ($F(5,72) = 18.69$, $p = .001$) had a significant main effect on plant height. These results were in line with the statistical analysis based on Tukey pairwise comparisons which confirmed that the fresh weight is significantly different between the wheat samples of the control ($M = 2.54$, $SE = 0.05$) and drought stress ($M = 0.50$, $SE = 0.05$) conditions. The mean difference was 2.03 g, $p < .001$ with the highest amount of fresh weight obtained under control conditions. Moreover, the highest reduction on fresh weight were found on ICARDA35531, ICARDA35015, and ICARDA32331 cultivars with 86.03%, 80.10%, 79.92% respectively; meaning that these cultivars have a sensitive genotype to drought stress. However, fresh weight reduction was minimal in varieties Atlas (79.82%), ICARDA 33847 (77.47%) and ICARDA31591 (70.90%); reflecting their ability to cope with the drought stress. These findings were consistent with the results of the Tukey's post-hoc comparison which was performed to determine which of the ICARDA bread wheat varieties had statistically significant fresh weight under control and drought stress. The outcome of this test reveal that only ICARDA 232331 genotype had significant fresh weight (p value of 0.04< 0.05) under drought stress while the Atlas, ICARDA 33847, and ICARDA31591, seemed to have significant fresh weight in optimum conditions ($p < 0.01$). A detailed comparison is provided in Table 4. 6.

Table. 4.5. Two-way ANOVA for fresh weight (g) per variety under control (100- 85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	32.73	5	**<0.001**
Treatment	121.06	1	**<0.001**
Variety * Treatment	6.06	5	**0.001**
Error	-	27	-

* Data in bold represent significant results at 0.05

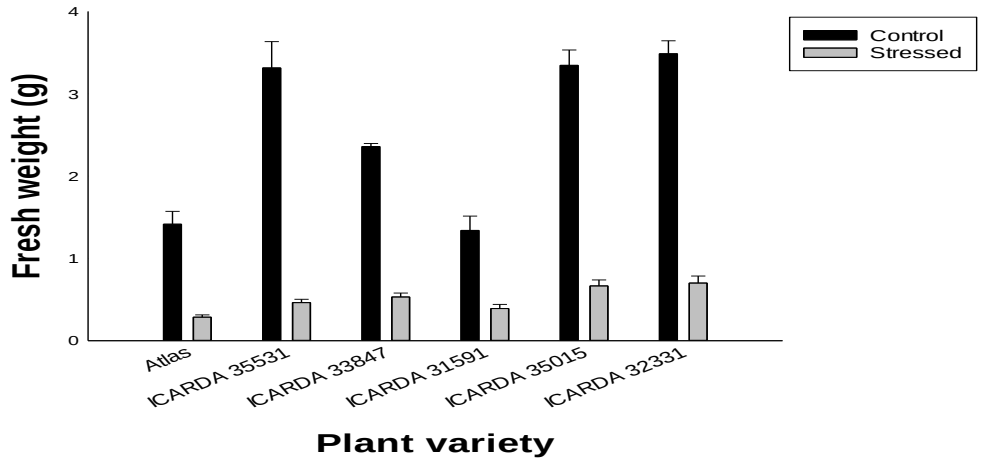


Figure 4.2. Fresh weight of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = fresh weight (g). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 2.54 (g) and standard deviation = 1.02. The overall mean under drought stress = 0.50 (g) and standard deviation = 0.20.

Table. 4.6. Post-hoc comparison for fresh weight (g) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean; lower Bound = mean difference minus margin of error; upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	-1.90±0.20	0.70	-0.32	0.47
		ICARDA 33847	-0.94±0.20	**<0.01***	-1.34	-0.55
		ICARDA 31591	0.08±0.20	0.70	-0.32	0.47
		ICARDA 35015	-1.93±0.20	**<0.01**	-2.33	-1.53
		ICARDA 32331	-2.07±0.20	**<0.01**	-2.47	-1.67
	ICARDA 35531	*ICARDA 33847*	0.96±0.20	**<0.01**	0.56	1.35
		ICARDA 31591	1.97±0.20	**<0.01**	1.58	2.37

		ICARDA 35015	-0.03±0.20	0.88	-0.43	0.36
		ICARDA 32331	-0.17±0.20	0.39	-0.57	0.22
	ICARDA 33847	*ICARDA 31591*	1.02±0.20	**<0.01**	0.62	1.41
		ICARDA 35015	-0.99±0.20	**<0.01**	-1.38	-0.59
		ICARDA 32331	-1.13±0.20	**<0.01**	-1.52	-0.73
	ICARDA 31591	*ICARDA 35015*	-2.01±0.20	**<0.01**	-2.40	-1.61
		ICARDA 32331	-2.15±0.20	**<0.01**	-2.54	-1.75
	ICARDA 35015	*ICARDA 32331*	-0.14±0.20	0.48	-0.54	0.26
Stressed	*Atlas*	*ICARDA 35531*	-0.18±0.20	0.38	-0.57	0.22
		ICARDA 33847	-0.25±0.20	0.22	-0.64	0.15
		ICARDA 31591	-0.10±0.20	0.60	-0.50	0.29
		ICARDA 35015	-0.38±0.20	0.06	-0.78	0.02
		ICARDA 32331	-0.41±0.20	**0.04**	-0.81	-0.02
	ICARDA 35531	*ICARDA 33847*	-0.07±0.20	0.73	-0.46	0.33
		ICARDA 31591	0.07±0.20	0.72	-0.32	0.47
		ICARDA 35015	-0.20±0.20	0.31	-0.60	0.19
		ICARDA 32331	-0.24±0.20	0.24	-0.63	0.16
	ICARDA 33847	*ICARDA 31591*	0.14±0.20	0.48	-0.25	0.54
		ICARDA 35015	-0.13±0.20	0.50	-0.53	0.26
		ICARDA 32331	-0.17±0.20	0.40	-0.56	0.23
	ICARDA 31591	*ICARDA 35015*	-0.28±0.20	0.17	-0.67	0.12
		ICARDA 32331	-0.31±0.20	0.12	-0.71	0.09
	ICARDA 35015	*ICARDA 32331*	-0.03±0.20	0.86	-0.43	0.36

*Data in bold represent significant results at 0.05

4.4.3. Dry weight (g) changes in response to drought

Comparable results were evident upon measuring plant dry weight, where the level of stress condition significantly affected plant dry weight per bread wheat variety differently (Figure 4.3, Table 4.8). The dry weight varied between 0.28 and 0.7 g with average 0.5±0.2 g, under drought stress conditions while it fluctuated between 0.24 and 0.67 g with an average of 0.39±0.17 g under control conditions. Like the fresh weight, the dry weight was significantly negatively affected by the drought stress ($p < .001$). According to Figure 4.4, the average plant dry weight under drought stress can be ordered as follows:

Table 4.7. Average dry weight (g) of the wheat cultivars, under control (100-85% WFC) and drought (25% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 31591	1.34	ICARDA 32331	0.19
ICARDA 35531	0.66	ICARDA 35015	0.19
ICARDA 35015	0.44	ICARDA 33847	0.19
ICARDA32331	0.38	ICARDA 35531	0.21
ICARDA 33847	0.38	ICARDA 31591	0.20
Atlas	0.24	Atlas	0.16

These rankings were not in line with those based on fresh weight. The difference in dry weight of the cultivars under control conditions reflect the impact of water on their development and also their different water content capacity. The variation in the order of the cultivars, based on dry weight, under control and water stress conditions highlighted the statistically proved significance of the variety ($F(5,72) = 18.71$, $p < .001$), treatment ($F(1,72) = 162.15$, $p < .001$), and the interaction between these two variables on dry weight ($F(5,72) = 14.34$, $p = .001$). Pairwise comparisons based on Tukey test were in coherent with the statistical results mentioned above and confirmed the significant difference between control ($M = 2.54$, $SE = 0.05$) and drought treatment ($M = 0.50$, $SE = 0.05$) on dry weight. The mean of this difference was found to be 2.03 g, $p < .001$ with the highest value obtained under control conditions.

The highest reduction percentage of the dry weight was observed in ICARDA35531, ICARDA35015, ICARDA 33847 with 68.45%, 55.81%, 50.00% respectively, reflecting their sensitive genotype. However, the lowest decrease of the dry weight was obtained in ICARDA

32331 (49.26%), Atlas (33.72%), and ICARDA31591 (16.67%), indicating their ability to cope with the drought stress.

Table. 4.8. Two- way ANOVA for dry weight (g) per variety under control (100- 85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	18.71	5	**<0.001**
Treatment	162.15	1	**<0.001**
Variety * Treatment	14.34	5	**<0.001**
Error	-	72	-

*Data in bold represent significant results at 0.05

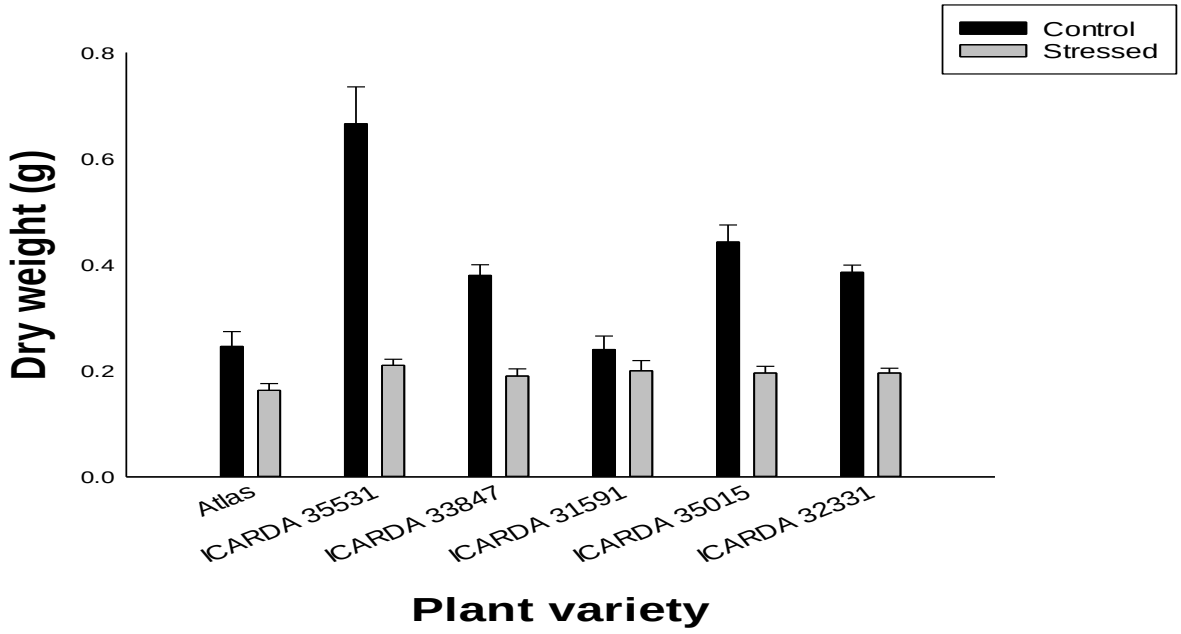


Figure 4.3. Dry weight of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = dry weight (g). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 0.39 (g) and standard deviation = 0.17. The overall mean under drought stress = 0.2 (g) and standard deviation = 0.03.

These results were consistent with the results of the Tukey's post-hoc comparison which was performed to determine which of the ICARDA bread wheat varieties had statistically significant dry weight under control and drought stress. The data revealed that ICARDA 32331, ICARDA31591, ICARDA35015, ICARDA35531, and ICARDA 33847 genotypes had significant dry weight under optimum growth conditions. No significance on dry weight was observed under stress conditions. A detailed comparison is provided in Table 4. 9.

Table. 4.9. Post- hoc comparison for dry weight (g) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	-0.42±0.04	**<0.001***	-0.497	-0.343
		ICARDA 33847	-0.134±0.04	**<0.001**	-0.21	-0.06
		ICARDA 31591	0.01±0.04	0.88	-0.07	0.08
		ICARDA 35015	-0.20±0.04	**<0.001**	-0.27	-0.12
		ICARDA 32331	-0.14±0.04	**<0.001**	-0.22	-0.06
	ICARDA 35531	*ICARDA 33847*	-0.03±0.04	0.49	-0.10	0.05
		ICARDA 31591	-0.04±0.04	0.34	-0.11	0.04
		ICARDA 35015	-0.03±0.04	0.40	-0.11	0.04
		ICARDA 32331	-0.03±0.04	0.40	-0.11	0.04
	ICARDA 33847	*ICARDA 31591*	0.14±0.04	**<0.001**	0.06	0.22
		ICARDA 35015	-0.06±0.04	0.11	-0.14	0.01
		ICARDA 32331	-0.01±0.04	0.88	-0.08	0.07
	ICARDA 31591	*ICARDA 35015*	-0.20±0.04	**<0.001**	-0.28	-0.13
		ICARDA 32331	-0.15±0.04	**<0.001**	-0.22	-0.07
	ICARDA 35015	*ICARDA 32331*	0.06±0.04	0.14	-0.02	0.13

	Atlas	*ICARDA 35531*	-0.05±0.04	0.23	-0.12	0.03
		ICARDA 33847	-0.03±0.04	0.49	-0.10	0.05
		ICARDA 31591	-0.04±0.04	0.34	-0.11	0.04
		ICARDA 35015	-0.03±0.04	0.40	-0.11	0.04
		ICARDA 32331	-0.03±0.04	0.40	-0.11	0.04
Stressed	*ICARDA 35531*	*ICARDA 33847*	0.02±0.04	0.61	-0.06	0.10
		ICARDA 31591	0.01±0.04	0.80	-0.07	0.09
		ICARDA 35015	0.01±0.04	0.71	-0.06	0.09
		ICARDA 32331	0.01±0.04	0.71	-0.06	0.09
	ICARDA 33847	*ICARDA 31591*	-0.01±0.04	0.80	-0.09	0.07
		ICARDA 35015	-0.01±0.04	0.88	-0.08	0.07
		ICARDA 32331	-0.01±0.04	0.88	-0.08	0.07
	ICARDA 31591	*ICARDA 35015*	-0.03±0.04	0.40	-0.11	0.04
		ICARDA 32331	-0.03±0.04	0.40	-0.11	0.04
	ICARDA 35015	*ICARDA 32331*	0.00±0.04	1.00	-0.08	0.08

*Data in bold represent significant results at 0.05

4.4.4. Effect of drought on wheat relative water content (RWC%)

Relative water content is one of the critical parameters in plant physiology which was found to be significantly affected by the drought stress ($p < .001$). RWC fluctuated between 42.17% and 70.05% with an average percent of 57.31±13.94% under drought stress (Table 4.10). Whereas it ranged from 78.59% to 88.76% with an average of 83.71±5.05 under control. RWC of the six wheat cultivars was reduced by 31.53% under drought stress.

Data illustrated in Figure 4.4 demonstrated that RWC of the six bread wheat varieties kept under drought stress took this order:

Table 4.10. Average relative water content (%) of the wheat cultivars, under control (100-85% WFC) and drought (25% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32331	88.76%	ICARDA 32331	70.05%
ICARDA 35015	86.68%	ICARDA 35015	69.45%
ICARDA 33847	83.91%	ICARDA 33847	61.45%
Atlas	82.70%	ICARDA 35531	53.10%
ICARDA 31591	81.60%	ICARDA 31591	47.23%
ICARDA 35531	78.59%	Atlas	42.17%

Two way ANOVA test showed that the variety ($F(5,72) = 14.09$, $p < .001$), treatment ($F(1,72) = 276.75$, $p < .001$) and the interaction between variety and treatment ($F(5,72) = 5.64$, $p = .001$) had a significant effect on WFC as shown in Table 4.11. Moreover, Tukey pairwise comparison showed that there was a significant difference in RWC between control ($M = 83.70$, $SE = 1.12$) and drought stress (($M = 57.31$, $SE = 1.12$) conditions. This difference has an average of 26.39 g, $p < .001$ with the highest RWC detected in wheat sample under optimum conditions.

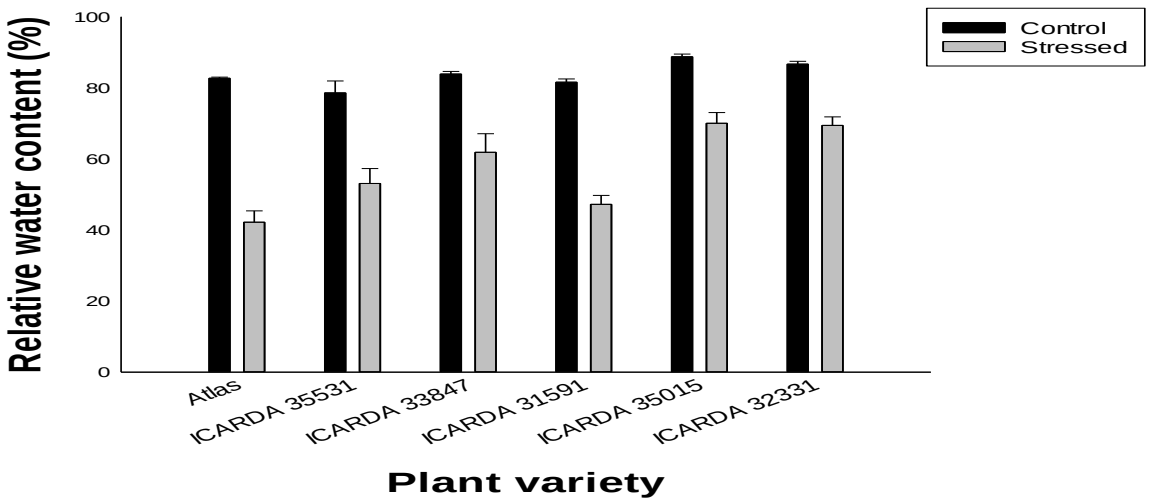


Figure 4.4. Relative water content of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = relative water content (%). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 83.71 (%) and standard deviation = 5.05. The overall mean under drought stress = 57.31 (%) and standard deviation = 13.94.

Table. 4.11. Two- way ANOVA for relative water content (%) per variety under control (100-85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	14.09	5	**<0.001**
Treatment	276.75	1	**<0.001**
Variety * Treatment	5.64	5	**<0.001**
Error	-	72	-

*Data in bold represent significant results at 0.05

RWC findings implied that the genotypes Atlas, ICARDA31591, and ICARDA35531 had the greatest decrease in RWC percent of 49%, 42.12%, 32.43% respectively, suggesting them as the least resistant to drought stress. In contrast, genotypes ICARDA 35015 (19.88%), ICARDA32331 (21.08%), and ICARDA33847 (26.25%) retained higher water level therefore had a minimal decrease in the RWC indicating their ability to cope with the drought stress.

To further explore which ICARDA bread wheat varieties had significant RWC under control and stress regime, Tukey's post-hoc comparison was performed. Under drought stress conditions, the post-hoc analysis revealed that ICARDA 35531, ICARDA 33847, ICARDA 35015 had significantly greater RWC under drought stress compared to the sensitive genotypes, Atlas, ICARDA31591, and ICARDA35531. A detailed comparison is provided in Table 4. 12.

Table. 4.12. Post- hoc comparison for relative water content (%) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean; lower Bound = mean difference minus margin of error; upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	4.10±3.89	0.29	-3.64	11.85
		ICARDA 33847	-1.21±3.89	0.76	-8.96	6.54
		ICARDA 31591	1.1±3.89	0.78	-6.65	8.84

		ICARDA 35015	-3.99±3.89	0.31	-11.73	3.76
		ICARDA 32331	-6.06±3.89	0.12	-13.80	1.69
	ICARDA 35531	*ICARDA 33847*	-5.32±3.89	0.18	-13.06	2.43
		ICARDA 31591	-3.01±3.89	0.44	-10.76	4.74
		ICARDA 35015	-8.08±3.89	**0.04***	-15.84	-0.34
		ICARDA 32331	-10.16±3.89	**0.01**	-17.91	-2.41
	ICARDA 33847	*ICARDA 31591*	2.31±3.89	0.56	-5.44	10.05
		ICARDA 35015	-2.77±3.89	0.48	-10.52	4.97
		ICARDA 32331	-4.85±3.89	0.22	-12.59	2.90
	ICARDA 31591	*ICARDA 35015*	-5.08±3.89	0.20	-12.83	2.67
		ICARDA 32331	-7.15±3.89	0.07	-14.90	0.60
	ICARDA 35015	*ICARDA 32331*	-2.07	0.60	-9.82	5.67
Stressed	*Atlas*	*ICARDA 35531*	-10.93±3.89	**0.01**	-18.68	-3.18
		ICARDA 33847	-19.71±3.89	<**0.001**	-27.45	-11.96
		ICARDA 31591	-5.06±3.89	0.20	-12.80	2.69
		ICARDA 35015	-27.28±3.89	<**0.001**	-35.02	-19.53
		ICARDA 32331	-27.88±3.89	<**0.001**	-35.62	-20.13
	ICARDA 35531	*ICARDA 33847*	-8.78±3.89	**0.03**	-16.52	-1.03
		ICARDA 31591	5.87±3.89	0.14	-1.87	13.62
		ICARDA 35015	-16.35±3.89	<**0.001**	-24.09	-8.60
		ICARDA 32331	-16.95±3.89	<**0.001**	-24.70	-9.20
	ICARDA 33847	*ICARDA 31591*	14.65±3.89	<**0.001**	6.90	22.40
		ICARDA 35015	-7.57±3.89	**0.06**	-15.32	0.18
		ICARDA 32331	-8.17±3.89	**0.04**	-15.92	-0.42

ICARDA 31591	*ICARDA 35015*	-22.22±3.89	**<0.001**	-29.97	-14.47
	ICARDA 32331	-22.82±3.89	**<0.001**	-30.57	-15.07
ICARDA 35015	*ICARDA 32331*	-0.60±3.89	0.88	-8.35	7.15

*Data in bold represent significant results at 0.05

4.4.5. Changes in Chlorophyll content (SPAD value) resulting from drought

Similar findings were also evident for chlorophyll concentration (SPAD value) as it depended on both plant variety and stress condition (Figure 4.5). ANOVA test showed that the variety ($F(5,24) = 4.03$, $p < .009$), treatment ($F(1, 24) = 222.88$, $p < .001$), and the interaction between these two variables ($F(5,24) = 2.92$, $p = .001$) had a significant effect on the chlorophyll content as shown in Table 4.13. The water stress regime had negatively affected the chlorophyll content for all the six wheat cultivars ($p < .001$) and reduced by 65.93%. Chlorophyll content for the six plant genotypes under drought stress conditions fluctuated between 2.43 and 7.84 SPAD value with an average of 5.54±2.68. Whereas under control conditions it ranged between 13.7 and 19.61 SPAD value with a mean of 16.27±2.97.

Table. 4.13. Two- way ANOVA for chlorophyll concentration (SPAD value) per variety under control (100- 85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	4.03	5	**0.009**
Treatment	222.88	1	**<0.001**
Variety * Treatment	2.92	5	**0.034**
Error	4.65	24	-

*Data in bold represent significant results at 0.05

According to data presented in Figure 4.5, the average chlorophyll content (SPAD value) under stress conditions was ranked as follows:

Table 4.14. Average chlorophyll content (SPAD value) of the wheat cultivars, under control (100-85% WFC) and drought (25% WFC) conditions, in descending order.

Optimal (control) conditions	Mean	Drought conditions	Mean
ICARDA 32331	19.61	ICARDA 33847	7.84
ICARDA35015	18.25	ICARDA 32331	7.59

ICARDA 31591	16.75	ICARDA 35015	6.28
Atlas	14.90	ICARDA 35531	6.12
ICARDA 35531	14.43	ICARDA 31591	2.99
ICARDA 33847	13.69	Atlas	2.43

These variations between the two growth conditions agreed with the statistically significant effect of the three variables presented in Table 4.13.

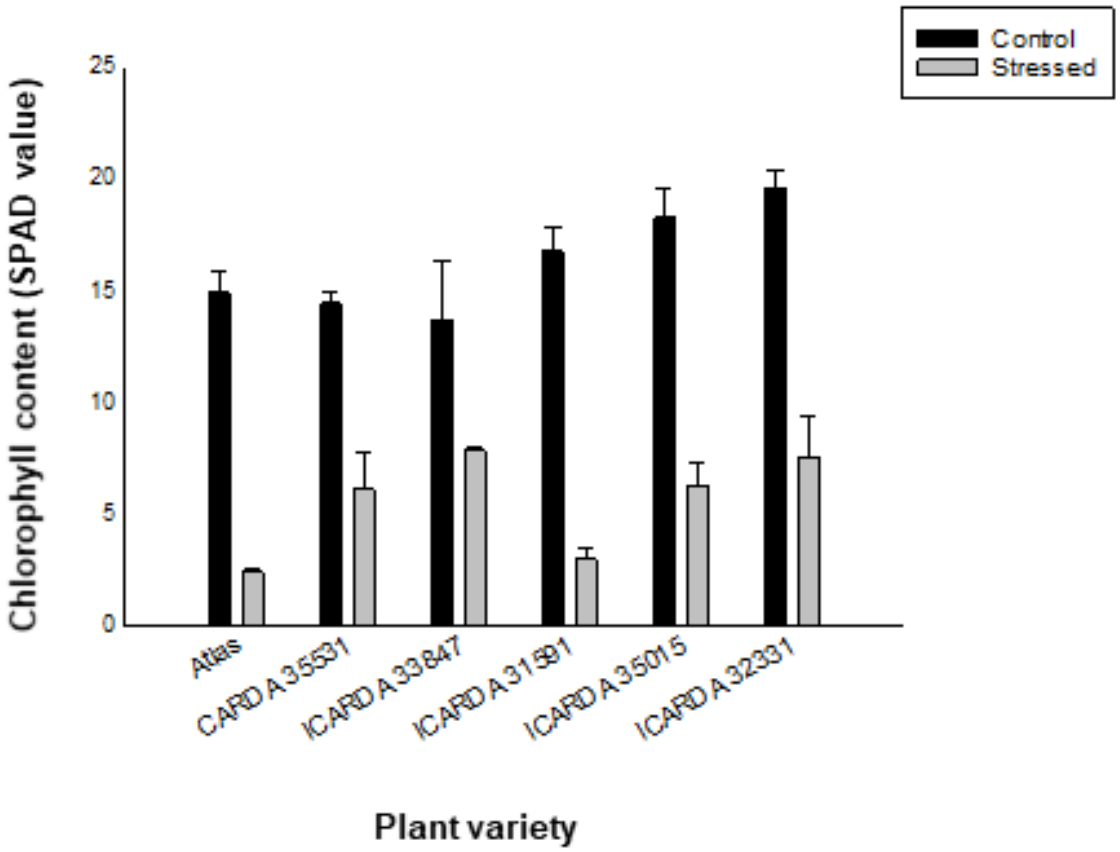


Figure 4. 5. Chlorophyll content of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = chlorophyll content (SPAD value). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 16.27 and standard deviation = 2.97. The overall mean under drought stress = 5.54 and standard deviation = 2.68.

As reduction in chlorophyll content (SPAD value reduction) reached its maximum in genotypes Atlas and ICARDA 31591 with 83.70% and 82.12%, respectively, they are classified as highly sensitive to drought stress. On the contrary, genotypes ICARDA 35015 (65.60%), ICARDA 32331 (61.31%), ICARDA35531 (57.54) and ICARDA 33847 (42.68%) had a minimal decrease in chlorophyll content under drought stress condition indicating their ability to cope with the drought stress.

To determine the significant difference in chlorophyll content between all pairs of varieties under optimal and drought conditions, Tukey's method was applied. The results showed that under drought stress conditions, ICARDA 35531, ICARDA 33847, ICARDA 35015, and ICARDA 32331 had significantly greater chlorophyll level compared to the sensitive genotypes, Atlas and ICARDA 31591. However, under control growth conditions, only ICARDA 32331 had significantly greater level of chlorophyll compared to Atlas genotype. More detailed comparison between the studied cultivars are presented in Table 4.15.

Table. 4.15. Post- hoc comparison for chorophyll concentration (SPAD value) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean; lower Bound = mean difference minus margin of error; upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	0.48±1.76	0.79	-3.16	4.11
		ICARDA 33847	1.22±1.76	0.50	-2.42	4.85
		ICARDA 31591	-1.85±1.76	0.30	-5.48	1.78
		ICARDA 35015	-3.35±1.76	0.07	-6.98	0.28
		ICARDA 32331	-4.71±1.76	**0.01***	-8.34	-1.08
	ICARDA 35531	*ICARDA 33847*	0.74±1.76	0.68	-2.89	4.37
		ICARDA 31591	-2.33±1.76	0.20	-5.96	1.31

		ICARDA 35015	-3.83±1.76	**0.04**	-7.46	-0.19
		ICARDA 32331	-5.19±1.76	**0.01**	-8.82	-1.55
	ICARDA 33847	*ICARDA 31591*	-3.07±1.76	0.09	-6.70	0.57
		ICARDA 35015	-4.57±1.76	**0.02**	-8.20	-0.93
		ICARDA 32331	-5.93±1.76	<**0.001**	-9.56	-2.29
	ICARDA 31591	*ICARDA 35015*	-1.50±1.76	0.40	-5.13	2.13
		ICARDA 32331	-2.86±1.76	0.12	-6.49	0.77
	ICARDA 35015	*ICARDA 32331*	-1.36±1.76	0.45	-4.99	2.27
Stressed	*Atlas*	*ICARDA 35531*	-3.70±1.76	**0.05**	-7.33	-0.06
		ICARDA 33847	-5.42±1.76	**0.01**	-9.05	-1.78
		ICARDA 31591	-0.57±1.76	0.75	-4.20	3.07
		ICARDA 35015	-3.85±1.76	**0.04**	-7.48	-0.22
		ICARDA 32331	-5.16±1.76	**0.01**	-8.79	-1.53
	ICARDA 35531	*ICARDA 33847*	-1.72±1.76	0.34	-5.35	1.91
		ICARDA 31591	3.13±1.76	0.09	-0.50	6.76
		ICARDA 35015	-0.15±1.76	0.93	-3.79	3.48
		ICARDA 32331	-1.46±1.76	0.41	-5.10	2.17
	ICARDA 33847	*ICARDA 31591*	4.85±1.76	**0.01**	1.22	8.48
		ICARDA 35015	1.57±1.76	0.38	-2.07	5.20
		ICARDA 32331	0.26±1.76	0.89	-3.38	3.89
	ICARDA 31591	*ICARDA 35015*	-3.28±1.76	0.07	-6.92	0.35
		ICARDA 32331	-4.59±1.76	**0.02**	-8.23	-0.96
	ICARDA 35015	*ICARDA 32331*	-1.31±1.76	0.46	-4.94	2.32

*Data in bold represent significant results at 0.05

4.4.6. Proline content changes significantly in drought conditions.

In contrast to the other traits studied above, only drought stress condition ($F(1, 24) = 759.97$, $p < .001$) was found to have a significant impact on proline concentration in all the six bread wheat genotypes (Table 4.16). No significance effect for the variety ($F(5,24) = 1.60$, $p < .196$) and the interaction between variety and treatment ($F(5,24) = 2.05$, $p = .107$) on proline content was detected. Typically, proline level was significantly increased in all bread wheat varieties in response to drought stress by 33.8 fold. The proline content fluctuated from 23.26 to 31.70 (µg/ g FW) with a mean of 28.4±4.7 under drought stress conditions, while it ranged from 0.25 to 1.29 (µg/g FW) with an average of 0.84±0.45 under control growth conditions. A significant difference in the proline concentration was obtained between the control ($M = 0.84$, $SE = 0.70$) and the drought stress regime ($M = 28.40$, $SE = 0.70$).

Table. 4.16. Two-way ANOVA for proline level (µg/g FW) per variety under control (100-85% WFC) and drought (25% WFC) conditions.

Model	F	df	*P-value*
Variety	1.61	5	0.196
Treatment	759.97	1	**<0.001**
Variety * Treatment	2.05	5	0.107
Error	-	24	-

*Data in bold represent significant results at 0.05

As shown in Figure 4.6, the average proline content under stress regime was ranked as follows

Table 4.17. Average proline content of the wheat cultivars, under control (100-85% WFC) and drought (25% WFC) conditions, in descending order.

Optimal (control) conditions	Mean (µg/g FW)	Drought conditions	Mean (µg/g FW)
Atlas	1.29	ICARDA 31591	31.70
ICARDA35015	1.16	ICARDA 33847	31.64
ICARDA 35531	1.02	ICARDA 35015	29.60
ICARDA 32331	0.76	Atlas	27.95
ICARDA 31591	0.58	ICARDA 32331	26.26
ICARDA 33847	0.25	ICARDA 35531	23,26

The maximum percentage of increase in proline content under drought stress was ranked as follows ICARDA 33847 (125.6 fold) > ICARDA 31591 (54.6 fold) > ICARDA 32331 (35-0 fold) > ICARDA 35015 (25.5 fold) > ICARDA 35531 (22.8 fold) > Altas (20.6 fold). Based on these data, the cultivars ICARDA 33847 and ICARDA 31591 were able to cope with drought stress, unlike Atlas variety. These findings were consistent with the results of Tukey's post-hoc comparison which showed that ICARDA 33847, ICARDA 31591, ICARDA 32331, ICARDA 35015 had a significant concentration of proline under drought stress. No significance in this biochemical feature was detected under optimum conditions. More details about the pairwise comparison are displayed in table 4.18.

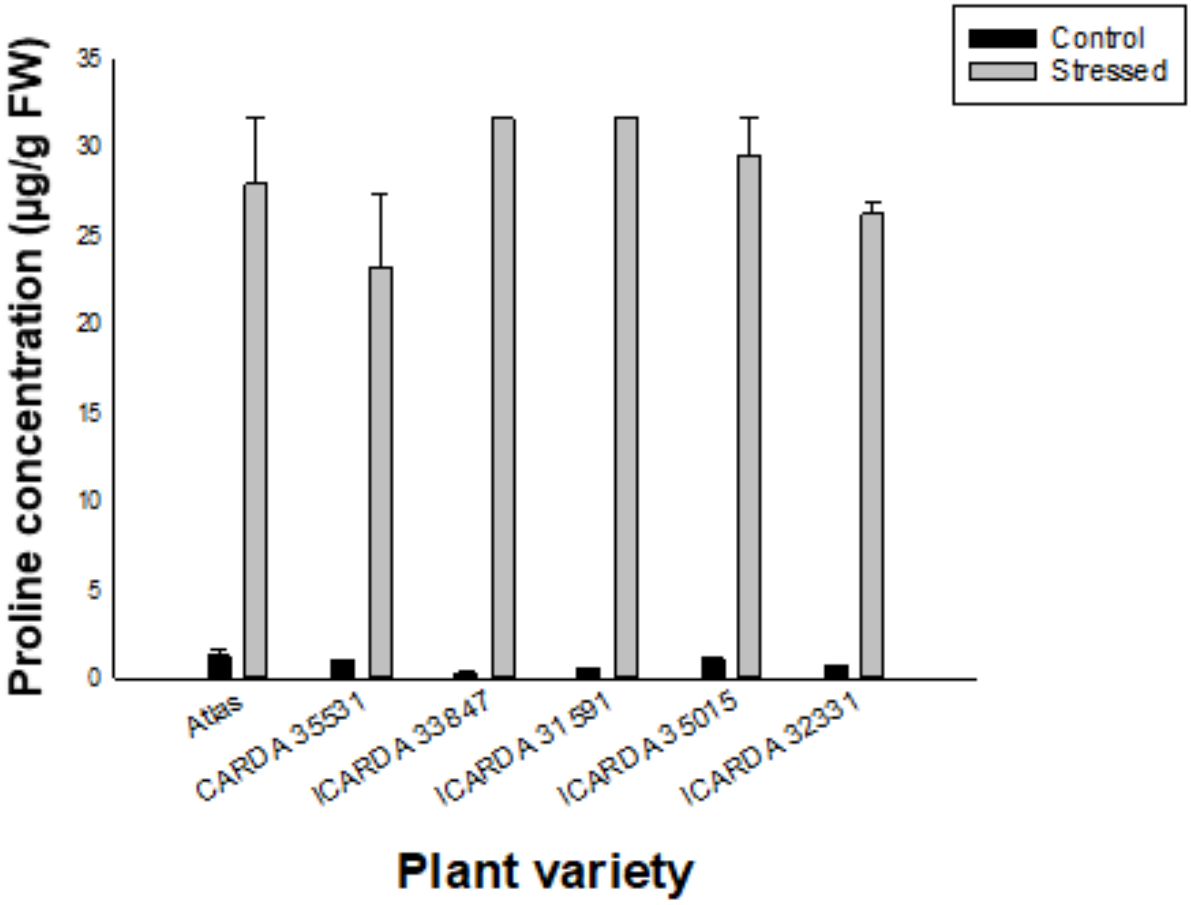


Figure 4. 6. Proline content of wheat cultivars under control (100- 85% WFC) and drought (25% WFC) conditions. X axis = wheat varieties. Y axis = proline content (µg/g FW). Black bar = wheat varieties under control condition. The experiment was performed in triplicate. Grey bar = wheat varieties under drought condition. The overall mean under control = 0.84 (µg/g

FW) and standard deviation = 0.45. The overall mean under drought stress = 28.40 (µg/g FW) and standard deviation = 4.70.

Table. 4.18. Post-hoc comparison for proline level (µg/g FW) per variety under control (100-85% WFC) and drought (25% WFC) conditions. SEM = standard error mean, Lower Bound = mean difference minus margin of error, Upper Bound = mean difference plus margin of error.

Treatment	Variety	Variety	Mean Difference ± S.E.M	*P*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	*Atlas*	*ICARDA 35531*	0.27±2.45	0.913	-4.784	5.324
		ICARDA 33847	1.043±2.45	0.67	-4.01	6.10
		ICARDA 31591	0.71±2.45	0.78	-4.35	5.76
		ICARDA 35015	0.13±2.45	0.96	-4.92	5.18
		ICARDA 32331	0.53±2.45	0.83	-4.52	5.59
	ICARDA 35531	*ICARDA 33847*	0.77±2.45	0.76	-4.28	5.83
		ICARDA 31591	0.44±2.45	0.86	-4.62	5.49
		ICARDA 35015	-0.14±2.45	0.96	-5.19	4.91
		ICARDA 32331	0.26±2.45	0.92	-4.79	5.32
	ICARDA 33847	*ICARDA 31591*	-0.34±2.45	0.89	-5.39	4.72
		ICARDA 35015	-0.91±2.45	0.71	-5.97	4.14
		ICARDA 32331	-0.51±2.45	0.84	-5.56	4.54
	ICARDA 31591	*ICARDA 35015*	-0.58±2.45	0.82	-5.63	4.48
		ICARDA 32331	-0.17±2.45	0.94	-5.23	4.88
	ICARDA 35015	*ICARDA 32331*	0.26±2.45	0.92	-4.79	5.32
Stressed	*Atlas*	*ICARDA 35531*	4.69±2.45	0.07	-0.36	9.75
		ICARDA 33847	-3.69±2.45	0.15	-8.74	1.36

	ICARDA 31591	-3.75±2.45	0.14	-8.80	1.31
	ICARDA 35015	-1.65±2.45	0.51	-6.71	3.40
	ICARDA 32331	1.69±2.45	0.50	-3.36	6.75
ICARDA 35531	*ICARDA 33847*	-8.38±2.45	**<0.01***	-13.44	-3.33
	ICARDA 31591	-8.44±2.45	**<0.01**	-13.49	-3.39
	ICARDA 35015	-6.35±2.45	**0.02**	-11.40	-1.29
	ICARDA 32331	-3.00±2.45	0.23	-8.05	2.05
ICARDA 33847	*ICARDA 31591*	-0.06±2.45	0.98	-5.11	5.00
	ICARDA 35015	2.04±2.45	0.41	-3.02	7.09
	ICARDA 32331	5.38±2.45	**0.04**	0.33	10.44
ICARDA 31591	*ICARDA 35015*	2.09±2.45	0.40	-2.96	7.15
	ICARDA 32331	5.44±2.45	**0.04**	0.39	10.49
ICARDA 35015	*ICARDA 32331*	3.35±2.45	0.18	-1.71	8.40

*Data in bold represent significant results at 0.05

4.4.7. Plant Genotypes Agglomerative Hierarchical Clustering (PGAHC)

In order to group bread wheat varieties according to their drought sensitive, tolerant, or moderate genotypes, cluster analysis relying on Euclidian distance by grouping similar data into clusters was performed. This helped distinguishing genotypes inter-relationships using all the physio-morphological and biochemical traits listed above of wheat cultivars under drought stress conditions. Agglomerative Hierarchical Clustering was inferred from the excel adds-on tool known as XLSTAT. This analysis was based on categorized the six bread wheat genotypes into 2 prominent clades based on their dissimilarities. Cluster C1 contained Atlas and ICARDA31591cultivars which are considered as drought-sensitive genotypes while cluster C2 encompassed the drought-tolerant genotypes, ICARDA32331 and ICARDA35015 which formed together a subcluster closely associated with a branch of ICARDA33847 that is placed next to a distinct branch of ICARDA35531. These latter, ICARDA35531 and ICARDA33847

were classified as drought-moderate plant genotypes (Figure 4.7). The clustering of the cultivars and their inter-relationship were in line with the data mentioned above.

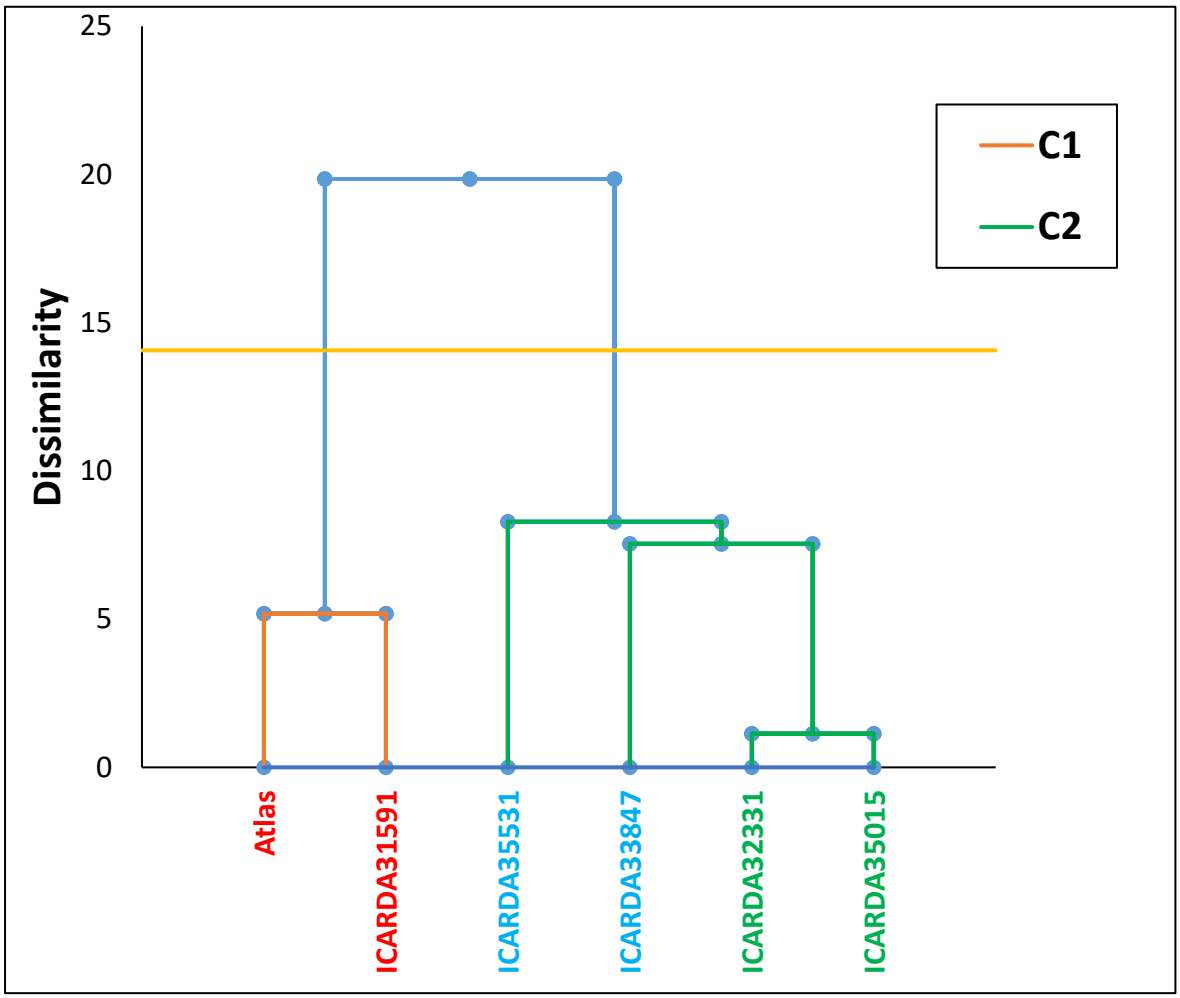


Figure 4.7. Hierarchical cluster analysis of 6 wheat genotypes. This clustering analysis was based on Euclidean distance of the measured morphological and physiological traits under control and stressed conditions. Genotypes denoted by red font represent drought-sensitive cultivars, blue font represent genotypes with moderate drought-sensitivity, while green font indicate wheat genotypes resistant to drought stress. C1 represents drought-sensitive clusters and C2 indicates drought-resistant plant clusters.

4.4.8. Exploring the correlation among different physio-morphological variables in different bread wheat varieties

Pearson's correlation coefficient analysis was performed among the physio-morphological and biochemical traits of bread wheat genotypes under stress and control growth conditions. The results of this analysis revealed that there is a strong positive correlation between plant height (PH), chlorophyl content (CHL), fresh weight (FW) and relative water content (RWC) under control conditions. There was also a positive moderate correlation between CHL and FW and RWC under control conditions.

Under stress conditions, there was a strong positive correlation between CHL and FW, RWC, and STI; FW and RWC and STI; RWC and STI. Moreover, there was a moderate correlation between PH and proline; DW and CHL; DW and FW; DW and RWC and STI. As shown in Table 4.19, STI was found to be highly correlated with RWC > FW > CHL under drought stress.

Table. 4.19. Pearson's correlations among morphological and physiological traits of six different bread wheat genotypes under control (100-85% WFC) and drought-stress (25% WFC) conditions.

Condition	Variables	PH	CHL	proline	FW	DW	RWC	STI
Control	**PH**	**1**	-0.733	-0.119	**-0.855**	-0.346	-0.767	-
	CHL	-0.733	**1**	0.179	0.412	-0.146	0.755	-
	proline	-0.119	0.179	**1**	0.118	0.152	-0.059	-
	FW	**-0.855**	**0.412**	0.118	**1**	0.772	0.381	-
	DW	-0.346	-0.146	0.152	**0.772**	**1**	-0.287	-
	RWC	-0.767	**0.755**	-0.059	0.381	-0.287	**1**	-
Stressed	**PH**	**1**	-0.102	0.474	-0.498	-0.523	-0.372	0.385
	CHL	-0.102	**1**	-0.159	**0.831**	0.472	**0.863**	**0.815**
	proline	0.474	-0.159	**1**	-0.077	-0.241	-0.014	0.021
	FW	-0.498	**0.831**	-0.077	**1**	0.470	**0.990**	**0.982**
	DW	-0.523	0.472	-0.241	0.470	**1**	0.396	0.308
	RWC	-0.372	**0.863**	-0.014	**0.990**	0.396	**1**	**0.992**
	STI	-0.385	**0.815**	0.021	**0.982**	0.308	**0.992**	**1**

*Values in bold are different from 0 with a significance level alpha=0.05. PH=Plant hight. CHL=Chlorophyl content. FW=Fresh weight. DW= Dry weight; Proline=Proline content; RWC= relative water content; STI= Stress tolerance index.

4.4.9. Classification of Wheat Genotypes Based on their Tolerance Indices for Drought Stress

Several drought stress indices were calculated in order to differentiate sensitive/tolerant plant genotypes relying on their relative water content data as it is difficult to determine drought tolerance genotype based on a single plant-stress index. The following plant stress-related indices were calculated: Yp and Ys which stand for RWC of the genotype under non-stress and stress conditions, tolerance index (TOL), relative stress index (RSI), mean productivity (MP), harmonic mean (HM), yield stability index (YSI), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), and yield index (YI).

Figure 4.8 represents differences in the STI for all six bread wheat varieties and proved that ICARDA32331, ICARDA35015, and ICARDA33847 had STIs higher than 0.69. Therefore, these plant genotypes are classified as drought-tolerant varieties. On the other hand, as Atlas, ICARDA35531, and ICARDA31591 had STIs lower than 0.69, they merit to be considered as drought-sensitive genotypes.

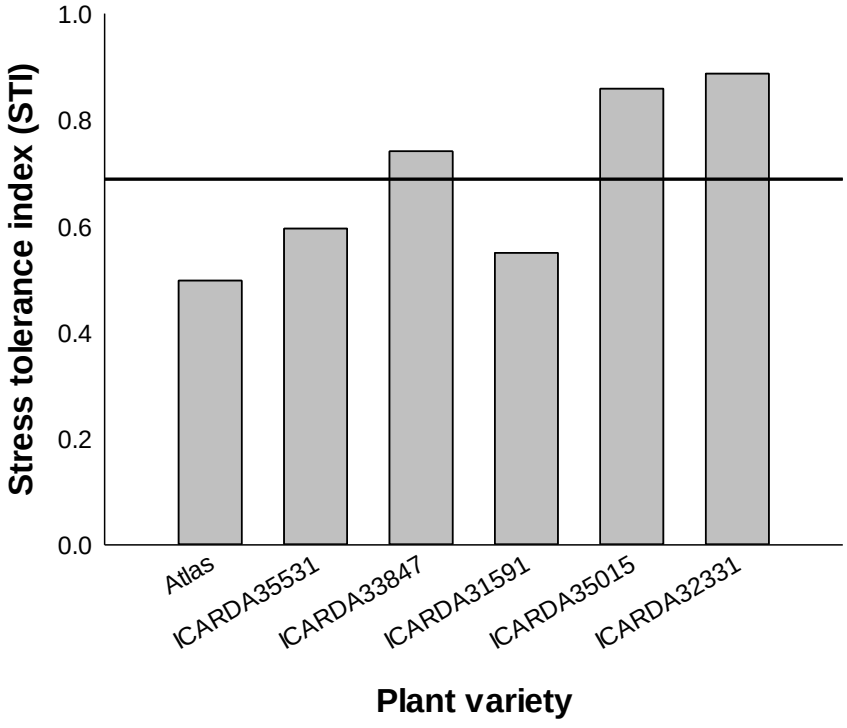


Figure 4.8. Bar chart represents stress tolerance index (STI) of six different bread wheat varieties kept under drought-stress conditions in the growth chamber. The black trendline represents the mean of STI of the six tested bread wheat genotypes.

The low values of SSI and TOL indices correspond to high stress-tolerance. Therefore, the tolerant genotypes of ICARDA35015 (SSI 0.63; TOL 17.23) and ICARDA32331 (SSI 0.67; TOL 18.71) were confirmed by their low SSI and TOL values. These results were consistent with the high values of HM, MP, STI, GMP, YI, YSI and RSI for ICARDA35015 and ICARDA32331 varieties, indicating their ability to tolerate drought-stress (Table 4.19). In contrast the low values of HM, MP, STI, GMP, YI, YSI and RSI obtained for Atlas and ICARDA31591 confirmed their sensitivity to water stress (Table 4.20).

The average sum of rank (ASR) was calculated using values of drought stress indices for RWC (Table 4.21) to confirm the assignment of the six wheat cultivars to drought-tolerant or sensitive genotypes. The high ASR value reflects the sensitivity of the plant to water stress. The lowest ASR value was obtained for ICARDA32331 genotype with ASR =14 while the highest value of ASR = 60 was detected in Atlas; thus ICARDA32331 cultivar merits to be considered as the best drought-tolerant variety, while Atlas is the most sensitive cultivar.

Table. 4.20. Screening of 12 different drought stress indices computed via iPASTIC of 6 bread wheat genotypes based on RWC.

Genotype	YP*	YS	RC	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
Atlas	82.70	42.17	49.01	40.53	62.44	59.05	55.86	1.55	0.50	0.74	0.51	0.74
ICARDA35531	78.59	53.10	32.43	25.49	65.85	64.60	63.38	1.03	0.60	0.93	0.68	0.99
ICARDA33847	83.91	61.88	26.25	22.03	72.90	72.06	71.23	0.83	0.74	1.08	0.74	1.08
ICARDA31591	81.60	47.23	42.12	34.37	64.42	62.08	59.83	1.34	0.55	0.82	0.58	0.85
ICARDA35015	86.68	69.45	19.88	17.23	78.07	77.59	77.11	0.63	0.86	1.21	0.80	1.17
ICARDA32331	88.76	70.05	21.08	18.71	79.41	78.85	78.30	0.67	0.89	1.22	0.79	1.15

*Note: Yp and Ys stands for yields (relative water content) of the genotype, RC: relative change due to stress, TOL: tolerance index, MP: Mean productivity, GMP: Geometric mean productivity, STI: stress tolerance index, HM: Harmonic mean, SSI: Stress susceptibility index, RSI: relative stress index, YSI: yield stability index, YI: yield index.

Table. 4.21. Rank of 12 different drought stress indices computed via iPASTIC of 6 bread wheat genotypes based on RWC.

Genotype	YP	YS	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI	ASR	AR	SD
Atlas	82.70	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	60.00	6.00	0.00
ICARDA35531	78.59	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	40.00	4.00	0.00
ICARDA33847	83.91	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	30.00	3.00	0.00
ICARDA31591	81.60	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	50.00	5.00	0.00
ICARDA35015	86.68	2.00	1.00	2.00	2.00	2.00	1.00	2.00	2.00	1.00	1.00	16.00	1.60	0.52
ICARDA32331	88.76	1.00	2.00	1.00	1.00	1.00	2.00	1.00	1.00	2.00	2.00	14.00	1.40	0.52

*Note: Yp and Ys stands for yields (relative water content) of the genotype, RC: relative change due to stress, TOL: tolerance index, MP: Mean productivity, GMP: Geometric mean productivity, STI: stress tolerance index, HM: Harmonic mean, SSI: Stress susceptibility index, RSI: relative stress index, YSI: yield stability index, YI: yield index, ASR: Average sum of rank, AR: Average rank, SD: Standard deviation

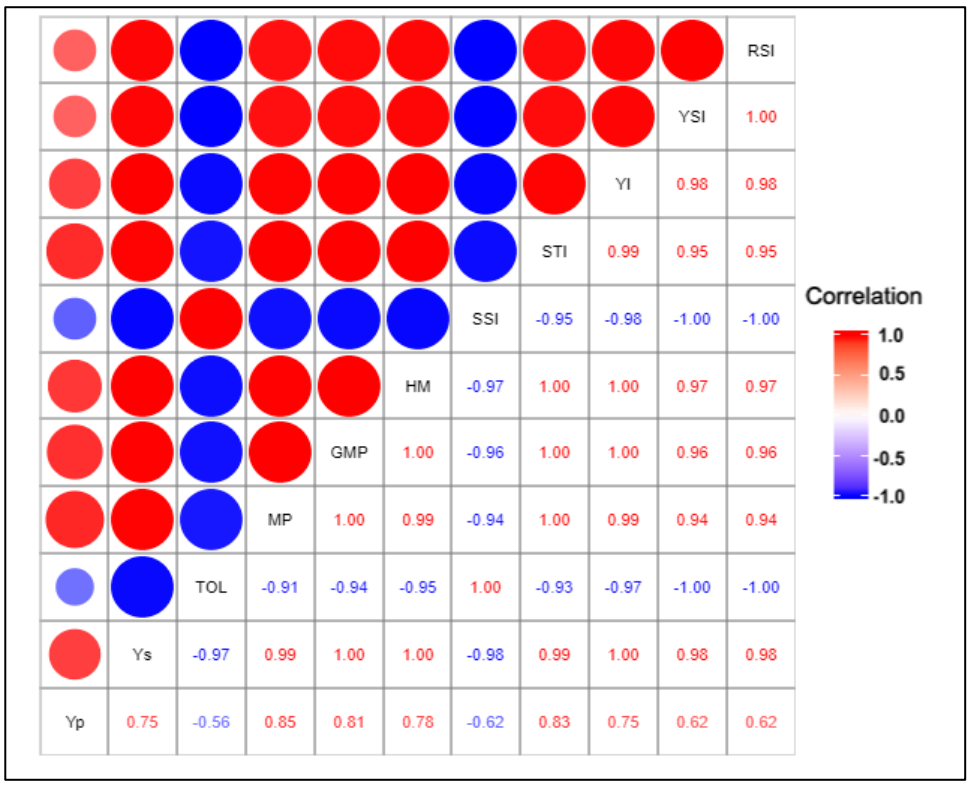


Figure 4.9. Heatmap representing the correlation amongst variable bread wheat drought-tolerance/sensitivity indices. Correlation intensity is represented by variability in circle size between two different indices. Note: Red circle (+1) means strong correlation, red gradient <+1 indicates moderate correlation, blue circle (-1) denotes no correlation, and blue gradient <0 and >-1 indicates weak correlation. *Note: Yp and Ys stands for yields (relative water content) of the ith genotype, RC: relative change due to stress, TOL: tolerance index, MP: Mean productivity, GMP: Geometric mean productivity, STI: stress tolerance index, HM: Harmonic mean, SSI: Stress susceptibility index, RSI: relative stress index, YSI: yield stability index, YI: yield index, ASR: Average sum of rank, AR: Average rank, SD: Standard deviation.

4.4.10. Exploring the correlation among different drought stress indices based on RWC in different bread wheat varieties

The correlation among all drought stress indices was explored for the first time based on RWC in the six bread wheat varieties. The heatmap in Figure (4.9) explicitly shows that MP, GMP, HM, STI, YI YSI, and RSI indices showed a positive and significant correlation with Yp and Ys. Consequently, we can adopt these stress indices as a representative and alternative indicator when selecting high-yielding genotypes under drought conditions. However, no correlation was obtained between the TOL and SSI with all the studied stress indices, though they are well

correlated to each other. The STI was considered one of the best index used in seedling stage to determine the drought tolerant genotype. This index was estimated previously based on the dry weight (Belay et al .2021).

4.5. Discussion

Drought stress is a well-studied topic that was established as one of the most critical factors influencing plant growth in semi-arid conditions. Maintaining a sufficient level of water during various stages of plant growth is critical for maintaining plant turgor, which is essential for healthy cellular growth, enlargement and overall healthy plant phenotype. In this study, all plant morphological and physiological parameters including plant height (cm), fresh weight (g), dry weight (g), relative water content (RWC %) and chlorophyll content (SPAD value) found to be negatively impacted (reduced) in response to experimentally induced drought stress during plant growth in seedling stage of six bread wheat genotypes. Typically, all measured plant traits were severely reduced due to exerted stress during growth except proline concentration (µg/g FW) that increased. For morphological plant traits such as PH, FW, DW, and physiological traits such as RWC and CHL, drought stress was accounted for a significant total reduction by about 41.08%, 80.1%, 51.09%, and 31.53%, and 65.93%, respectively, compared to the control growth conditions in all bread wheat varieties. However, proline content was the exception as it increased in response to drought stress.

This means that wheat growth at the seedling stage has been disrupted as it is critically dependent on the correct level of water intake. The results about PH, FW, and DW reduction agree with previously published findings. Under drought stress conditions PH, FW, and DW were previously reported to decrease when water uptake is deficit in all bread wheat varieties tested (Mujtaba, Faisal et al. 2016, Faisal, Mujtaba et al. 2017, Ahmed, Sajjad et al. 2019, Ahmed, Zeng et al. 2020, Ahmed, Zeng et al. 2022). Without the optimum level of water, the growing plant-especially at the seedling stage-experiences a great disruption in nutrients uptake which results in retarded and reduced growth (Elemike, Uzoh et al. 2019, Seleiman, Al-Suhaibani et al. 2021). Limited amounts of water causes impairment in the cells division due to changing the osmotic potential, which in turn results in reduced plant growth and decreased PH, FW, and DW (Taiz, Zeiger et al. 2015, Bandeppa, Paul et al. 2018, Ahmed, Zeng et al. 2022). As plants need a specific level of water consumption that must surpasses its demand for growth and metabolic functions and transpiration, experiencing water deficit means that water intake does not meet the minimum requirement needed by the plant to perform these processes.

As a result, the water loss due to transpiration exceeds the amount of water absorbed by the roots (Goche, Shargie et al. 2020). To overcome this, the plant develops a coping strategy to minimize water loss during transpiration. To achieve so, the plant leaves roll, get yellowish in colour, and it could worsen as leaves get scorched, or completely and permanently wilted. During the seedling stage- as a critically important stage during plant growth cycle- reduction in water intake to sub-optimum level causes disruption and decrease in plant cell wall expansion plant turgor (Seleiman, Al-Suhaibani et al. 2021). Another strategy that the plant adopts to cope with drought stress is by undergoing osmotic adjustments such as increasing the sugar level of the different parts of the plant including roots and shoots (Miranda, Da Silva et al. 2021).

The amount of water retained by plant; denoted as RWC is regarded as the most critical variable to assess plant tolerance to drought stress during growth. Current work demonstrated that the RWC of all wheat genotypes had significantly reduced to about 31.53% in total. This was previously reported by several researchers (Ahmed et al. 2019; Ahmed et al. 2020; Ahmed et al. 2022). Interestingly, the greatest reduction of RWC was found in drought sensitive wheat varieties (Atlas, ICARDA31591, and ICARDA35531), whereas RWC was higher in drought-tolerant wheat varieties (ICARDA 35015, ICARDA32331, and ICARDA33847). Similar findings were reported by Belay et al. (2021) and Ghaffar et al. (2023). This can be explained as a result of reduced water uptake while high stomatal water loss in the leaves (Climent et al. 2021; Ghaffar et al. 2023), diminished plant vigour (Prathyusha and Chaitanya 2019). Typically, the plant performs osmotic regulation in response to water scarcity conditions. This includes reduction in the osmotic potential for water absorption due to accumulation of solutes such as proline and carbohydrate which then decreases soil water potential and thereby soil water uptake by the plant (Martin et al. 1993). Moreover, plants stomatal closure is another adaptive defensive mechanism to limit the amount of evaporation through plant leaves under dehydration, which results in decreased photosynthesis rate in stressed plants (Cornic 2000; Keyvan 2010).

Chlorophyll content is another essential factor for evaluating plant quality and can be measured using non-invasive screening using a SPAD meter. Under water scarcity conditions, chlorophyll content was reduced (by 65.93%). This means that essential physiological process such as photosynthesis (Hussain et al. 2019) will be greatly disrupted which in turn has a detrimental effect on the plant growth. Findings of the current study comply with previous

research (Ahmed et al. 2022). Among such negative impacts of reduction in chlorophyll content in response to drought stress is the increased production of the toxic reactive oxygen species (ROS) (Ahmed et al. 2022). Overproduction of ROS by the chloroplast tends to oxidise neighbouring macromolecules, causing considerable damage to the structural and functional integrity of the chloroplast and consequently have detrimental impacts on plant growth, cell division and cell expansion. Other subcellular compartments are also alerted by ROS to reset the cell's metabolic homeostasis (Li and Kim 2022). The reduction in chlorophyll interrupts the photosynthesis machinery and their by-products such as oxygen, which is necessary for photorespiration, where RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) oxygenates Ribulose-1,5-bisphosphate (RuBP), resulting in the overproduction of ROS by peroxisomes and oxidative phosphorylation by mitochondria (Li and Kim 2022).Reduced or damaged chlorophyll content accompanied by increased levels of ROS in stressed plants could also cause lipid peroxidation (Kalaji et al. 2016, Ahmed et al. 2022).

Measuring the level of proline (µg/l) in plant shoots is a critically important component when judging plant genotypes sensitivity/resistance to drought stress. Hence, in this present study, proline content of plant was found to be significantly increased by 33.8-fold in total in stressed plants regardless of the genotype. These findings agree with those previously reported in wheat (Sultan et al. 2012, Nemati et al. 2019), tall fescue (Man et al. 2011), cotton (Parida et al. 2008), barely (Bandurska et al. 2017). This highlights the importance of this amino acid, proline, in defending plant growth during the seedling stage under water scarcity. Proline over-synthesis is regarded as a plant defensive mechanism against abiotic stress including drought stress in a great variety of researched plants (for review see Ghosh et al. (2022)). Plants with high proline content under abiotic stress show improved regulation of cellular osmolality, internal homeostasis such as balanced level of ions, and maintenance of enzymatic and none-enzymatic level of antioxidants (reviewed by Ghosh et al. (2022)). Further, proline protects plant cells against oxidative stress caused by free radicals and ROS which was found to increase upon stressful conditions such as dehydration (Smirnoff and Cumbes 1989) and reviewed by (Matysik et al. (2002); Ghosh et al. (2022)). Among the adverse impacts of drought stress on plant cells is the presence of singles oxygen ($^1O_2$) which is characterised by its high reactivity to oxidize biological molecules including DNA, amino acids, and Lipids (Adam 1981; Matysik et al. 2002). In turn, this reaction results in the production of free radicals known as ROX compounds such as hydroxyl radical and the reduced superoxide radical which are also highly reactive and exert stress on cells (Adam 1981) including plant cells (Matysik et al. 2002). Such

stress causes further production of ROS compounds, therefore proline levels are elevated to protect essential molecules from (photo)oxidative degradation and mitigate the negative impact of dehydration on plant cells. Proline is considered to be an effective scavenger of hydroxyl radicals ($^{\circ}OH$) (at a rate of 4.8 x 10^8 M^{-1} S^{-1}) and singlet oxygen ($^1O_2$) (Smirnoff and Cumbe 1989; Matysik et al. 2002; Davies 2005) as it deactivates $^1O_2$ by producing peroxide anion $[OeO]^{2-}$ or superoxide radical $O_2^{\circ-}$ or by physical quenching (Alia et al. 2001). However, Signorelli et al. (2013) proved that proline cannot quench $^1O_2$ in an aqueous buffer and pointed out that the study by Alia et al. (2001) on the role of proline in singlet oxygen production, was performed in ethanol and showed no evidence that proline is a physical or chemical quencher. Signorelli et al (2013) call for a reconsideration of the role of proline as an effective non-enzymatic antioxidant in plants, particularly if its reaction with $^1O_2$ leads to generate other ROS products. Additionally, proline acts as an essential regulator for the pH in the cytosol due to its buffering capacity, helping to stabilise pH fluctuations in case of abiotic stress (Venekamp et al. 1989; Matysik et al. 2002). Moreover, pH regulation involves other metabolic pathways linked to proline metabolism. Proline synthesis requires NADP(H), which is essential for redox maintenance and pH balance (Aliaand Matysik 2001; Matysik, et al. 2002). Therefore, improving metabolism, photosynthesis, and energy production in the cell (Ali et al. 2007; Ghosh et al. 2022). Hence, it improves overall plant growth under stressful conditions including drought stress.

There was significantly high association between FW, DW, and CHL level under both growth conditions. Similarly, PH and Proline, DW and CHL, and DW, FW, RWC, and STI were positively highly correlated under drought stress conditions in all studied bread wheat genotypes. Increasing osmoregulatory components such as proline is a common physiological response to drought stress, preventing water loss by maintaining cell turgidity and thereby protecting the plant's cellular function and metabolic activities (Keyvan 2010). This allows the plant to complete its growth phase, including main stem elongation (PH), which often correlates positively with proline content, as in the present study (Mahdavi et al. 2023). It has been shown that plants that maintain a high chlorophyll content under drought stress often have good photosynthetic machinery that ensures efficient growth leading to a high amount of Dw biomass (Mwadizingeni et al. 2016). This reflects the positive correlation between these physio-morphological parameters: chlorophyll and DW.

RWC is an indicator of water status, which is vital for the plant to continue its life cycle. This characteristic gives an idea of the plant's ability to maintain growth and cope effectively with drought. Consequently, a correlation is often established between RWC and plant biomass, including FW and DW. The interconnection between RWC, FW and DW has been shown to correlate with the stress tolerance index, highlighting that cultivars with this association of traits are drought tolerant, like in this present study (Ghanem and Al-Farouk 2024).

This implies that all these plant traits are important and highly related when investigating the impact of drought conditions on bread wheat of different genotypes. Such high correlation between morphological traits and physiological variables confirms the overall trend and destiny of wheat plant when experiencing drought conditions. Data driven from current study confirm previous findings by (Ahmed et al. 2019; Ahmed et al. 2020; Ahmed et al. 2022).

The classification of bread wheat genotypes in current study into drought-tolerant or sensitive cultivar was based on the STI (stress tolerance index) index as a standardized mean to complete such differentiation. This index was reported to be the most accurate and best plant stress index to classify the plant based on its sensitivity to drought stress (Pour-Aboughadareh et al. 2019). Accordingly, bread wheat genotypes ICARDA32331, ICARDA35015, and ICARDA33847 as drought-tolerant cultivars thanks to having STIs higher than 0.69. This STIs values lie within the range reported by previous researchers confirming this conclusion. In contrast, bread wheat varieties Atlas, ICARDA35531, and ICARDA31591 had STIs lower than 0.69, therefore, they are considered as drought-sensitive genotypes compared to the tolerant varieties. This huge variability to drought tolerance between varities in this present work and those in other studies (Belay et al. 2021; Pour-Aboughadareh et al. 2019) can be attibuted to profound genetic differences among wheat cultivars investigated here.

To conclude, considering various plant traits investigated in this study, the genotypes ICARDA32331, ICARDA35015, and ICARDA33847 were distinguished as drought-tolerant wheat varieties, and Atlas, ICARDA35531, and ICARDA31591 as drought-sensitive cultivars. Overall, this study provides a proof for wheat response towards exerted drought stress in the six different genotypes, differentiating them into drought sensitive/tolerant varieties based on differences in their morphological and physiological traits relying on their STI index and correlation among variables. This work sets the basis and provide valuable insights for wheat variety selection when considering the plant quality and tolerance to drought stress; the most important plant stressor in semi aride areas as in Saudi Arabia. The

results of this work are useful for the breeding program for drought-tolerant cultivars with high grain yields. To overcome the limitation of this study to shoots, further investigation into the morphological structure of the roots is needed, as this is also associated with yield and quality of these cultivars and their performance under drought stress conditions.

Chapter 5. Comparative transcriptomics analyses of two wheat cultivars in response to drought stress during seedling stage

5.1. Abstract

With global warming, drought is one of the most detrimental and complex abiotic stress affecting wheat (*Triticum aestivum* L.) production. Screening for drought tolerance associated genes is crucial for the breeding approach. In this context, two wheat (*Triticum aestivum* L) cultivars, Atlas and ICARDA32331, with sensitive and tolerant genotypes to drought stress were subjected to a comparative transcriptome study. RNA-Seq analysis revealed that the two cultivars shared 5068 differentially expressed genes (DEGs) in response to drought stress and distinguish ICARDA32331 from Atlas cultivars by 8676 and 10284 DEGs, respectively. Under drought conditions, ICARDA32331 and Atlas had 6126 and 6875 up-regulated genes out of 13744 and 15352 DEGs respectively. Gene ontology terms in ICARDA are mainly related to lipid and cell wall metabolism, oxidoreductase activity, lipid transport and localisation as molecular function (MF); apoplast, cell wall and external encapsulating structure as cellular component (CC), and biological process (BP) of lipid transport and localisation. These enriched genes were found to be up-regulated in ICARDA while the most significant degree of DEGs enrichment in Atlas cultivar were those related to photosynthesis and the chloroplastic component, but most of BP, CC, and MF associated genes were down-regulated, reflecting the sensitivity of the chloroplast and photosynthesis activity to drought in Atlas. The cultivars ICARDA32331 and Atlas had an over expression of DEGs associated with dehydrin and proline biosynthesis, indicating that they had undergone osmotic adjustment to adapt to drought. These results are in line with the high level of proline in both cultivars under drought conditions, which questions the role of proline as a marker of the drought tolerant genotype. Transcriptomic data highlight the role of lipid metabolism and the importance of nitric oxide in drought tolerance mechanism and call for further investigations on the crosstalk role of nitric oxide that seems to be a promotor for several protective mechanisms. A high number of SNPs and InDels were found in this transcriptomic study, which is a consequence of the genetic diversity of chosen wheat cultivars. This study helps to gain better understanding of the complex regulatory mechanism of wheat for drought stress by highlighting the role of lipid metabolism in drought tolerance and provides new molecular factors that can be targeted for drought tolerance breeding programme.

5.2. Introduction

The plant's response to water scarcity is complex, as various genes associated with several metabolic and molecular networks are regulated at the molecular level by the activation or suppression of genes and gene networks. Understanding the regulatory response to drought is crucial to maintaining sustainable wheat production through genetic improvement and selection of cultivars tolerant to abiotic stress. With advances in RNA-Seq and high-throughput sequencing technologies that have become more affordable in recent years, and the availability of the *Triticum aestivium* genome sequence, many transcriptional regulatory factors and genes expressed in response to drought have been identified (Aprile et al. 2009; Li et al. 2012; Aprile et al. 2013Hübner et al. 2015; Liu et al. 2015; Ma et al. 2017). Gene expression is regulated by transcription factors (TF) that bind directly to specific DNA sequences, the cis-acting element, placed downstream of the promoter of its genes. This binding leads to activation or repression of the expression of the targeted genes (Zhao et al. 2018). These TFs induced by drought stress in wheat include C-repeat-binding factor (CBF), myeloblastosis oncogene (MYB), WRKY, AP2/ERF, dehydration-responsive element binding protein (DREB), NAC, and HD-ZIP (Ramanjuli and Bartels 2002; Sakuma et al. 2006; Ding et al. 2009; Moumeni et al. 2011; Hossain et al. 2012; Nuruzzaman et al. 2013; Banerjee and Roychoudhury 2017;Lv et al. 2018). In addition, several metabolic pathways are known to be activated at different growth stages in response to drought stress, such as carbohydrate, amino acid, protein folding, lipid, secondary metabolite biosynthesis, and Mitogen-activated protein kinase (MAPK) signalling pathway (Yildirim and Kaya 2017; Dudhate et al. 2018Chaichi et al. 2019). Other pathways were also found to be significantly enriched in wheat (*Triticum aestivum* L.) under drought stress such as glycolysis and gluconeogenesis, plant-pathogen interaction, starch and sucrose metabolism, plant hormone signal transduction, phenylpropanoid biosynthetic pathways, and photosynthesis (Zhang et al. 2021; Lv et al. 2022). Among the most significant differential gene expressions (DEGs) in most cereal crops, including wheat, subjected to drought stress are those related to amino acids (eg. proline, glutamic acid, phenylalanine, etc.), which are known to play an important role in cell osmotic balance and acts as ROS scavengers and signalling molecules (You et al. 2019). The content of free amino acids (eg. proline, phenylalanine, etc.), generated by amino acid metabolism, has been used as indicator of the level of drought resistance in crops (Parry et al. 2005; Verdoy et al. 2006; Li et al. 2024). Amino acid composition seems to have an influence on cereal grains quality, with a better taste quality associated with free glutamic acids (Kawakatsu and Takaiwa 2010).Wheat is known for its wide variety of amino acids that

are metabolised into glucose, and increasing the quantity of these glucogenic amino acid leads to an eventual rise in sugar levels, and therefore to greater resistance of wheat to abiotic stress (Živanović et al. 2020; Li et al. 2024). These are coherent with the significant enrichment of carbohydrate metabolism, including glycolysis and gluconeogenesis, which generates energy, osmo-regulatory and osmo-protective substances in response to drought stress in several plants (eg. wheat, sorghum, rice, barley, maize, sesame) (Fracasso et al. 2016; Chu et al. 2021; Jiang et al. 2023; Sahoo et al. 2023). Water-soluble carbohydrates (eg. glucose, sucrose etc.) serve to protect cell turgidity and defend proteins and membranes against stress-induced damage (Zhou and Yu, 2010). Glycolysis takes place in the cytoplasm and consists of converting glucose into pyruvate, thus generating the ATP and NADH required for cell function. This process marks the initial stage of cellular respiration. In contrast, glycogenesis is the use of non-carbohydrates (eg. amino acids, pyruvate, lactate, etc.) to synthesis glucose; this pathway is activated and given high priority over glycolysis by the plant so that it can carry out its essential functions during drought. Moreover, the accumulation of certain carbohydrates also serves as signalling mocelules helping the plant to cope with water shortages. Significant enrichment in lipid metabolism was also obtained in wheat including fatty acid elongation and cutin, suberin, and wax biosynthesis pathways (Fu et al. 2024). Drought stress affects not only the primary metabolites that are essential for plant growth and survival, but also secondary metabolites (SMs). A large number of SMs are formed from primary metabolites, and their accumulation in the plant cell is often associated with the plant's adaptation to stress. SMs are known for their ability to regulate various enzymatic activities and to act as signalling molecules in drought stress such as flavonoids and plant hormones (ABA) (Campo et al. 2014; Su et al. 2023). They also enable the plant to interact and communicate with other organisms/species. Alteration of several essential physiological processes in plant leads to the biosynthesis and accumulation of SMs (eg. flavonoids, terpene, phenolic acids, etc.), which are involved in ROS scavenging, lipid peroxidation, cell wall reinforcement, acceleration of energy production via stimulation of the tricarboxylic acid cycle and glycolysis, and enhancement of glutamic acid-mediated proline biosynthesis (Ashraf et al. 2018; Qu et al. 2019; Yadav et al. 2021).

Moreover, other mechanisms are activated by the plant to deal with stress such as hormonal regulation (Guóth et al. 2009), oxidation-reduction (Gilbert 1993), reserve translocation (Wardlaw and Willenbrink 2000), and extending photosynthesis for a longer period known as stay green character (Nawaz et al. 2013). Differential gene expression (DEGs) induced by drought in wheat include those associated with heat shock protein, dehydrin, glutathione

transferase, cytochrome, transcription factor, etc. (Goswami et al. 2015; Kumar et al. 2015; Joshi et al. 2016; Kulkarni et al. 2017). The variation in DEGs and activated pathways observed in previous studies confirms that each genotype has specific mechanisms for addressing drought stress (Akpinar et al. 2012; Chu et al. 2021). The molecular and metabolic response of wheat growing under drought conditions depends on genotype, physiology, and environmental factors.

5.3. Aims and objectives

The aim of the present study was to identify candidate genes involved in drought stress at the seedling stage of the drought-tolerant (ICARDA) and drought-sensitive (Atlas) wheat cultivars identified in Chapter 4. These cultivars were subjected to full irrigation at 100% field capacity (FC) and drought stress with watering suspended for 2 weeks, from the development of the third leaf to the appearance of the first tillers, until FC had reached 25%. An RNA-Seq study was carried out on these cultivars and the genes differentially expressed between the control and stress samples were identified and the difference in drought tolerance mechanisms between these cultivars was explored. This chapter provides a new insight into drought tolerance genes and metabolic pathways.

5.4. Results

5.4.1. Overview about sequencing data

Seedling leaf tissues of two wheat cultivars, Atlas and ICARDA 32331, grown under optimal (90-75% WFC) and drought (25% WFC) conditions, were subjected to total RNA extraction and RNA-Seq analysis. Four biological replicates were included in this study. Table 5.1 provides an overview of the generated transcriptome sequences, including raw bases, clean reads, and all quality control statistical analyses (error rate, GC content distribution), leading to the final sequences. A gene is considered differentially expressed when its expression differs by more than two-fold in the two groups (control and drought stressed groups). To exclude the possibility that the difference in gene expression is derived from various errors or false positives due to a high number of transcripts, it was necessary to introduce and calibrate padj to the P-value of the hypothesis test to control the proportion of false positives (Young et al. 2010). Four biological replicates were included for the transcriptomic analysis. Gene expression level correlation between samples has an important role in ensuring the reliability and repeatability of the experiment that enables the analysis of differential gene expression to be estimated. In this regard, the Pearson correlation between the samples was estimated, confirming the accuracy of the data (Figure 5.1).

Inter and intra group sample differences were evaluated using Principal Component Analysis, which was performed on the basis of gene expression value (FPKM) of all samples. As shown in Figure 5.2, all samples from the same varieties, Atlas and ICARDA, were grouped together and the cultivars were distinguished from each other.

Table 5.1. Quality of transcriptomics data of seedling leaf tissues of two wheat cultivars, Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. RNA Seq libraries were prepared by Novogene Ltd, Cambridge, UK (https://www.novogene.com) using Illumina NovaSeq platforms with paired-end 150 bp sequencing method. The sequences were aligned with the genome of *Triticum aestivium* (accession number JAGHKL000000000.1) using HISAT2 algorithm. Abbreviations in sample names: C = control condition; D = drought conditions; 1, 2, 3, 4 = biological replicates.

Sample	library	raw_reads	raw_bases	clean_reads	clean_bases	error_rate	Q20	Q30	GC_pct
Atl_C_1	ERAS230001459-2r	99433436	14.92G	97653418	14.65G	0.03	96.73	92.03	56.26
Atl_C_2	ERAS230001460-2r	92213788	13.83G	90652330	13.6G	0.03	96.33	91.29	57.94
Atl_C_3	ERAS230001461-2r	1,24E+08	18.55G	1,2E+08	18.06G	0.03	97.03	92.83	56.86
Atl_C_4	ERAS230001462-2r	1,27E+08	18.98G	1,22E+08	18.32G	0.03	96.16	91.24	56.79
Atl_D_1	ERAS230001463-2r	1,19E+08	17.78G	1,16E+08	17.35G	0.03	95.74	90.72	53.94
Atl_D_2	ERAS230001464-2r	1,16E+08	17.38G	1,14E+08	17.09G	0.03	96.84	92.07	54.56
Atl_D_3	ERAS230001465-2r	1,14E+08	17.15G	1,13E+08	16.92G	0.03	96.95	92.21	54.36
Atl_D_4	ERAS230001466-2r	81267254	12.19G	80307276	12.05G	0.03	96.84	92.05	55.57
ICA_C_1	ERAS230001467-2r	1,14E+08	17.13G	1,12E+08	16.74G	0.03	97.66	93.87	57.73
ICA_C_2	ERAS230001468-2r	1,14E+08	17.11G	1,12E+08	16.8G	0.03	97.67	93.84	57.82
ICA_C_3	ERAS230001469-2r	1,13E+08	17.01G	1,11E+08	16.67G	0.03	97.59	93.76	57.47
ICA_C_4	ERAS230001470-2r	1,22E+08	18.34G	1,21E+08	18.08G	0.03	97.67	93.94	58.27
ICA_D_1	ERAS230001471-2r	1,26E+08	18.89G	1,24E+08	18.58G	0.03	97.45	93.49	54.64
ICA_D_2	ERAS230001472-2r	1,14E+08	17.1G	1,12E+08	16.87G	0.03	97.44	93.55	55
ICA_D_3	ERAS230001473-1r	90372386	13.56G	88721856	13.31G	0.03	97.45	93.68	55.25
ICA_D_4	ERAS230001474-2r	1E+08	15.04G	98483944	14.77G	0.03	97.24	93.22	54.95

raw_reads: Reads count from the raw data, four rows as a unit, with statistics of reads count for every sequencing. Raw_bases: Base number of raw data. (number of raw reads) * (sequence length), converting unit to G. clean_reads: Base number of raw data after filtering. (number of clean reads) * (sequence length), converting unit to G. clean_bases: (clean base=clean reads*150bp) number multiply read length, saved in G unit. Error_rate: Average sequencing error rate, which is calculated by Qphred=-10log10€. Q20: The percentage of the bases whose Q Phred values is greater than 20. (Number of bases with Q Phred value > 20) / (Number of total bases) *100. Q30: The percentage of the bases whose Q Phred values is greater than 30. (Number of bases with Q Phred value > 30) / (Number of total bases) *100. GC_pct: The percentage of G&C base numbers of total bases.(G&C base number) / (Total base number)*100.

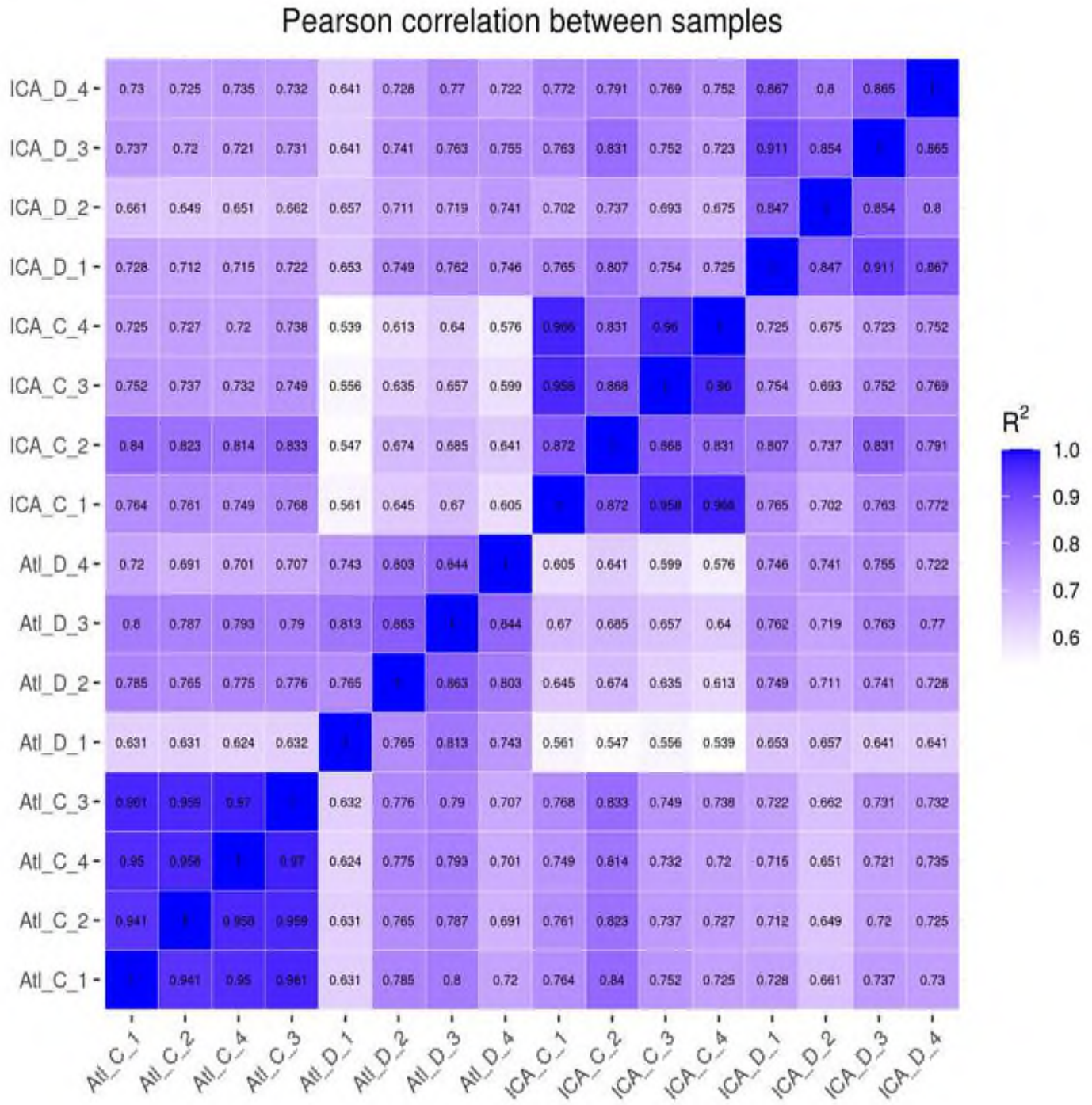


Figure 5.1. Pearson's correlation matrix showing the correlation of the gene expression levels (RPKM or FPKM) between seedling leaf tissues of two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. R language was used to calculate Pearson correlation coefficient. $R^2 > 0.8$ = high similarity between the samples (closer expression pattern). Abbreviations in sample names: C = control condition; D = drought condition; 1, 2, 3, 4 = biological replicates.

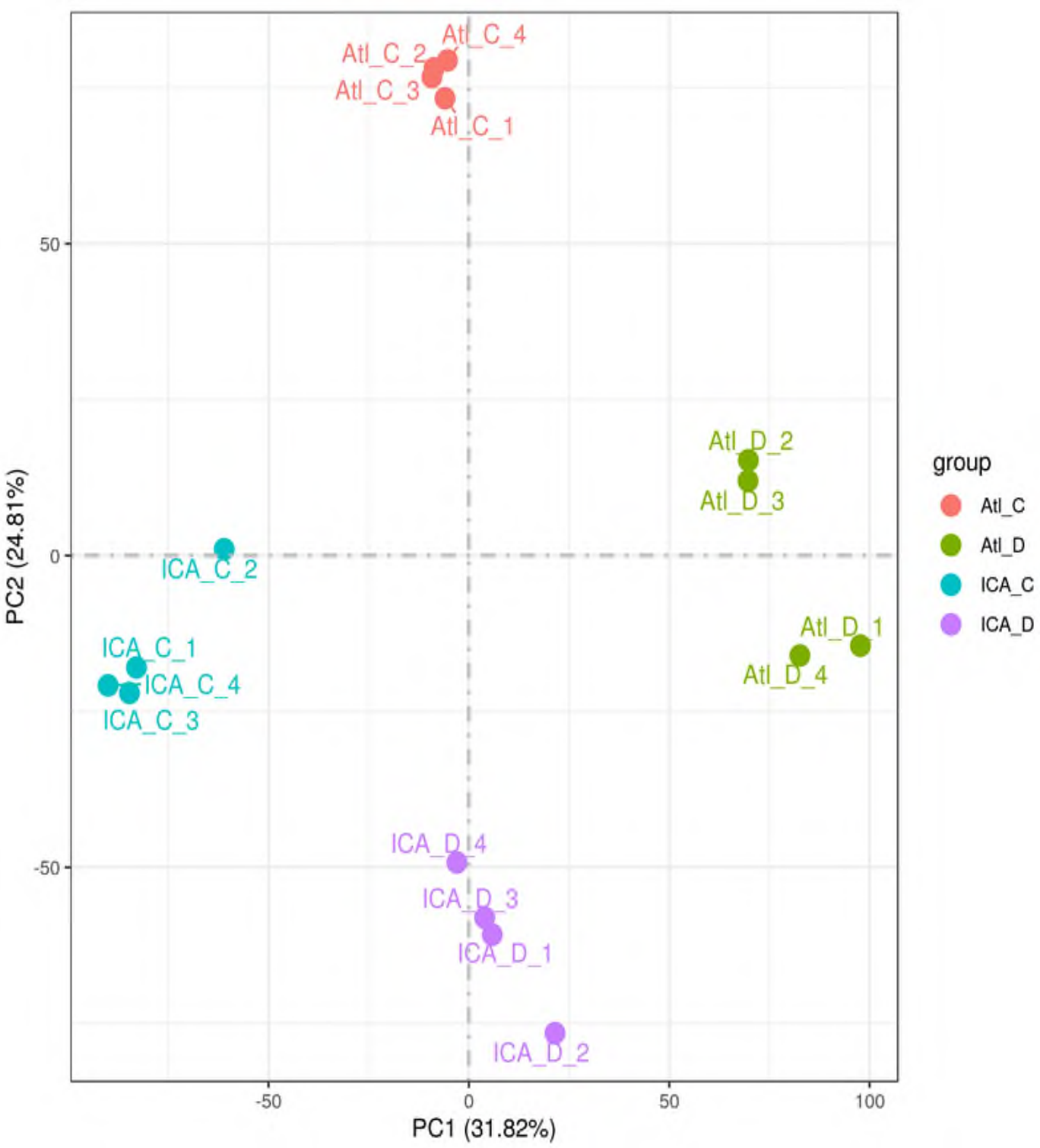
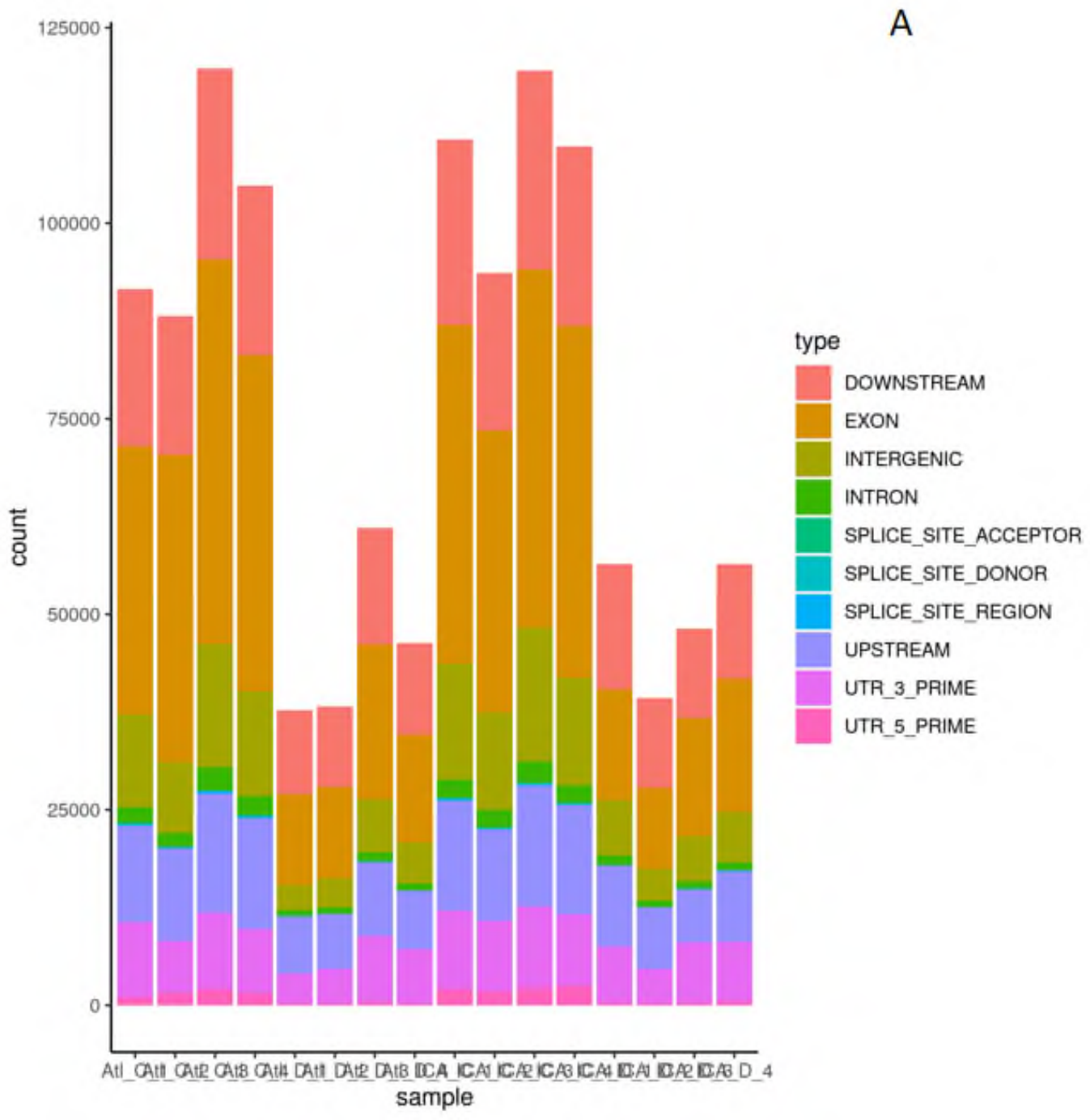
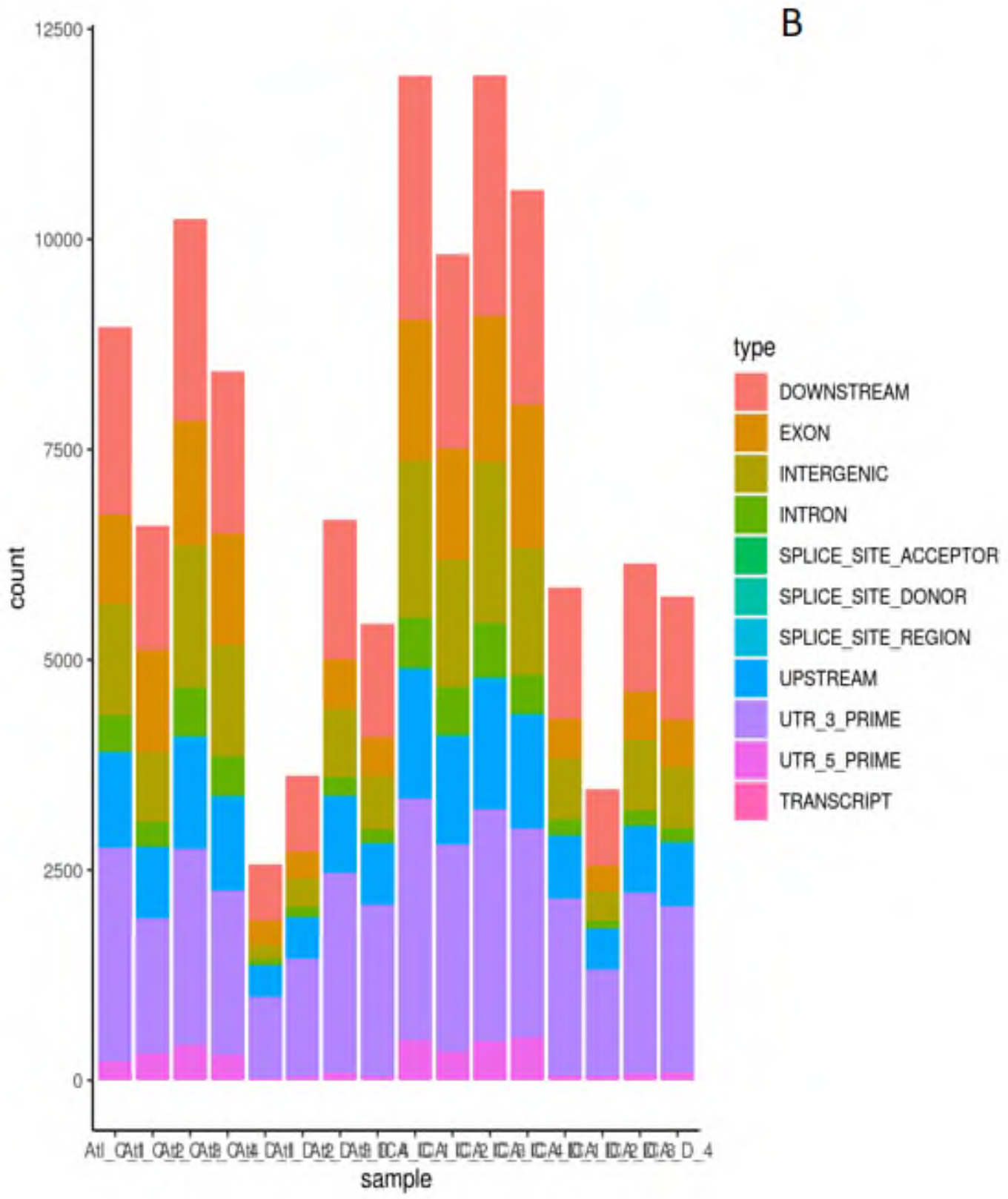


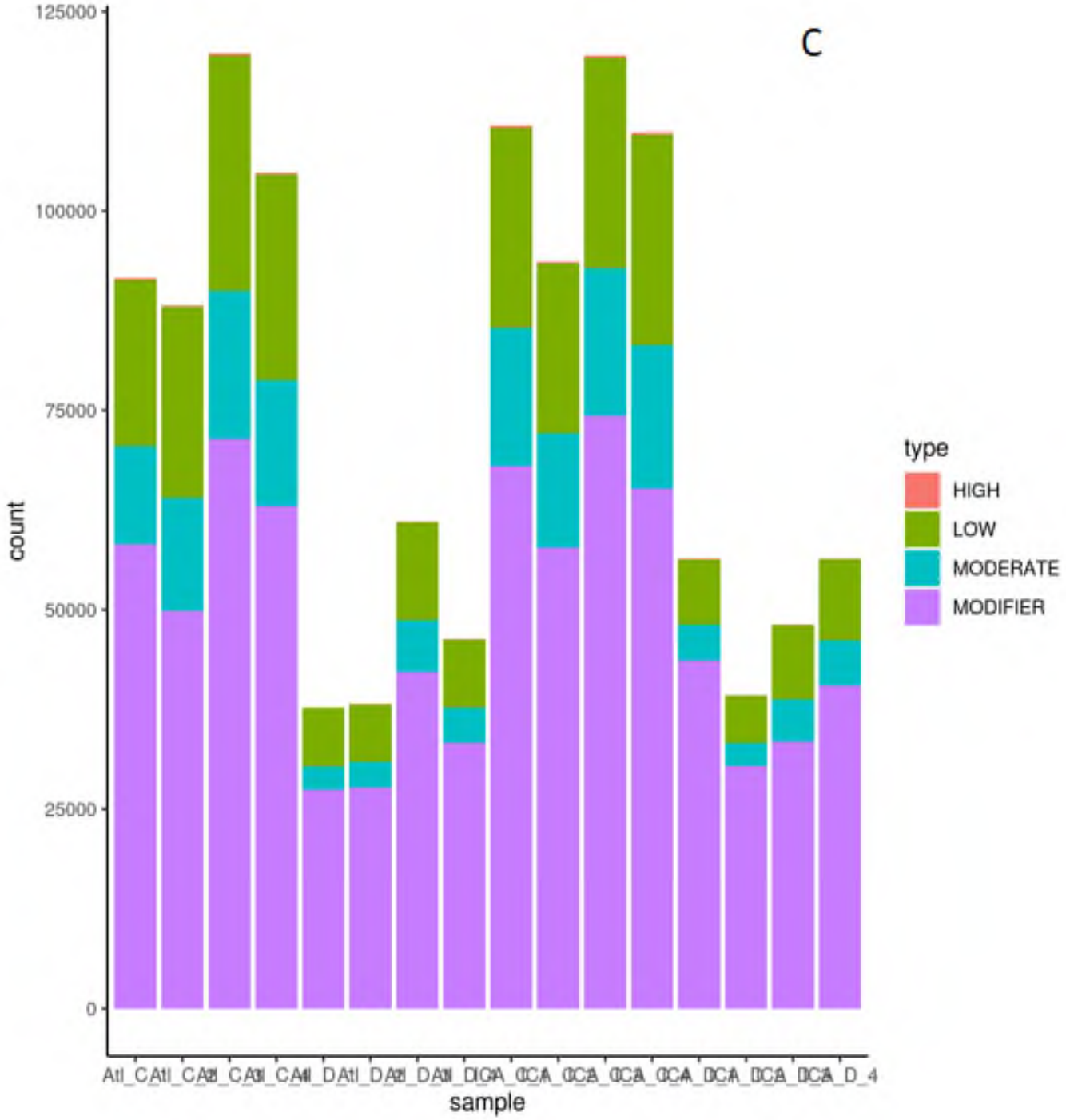
Figure 5.2. Two-dimensional principal component analysis (PCA) for seedling leaf tissues of two wheat cultivars, Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. PCA analysis was performed based on the gene expression value (FPKM) of all sample showing the intergroup differences and intragroup sample duplication. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; 1, 2, 3, 4 = biological replicates.

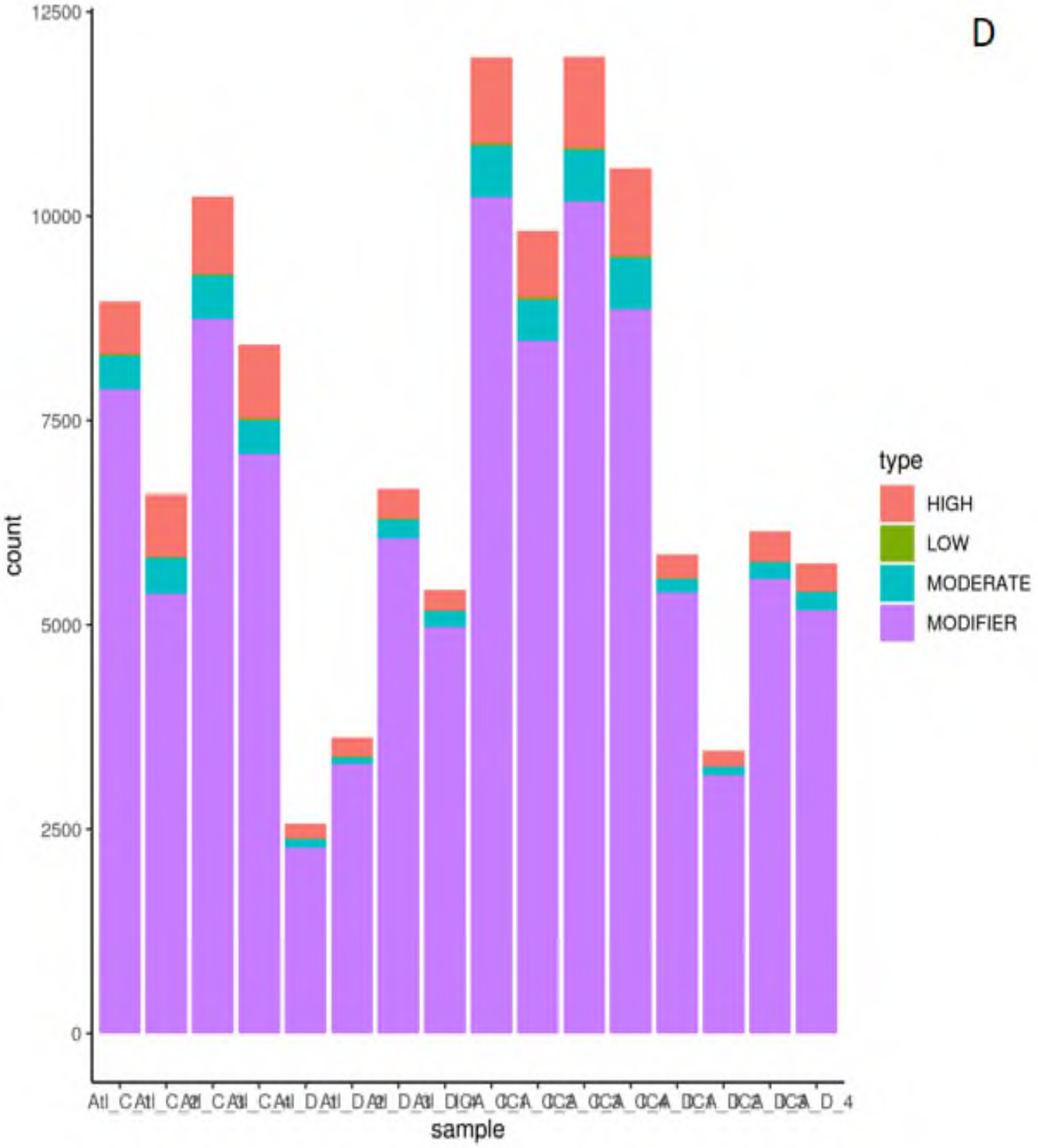
5.4.2. SNP and INDEL analysesA single nucleotide polymorphism (SNP) and the insertion or deletion of one or more nucleotides, known as an INDEL help to better understand genome evolution and wheat adaptation to the environment. Each wheat cultivar has a unique genotype with particular variations in SNPs and INDELs that are due to evolutionary processes and selective environmental factors (e.g. climate, soil conditions, etc.) (Yang et al. 2023). The difference between SNPs and INDELs can also be caused by natural biological processes such as recombination or DNA repair (Yang et al. 2023). SNP and INDEL detected in the cultivars Atlas and ICARDA were mapped along the reference genome of *Triticum aestivium* and found to be uniformly distributed in the reference sequence. Under 100% field capacity condition (FC) conditions, quadruplicate biological samples of ICARDA showed 55774, 46580, 60454, 55261 SNP and 5570, 4542, 5614, 4908 INDEL while Atlas had 44264, 42205, 59199, 50873 SNP and 4080, 3006, 4791, 3937 INDEL. These SNPs and INDELs variations between Atlas and ICARDA cultivars are due to their genetic make-up and diversity. Under drought conditions, the number of variant sites decreased in ICARDA to 23484, 15541, 22651, 25017 SNP and 2537, 1458, 2750, 2558 INDEL. Each wheat cultivar has a unique genotype with particular variations in SNPs and INDELs that are due to evolutionary processes and selective environmental factors (e.g. climate, soil conditions, etc.) (Yang et al. 2023). The difference between SNPs and INDELs can also be caused by natural biological processes such as recombination or DNA repair (Yang et al. 2023). The reduction in the number of variant sites was also noted in the Atlas cultivar with 14888, 15882, 27618, 20631 SNP and 1055, 1537, 2937, 2399 INDEL. SNPs and INDELs can occur in different regions of the genome (e.g. promoter or regulatory regions, etc.), affecting gene expression levels (DEGs) and consequently leading to changes in protein structure and function (Yang et al. 2023). In this context, statistical analyses of the variant site, related to SNP and INDEL provided further insight into the function, impact, and location of mutation sites. SNP and INDEL regions were distributed downstream and upstream, in the exon and intron, in the intergenic space, within the UTR 3'and UTR 5' regions and the splice region (including acceptor and donor splice sites), as well as in the transcript. However, SNP- related mutations appear to be more localised in the exon and downstream, while INDEL are found more in downstream and 3' UTR region, as shown in Figure 5.3. The function of SNP was determined as missense, nonsense and silent mutations, with a predominance of missense and silent mutations. A missense mutation can alter the function of the protein as a substitution of a single base pair changes the genetic code to produce the usual amino acid, unlike silent mutation. The predominant impact of SNPs and INDELs in Atlas and ICARDA cultivars is a modifier impact that can affect the phenotype and/or

molecular expression of other genes. The predominance of a missense mutation with a modifier impact in the studied cultivars is in condordance with the significant number of DEGs found under drought stress condition (see below). Identifying SNPs and INDEL provides a better understanding of the genetic basis of DEGs and their impact on phenotypes, as well as the potential causal variants that contribute to a particular trait or phenotype and its underlying pathway and mechanism.The data in this chapter can be used at a later date for further analysis of the variant/genes and their relationship to drought stress.




B
12500
10000
7500
5000
2500
0
count
sample
type
DOWNSTREAM
EXON
INTERGENIC
INTRON
SPLICE_SITE_ACCEPTOR
SPLICE_SITE_DONOR
SPLICE_SITE_REGION
UPSTREAM
UTR_3_PRIME
UTR_5_PRIME
TRANSCRIPT


C
125000
100000
75000
50000
25000
0
count
sample
type
HIGH
LOW
MODERATE
MODIFIER


D
12500
10000
7500
5000
2500
0
count
sample
type
HIGH
LOW
MODERATE
MODIFIER

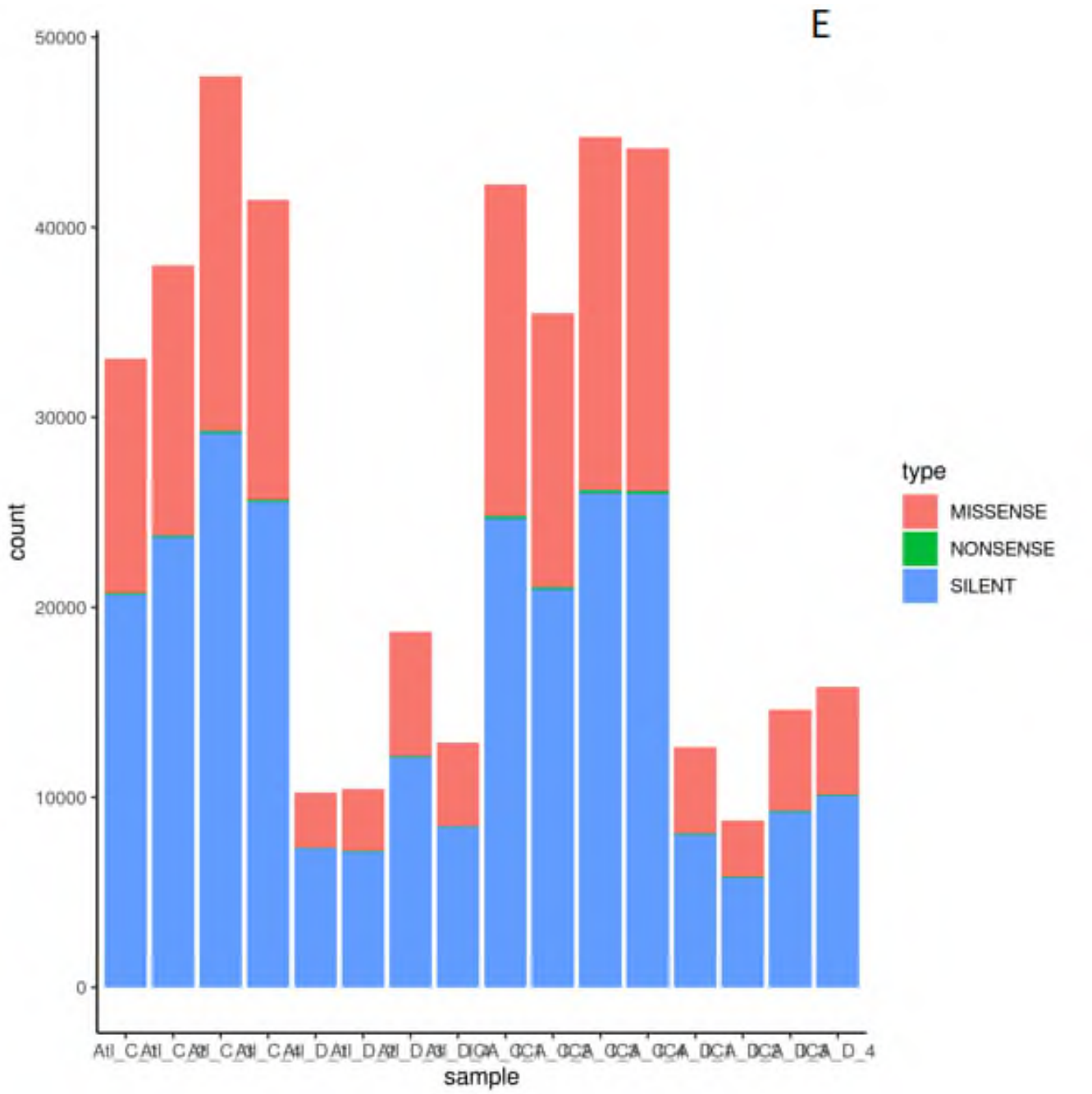


Figure 5.3. Single nucleotide polymorphism (SNP) and INDEL statistical analyses for seedling leaf tissues of two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; 1, 2, 3, 4 = biological replicates. A. SNP-region, B. INDEL-region, C. SNP-impact, D. INDEL-impact, E. SNP-function. The four levels of impact (high, middle, low, modifier) are pre-defined categories to find more significant variant. High impact mutations are variations in splice sites, and start and stop codons. Moderate impact mutations are non-synonymous variations that alter the amino acid sequence of a protein, unlike low impact mutations. The latter occur in coding

regions, and start and stop condons. Modifier impact is usually variations in non-coding region, and can modify the expressivity of a phenotype. Most of SNP and INDEL have the modifier impact category for the genome. X axis = sample names; Y axis = number of variants.

5.4.3. Differential Gene Expression Analysis (DEGs)

In order to discover the underlying molecular mechanism and genetic machinery of the drought-tolerant and drought-sensitive genotypes, the qualitative and quantitative variations in the transcripts of Atlas and ICARDA cultivars were compared. The mRNA transcript abundance, denoted by FPKM values, within the genotype was quantified and compared. The box plot of log2FPKM values revealed a slight variation in the distribution of gene expression within and between the two genotypes under control and drought stress conditions (Figure 5.4). For both genotypes and irrespective of growth conditions, log2 (FPKM) values were greater than 5, indicating that most DEGs were highly expressed. The overall distribution of DEGs in Atlas and ICARDA under drought stress was similar, as shown in Figure 5.4, indicating that these DEGs were evenly distributedin both cultivars.

II.3.A. DEGs profile of Altas and ICARDA under control and drought conditions

A total of 15352 and 13744 DEGs were detected for Atl_C_vs_Atl_D and ICA_C_vs_ ICA_D, while 7348 DEGs were obtained for ICA_D_vs_Atl_D, respectively (Figure 5.5). The 7348 DEGS found in Atlas and ICARDA under drought stress included 3886 up-regulated and 3462 down-regulated DEGs. The number of up-regulated DEGs was 6875, 6126 and 11316, while the number of down-regulated DEGs was 8477, 7618 and 6415 for the set Atl_D_vs_Atl_C, ICA_D_vs_ICA_C and ICA_C_vs_Atl_C, respectively (Figure 5.5). More details on the threshold used for this analysis are presented in Table 5.2.

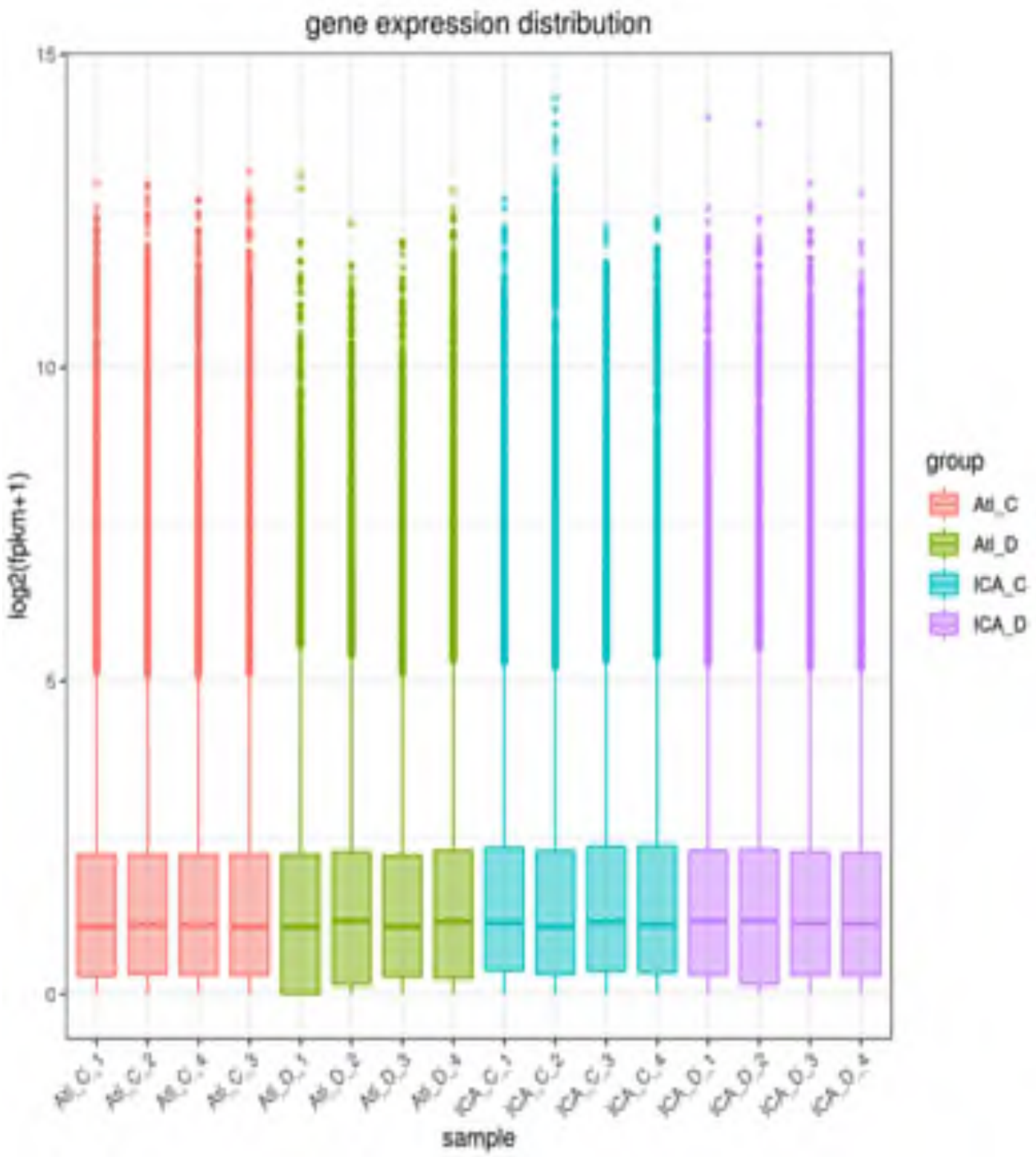

gene expression distribution
log2(fpkm+1)
15
10
5
0
sample
group
Ati_C
Ati_D
ICA_C
ICA_D

Figure 5.4. Distribution of gene expression levels across seedling leaf tissues of two wheat cultivars, Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions, plotted in a box plot representation. Four biological replicates were used. Parameters of box plots are indicated, including maximum, upper quartile, mid-value, lower quartile and minimum. Abbreviations in sample names: C = control condition; D = drought condition; 1, 2, 3, 4 = biological replicates. X axis represents the name of the sample; Y axis indicates the log2(FPKM+1). The final FPKM is the mean value of biological replicates.

Table 5.2. Differential gene statistics for two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; vs = versus. All: the total number of differential genes in the compare group; up: the up-regulation number of differential genes in the compare group; down: the down-regulation number of differential genes in the compare group. Threshold: The compare group software and thresholds for differential gene screening.

Compare	all	up	down	threshold
Atl_DvsAtl_C	15352	6875	8477	DESeq2 padj<=0.05 |log2FoldChange|>=1.0
ICA_DvsICA_C	13744	6126	7618	DESeq2 padj<=0.05 |log2FoldChange|>=1.0
ICA_DvsAtl_D	7348	3886	3462	DESeq2 padj<=0.05 |log2FoldChange|>=1.0
ICA_CvsAtl_C	17731	11316	6415	DESeq2 padj<=0.05 |log2FoldChange|>=1.0

The total number of DEGs obtained between the intra and inter compared treatment group and the control group showed that ICARDA and Atlas had 8676 and 10284, respectively. Both cultivars share 5068 DEGs (Figure 5.6). More details about the co-expressed genes in these cultivars are provided in Figure 5.7. The overall distribution of DEGs for each cultivar under drought and control conditions are shown in Figure 5.8.

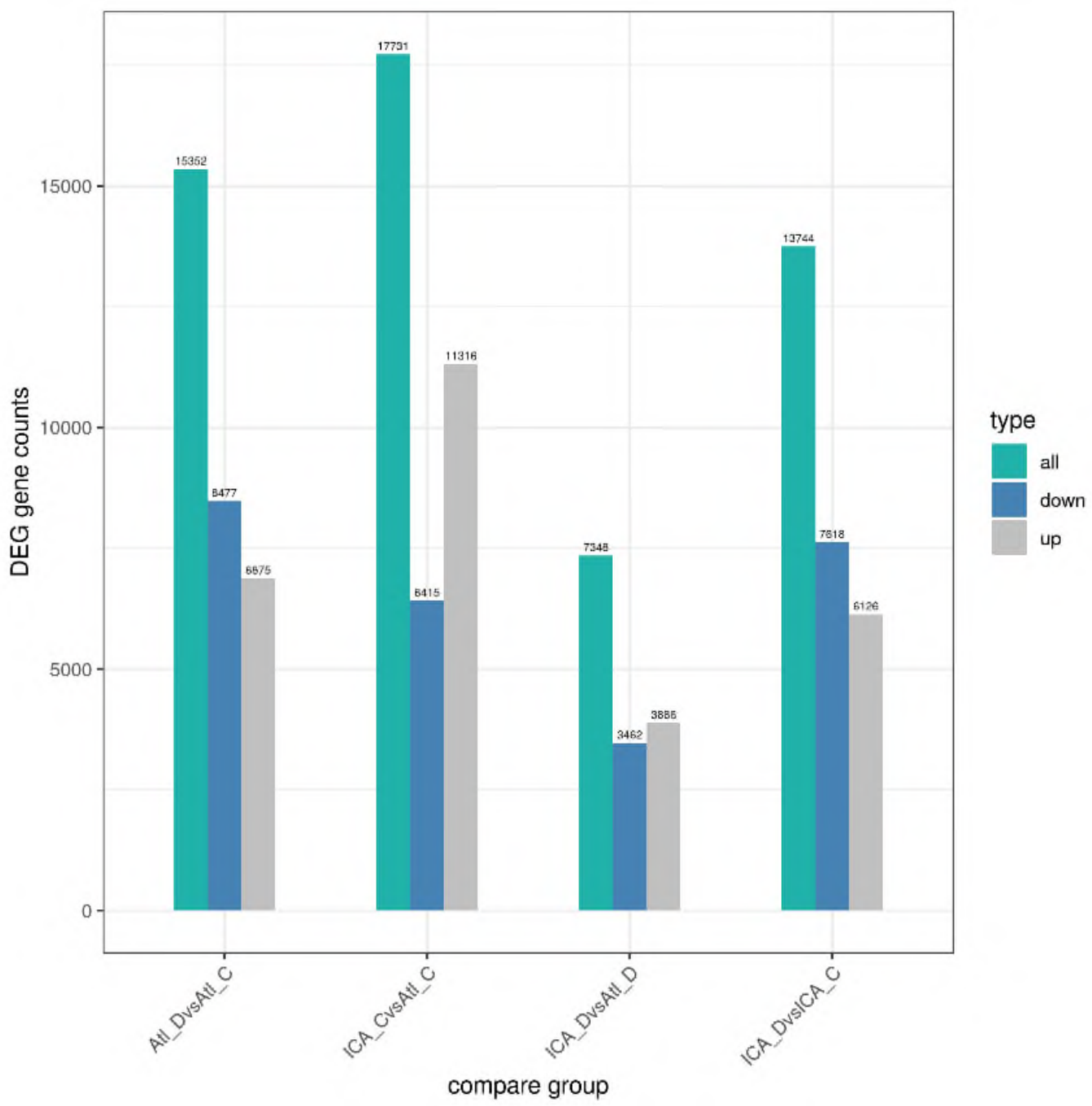


Figure 5.5. Histogram of the number of DEGs for seedling leaf tissues of two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; vs = versus. X axis is the compared group; Y axis is DEG gene counts.

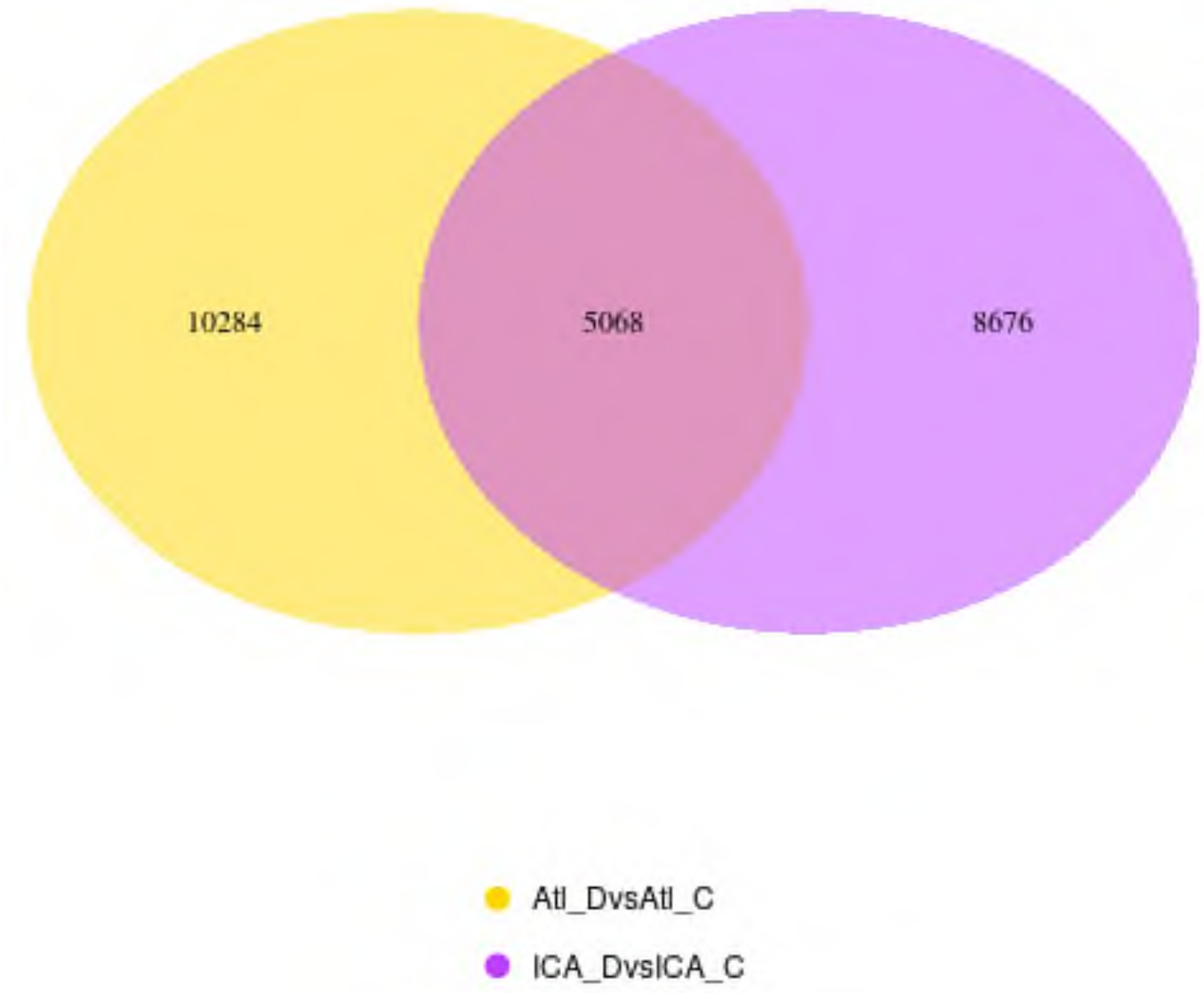
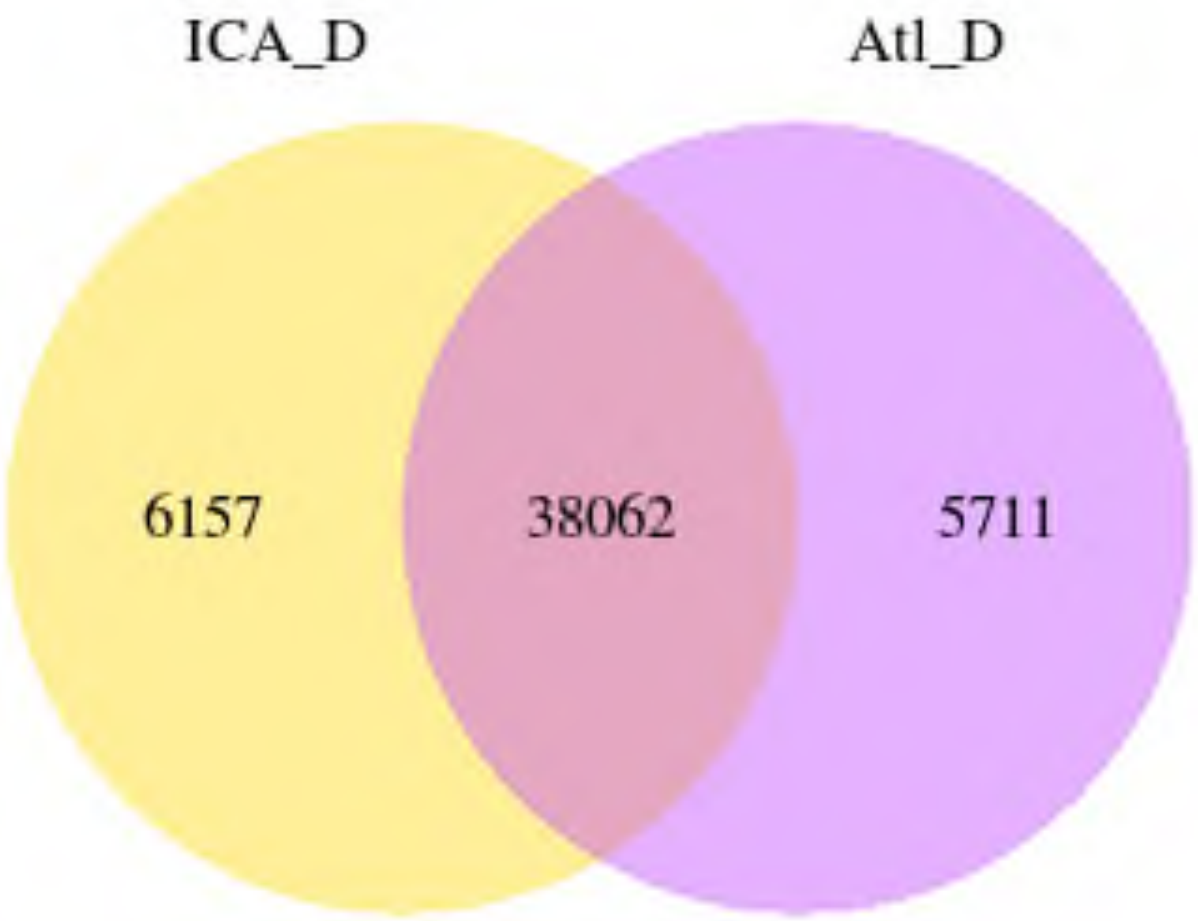
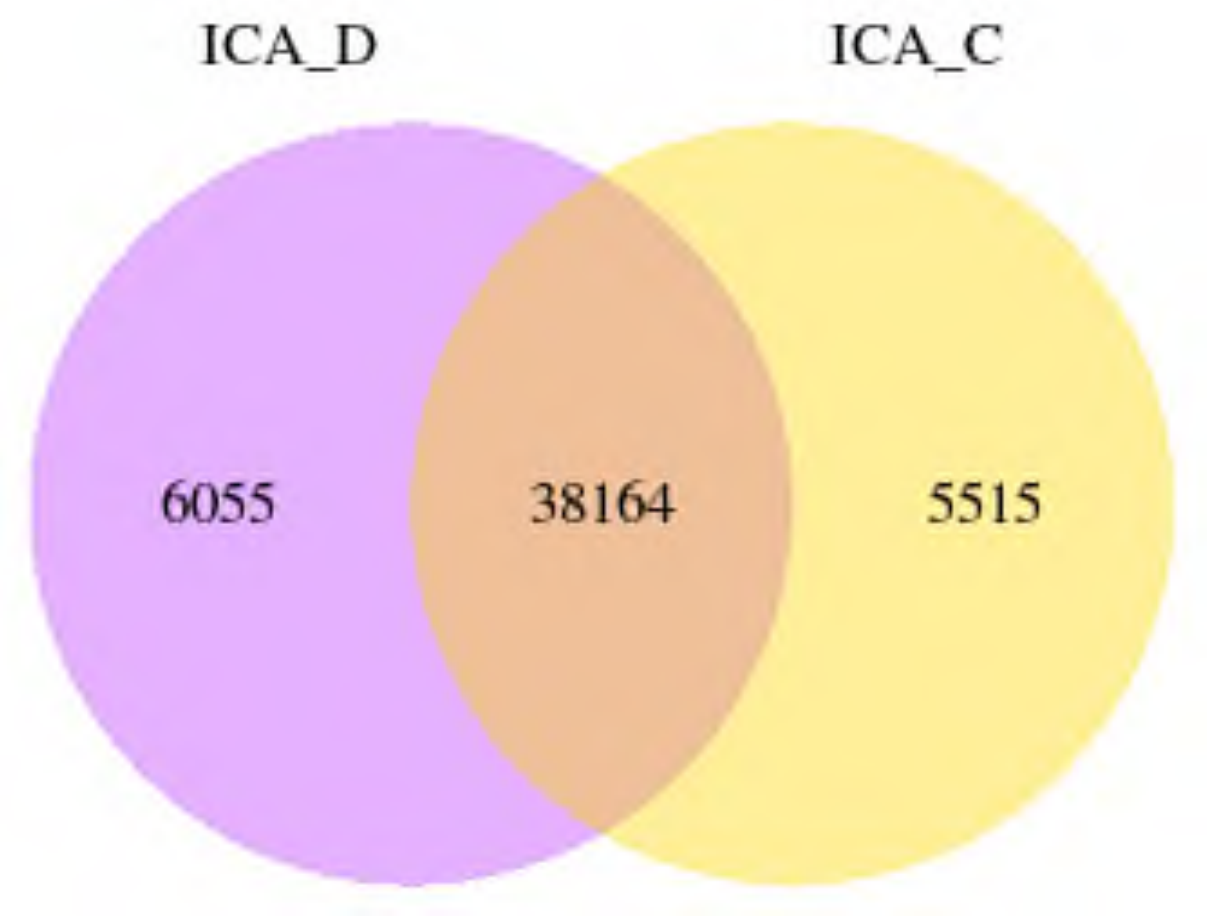
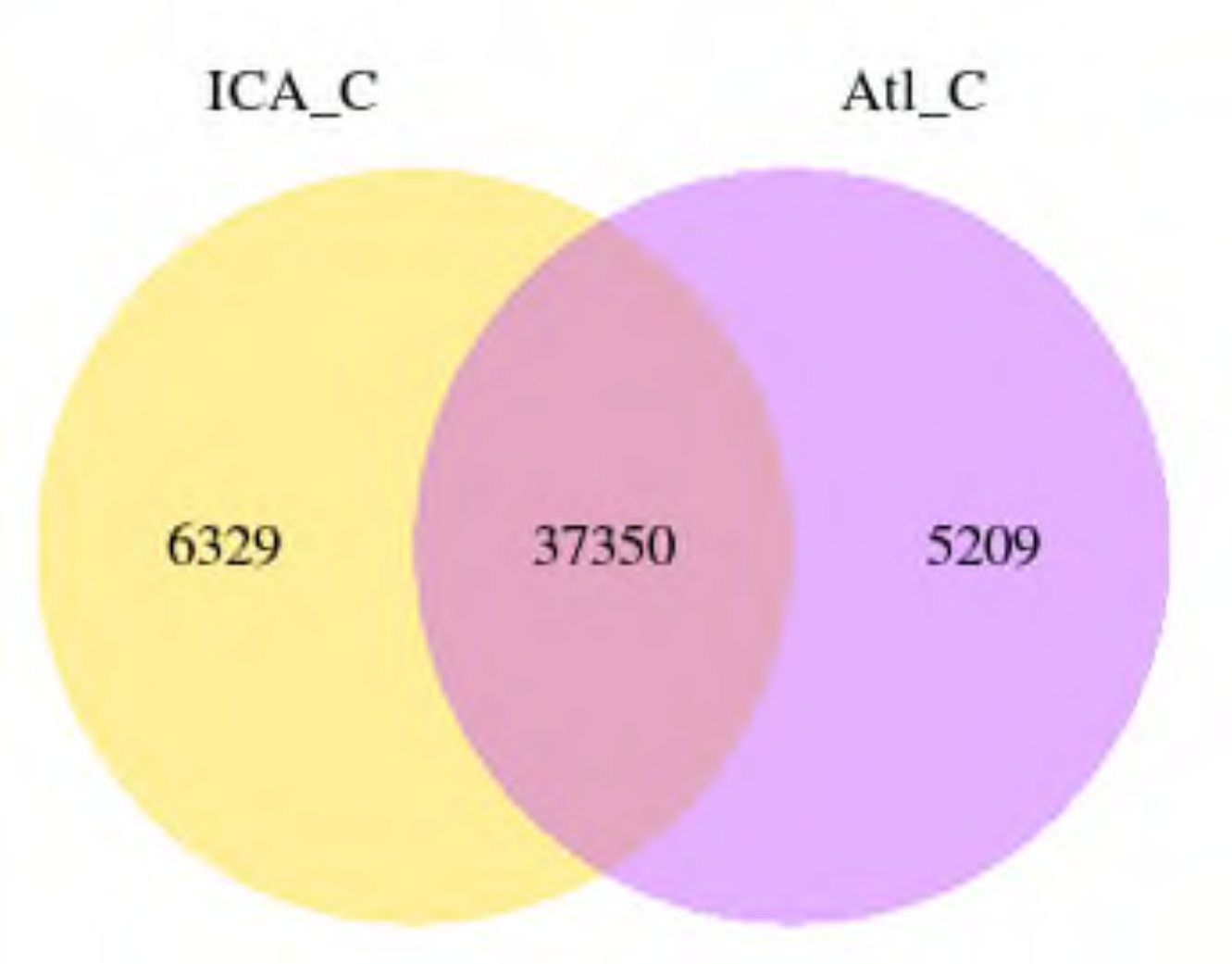


Figure 5.6. Venn Diagram showing a comparison of the number of differential genes between two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; vs = versus.


ICA_D
Atl_D
6157
38062
5711


ICA_D
ICA_C
6055
38164
5515


Atl_D
Atl_C
6558
37215
5344


ICA_C
Atl_C
6329
37350
5209

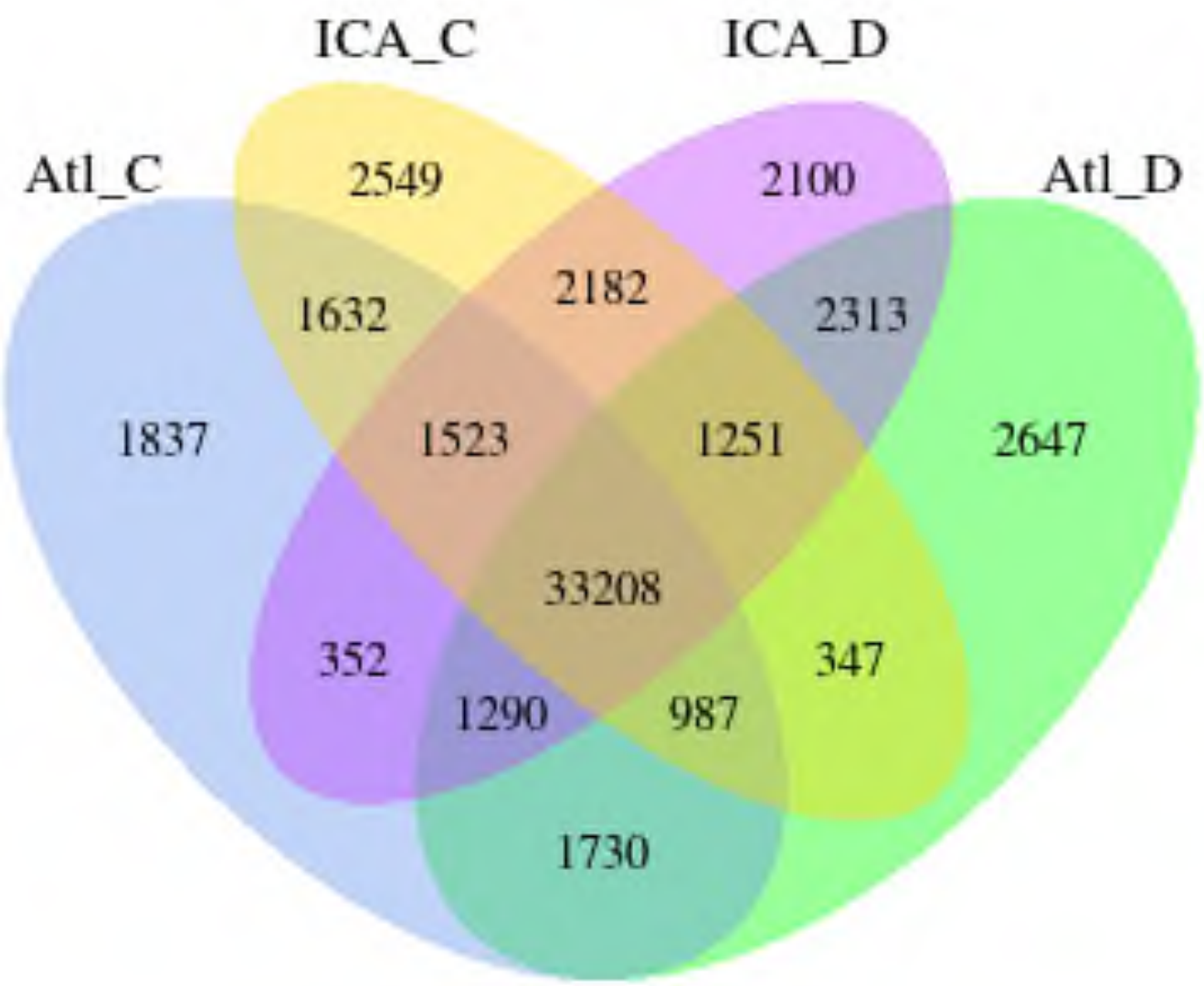
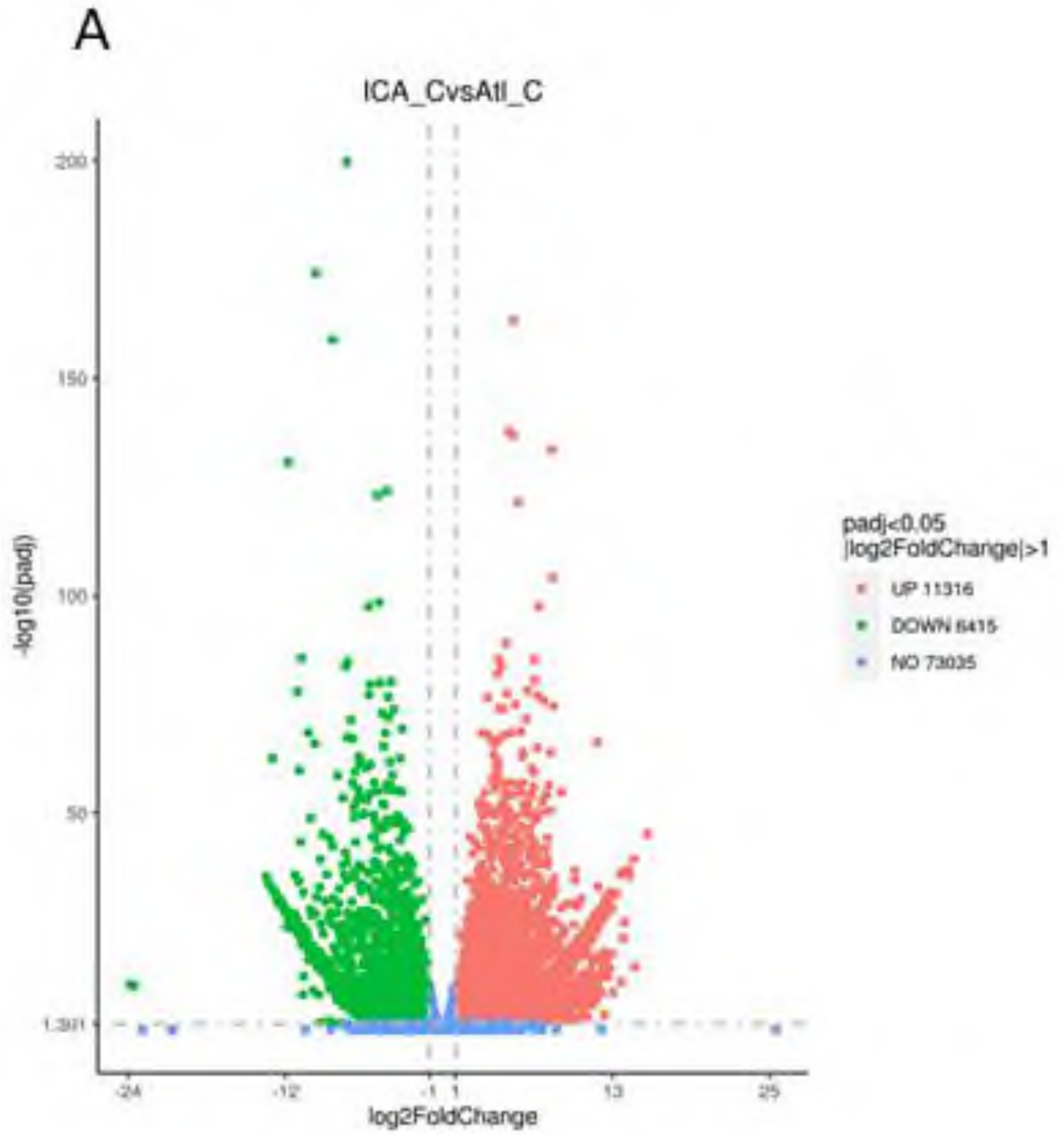
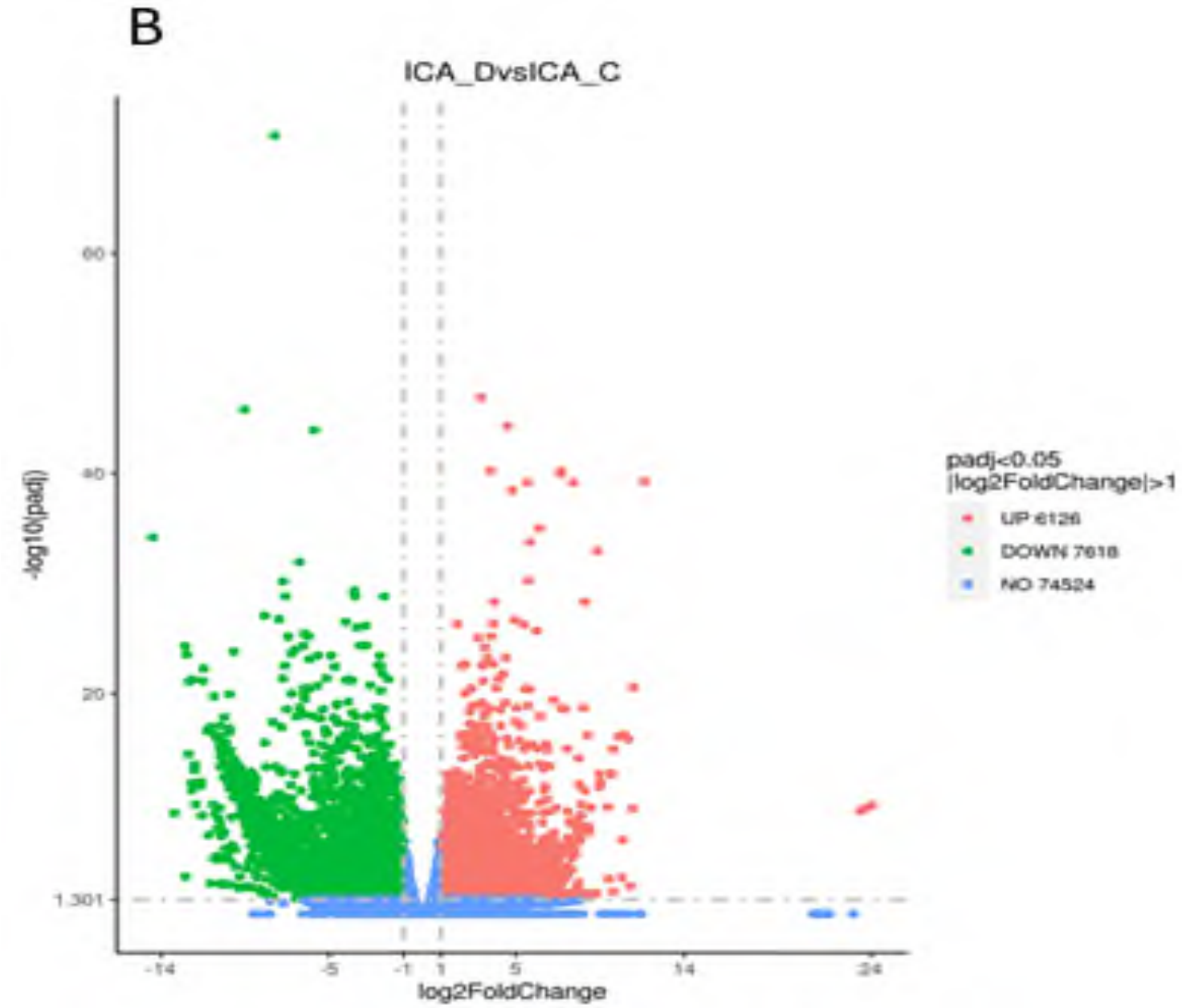
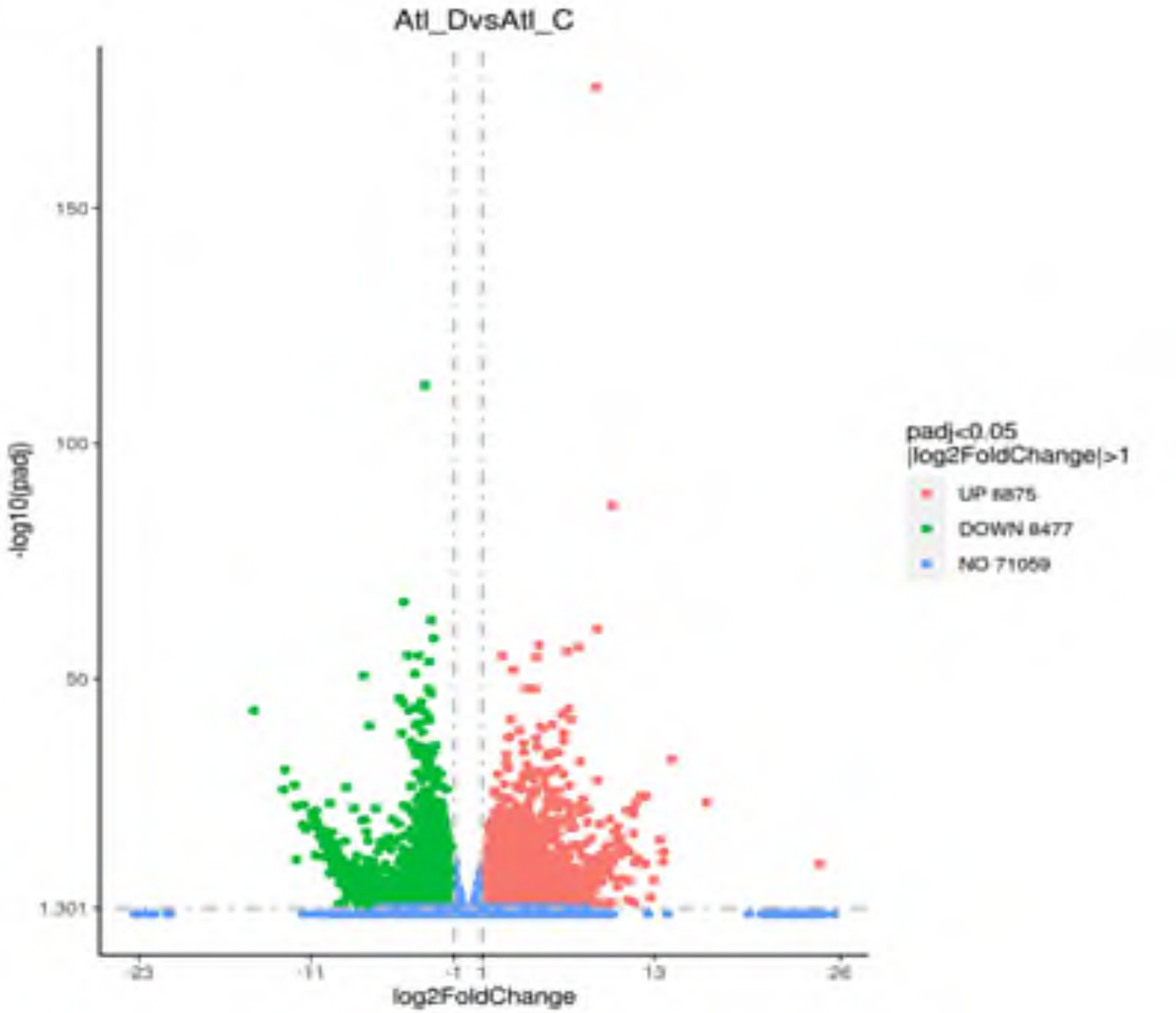


Figure 5.7. Number of genes that are uniquely expressed within each wheat cultivars (seedling leaf tissues), Atlas (Atl) and ICARDA 32331 (ICA), and the co-expressed in two or more cultivars presented in Venn Diagram. These cultivars were grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; vs = versus.


A
ICA_CvsAtl_C
200
150
100
50
1.391
-log10(padj)
-24
-12
-1
1
13
25
log2FoldChange
padj<0.05
|log2FoldChange|>1
UP 11316
DOWN 6415
NO 73035


B
ICA_DvsICA_C
-log10(padj)
60
40
20
1.301
-14
-5
-1
1
5
14
24
log2FoldChange
padj<0.05
|log2FoldChange|>1
UP 6126
DOWN 7618
NO 74524

C


Atl_DvsAtl_C
-log10(padj)
150
100
50
1.301
-23
-11
-1
1
13
26
log2FoldChange
padj<0.05
|log2FoldChange|>1
UP 8875
DOWN 8477
NO 71059

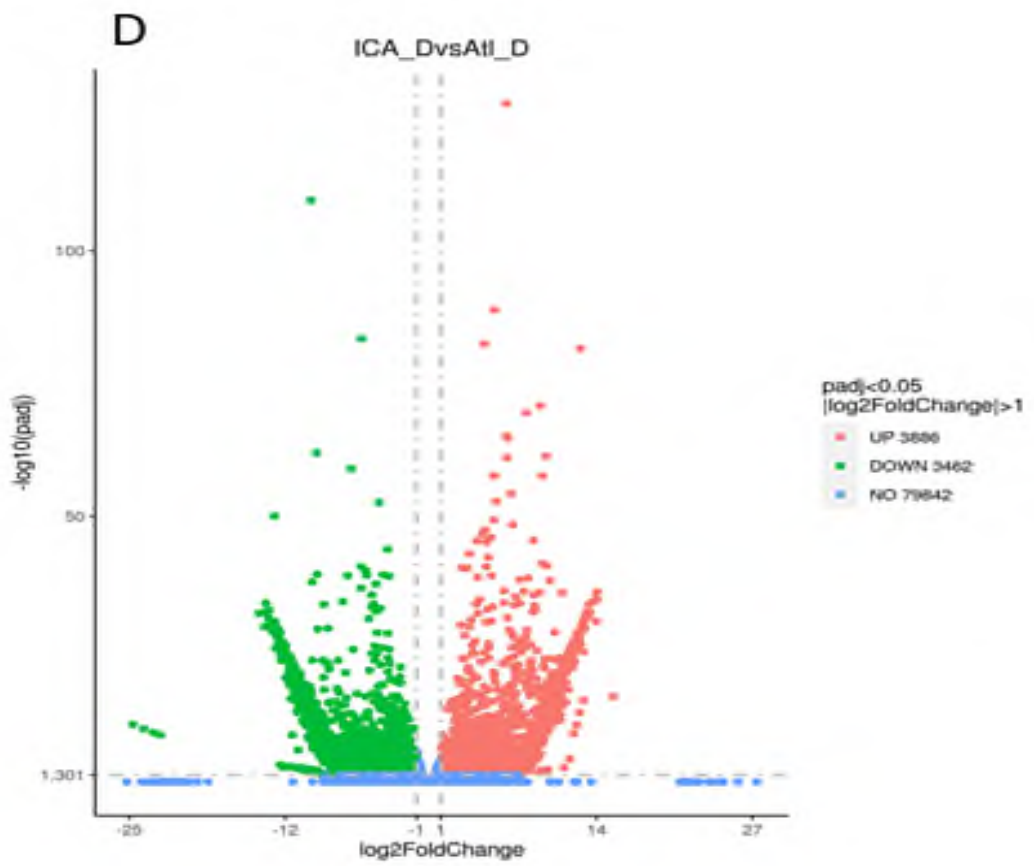


Figure 5.8. Differential gene expression volcano map for seedling leaf tissues of wheat cultivars, Atlas (Atl) and ICARDA 32331 (ICA) grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition; vs = versus. The X axis is log2FoldChange in gene expression between different samples. Y axis is -log10padj or -

log10pvalue shows the statistical significance of the differences. Red dots are up-regulation genes and green dots represent down-regulation genes. The blue dashed line indicates the threshold line for differential gene screening criteria.

Cluster analysis based on all differentially expressed genes in the comparison group (drought and control) within ICARDA and Atlas showed that these cultivars were distributed in two distant clusters according to growth conditions. Cluster 1 contains 2 distinct sub-clusters housing the Atlas and ICARDA samples respectively, each containing cluster genes with similar expression profiles. However, cluster 2 includes both varieties, under control conditions, grouped in separate sub-clusters, reflecting a similar gene expression profile within the cluster but different from those expressed under stress conditions (Figure 5.9).

There were significant differences in gene expression for all groups compared under different growth conditions. The ICARDA genotype showed several up-regulated DEGs whereas these genes were down-regulated in Atlas under drought stress conditions. In this context, the universal stress protein (USP)-associated gene, PF00582, located on chromosome 1B, was significantly expressed in ICARDA under drought stress conditions, in contrast to Atlas. This protein has been found in archaea, bacteria, fungi, protozoa and plants, and enhances the biological organism's ability to resist different environmental stresses (Kvint et al. 2003; Siegele 2005; Isokpehi et al. 2011). Environmental stressors that induce USP expression include drought, high and low temperatures, salinity and the presence of toxic chemicals. This protein has a conserved domain of 140-160 residues and has been identified in *Arabidopsis thaliana* (Kerk et al. 2003), *Viridiplantae* (Isokpehi et al. 2011), barley (La et al 2005; Gupta et al. 2003), citrus (Chen et al. 2006), peanut (Liang et al. 2009) and wheat (Lazo et al. 2004; Gupta et al. 2003) in response to drought stress. In the same chromosome 1B, another DEG was found to be overexpressed in ICARDA under water-limited conditions, which is the gene associated with ferrochelatase. The latter has been identified in barley and appears to improve photosynthesis and reduce drought-induced photo-oxidative damage in transgenic barley (Nagahatenna et al. 2020). It confers salinity resistance in *Arabidopsis thaliana* (Zhao et al. 2017) and has been shown to be involved in heme biosynthesis, mediating intracellular signalling and stimulating the ROS detoxification system under drought conditions (Nagahatenna et al. 2015).

However, the ABA-WDS (ABA-water deficit stress) protein was highly expressed in Atlas, in contrast to ICARDA, although both cultivars were subjected to the same stress. The ABA-WDS domains, located on chromosome 3D, belong to the ASR (abscisic acid stress ripening protein)

gene family. They play an important role in plant growth, senescence and fruit ripening, as well as in the direct protection of most plants against abiotic stress conditions such as heat, salt and cold, particularly in wheat, barley, *Oryza sativa*, tomato, Zea mays and plantain (Yacoubi et al. 2021; Hu et al. 2013; Gonzalez et al. 2014; Goldgur et al. 2007; Hamdi et al. 2020; Arenhart et al. 2016; Virlouvet et l. 2011).

In Atlas, drought induced expression of the dehydrin-associated gene located on chromosomes 3B, 3D, 5A, 5B, 5D, 6A, 6B, 6D. Several studies have shown that dehydrin is induced under abiotic stress (drought, salt, heat, cold, ABA, wounding, etc.) in wheat and that its expression is linked to the ABA-dependent pathway and confers some drought and salinity tolerance to the plant (Habib et al. 2022, Hassan et al. 2015). Dihydrin has been found mainly in plasma membranes, cytosol and nucleus (Danyluk et al. 1998). Drought stress in Atlas down-regulated the expression of Myo-inositol-1-phosphate synthase (MIPS, PF01658) and N-terminal C2 in EEIG1 and EHBP1 (PF10358) proteins placed in chromosomes 4D and 5A, respectively. MIPS acts as an enhancer of tolerance to abiotic stress and pathogens. It therefore plays a protective role and controls plant growth and immunity via the ethylene signalling pathway (Sharma et al. 2020). Overexpression of this gene has been associated with increased tolerance to abiotic and biotic stresses in several plants (Joshi et al. 2013; Zhai et al. 2016) and in transgenic sweet potato (Zhai et al. 2016), respectively. Nevertheless, down-regulation of MIPS is associated with increased leaf senescence, decreased apical dominance and reduced yield according to Brearley et al. (1998). In transgenic plants, down-regulation of MIPS was found to negatively affect ascorbate levels, inhibit seed germination and generate high sensitivity to ABA (Ali et al. 2013; Nunes et al. 2006; Sharma et al. 2020). The down-regulation of this gene in Atlas under drought stress is consistent with its high leaf senescence and low grain yield, confirming its sensitivity to drought stress. The transcriptomic study showed that the gene associated with the N-terminal C2 domain of the EEIG1 and EHBP1 proteins (PF10358) was down-regulated under water-limited conditions. The NT-C2 domain was found in two plant proteins, RPG and PMI1, which are involved in the modulation of root hair growth of *Rhizobium* (Arrighi et al. 2008) and it seemed to be crucial for the intracellular motility or re-localisation of certain cellular organelles such as chloroplasts and vesicles (DeBlasio et al. 2005; Arrighi et al. 2008). The NT-C2 domain has also been detected in several eukaryotic proteins of unknown function. The EHBP1 was found to have an endocytotic function (Guilherme et al. 2004a; Guilherme et al. 2004b; Naslavsky and Caplan 2005) and binds specifically to PtdIns(4)P and PtdIns(4,5)P2 located in the membrane of endocytic vesicles (Zhang and Aravind 2010). The process of

endocytic trafficking is vital for plants, including wheat, and crucial for normal growth. It has also been shown to be important for stress signalling (Tripathy et al. 2021). Its down-regulation in Atlas under drought reflects the sensitivity of this cultivar to drought.

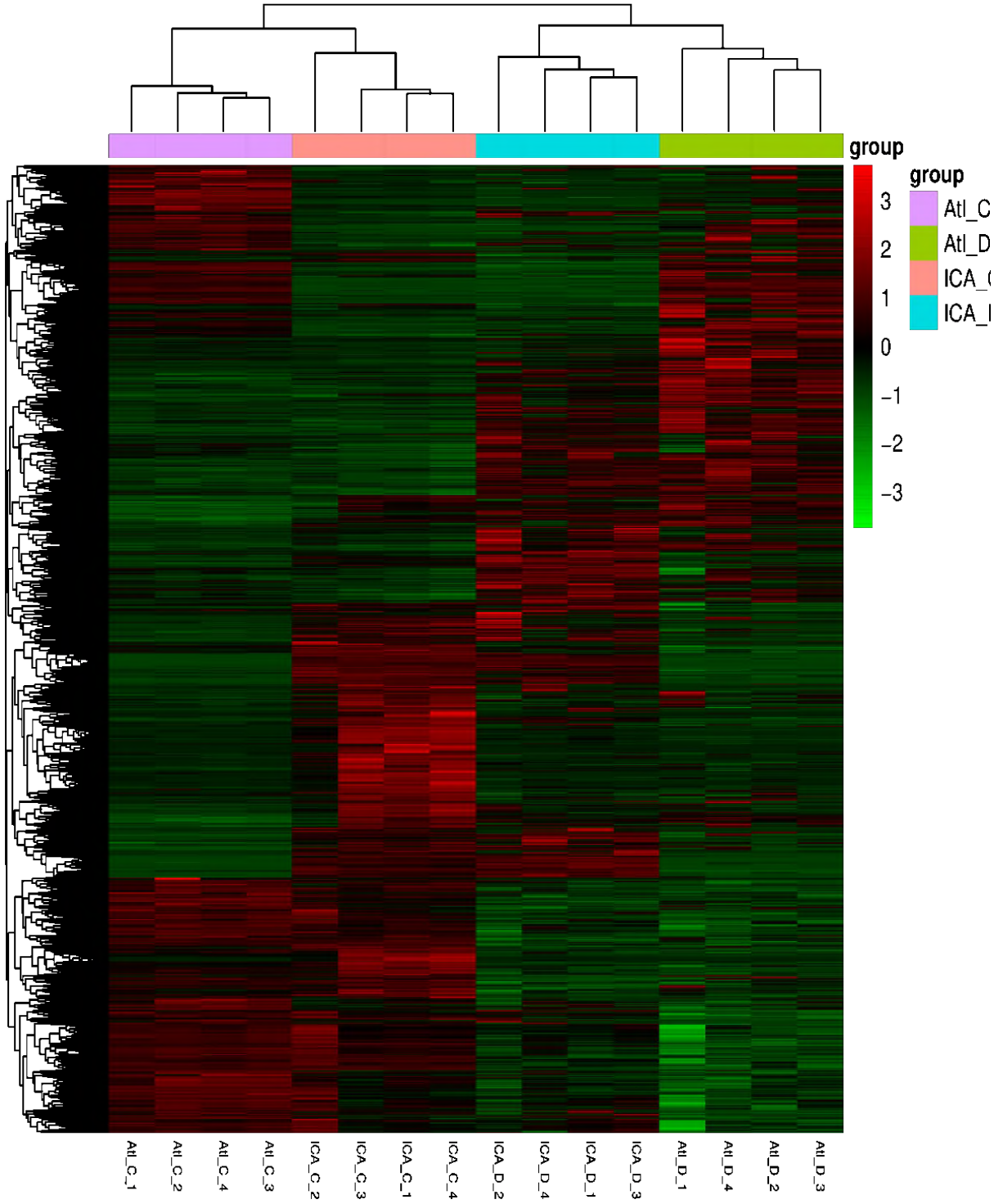


Figure 5.9. Differential expression gene clustering heatmap for two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought

(25% WFC) conditions, showing inter group and sample clustering. Four biological replicates were used. Abbreviations in sample names: C = control condition; D = drought condition. The color in each grid reflects the value obtained after homogenizing the expression data rows (generally between – 2 and 2). The mainstream hierarchical clustering to cluster the fpkm values of genes, and homogenized the row (Z-score) was used. Samples with similar expression patterns in the heatmap are grouped together. The heatmap shows inter and intra group clustering.

5.4.4. Gene ontology enrichment analysis

Differentially expressed genes were associated with their biological functions or corresponding pathways by enrichment analysis. Gene oncology (GO) analysis was performed and enabled DEGs to be classified into three main categories: cellular component (CC), molecular function (MF) and biological process (BP) . In this thesis, only the 30 most significant GO terms are described and presented below (Figures 5.10, 5.11 and 5.12).

Significantly enriched GO term in ICA_D_vs_Atlas_D comprise DEGs associated with the catabolic process of purine-containing compounds and carbon fixation, as BP (Figure 5.10). Genes related to carbon fixation, porphyrin-containing compound biosynthetic process, pigment biosynthetic process, tetrapyrrole biosynthetic process, porphyrin-containing compound metabolic process, tetrapyrrole metabolic process, photosynthesis and photorespiration process were highly up-regulated (Figures 5.11 and and 5.12). For MF, this set of cultivars showed the most dominant DEGs associated with carboxy-lyase activity, binding pattern including polysaccharide binding, carbon-carbon-lyase activity, and ribulose-bisphosphate carboxylase activity (Figure 5.10). The latter were found to be up-regulated in addition to phosphoenolpyruvate carboxylase activity (Figures 5.11 and and 5.12). In CC, DEGs associated with thylakoid, chloroplast and plastid membrane were significantly up-regulated (Figures 5.10, 5.11 and and 5.12).

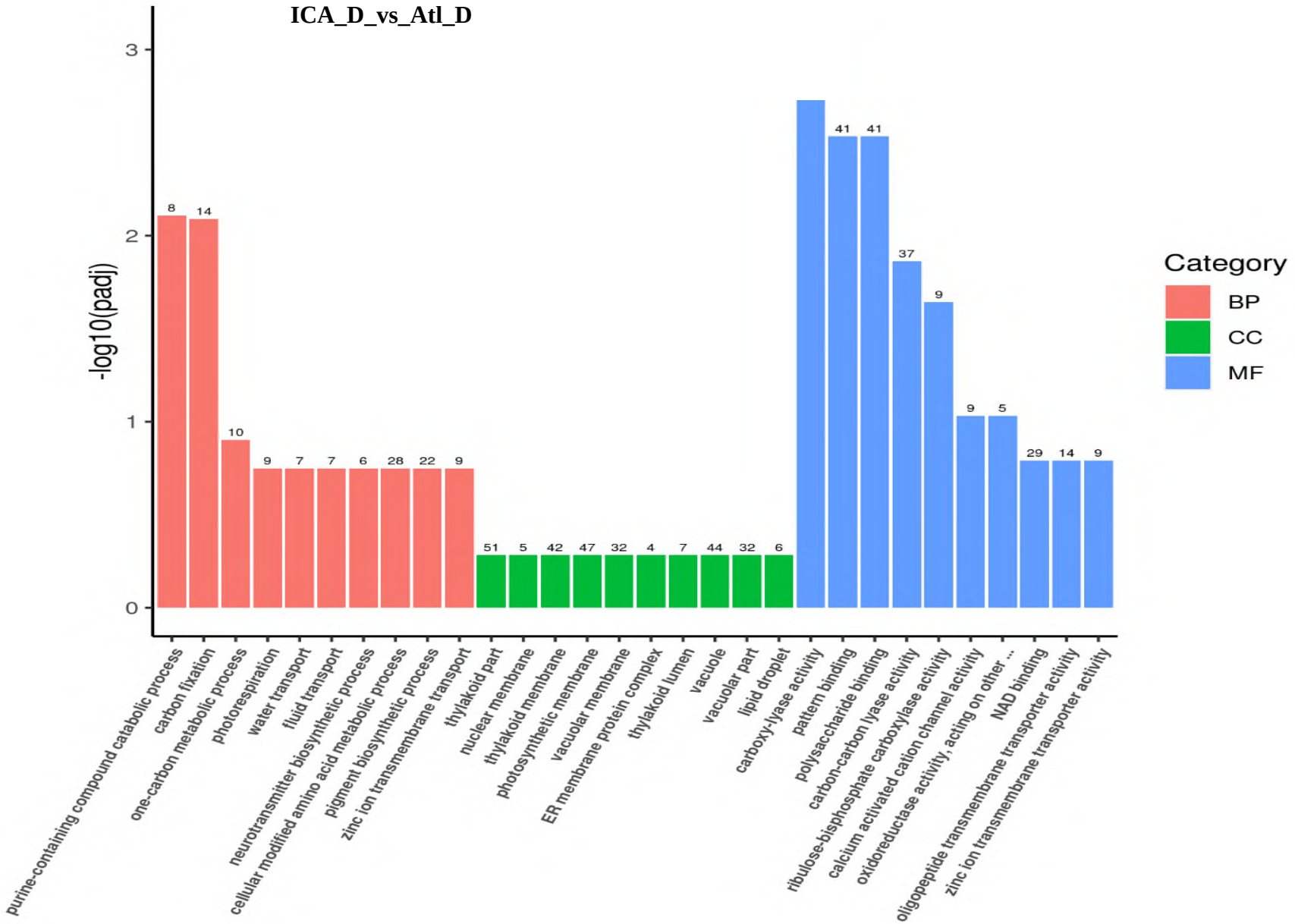
Comparison between the same cultivars under control and drought conditions showed that Atlas (Atlas_D_vs_Atlas_C) had photosynthesis, photosynthesis light reaction, and plastid organization as the most enriched BP category, but with significantly down regulated DEGs under drought stress. These results were coherent with the significant enrichment of CC category with DEGs related to the photosystem and chloroplast components (thylakoid, stroma and plastid envelope), which were found to be significantly down regulated. MF category was

significantly enriched with DEGs belonging to chlorophyll binding, carbon-carbon lyaseantioxidant, ribulose-biphosphate carboxylase, ATP-dependent peptidase, and carboxy-lyase activities(Figure 5.10). However, these DEGs were down regulated (Figures 5.11 and 5.12). These results were coherent with the low chlorophyll content of the Atlas cultivar under drought stress conditions.

The most significantly enriched GO terms in the DEGs of ICARDA cultivar under control and stress conditions (ICA_D_vc_ICA_C), were found to be lipid transport and localisation as BP category (Figure 5.10). A high number of DEGs associated with lipid transport and localisation were found to be significantly up-regulated (Figures 5.11 and 5.12). In CC, DEGs related to the apoplast, cell wall, external encapsulating structure, and lipid droplet were found to be up-regulated, while photosystem, endoplasmic reticulum membrane and sub-compartment, and signal peptidase complex were down regulated (Figures 5.11 and 5.12). Based on these results, ICARDA has prioritised the production of storage lipids and strengthening the fluidity of its cell wall over protein maturation, in order to maintain its survival and strengthen its adaptation to drought. The cell wall and external encapsulation structure surround the whole plant cell, while the apoplast is the space outside the plasma membrane that plays an important role in the dissemination of solvents throughout the plant tissue. Lipid droplets (LDs) are neutral lipid storage organelles in the cytosol (oleosomes or spherosomes or oil bodies) and in the plastids (plastoglobules) (Bréhélin et al. 2007) and are known to be mostly triacylglycerols (TAGs) and sterol esters. TAGs are the densest energetic molecules and their degradation into sucrose provides the energy required for the seedling stage. TAGs conversion begins at the end of germination stage (Eastmond et al., 2000; Graham, 2008). The presence of LDs in stomata of *Arabidopsis thaliana* highlights their role in regulation of stomatal opening, and especially under heat stress (Aubert et al. 2010; Ge et al. 2022) by supplying energy from TAGs breakdown and the β-oxidation pathway (McLachlan et al. 2016). Previous studies have reported that TAGs accumulation in plant tissues occurs during heat, cold, salt, high light and drought stresses (Higashi and Saito, 2019; Bouchnak et al. 2023). It has been reported that TAGs reached a high amount 6h after *Arabidopsis* leaves were exposed to heat stress, but no change in total fatty acid content was noted (Mueller et al., 2015; Yurchenko et al., 2018), indicating that the heat stress is the strongest inducer of TAGs (Mueller et al., 2015). The increase of LDs number in leaves paralleled those of TAGs under abiotic stress Doner et al., 2021). Abiotic stress such as drought, heat or cold cause membrane lipid peroxidation or membrane deformations that activate several metabolic pathways and up-regulate the

expression of lipid membrane and LDs associated genes to rapidly remodel the membrane (Li al. 2015), as observed in ICARDA cultivars. Drought stress appers to have affected the fluidity of ICARDA lipid membrane, inducing overexpression of genes associated with cell wall (apoplast, cell wall, external encapsulating structure, and lipid droplet), the product of which contributes to lipid membrane stabilisation, cell homeostasis regulation, and plant energy enhancement (Bouchnak et al. 2023). Sgnificant level of enrichment of MF category with DEGs belong to binding chitinase activity, carbohydrate transmembrane transporter activity, oxidoreductase activity, glutathione transferase activity, and symporter activity. All these later were down regulated except oxidoreductase activity (Figures 5.10, 5.11, and and 5.12). Under drought stress, plants may prioritise certain metabolisms necessary for survival over others. This shift may include over-expression of certain genes whose products are required for vital function and/or adaptation (eg. oxidoreductase activity) and down-regulation of other genes or pathways, as is the case for the ICARDA cultivar. According to major categories of biological processes, cell components, molecular functions and categories of up and down expressed genes, drought stress on ICARDA had up-regulated DEGs related mainly to lipid and cell wall metabolism and oxidoreductase activity (Figure 5.11) while in Atlas had down regulated several biological processes mainly related to 225 photosynthesis.

The network and hierarchy of enriched differentially expressed genes, associated with biological process, molecular function and cellular component, are presented in more detail in a directed acyclic graph provided as Appendix 1.


ICA_D_vs_Atl_D
-log10(padj)
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1
2
3
8
14
10
9
7
7
6
28
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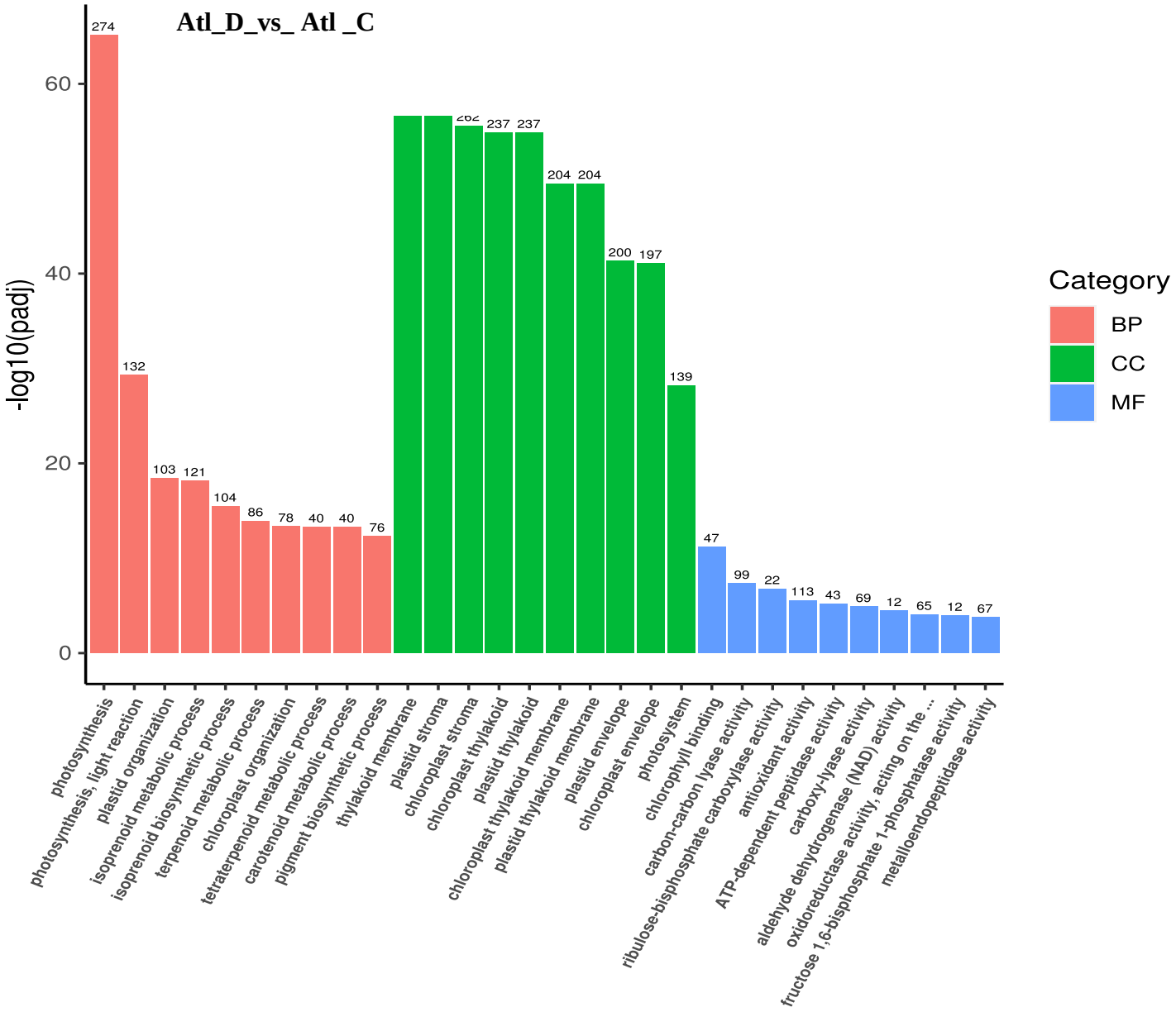
9
51
5
42
47
32
4
7
44
32
6
41
41
37
9
9
5
29
14
9
Category
BP
CC
MF
purine-containing compound catabolic process
carbon fixation
one-carbon metabolic process
photorespiration
water transport
fluid transport
neurotransmitter biosynthetic process
cellular modified amino acid metabolic process
pigment biosynthetic process
zinc ion transmembrane transport
thylakoid part
nuclear membrane
thylakoid membrane
photosynthetic membrane
vacuolar membrane
ER membrane protein complex
thylakoid lumen
vacuole
vacuolar part
lipid droplet
carboxy-lyase activity
pattern binding
polysaccharide binding
carbon-carbon lyase activity
ribulose-bisphosphate carboxylase activity
calcium activated cation channel activity
oxidoreductase activity, acting on other ...
NAD binding
oligopeptide transmembrane transporter activity
zinc ion transmembrane transporter activity


Atl_D_vs_ Atl _C
-log10(padj)
0
20
40
60
274
132
103
121
104
86
78
40
40
76
237
237
204
204
200
197
139
47
99
22
113
43
69
12
65
12
67
photosynthesis
photosynthesis, light reaction
plastid organization
isoprenoid metabolic process
isoprenoid biosynthetic process
terpenoid metabolic process
chloroplast organization
tetraterpenoid metabolic process
carotenoid metabolic process
pigment biosynthetic process
thylakoid membrane
plastid stroma
chloroplast stroma
chloroplast thylakoid
plastid thylakoid
chloroplast thylakoid membrane
plastid thylakoid membrane
plastid envelope
chloroplast envelope
photosystem
chlorophyll binding
carbon-carbon lyase activity
ribulose-bisphosphate carboxylase activity
antioxidant activity
ATP-dependent peptidase activity
carboxy-lyase activity
aldehyde dehydrogenase (NAD) activity
oxidoreductase activity, acting on the ...
fructose 1,6-bisphosphate 1-phosphatase activity
metalloendopeptidase activity
Category
BP
CC
MF

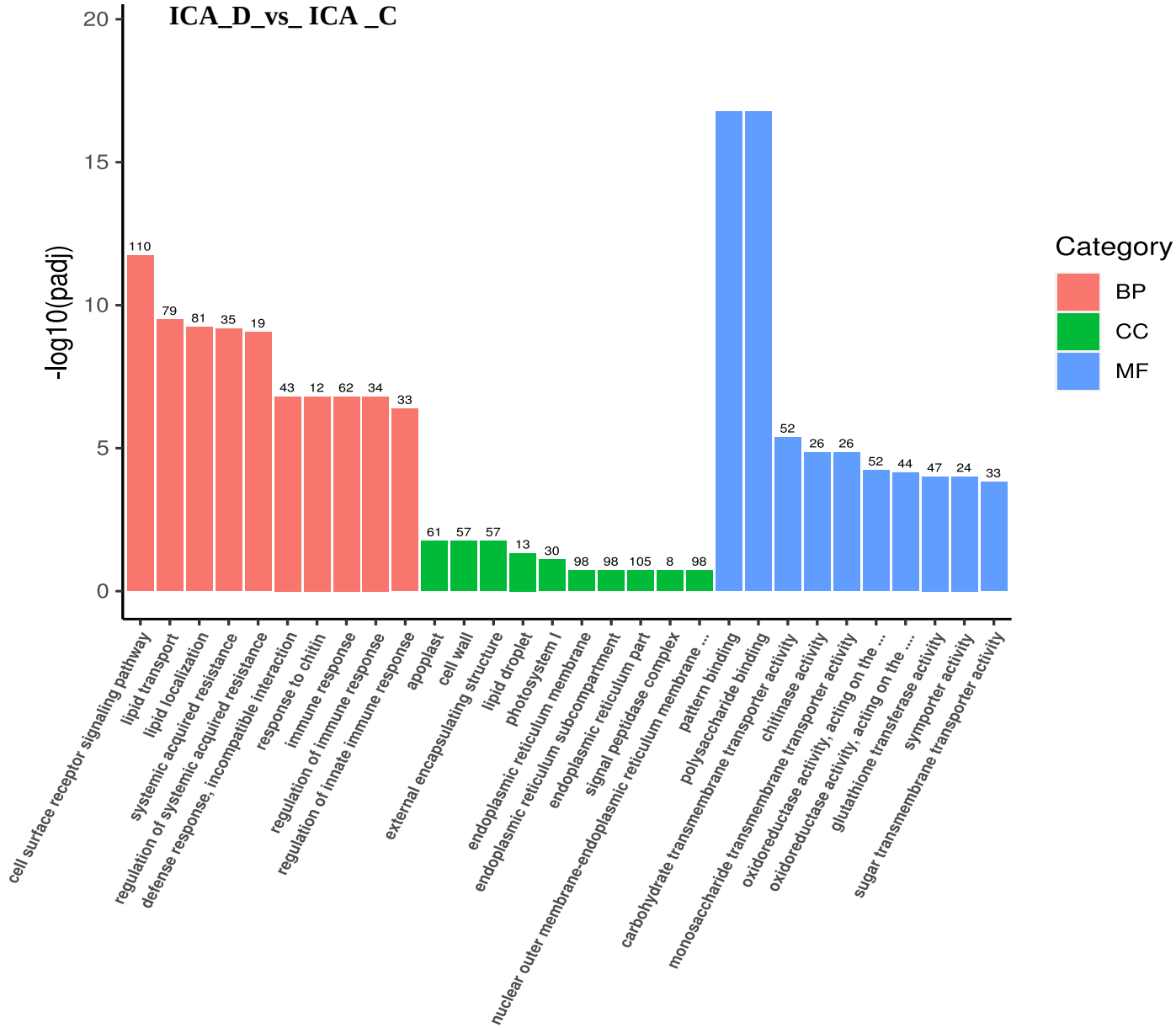
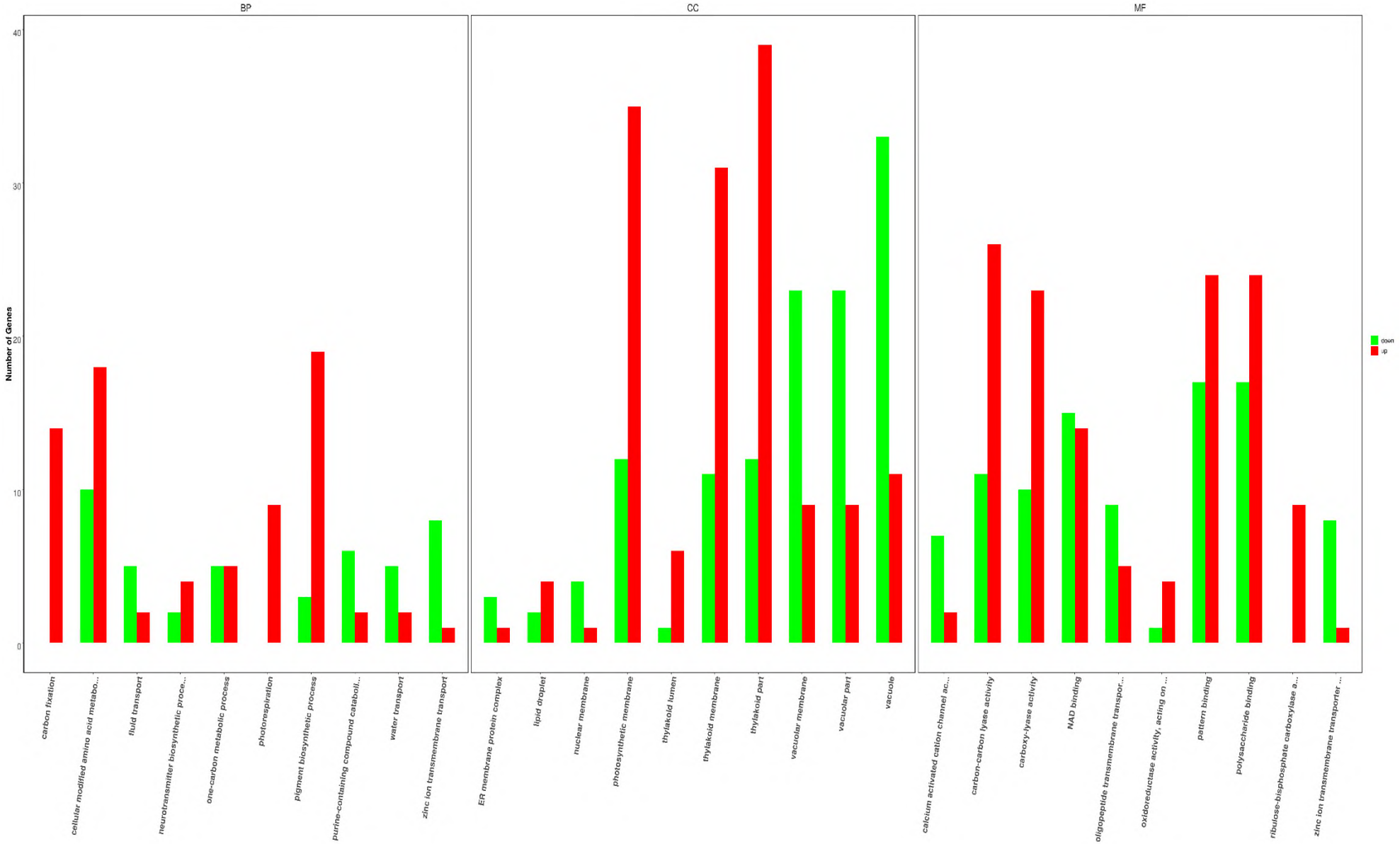
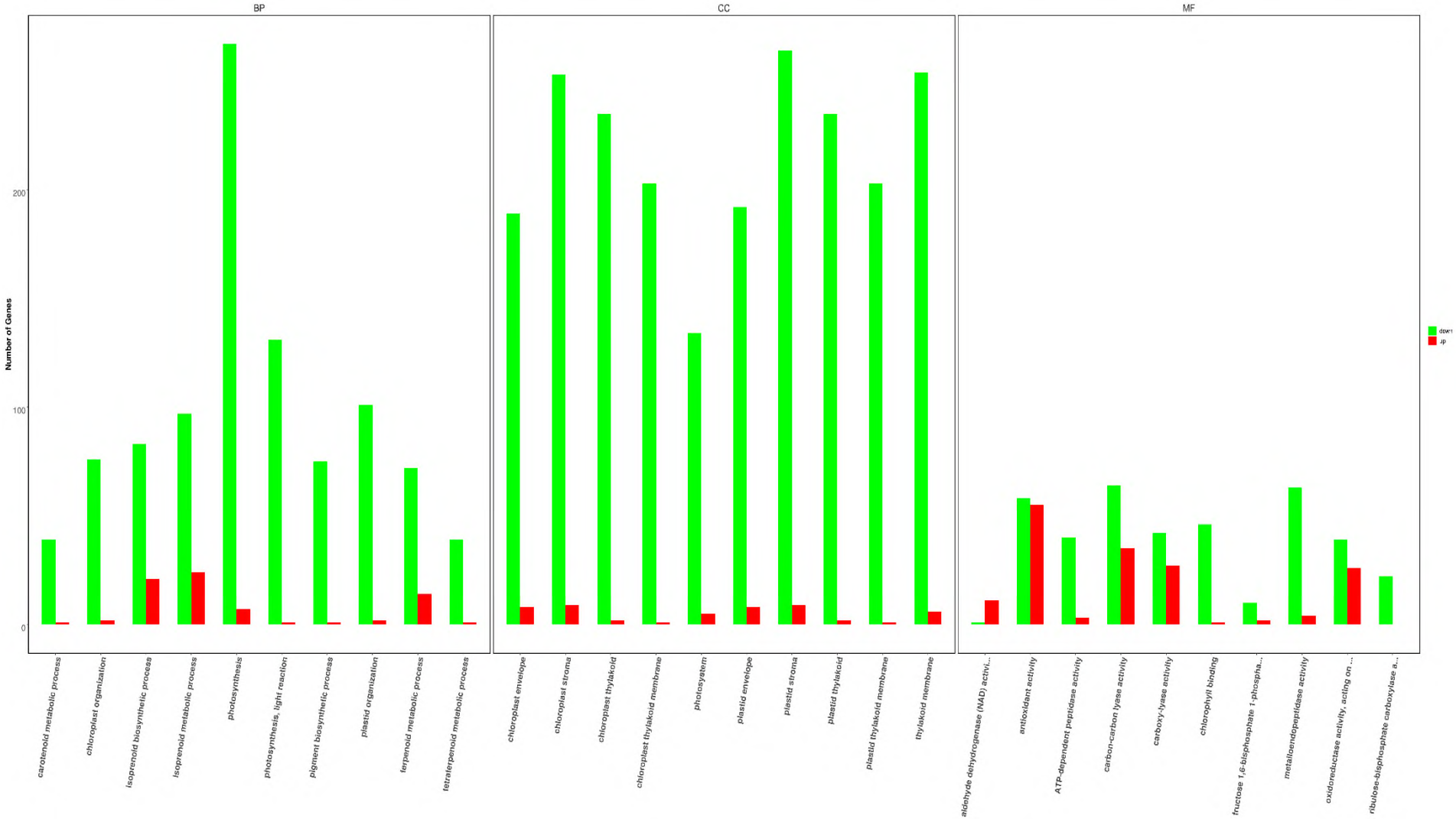

ICA_D_vs_ ICA _C
-log10(padj)
20
15
10
5
0
110
79
81
35
19
43
12
62
34
33
61
57
57
13
30
98
98
105
8
98
52
26
26
52
44
47
24
33
Category
BP
CC
MF
cell surface receptor signaling pathway
lipid transport
lipid localization
systemic acquired resistance
regulation of systemic acquired resistance
defense response, incompatible interaction
response to chitin
immune response
regulation of immune response
regulation of innate immune response
apoplast
external encapsulating structure
cell wall
lipid droplet
photosystem I
endoplasmic reticulum membrane
endoplasmic reticulum subcompartment
endoplasmic reticulum part
endoplasmic reticulum
signal peptidase complex
nuclear outer membrane-endoplasmic reticulum membrane ...
pattern binding
polysaccharide binding
carbohydrate transmembrane transporter activity
monosaccharide transmembrane transporter activity
chitinase activity
oxidoreductase activity, acting on the ...
oxidoreductase activity, acting on the ....
glutathione transferase activity
symporter activity
sugar transmembrane transporter activity

Figure 5.10. Gene ontology enrichment histogram for two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions, showing different functional categories. Four biological replicates were used. Abbreviations: C = control condition; D = drought condition; vs = versus; CC = cellular components; MF = molecular function; BP = biological processes. X axis = the most significant 30 GO Term; Y axis = level of significance.

ICA_D_vs_Atl_D


BP
CC
MF
Number of Genes
0
10
20
30
40
down
up
carbon fixation
cellular modified amino acid metabo...
fluid transport
neurotransmitter biosynthetic proce...
one-carbon metabolic process
photorespiration
pigment biosynthetic process
purine-containing compound catabol...
water transport
zinc ion transmembrane transport
ER membrane protein complex
lipid droplet
nuclear membrane
photosynthetic membrane
thylakoid lumen
thylakoid membrane
thylakoid part
vacuolar membrane
vacuolar part
vacuole
calcium activated cation channel ac...
carbon-carbon lyase activity
carboxy-lyase activity
NAD binding
oligopeptide transmembrane transpor...
oxidoreductase activity, acting on ...
pattern binding
polysaccharide binding
ribulose-bisphosphate carboxylase a...
zinc ion transmembrane transporter ...

Atl_D_vs_ Atl _C


BP
CC
MF
Number of Genes
200
100
0
down
up
carotenoid metabolic process
chloroplast organization
isoprenoid biosynthetic process
isoprenoid metabolic process
photosynthesis
photosynthesis, light reaction
pigment biosynthetic process
plastid organization
terpenoid metabolic process
tetraterpenoid metabolic process
chloroplast envelope
chloroplast stroma
chloroplast thylakoid
chloroplast thylakoid membrane
photosystem
plastid envelope
plastid stroma
plastid thylakoid
plastid thylakoid membrane
thylakoid membrane
aldehyde dehydrogenase (NAD) activi...
antioxidant activity
ATP-dependent peptidase activity
carbon-carbon lyase activity
carboxy-lyase activity
chlorophyll binding
fructose 1,6-bisphosphate 1-phospha...
metalloendopeptidase activity
oxidoreductase activity, acting on ...
ribulose-bisphosphate carboxylase a...

ICA_D_vs_ ICA _C

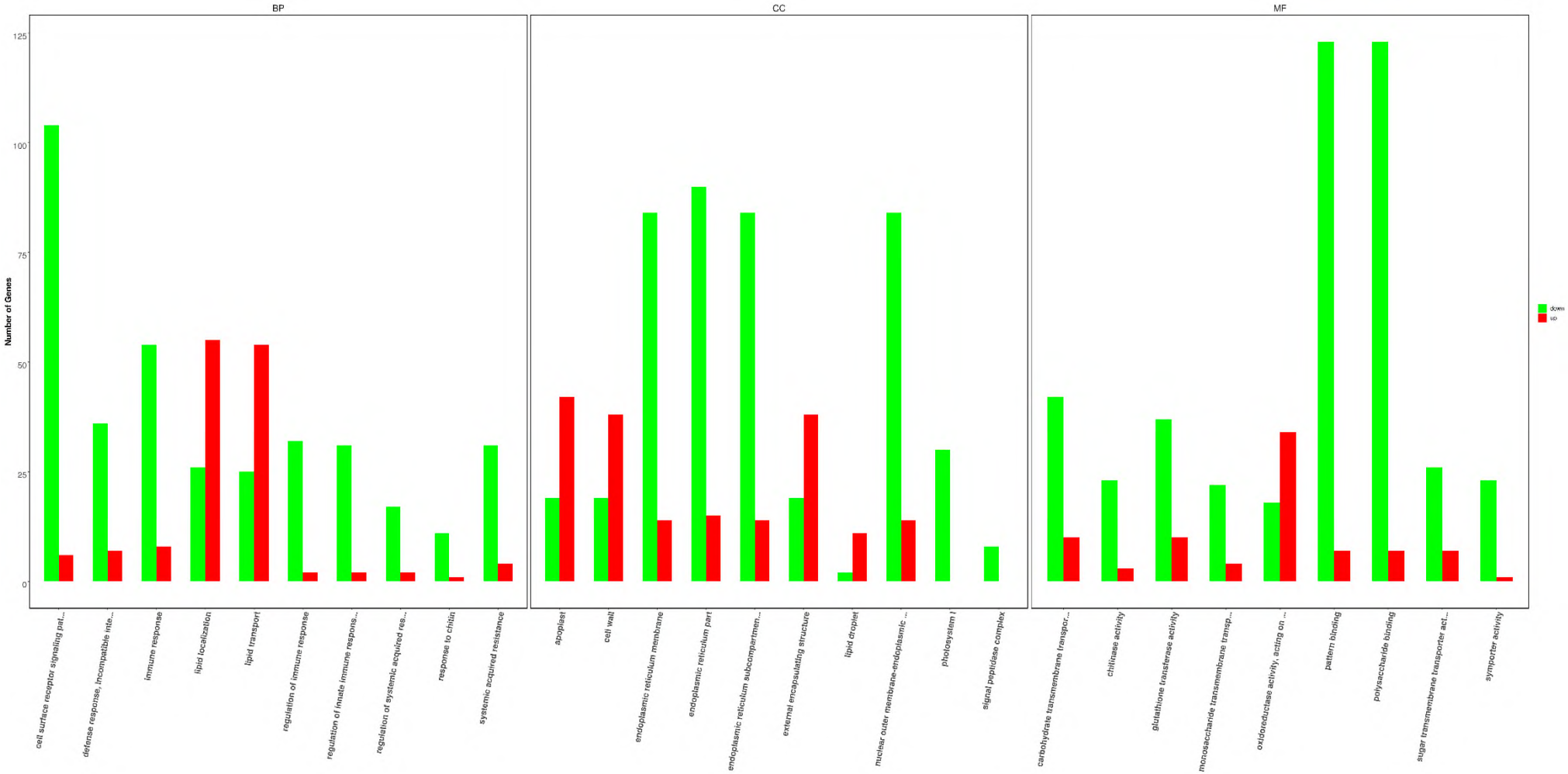
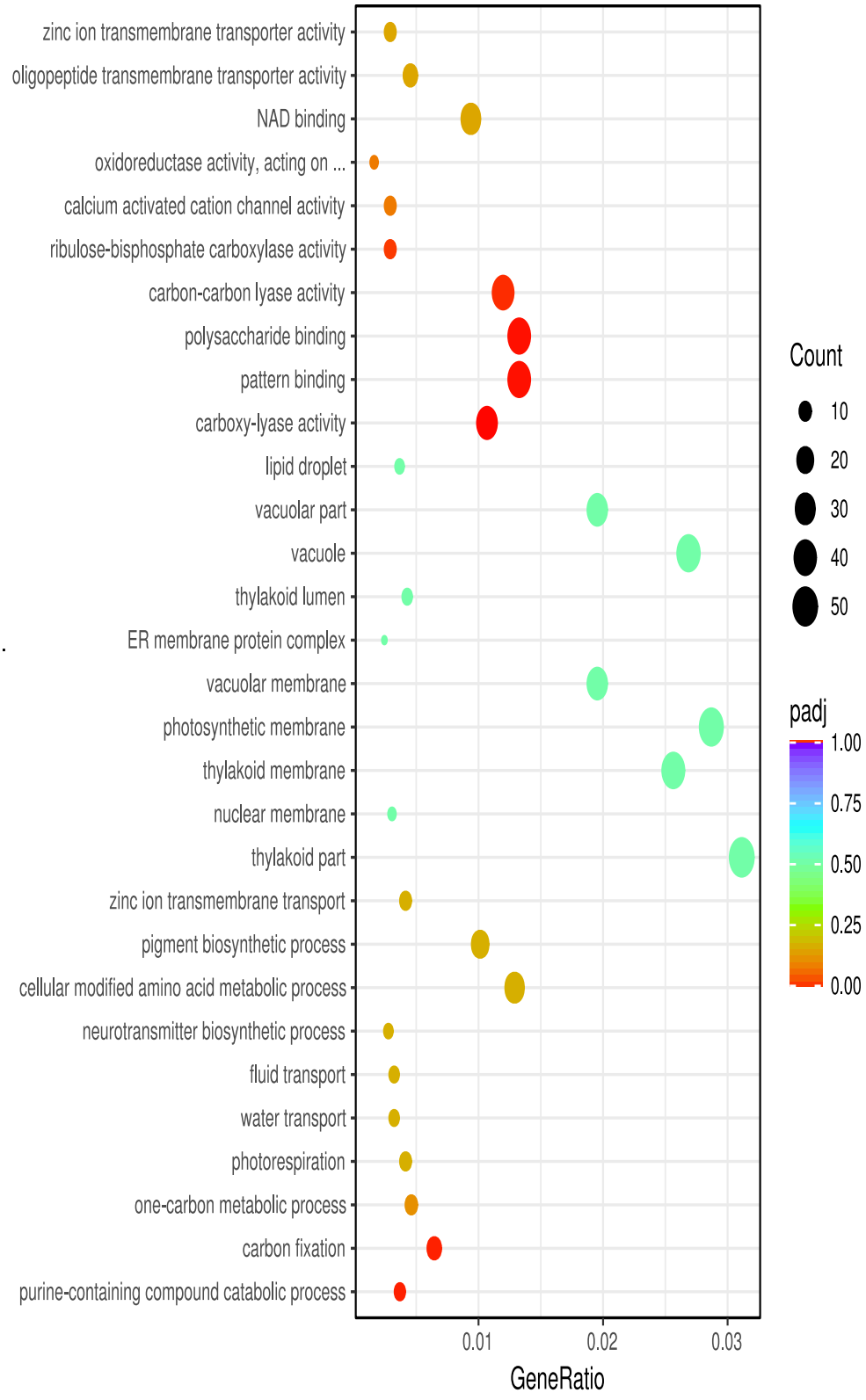

BP
CC
MF
Number of Genes
125
100
75
50
25
0
down
up
cell surface receptor signaling pat...
defense response, incompatible inte...
immune response
lipid localization
lipid transport
regulation of immune response
regulation of innate immune respons...
regulation of systemic acquired res...
response to chitin
systemic acquired resistance
apoplast
cell wall
endoplasmic reticulum membrane
endoplasmic reticulum part
endoplasmic reticulum subcompartmen...
external encapsulating structure
lipid droplet
nuclear outer membrane-endoplasmic ...
photosystem I
signal peptidase complex
carbohydrate transmembrane transpor...
chitinase activity
glutathione transferase activity
monosaccharide transmembrane transp...
oxidoreductase activity, acting on ...
pattern binding
polysaccharide binding
sugar transmembrane transporter act...
symporter activity

Figure 5.11. Gene ontology (GO) histogram for two wheat cultivars (seedling leaf tissues), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Abbreviations: C = control condition; D = drought condition; vs = versus; CC = cellular components; MF = molecular function; BP = biological processes. Green colour = down regulated genes; red colour = up-regulated genes. X axis = the most significant 30 GO Term; Y axis = level of significance.

ICA_D_vs_Atl_D


zinc ion transmembrane transporter activity
oligopeptide transmembrane transporter activity
NAD binding
oxidoreductase activity, acting on ...
calcium activated cation channel activity
ribulose-bisphosphate carboxylase activity
carbon-carbon lyase activity
polysaccharide binding
pattern binding
carboxy-lyase activity
lipid droplet
vacuolar part
vacuole
thylakoid lumen
ER membrane protein complex
vacuolar membrane
photosynthetic membrane
thylakoid membrane
nuclear membrane
thylakoid part
zinc ion transmembrane transport
pigment biosynthetic process
cellular modified amino acid metabolic process
neurotransmitter biosynthetic process
fluid transport
water transport
photorespiration
one-carbon metabolic process
carbon fixation
purine-containing compound catabolic process
0.01
0.02
0.03
GeneRatio
Count
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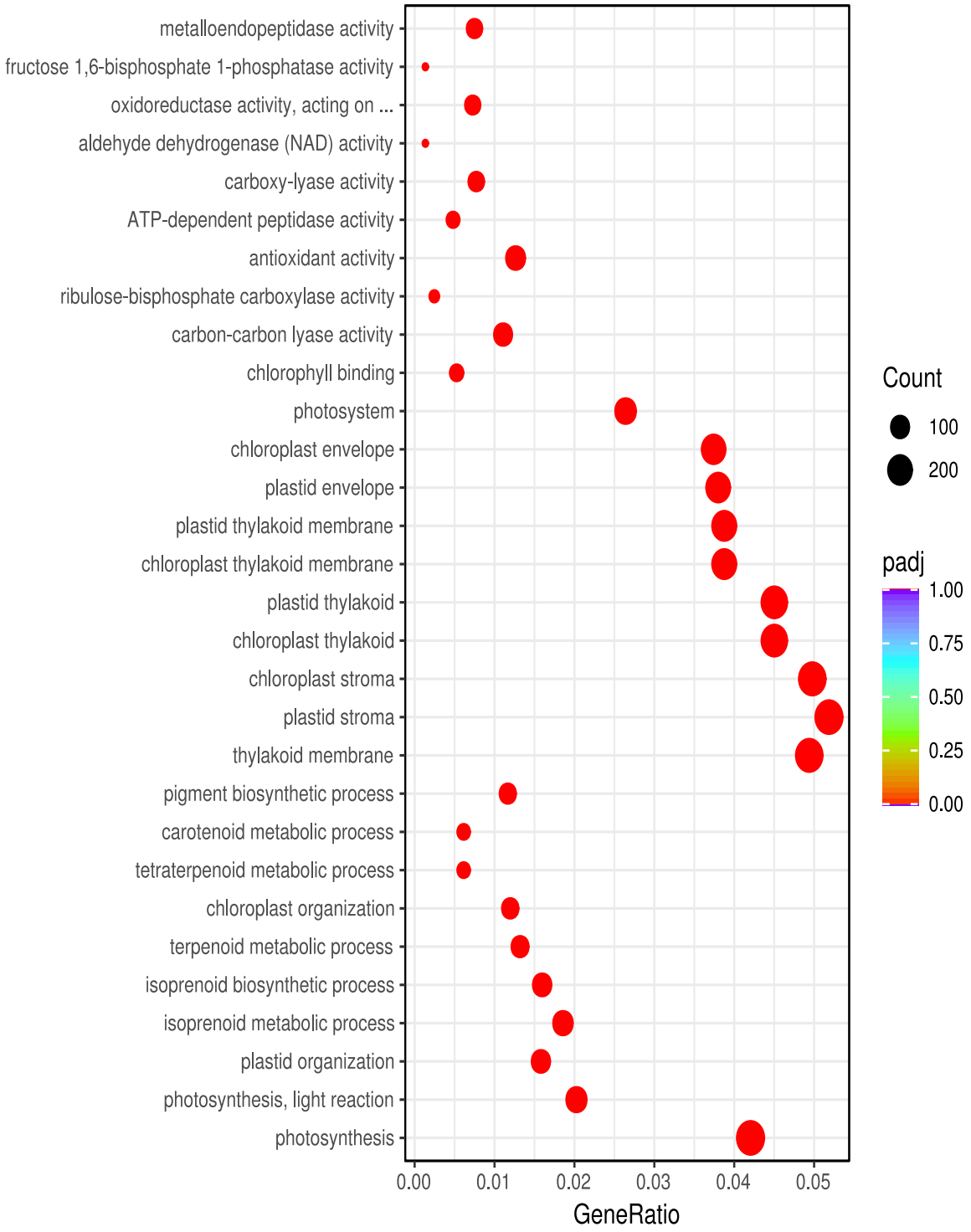
20
30
40
50
padj
1.00
0.75
0.50
0.25
0.00

Atl_D_vs_Atl_C


metalloendopeptidase activity
fructose 1,6-bisphosphate 1-phosphatase activity
oxidoreductase activity, acting on ...
aldehyde dehydrogenase (NAD) activity
carboxy-lyase activity
ATP-dependent peptidase activity
antioxidant activity
ribulose-bisphosphate carboxylase activity
carbon-carbon lyase activity
chlorophyll binding
photosystem
chloroplast envelope
plastid envelope
plastid thylakoid membrane
chloroplast thylakoid membrane
plastid thylakoid
chloroplast thylakoid
chloroplast stroma
plastid stroma
thylakoid membrane
pigment biosynthetic process
carotenoid metabolic process
tetraterpenoid metabolic process
chloroplast organization
terpenoid metabolic process
isoprenoid biosynthetic process
isoprenoid metabolic process
plastid organization
photosynthesis, light reaction
photosynthesis
0.00
0.01
0.02
0.03
0.04
0.05
GeneRatio
Count
100
200
padj
1.00
0.75
0.50
0.25
0.00

ICA_D_vs_ICA_C

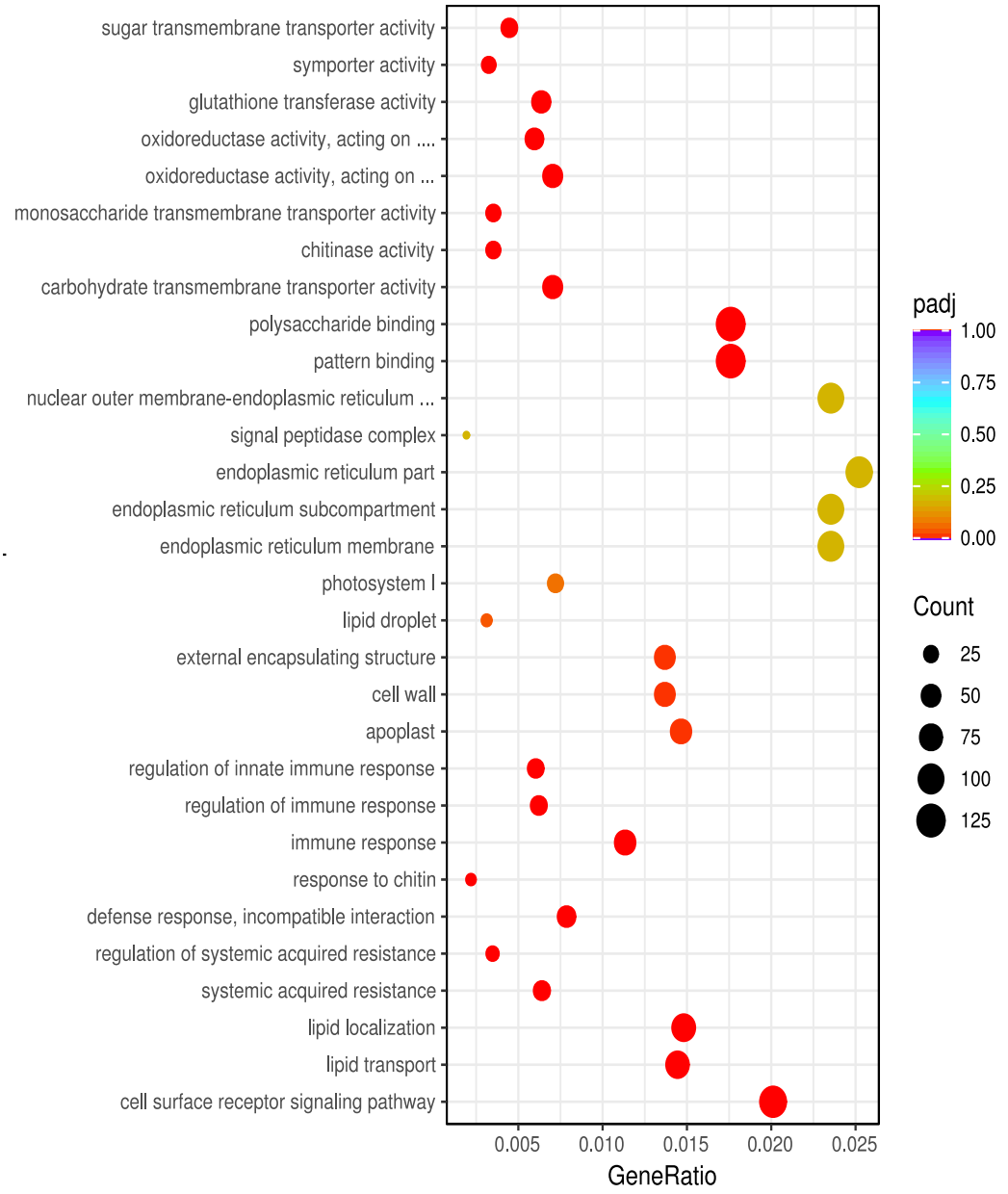


Figure 5.12. Gene ontology (GO) enrichment Scatter Plot for two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used.The size of a point represents the number of genes annotated to a specific GO Term, and the color from red to purple represents the significant level of the enrichment. C = control condition; D = drought condition; vs =

versus. X axis represents the ratio of the differential gene number to the total number of differential genes on the GO Term; Y axis represents GO Term.

5.4.5. KEGG pathway enrichment

Significantly enriched metabolic or signal transduction pathways associated with DEGs were identified using Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Kanehisa et al. 2000), comparing the whole genome background. KEGG pathways with padj < 0.05 represent significant enrichment. Comparative analysis between the KEGG pathways of Atlas and ICARDA under drought stress (Atlas_D_vs_ICA_D) showed that cysteine and methionine metabolism, monobactam biosynthesis, glutathione metabolism, glyoxylate and dicarboxylate metabolism, and porphyrin metabolism were the most significantly enriched pathways (Figure 5.13). The cysteine and methionine metabolism pathway and monobactam biosynthesis were found to have a significant level of enrichment (Figure 5.13).

The results showed that the two cultivars could be distinguished from each other on the basis of KEGG-enriched pathways, although they shared some common metabolic pathways, but with some variation in the number of genes expressed and the level of significance. Twenty-two and twenty-three pathways were significantly affected by drought in the cultivars Atlas (Atl_D_vsAt_C) and ICARDA (ICA_D_vs_IC_C), respectively.

The cultivar Atlas (Atlas_D_vs_Atlas_C) showed carbon fixation in photosynthetic organisms, photosynthesis, glyoxylate and dicarboxylate metabolism, photosynthesis — antenna proteins as the 5 most significantly enriched pathways (Figure 5.13). These results are consistent with the scatter plot data in which glycolysis and gluconeogenesis and MAPK signalling pathways also show a significant level of enrichment with the highest Gene ratio (Figure 5.13). The ratio is the number of differential genes related to the KEGG pathway to the total number of differential genes. Appendix 2a provides more details on significant KEGG enrichment. In this cultivar, most of the genes associated with the first 22 significant enrichment of the KEGG pathways were more down-regulated than up-regulated, except for those linked to galactose metabolism (70 vs 17).

However, ICARDA had the MAPK-plant signalling pathway, galactose metabolism, fatty acid elongation, amino sugar and nucleotide sugar metabolism, glycerophospholipid metabolism. These results are consistent with the scatter plot data in which phenylpropanoid biosynthesis and glutathione metabolism also show a significant level of enrichment (Figure 5.13). Appendix

2b provides more details on significant KEGG enrichment and number of up and down regulated DEGs. In this cultivar, the genes associated with galactose metabolism, fatty acids elongation, and valine, leucine, isoleucine degradation pathway were more up-regulated than down-regulated (Appendix 2b).

5.4.5.1. KEGG pathways that are enriched for up-regulated genes

In the section below, KEGG pathways that are enriched for up-regulated genes in ICARDA and Atlas are discussed in detail, though most of the studied pathways found in this chapter have overexpressed and under-expressed genes. Several metabolic pathways were impacted by the drought in the cultivars ICARDA and Atlas compared to the control group.

In ICARDA, drought has up-regulated several (i) amino acid metabolism associated with glycine, serine and threonine, valine, leucine and isoleucine degradation, tryptophan, arginine and proline, which were ranked among the top 9 significant KEGG enriched for up-regulated genes. Changes in the expression of genes related to (ii) carbohydrate metabolism associated with galactose and pyruvate metabolism, and glycolysis / gluconeogenesis pathway was also detected in ICARDA. In addition, an up-regulation of certain genes was observed in (iii) nucleic acid metabolism such as nucleotide and pyrimidine metabolism. Furthermore, (iv) lipid metabolism appears to be affected by drought as genes involved in fatty acid elongation, cutin, suberine and wax biosynthesis, fatty acid degradation was over-expressed (Table 5.3).

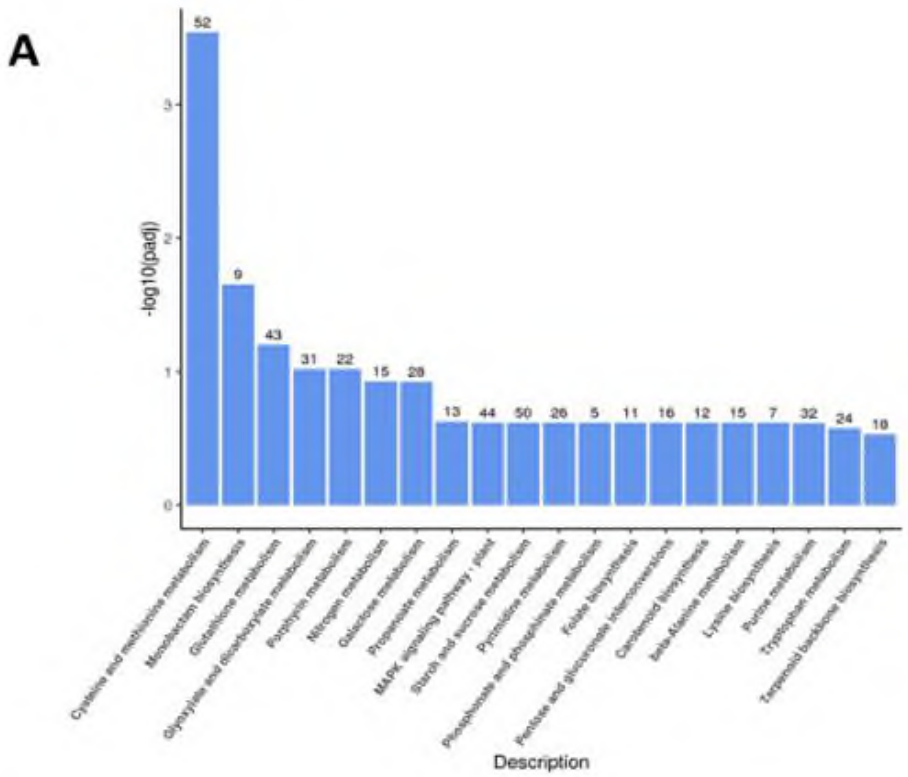
Among the top 20 KEGG pathways that show significant enrichment for up-regulated genes in Atlas are those associated with (i) amino acid metabolism, such as valine, leucine and isoleucine degradation, arginine and proline, cysteine and methionine metabolism. (ii) Carbohydrate metabolism related to galactose, N-glycan and various types of N-glycan biosynthesis, and glycolysis / gluconeogenesis metabolism. (iii) Nucleic acid metabolism, such as biosynthesis of nucleotide sugars. (iv) Lipid metabolism related to fatty acid elongation, and ether lipid metabolism (Table 5.3).

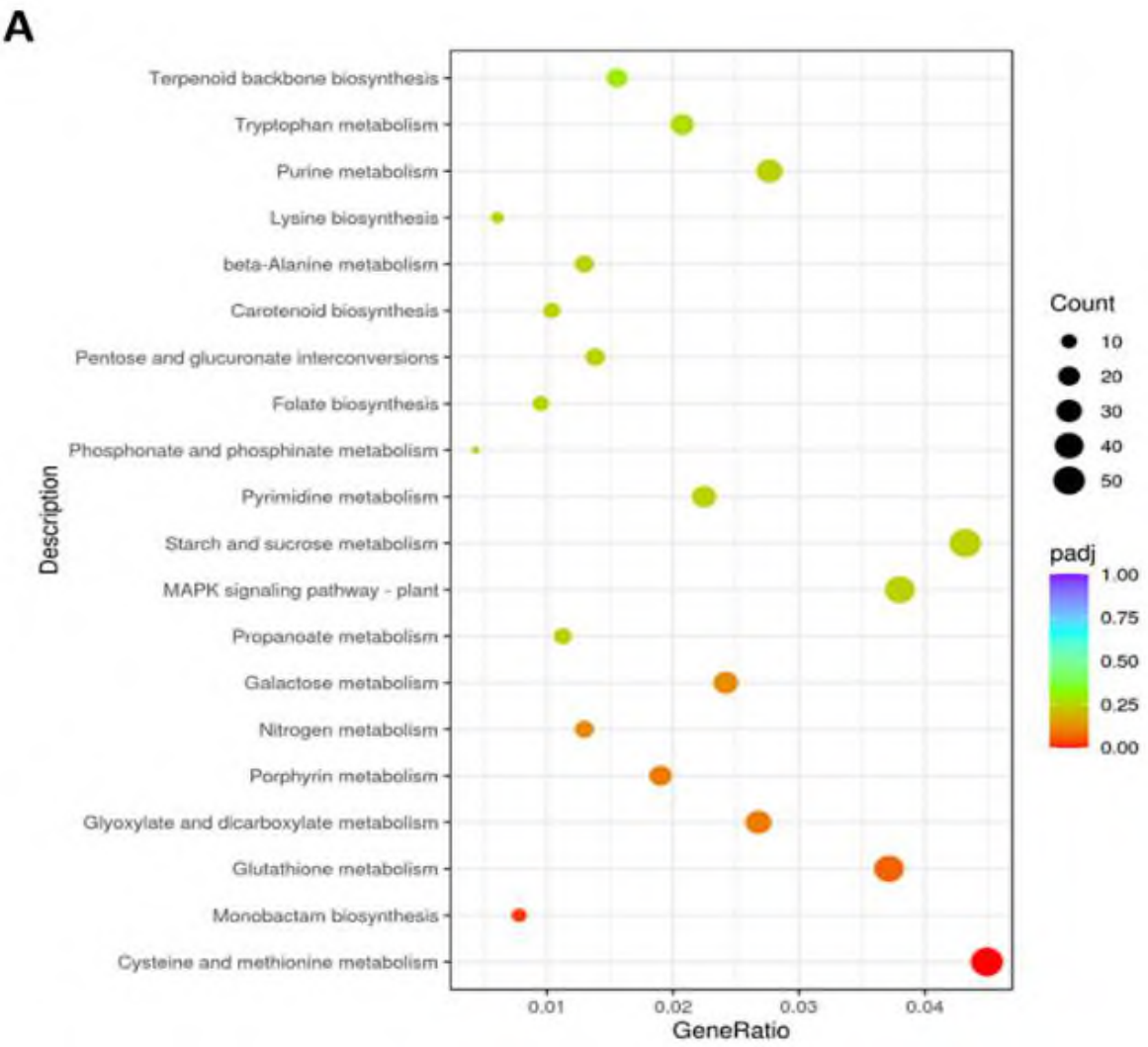
5.4.5.1.A. amino acids metabolism

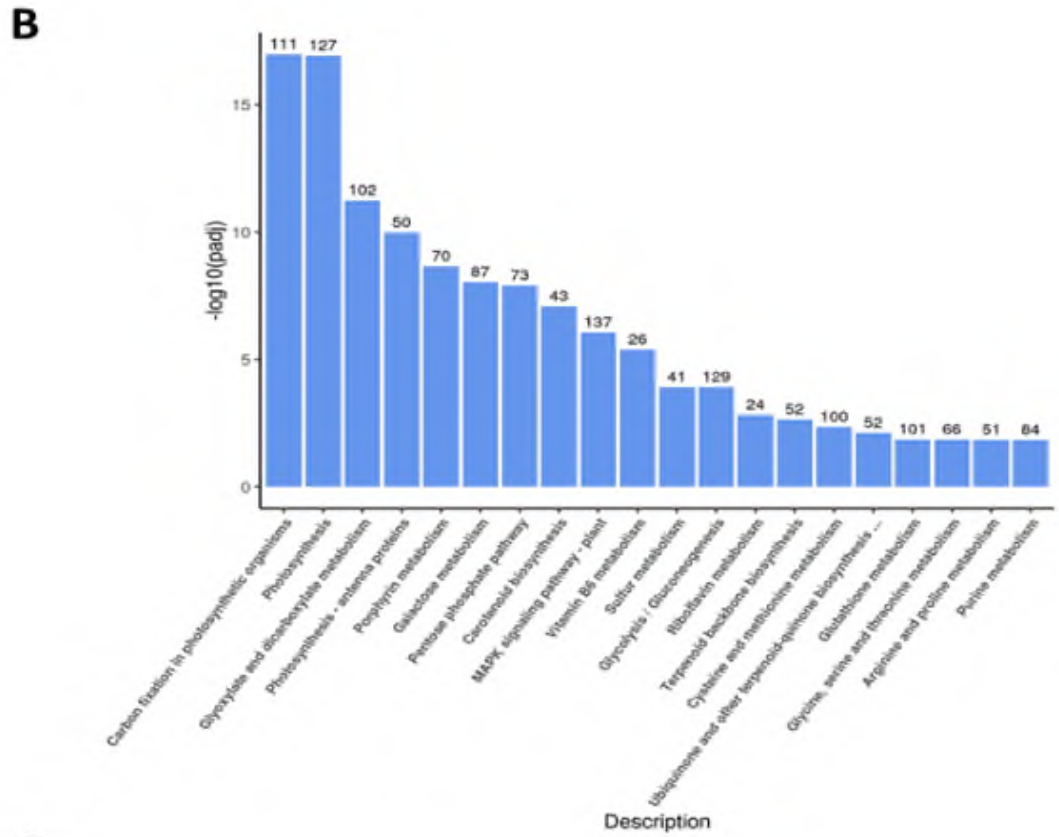
Glycine, serine and threonine metabolic pathway is involved in several plant biological processes associated with growth, development and phytohormone regulation (Muthuramalingam et al. 2018). It is a dynamic pathway that changes in response to environmental factors and the physiological state of the plant. It plays an important role in

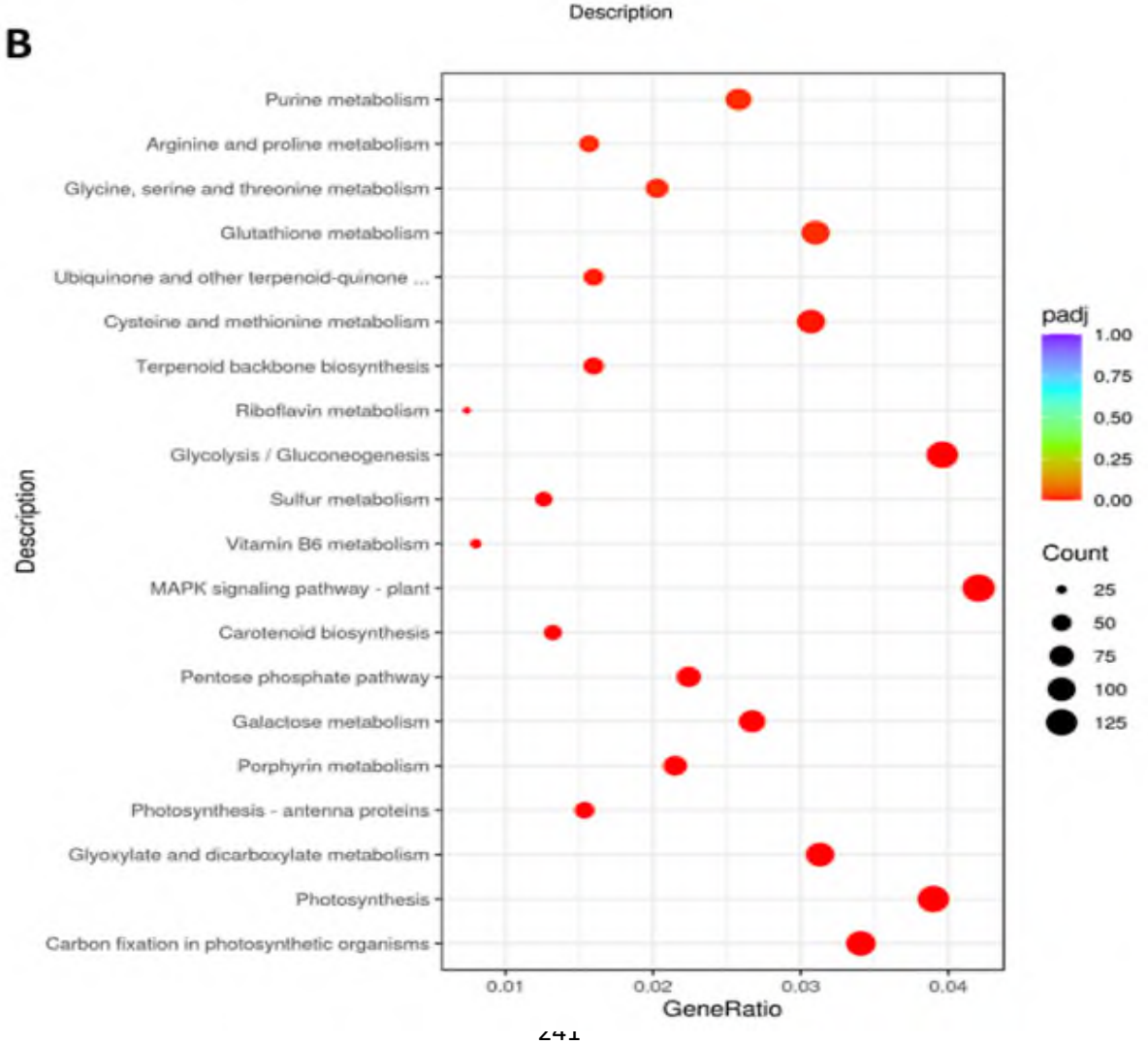
defence mechanisms against abiotic stress and is found to be connected to other amino acid metabolism such as arginine and proline, alanine, aspartate and glutamate, as well as glucolysis/gluconeogenesis, glyoxylate metabolism, glycerophosholipid metabolism among KEGG pathways, as shown in Appendix 3.

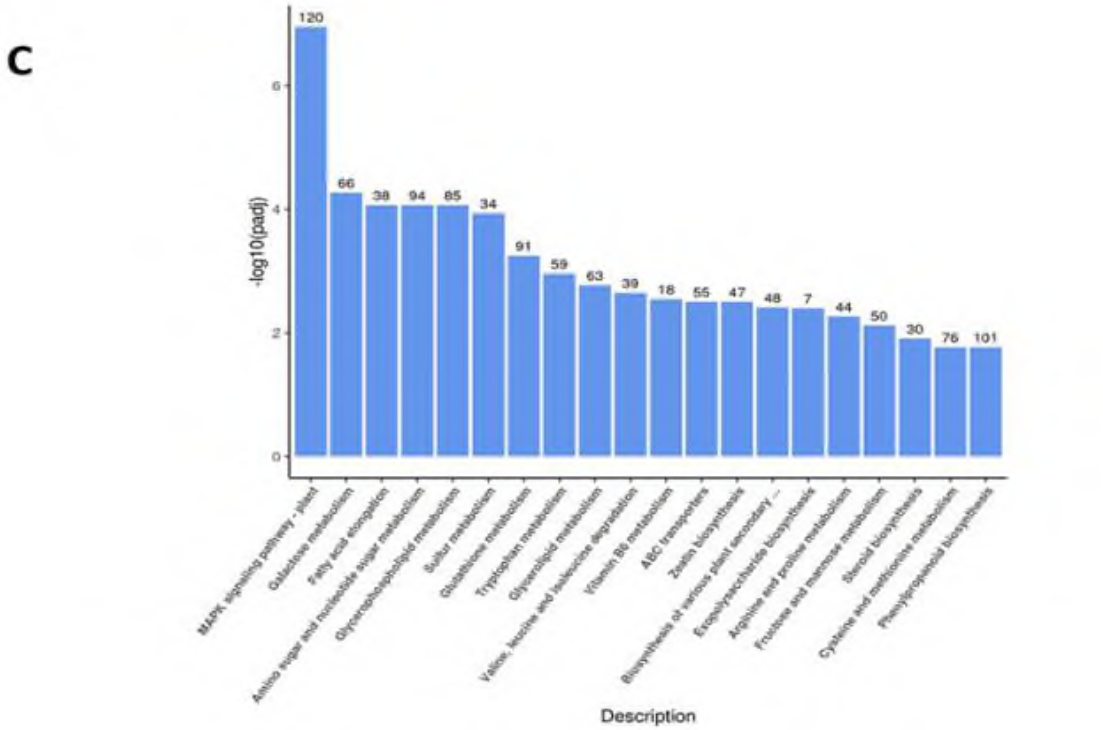
Based on this study, glycine, serine and threonine metabolism are the predominant amino acid pathways significantly affected by drought stress in ICARDA (Table 5.3) which affected 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, glycerate dehydrogenase, glyoxylate/hydroxypyruvate reductase, glutamate—glyoxylate aminotransferase, threonine aldolase, homoserine kinase, and aldehyde dehydrogenase family 7 member A1. These enzymes are involved in different metabolic pathways (Appendix 3a). In Atlas, glycine, serine and threonine metabolism pathway showed a significant enrichment for down regulated genes (Appendix 3b). These results are in line with previous studies on the response of barley, millet and wheat varieties to drought and salt stress (Liang et al. 2017; Li et al. 2021; Wang et al. 2023) reporting up-regulation of genes associated with glycine, serine and threonine metabolism under abiotic stress. Drought has been shown to induce an accumulation of photorespiratory amino acids such as glycine and serine in *Arabidospis thaliana*, barley (*Hordeum vulgare*) and rice (*Oryza sativa*) (Li et al. 2015; Templer et al. 2017) while in wheat, methionine, tryptophan, proline and cysteine are most produced under drought stress (Bowne et al. 2012; Islam et al. 2015).


A
-log10(padj)
0
1
2
3
52
9
43
31
22
15
28
13
44
50
26
5
11
16
12
15
7
32
24
18
Cysteine and methionine metabolism
Monobactam biosynthesis
Glutathione metabolism
Glyoxylate and dicarboxylate metabolism
Porphyrin metabolism
Nitrogen metabolism
Galactose metabolism
Propanoate metabolism
MAPK signaling pathway - plant
Starch and sucrose metabolism
Pyrimidine metabolism
Phosphonate and phosphinate metabolism
Folate biosynthesis
Pentose and glucuronate interconversions
Carotenoid biosynthesis
beta-Alanine metabolism
Lysine biosynthesis
Purine metabolism
Tryptophan metabolism
Terpenoid backbone biosynthesis
Description


A
Terpenoid backbone biosynthesis
Tryptophan metabolism
Purine metabolism
Lysine biosynthesis
beta-Alanine metabolism
Carotenoid biosynthesis
Pentose and glucuronate interconversions
Folate biosynthesis
Phosphonate and phosphinate metabolism
Pyrimidine metabolism
Starch and sucrose metabolism
MAPK signaling pathway - plant
Propanoate metabolism
Galactose metabolism
Nitrogen metabolism
Porphyrin metabolism
Glyoxylate and dicarboxylate metabolism
Glutathione metabolism
Monobactam biosynthesis
Cysteine and methionine metabolism
Description
0.01
0.02
0.03
0.04
GeneRatio
Count
10
20
30
40
50
padj
1.00
0.75
0.50
0.25
0.00


B
-log10(padj)
15
10
5
0
111
127
102
50
70
87
73
43
137
26
41
129
24
52
100
52
101
66
51
84
Carbon fixation in photosynthetic organisms
Photosynthesis
Glyoxylate and dicarboxylate metabolism
Photosynthesis - antenna proteins
Porphyrin metabolism
Galactose metabolism
Pentose phosphate pathway
Carotenoid biosynthesis
MAPK signaling pathway - plant
Vitamin B6 metabolism
Sulfur metabolism
Glycolysis / Gluconeogenesis
Riboflavin metabolism
Terpenoid backbone biosynthesis
Cysteine and methionine metabolism
Ubiquinone and other terpenoid-quinone biosynthesis ...
Glutathione metabolism
Glycine, serine and threonine metabolism
Arginine and proline metabolism
Purine metabolism
Description


B
Description
Purine metabolism
Arginine and proline metabolism
Glycine, serine and threonine metabolism
Glutathione metabolism
Ubiquinone and other terpenoid-quinone ...
Cysteine and methionine metabolism
Terpenoid backbone biosynthesis
Riboflavin metabolism
Glycolysis / Gluconeogenesis
Sulfur metabolism
Vitamin B6 metabolism
MAPK signaling pathway - plant
Carotenoid biosynthesis
Pentose phosphate pathway
Galactose metabolism
Porphyrin metabolism
Photosynthesis - antenna proteins
Glyoxylate and dicarboxylate metabolism
Photosynthesis
Carbon fixation in photosynthetic organisms
0.01
0.02
0.03
0.04
GeneRatio
padj
1.00
0.75
0.50
0.25
0.00
Count
25
50
75
100
125


C
-log10(padj)
0
2
4
6
120
66
38
94
85
34
91
59
63
39
18
55
47
48
7
44
50
30
76
101
MAPK signaling pathway - plant
Galactose metabolism
Fatty acid elongation
Amino sugar and nucleotide sugar metabolism
Glycerophospholipid metabolism
Sulfur metabolism
Glutathione metabolism
Tryptophan metabolism
Glycerolipid metabolism
Valine, leucine and isoleucine degradation
Vitamin B6 metabolism
ABC transporters
Zeatin biosynthesis
Biosynthesis of various plant secondary ...
Exopolysaccharide biosynthesis
Arginine and proline metabolism
Fructose and mannose metabolism
Steroid biosynthesis
Cysteine and methionine metabolism
Phenylpropanoid biosynthesis
Description

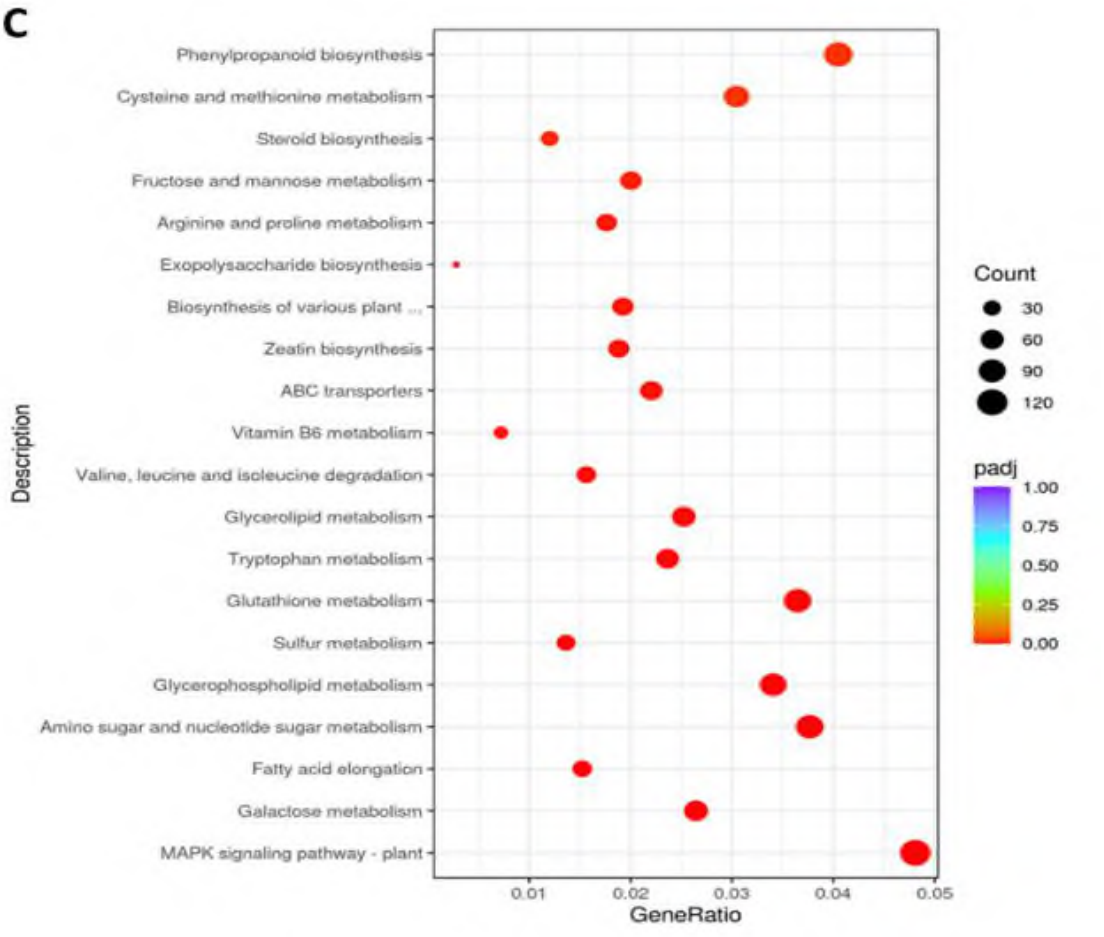

C
Phenylpropanoid biosynthesis
Cysteine and methionine metabolism
Steroid biosynthesis
Fructose and mannose metabolism
Arginine and proline metabolism
Exopolysaccharide biosynthesis
Biosynthesis of various plant ...
Zeatin biosynthesis
ABC transporters
Vitamin B6 metabolism
Valine, leucine and isoleucine degradation
Glycerolipid metabolism
Tryptophan metabolism
Glutathione metabolism
Sulfur metabolism
Glycerophospholipid metabolism
Amino sugar and nucleotide sugar metabolism
Fatty acid elongation
Galactose metabolism
MAPK signaling pathway - plant
Description
0.01
0.02
0.03
0.04
0.05
GeneRatio
Count
30
60
90
120
padj
1.00
0.75
0.50
0.25
0.00

Figure 5.13. KEGG enrichment analysis for two wheat cultivars (leaves), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used. Red colour = KEGG node including up-regulated genes; green colour = KEGG node including down regulated genes. The upper part of the figure is the KEGG enrichment analysis histogram; X axis is the most significant 20 KEGG pathways; Y axis is the significance level of the KEGG pathway enrichment. The lower part of the figure is the KEGG enrichment scatter plot showing the most significant 20 KEGG pathways; X axis is s the ratio of the number of differential genes on the KEGG pathway to the total number of differential genes; Y axis is KEGG pathway. A: Atl_D_vs_ICA_D; B: Atl_D_vs_Atl_C; C: ICA_D_vs_ICA_C (C = control condition; D = drought condition; vs = versus).

Table 5.3. KEGG pathways that are enriched for up-regulated genes for two wheat cultivars (seedling leaf tissues), Atlas (Atl) and ICARDA 32331 (ICA), grown under optimal (100-85% WFC) and drought (25% WFC) conditions. Four biological replicates were used.

KEGG_ID	Description	GeneRatio	BgRatio	pvalue	padj	Count
ICA_D_vs_ICA_C						
ath00052	Galactose metabolism	43/1080	231/15598	2,11E+05	2,53E+07	43
ath00062	Fatty acid elongation	25/1080	112/15598	1,37E+07	8,25E+08	25
ath00280	Valine, leucine and isoleucine degradation	26/1080	138/15598	2,50E+08	9,99E+09	26
ath00564	Glycerophospholipid metabolism	43/1080	331/15598	4,84E+09	0.00145227694811615	43
ath00380	Tryptophan metabolism	32/1080	227/15598	9,49E+09	0.00227710162808094	32
ath00330	Arginine and proline metabolism	26/1080	171/15598	0.000121523054343983	0.00243046108687965	26
ath00901	Indole alkaloid biosynthesis	6/1080	17/15598	0.000692320677599285	0.0118683544731306	6
ath00260	Glycine, serine and threonine metabolism	29/1080	227/15598	0.00102131929489614	0.015319789423442	29
ath00999	Biosynthesis of various plant secondary metabolites	25/1080	187/15598	0.00115908540953366	0.0154544721271155	25
ath00073	Cutin, suberine and wax biosynthesis	17/1080	113/15598	0.00193917295220056	0.0232700754264067	17
ath00071	Fatty acid degradation	22/1080	165/15598	0.0023016337599061	0.0251087319262483	22

ath00240	Pyrimidine metabolism	27/1080	225/15598	0.00366743413579093	0.0341291677331579	27
ath00440	Phosphonate and phosphinate metabolism	6/1080	23/15598	0.00394666455995676	0.0341291677331579	6
ath00620	Pyruvate metabolism	35/1080	316/15598	0.00398173623553509	0.0341291677331579	35
ath00561	Glycerolipid metabolism	29/1080	251/15598	0.00461353832044863	0.0350063382334426	29
ath00965	Betalain biosynthesis	6/1080	24/15598	0.00495882798553014	0.0350063382334426	6
ath02010	ABC transporters	26/1080	219/15598	0.0049592312497377	0.0350063382334426	26
ath00010	Glycolysis / Gluconeogenesis	45/1080	441/15598	0.00573825076704532	0.0377545470261567	45
ath01232	Nucleotide metabolism	30/1080	267/15598	0.00597780327914148	0.0377545470261567	30
Atlas_D_vs_Atlas_C						
KEGGID	Description	GeneRatio	BgRatio	pvalue	padj	Count
ath00052	Galactose metabolism	70/1471	220/15309	2,78E-10	3,37E-06	70
ath04016	MAPK 245ignaling245 pathway – plant	73/1471	425/15309	6,09E+07	3,69E+09	73
ath00330	Arginine and proline metabolism	34/1471	165/15309	1,45E+09	0.000583138055313122	34
ath00510	N-Glycan biosynthesis	30/1471	141/15309	2,38E+09	0.000648288778621006	30
ath00280	Valine, leucine and isoleucine degradation	29/1471	135/15309	2,68E+09	0.000648288778621006	29
ath00564	Glycerophospholipid metabolism	54/1471	325/15309	4,48E+09	0.000902884106165729	54

ath00940	Phenylpropanoid biosynthesis	63/1471	419/15309	0.00022082328365235	0.00381708818884777	63
ath01250	Biosynthesis of nucleotide sugars	41/1471	243/15309	0.000254326102518928	0.00384668230059878	41
ath00941	Flavonoid biosynthesis	29/1471	159/15309	0.000540139141523336	0.00726187068048041	29
ath00062	Fatty acid elongation	22/1471	109/15309	0.00060229863251663	0.00728781345345123	22
ath00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	14/1471	60/15309	0.00135515344223215	0.0149066878645537	14
ath00270	Cysteine and methionine metabolism	51/1471	348/15309	0.00149734144273992	0.0150981928809609	51
ath00010	Glycolysis / Gluconeogenesis	60/1471	431/15309	0.00213366827967414	0.01985952783389	60
ath00565	Ether lipid metabolism	16/1471	78/15309	0.00268769211305639	0.0232293389771302	16
ath00100	Steroid biosynthesis	19/1471	103/15309	0.00403247954462111	0.0325286683266103	19
ath00440	Phosphonate and phosphinate metabolism	7/1471	23/15309	0.00459351258004478	0.0347384388865887	7
ath00513	Various types of N-glycan biosynthesis	20/1471	113/15309	0.00524469019617429	0.0373298537492405	20
Atlas_D_vs_ICA_D						
KEGGID	Description	GeneRatio	BgRatio	pvalue	padj	Count
ath00860	Porphyrin metabolism	20/638	157/15206	9,33E+08	0.00109103844567443	20
ath00710	Carbon fixation in photosynthetic organisms	22/638	231/15206	0.000295798540994884	0.0173042146482007	22

ath00480	Glutathione metabolism	29/638	354/15206	0.000468260869520934	0.0182621739113164	29

Abbreviations: C = control condition; D = drought condition; vs = versus; GeneRatio = ratio between the number of differentially expressed genes in each GO term and all differentially expressed genes that can be found in GO database; BgRatio = ratio of all genes concerning this GO term to all genes; pvalue: Statistics category term; abbreviation for probability value; padj: Adjusted p-value. Generally, GO Terms with Corrected_pValue < 0.05 are significant enrichment; Count: The number of difference gene annotated to GO number.

The metabolic pathway of arginine and proline was significantly affected by drought in the cultivar ICARDA. Proline is used as a biomarker for drought stress because of its osmoprotective role. Up-regulation of several genes was detected in this pathway (Table 5.3), which is also connected to other amino acid metabolic pathways such as alanine, aspartate and glutamate, B-alanine, cysteine and methionine (Appendix 4a). Four over-expressed enzymes whose genes do not match those of the *Triticum aestivium* genome were found to be associated with delta-1-pyrroline-5-carboxylate synthetase [EC:2.7.2.11; 1.2.1.41], N-carbamoylputrescine amidase [EC:3.5.1.53], 1-pyrroline-5-carboxylate dehydrogenase [EC:1.2.1.88], and nitric-oxide synthase [NOA1, EC:1.14.13.39]. These results were based on a comparison of the gene sequences with the KEGG database. All these genes are involved in the biosynthesis of amino acids such as proline, arginine, alanine, and aspartate and glutamate metabolism. Interestingly, nitric-oxide synthase is known for its beneficial role for plant by conferring certain resistance to abiotic and biotic stress against pathogen infection (Shi et al. 2012). However, in Atlas, the gene products nitric-oxide synthase (NOA1) and arginine decarboxylase (ADC1-2) were down regulated (Appendix 4b). Up-regulation of genes associated with proline and arginine biosynthesis is common in wheat cultivars and endows some resistance to abiotic stress (drought and salt) as shown in previous studies (Xiong et al. 2017; Chaichi et al. 2019; Lv et al. 2020).

Tryptophan metabolism is often altered in the presence of drought stress (Dubouzet et al. 2007; Bowne et al. 2012; Witt et al. 2012). Tryptophan is an aromatic amino acid formed via the shikimate pathway and is a precursor of numerous secondary metabolites such as terpenoids (Korkina 2007; Less and Galili 2008). Up-regulation of the following gene products was detected: aldehyde dehydrogenase (ALDH) [EC:1.2.1.3], L-amino-aromatic acid/L-tryptophan decarboxylase [EC:4.1.1.28 4.1.1.105], acetylserotonin O-methyltransferase, plant [EC:2.1.1.4], benzaldehyde dehydrogenase (NADP+) / indole-3-acetaldehyde oxidase [EC:1.2.1.28 1.2.3.7], aromatic desulfoglucosinolate sulfotransferase [EC:2. 8.2.24], 2-oxoglutarate-dependent dioxygenase [EC:1.14.11.-], catalase [EC:1.11.1.6, and acetyl-CoA C-acetyltransferase [EC:2.3.1.9] (Appendix 5). Genes associated with indole-3-acetaldehyde oxidase and catalase are pivotal in the regulation of tryptophan biosynthesis and drought tolerance, as suggested by Li et al. (2023). Increasing amino acid levels is a normal strategy for many plants to survive stress, which reflects the ability of ICARDA to cope with drought stress. However, the tryptophan metabolic pathway has no significant enrichment in Atlas. Under drought conditions, tryptophan was found to be one of the amino acids important for

maize's response to this abiotic stress, as it strengthens resistance via flavonoid biosynthesis and the IAA and ABA 249ignaling249 pathways (Li et al. 2023). The up-regulation of tryptophan-associated genes in ICARDA reflects its drought tolerance genotype. These findings are consistent with previous studies on wheat cultivars (Chaichi et al. 2019; Luo et al. 2019).

The following gene products were enriched in the branched amino acids, valine, leucine and isoleucine metabolic pathways of ICARDA and Atlas cultivars: branched-chain amino acid aminotransferase (BCAT1-2, 3,5,7), 2-oxoisovalerate dehydrogenase E1 component subunit alpha (BCDH_beta1 and DIN4), 3-methylcrotonyl-CoA carboxylase alpha subunit (MCCA, MCCB), acetyl-CoA C-acetyltransferase (AACT1, ACAT2), 3-hydroxyisobutyryl-CoA hydrolase (CHY1), alanine-glyoxylate transaminase / I-3-amino-2-methylpropionate-pyruvate transaminase (AGT 2-3, PYD4, [EC:2.6.1.44 2.6.1.40], aldehyde dehydrogenase (ALDH) [EC:1.2.1.3] (Appendix 6a, b). Several compounds including amino acids are implicated in the osmotic adjustment of plant cells and are often over expressed under drought stress. The change in gene expression observed in this biological pathway in ICARDA, under water-limited conditions, was intended to help the plant achieve osmotic equilibrium, as revealed by Rhodes and Hanson (1993). Changes in the genes of this biological pathway have also been reported in previous studies (You et al. 2019; Hu et al. 2022; Yang et al. 2022; Wang et al. 2023).

Cysteine and methionine metabolisms were found to be significantly enriched with up-regulated genes in Atlas. The significant up-regulated DEGs are associated with branched-chain amino acid aminotransferase (BCAT1-3, 7, ATBCAT5), alanine-glyoxylate transaminase / (R)-3-amino-2-methylpropionate-pyruvate transaminase (AGT 2, 3, PYD4),

1-aminocyclopropane-1-carboxylate synthase (1/2/6 ACS1-2, 4-9, 11). The gene product, aminocyclopropanecarboxylate oxidase (EFE, ACO1-2), involved in the conversion of 1-aminocyclopropoane-1-carboxylate to ethylene was overexpressed, as well as the methionine-gamma-lyase (MGL) responsible for transforming L-methionine to methanethiol (Appendix 7). Cysteine and methionine metabolism were found to be activated in the durum-tolerant genotype (DBA Aurora), and in barley seedlings and chickpea subjected to drought stress (Kudapa et al. 2018; Liu et al. 2020; Xiong et al. 2023). However, this metabolic pathway was not significantly enriched in ICARDA.

5.4.5.1.B. Glycolysis and gluconeogenesis pathways

Drought also enhanced sugar metabolism in ICARDA and Atlas, mainly linked to galactose and the glycolysis and gluconeogenesis pathways. Accumulation of nutrients such as amino acids and sugars improves plant tolerance to drought stress by aiding osmotic balance and improving membrane stability and ROS detoxification capacity (Iqbal et al. 2011; Loutfy et al. 2012; Marcin′ska et al. 2013, Guo et al. 2018). Changes in the galactose metabolism was manifested by over-expression of alpha-galactosidase [EC:3.2.1.22, beta-galactosidase [EC:3.2.1.23], inositol 3-alpha-galactosyltransferase [EC:2. 4.1.123], hexokinase [EC:2.7.1.1, raffinose synthase [EC:2.4.1.82], beta-fructofuranosidase [EC:3.2.1.26], alpha-glucosidase [EC:3.2.1.20] and 6-phosphofructokinase 1 [EC:2.7.1.11] (Appendix 8). These results are consistent with previous study on wheat response to salt stress showing that galactose metabolism was one of the most significantly regulated pathways in roots and shoots under salt-stress (Alyahya and Taybi 2023).

The glycolysis and gluconeogenesis pathways are very important for the pl’nt's development and acclimation to external stress. Glycolysis supplies energy to the plant by converting glucose into pyruvate. The products of glycolysis are the precursors of the pl’nt's primary metabolites, while gluconeogenesis is considered to be an interface between the metabolism of organic acids, amino acids, lipids and sugars (Walker et al. 2021). Changes in glycolysis and gluconeogenesis pathways have been recorded in several plants subjected to abiotic stress (Zhu et al. 2019). In the present study, key enzymes involved in glycolysis were found to be up-regulated in ICARDA and Altas varieties under drought stress, including phosphoglycerate mutase. These results are consistent with the previous study by Shu et al. (2011) on rice seedlings. The following gene products were enriched and over-expressed in the glycolysis and gluconeogenesis pathway in response to drought stress: hexokinase [EC:2.7.1.1], glucose-6-phosphate 1-epimerase [EC:5.1.3.15], fructose-1,6-bisphosphatase [EC:3.1.3.11], diphosphate-dependent phosphofructokinase [EC:2.7.1.90], fructose-bisphosphate aldolase, class I [EC:4.1.2.13], glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) [EC:1.2.1.12], phosphoglycerate kinase [EC:2.7.2.3, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase [EC:5.4.2.12], pyruvate kinase [EC:2.7.1.40, pyruvate dehydrogenase E1 component subunit alpha [EC:1.2.4.1, S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase [EC:1.1.1.284 1.1.1.1], aldehyde dehydrogenase (ALDH) [EC:1.2.1.3]. As shown in Appendix 9, the acetyl CoA produced as a result of the glycolysis process is used by the tricarboxylic acid cycle and, consequently, more ATP is generated, enabling the plant to

combat drought stress (Shu et al. 2011). The significant regulation of glycolysis and gluconeogenesis pathways in current cultivars is consistent with previous transcriptomic studies in wheat, soybean and *Medicago falcata* (Zhang et al. 2021; Wang et al, 2022; Li et al. 2022).

5.4.5.1.C. N-glycan metabolism

The pathway of N-glycan biosynthesis showed a significant enrichment with up-regulated genes in Atlas. Glycan has an important function in protein folding and is considered a signal molecule in the stress response, in addition to its role as the pl’nt's metabolic energy (Strasser 2006). Compared to the control group, several genes were up-regulated under drought stress in Altas. These genes encode for: beta-1,4-N-acetylglucosaminyl transferase ,dolichol-phosphate mannosyltransferase (DPMS1), alpha-1,3-mannosyltransferase (ALG3), ALG6, ALG12, ALG8, dolichyl-diphosphooligosaccharide---protein glycosyltransferase subunit MAGT1/TUSC3 (OST), dolichyl-diphosphooligosaccharide---protein glycosyltransferase (STT), mannosyl-oligosaccharide glucosidase (GCS1), mannosyl-oligosaccharide alpha-1,3-glucosidase (RSW3), alpha-1,3-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (CGL1), beta-1,4-mannosyl-glycoprotein beta-1,4-N-acetylglucosaminyltransferase (Appendix 23a). Beta-1,4-N-acetylglucosaminyl transferase is the key enzyme for N-glycan complex formation (Seifert et al. 2021). These results are consistent with previous studies (which showed that N-glycan metabolism was activated in sorghum, onion and banana under drought stress Fracasso et al. 2016; Muthusamy et al. 2016; Ghodke et al. 2020). However, this pathway was enriched by down-regulated gene in ICARDA (Appendix 10b).

5.4.5.1.D. Biosynthesis of cutin, suberin and wax and fatty acid elongation pathways

Cutin and wax are components of the cuticle covering the leaves and stems of plants, which is in direct contact with the environment. Cutin is made up of hydroxy and epoxy-hydroxy fatty acids with a chain length of C16-18 carbons, while wax, the intracuticular and epicuticular layer above cutin, is made up of a very long chain of fatty acids and derivatives (Jeffree 1996; Nawrath 2002). The cuticle is known for its role in regulating gas exchange and the absorption of non-volatile products, acting as a barrier for water and solutes, and protecting against abiotic (irradiation) and biotic (e.g. pathogens, herbivores) damage (Kerstiens 1996; Schönherr and Baur 1996; Grace and Gardingen 1996; Kerstiens 1996). While the wax layer plays an important role in plant-insect interaction (Eigenbrode 1996), cutin is more important for the

plant-fungus association (Kerstiens 1996). Suberine is a heteropolymer present in plant tissues in the form of lamellae and protects the plant from loss of water and nutrients in the event of abiotic stress. A low relative water content was obtained in suberine-deficient mutant *Arabidopsis thaliana* leaves (de Silva et al. 2021), demonstrating the role of suberine in protecting the plant against dehydration. In addition, drought induces an increase in suberin biosynthesis to prevent the plant from releasing nutrients. This phenomenon has also been detected in the presence of osmotic and salt stress (Kreszies et al. 2019; Barberon et al. 2016, Kim et al. 2022). Based on RNA-Seq data, drought stress on ICARDA had induced changes in the cutin, suberin and wax biosynthesis pathway. Several genes involved in the biosynthesis of unsaturated fatty acids were up-regulated and associated with the following enzymes (RWP1, FACT), (CLO4, RD20, ATS1, ATPXG2). The products of these genes are involved in the synthesis of 16-feruloyloxypalmitic acid, 9, 10, 18-trihydroxystearate and polyhydroxy α, ω dicarboxylic acid, which are important in cutin and suberin biosynthesis. For wax synthesis, there was overexpression of the CER1-associated gene involved in a long-chain dike tone and the gene encoding MS2 and FAR 1, 4 5, 6, 7 and 8 for the construction of a long-chain wax ester (Appendix 11a). These findings are coherent with the changes observed in the enriched fatty acid elongation pathway in the endoplasmic reticulum, where the fatty acid has a long chain of carbon atom >16 (C20-34) (Kunst and Samuels 2009). Three out of five serial enzymes for the fatty acids elongation process were found to be up-regulated in ICARDA: 3-ketoacyl-CoA synthetases (KCS), 17 beta-estradiol 17-dehydrogenase / very-long-chain 3-oxoacyl-CoA reductase, and very-long-chain enoyl-CoA reductase (Appendix 11a). The results are in line with the high RWC found in ICARDA, reflecting its ability to adjust its physiology to be more tolerant to drought stress. Overexpression of cutin and wax helps the plant to protect its leaves from overheating and reduces the amount of damaging UV light that penetrates and affects deeper areas of the leaf, resulting in reduced water loss through transpiration (Mittler 2006; Shepherd and Griffiths 2006; Bandurska et al. 2013). However, cutin, suberin and wax biosynthesis pathway has no significant enrichment in Atlas unlike the fatty acid elongation pathway which was enriched for up regulated genes, like ICARDA (Appendix 11b). These results are in concordance with the up- regulated expression of genes associated with cutin, suberin and wax biosynthesis in barley, cotton, chieh-qua and wheat under drought stress (Wang et al. 2019; Luan et al. 2017; Yang et al. 2022; Shellakkutti et al. 2022; Zheng et al. 2021; Wen et al. 2021).

5.4.5.1.E. Nucleic acid metabolism

Under drought stress conditions, nucleic acid metabolism including purine and pyrimidine, and nucleotide sugars were significantly enriched by up-regulated genes in ICARDA and Atlas, respectively. In the purine and pyrimidine metabolic pathways, up-regulation of several genes was detected in ICARDA. Among the up-regulated genes in the purine pathway, the gene encode for allantoinase (ALN) involved in the conversion of allantoate to S-allantoin was overexpressed, while the allantoin metabolism pathway was not enriched. This response to drought stress has also been reported in rice, where treatment with exogenous allantoin enhanced drought resistance (Lu et al. 2022). However, in Atlas, these metabolic pathways were enriched with down-regulated genes while the biosynthesis of nucleotide sugar was enriched with up-regulated gene. Drought induction of purine and pyrimidine has previously been reported in other drought-tolerant plants such as rice and wheat (Bhoite et al. 2021; Lu et al. 2022).

5.4.5.1.F. Biosynthesis of various secondary plant metabolites

Secondary metabolites are produced in response to environmental factors and provide the organism with a degree of resistance to biotic and abiotic stresses. Based on transcriptomic data of the ICARDA cultivar, the podophyllotoxin biosynthesis pathway was enriched. Podophyllotoxin (PPT) is a non-alkaloid lignin toxin derived from the root of *Podophyllum* that are anticancer, antiviral and vesicant compounds (Damayanthi and Lown 1998; Gordaliza et al. 2004; Xu et al. 2009). The following gene products, involved in podophyllotoxin biosynthesis, were up-regulated: nicotianamine synthase [EC:2.5.1.43]’’3"-deamin’’3"-oxonicotianamine reductase [EC:1.1.1.285], beta-glucosidase [EC:3.2.1.21, pinoresinol/lariciresinol reductase [EC:1.23.1.1 1.23.1.2 1.23.1.3 1.23.1.4] (Appendix 13a). These results are consistent with a previous study (Kumari et al. 2022). PPT was overexpressed in *Sinopodophyllum hexandrum*, a medicinal plant, under drought conditions, along with up-regulation of key genes in the phenylpropanoid metabolic pathway (Kumari et al. 2022). However, change in the expression of the genes associated with phenylpropanoid metabolic pathway was noted in ICARDA, though it has not significant enrichment (Appendix 13b), unlike Atlas cultivars (Appendix 13c). Kumari et al. (2022) indicated that increased PPT may confer some resistance to drought stress on the plant. No studies have been carried out on TPP biosynthesis in wheat. The biosynthesis of various secondary plant metabolites KEEG pathway has no significant enrichment in Atlas.

5.5. Disscussion

Drought is the major abiotic stress limiting wheat crop and quality worldwide by altering the agronomic traits of the plant (e.g. fresh biomass, grain yield, etc.) and consequently threatens the global food security (Zhang et al. 2018). Studies on the physio-morphological and biochemical features of wheat in response to drought stress at different growth stages with varying drought intensity have proven to be very useful for selecting drought tolerant genotypes. However further research studies at molecular level are needed to decipher the key molecular factors of drought tolerance in wheat, especially as the traditional breeding method had some limitations in enhancing traits (Iquebal et al. 2019). With the availability and accessibility to wheat genome sequence and transcriptomic data, several genes and transcriptional factors have been identified, such as TaNAC071-A, TaPYL1-1B, TaSNAC8-6A and TaSAP5 (Zhang et al. 2017; Mao et al. 2020, 2022). Little is known about the mechanisms of tolerance in wheat further research investigations are needed. In the present study, comparative transcriptional analysis between two cultivars Altas and ICARDA supported their classification into drought-sensitive and drought-tolerant genotypes that was based on phenotypic data (chapters 3 and 4).

The cellular component of significant DEGs associated proteins for ICARDA in response to drought stress was mainly related to the apoplast, cell wall and external encapsulating structure. These latter are associated with lipid and cell wall metabolism, oxidoreductase activity, lipid transport and signaling, which were found to be upregulated. These findings are consistent with the significant enrichment of lipid metabolic pathways, including the fatty acid elongation, cutin, suberin, and wax biosynthesis pathway. The following enzymes were found to be overexpressed in this enriched pathway: 3-ketoacyl-CoA synthetases (KCS), 17 beta-estradiol 17-dehydrogenase / very-long-chain 3-oxoacyl-CoA reductase, very-long-chain enoyl-CoA reductase, 16-feruloyloxypalmitic acid, 9, 10, 18-trihydroxystearate and polyhydroxy α, ω dicarboxylic acid. The activation of cutin and suberin, which are components of the cuticle covering the leaves and stems of plants, is in concordance with the high RWC found in ICARDA, reflecting its ability to adjust its physiology to be more tolerant to drought stress. These results agree with that of de Silva et al. (2021) who showed that suberin-deficient mutant *Arabidopsis thaliana* leaves had a low relative water content, highlighting the role of suberin in protecting the plant against dehydration. Activation of this pathway in response to drought stress has already been reported in barley, cotton, chieh-qua and wheat under drought stress (Wang et al. 2019; Luan et al. 2017; Yang et al. 2022; Shellakkutti et al. 2022; Zheng et al.

2021; Wen et al. 2021). A part from the lipid metabolism, the top 9 significant KEGG enriched pathway for up-regulated genes only in ICARDA and/or down regulated gene in Atlas were associated mainly with (i) amino acids metabolic pathway (glycine, serine and threonine, tryptophan, pathways), (ii) carbohydrate metabolisms (galactose and pyruvate metabolisms), and (iii) nucleic acid (nucleotide and pyrimidine metabolisms). Activation of these pathways in ICARDA as response to drought stress are coherent with previous studies in different plants and varieties as detailed in section II.5.1.

However, the cellular components of the proteins associated with the significant DEGs in Atlas were linked to photosystem and chloroplast components such as the thylakoid, stroma and plastid envelope. The molecular function of these DEGs are carbon-carbon lyase activity, chlorophyll fixation, antioxidant activity and ribulose-biphosphate carboxylase, and carboxy-lyase activity. The biological process of these DEGs are photosynthesis, isoprenoid metabolism, terpenoids and tetraterpenoids metabolic process, pigment and biosynthesis process, and chloroplast organisation. However, all the enriched GO terms (BP, CC, and MF) were down regulated. These data reflect the sensitivity of chloroplast components and photosynthesis activities to the drought stress in the Atlas cultivar, which is coherent with its low chlorophyll content under drought conditions (chapters 3 and 4). The transcriptomic data are consistent with the biochemical features related mainly to low relative water content, as well as with the stress susceptibility and tolerance index, which are in good agreement with the sensitivity of Atlas (chapter 4). However, the cultivar Atlas showed a high concentration of proline under drought stress which was higher than the drought tolerant cultivar ICARDA. The results of the *in vitro* test of proline for Atlas and ICARDA were coherent with the enriched proline pathway for up-regulated genes (section II.5.1.A). However, gene associated with nitric-oxide synthase (NOA1) was down regulated in Atlas. Nitric oxide is known to be plant by conferring resistance to abiotic (heat stress) and biotic stress against pathogenic infections (Shi et al. 2012; Sehar et al, 2023). Nitric oxide has been shown to regulate in wheat the level of ethylene assimilation and the antioxidant system by increasing the osmolytes under heat stress and consequently increasing photosynthesis activity, leading to more tolerance to heat stress (Sehar et al. 2023). Under abiotic stress, proline is over-expressed and accumulated in the plant to maintain cell turgidity via osmotic balance, restore cell homeostasis and redox stability, calibrate the antioxidant system and modulate cell structure and proteins (Fatma et al. 2021; Guatam et al. 2022; Iqbal et al.2022; Sehar et al, 2022a, b). This is coherent with the high level of proline and overexpression of the dehydrin-associated gene in Atlas. These proteins are

induced in wheat under abiotic stress (drought, salt, heat, cold, wounding, etc.) and their expression is linked to the ABA-dependent pathway. ABA enhances the wheat seedling, which justifies the well-developed morphology of Atlas under drought stress (Wei et al. 2015). Dehydrins belong to a large family of transcriptional factors known as Dehydration responsive element binding proteins (DREBs) which have several protective functions (Allagulova et al. 2003). These results are coherent with the significant increase in proline content in this cultivar under drought conditions (chapter 4). The accumulation of proline and dehydrins in Atlas indicates that it has undergone osmotic adjustment to adapt to water-limited conditions. Osmotic regulation is one of the most fundamental mechanisms that plants adopt to adapt and cope with water deficit (Sanders et al. 2012). However, all these protective mechanisms are not operational without chemical or gaseous signaling molecules such as phytohormones. Ethylene is one of the most important chemical messengers in plant that play an important role in improving heat tolerance by reducing oxidative stress and maintaining chlorophyll level (Wu et al. 2019). Under drought stress, ethylene is overproduced up to a concentration of 25 g/l, above which it becomes toxic to the plant and causes chlorosis, leaf abscission and senescence, following the decline of root and shoot growth (Apelbaum and Yang 1981; Singh, et al. 2015). In wheat, the amount of ethylene is doubled under severe drought stress and leads to a decrease in grain filling rate and final grain weight (Yang et al. 2006).

Nitric oxide is involved in different biological process in plants (Gautam et al, 2022; Iqbal et al. 2022) such as seed germination (Paopova et al 2010), senescence (Mir et al. 2023), fruit ripening (González-Gordo et al. 2023), it has also been found to be associated with the abiotic stress response (kaya et al. 2023; Iqbal et al. 2022). Several studies have investigated the regulatory role of nitric oxide in phytohormone associated with plant growth and stress (Sehar et al. 2022; Mir et al. 2022; Sehar et al. 2019). Gayatri et al. (2013) highlighted the possible role of nitric oxide as a crossta256ignalinging element for various compounds that activate metabolic pathway and plant hormone such as abscisic acid. The down regulation of nitric oxide synthase in Atlas could disrupt the activation of various pathways involved in abiotic stress tolerance and /or lead to a lack of optimizing of ethylene level which might turned to be toxic for Atlas (chapters 3 and 5) and consequently a decrease in final grain weight as obtained for Atlas (chapter 3). This scenario is supported by the well-developed agronomic traits of Atlas under drought stress (increased plant height, dry and fresh weight, number of leaves and tillers) compared with the control group (chapter 3). The enhancement in Atlas growth might be due to an adequate ethylene level at the start of drought stress. Ethylene is known for to promote

root proliferation and development of leaves, flowers and fruit tissues. However, this hormone should 257ignalingsed so that it become not toxic to the plant. This conclusion is underpinned by the overexpression of ethylene associated gene in Atlas and down regulation of nitric oxide associated gene. Further molecular studies ares needed to confirm this hypothesis.

5.6. Conclusion

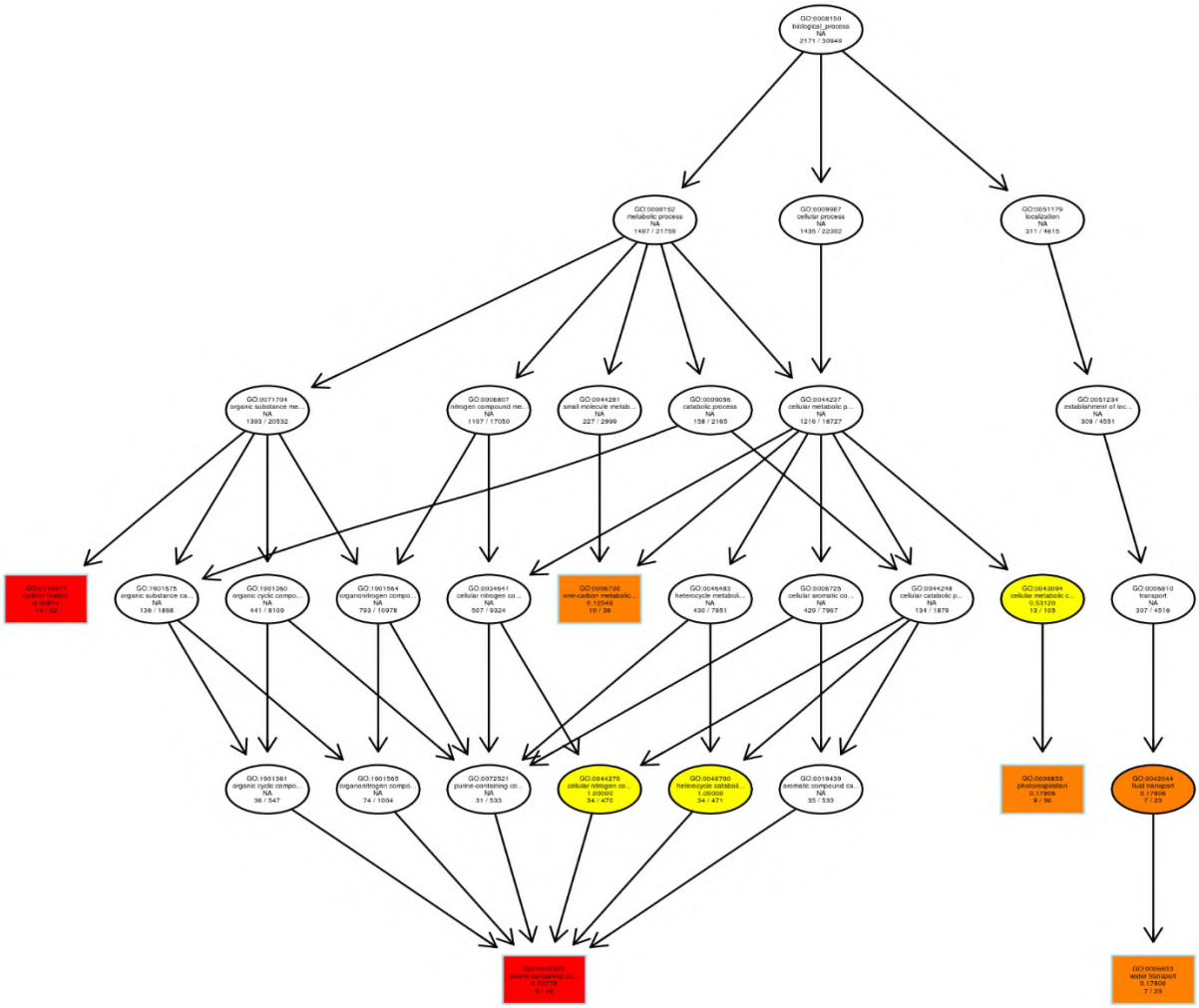
Transcriptomic study showed that the cultivars Atlas and ICARDA have drought-sensitive and drought-resistant genotypes. The gene ontology terms in ICARDA are mainly related to lipid and cell wall metabolism, oxidoreductase activity, lipid transport a257ignaling257257257ion as molecular function; apoplast, cell wall and external encapsulating structure as cellular component (CC), and biological process (BP) of lipid transport and localisation. These enriched genes were found to be up-regulated in ICARDA and consistent with the significant enrichment of the fatty acid elongation, cutin, suberin and wax biosynthesis pathway. These results are in line with the high relative water content in ICARDA, which makes this cultivar tolerant to drought and capable of successfully completing its growth cycle, generating a stable grain yield. However, in the Atlas cultivar, the most significant enriched DEGs were related to photosynthesis and the chloroplastic component. All DEGs associated with the enriched BP, CC, and MF were down-regulated. These results highlight the sensitivity of the chloroplast and photosynthesis to the drought, which is in concordance with the low chlorophyll content of Atlas under water-limited conditions. However, Atlas had well-developed agronomic traits and a high level of proline, which can be explained by the arginine and proline metabolic pathway being enriched for up-regulated genes and by the overexpression of the enzyme associated with ethylene biosynthesis. However, RNA-Seq data showed that the gene associated with nitric oxide is down-regulated in Atlas, leading to a lack of optimization of ethylene levels under drought stress. Ethylene can be toxic when it reaches a certain level in the plant, and therefore adversely affects grain fillings, making Atlas a sensitive genotype. Based on RNA-Seq, the low relative water content of Atlas under drought can be explained by the lack of significant enrichment of cutin, suberin and wax biosynthesis pathway, unlike ICARDA. Moreover, a high number of SNPs and InDels were found in this transcriptomic study, which will greatly help in better understanding the evolution and genetic diversity of wheat. In addition, large number of genes associated with protein with unknown function were detected in response to drought stress in Atlas and ICARDA cultivars, which could be new genes expressed in the presence of drought stress. This study helps provide a better understanding of the complex regulatory

mechanism of wheat for drought stress, and provide new molecular factors that can be targeted for drought tolerance breeding programme.

Appendix 1. Directed acyclic graph for two wheat cultivars (leaves), Atlas and ICARDA 32331 (ICA) grown under optimal (90-75% WFC) and drought (25% WFC) conditions. The graph shows the network and hierarchy of enriched differentially expressed genes, associated with biological process (BP), molecular function (MF) and cellular component (CC). Four biological replicates were used. The top 5 results of GO enrichment analysis are chosen as the main nodes (shown by box). Red colour = up-regulated genes; yellow colour = down and up-regulated genes. Abbreviations: C = control condition; D = drought condition; vs = versus; branch = hierarchical connection; darker shades = higher enrichment degree.

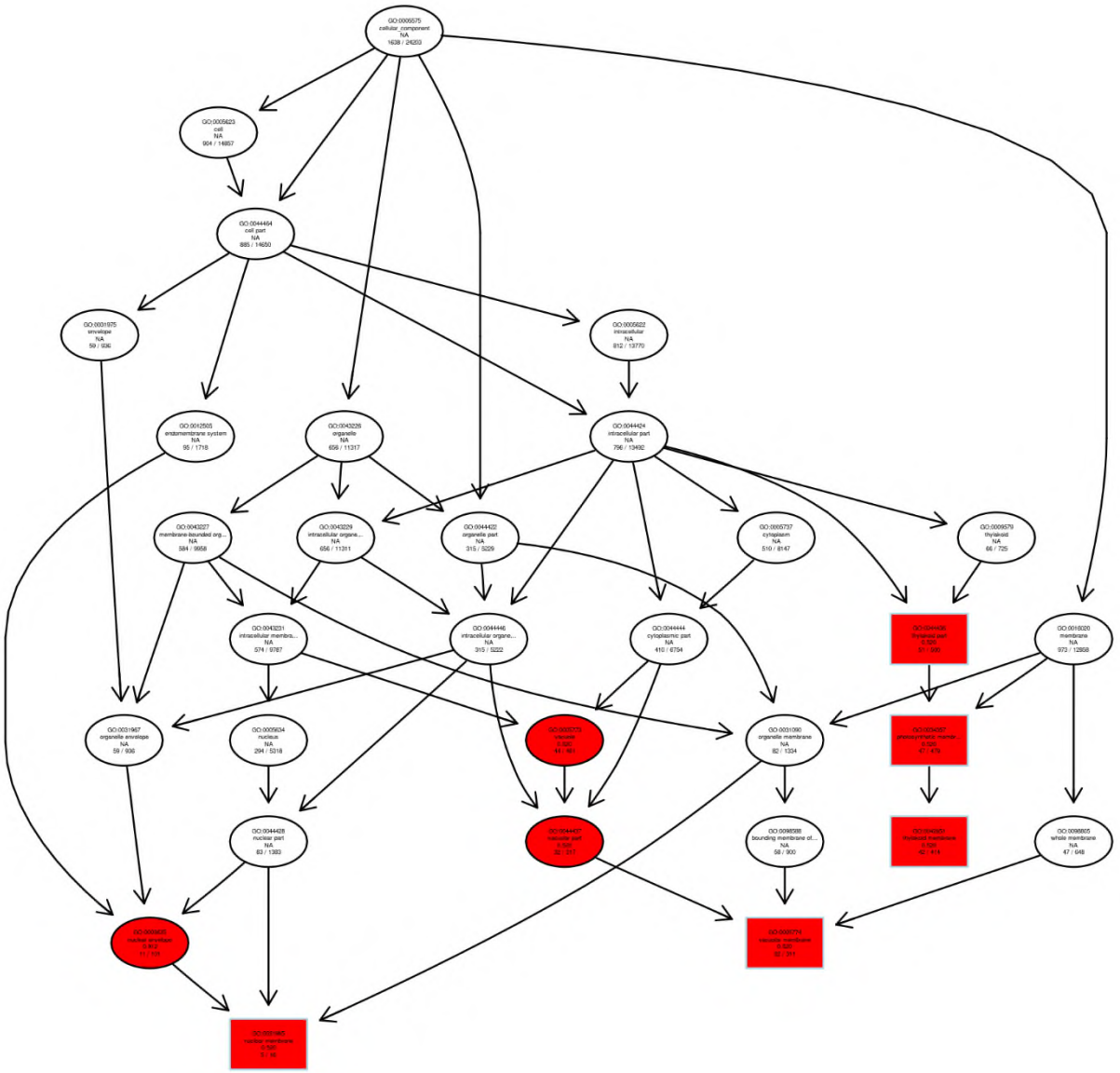
BP

Atl_D_vs_ICA_D



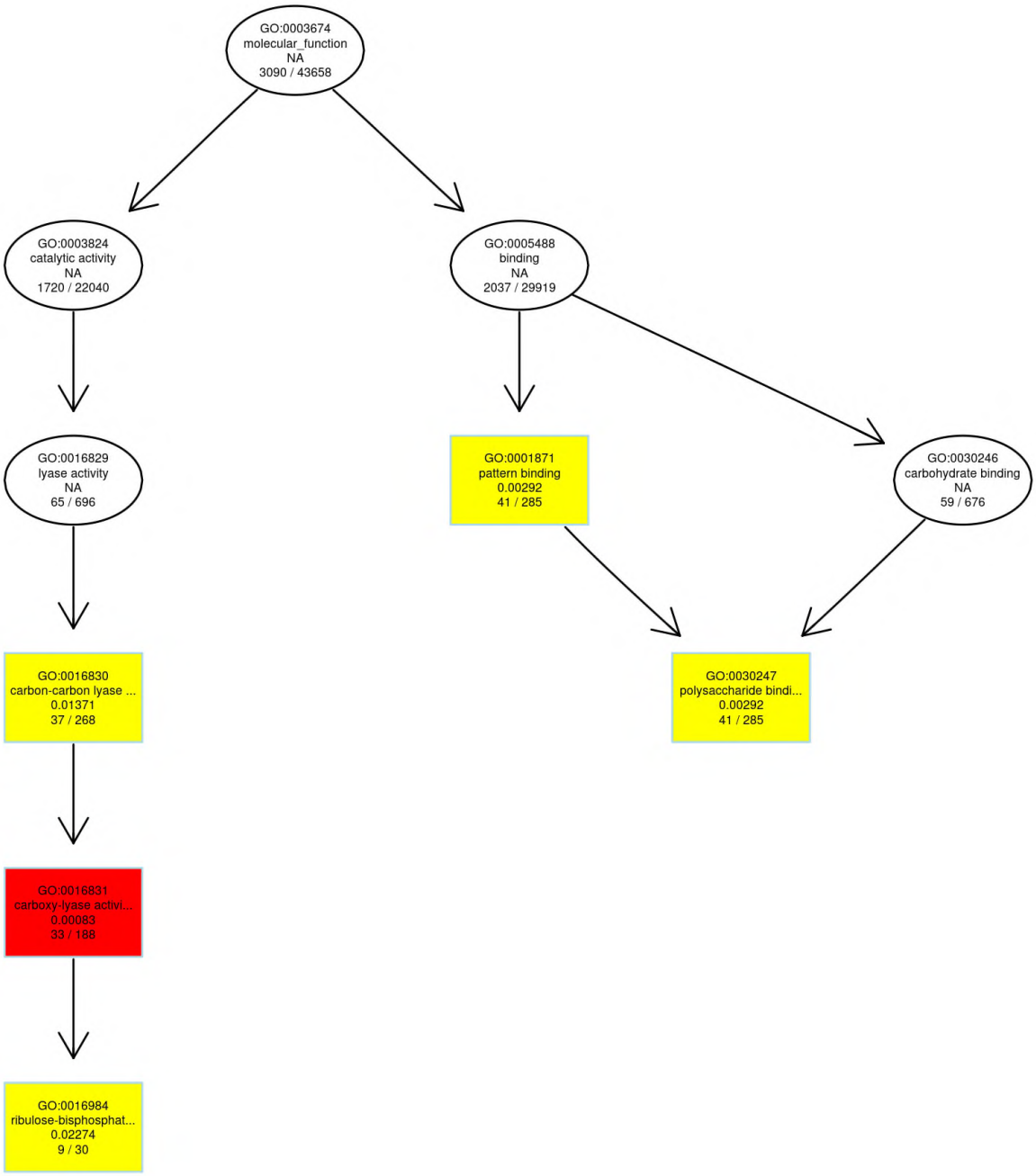
CC

Atl_D_vs_ICA_D



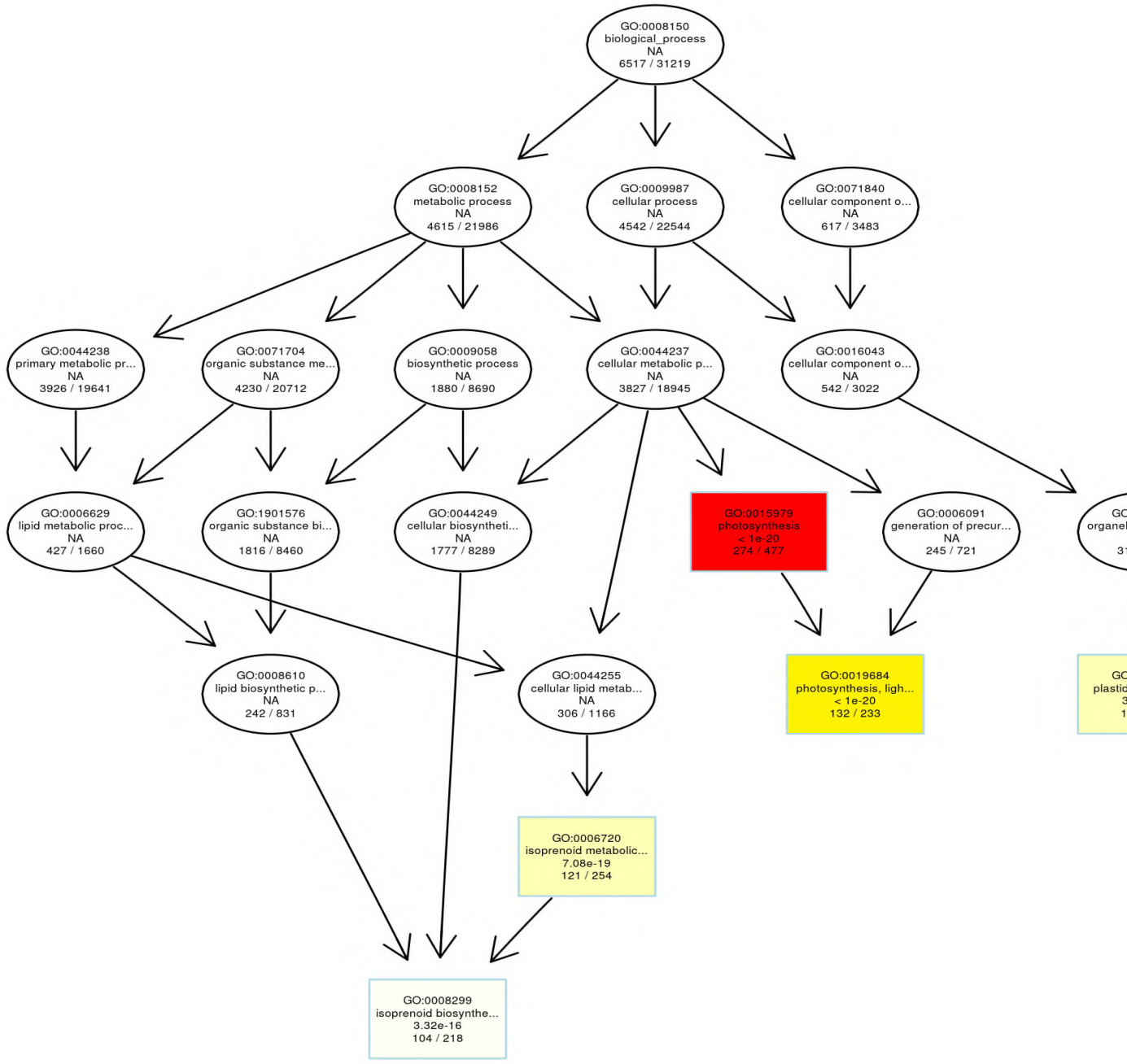
MF

Atl_D_vs_ICA_D



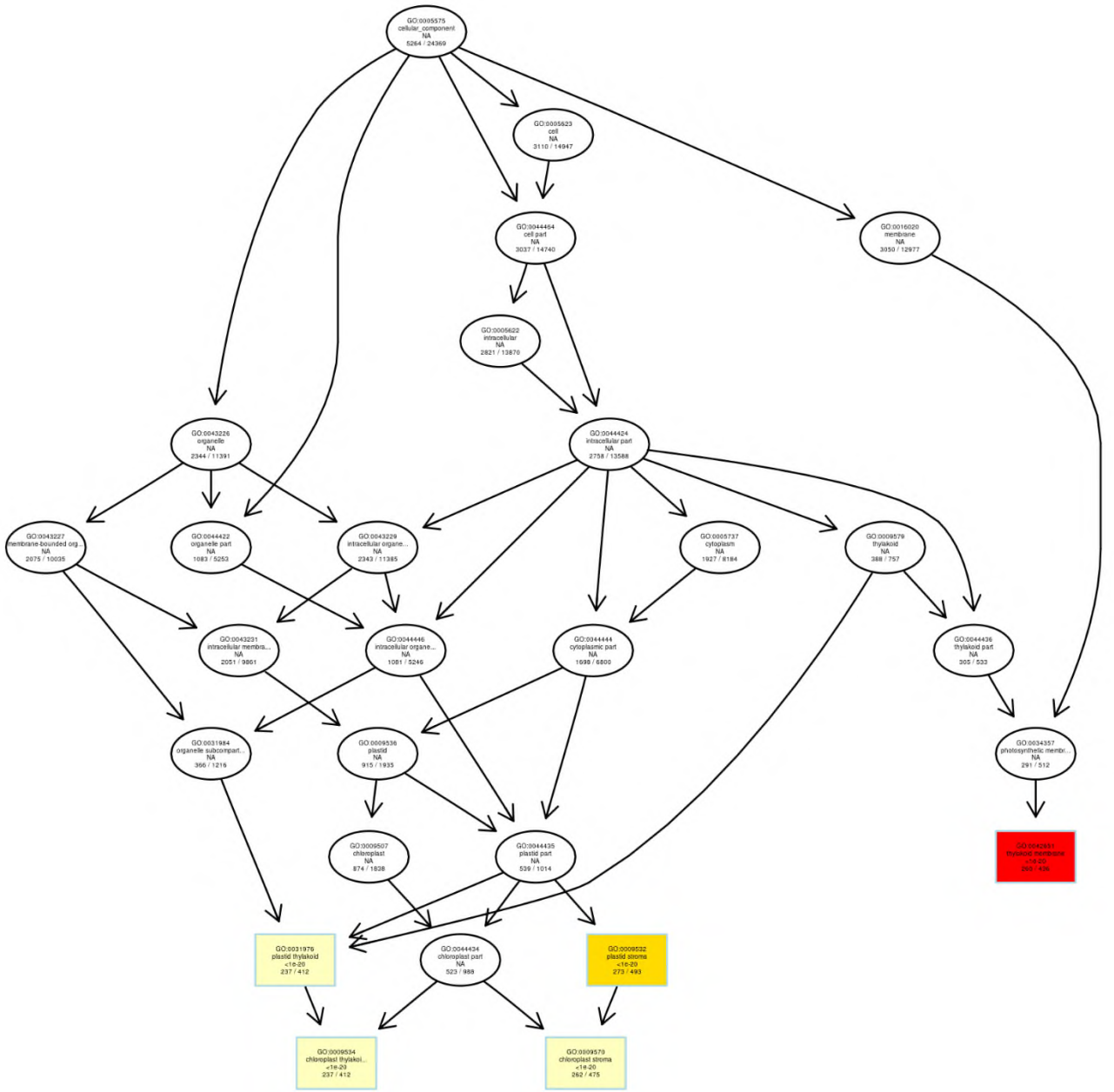
BP

Atl_D_vs_Atl_C



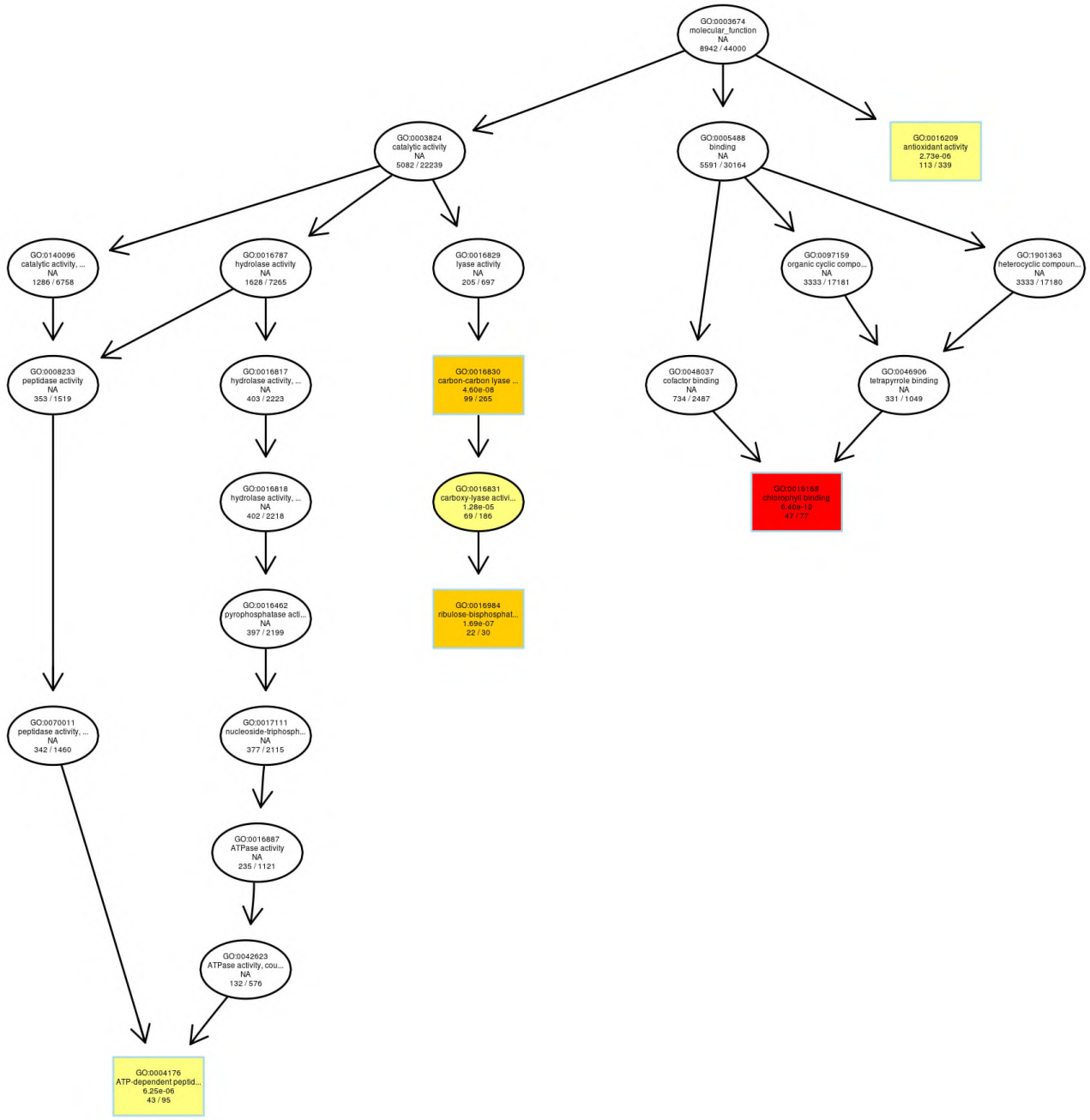
CC

Atl_D_vs_Atl_C



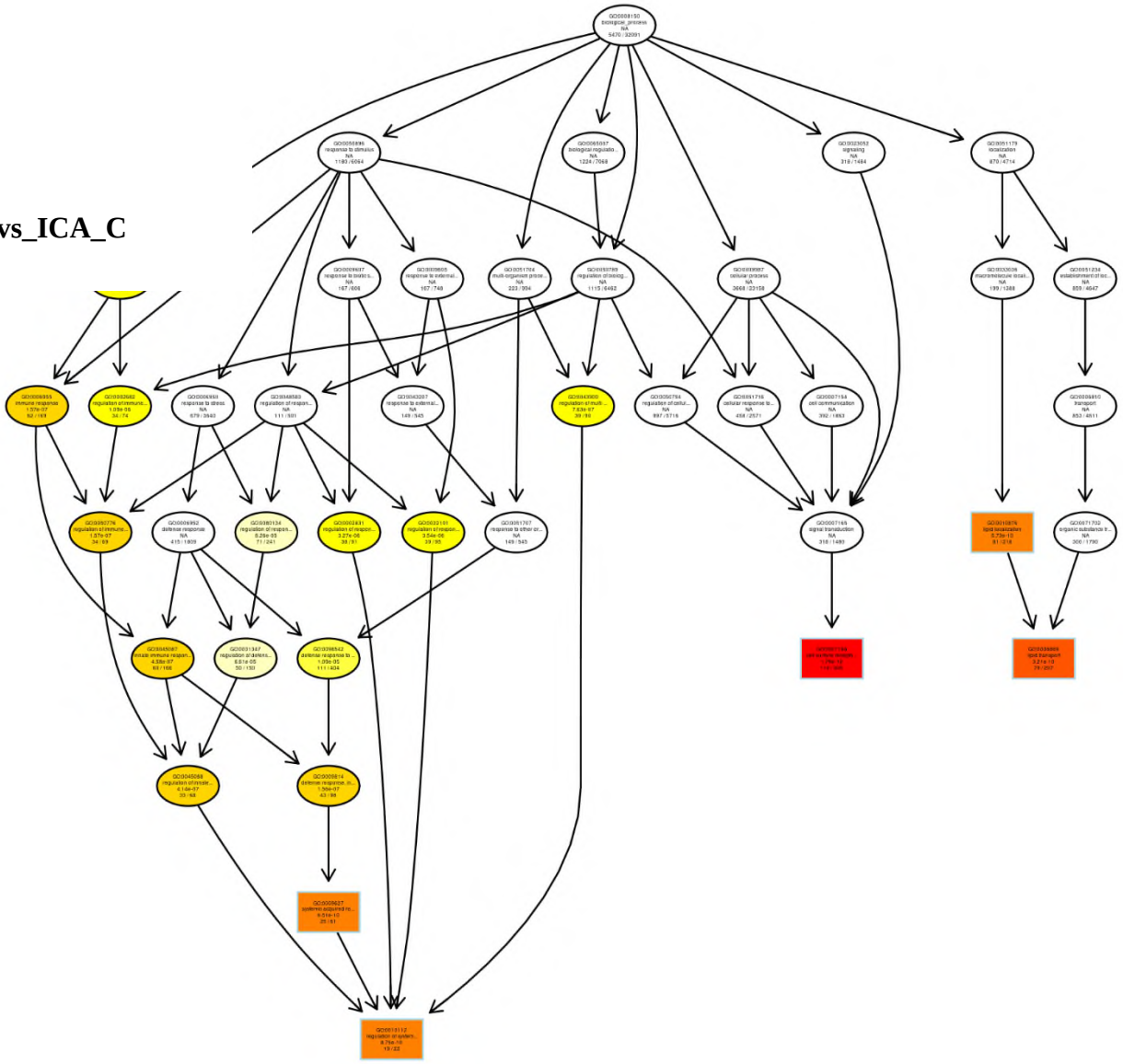
MF

Atl_D_vs_Atl_C



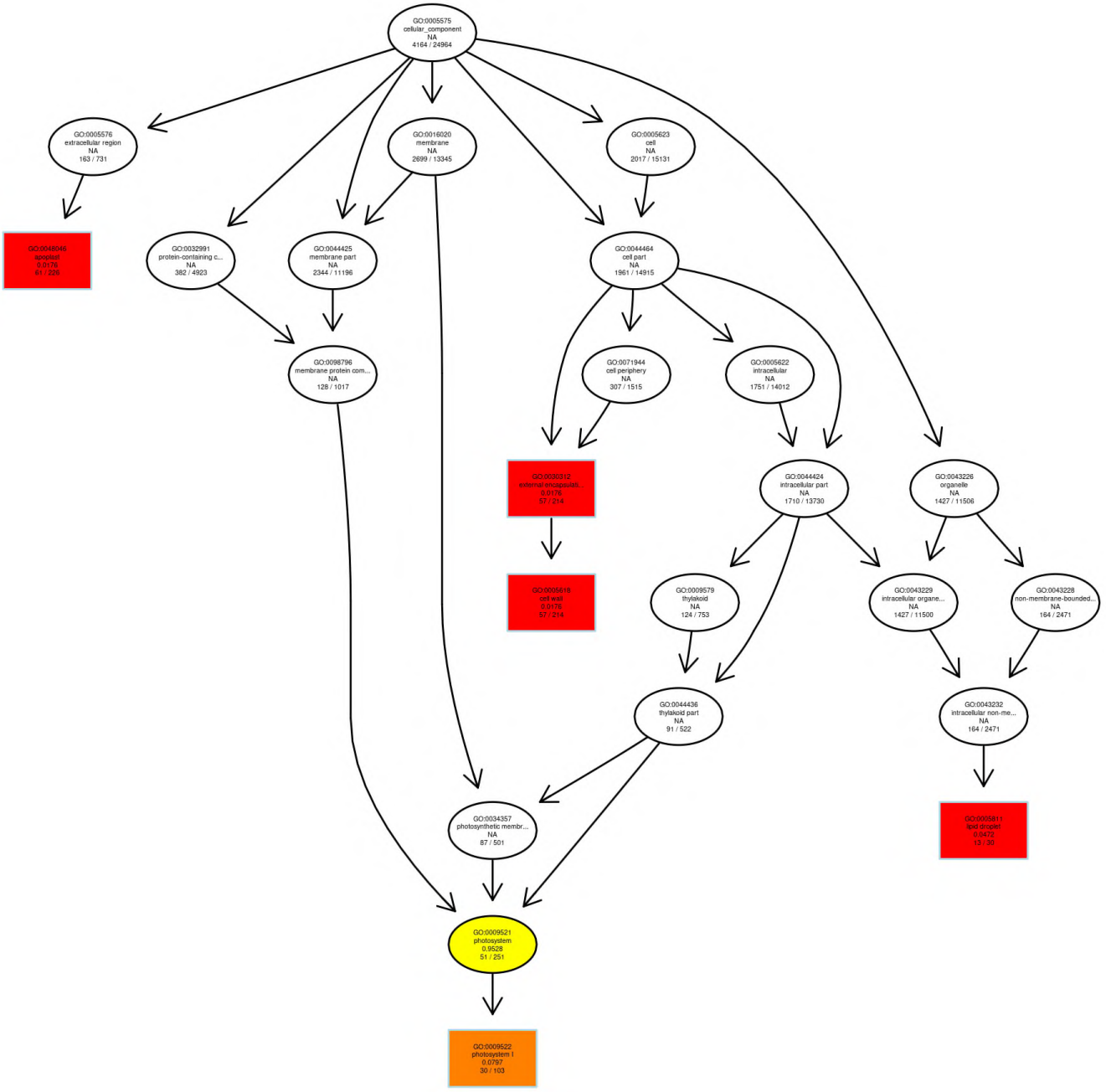
BP

ICA_D_vs_ICA_C



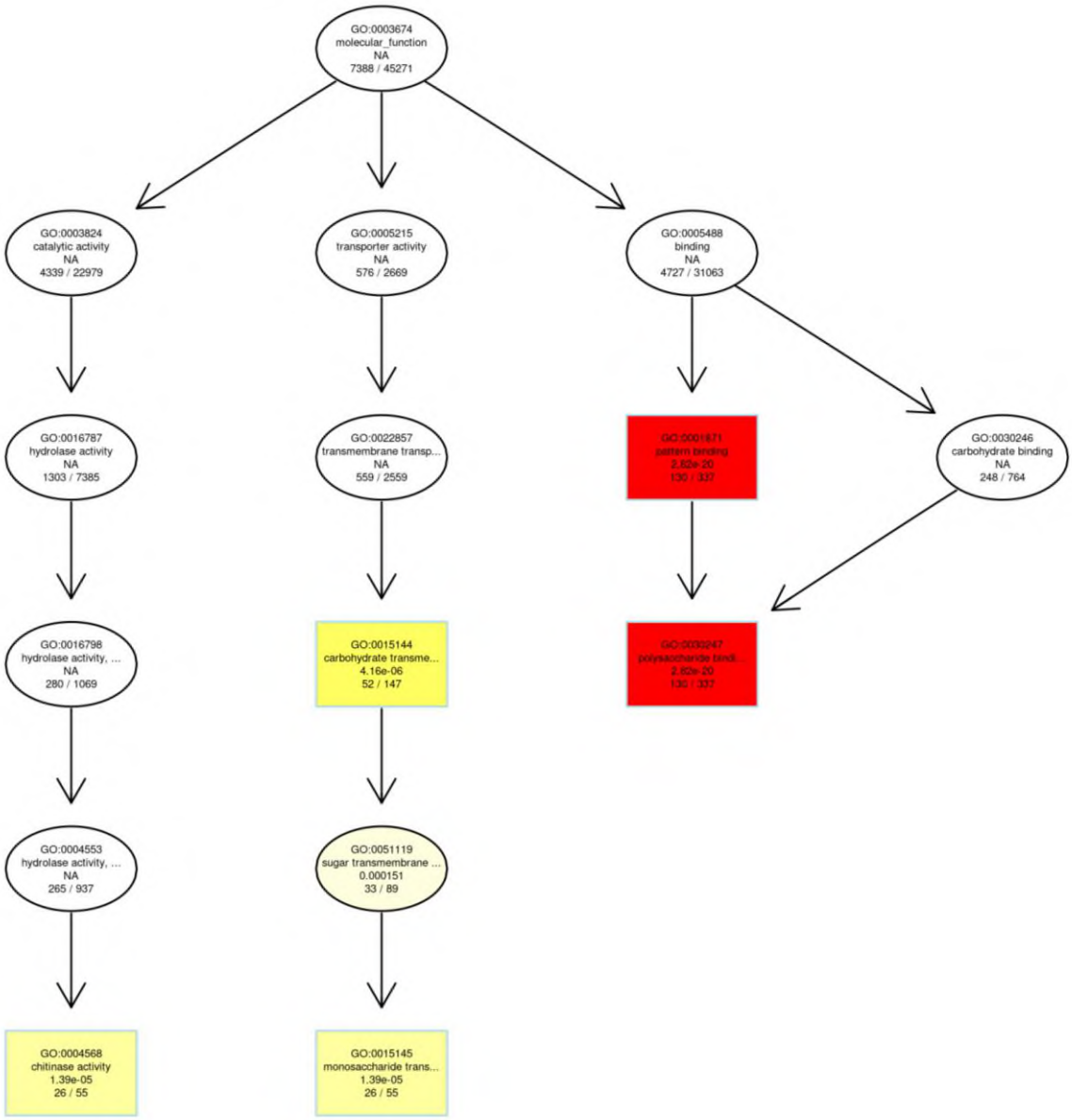
CC

ICA_D_vs_ICA_C



MF

ICA_D_vs_ICA_C

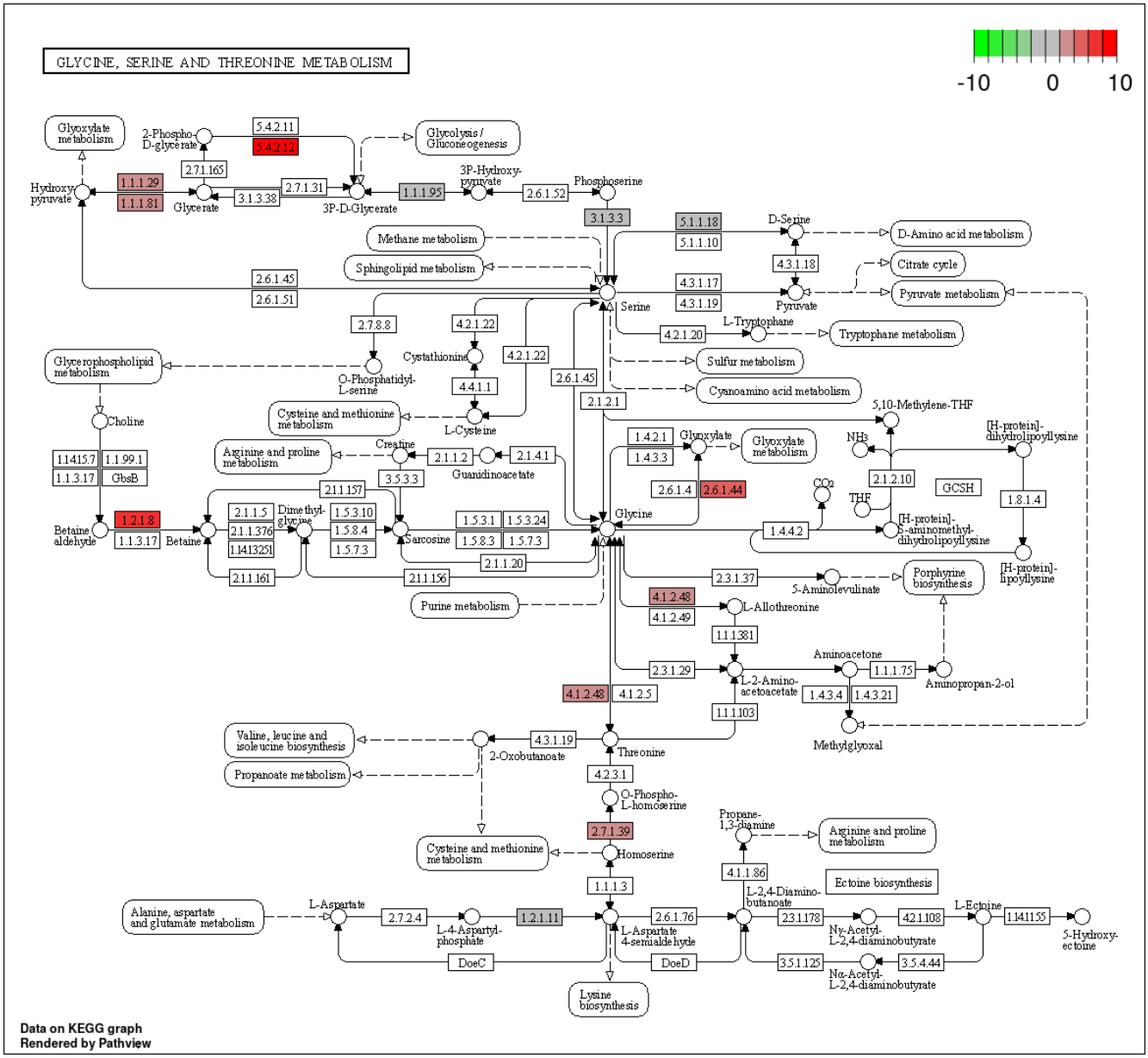


Appendix 2. Significant KEGG pathway enrichment in Atlas under control and drought conditions

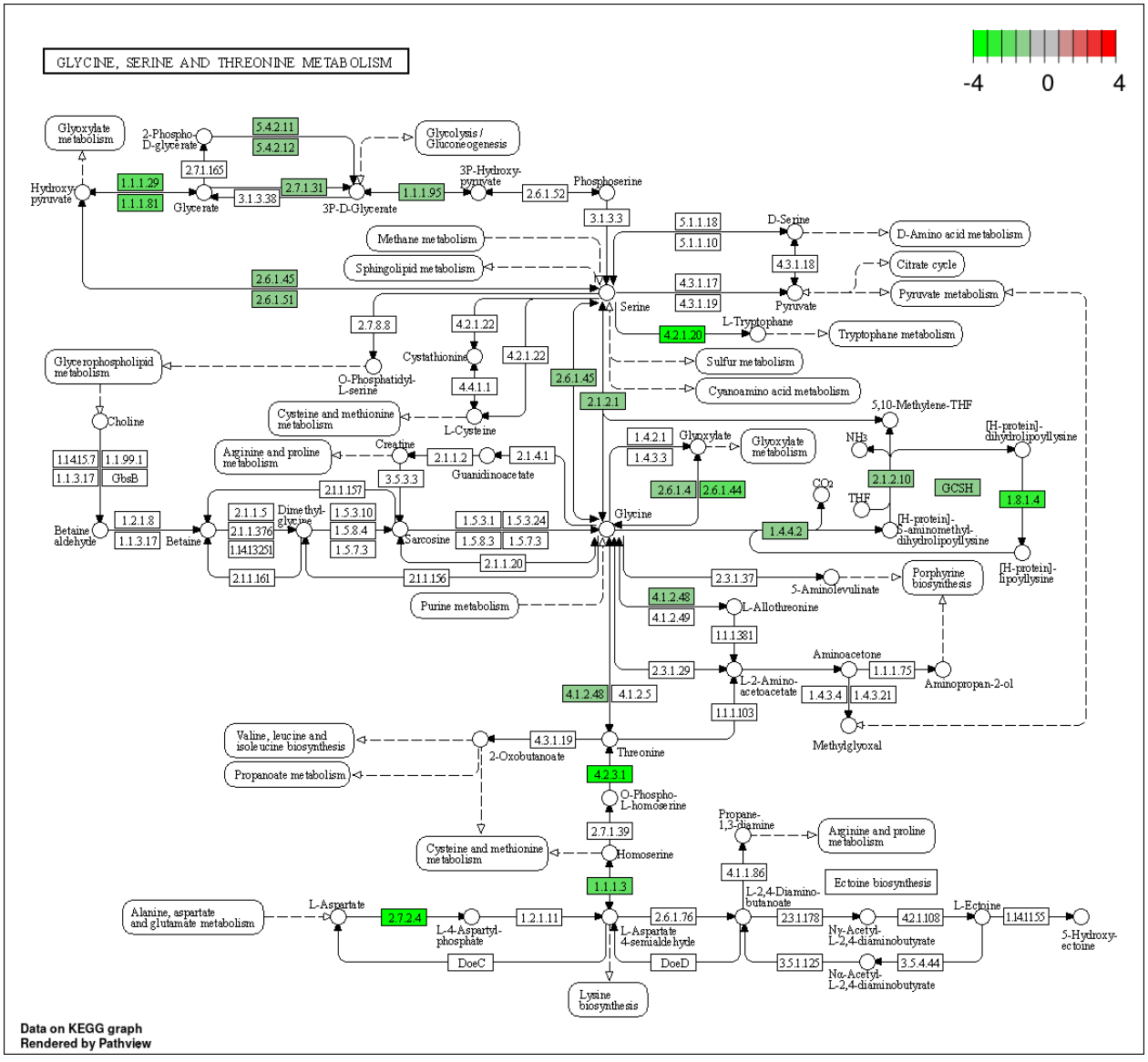
KEGGID	Description	GeneRatio	BgRatio	pvalue	padj	Count	Up	Down
ath00710	Carbon fixation in photosynthetic organisms	111/3257	231/15309	8.47E-20	1.06E-17	111	25	86
ath00195	Photosynthesis	127/3257	282/15309	1.93E-19	1.20E-17	127	4	123
ath00630	Glyoxylate and dicarboxylate metabolism	102/3257	242/15309	1.38E-13	5.74E-12	102	21	81
ath00196	Photosynthesis - antenna proteins	50/3257	92/15309	3.35E-12	1.05E-10	50	2	48
ath00860	Porphyrin metabolism	70/3257	159/15309	8.79E-11	2.20E-09	70	2	68
ath00052	Galactose metabolism	87/3257	220/15309	4.53E-10	9.43E-09	87	70	17
ath00030	Pentose phosphate pathway	73/3257	175/15309	7.01E-10	1.25E-08	73	26	47
ath00906	Carotenoid biosynthesis	43/3257	87/15309	5.36E-09	8.38E-08	43	3	40
ath04016	MAPK signaling pathway - plant	137/3257	425/15309	6.41E-08	8.91E-07	137	73	64
ath00750	Vitamin B6 metabolism	26/3257	47/15309	3.32E-07	4.15E-06	26	8	18
ath00920	Sulfur metabolism	41/3257	102/15309	1.09E-05	0.000122	41	2	39
ath00010	Glycolysis / Gluconeogenesis	129/3257	431/15309	1.17E-05	0.000122	129	60	69
ath00740	Riboflavin metabolism	24/3257	55/15309	0.000159	0.001532	24	3	21

ath00900	Terpenoid backbone biosynthesis	52/3257	155/15309	0.000255	0.002275	52	12	40
ath00270	Cysteine and methionine metabolism	100/3257	348/15309	0.000544	0.004534	100	51	49
ath00130	Ubiquinone and other terpenoid-quinone biosynthesis	52/3257	163/15309	0.000969	0.007572	52	22	30
ath00480	Glutathione metabolism	101/3257	365/15309	0.002001	0.014007	101	48	53
ath00260	Glycine, serine and threonine metabolism	66/3257	223/15309	0.002017	0.014007	66	26	40
ath00330	Arginine and proline metabolism	51/3257	165/15309	0.00231	0.01482	51	34	17
ath00230	Purine metabolism	84/3257	297/15309	0.002371	0.01482	84	30	54
ath00650	Butanoate metabolism	29/3257	84/15309	0.003422	0.020368	29	12	17
ath00051	Fructose and mannose metabolism	59/3257	202/15309	0.004631	0.026312	59	26	33

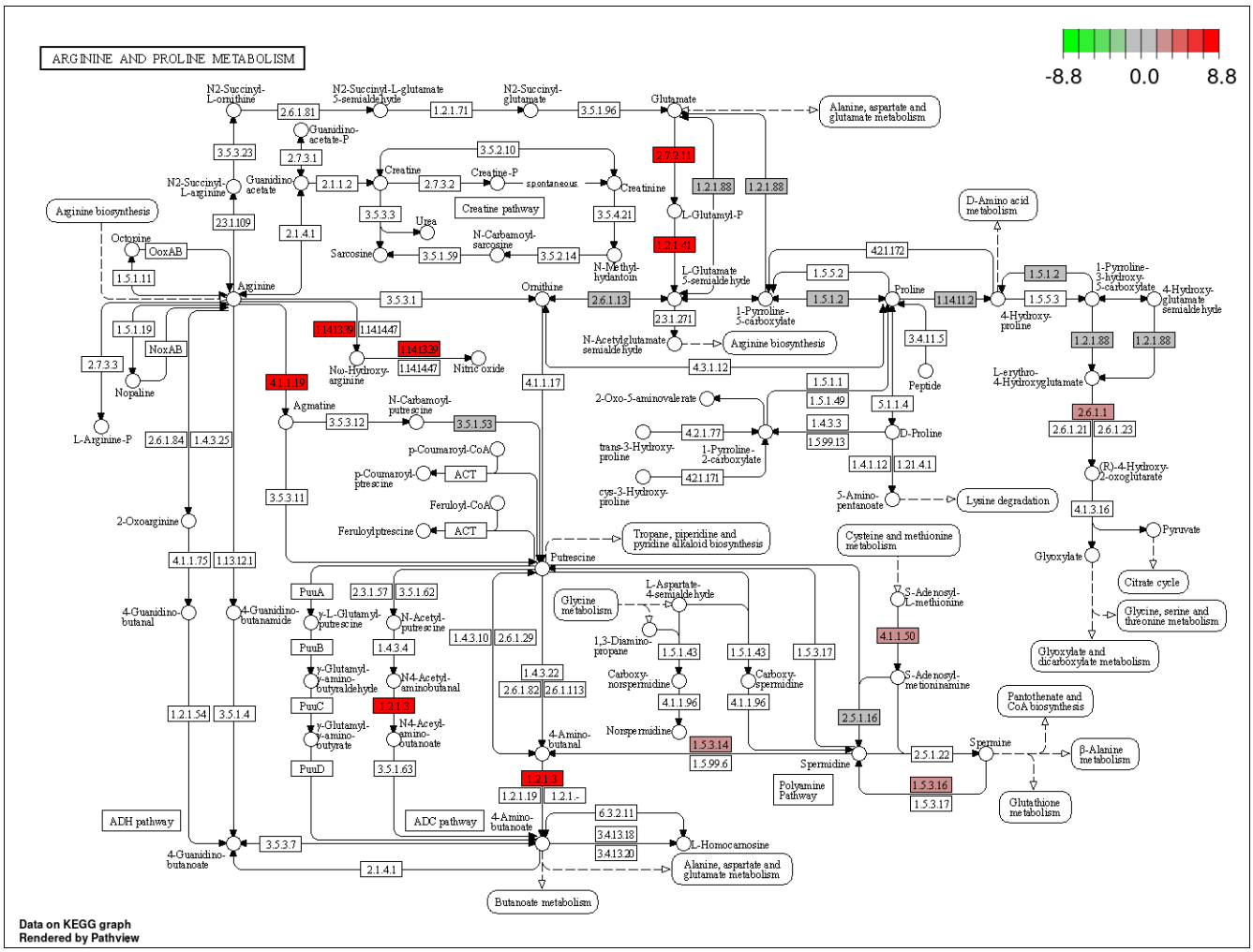
Appendix 3a. Glycine, serine and threonine metabolism enriched with up-regulated gene in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



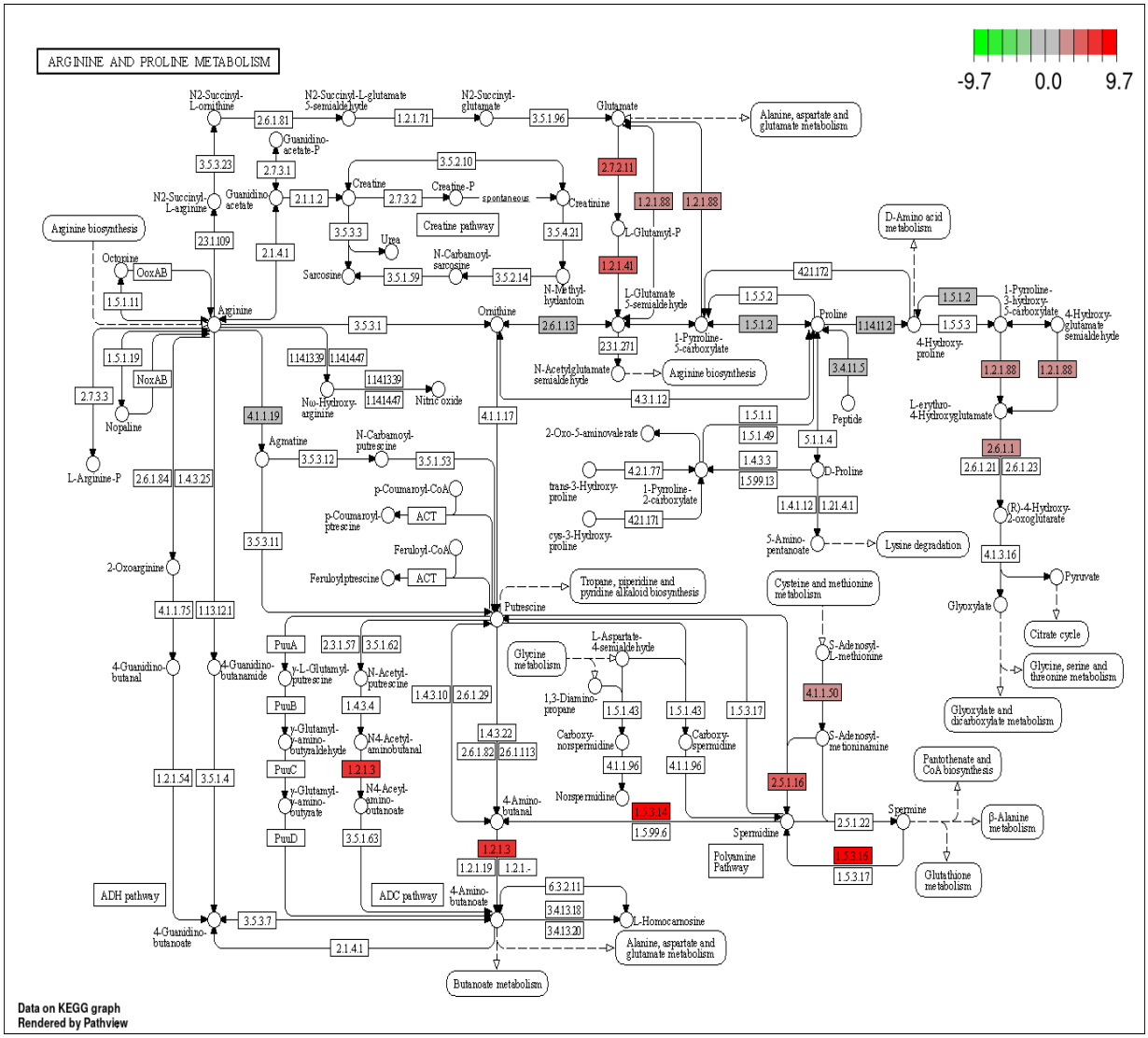
Appendix 3b. Glycine, serine and threonine metabolism enriched with down regulated gene in wheat cultivar Atlas (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



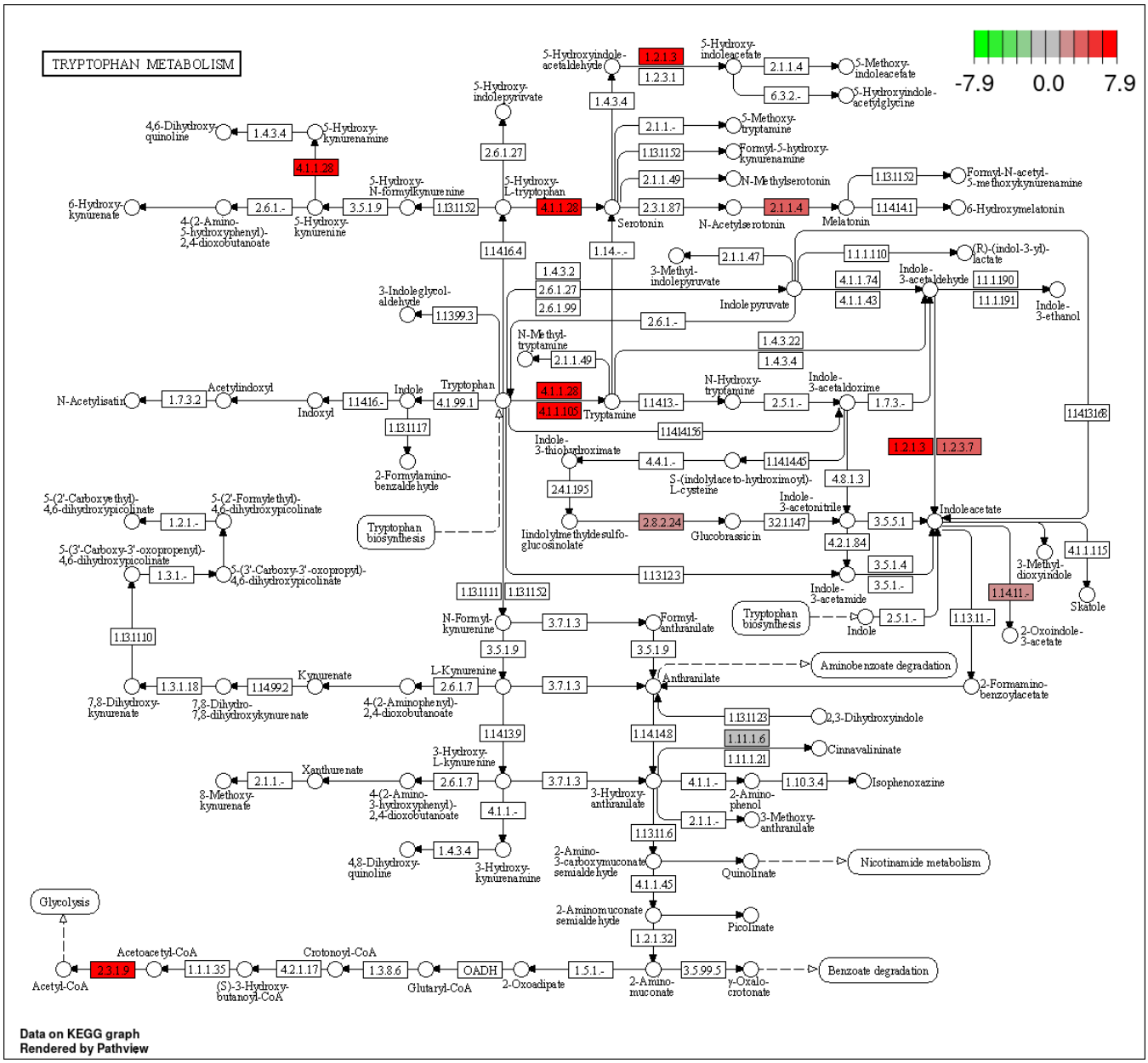
Appendix 4a. Arginin and proline metabolism enriched with up-regulated gene in ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



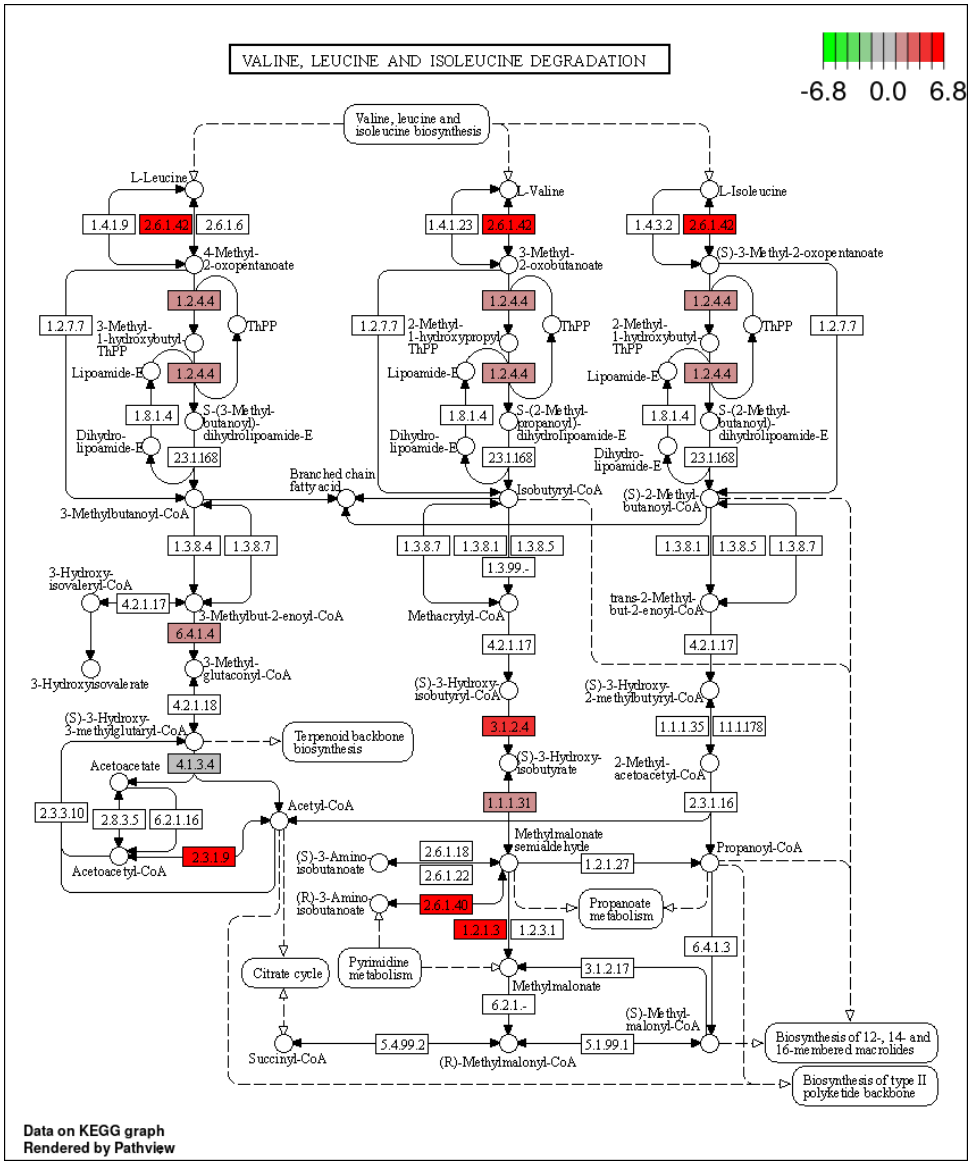
Appendix 4b. Arginin and proline metabolism enriched with up-regulated gene in Atlas (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



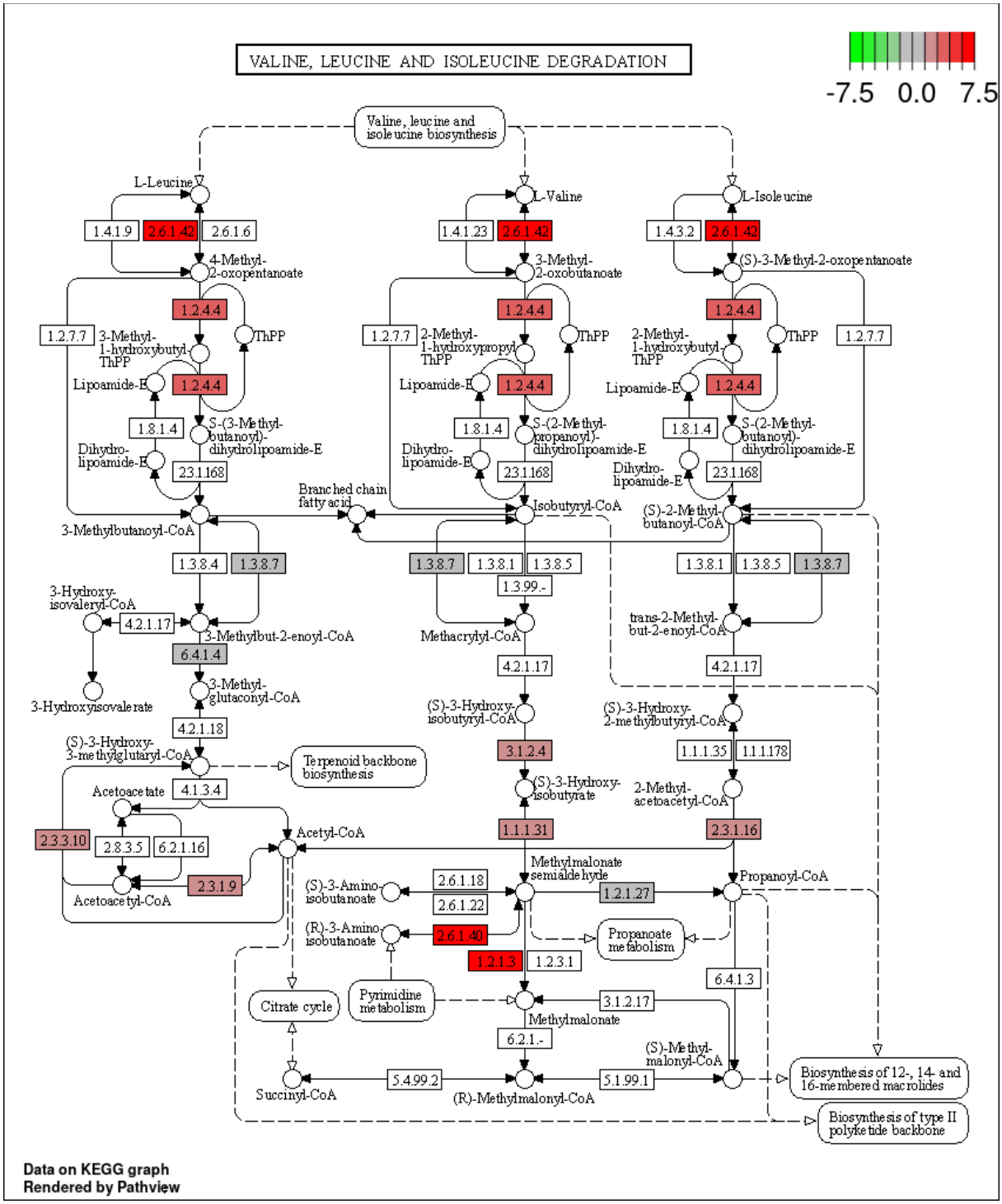
Appendix 5. Tryptophan metabolism enriched with up-regulated gene in ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



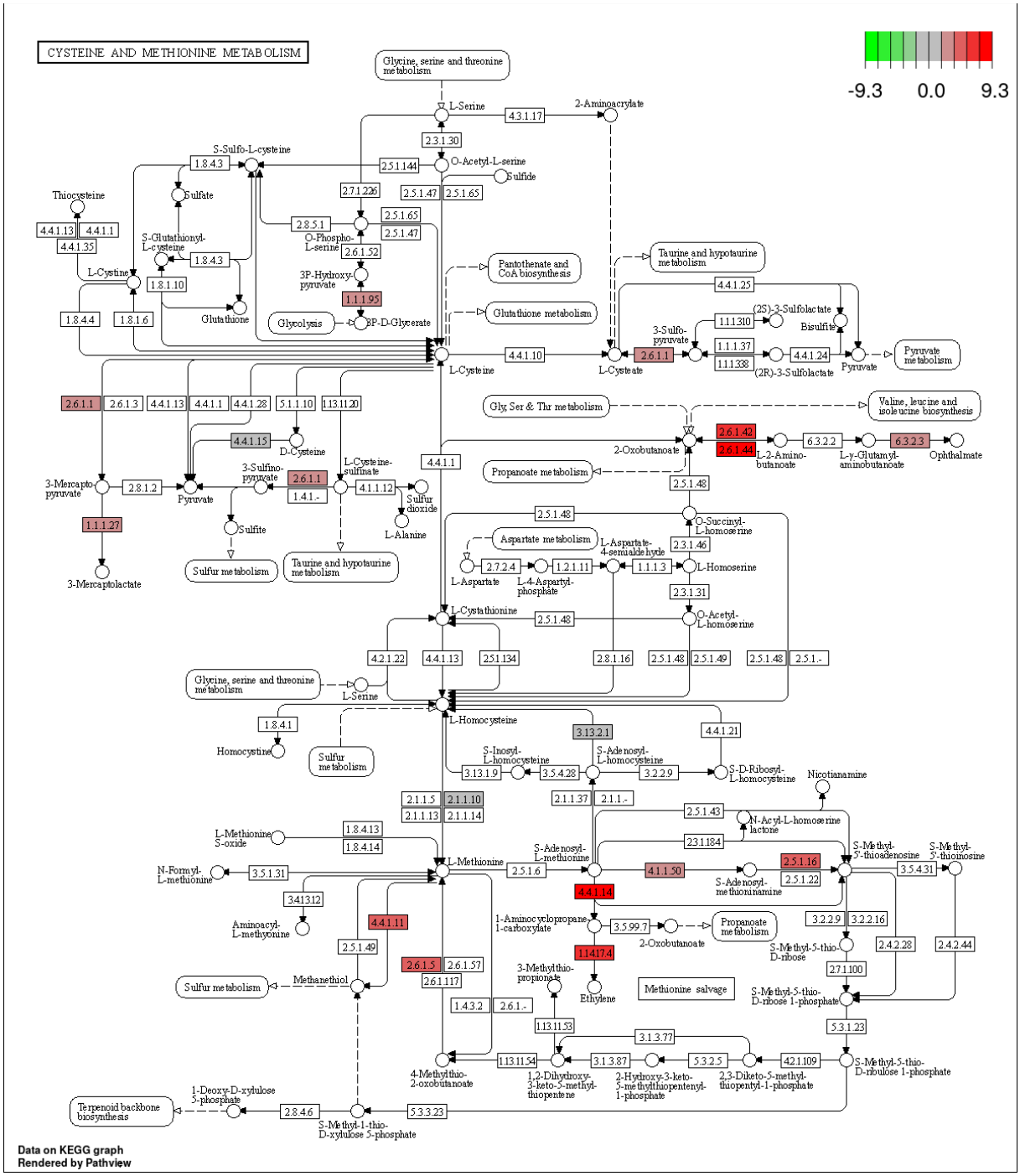
Appendix 6a. Valine, leucine and Isoleucine metabolism enriched with up-regulated gene in ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



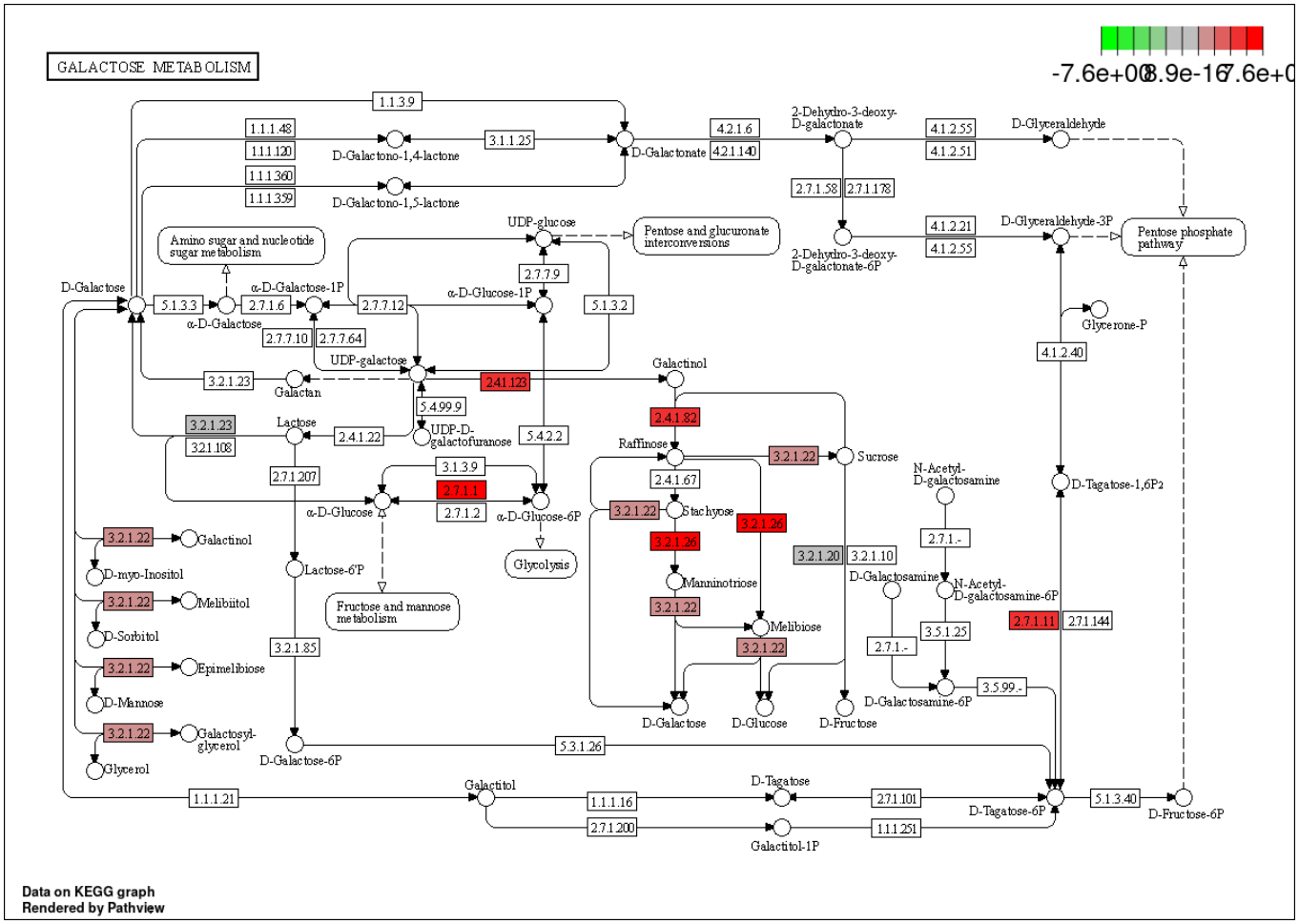
Appendix 6b. Valine, leucine and Isoleucine metabolism enriched with up-regulated gene in Atlas (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



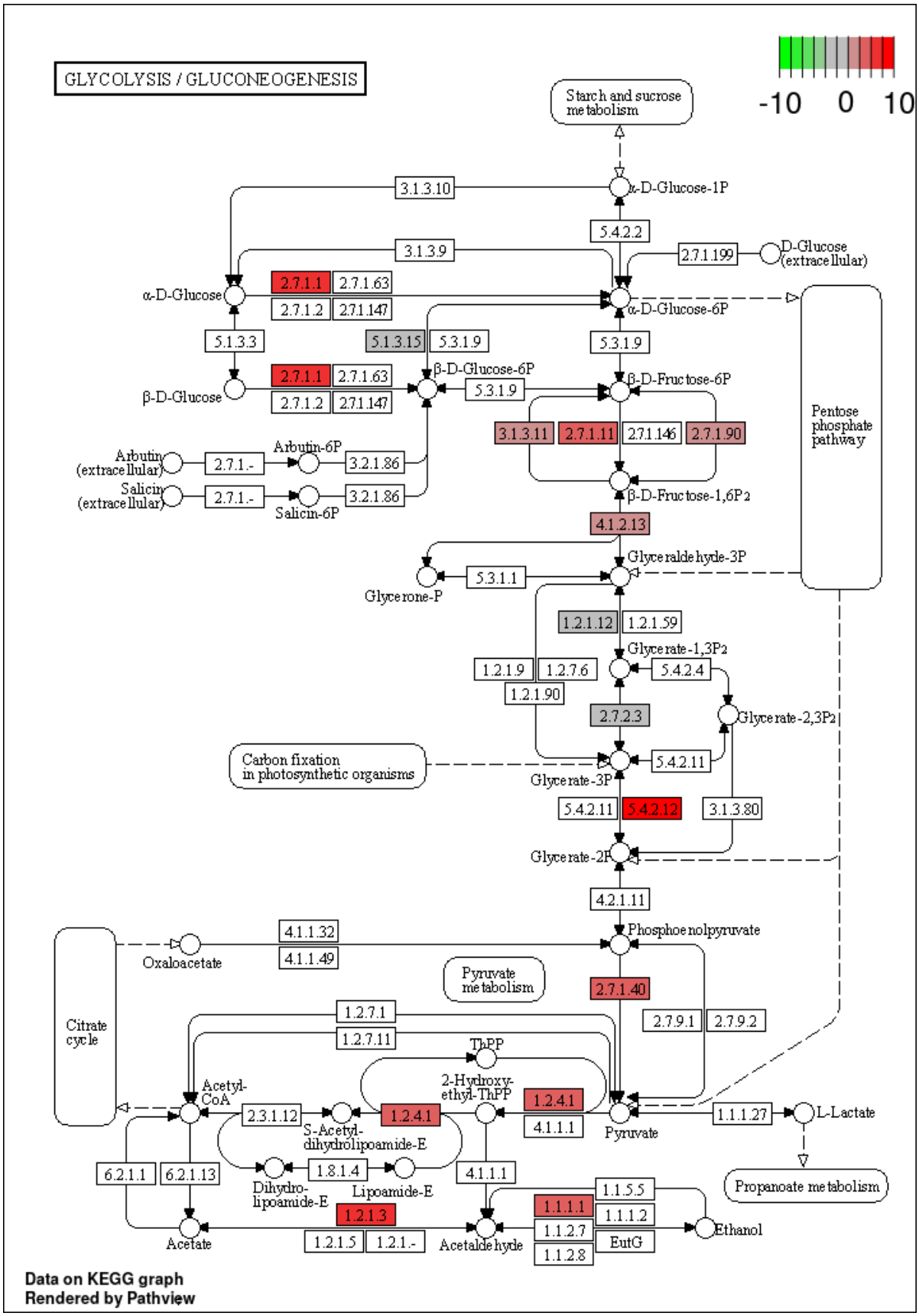
Appendix 7. Cysteine and methionine metabolism enriched with up-regulated gene in Atlas (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



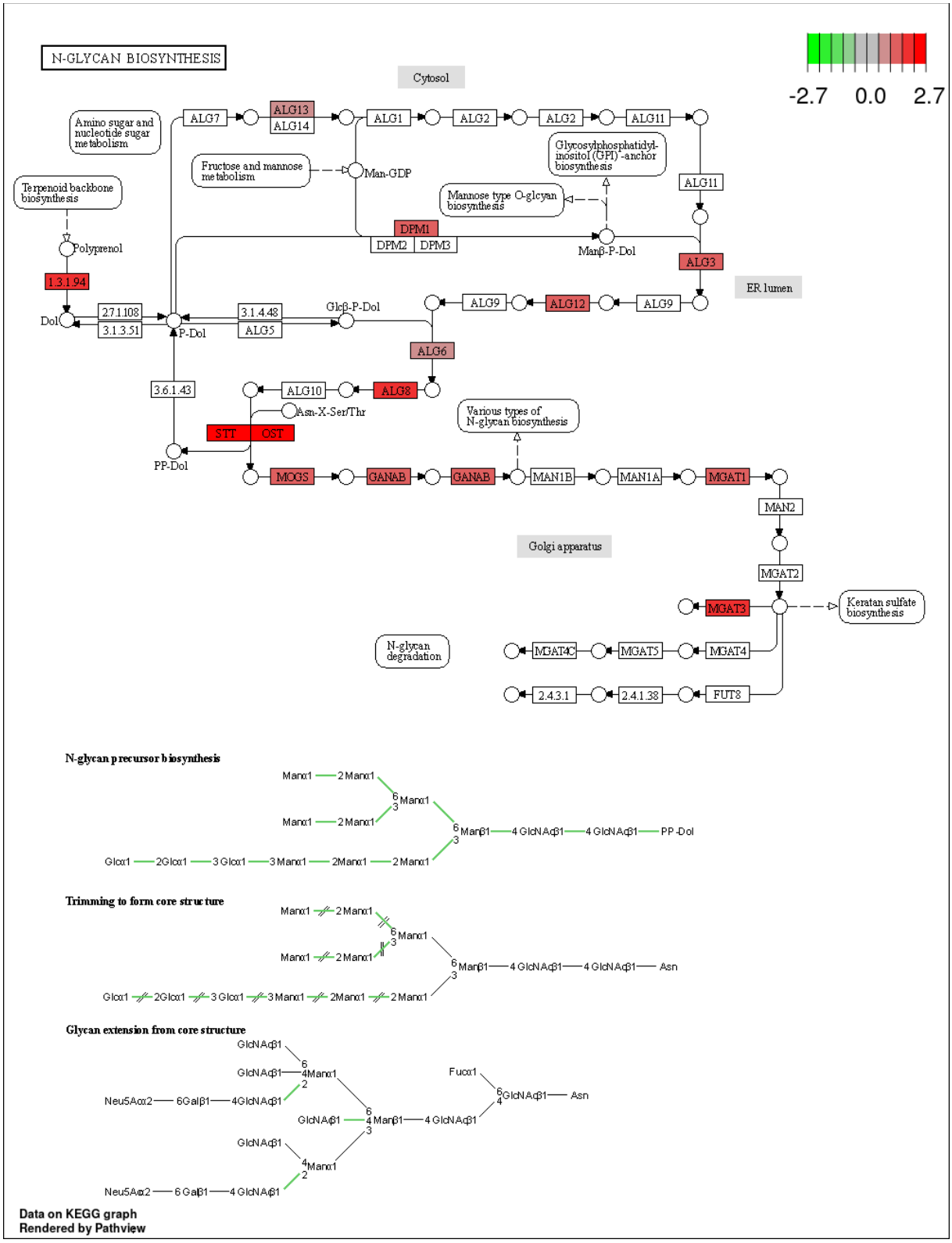
Appendix 8. Galactose metabolism enriched with up-regulated gene in wheat cultivars ICARDA and Atlas(leaves). Red colour = up-regulated genes; green colour = down regulated genes.



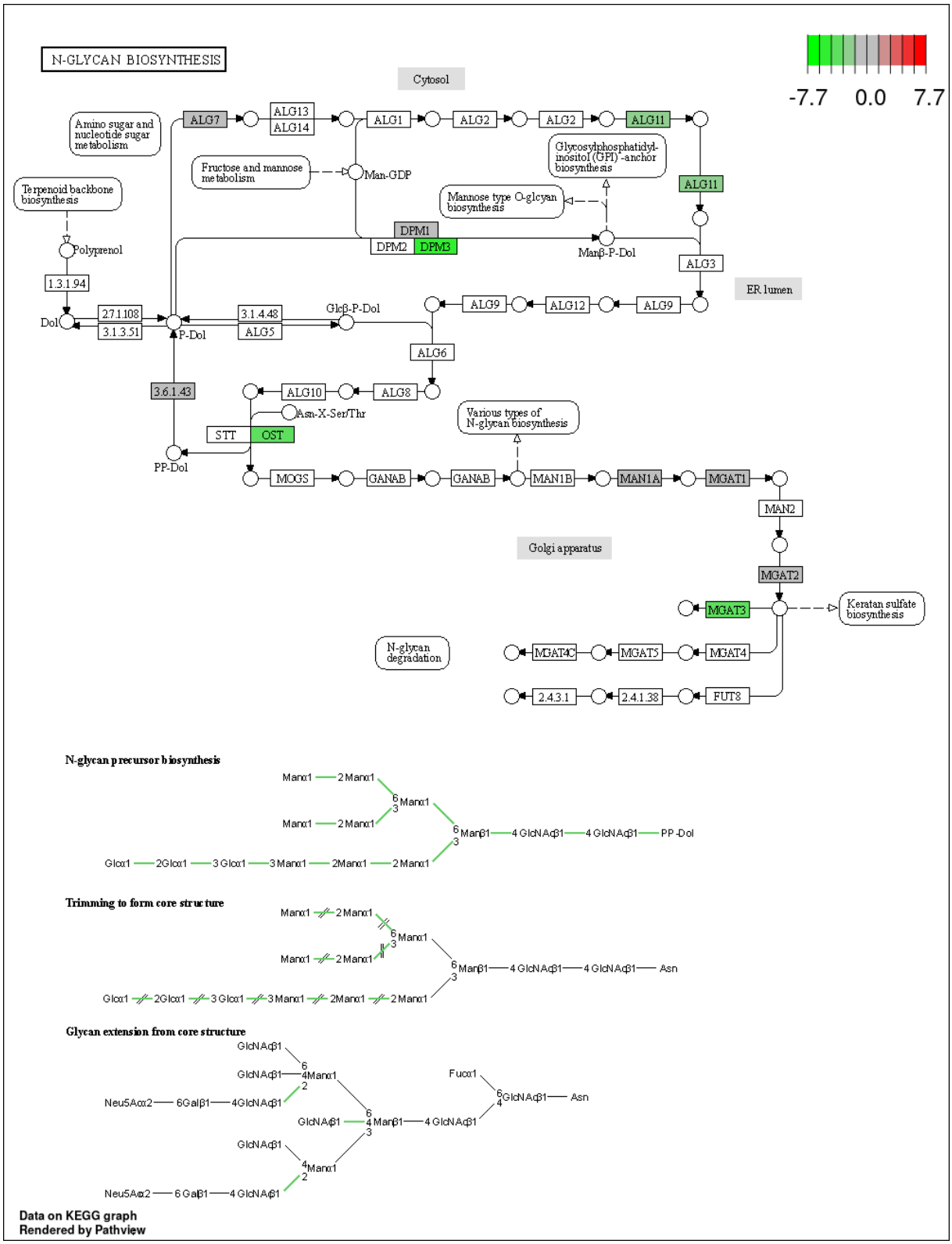
Appendix 9. Glycolysis and gluconeogenesis pathways enriched with up-regulated genes in wheat cultivars (leaves) ICARDA and Atlas. Red colour = up-regulated genes; green colour = down regulated genes.



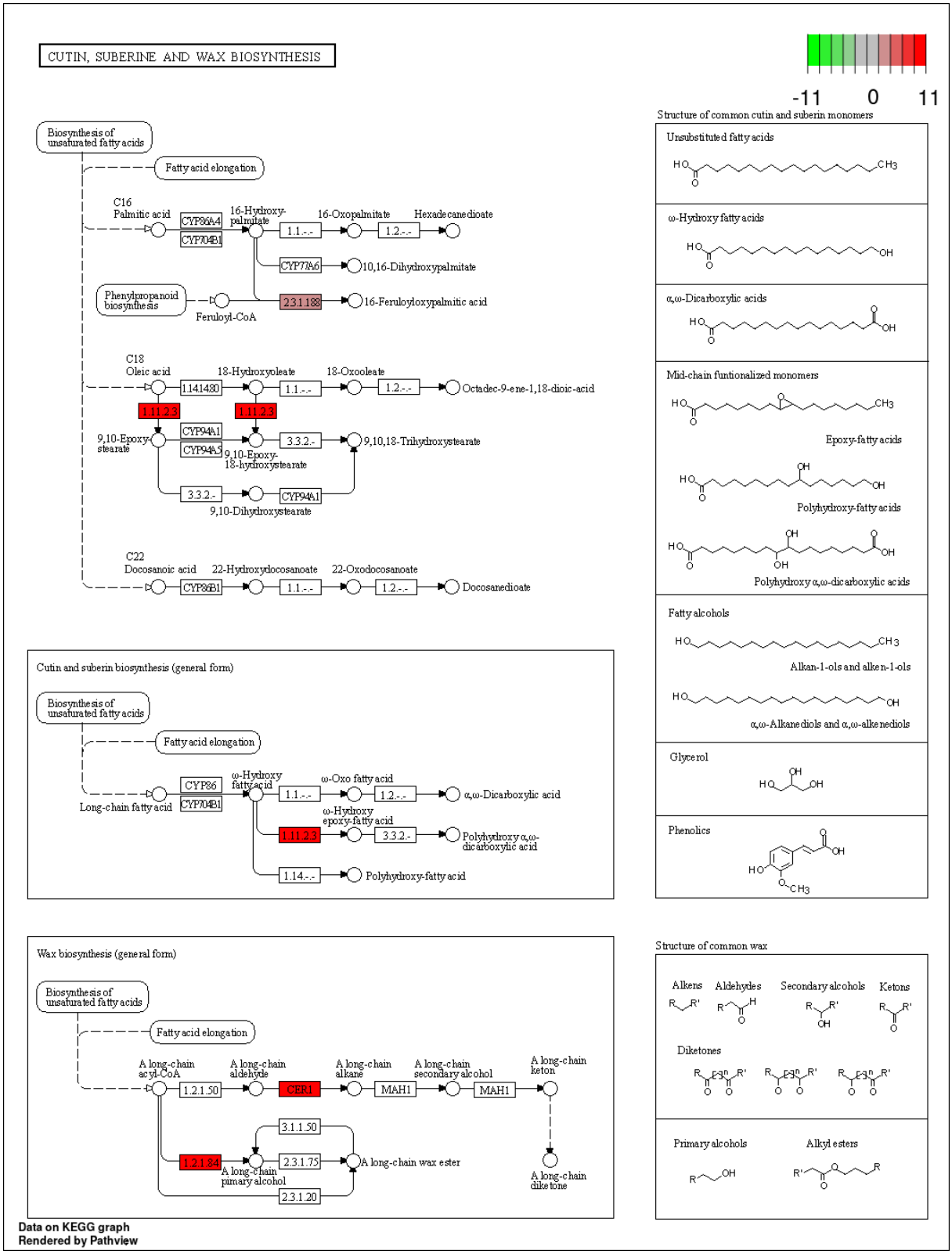
Appendix 10a. N-Glycan pathway enriched with up-regulated genes in wheat cultivar Atlas (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



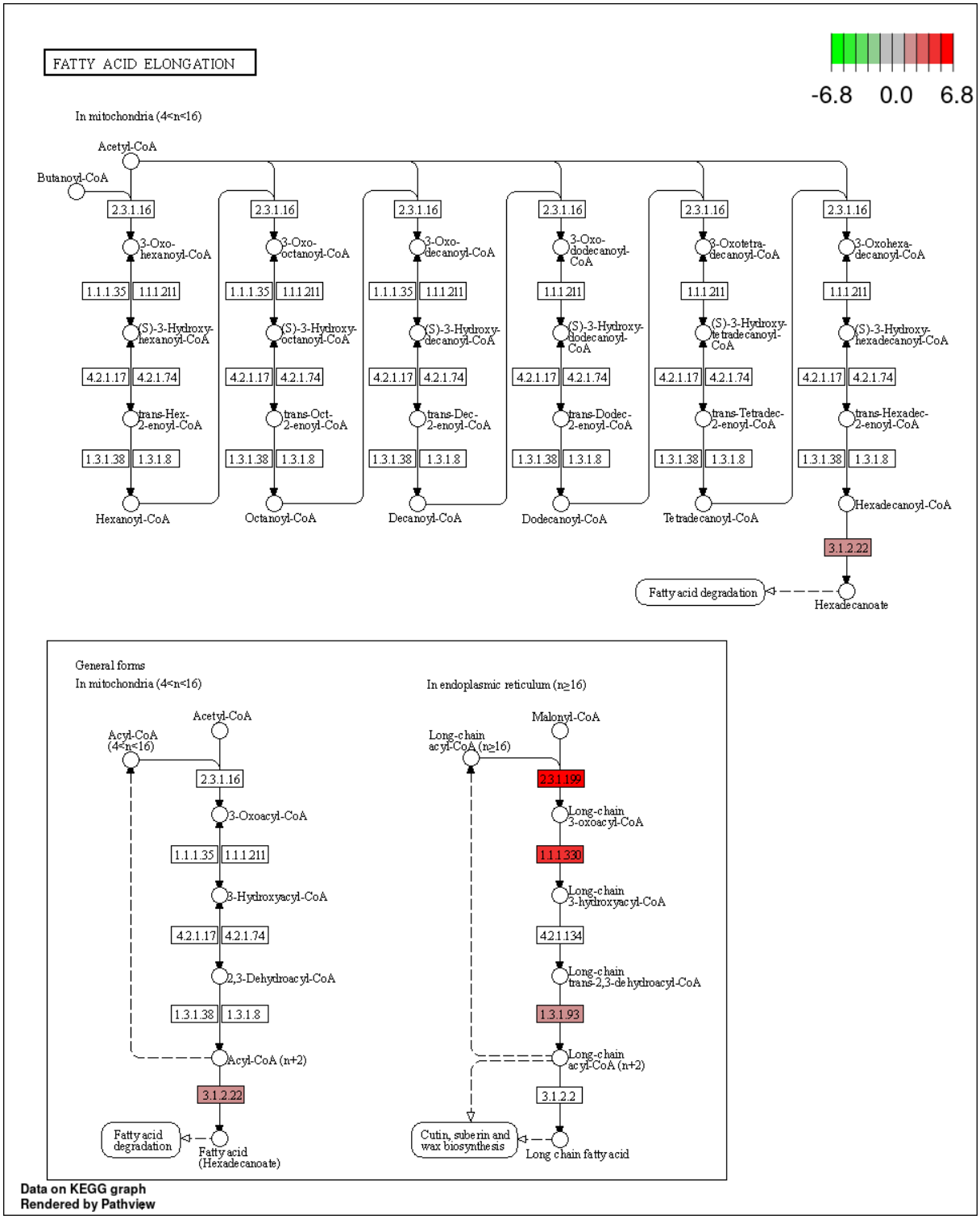
Appendix 10b. N-Glycan pathway enriched with down regulated genes in ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



Appendix 11a. (a) Biosynthesis of cutin, suberin and wax and fatty acid elongation pathway enriched with up- regulated genes in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.

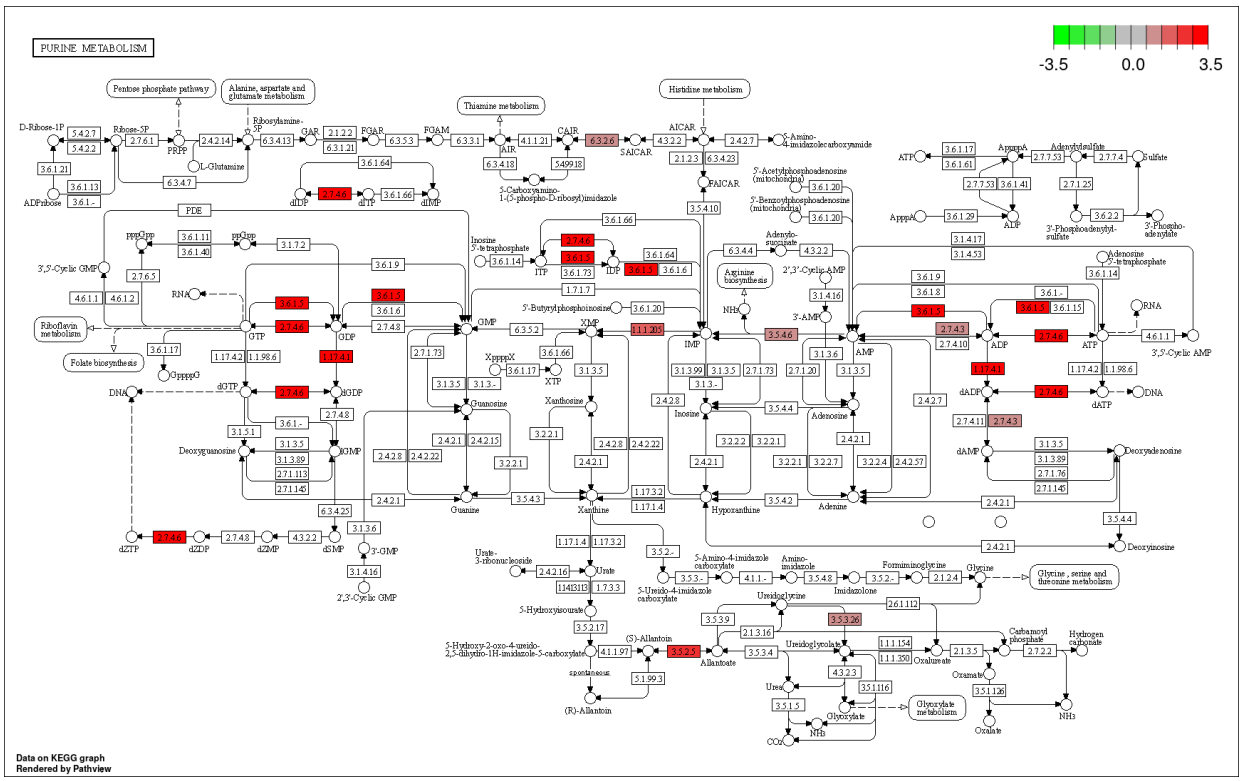


Appendix 11a. (b) Fatty acid elongation pathway enriched with up- regulated genes in wheat cultivars (leaves) ICARDA and Atlas. Red colour = up-regulated genes; green colour = down regulated genes.

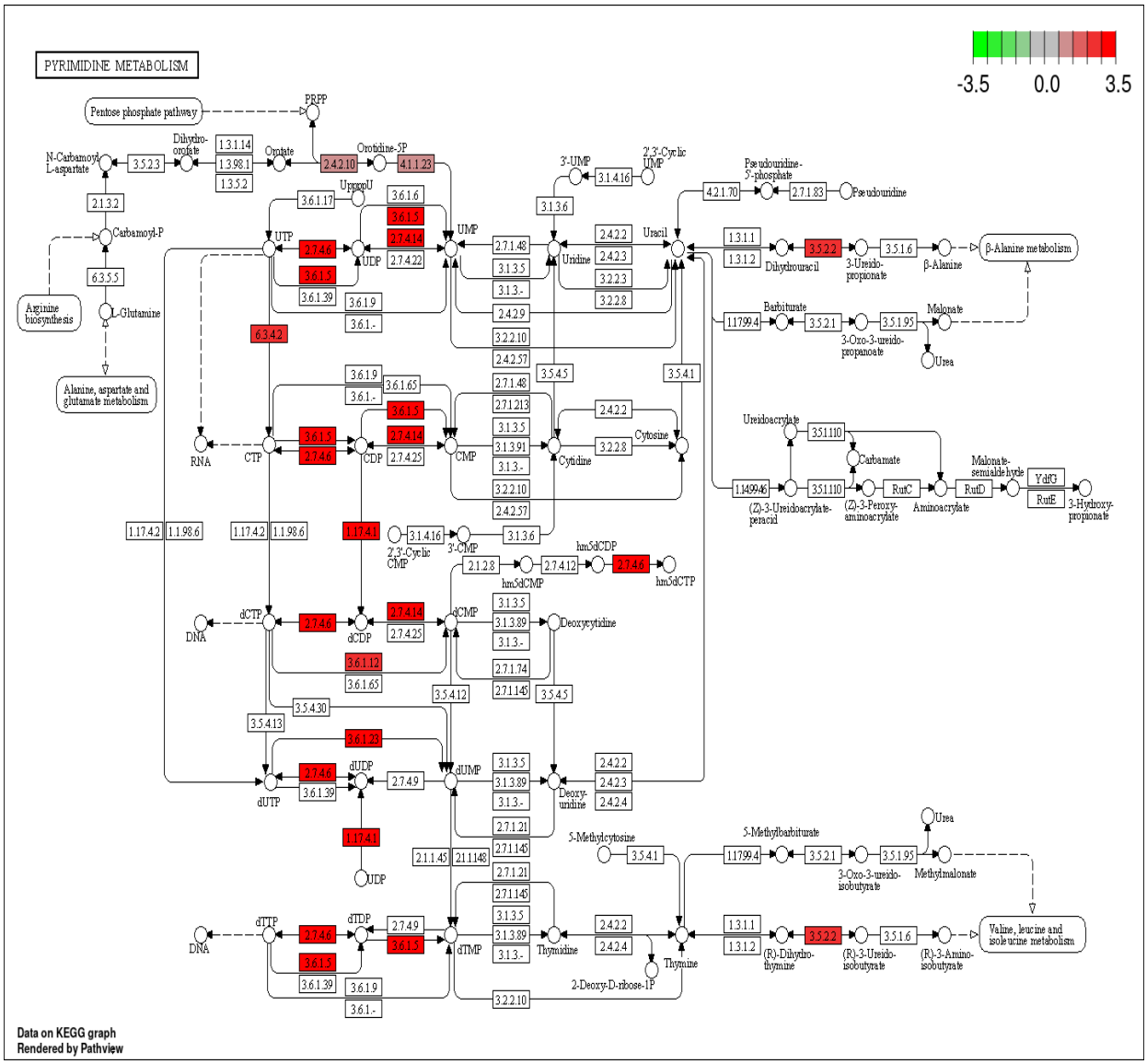


Appendix 12a. Purine pathway enriched with up- regulated genes in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.

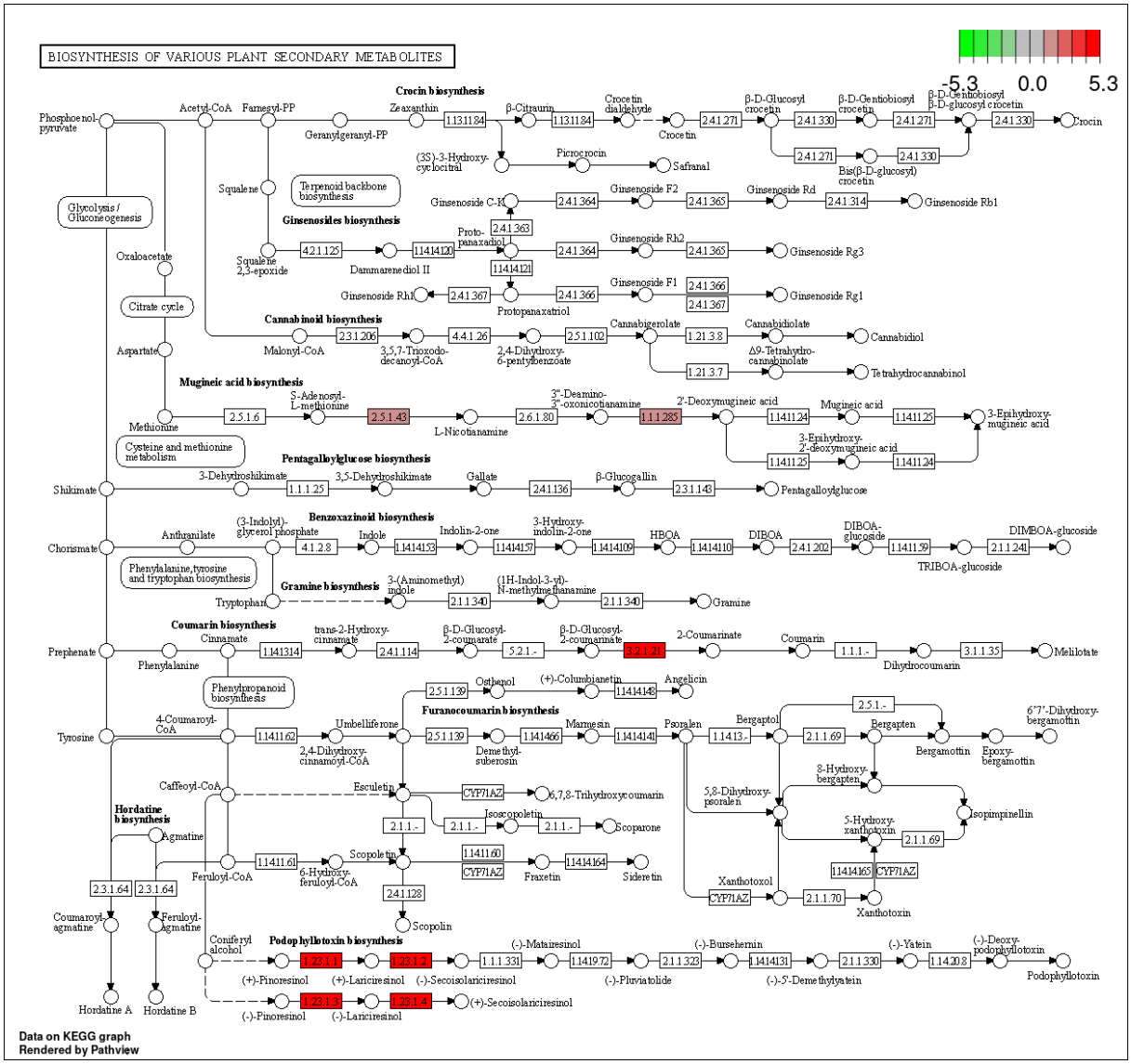
.



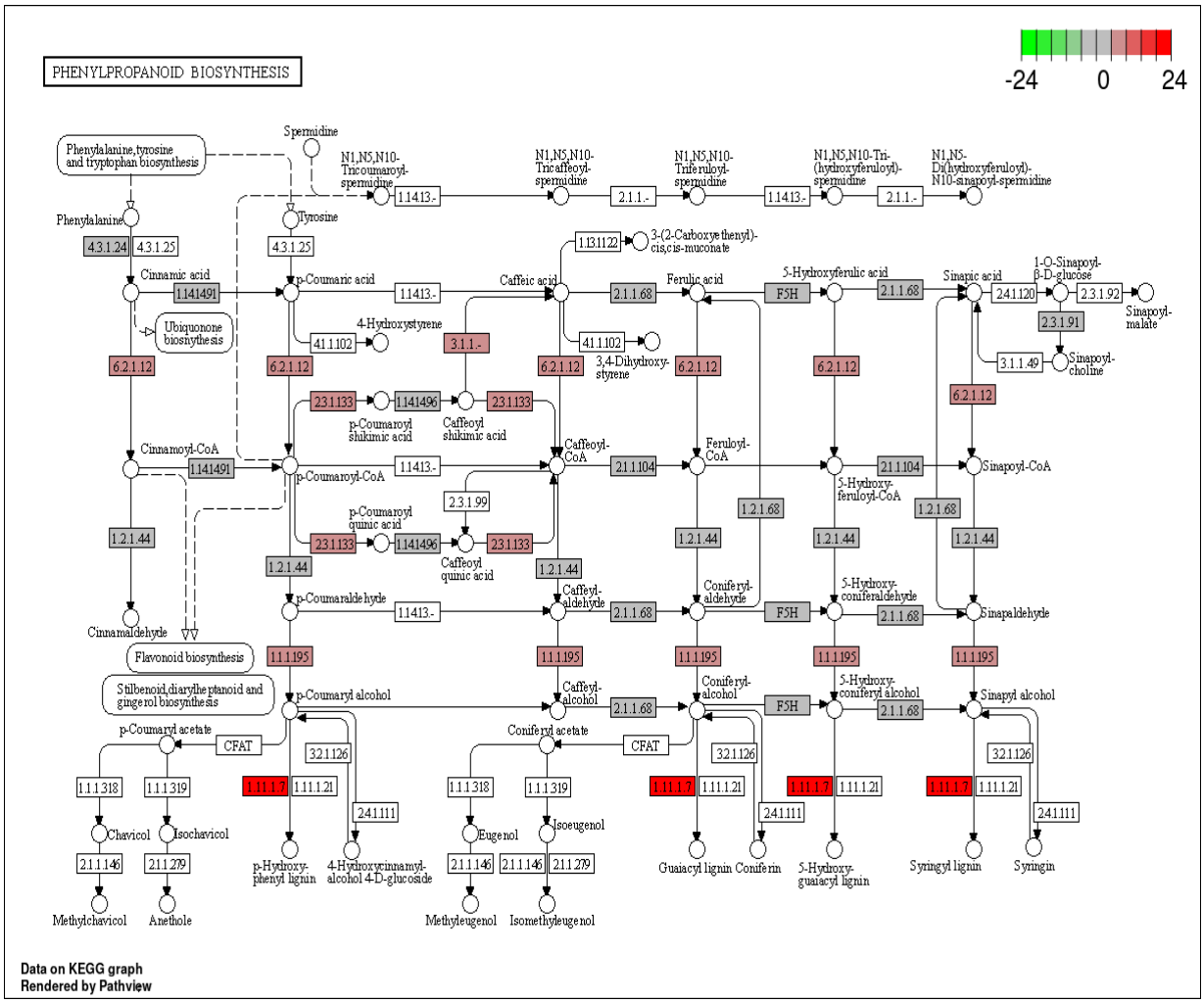
Appendix 12b. Pyrimidine pathway enriched with up- regulated genes in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



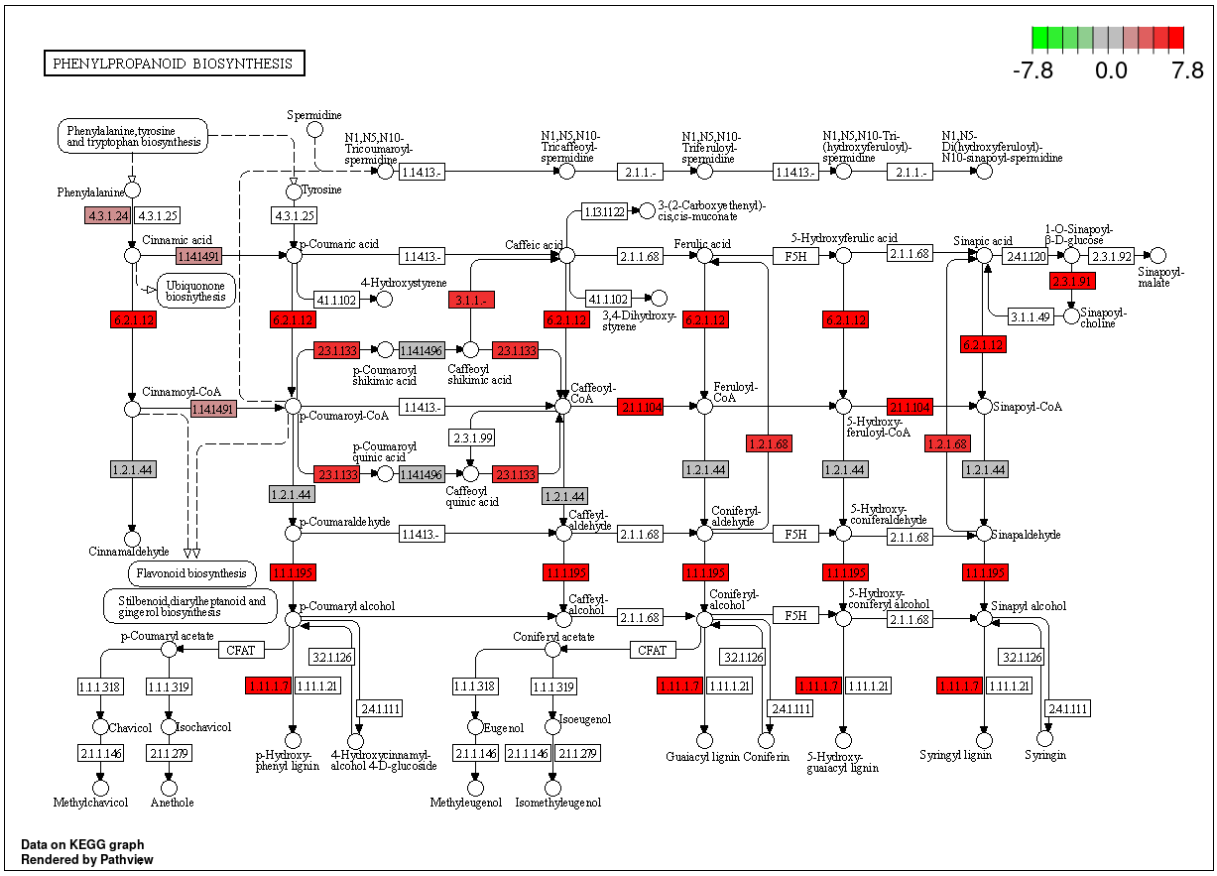
Appendix 13a. Biosynthesis of various secondary plant metabolites pathway enriched with up-regulated genes in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



Appendix 13b. Phenylpropanoid metabolic pathway in wheat cultivar ICARDA (leaves). Red colour = up-regulated genes; green colour = down regulated genes.



Appendix 13c. Phenylpropanoid metabolic pathway enriched with up-regulated genes in wheat cultivar Atlas (leaves. Red colour = up-regulated genes; green colour = down regulated genes.



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