TRANSCRIPTOMIC ANALYSIS OF SEED DORMANCY IN WHEAT

by

Riya Kalota

A Thesis submitted to the Faculty of Graduate Studies

of The University of Manitoba

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg

Copyright © 2024 by Riya Kalota

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Dr. Belay Ayele for his invaluable guidance, unwavering support, and insightful feedback throughout my academic journey. His guidance and mentorship have not only enriched my academic knowledge but has also taught me that striving for perfection is not always necessary rather it is the willingness to learn from mistakes that truly fosters growth and development. I am also deeply thankful to Dr. Marta Izydorczyk and Dr. Santosh Kumar for their valuable contributions as my committee members. Their expertise and valuable suggestions have been incredibly beneficial to me throughout these years.

I owe a special thanks to the postdoctoral fellow, Dr. Deepak Sharma for guiding and directing me during my research. Thanks to all the former and present members of Dr. Ayele's lab, Dr. Anh Pham, Dr. Zahoor, Dr. Murali, Dr. Manisha, Dr. Uzzal, Gurkamal, Ginny, Gurnoor, Rui and Prabhleen for their support and motivation. Thanks for being there!

Finally, I would like to thank all my family members and friends for their encouragement and care throughout this journey. Specifically, I wish to express my heartfelt appreciation to my grandparents, parents and my sister for their unwavering support and love that has been instrumental in my achievement, and I am deeply indebted to them.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Kalota, Riya. M.Sc. The University of Manitoba, February 2024. Transcriptomic analysis of seed dormancy in wheat. M.Sc. supervisor: Dr. Belay T. Ayele.

Wheat production is negatively affected by various factors, including preharvest sprouting (PHS), which is defined as the germination of grains on the mother plant due to humid and rainy weather conditions. Seed dormancy is an adaptive trait that has an influence on the incidence of PHS. The presence of low dormancy in wheat seeds leads to PHS and therefore significant losses in yield and quality. Therefore, it is essential to induce an adequate level of seed dormancy to prevent PHS. To better understand the molecular mechanisms regulating seed dormancy in wheat, this study performed comparative genome-wide transcriptomic analysis between dormant and non-dormant (after-ripened) seeds of a highly dormant wheat genotype. The findings of this thesis projects indicate that after-ripening effectively breaks seed dormancy and causes differential expression of a large number of genes during imbibition. Gene ontology enrichment analysis of the differentially expressed genes (DEGs) was performed to identify the biological processes regulating seed dormancy. The study also examined the expression patterns of abscisic acid (ABA) and gibberellin (GA), the two major hormones that control seed dormancy, and the findings showed close association between the expression patterns of specific genes involved in ABA and GA metabolism and signaling and the level of seed dormancy.

FOREWORD

This thesis is written in manuscript style. A general introduction about the research project and a literature review precedes the manuscript. An abstract, introduction, materials and methods, results and discussion form a complete manuscript. The manuscript is followed by a general discussion and conclusions, a list of references and appendices.

1.0 GENERAL INTRODUCTION

Wheat is one of the most important cereal crops worldwide, serving numerous purposes such as food, feed, and industrial applications. Its agronomic adaptability, convenience of grain storage, ease of converting grain into flour, and unique viscoelastic properties makes it an ideal global food crop (Giraldo et al., 2019; Shavanov and Shigapov, 2020). However, the production and quality of wheat crop is negatively affected by several biotic and abiotic factors. One of the major factors significantly impacting global wheat production is preharvest sprouting (PHS), involving the germination of grains in the spikes prior to harvest due to exposure of rain and high humidity (Gao and Ayele, 2014). PHS is associated with elevated α-amylase activity in seeds, resulting in the breakdown of starch, thereby reducing the overall quality of the wheat grains (Olaerts et al., 2016) and therefore resulting in huge economic losses to farmers and food processors. In Canada, the economic loss associated with PHS is estimated to be approximately $100 million (Clarke et al., 2005).

The phenomenon of PHS is closely linked with seed dormancy, an inherited characteristic that prevents the germination of viable seeds under favourable environmental conditions (Gao and Ayele, 2014). Cereal crops that exhibit insufficient or low level of dormancy at the time of harvest are susceptible to PHS damage (Rodríguez et al., 2015). Dormancy and PHS traits are influenced by several endogenous and exogenous factors such as phytohormones, water, light, temperature, and after-ripening (Finkelstein et al., 2008; Ali et al., 2019). Seed dormancy and germination are primarily regulated by two phytohormones, gibberellin (GA) and abscisic acid (ABA). The plant hormone ABA plays a pivotal role via inducing seed dormancy, and thereby inhibiting germination. In contrast, GA functions as a negative regulator of dormancy as it promotes dormancy release and facilitates the initiation of germination (Finkelstein et al., 2008). Therefore, these two hormones regulate dormancy induction/release and germination through their antagonistic interaction. Previous studies

showed that the expression patterns of ABA and GA metabolism genes as well as their signaling genes are related to dormancy and its decay in wheat seeds (Liu et al. 2013; Kashiwakura et al. 2016; Izydorczyk et al., 2018; Tuan et al., 2021). In addition, the developmental changes from dormancy to germination in wheat seeds due to dormancy breaking factors such as after-ripening are regulated by changes in the expression patterns of genes involved in several other biological processes or molecular functions, including, jasmonate responses, cell wall modification, nitrogen metabolism, carbohydrate metabolism, and histone modifications (Barrero et al., 2009; Gao et al., 2012).

Considering the losses caused by PHS, a comprehensive understanding of the mechanisms governing seed dormancy becomes crucial in developing PHS-resistant wheat cultivars. The use of advanced approaches such as genome-wide transcriptomics and plant materials with diverse genetic background has the potential to provides important information in identifying biological processes, regulatory elements and candidate genes involved in the regulation dormancy. Genome-wide transcriptomic studies use the application of next generation sequencing technologies (NGS) such as RNA sequencing (RNA-seq) that allows to examine differential expression of genes between dormant and non-dormant seeds. As the result, this approach has the advantage of detecting both known and novel transcripts in a single assay (Byron et al., 2016). In addition, availability of the wheat reference genome sequence (International Wheat Genome Sequencing Consortium [IWGSC] RefSeq v1.0 and v2.1) plays an important role in transcriptomic studies of different traits such as dormancy as it facilitates the annotation of differentially expressed genes and identifying the biological processes they represent. The specific objectives of this study are:

1. To identify biological processes/molecular functions involved in the regulation of dormancy release by after-ripening.

2. To investigate changes in the expression pattern of ABA and GA metabolism and signaling genes due to after-ripening.

2.0 LITERATURE REVIEW

2.1 Wheat: a major cereal crop

Wheat (*Triticum aestivum* L., 2n = 6x = 42, genome AABBDD) is among the world's earliest domesticated crop species, first cultivated in southeast Turkey at least 10,000 years ago (Faris, 2014). This prehistoric crop is a staple food for millions of people and is grown on more than 218 million hectares (540 million acres) globally (Giraldo et al., 2019). Wheat evolved through a series of complex processes, including, polyploidization, domestication, natural hybridization, and mutation from its ancient form (einkorn wheat) into the two main types of wheat grown today; bread wheat (*T. aestivum* L.) and durum wheat (*T. turgidum* L.) (Peng et al., 2011; Feldmam and Levy, 2012), with varying characteristics in terms of their genomic content, grain composition, and end use. Wheat is a highly versatile cereal grain that can grow in a wide geographical range, spanning from equatorial to temperate regions, including latitudes as high as 60°N and 44°S, and altitudes up to 3000 meters above sea level (Enghiad et al., 2017; de Sousa et al., 2021). This adaptability is a result of the genetic diversity present within the wheat gene pool. Wheat is conventionally categorized into two distinct types: winter wheat, which requires vernalization (planted in the fall and harvested in the summer) and spring wheat without this requirement (planted in the spring and harvested in late summer) (Yan et al., 2015).

Wheat has diverse applications and is used for various purposes, including human consumption, animal feed, biofuel production, and bioplastics. Over two-thirds of global wheat is consumed as food, with 20% used for livestock feed, and 3-5% each allocated for seed, industrial use, and other purposes (Shiferaw et al., 2013). It contributes substantially to global food security, providing a principal source of nutrition for approximately 36% of the world's population (Grote et al., 2021). Wheat accounts for around 55% of the carbohydrates and 21% of the dietary calories consumed globally (Khalid et al., 2023). Additionally, the demand for

wheat is increasing globally due to the unique properties of gluten proteins found in it. Gluten facilitates the production of processed foods, which are becoming more popular with the global industrialization process and westernization of diets. Wheat endosperm proteins, specifically gliadins and glutenins, are the main components of gluten in wheat flour (Dhaka and Khatkar, 2015). These proteins play a crucial role in breadmaking, as they determine the elasticity and extensibility of the dough to be processed into various bakery products, including bread, cakes, biscuits, pasta, and more (Peña, 2002).

Global wheat production has grown significantly since the 1990s. Global yield averages increased from just a little over 1 ton/ha in the early 1960s to around 3.5 tons/ha currently (Erenstein et al., 2022). In 2022, the global production of wheat amounted to over 781 million metric tonnes, exhibiting an increase as compared to the previous marketing year (Erenstein et al., 2022). However, food insecurity is the biggest concern because of the influence of climate change on wheat production at the global, regional, and local levels. The current population growth is expected to increase wheat demand from 700 million tonnes in 2021 to over 900 million tonnes in 2050 (Grote et al., 2021). Therefore, continued research efforts are needed to improve the yield and productivity of wheat, which includes developing new wheat varieties with improved resistance to diseases and pests, implementing sustainable agricultural practices to conserve water and maintain soil fertility, and promoting genetic diversity in wheat crops.

2.2 Wheat production in Canada

Wheat cultivation in North America, particularly in Canada, has a long history dating back to the late 15th and 16th centuries. The cultivation of wheat in Canada was first initiated by French settlers at Port Royal, Nova Scotia, in 1605, with the first exports occurring in 1654 (Hopper, 1923; Campbell, 2015). Early wheat farming in Western Canada faced challenges due to unsuitable cultivars and harsh winters. At first, various trials were conducted using the

European cultivars, but some could not survive severe winters, while others matured too late for the short growing season. In 1812, a larger plantation was initiated by settlers from Scotland in Selkirk, Manitoba. They brought wheat seeds from their home nations for cultivation in Canada, but their attempts were failed as it faced serious agronomic challenges including late maturity, susceptibility to frost damage, lodging, stem rust, and shattering tendency (Buller, 1919; Newman 1928; McCallum and DePauw, 2008). Later, a Ukrainian cultivar, Red Fife gained popularity in Canada for its high productivity and good milling and baking qualities (Symko, 1999). However, it also suffered from freezing due to early frosts and it is believed that Red Fife is the Galician cultivar from central Europe (Symko, 1999). Later, Sir Charles Saunders developed the Marquis cultivar by crossing Hard Red Calcutta with Red Fife in 1904 (Symko, 1999). It was distributed in 1909 and gained rapid popularity across Canada for excellent yield and superior milling and baking qualities (Morrison, 1960; Morrison, 2008). Western wheat production soared from 2 million tonnes in 1904 to 7.7 million tonnes in 1913 (Campbell, 2015). Red Fife and Marquis established Canada's reputation for high-quality hard red spring wheat (McCallum and DePauw, 2008). The cultivation of hard red spring wheat further contributed to the growth of the wheat industry in Canada.

Though Canadian wheat production has fluctuated considerably in recent years, it has shown a steady increase from 1971 to 2020, reaching 35 million tonnes in 2020 (Statistics Canada, 2021). However, despite the extreme dry heat conditions that occurred during the 2021 growing season, Canadian farmers produced about 22 million tonnes of wheat, and Manitoba accounted for 17% of total production (Government of Manitoba, 2021). The prairie provinces, comprising Alberta, Saskatchewan, and Manitoba, represent the most important agricultural areas in Canada. In year 2022, Saskatchewan and Alberta produced 14.8 and 11.3 million tonnes of the wheat, respectively, while Manitoba produced 4.8 million tonnes (Statistics Canada, 2022a). Canadian farmers reported planting approximately 25.4 million acres of wheat

in 2022, which reflects an increase of 8.7% compared to the previous year (Statistics Canada, 2022b). Consistently, Canada's wheat production surged by 51.7% in 2022, reaching 33.8 million tonnes, representing a significant increase in production as compared to 2021 (Statistics Canada, 2022a).

2.3 Evolution and origin of wheat

The evolution of wheat from wild species to modern cultivars was a complex process that involved multiple hybridization events, genetic mutations, and environmental pressures. Genetics and archaeological studies suggest that the origins of modern wheat can be traced back to the Karacadag mountain region in southeastern Turkey (Heun et al., 1997). Emmer and einkorn wheats are indeed two of the eight founder crops that marked the beginning of agriculture, along with barley, lentils, peas, chickpeas, bitter vetch, and flax (Abbo et al., 2013). Wild emmer wheat (*T. dicoccoides*, 2n = 4x = 28, genome AABB) is a tetraploid derived from wild diploid wheat (*T. urartu*, 2n = 2x = 14, genome AA) and Goat Grass 1 (*Aegilops speltoides*, 2n = 2x = 14, genome BB) through a hybridisation event around 0.5 million years ago (Dvorak and Akhunov, 2005; Peng et al., 2011; Rahman et al., 2020). The earliest documented use of emmer dates to about 23,000 years ago, when it was collected from wild areas by inhabitants of the Ohalo II archaeological site in Israel (Feldman and Kislev 2007). Later, subconscious selection gradually led to the development of a cultivated emmer (*T. dicoccum*, 2n = 4x = 28, genome AABB) that underwent spontaneous hybridization with Goat Grass 2 (*Ae. tauschii*, 2n = 2x = 14, genome DD) around 9,000 years before present (BP), leading to the emergence of an early spelt (*T. spelta*, 2n = 6x = 42, genome AuAuBBDD) (Matsuoka and Nasuda, 2004; Peng et al., 2011). Early findings indicated that the cultivation of emmer wheat may be traced back to the southern Levant, including sites such as Netiv Hagdud and Tell Aswad, both of which are Pre-Pottery Neolithic A sites (Feldman and Kislev 2007).

Around 8,500 years ago, a natural mutation occurred in both emmer and spelt wheat that changed the ears to a more easily threshed type (Peng et al., 2011) This mutation was advantageous for farmers as it made the process of separating the wheat grain from the chaff easier. Over time, this trait was selected for and became fixed in some populations, which eventually evolved into free-threshing durum (*T. durum*, 2n = 4x = 28, genome AABB) and bread wheat (*T. aestivum*, 2n = 6x = 42, genome AABBDD) (Peng et al. 2011). Overall, the evolution of wheat from wild species to modern cultivars was a gradual process that involved both natural and human-driven factors. Over time, wild grasses were domesticated and selected for traits that were advantageous for human consumption and agricultural production. Einkorn, on the other hand, is found in the northern Levant, including sites such as Abu Hureyra in Syria. The wild ancestor of einkorn is believed to be *T. boeoticum*, a wild grass that grows in the same region (Faris, 2014).

2.4 Domestication of Wheat

Wheat was domesticated around 10,000 years ago in the Fertile Crescent, which includes parts of the modern Middle East. This period, which is known as the "Neolithic revolution" or the "agricultural revolution," marks a significant transition from a lifestyle primarily based on hunting and gathering to one that is more centred around sedentary agriculture. During the domestication process of wheat, the plant's phenotype has experienced both rapid changes (in a few hundred years), and slow changes (over thousands of years). These changes include wheat's primary domestication trait of non-shattering, and secondary traits that include glume reduction to enhance threshing, changes in plant architecture and size of ear and kernel, dormancy loss, and decrease in grain protein and increase in grain carbohydrate content (Flint-Garcia, 2013). According to historical records of wheat evolution, it is widely accepted that

only wild einkorn and wild emmer wheat went through the process of early domestication selection (Peng et al., 2011).

The domestication of diploid einkorn wheat (*T. monococcum*) from its wild progenitor, *T. boeoticum*, is considered one of the earliest examples of crop domestication in the Fertile Crescent. Wild einkorn, a type of diploid wheat, was widely distributed across the Middle East, often growing as a weed near roadsides and fields (Zohary et al., 2012). The domestication process of einkorn wheat was facilitated by the occurrence of a genetic mutation that led to a non-brittle rachis (Faris, 2014). This trait revolutionized the harvesting process, enabling more efficient gathering of grains. Research utilizing amplified fragment length polymorphism (AFLP) DNA fingerprinting identified the Karacadag region in southeastern Turkey, located in the northern Levantine Corridor, as the probable site of einkorn domestication (Heun et al., 1997; Faris, 2014). Today, einkorn wheat is grown as a relic crop in certain Mediterranean countries for animal feed (Faris, 2014). Domestication of emmer wheat represents another key milestone in agricultural history, as it is the only true wild polyploid wheat of the lineage and the ancestor of modern durum and bread wheat varieties (Faris, 2014). The specific location of emmer wheat domestication has been a subject of debate, but genetic evidence points to the southern Levant and southeastern Turkey as possible domestication centres (Faris, 2014). It is estimated that domesticated emmer wheat types with naked, free-threshing grains emerged in these regions around 9,500 to 9,000 BP (Peng et al., 2011). For over a millennium since its domestication, emmer wheat was cultivated and grown alongside wild emmer wheat in a complex cropping system at numerous Levantine sites (Feldman and Kislev, 2007; Rahman et al., 2020). This coexistence facilitated spontaneous and uncontrolled hybridizations, resulting in the transfer of genes including the non-brittleness gene, between the wild and domesticated populations (Feldman and Kislev, 2007, Rahman et al., 2020). Subsequently, domesticated emmer wheat exhibited polymorphic populations with

a wide range of genetic diversity and traits (Feldman and Kislev, 2007). While the diploid and tetraploid species einkorn and emmer experienced most of the morphological changes related to domestication, neither of them is considered a significant crop in present times. Instead, they have been replaced by the allohexaploid species known as *T. aestivum*. Today, modern wheat varieties of *T. aestivum* are widely distributed and serve as a primary source of calorie globally.

2.5 Factors affecting wheat production

Wheat is widely recognized as the "King of Cereals" due to its significant global importance as a staple crop. However, the global wheat production is facing threats from both abiotic and biotic stresses. Abiotic stress refers to environmental factors that negatively affect plant growth and productivity. On the other hand, biotic stress refers to biological factors like diseases, insects, and pests that can harm crop plants (Abdulbaki et al., 2022). These biotic and abiotic stress factors induce physiological and metabolic changes in plants, thereby affecting plant growth. In general, wheat cultivation around the world faces a range of abiotic stresses such as drought, salinity, extreme temperatures and preharvest sprouting (PHS) (Mamrutha et al., 2022). For example, in 2021, wheat production in Canada experienced a significant decline of 38.5 percent, reaching 21.7 million tonnes, as compared to its production in the previous year, 2020 (Statistics Canada, 2021). This decline in wheat yield is attributed primarily to the adverse effects of extreme hot and dry weather conditions in the Prairie regions during the 2021 growing season. PHS is another major abiotic constraint that affects the global production of high-quality grains. Worldwide losses associated with PHS have been estimated at more than a billion dollars annually (Derera, 1990). In Canada, PHS is estimated to cost the wheat industry an average of $100 million loss (Clarke et al., 2005; Miao et al., 2013). Therefore, effective and reliable methods of introducing PHS resistance in wheat cultivars play critical roles in improving wheat yield quality.

2.6 Preharvest sprouting in wheat and evaluation methods

PHS is defined as the germination of grains in the spikes before harvest due to exposure to rain and high humidity (Gao and Ayele, 2014). PHS affects the production of wheat around the world including Australia, Canada, China, Germany, India, Japan, and the USA (Derera, 1990; Zhang et al., 2014; Dhariwal et al., 2021). It causes significant reduction in grain yield, test weight, quality, and seed viability and vigour, resulting in major economic losses. The yield and quality loss due to PHS is associated with its effect in activating hydrolytic enzymes such as α-amylase, lipases, and proteases in the seeds, causing a cascade of physiological events (Olaerts et al., 2016). The increased enzyme activity causes the degradation of carbohydrates and proteins stored in the endosperm, lowering yield and test weight. Grain yield reductions dur to PHS take place during threshing as the plumules, radicles, and lightweight kernels are separated out by winnowing (Derera, 1979). The enzymatic breakdown of starch granules also produces a honeycomb-like structure, which reduces gelation and pasting ability, and affects grain texture and end product quality (Naguleswaran et al., 2012).

There are various visual symptoms of PHS, including premature kernel swelling, germ discolouration, seed coat splitting, and root and shoot emergence from germinating seeds (Groos et al., 2002; Simsek et al., 2014). However, relying solely on visual estimation to determine sprouting damage in wheat is not reliable since a significant amount of the damage occurs before the germination of grains becomes visible (Jensen et al., 1984; Barnard et al., 2005). As a result, several accepted indicators and evaluation methods of PHS have been developed. PHS is characterized by four primary characteristics: Hagberg falling number, sprouting index, weighted germination index, and germination rate (Cabral et al., 2014; Miao et al., 2019; Tai et al., 2021). A low falling number suggests high amylase activity, which is a sign of low grain quality (Ral et al., 2016; Newberry et al., 2018; Tai et al., 2021). On the other hand, sprouting index, weighted germination index, and germination rate values are directly

correlated with PHS (Nakamura et al., 2011; Lin et al., 2015; Abe et al., 2019; Tai et al., 2021). Germination tests conducted using individual seeds or intact spikes only provide information about the grain's ability to germinate. Even if some grains do not exhibit obvious signs of germination, they may still exhibit high α -amylase activity in the endosperm that induces starch degradation. The falling number test serves as a preferred method for assessing α-amylase activity and PHS damage that degrade quality (Wang, 2021). Falling number test is based on the viscosity of a mixture consisting of flour derived from sampled grains and water. It is important to note that this assessment does not distinguish damage due to other potential factors, such as late-maturity α-amylase (LMA). Low falling number indicates starch degradation due to increased α-amylase, leading to poor end use quality (Wheat Grain Quality - Falling Number and Pre-Harvest Sprouting Resistance, 2022).

The occurrence of PHS is influenced by a variety of environmental and genetic factors. The genetic factors influencing PHS are linked to various developmental, physiological, and morphological characteristics of the spike and the seed, including pericarp colour and permeability to water and/or oxygen, seed dormancy, enzymatic activity such as that of α-amylase and levels and signaling of plant hormones ABA, and GA, all of which play a role during this process (Ali et al., 2019; Sohn et al., 2021). Other factors such as hairiness, ear morphology, waxiness, and germination inhibitory compounds have also been shown to be associated with resistance/susceptibility to PHS (King and von Wettstein-Knowles, 2000; Gatford et al., 2002; Ali et al., 2019; Sohn et al., 2021). Previous studies have reported the presence of water-soluble compounds in the husks of *T. aestivum* and *T. tauschii*, as well as in the endosperm of *Uniola paniculata* (Westra and Loomis, 1966; Gatford et al., 2002; Kato et al., 2002; Duclos et al., 2013). A study conducted in barley revealed that the glaucous wild type lines exhibit higher water repellency, leading to reduced wetting and a significant decrease in-ear sprouting as compared to non-glaucous or less waxy lines (King and von Wettstein-

Knowles, 2000). Among all these factors, seed dormancy is the primary genetic factor that controls PHS, and it contributes to about 60% of variation in PHS resistance (DePauw and McCaig, 1991; Cheng et al., 2021). Therefore, researchers have been interested in comprehending the molecular mechanisms of seed dormancy and therefore developing molecular tools for improving PHS resistance.

2.7 Seed development

The development of seed is a crucial stage in the life cycle of flowering plants, ensuring the survival and dispersal of the species. In angiosperms, a mature seed consists of an embryo along with other filial structures like cotyledons and endosperm that are filled with stored nutrients (Dante et al., 2014; Sabelli, 2012). These structures support embryo development and in certain species, assist in seedling growth during germination (Sabelli, 2012).

The process of seed development follows distinct sequential phases that are necessary to form a fully functional reproductive structure (Sabelli, 2012). Typically, seed development in angiosperms begins with a double fertilization process, where one of the two haploid pollen nuclei fertilizes the haploid egg cell, and the other one fertilizes the diploid central cell within the female gametophyte in the ovule (Sabelli, 2012). This syngamic process produces a diploid zygote that later develops into the embryo and triploid primary endosperm nucleus, respectively (Sabelli, 2012). After double fertilization, the process of early morphogenesis starts with the division of the zygote into the apical and basal cells in the embryo sac, which gives rise to the embryonic shoot and root systems, respectively (Sabelli, 2012). Endosperm development involves three phases: syncytial, cellularization and differentiation. During the syncytial phase, the endosperm goes through several rounds of division without cytokinesis, which leads to the formation of a multinucleate cell called a syncytium. Once the syncytial phase ends, the endosperm undergoes a transformative process known as the cellularization

phase. This crucial stage involves the division of the syncytium into individual cells by forming cell walls. Following this phase, the endosperm cells differentiate into a variety of cell types, each with a specific function to support the embryo's growth and development. The endosperm of cereal grains is composed of four main cell types: transfer cells, aleurone cells, starchy endosperm cells, and embryo-surrounding region (ESR) cells (Sabelli and Larkins, 2009). Upon completing morphogenesis and patterning stages, both embryo and endosperm enter a maturation phase which comprises accumulation of storage reserves, dormancy induction, arrest of embryo growth, desiccation-induced water loss and acquisition of desiccation tolerance (Bewley and Black, 1994).

2.8 Seed germination and the associated physiological events

Seed germination is a critical stage in plant development and plays a crucial role in determining plant productivity. It is the process by which a seed begins to grow and develop into a seedling. The process of seed germination begins with the absorption of water by the seed, and it is completed with protrusion of the radicle through the covering structures (Bewley, 1997). Seed germination is characterized by three distinct phases of water uptake, known as triphasic water uptake. Phase I is marked by the rapid uptake of water by the dry seed, which causes significant changes in the seed's physical characteristics, including swelling and changes in seed shape. As seeds absorb water, their cell membranes undergo structural changes, resulting in the instant release of solutes and low molecular weight metabolites into the imbibition media (Bewley, 1997). However, after a brief period of hydration, the membrane structure undergoes repair and is restored to its functional state. This phase of water uptake is marked by the activation of respiratory process and the synthesis of proteins using pre-existing mRNAs (messenger ribonucleic acid) in the dry seeds. In addition, first phase of water uptake involves the repair

of cellular components including cell membranes, organelles, DNA (deoxyribonucleic acid) and mitochondria which have been damaged during the desiccation phase (Bewley, 1997).

Phase II of water uptake begins when the rate of water absorption by the germinating seed slows down and reaches a stable level of water content. Metabolic activities shift from those associated with seed development to those involved in germination. Several events occur during this stage of water uptake such as DNA restoration, synthesis of new mitochondria and proteins, embryo growth and softening of seed covering layers (Bewley, 1997). The emergence of the radicle through the testa or seed coat signifies the transition from phase II to phase III of water uptake. Phase III marks post germination stage which is distinguished by the mobilization of reserves stored in storage organs like the endosperm in cereal crops. This mobilization of storage reserves leads to increased water uptake and subsequent growth of the seedling (Nonogaki et al., 2010). Furthermore, the third phase includes important processes such as DNA synthesis, cell division, and radicle cells elongation (Bewley, 1997; Nonogaki et al., 2010). Dormant seeds do not enter the third stage of water uptake because they do not complete germination.

2.9 Seed dormancy

Seed dormancy is the inability of seeds to germinate under conditions that are favourable for germination (Gao and Ayele, 2014). In general, dormancy is an advantageous adaptive trait since it prevents seeds from germinating when environmental conditions are temporarily favourable but unsuitable for seedling establishment and growth. It is widely recognized as a beneficial mechanism that increases the chances of survival of plant species by optimizing the distribution of germination in both time and space. (Foley and Fennimore, 1998; Nautiyal et al., 2023). The question of whether a seed should remain dormant or germinate under specific environmental conditions is of great importance in two ways: firstly, it determines the species

ability to survive under specific ecological conditions, and secondly, it has economic and agricultural implications (Mbi et al., 2022). From an ecological perspective, dormancy is a crucial survival mechanism that facilitates the propagation and dispersal of seeds to establish plant populations (Hilhorst, 1995). Additionally, it allows for heterogeneous and asynchronous seed germination over years, which aids in the survival of certain species. This is because individual seeds in a seed population typically have varying levels of dormancy, causing their germination to be spread out over time. Dormancy induced delay in germination helps in avoiding unfavourable environmental conditions, such as drought and reduces intra-specific competition for ecosystem resources (Mbi et al., 2022).

Seed dormancy is an important survival mechanism for wild plants in their natural environment; however, strong dormancy is undesirable trait in the production of many field crops because it negatively affects rapid and uniform germination and seedling establishment. As a result, domestication of cereal crops from the wild ancestors was aimed at promoting a uniform and rapid seed germination and seedling establishment (Simpson, 1990). Moreover, many industrial processes involving seed germination, such as malting and sprouting, require grains with low levels of dormancy to ensure a uniform germination (Gubler et al., 2005). However, this resulted in a low level of dormancy in the seeds, thereby, favouring PHS, which is a serious problem that affects grain yield and quality as well as seed viability and vigour (Rodríguez et al., 2015). Therefore, all these problems linked with low level of seed dormancy highlight the importance of incorporating a moderate or an intermediate level of dormancy into superior wheat cultivars.

2.10 Types of seed dormancy

Dormancy can be categorized into primary and secondary categories based on the timing of development. The phenomenon of primary seed dormancy occurs when the seed is released

from the mother plant already in a dormant state (Buijs, 2020). Secondary dormancy occurs when the seed becomes dormant after being released from the mother plant due to unfavourable environmental factors (Buijs, 2020). For example, induction of secondary dormancy due to high temperature and hypoxia occurs in seeds of numerous cereal species (Grahl, 1965; Simpson, 1990).

In 2004, Baskin and Baskin proposed a hierarchical classification system for primary seed dormancy based on a comprehensive system developed by seed physiologist Marianna G. Nikolaeva in 1969 (Baskin and Baskin, 2004). This system is suggested as an internationally acceptable classification of seed dormancy and includes modifications to Nikolaeva's original system. The modified system comprises three hierarchical layers: class, level, and type; where a level can only contain types and a class can contain both levels and types (Baskin and Baskin, 2004). This system contains five different classes of dormancy: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY), combinational dormancy (PY+PD) (Baskin and Baskin, 2004). PD is primarily controlled by physiological processes within the seed, and it occurs when a dormant embryo requires specific environmental conditions such as temperature or light to break dormancy and initiate germination. Furthermore, PD consists of three levels, which includes deep physiological dormancy (PD-D), non-deep physiological dormancy (PD-N) and intermediate physiological dormancy (PD-I). PD-N is considered as the most common type of seed dormancy and is found in gymnosperms as well as all major clades of angiosperms. While MD is common in seeds that have underdeveloped embryo. However, it is important to note that these embryos are not physiologically dormant rather they require more time to mature before the seed can germinate. In contrast to MD, MPD is characterized by embryos that are physiologically dormant and require a significantly long period of dormancy-breaking treatment. PY is characterized by the presence of water impermeable seed coat ‘testa’ and fruit

coat ‘pericarp’, while combinational dormancy is governed by both the physical and physiological characteristics of the seed (Baskin and Baskin, 2004).

PD-N is the most common type of seed dormancy (Baskin and Baskin, 2004). There are two types of PD-N, embryo and seed coat-imposed dormancy, based on the cause of dormancy (Bewely and Black, 1994). Seed coat-imposed dormancy occurs due to structures surrounding the embryo such as endosperm, pericarp or seed coat and extra floral organs, which affect the absorption of water and gases that are essential for germination. Seed coat dormancy is also associated with seed coat colour. Several studies have shown that the red grained wheat genotypes have a higher level of dormancy than the white grained genotypes, suggesting the presence of genes for grain dormancy in the pericarp/seed coat of red grained wheats (Zhang et al., 2008). Embryo dormancy is mainly related to endogenous hormonal balance and the plant hormone ABA play an important role in the induction and maintenance of seed dormancy (Derera et al., 1977; Rodríguez-Gacio et al., 2009).

2.11 Regulation of seed dormancy and germination

2.11.1 Plant hormones

Phytohormones are molecules that are produced in plants at very low concentrations and regulate plant growth and development as well as adaptation to adverse environmental conditions (Smith et al., 2017). Plant hormones produced by plants include ABA, GA, auxins, cytokinins (CK), ethylene, salicylic acid (SA), jasmonates (JA), brassinosteroids (BR) and strigolactones (Verma et al., 2016; Wani et al., 2016). Among these, GA and ABA are considered the major regulators of seed dormancy and germination (Tuan et al., 2018). Previous studies reported the importance of both metabolic and signaling pathways of these two hormones and their interplay in the regulation of dormancy and germination in cereal seeds.

2.11.1.1 ABA Metabolism

ABA, a 15-carbon terpene molecule, was discovered in the 1960s and recognized as a growth-inhibiting substance linked to fruit abscission in cotton and bud dormancy in maple, which was referred to as dormin and abscisin II, respectively (Cutler, 2005). ABA biosynthesis takes place mostly in the plastid and partly in the cytosol (Seo and Koshiba, 2002; Cutler et al., 2010). ABA is synthesised by the cleavage of C40 epoxycarotenoid precursors into an intermediate xanthoxin, which is transformed into ABA via ABA-aldehyde. Carotenoids, like other isoprenoids, are derived from isopentenyl diphosphate (IDP), which is converted through a series of reactions to geranylgeranyl pyrophosphate (GGPP) (Seo and Koshiba, 2002; Nambara and Marion-Poll, 2005). GGPP then forms phytoene, a C40 molecule that serves as the precursor for the synthesis of a wide variety of carotenoids including lycopene, beta-carotene, and zeaxanthin (Seo and Koshiba, 2002). The process of ABA biosynthesis from zeaxanthin involves three major steps. The first step involves the epoxidation of zeaxanthin to violaxanthin by the enzyme zeaxanthin epoxidase (ZEP) and this reaction can be reversed by violaxanthin de-epoxidase (VDE) to form zeaxanthin (Marin et al., 1996; Nambara and Marion-Poll, 2005). Violaxanthin then forms 9'-*cis*-violaxanthin and 9'-*cis*-neoxanthin by neoxanthin synthase (NSY) and an unknown isomerase. The next step of ABA biosynthesis involves the conversion of the cis-isomers of violaxanthin and neoxanthin into xanthoxin, a C15 precursor molecule and a C25 metabolite, respectively, by 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Schwartz et al., 1997; Schwartz et al., 2003). NCED is considered as the key rate-limiting enzyme in ABA biosynthesis and facilitates the catalysis of the first irreversible step in the ABA biosynthetic pathway (Qin and Zeevaart,1999; Thompson et al.,2000; Endo et al., 2008). The third regulatory step involves the transportation of xanthoxin to the cytosol, where it is transformed into ABA-aldehyde by ABA deficient 2 (ABA2), an enzyme that belongs to short-chain dehydrogenase/reductase family (Seo and Koshiba, 2002; Nambara and Marion-Poll,

2005). Finally, ABA-aldehyde oxidase 3 (AAO3) catalyses the oxidation of ABA-aldehyde to form active ABA (Nambara and Marion-Poll, 2005). The rate of catabolism in plants also plays a role in determining the level of bioactive ABA. ABA is catabolized mainly by ABA 8'-hydroxylase, which catalyzes ABA hydroxylation at the 8′ position (Saito et al., 2004; Schwartz et al., 2003).

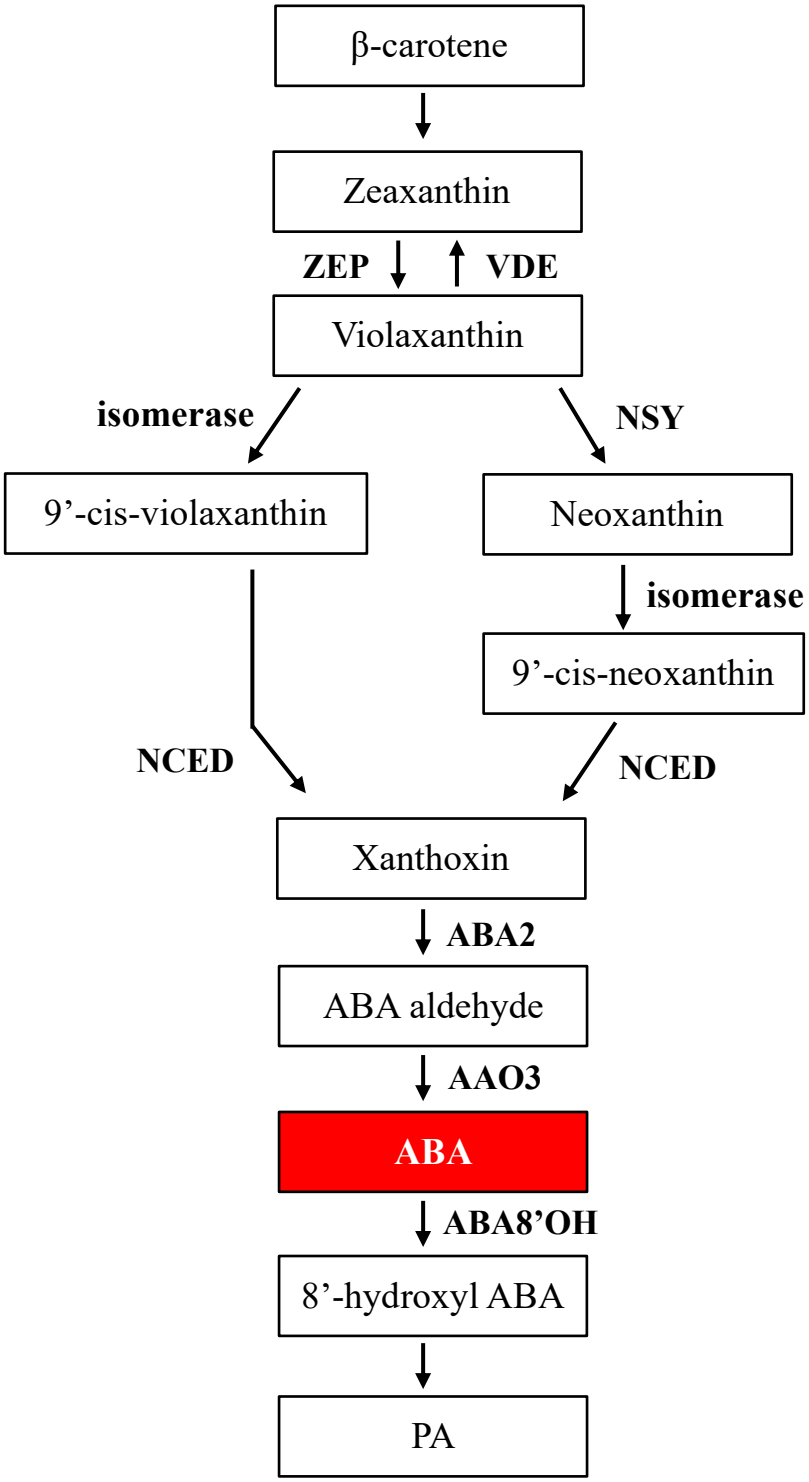


Figure 2.1. ABA biosynthesis and catabolism pathway in plants. ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NSY, Neoxanthin synthase; NCED, 9-cis-epoxycarotenoid dioxygenase; ABA2, ABA deficient 2; AAO3, abscisic aldehyde oxidase 3; ABA8'OH, ABA 8′ hydroxylase; PA, Phaseic acid. (Adapted from Nambara and Marion-Poll, 2005).

2.11.1.2 Role of ABA metabolism in the regulation of seed dormancy

ABA plays an important role in the regulation of seed dormancy and germination. However, it is important to note that only ABA de novo synthesized in zygotic tissues during the later stages of seed maturation plays a predominant role in inducing dormancy (Tuan et al., 2018). In wheat, there are two distinct peaks of embryonic ABA accumulation during seed development, which take place during the middle and later stages of seed maturation (Suzuki et al., 2000; Tuan et al., 2018). The first peak appears approximately 25 days after pollination (DAP) and is associated with the development of ABA sensitivity in embryo. However, the first peak alone is not sufficient to induce dormancy because seeds which exhibit high ABA levels only around 25 DAP are non-dormant. The second peak occurs around 35 DAP and persists until 40 DAP in dormant wheat lines, demonstrating the importance of ABA in the induction and maintenance of seed dormancy during the middle to late stages of seed maturation (Suzuki et al., 2000; Ali et al., 2019). A previous study has shown that imbibition of mature barley seeds decreases ABA levels in both dormant and non-dormant seeds (after-ripened), but the decrease in ABA level is more in non-dormant seeds, leading to their germination (Millar et al., 2006)

Research has established a direct correlation between changes in seed ABA levels and expression profiles of the *NCED* and *CYP707A* in seeds of several species such as Arabidopsis, barley, maize and wheat (Finkelstein et al., 2008; Nambara et al., 2010; Tuan et al., 2018). So far, two *NCED* genes (*TaNCED1* and *TaNCED2*) have been identified in wheat and the expression pattern of these genes are known to be correlated with seed dormancy (Zhang et al., 2014; Son et al., 2016; Izydorczyk et al., 2018). For instance, a study in wheat showed higher expression of *TaNCED2* in imbibed seeds of the dormant genotype as compared to those from a non-dormant genotype (Son et al., 2016). Another study showed imbibing wheat seeds at supraoptiomal temperature inhibits germination by increasing embryonic ABA level via upregulation of ABA biosynthetic genes (*TaNCED1* and *TaNCED2*) (Izydorczyk et al., 2018).

Additionally, ABA catabolism, catalysed by ABA8′OH and encoded by cytochrome P450 monooxygenase 707A (*CYP707A*) gene family play a vital role in regulating ABA level (Okamoto et al., 2006). For example, mutations in the two homeologs of *ABA80OH* in wheat (*TaABA80OH1A and TaABA80OH1D*) led to a rise in ABA level in the embryos during the mid and late phases of seed development (40–60 DAA), which results in a higher level of seed dormancy (Chono et al., 2013; Tuan et al., 2018). In wheat, two *CYP707A* (*TaCYP707A1* and *TaCYP707A2*) genes have been identified with their expression pattern linked to seed dormancy (Chono et al., 2013; Iehisa et al., 2014; Kashiwakura et al., 2016; Son et al., 2016; Izydorczyk et al., 2018). Imbibition of wheat seeds at low temperature (15 °C) leads to release of dormancy due to a reduction in embryonic ABA level via increased expression of *CYP707A* genes (Kashiwakura et al., 2016). Another study showed imbibing wheat seeds at suboptimal temperatures, inhibits germination by increasing ABA level via upregulation of *TaNCEDs* and downregulation of *TaCYP707A* in both embryo and endosperm (Izydorczyk et al., 2018). Maintenance of dormancy in imbibed wheat seeds is also reported to be mediated by the expression patterns of *TaNCED2* and *TaCYP707A1* genes (Tuan et al., 2021; Rehal et al., 2022).

2.11.1.3 ABA signaling and its role in the regulation of seed dormancy

ABA signaling pathway consists of three key components: the pyrabactin resistance/pyrabactin like/regulatory components of ABA receptors (PYR/PYL/RCAR), protein phosphatase 2Cs (PP2Cs) and SNF1-related protein kinase2s (SnRK2s) (Tuan et al., 2018). ABA binds to the receptor which interacts with PP2Cs, a negative regulator of ABA signaling, and inhibits its function. Inhibition of PP2C activates SnRK2s, a positive regulator of downstream components (Raghavendra et al., 2010), which in turn activates ABRE-binding protein/ABRE binding factor (AREB/ABF) transcription factors including ABI3, ABI4 and ABI5, which are critical

for the regulation of ABA responsive genes in seeds (Nambara et al., 2010; Tuan et al., 2018). The differences in dormancy dynamics cannot be attributed solely to the variations in endogenous ABA content, without considering ABA sensitivity (Kawakami et al., 1997; Rikiishi and Maekawa, 2010; Schramm et al., 2012; Rodríguez et al., 2015). Previous studies have shown decreases in both ABA content and ABA sensitivity in various non-dormant wheat mutants (Kawakami et al., 1997; Rodríguez et al., 2015). Moreover, variations in seed dormancy among varieties within different cereal crops such as wheat, sorghum and rice do not typically correlate with differences in ABA content (Walker-Simmons, 1987; Steinbach et al., 1995; Gianinetti and Vernieri, 2007; Tuan et al., 2018). For example, seeds of the Zak ERA8 (ENHANCED RESPONSE to ABA8) mutant of wheat exhibit a higher level of dormancy relative to the Zak wild type, despite embryos of both lines contain a similar level of endogenous ABA (Martinez et al., 2016), indicating that seed dormancy in these two lines is closely linked to sensitivity of their embryos to ABA.

The varying levels of embryo sensitivity to ABA can be due to differences in the efficiency of the molecular components involved in the ABA signaling pathway (Benech-Arnold et al., 2006; Rodríguez et al., 2015). Previous studies in Arabidopsis have shown the important roles played by the fourteen members of the PYR/PYL/RCAR protein family in seed development. Specifically, the *pyr1/prl1/prl2/prl4* quadruple mutants and *pyl* duodecuple mutants have been observed to exhibit decreased seed dormancy and response to ABA (Ma et al., 2009, Zhao et al., 2018; Ali et al., 2022). A study in sorghum showed enhanced expression of *ABI3*, *ABI4*, *ABI5*, *PKABA1* (wheat ortholog of *SnRK2*) transcripts and ABI5 protein in dormant immature grains (30 DAP, before physiological maturity) in comparison to the grains of lines that exhibit less dormancy (Rodríguez et al., 2009). It has also been shown that imbibing wheat seeds under supraoptimal temperature delays germination by increasing the expression of genes involved in ABA signaling such as *PYL5, SnRK2, ABI3,* and *ABI5*

(Izydorczyk et al., 2018). Furthermore, maintenance of dormancy in imbibed wheat seeds has been found to be regulated by upregulation of ABA signaling genes (*TaPYL2*, *TaSnRK2*, *TaABI3*, *TaABI4* and *TaABI5*) (Tuan et al., 2021; Rehal et al., 2022). Studies have also uncovered the role of *Delay of Germination 1* (*DOG1*)-like genes in imparting some level of seed dormancy in wheat (Ashikawa et al., 2013, 2014). For instance, it has been shown that the DOG1 interacts with a subset of clade A PP2C phosphatases and this interaction inhibits the phosphatase activity of PP2Cs, which is essential for maintaining seed dormancy (Née et al., 2017). Further studies are required to validate the function of DOG-1 as a transcriptional factor. In general, dormant wheat seeds exhibit enhanced sensitivity to ABA compared to non-dormant seeds (Tuan et al., 2018).

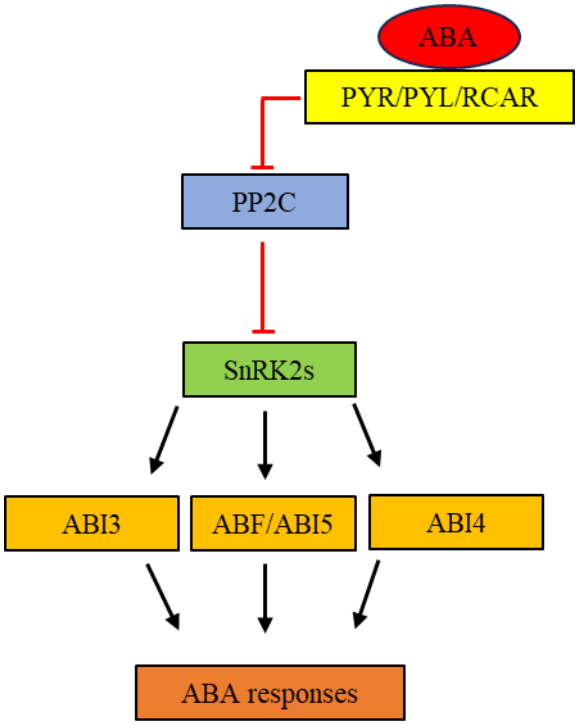


Figure 2.2. A simplified diagram of ABA signaling pathway. PYR/PYL/RCAR, pyrabactin resistance/pyrabactin-like/regulatory components of ABA receptors; PP2C, protein phosphatase 2C; SnRK2, SNF1-related protein kinase2; ABI3, abscisic acid insensitive 3; ABI4, abscisic acid insensitive 4; ABI5, abscisic acid insensitive 5; ABF, ABRE binding factor. (Adapted from Tuan et al., 2018).

2.11.1.4 GA metabolism and signalling

GA is a tetracyclic di-terpenoid compound stimulating plant growth and development (Yamaguchi, 2008). Over 136 GAs have been identified from plants, fungi and bacteria to date (MacMillan, 2001; Gao and Chu, 2020). However, there are only a few GAs that are biologically active such as GA_1, GA_3, GA_4, and GA_7, which regulate plant growth and development. GA is known to promote seed germination, shoot growth, flowering, and internode elongation (Gupta and Chakrabarty, 2013). The level of bioactive GAs in a plant tissue is controlled by both the rates of their biosynthesis and catabolism (Yamaguchi, 2008). The metabolism of GAs involves a number of reactions involving several enzymes; however, the level of GA in plants is regulated mainly GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox) that catalyse reactions in the late stage of GA biosynthesis and GA 2-oxidase (GA2ox) that catalyse the main GA inactivation pathway (Sun, 2008).

The biosynthesis pathway of GA can be categorized into three main stages. The first stage includes the conversion of geranyl-geranyl diphosphate (GGDP) to *ent*-kaurene via *ent*-copalyl diphosphate (CDP) by two terpene synthases (TPSs), namely *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS). The second stage involves the conversion of *ent*-kaurene to GA_{12}. This process involves six oxidation steps catalysed by cytochrome P450 monooxygenases (P450s) known as *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO) that introduce oxygen atoms into the *ent*-kaurene structure and eventually forming GA_{12} (Helliwell et al., 1999: Nelson et al. 2004; Yamaguchi, 2008). During the last stage, GA_{12} is 13-hydroxylated by GA13-oxidase *(*GA13ox) to produce GA_{53}. Both GA_{12} and GA_{53} serve as substrates to produce bioactive GAs, GA_4 and GA_1, respectively via a series of oxidation reactions catalysed by 2-oxoglutarate-dependent dioxygenases (2ODDs) enzymes, namely GA20ox and GA3ox (Sun, 2008). In addition to GA biosynthesis, GA inactivation is equally essential for regulating the levels of active GAs within

plants, thereby influencing various physiological processes. GA inactivation predominantly takes place via 2β-hydroxylation of GAs by the action of a 2ODD enzyme, GA2ox (Hedden and Thomas, 2012). The GA2ox enzyme exhibit the capacity to act on a range of GAs, including bioactive forms and their immediate precursors such as GA_9 and GA_{20} (Regnault et al., 2014). Studies has uncovered a distinct class of GA2oxs with a specific affinity for C20-GAs, providing new insights into the diversity of GA catabolic pathways (Regnault et al., 2014).

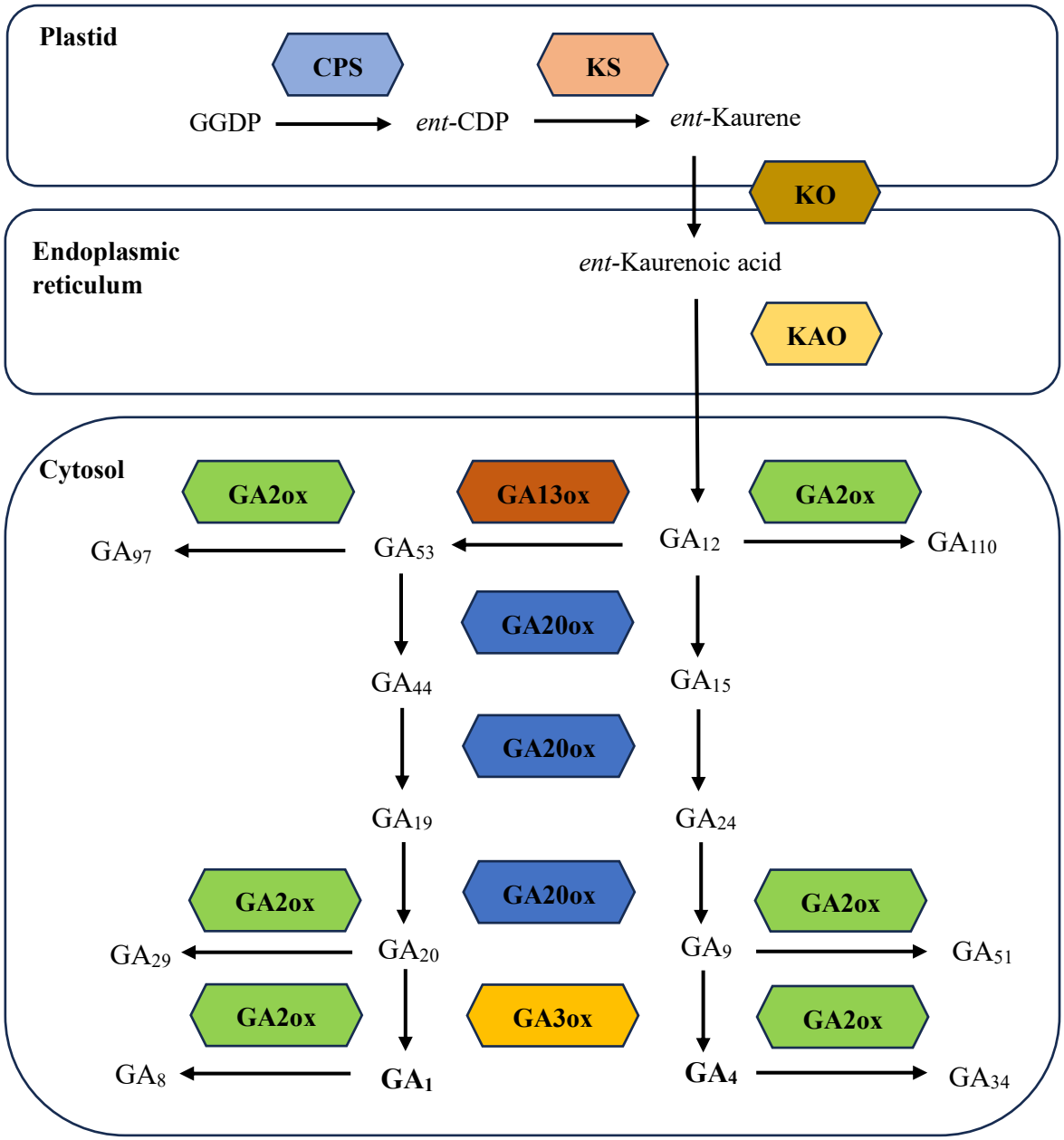


Figure 2.3. Gibberellin metabolism pathway in plants. GGDP, geranylgeranyl diphosphate; ent-CDP, ent-copalyl diphosphate; CPS, ent-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, ent-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA2ox, GA 2-oxidase; GA3ox, GA 3-oxidase. (Adapted from Yamaguchi, 2018).

Molecular and genetic studies have also enhanced our understanding of GA signaling mechanisms in plants. The presence of bioactive GA triggers the GA signaling in plants by binding to its receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) (Ueguchi-Tanaka et al., 2005). DELLA proteins are plant specific proteins, which function as negative regulators

GA signaling (Lacascio et al., 2013). The binding of GA to GID1 forms a GA-GID1-DELLA complex, which further associates with the $SCF^{SLY1/GID2}$ ubiquitin E3 ligase complex that leads to the degradation of DELLA via ubiquitination (Ueguchi-Tanaka et al., 2005; Griffiths et al., 2006). In the absence of bioactive GA, the DELLA proteins suppress GA dependent plant responses such as growth and germination (Hussain et al., 2003). GAMYB, a GA-regulated MYB transcription factor, is activated by the degradation of DELLA through GA action (Tuan et al., 2018). GAYMB proteins stimulate *α-amylase* expression in barley and rice aleurone layers by interacting with GA responsive elements (TAACAAA) found in its promoter region (Gubler et al., 1999; Sun and Gubler, 2004; Tuan et al., 2018). In addition, a study in Arabidopsis suggests that GAMYB may play a role in regulating GATA-type zinc finger transcription factor, which positively controls seed dormancy (Ravindran et al., 2017).

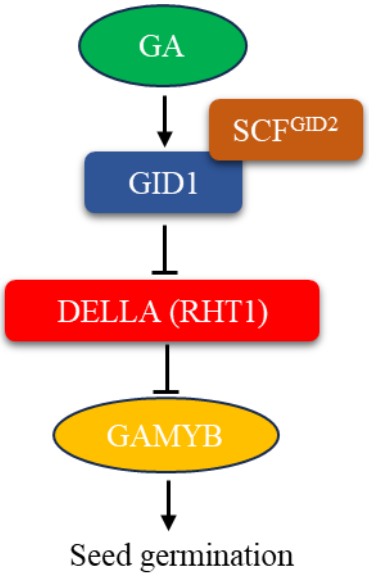


Figure 2.4. A simplified diagram of GA signaling pathway. GA, gibberellin; GID1, gibberellin insensitive dwarf 1; SCF, S-phase-kinase-associated protein 1, Cullin-1/Cdc53, and an F-Box protein; gibberellin insensitive dwarf 2; GID2, gibberellin insensitive dwarf 2; RHT, reduced height; GAMYB, GA regulated MYB transcriptional regulator. (Adapted from Tuan et al., 2018)

2.11.1.5 Role of GA in the regulation of seed dormancy

GA is known to have a negative effect on dormancy level and stimulates germination via increasing the growth potential of the embryo (Debeaujon and Koornneef, 2000; Kucera et al., 2005; Tuan et al., 2018). According to the hormone balance theory, loss of seed dormancy is linked to a decrease in the levels of ABA and an increase in GA levels (Karssen and Lacka, 1986; Ali-Rachedi et al., 2004; Finch-Savage and Leubner-Metzger, 2006). A previous study observed an increase in bioactive GA_1 during imbibition of after-ripened (AR) seeds compared to dormant seeds in barley, when grains were germinating (Jacobsen et al., 2002). A study on Arabidopsis seeds by Ariizumi et al. (2013) examined the impact of after-ripening on GA levels using the highly dormant GA-insensitive *sleepy1-2* mutant and the results of this study indicated that after-ripening led to an increase in GA levels.

Molecular mechanisms by which GA regulates seed germination and dormancy have been elucidated through mutational and transcriptomic studies. For example, analysis of loss of function mutation of *GA20ox2* in rice resulted in lower GA levels in seeds and increased dormancy (Ye et al., 2015). A previous study has also shown increase in expression of *GA20ox* and GA_4 levels in imbibing non-dormant seeds of sorghum (Rodríguez et al., 2012). Moreover, expressing a runner bean GA catabolism *GA2ox* in wheat has been shown to cause a decrease in GA level and increased seed dormancy (Appleford et al., 2007). Other studies in wheat have also shown upregulation of the GA biosynthetic genes, *GA20ox* and *GA3ox*, during imbibition of AR seeds, thereby improving their germination (Gao et al., 2012; Liu et al., 2013). Moreover, imbibition of dormant wheat seeds at low temperatures (15°C) causes dormancy loss by upregulating TaGA*3ox2*, leading to an increase in GA level in the embryo (Kashiwakura et al., 2016). On the other hand, imbibing non-dormant wheat seeds at suboptimal temperature inhibits germination by reducing GA content in both embryo and endosperm due to

downregulation of GA biosynthetic genes, *TaGA20oxs* and *TaGA3ox2* (Izydorczyk et al., 2018).

Seed dormancy or germination can be affected by variations in seed sensitivity to GA, which is regulated by the expression of genes that encode the components of GA signaling (Tuttle et al., 2015; Tuan et al., 2018). Previous research in Arabidospsis have found that *sly1* mutant and the *gid1a gid1b gid1c* triple mutants showed reduced or failed germination that cannot be resecued by application of exogenous GA (Steber et al., 1998, Griffiths et al., 2006, Ariizumi and Steber 2007; Iuchi et al., 2007; Hauvermale et al., 2015). On the other hand, the mutation in *GID1* and *GID2* of rice has been found to suppress the activity of α-amylase, without affecting seed germination (Ueguchi-Tanaka et al., 2005). Expression analysis of *GID1* and *GID2* in the seeds of cereals, including wheat, barley, and rice, revealed no correlation with the seed germination phenotype (Barrero et al., 2009; Ueguchi-Tanaka et al., 2005; Izydorczyk et al., 2018; Tuan et al., 2018) However, a recent study in wheat showed higher expression of *TaGID1* and *TaGID2* in less dormant seed than those that exhibit strong dormancy , suggesting increased seed GA sensitivity (Tuan et al., 2021). Also, the role of GA is to counteract the negative effect of DELLA on germination (Hauvermale et al., 2012). For instance, *ga1-3* mutant seeds in which GA biosynthesis is inhibited are known to exhibit high level of DELLA protein, which suppresses germination. However, when these seeds are treated with GA, DELLA proteolysis occurs and germination is triggered (Ariizumi and Steber, 2007). A study in wheat showed imbibing non-dormant seeds at suboptimal and supraoptimal temperature inhibits germination via increasing the expression of *RHT1,* a gene encoding the wheat DELLA protein, in the embryo (Izydorczyk et al., 2018). Another study showed lack of correlation between the expression patterns of GA signalling genes, *RHT1* and *GAMYB*, and dormancy phenotype of wheat seeds (Tuan et al., 2021).

2.11.2 Environmental factors regulating seed dormancy

Seeds are known to act as environmental sensors, and their depth of dormancy varies in response to the environmental signals (Footitt et al., 2011). Environmental factors such as light, temperature, moisture, nitrate, and soil temperature play important roles in the regulation of dormancy and germination. Among these factors, light and temperature are considered as the most important environmental factor regulating dormancy and germination (Tognacca et al., 2021).

2.11.2.1 Light

Light is an important environmental cue that regulates the cycle of germination and dormancy. In cereals like barley and wheat, blue light promotes dormancy, while in Arabidopsis, white and red light promotes germination (Gubler et al., 2008). The inhibitory action of blue light on the germination of barley and wheat grains is linked with an increase in embryonic ABA content due to upregulation of ABA biosynthetic genes (*NCED1* and *NCED2*) and downregulation of ABA catabolic gene (*CYP707A1*) (Gubler et al., 2008; Jacobsen et al., 2013). In barley, blue light also reduces GA content, and this has been shown to be associated with upregulation of *GA2ox3* and *GA2ox5* and downregulation of *GA3ox* (Gubler et al., 2008; Hoang et al., 2014). Several studies on Arabidopsis have also revealed the role of red/far-red light on seed germination. This effect of red/far-red light is mediated by interactions between phytochrome (phy) and PIF1 (PHYTOCHROME-INTERACTING FACTORs), which ultimately control the ABA and GA metabolism and signaling pathways (de Wit et al. 2016; Wei et al., 2023). A study in lettuce seeds also showed that red light promotes germination by reducing ABA levels through downregulation of ABA biosynthetic genes, *NCED2* and *NCED4* and upregulation of the ABA catabolic gene, *ABA8ox4* (Sawada et al., 2008).

2.11.2.2 Temperature

Temperature is one of the environmental factors regulating dormancy and germination. Temperature is known to regulate the expression of genes that control the production of GA and ABA (Toh et al., 2008). Suboptimal and supraoptimal temperature can inhibit seed germination by increasing the ABA content through upregulation of genes involved in ABA biosynthesis and ABA signaling (Izydorczyk et al., 2018). In addition, suboptimal temperature decreases the GA content in seeds via downregulation of the GA biosynthetic genes as well as inhibit GA signaling (Izydorczyk et al., 2018). Moreover, the effect of temperature depends on the developmental phase of the seeds. In wheat and barley, seed treatment with low temperature increases seed germination, whereas low temperature during grain filling induces dormancy (Nyachiro et al., 2002; Gualano and Benech-Arnold, 2009; Tuan et al., 2021).

2.11.2.3 After-ripening

Seed after-ripening occurs during a period of dry storage of seeds (Meimoun et al., 2014). Dry after-ripening does not result in an immediate transition from dormancy to full germination. Instead, it makes the seeds more responsive to favourable germination conditions while reducing their responsiveness to inhibitory conditions (Iglesias et al., 2011). Specific environmental conditions that influence after-ripening of seeds vary with species. For instance, cereal species like rice, wild oat, and barley require warm and dry conditions for effective after-ripening to occur. Previous studies have shown that after-ripening has little impact on ABA content in dry seeds but induces reduction of ABA levels after imbibition (Millar et al., 2006; Gubler et al., 2008; Iglesias et al., 2011). Studies in barley and Arabidopsis have shown that upregulation of the ABA catabolic gene, *CYP707A1*, and lower levels of ABA in AR than dormant seeds (Millar et al., 2006; Okamoto et al., 2006; Barrero et al., 2009). Moreover, after-ripening causes upregulation of *TaCYP707A* and downregulation of *TaNCED1* (Jacobsen et al.,

2013). Other studies have also shown the effect of after-ripening in decreasing the expression of ABA signaling genes, which suggests a decrease in ABA sensitivity (Liu et al., 2013). Another mechanism that has been associated with after-ripening is the generation of reactive oxygen species, which lead to post-translational modification of mRNA and proteins through oxidation (Bazin et al., 2011; El-Maarouf-Bouteau et al., 2013; Gao et al., 2013). This can in turn affect the regulation of gene expression and other cellular processes, potentially contributing to the effect of after-ripening on dormancy release.

2.12 Wheat genome and transcriptomics

A genome contains the entire set of genetic instructions of an organism's DNA, and it remains stable throughout the life of a given organism (Keller, 2011). It represents the blueprint for all the proteins and functional RNA (ribonucleic acid) molecules that can be produced (Keller, 2011). On the other hand, the content of a transcriptome is dynamic and can vary depending on various factors such as tissue type, developmental stage, environmental conditions, and cellular responses (Brady et al., 2006; Zapalska-Sozoniuk et al., 2019). This dynamic nature of the transcriptome allows organisms to respond and adapt to changes in their environment, development, and physiological processes (Brady et al., 2006). Transcriptomics involves analysis of all RNA molecules/transcripts produced by the genome at a specific time or under specific conditions. Transcriptomic study in plants, thus, provide insights into gene expression patterns, identify novel genes, and understand the molecular mechanisms underlying growth, development, and responses to various stresses. Transcriptomic studies involve the application of different approaches such as genome-wide RNA sequencing (RNA-seq), microarray analysis, and quantitative polymerase chain reaction (qPCR).

The availability of an accurate reference genome sequence of an organism via the application of high-throughput next generation sequencing technologies (NGS) is crucial for

advancing genomic and transcriptomic studies and fully harnessing NGS data. This holds particularly true for crops such as wheat that possess complex and large genomes. The common wheat genome is exceptionally large compared to other plant genomes, and it is estimated to be around 17 gigabases (Gb) in size (Uauy, 2017). This is approximately 130 times larger than the genome of Arabidopsis (~135-megabases), a widely studied model plant (Arabidopsis Genome Initiative, 2000). To tackle the challenges posed by the complexity and size of a wheat genome, the IWGSC was established in 2005 (Guan et al., 2020). The IWGSC employed a novel strategy to overcome the complexity and size constraints of the wheat genome prior to sequencing. They isolated individual wheat chromosomes and chromosome arms using flow cytometry and sequenced them separately (Eversole et al., 2014; Mayer et al., 2014). This approach allowed for a more manageable sequencing process. Initially, the IWGSC released a physical map and survey sequences, followed by the first version of the wheat reference sequence in 2017 (https://www.wheatgenome.org/about/roadmap). Subsequent updates to the wheat whole-genome assembly were made by the IWGSC, resulting in the latest version, IWGSC RefSeq v2.1. The updated assembly utilized advanced sequencing technologies and assembly methods such as whole-genome optical mapping and long-read PacBio contigs (Zhu et al., 2021). These improvements enhanced the accuracy and completeness of the wheat genome assembly. Generation of RefSeq v2.1 involved better placement of scaffolds within the genome including anchoring previously unassigned scaffolds and closing gaps (Zhu et al., 2021). Genome assemblies like IWGSC RefSeq v2.1 serve as a robust reference framework for transcriptomic analysis in wheat. They enable precise gene annotation, identification of differentially expressed genes (DEGs), analysis of alternative splicing events, discovery of novel transcripts, and construction of gene co-expression networks. These resources empower wheat researchers and breeders to gain a comprehensive understanding of gene expression

dynamics, functional gene networks, and regulatory mechanisms. Ultimately, this knowledge will contribute to targeted breeding efforts for improved agronomic traits of wheat.

2.13 Gene expression analysis: genome-wide and targeted

Gene expression analysis is a fundamental tool in modern biology that allows researchers to gain insights into the transcriptional behaviour of biological systems. Researchers can uncover the molecular mechanisms underlying various biological processes that govern plant growth, development, and responses to environmental stimuli by studying gene expression patterns.

There are two main approaches to study gene expression: genome-wide and targeted (Bartlett, 2002; VanGuilder et al., 2008; Dekkers et al., 2012; Kogenaru et al., 2012; Mo et al., 2012; O'brien et al., 2012). In genome-wide approaches, researchers aim to capture the entire transcriptome of a biological system, even when the specific genes of interest are not known. This is achieved through the use of approaches such as microarrays or RNA-seq (Kogenaru et al., 2012). Microarrays involve the attachment of an array of oligonucleotide probes to a solid substrate and the RNA molecules present in the sample are converted into labeled complementary DNA (cDNA). The labeled cDNA is hybridized with cDNA sequences on the probes (Govindarajan et al., 2012). The amount of hybridization is measured using a fluorescence scanner, which provides information about the expression levels of specific genes. DNA microarrays allow for the simultaneous analysis of multiple genes and have been widely used in various biological studies (Bumgarner, 2013). RNA-seq, on the other hand, utilizes NGS technologies to sequence and quantify the RNA molecules in a sample (Wang et al., 2009). This method provides a more comprehensive view of the transcriptome, enabling the detection of known and novel transcripts. Understanding the cell transcriptome is crucial as it bridges the gap between the genetic information encoded in the genome and the observable characteristics/ phenotype (Wang et al., 2009). By profiling the transcriptome, researchers can

identify genes differentially expressed between different tissues or under different experimental conditions. This information helps uncover the regulatory networks and molecular pathways that drive specific cellular behaviours. In cases where the genes of interest are already known, more targeted methodologies can be employed for gene expression analysis such as qPCR, a highly sensitive technique that measures the expression levels of target genes (Bartlett, 2002). For years, qPCR has provided a reliable and robust approach to quantitatively examine gene expression in a precise and targeted manner. It is commonly used for validating gene expression results obtained from genome-wide analysis or when studying a small number of specific genes (VanGuilder et al., 2008). Overall, gene expression analysis plays a crucial role in understanding the dynamic nature of gene regulation and its impact on cellular processes.

2.13.1 Data generation through RNA sequencing

RNA-seq involves multiple steps to prepare the RNA samples for NGS. This process begins with extracting and isolating RNA from a biological sample such as a frozen tissue sample. The next step involves library construction from the RNA samples, which starts by depleting ribosomal RNA (rRNA) or enriching mRNA, as rRNA makes up most of the total RNA in cells or tissues (Shi et al., 2021). The extracted RNA is further converted into cDNA using reverse transcription (RT). After cDNA synthesis, the subsequent steps involve the cleavage and amplification of cDNA molecules and the binding of sequencing adapters (Prakash and Haeseler, 2017). Adapter sequences are short oligonucleotides ligated to both ends of the cDNA during library preparation to ensure proper attachment of the DNA fragments to the sequencing flow cell (Jiang et al., 2014; Akintunde et al., 2023). It can include additional functional elements like barcode/index for multiplexing and a sequencing-priming site for paired-end sequencing (Pereira et al., 2017). Achieving optimal results and accuracy in RNA-

seq experiments requires accurate quantification of the NGS libraries. This can be conducted through qPCR and use of various commercial kits (Pereira et al., 2017). The RNA-seq library is then used as the input for a sequencing platform, which generates reads (Kukurba and Montgomery, 2015).

2.13.2 RNA-seq Data Analysis: transforming raw data into meaningful interpretation

Strict quality control measures need to be implemented to carefully evaluate the quality of the raw sequencing reads and detect any possible errors in the experimental setup. Raw read data often exhibit biases and artifacts that arise from experimental and sequencing protocols (Deshpande et al., 2023). These factors can significantly impact the accuracy of read alignments and downstream analysis. Therefore, it is important to generate summary of statistical analysis that evaluate the overall quality of the generated data. The statistical analysis involves assessing the distribution of nucleotide and quality score, along with sequence properties such as GC-content, levels of sequence ambiguity, overrepresented k-mers, and PCR artifacts **(**Pfeifer, 2016). Several popular tools can be used for conducting the quality assessment. These include FastQC and HTSeq (Anders et al., 2014), Kraken (Davis et al., 2013), NGSQC (Dai et al., 2010), PRINSEQ (https://prinseq.sourceforge.net/), qrqc (https://github.com/vsbuffalo/qrqc), RSeQC (Wang et al., 2012), and SAMStat (Lassmann et al., 2010). Based on the results of the analysis, low-quality bases are removed, adapter sequences are trimmed, and any sequencing-related anomalies or biases are filtered out. Various tools such as Trimmomatic (Bolger et al., 2014), BBDuk, Cutadapt (Magoč and Salzberg, 2011), Sickle (Joshi and Fass, 2011), and FASTX-toolkit (Hannon, 2010) have been used to successfully achieve this task.

2.13.3 Read Alignment

RNA-seq data does not provide specific information about the arrangement and origin of the reads such as details about the specific genomic region, homologous sequence, or strand of the genome from which the reads originate (Deshpande et al., 2023). However, the genomic locations from which the reads originated can be determined through computational alignment (Brown, 2002). Read alignment aims to efficiently map short sequencing reads to a large reference genome. Read alignment allows us to identify the genomic loci from which the reads originate while also considering errors in the sequence reads. The conventional alignment tools such as Bowtie (Langmead et al., 2009) and Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009) have been extensively employed to map DNA sequencing reads. However, these methods are commonly considered not suitable for mapping RNA-seq reads. Aligning RNA-seq reads poses extra difficulties due to the presence of reads that span splice junctions. To overcome these challenges, various computational tools and algorithms, which can accurately align the reads by considering the presence of spliced junctions and alternative splicing events, have been developed. Some examples of these tools include GSNAP (Wu and Nacu, 2010), TopHat (Trapnell et al., 2009), STAR (Dobin et al., 2012), HISAT2 (Kim et al., 2019) and MapSplice (Wang et al., 2010). HISAT2 is a widely used RNA-seq alignment tool that succeeded both HISAT and TopHat2. HISAT2 is a high-speed spliced mapper specifically designed for mapping RNA-seq data to the reference genome (https://daehwankimlab.github.io/hisat2/). It considers the presence of spliced junctions and the possibility of reads being split across multiple exons with intronic gaps. It employs advanced indexing techniques including the Burrows-Wheeler transform (BWT) and Ferragina-Manzini (FM) index to efficiently store and compress the genome, and thereby reducing the storage space required (Kim et al., 2019). Several RNA-seq studies in wheat

utilized HISAT2 to align the generated reads to the Chinese Spring reference genome (Wang et al., 2021; Gong et al., 2022; Sun et al., 2023).

2.13.4 Transcript assembly and quantification

Once the RNA-seq reads have been aligned, the next step is to assemble them into transcripts. There are two primary methods for transcriptome assembly: de novo and reference-guided assembly. De novo assemblers like Trinity (Grabherr et al., 2011) and Oases (Schulz et al., 2012) identify overlaps between reads, and construct complete transcripts without aligning the reads to a genome (Kovaka et al., 2019). However, this approach is challenging due to the presence of paralogous genes and overlapping isoforms, resulting in fragmented and error-prone transcriptomes (Kovaka et al., 2019). On the other hand, reference-guided assemblers like Cufflinks (Trapnell et al., 2010), Bayesembler (Maretty et al., 2014), StringTie (Pertea et al., 2015), TransComb (Liu et al., 2016), and Scallop (Shao and Kingsford, 2017) utilize an existing genome (Kovaka et al., 2019). The RNA-seq reads are aligned to this genome using spliced aligners such as HISAT2 or STAR (Kovaka et al., 2019). These assemblers can generate more accurate transcript reconstructions by leveraging the reference genome.

The transcript reconstruction software has the ability to estimate gene expression levels (Kukurba and Montgomery, 2015). For example, StringTie performs gene assembly for each dataset separately while estimating expression levels for both genes and isoforms during the assembly process (Pertea et al., 2016). StringTie uses advanced computational methods to reconstruct transcript structures and estimate transcript abundance from bulk RNA-Seq reads aligned to a reference genome. Its input consists of spliced alignments in coordinate-sorted SAM/BAM/CRAM format and the output is gene transfer format (GTF) which includes assembled transcript structures, their estimated expression levels (fragments per kilobase per million mapped fragments/ transcripts per kilobase million; FPKM/TPM) and base coverage

values (https://ccb.jhu.edu/software/stringtie/). In addition, normalizing read counts is crucial to account for systematic variability that can affect gene expression analysis. Such variability includes differences in library fragment size, bias in sequence composition, and variations in read depth (Oshlack and Wakefield, 2009; Roberts et al., 2011). One way to normalize RNA-seq data is by utilizing the reads per kilobase of transcript per million mapped (RPKM) metrics. This metric considers the transcript length and the total number of reads mapped in the sample (Kukurba and Montgomery, 2015). On the other hand, the FPKM metric is used for normalization of paired-end data, measuring the fragments per kilobase of transcript per million mapped (Trapnell et al., 2010; Kukurba and Montgomery, 2015)

2.13.5 Differential gene expression

The primary objective of gene expression studies is to detect transcripts that exhibit differential expression under two or more conditions (Glaus et al., 2012). The count-based nature of RNA-seq data presents difficulties in accurately modelling gene expression variability (Kukurba and Montgomery, 2015). Early studies suggested that read counts followed a Poisson distribution, which became the foundation for modelling RNA-seq count data (Marioni et al., 2008; Kukurba and Montgomery, 2015). However, models using the Poisson distribution failed to account for biological variability across samples, leading to a high false discovery rate due to underscoring of sampling error (Anders and Huber, 2010; Robinson and Oshlack, 2010; Kukurba and Montgomery, 2015). To address this limitation, the negative binomial (NB) distribution was introduced. The NB distribution extends the Poisson distribution by incorporating an additional dispersion parameter for extra variability observed in biological replicates (Di, 2015). This methodology effectively models count across samples and provides accurate measures of statistical significance. By using the NB distribution, variability in gene expression can be captured better and reduce false discovery rates in RNA-seq data analysis.

The best-performing tools for differential gene expression analysis are edgeR (Robinson et al., 2010), DESeq (Love et al., 2014), DESeq2 (Anders and Huber, 2010), Limma (Ritichie et al., 2015), and baySeq (Hardcastle and Kelly, 2010). All these tools are based on the negative binomial or log-normal distributions and utilize various statistical methods to improve the ability to detect significant changes based on limited replicates and handle the unique characteristics of count data. However, careful interpretation of the biological conclusions obtained from these methodologies is essential. Variability in library construction and inherent variability in biological samples can significantly impact the number of false positives. Therefore, it is essential to include biological replicates in the RNA-seq dataset as they play a vital role in accurately measuring the intrinsic variability of the sample. The edgeR tool implements the NB distribution for differential gene expression analysis, and it is recommended for experiments with fewer than 12 replicates and provides better control over false positives (Schurch et al., 2016). It identifies genes exhibiting statistically significant differential expression by applying statistical tests. For example, a comparative RNA-seq study that involved the use of edgeR tool identified 8,013 DEGs between drought susceptible and drought tolerant wheat genotypes (Iquebal et al., 2019). Whereas another RNA-Seq study, which used the DESeq2 R package, reported a total of 3,106 DEGs between PHS-sensitive and PHS-resistant rice varieties under high humidity conditions (Liu et al., 2022). The DEGs can be further validated using independent techniques such as qPCR. In addition, functional analysis methodologies such as gene ontology enrichment analysis and pathway analysis can be employed to gain a comprehensive understanding of the biological significance of these genes. These methodologies facilitate identifying biological processes, molecular functions, cellular components, and pathways associated with the genes exhibiting differential expression.

2.13.6 Gene set enrichment analysis

Conventional gene expression analysis involves comparing the expression levels of individual genes in different samples using statistical tests and adjusting for multiple comparisons. However, this approach often yielded false negatives due to the implementation of conservative adjustment methods. Furthermore, the utilization of arbitrary threshold values led to varying interpretations and the approach overlooks the valuable information available in public databases about gene sets and their biological functions (Subramanian et al., 2005; Maleki et al., 2020). To address these limitations, gene set analysis, also known as enrichment analysis, was developed. Gene set analysis focuses on identifying the enrichment or depletion of expression levels within specific gene sets of interest (Maleki et al., 2020). This approach seeks to overcome the shortcomings of single-gene analysis and provides valuable insights from gene expression data by considering groups of functionally related genes rather than individual genes alone. Moreover, the single-gene approach struggled in accurately identifying differences in gene expression from variations caused by biological variability when measured values for a single gene across treatments are subtle (Subramanian et al., 2005; Maleki et al., 2020). In contrast, gene set analysis showed better results in detecting subtle but consistent changes in the gene expression patterns within a gene set (Maleki et al., 2020). The application of enrichment analysis bioinformatic methods utilizing gene annotation databases has led to the development of various powerful tools. Examples of such tools include the DAVID online analysis tool (Sherman et al., 2022), the R Cluster-Profiler package (Wu et al., 2021), and Meta-scape (Zhou et al., 2019) among others. These tools have become integral in analysing gene function and exploring biological knowledge derived from high-throughput sequencing technologies. Most of the tools currently used rely on enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). These tools utilize the curated

information provided by GO and KEGG to identify overrepresented biological processes, molecular functions, cellular components, and pathways associated with a specific set of genes.

2.13.7 Gene Ontology

The GO project is an initiative that aims to annotate genes across different species. It provides a curated set of terms that describe the function, biological process, or cellular component of a gene product. The GO project (http://www.geneontology.org/) led by the GO Consortium, has achieved remarkable success in systematically characterizing the biological characteristics of genes, paving the way for effortless integration, retrieval, and analysis of genetic data. The GO project was initiated in 1998 by researchers who were investigating the genetic makeup of three model organisms: fruit fly (*Drosophila melanogaster*), mouse (*Mus musculus*), and yeast (*Saccharomyces cerevisiae*). Over time, the GO Consortium has witnessed the inclusion of databases for other organisms such as wheat, which has significantly contributed to the expansion and development of the GO project (The Gene Ontology Consortium, 2004). The primary objective of GO is to conduct enrichment analysis for sets of genes. If we have a group of genes that are upregulated in specific conditions, an enrichment analysis can be conducted to identify which GO terms are over-represented or under-represented in the identified group of genes. For instance, the GO enrichment analysis of genes differentially expressed between PHS sensitive wheat cultivar 'Long 13-3778' and its PHS resistant mutants indicates a major difference in chitin catabolism activity (Li et al. 2021). A recent RNA-seq based transcriptomic analysis in wheat showed the enrichment of gene sets that exhibit higher expression levels in PHS resistant as compared to PHS susceptible cultivar in specific GO categories including response to reactive oxygen species, trehalose biosynthesis, abscisic acid transport, endopeptidase inhibitor activity, and response to oxidative stress (Lee et al. 2021). Another RNA-seq based study in wheat reported the enrichment of DEGs in PHS resistant isolines at

15, 25, and 35 days post anthesis (DPA) in cell, cell part, organelle, membrane and membrane part, catalytic activity, binding and transporter activity, metabolic process, cellular process, response to stimulus, and biological regulation GO terms (Wang et al. 2021). Enrichment analysis of DEGs between PHS-sensitive and PHS-resistant rice varieties also revealed their enrichment in nutrient reservoir activity, signal transducer activity, response to stimulus, and transporter activity GO terms (Liu et al., 2022).

2.13.8 Kyoto Encyclopedia of Genes and Genomes

The KEGG database serves as a valuable resource for comprehending high-level functions and utilities of the biological systems ranging from cells to organisms and ecosystems based on molecular-level data, particularly those generated through genome sequencing and other high-throughput experimental techniques (Kanehisa and Goto, 2000; Kanehisa, 2002). The KEGG project aims to establish connections between genomic information and higher-level functional insights by systematically organizing current knowledge on cellular processes and standardizing gene annotations (Kanehisa and Goto, 2000). Within the KEGG pathway databases, one can find an array of ortholog group tables that include primary, sub-category, and secondary pathways. These pathways are encoded by genes located on chromosomes, thereby enabling us to make predictions about gene function (Kanehisa and Goto, 2000). Thus, KEGG pathway is widely recognized as one of the most effective methods in enrichment analysis platforms. A recent RNA-seq study analyzed DEGs between rice seeds imbibed for 6 hours after imbibition (HAI) and 24 HAI, and the study identified metabolic pathways, biosynthesis of secondary metabolites, and ribosome pathways as enriched in gene sets upregulated in 24 h imbibed seeds compared to those imbibed for 6 h. On the other hand, pathways of plant hormone signal transduction and spliceosome showed significant enrichment in gene sets downregulated in the same seed sample (Li et al., 2022). Furthermore, DEGs

derived from the response of two rice varieties (PHS-sensitive and PHS-resistant) to high humidity are found to be associated with plant hormone signal transduction, carbon metabolism, starch and sucrose metabolism, and phenylpropanoid biosynthesis pathways (Liu et al., 2022). Similarly, a KEGG pathway enrichment analysis of key DEGs between PHS resistance and susceptible near isogenic lines of wheat suggested that the DEGs to be linked with pathways such as MAPK signaling pathway, purine metabolism, fatty acid biosynthesis, starch and sucrose metabolism, phenylpropanoid biosynthesis, biosynthesis of secondary metabolite, and plant hormone signal transduction (Wang et al., 2021). To summarize, previous studies have shown that seed dormancy and germination are regulated by a wide range of biological pathways mainly plant hormone signal transduction, secondary metabolite biosynthesis, MAPK signaling, and spliceosome pathways.

2.14 Transcriptomic analysis of dormancy release by after-ripening

Several studies have been performed to understand changes in gene expression during seed imbibition and dormancy release due to after-ripening. Dry after-ripening has been shown to induce changes in gene transcript abundance in seeds of various plant species including *Nicotiana tobacum*, barley, and wheat. These changes in transcript abundance may arise from transcriptional regulation or differential mRNA turnover (Rajjou et al., 2004; Bove et al., 2005; Leubner-Metzger, 2005; Bazin et al., 2011; Nelson and Steber, 2017). Regardless of the underlying mechanisms involved in modifying the transcriptome during dry seed after-ripening, it is crucial to assess whether such alterations can affect the germination potential of seeds.

Under favourable environmental conditions, changes in transcript abundance that occur during dry seed storage regulate the ability to germinate upon seed imbibition, and differences in gene expression have been observed between dormant and AR seeds of Arabidopsis, wheat,

and barley (Cadman et al., 2006; Barrero et al., 2009; Gao et al., 2012; Liu et al., 2013). Transcriptomic analysis of dormant and AR imbibed seeds of Arabidopsis with differing level of dormancy revealed differential expression of genes related to seed dormancy or germination such as *PHYTOCHROME-INTERACTING FACTOR 6* (*PIF6*), *GIBBERELLIN 3-OXIDASE 2 (GA3ox2*), *ALTERED SEED GERMINATION 5* (*ASG5*), and late embryogenesis abundant (LEA) proteins encoding genes (Yazdanpanah et al., 2017). A recent study has shown that after-ripening treatment of GA-insensitive mutant (*sly1-2*) seeds significantly downregulated the expression of two positive regulators of seed dormancy, DELLA *GAI* (*GA-INSENSITIVE*) and the histone deacetylase *HDA6/SIL1* (*MODIFIERS OF SILENCING1*). Furthermore, the same study demonstrated that an increase in germination potential by after-ripening is linked to the expression of genes related to protein translation (Nelson et al., 2017).

Comparative transcriptomics analysis was performed using dormant and AR barley seeds, and the results revealed differential expression of genes related to jasmonate responses, cell wall modification, nitrate and nitrite reduction, and stability of mRNA that may act downstream of ABA signalling in the coleorhiza of imbibing seeds (Barrero et al., 2009). Moreover, after-ripening was found to stimulate ABA catabolism and reduce ABA sensitivity through differential expressions of genes involved in these processes. Transcriptomic study in wheat has revealed upregulation of genes involved in histone modification, nitrogen metabolism, carbohydrate metabolism, jasmonate biosynthesis, cytoplasmic membrane-bound vesicle, and cell wall modification during imbibition of AR seeds (Gao et al., 2012). For example, genes encoding xyloglucan endo-transglycosylase 7, basic chitinase, and beta-D-xylosidases were found to be upregulated in AR than dormant seeds, thus revealing that breakage of seed dormancy due to after-ripening is mediated by activation of the cell wall loosening process (Gao et al., 2012). Another study in wheat showed that dormancy release by after-ripening is associated with transcriptional activation and repression of specific BR, ET,

CK, and SA related gene sets (Chitnis et al., 2014). However, there is still a lack of comprehensive understanding regarding the molecular mechanisms that regulate dormancy breakage in wheat.

3.0 TRANSCRIPTOMIC ANALYSIS OF SEED DORMANCY IN WHEAT

Abstract

Preharvest sprouting, germination of grains in the spikes before harvest due to exposure to rain and high humidity, is highly influenced by the level of seed dormancy. In this study, we employed genome-wide transcriptomic analysis to get a better understanding of the molecular mechanisms regulating seed dormancy in a highly dormant wheat genotype. After-ripening of the dormant seeds led to the decay of dormancy. A total of 33,905 differentially expressed genes (DEGs) were identified between dry and imbibed samples of the dormant and after-ripened seeds. Gene ontology (GO) enrichment analysis showed that the GO terms enriched with the DEGs include those related to response to auxin, endopeptidase and peptidase inhibitor activity, glutathione metabolic process, and response to endogenous stimulus. Specific genes involved in abscisic acid (ABA) biosynthesis such as *NCED1* and *NCED3* showed upregulation during imbibition of dormant seeds relative to the after-ripened seeds while those involved in ABA catabolism such as *CYP707A1* and *CYP707A3* exhibited downregulation in the same seed samples. Our results also observed upregulation of genes involved in ABA signaling such as *SnRK3*, *SnRK10*, and *ABI5* during imbibition of dormant seeds as compared to the after-ripened seeds. In contrast, genes involved in gibberellin (GA) biosynthesis such as *TaGA3ox* and *TaGA20ox* were downregulated in the dormant seeds. The findings of this study also suggest post-transcriptional regulation of genes involved in GA signaling such as *TaGID1* and *TaGAMYB*.

3.1 Introduction

Seed dormancy is regarded as the key genetic factor that determines resistance to preharvest sprouting (PHS). Domestication and conventional breeding of cereal crops such as wheat from the wild ancestors was aimed at promoting a uniform and rapid seed germination as well as the establishment of seedlings (Simpson, 1990). This resulted in a low level of dormancy in the seeds, thereby, favouring PHS. Therefore, it is necessary for grains to have a moderate/optimum level of seed dormancy to prevent sprouting damage (Gao and Ayele, 2014). The establishment of seed dormancy occurs during embryo maturation and can be alleviated by various methods such as after-ripening (Nelson et al., 2017; Ali et al., 2022). The time needed for dormancy loss by after-ripening varies depending on the degree of seed dormancy (Nelson et al., 2017).

A plant's life cycle starts from a seed, which serves as the fundamental unit of propagation. Seed germination is a crucial process necessary for the maintenance of plant species and plays a key role in determining both crop yield and quality. Germination is a three-phase process that starts with the absorption of water and concludes with the emergence of radicle through the seed coat. Phase I involves the rapid uptake of water by the seed, followed by phase II which involves high metabolic processes and restricted water uptake. The Third phase involves rapid water absorption, reserve mobilization and root growth (Bewley, 1997). Seed germination and dormancy are controlled by various internal and environmental factors, including temperature, water, light as well as plant hormones (Nautiyal et al., 2023).

The transition from dormancy to germination of wheat seeds is controlled by differential expression of genes related to various biological processes and molecular functions. For example, a microarray analysis of dormant and after-ripened (AR) seeds in barley revealed that after-ripening alters the expression of genes representing on various biological processes, including jasmonate responses, cell wall modification, nitrate and nitrite reduction, and mRNA

stability (Barrero et al., 2009). A large-scale gene expression study in wheat revealed that after-ripening causes an increase in the expression of genes related to histone modification, nitrogen metabolism, carbohydrate metabolism, jasmonate biosynthesis, cytoplasmic membrane-bound vesicle, and cell wall modification in imbibed AR seeds (Gao et al., 2012). RNA-seq study in wheat also showed the enrichment of genes differentially expressed between a PHS resistant mutant and the wild-type line in chitin catabolic pathway (Li et al. 2021). Other recent RNA-seq studies in wheat reported the involvement of several biological processes and molecular functions including response to reactive oxygen species, trehalose biosynthesis, abscisic acid transport, endopeptidase inhibitor activity, catalytic activity, binding and transporter activity, and response to oxidative stress in regulating seed dormancy (Lee et al. 2021; Wang et al., 2021).

It is widely recognised that abscisic acid (ABA) and gibberellin (GA) play a crucial role in controlling seed dormancy and germination (Tuan et al., 2018). Several studies have shown that expression of ABA biosynthesis genes, in particular the *NCEDs* and ABA catabolism genes, *CYP707As*, regulating ABA level and seed dormancy. The level of dormancy in seeds developed at different seed development temperatures is shown to be regulated by the expression level of *TaNCED2* and *TaCYP707A1* genes (Tuan et al., 2021). Imbibition of dormant seeds as compared to non-dormant seeds was found to be linked with upregulation of *TaNCED2* and downregulation of *TaCYP707A1* (Son et al., 2016). In addition, the same study reported that ectopic expression of *TaNCED2A* in Arabidopsis resulted in a substantial increase of ABA level in seeds. Moreover, imbibing wheat seeds at high temperatures and at low temperatures inhibit germination by increasing ABA levels, which is regulated by the expression levels of *TaNCED2* and *TaCYP707A1* genes (Izydorczyk et al., 2018). A study in barley also showed upregulation of *HvCYP707A1* during imbibition of non-dormant seeds relative to dormant seeds, leading to reduction in ABA level (Millar et al., 2006).

Seed dormancy is also controlled by how responsive the seed is to ABA. ABA signaling pathway consists of three major components: the pyrabactin resistance (PYR)/pyrabactin like (PYL)/regulatory components of ABA receptors (RCAR), protein phosphatase 2Cs (PP2Cs) and SNF1-related protein kinase2s (SnRK2s) along with downstream transcription factors including ABI3, ABI4 and ABI5 (Tuan et al., 2018). Because of their downregulation in AR seeds relative to dormant seeds, a previous study in wheat suggested that *SnRK2* and *ABI5* genes play a role in regulating the loss of ABA sensitivity due to after-ripening (Liu et al., 2013). Moreover, the expression of the *TaABF1* (wheat homolog of *AtABI5*) was found to increase during the imbibition of dormant wheat grains (Johnson et al., 2000). Recent studies have also shown increase in the expression of genes encoding *PYL5, SnRK2, ABI3,* and *ABI5* in dormant seeds during imbibition (Izydorczyk et al., 2018; Tuan et al., 2021). A study in sorghum also found increased expression level of *ABI5* in the seeds of a highly dormancy cultivar as compared to seeds of a cultivar with low level of dormancy (Rodríguez et al., 2009).

GA stimulates dormancy alleviation and germination. The amount of bioactive GAs is controlled by the rate of its biosynthesis and catabolism (Yamaguchi, 2008). Previous studies on different plant species such as Arabidopsis (Finch-Savage et al., 2007) and wheat (Gao et al., 2012; Liu et al., 2013) have shown after-ripening mediated increases in expression levels of GA biosynthetic genes (*GA20ox* and *GA3ox*) and decrease in expression level of GA catabolic gene (*GA2ox*) in non-dormant seeds. A study in sorghum also showed upregulation of *GA20ox* and an increase in the level of GA during imbibition of non-dormant seeds (Rodríguez et al., 2012). In addition, imbibition of dormant wheat seeds at low temperatures (15°C) causes upregulation of *TaGA3ox2*, an increase in GA level, and a decline in dormancy level (Kashiwakura et al., 2016). Imbibing wheat seeds at suboptimal temperature was shown to inhibit germination by decreasing the expression of *TaGA20ox* and *TaGA3ox2* and GA level (Izydorczyk et al., 2018). In addition, increases in GA levels have been shown during imbibition

of AR seeds (Jacobsen et al., 2002; Ariizumi et al., 2013). Seed dormancy is also controlled by how the seed responds to GA. The GA signalling pathway involves its receptor protein called GIBBERELLIN INSENSITIVE DWARF1 (GID1). DELLA protein, a negative regulator of GA signalling, is another important player in GA signaling, and an F-box protein GID2/SLY1 plays a crucial role by breaking down the DELLA protein, thereby activating the downstream transcription factor GAMYB that controls the expression of GA responsive genes (Tuan et al., 2018). However, previous reports showed a lack of correlation between the germination or dormancy phenotype of cereal seeds and the expression pattern of *GID1* (Barrero et al., 2009; Liu et al., 2013; Izydorczyk et al., 2018; Tuan et al., 2018) and *GAMYB* (Tuan et al., 2021). In addition, mutations in *GAMYB* and *GID1* genes of rice have no impact on germination but can inhibit α-amylase activity (Sasaki et al., 2003; Kaneko et al., 2004; Ueguchi-Tanaka et al., 2005). Previous studies provided insights into molecular mechanisms regulating seed dormancy in wheat. However, more study is needed to identify more candidate genes and biological processes involved in regulating seed dormancy.

3.2 Materials and methods

3.2.1 Plant materials and growth conditions

For this study, wheat genotype RL4137 was used. RL4137 is characterized by its high resistance to PHS, and it has been widely used as a parent in Western Canadian Spring wheat breeding programs. Canada Western Red Spring (CWRS) wheat cultivars with RL4137 in their ancestry showed lower PHS score compared to those CWRS wheat cultivars without it (DePauw et al., 2009). RL4137 originated from the cross RL2520/Thatcher *6/Kenya Farmer and its high level of dormancy is mediated by two factors: one factor is the red colour of the seed coat while the other one is linked to factors independent of the seed coat colour (DePauw et al., 2009). Seeds of RL4137 were imbibed and transplanted to pots in a growth chamber

under 22/18ºC (day/night) and 16/8 h photoperiod. Seeds were harvested at harvest maturity; portion of the seeds were AR for 12 months while the remaining portion were stored at -80ºC to maintain dormancy.

3.2.2 Germination Assay

Mature dry seeds of RL4137 were surface sterilized with 70% ethanol for 1 min followed by 5% sodium hypochlorite treatment for 20 min on MaxQ™ 4000 Benchtop orbital shaker (Thermofisher Scientific, Waltham, MA, USA). Afterward, the seeds were washed 5 times with sterile water to eliminate traces of sodium hypochlorite. The seeds were then imbibed with 7 mL sterile water in a 9 cm sterile Petri-plate with two layers of Whatman# 1 filter paper kept under darkness at room temperature (25 seeds per plate, 3 replicates). Germination was monitored daily for 7 days and seeds were marked as germinated when protrusion of coleorhiza through the seed coat was visible (Gao et al., 2012). Embryonic tissues were excised from dormant and AR seeds at 0, 12, and 24 HAI and stored at -80ºC until further use.

3.2.3 RNA extraction, cDNA library preparation, and RNA-seq

Total RNA was extracted from the embryos of dry, 12 h and 24 h imbibed dormant and AR seeds as described previously (Mukherjee et al., 2015) and then digested with DNAse (DNA-free Kit; Ambion, Austin, TX, USA) to remove genomic DNA contamination. The quality and purity of the RNA samples were verified by gel electrophoresis and spectrophotometer analysis. The quality and integrity of RNA samples were further examined using Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Total RNA samples with RIN value >8.0 were used for isolating mRNA samples, which were then subjected to preparation of cDNA library using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following the manufacturer's protocol. Briefly, mRNA

was isolated from total RNA using poly-T oligo-attached magnetic beads, followed by fragmentation in NEBNext First Strand Synthesis Reaction Buffer. The first strand cDNA synthesis was performed using a random hexamer primer and M-MuLV Reverse Transcriptase (RNase H), followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. The subsequent steps involved conversion of the remaining overhangs to blunt ends, 3' adenylation, and adapter ligation. The cDNA fragments of 150-200 bp in length were purified with AMPure XP system (Beckman Coulter, Beverly, MA, USA) and amplified. The cDNA library quality was assessed using the high-sensitivity protocol of Qubit 2.0 Fluorometer, Bioanalyzer 2100 (Agilent) and qPCR. Subsequently, the cDNA libraries were sequenced using the Illumina NovaSeq 6000 platform. Paired-end (150 bp) sequencing was conducted with the generation of approximately 9.5 GB of data per sample.

3.2.4 Pre-processing and RNA-seq read alignment

The quality of the sequencing data was assessed using FastQC tool (Andrews, 2010: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Preprocessing of the data was performed using Trimmomatic tool (Bolger et al., 2014). Low-quality reads with a Phred quality score of ≤ 20, reads containing ambiguous bases 'N', adaptor sequences and low-quality bases from 5′ and 3′ ends were trimmed to improve the quality of the sequencing data. The high-quality filtered reads were then mapped to the Chinese Spring wheat reference genome (RefSeq v2.1, IWGSC) using Hisat2 v2.0.5 tool (Pertea et al. 2016; Wei et al., 2023) and annotated files were directly downloaded from the genome website (IWGSC Annotation v2.1; https://urgi.versailles.inrae.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v2.1/). The mapped reads of each sample were assembled by StringTie v1.3.3b (Pertea et al. 2015). The FPKM of each gene was calculated based on the length of the gene and the read count mapped to this gene.

3.2.5 Differential expression analysis

The abundance of each transcript was calculated using FPKM. Analysis of the differentially expressed genes between the samples was performed using the edgeR v3.22.5 (Robinson et al., 2010) The P values were adjusted using the Benjamini and Hochberg method, and the differentially expressed genes (DEGs) were identified based on the log2 fold change (log2FC) ≥ + 1 or ≤ - 1 (≥ + 2 or ≤ - 2-fold on linear-scale) in expression and adjusted p-value of ≤ 0.05.

3.2.6 GO enrichment analysis of differentially expressed genes

GO enrichment analysis of the DEGs was performed using the clusterProfiler v3.8.1 (Wu et al., 2021). GO terms with adjusted P value < 0.05 were considered significantly enriched with DEGs.

3.2.7 Identification of ABA and GA metabolism and signaling genes

Genes involved in ABA and GA metabolism and signalling were identified using the cDNA sequences of the corresponding genes of the model plant Arabidopsis, which were obtained from Arabidopsis Information Resource (TAIR) database. The cDNA sequences of the respective genes were used to search the wheat homologs in the wheat genome database using BLAST (https://plants.ensembl.org/Triticum_aestivum/Tools/Blast; Bolser at al., 2016)

3.2.8 Expression analysis of the target genes

The targeted genes with ≥ 1-fold change on log2-scale (≥2-fold on linear-scale) and P ≤ 0.05 were considered as differentially expressed between the two comparisons. The negative fold changes indicated downregulated genes, while the positive fold changes indicated upregulated genes.

3.2.9 Validation of RNA-seq data using qPCR

3.2.9.1 Primer design

The primers for validation of the expression patterns of randomly selected DEGs were designed from the conserved coding regions of the three homoeologous genes using the online PrimerQuest Tool on IDT website (https://sg.idtdna.com/Primerquest/Home/Index). The specificity of the primers was determined by BLAST search against GenBank database and melting curve and qPCR analysis. The sequences of the primers used for qPCR analysis are shown in Appendix 1.

3.2.9.2 cDNA synthesis

Total RNA was isolated from the sample, followed by digestion with DNase (Ambion, Austin, TX, USA) to eliminate genomic DNA. After digestion, cDNA was synthesized by using iScript Reverse transcriptase supermix (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. The cDNA samples were then diluted 20x to be used for qPCR analysis.

3.2.9.3 qPCR assay

The qPCR assays were conducted using CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). The qPCR reaction mixture consisted of 5 µL diluted cDNA, 1.2 µL forward primer (5 µM; final concentration 300 nM), 1.2 µL reverse primer (5 µM; final concentration 300 nM), 10 µL of Eva Green Supermix (Bio-Rad), and 2.6 µL sterile water, with a total reaction volume of 20 µL. The thermal cycling conditions used were as follows: initial denaturation and DNA polymerase activation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec. The Livak and Schmittgen (2001) method was employed to determine the relative transcript levels of the candidate genes, and the wheat *β-Actin* gene was used as a reference gene for normalization**.**

3.3 Results

3.3.1 Effect of after-ripening on the germination of dormant seeds

A comparative study was conducted to investigate the germination potential of dormant and AR seeds. After 24 h imbibition, the coleorhiza was visible in 93 % of the AR seeds, indicating successful germination of the dormant seeds due to after-ripening. In contrast, none of the dormant seeds germinated.

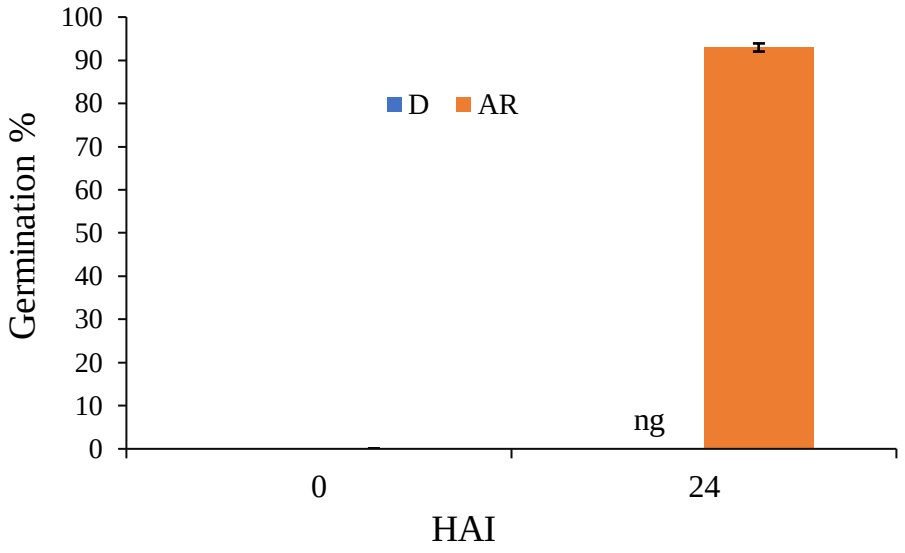


Figure 3.1. Germination percentage of dormant (D) and after-ripened (AR) seeds at 0 and 24 hours after imbibition (HAI). Data are mean and standard error (SE) of 3 replicates. ng, no germination.

3.3.2 Comparative analysis of differentially expressed genes

Comparative transcriptomic analysis was performed to understand the changes in gene expression during imbibition and between the dry and imbibed seeds of the dormant and AR samples. Three sets of comparisons were conducted in this study. The first comparison aimed to identify DEGs within dormant seeds during imbibition (Figure 3.2a) and the second comparison also to identify DEGs within AR seeds during their imbibition (Figure 3.2b). The

third set comparison examined DEGs between dry and imbibed dormant and AR seeds (Figure 3.2c).

The first set of comparison revealed that a total of 33,885 genes exhibited differential expression among dry and imbibed dormant seeds (Figure 3.2a). A total of 29,289 DEGs were differentially expressed between dry and 12 HAI dormant seeds (D_0 vs D_12) (Figure 3.2a). Of these, 10,274 genes were upregulated and 19,015 genes were downregulated in D_0 seeds (Appendix 2a). Our analysis also revealed 22,131 DEGs between dry and 24 HAI dormant seeds (D_0 vs D_24); 10,421 genes were upregulated and 11,710 genes were downregulated in the dry dormant seeds relative to 24 h imbibed dormant seeds (Figure 3.2a; Appendix 2b). During the transition from 12 to 24 h imbibition of dormant seeds (D_12 vs D_24), a total of 1,158 DEGs were detected; 752 genes upregulated and 406 genes downregulated in 12 h imbibed dormant seeds (Figure 3.2a; Appendix 2c). A total of 17,750 DEGs were found to be common between dry and 12 HAI, and dry and 24 HAI dormant seed (D_0 vs D_12 and D_0 vs D_24) comparisons (Figure 3.2a). Only 606 DEGs were shared between dry and 24 HAI, and 12 HAI and 24 HAI dormant seed (D_0 vs D_24 and D_12 vs D_24) comparisons while 695 DEGs were common between the comparisons between dry and 12 HAI and 12 HAI and 24 HAI dormant seeds (D_0 vs D_12 and D_12 vs D_24) comparisons (Figure 3.2a). Additionally, a total of 358 DEGs were uniquely expressed among all three sets of comparisons (D_0 vs D_12, D_12 vs D_24 and D_0 vs D_24) (Figure 3.2a).

The second set comparison revealed a total of 55,458 DEGs among dry and imbibed AR seeds (Figure 3.2b). Our data showed a total of 33,477 DEGs between dry and 12 HAI AR seeds (AR_0 vs AR_12) (Figure 3.2b). Of these genes, 12,722 genes were upregulated and 20,755 genes were downregulated in dry AR seeds relative to 12 h imbibed AR seeds (Appendix 3a). A total 46,317 DEGs were found between dry and 24 HAI AR seeds (AR_0 vs AR_24) out of which 15,929 genes were upregulated and 30,389 genes were downregulated in

dry AR seeds as compared to 24 HAI AR seeds (Figure 3.2b; Appendix 3b). During the transition from 12 to 24 h imbibition (AR_12 vs AR_24), a total of 21,445 genes were differentially expressed; 6,332 genes upregulated, and 15,113 genes downregulated in 12 HAI AR seeds as compared to 24 HAI seeds (Figure 3.2b; Appendix 3c). A total of 27,134 DEGs were found to be common between dry and 12 HAI AR, and dry and 24 HAI AR (AR_0 vs AR_12 and AR_0 vs AR_24) seeds (Figure 3.2b). A total of 16,536 shared DEGs were found between the comparisons of dry AR and 24 HAI AR, and 12 HAI AR and 24 HAI AR seeds (AR_0 vs AR_24 and AR_12 vs AR_24) (Figure 3.2b). There were a total 8,709 DEGs that were common between the comparisons of dry AR and 12 HAI AR, and 12 HAI AR and 24 HAI AR seeds (AR_0 vs AR_12 and AR_12 vs AR_24) (Figure 3.2b). Additionally, a total of 6,598 DEGs were uniquely detected among all three sets of comparisons (Figure 3.2b).

Analysis of the third set comparison revealed a total of 33,905 DEGs between dry and imbibed dormant and AR seeds. Only 82 genes were differentially expressed between dry dormant and dry AR seeds (D_0 vs AR_0) (Figure 3.2c). Our analysis showed that a total of 43 genes were upregulated, and 39 genes were downregulated in the dry dormant seeds as compared to dry AR seeds (Appendix 4a). The number of DEGs between the two seed samples increased to 12,081 and 29,230 at 12 HAI (D_12 vs AR_12) and at 24 HAI (D_24 vs AR_24), respectively (Figure 3.2c). Our comparative analysis further revealed upregulation of 6,413 and 9,737 genes and downregulation of 5,668 and 19,493 genes in dormant seeds relative to AR seeds at 12 HAI and 24 HAI, respectively (Appendix 4b, c). Only 24 DEGS were found to be shared between D_0 vs AR_0 and D_12 vs AR_12 comparisons. Comparisons between D_12 vs AR_12 and D_24 vs AR_24 exhibited 7,444 DEGs while only 36 DEGs are shared between D_0 vs AR_0 and D_24 vs AR_24 comparisons. Only 16 DEGs were found to overlap among all three sets of comparisons (Figure 3.2c).

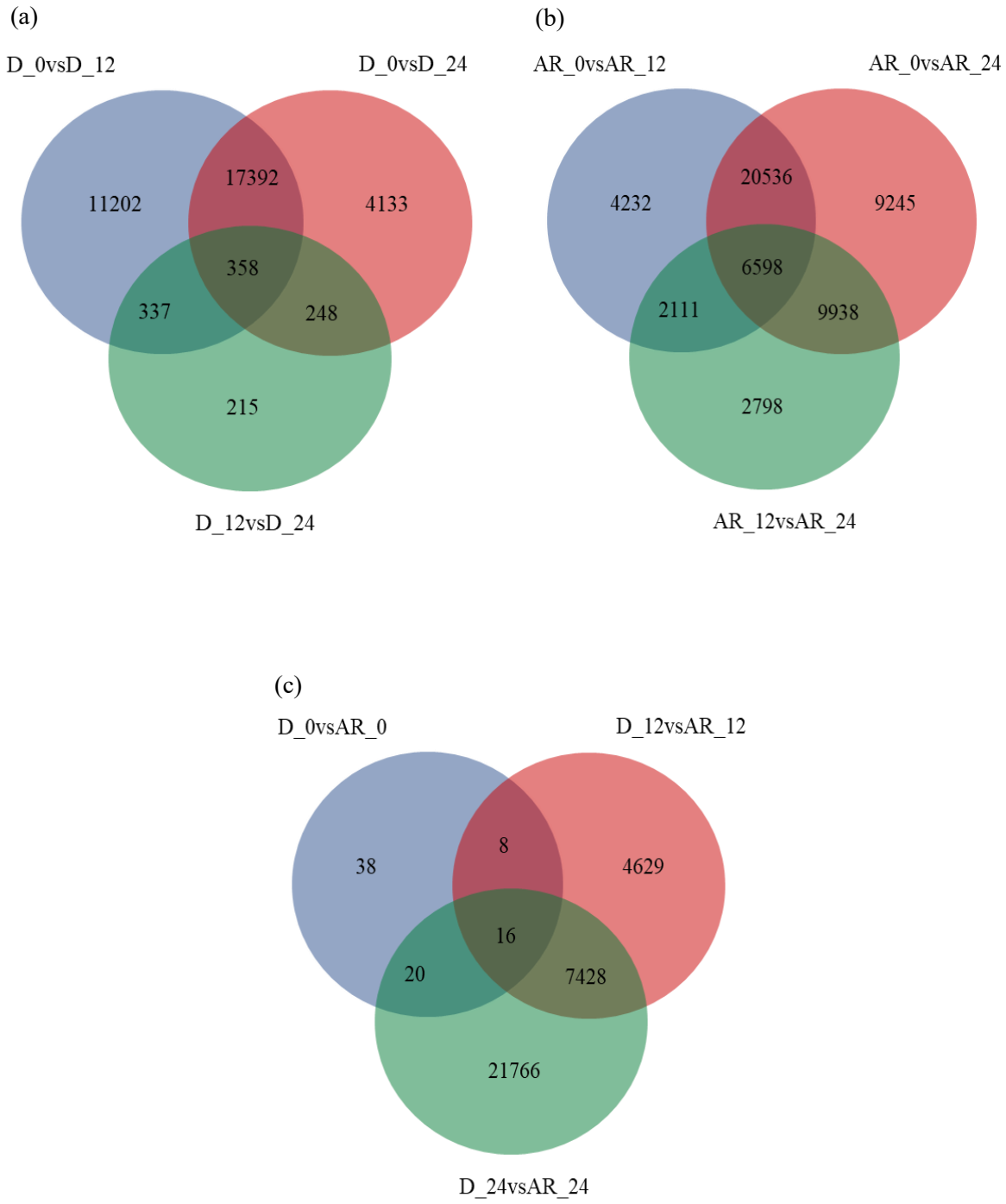


Figure 3.2. Venn diagrams showing the number of unique and shared DEGs among the different samples of dormant seeds at 0, 12 and 24 HAI (a), after-ripened (AR) seeds at 0, 12 and 24 HAI (b), dormant vs. AR seeds imbibed at 0, 12 and 24 HAI (c). Each circle represents the total number of DEGs for each comparison and the overlapping area represents DEGs shared by the different comparisons.

3.3.3 Gene ontology enrichment analysis of differentially expressed genes

3.3.3.1 Genes differentially expressed between dry dormant and after-ripened seeds

Only 82 differentially expressed genes were found between dry dormant and AR seeds, out of which 39 were downregulated and 43 were upregulated in dormant seeds (Appendix 4a). Given the small number of DEGs observed, no GO enrichment analysis was performed.

3.3.3.2 Genes differentially expressed between dormant and AR seeds at 12 h imbibition

Regarding comparison between dormant and AR seeds imbibed for 12 h (D_12 vs AR_12), 163 DEGs were enriched in GO terms related to biological processes including transmembrane transport, organic substance metabolic process, response to stimulus, and cellular processes (Figure 3.3). Specifically, many of the DEGs are enriched in response to auxin, cell wall biogenesis, glutathione metabolic process, and cellular modified amino acid metabolic process GO terms (Figure 3.3).

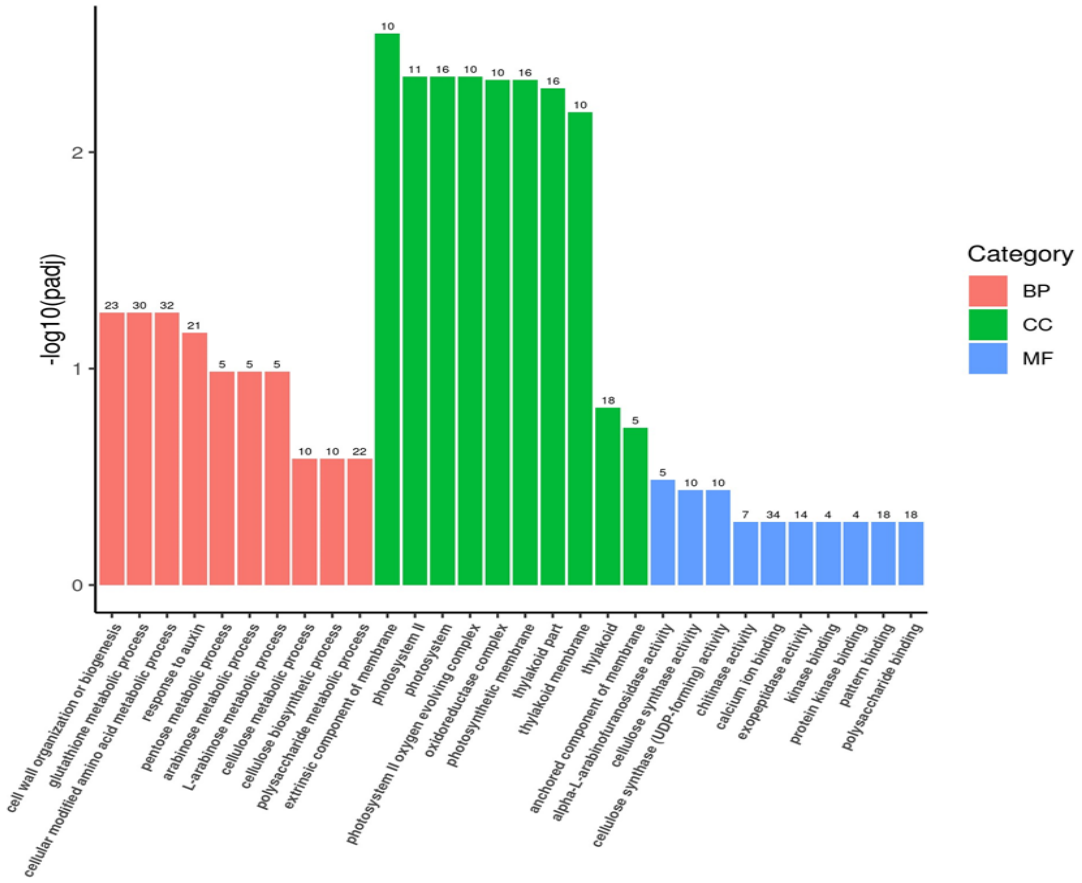


Figure 3.3. Gene ontologies enriched with genes differentially expressed between dormant and after-ripened seeds at 12 HAI. Different colours of the bar graphs represent the three main gene ontology domains: biological process (BP), cellular component (CC) and molecular function (MF).

Genes downregulated in D_12 as compared to AR_12 samples are primarily enriched in glutathione metabolic process, response to stimulus, including response to auxin, response to hormone, response to organic substance, and response to oxidative stress GO terms (Figure 3.4). Genes upregulated in D_12 as compared to AR_12 samples are enriched in GO terms related to photosynthesis, pentose metabolic process, and gene silencing (Figure 3.5).

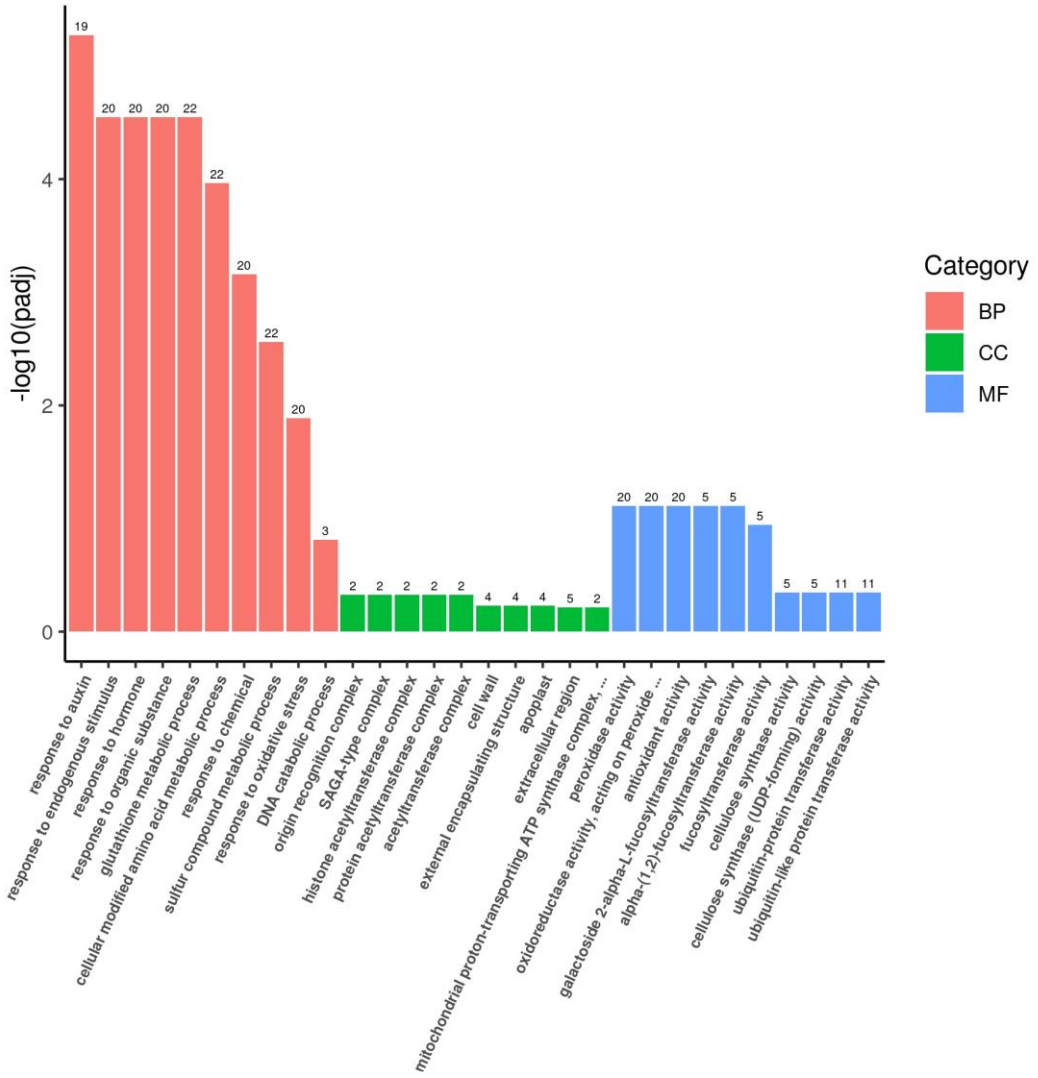


Figure 3.4. Gene ontologies enriched with genes downregulated in dormant as compared to after-ripened seeds at 12 HAI. Graph descriptions are as shown in Figure 3.3.

Under the cellular component domain of GO, 122 DEGs between D_12 and AR_12 seeds are found to be enriched in the broad categories of protein containing complex and cell anatomical entity (Fig. 3.3). Specifically, extrinsic component of membrane, photosystem, oxidoreductase complex, and photosynthetic membrane are among the topmost GO terms enriched with the DEGs (Figure 3.3). The same enriched GO terms are found to be enriched with genes upregulated in D_12 as compared to AR_12 samples (Figure 3.5). The functional GO terms enriched with genes downregulated in D_12 as compared to AR_12 samples genes

include origin recognition complex, cell wall, SAGA -type complex, and histone acetyltransferase complex (Figure 3.4).

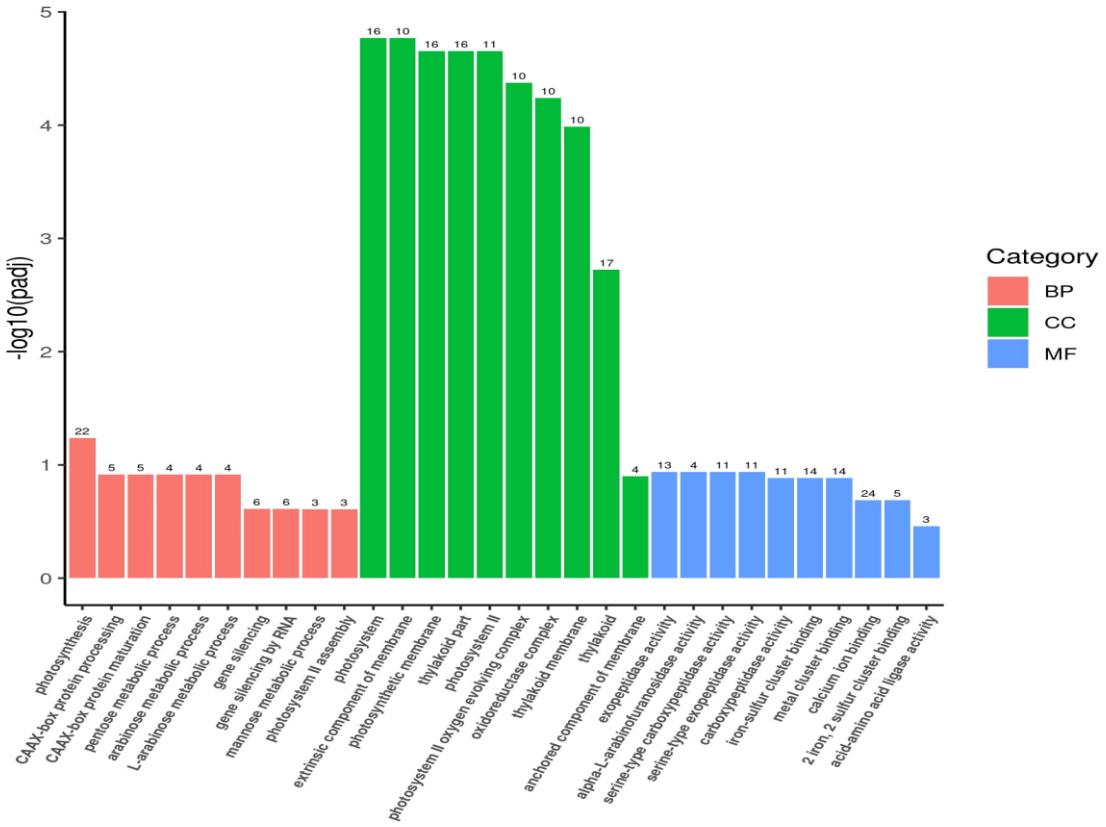


Figure 3.5. Gene ontologies enriched with genes upregulated in dormant as compared to after-ripened seeds at 12 HAI. Graph descriptions are as shown in Figure 3.3.

With respect to the molecular function domain, 124 DEGs between D_12 and AR_12 seeds are enriched in the catalytic activity and binding activity GO terms. Many of the 124 DEGs are specifically enriched in the alpha-L-arabinofuranosidase activity, cellulose synthase activity, chitinase activity, calcium ion binding, exopeptidase activity, and kinase binding GO terms (Figure 3.3). Peroxidase activity, oxidoreductase activity, antioxidant activity, and some catalytic activity terms are among GO terms enriched with genes downregulated in D_12 relative to AR_12 seeds (Figure 3.4). Genes upregulated in the same D_12 seeds are enriched in catalytic activity and binding activity GO terms (Figure 3.5).

3.3.3.3 Genes differentially expressed between dormant and AR seeds at 24 h imbibition

Regarding comparison between dormant and AR seeds imbibed for 24 h (D_24 vs AR_24), a total of 173 DEGs are enriched with specific biological processes GO terms, which include the broad categories of cellular process, organic substance metabolic process, and growth (Figure 3.6). In particular, the DEGs are mainly found to be enriched in positive regulation of biological process, microtubule-based process, oligosaccharide metabolic process, and growth GO terms (Fig. 3.6). The genes down regulated in D_24 relative to AR_24 seeds are primarily enriched in the positive regulation of biological process, growth, response to auxin, and movement of cell GO terms (Figure 3.7). Whereas genes upregulated in the same D_24 seed samples are enriched in the GO categories of generation of precursor metabolites and energy, cellular respiration, aerobic respiration, and electron transport chain (Figure 3.8).

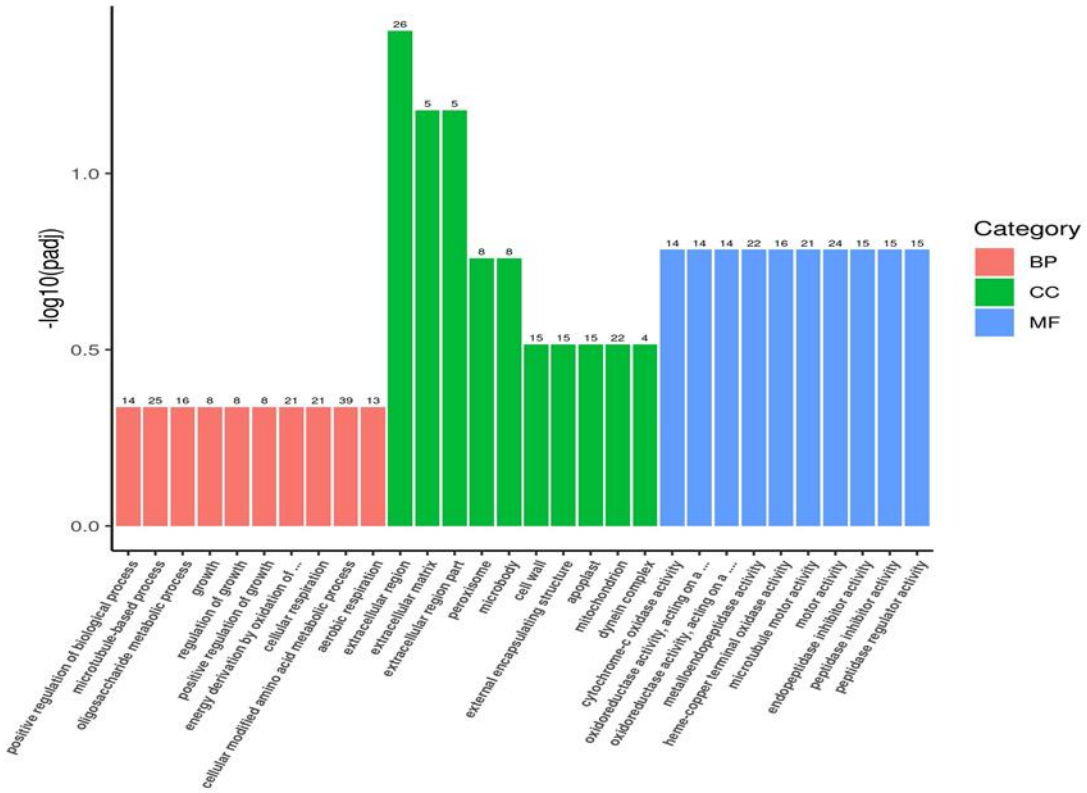


Figure 3.6. Gene ontologies enriched with genes differentially expressed between dormant and after-ripened seeds at 24 HAI. Graph descriptions are as shown in Figure 3.3.

When considering the cellular component GO domain, 123 DEGs between D_24 and AR_24 seeds are identified to be related to cellular anatomical entity; extracellular region, extra cellular matrix, peroxisome, microbody, and microbody are among the GO terms enriched with the DEGs (Figure 3.6). Gens downregulated in D_24 as compared to AR_24 seeds are primarily enriched in the extracellular region, mitochondrion, cell wall, and apoplast GO terms (Figure 3.7) while genes upregulated in the same seed sample are enriched GO terms related to extra cellular matrix, extracellular region part, peroxisomal membrane, and microbody membrane (Figure 3.8).

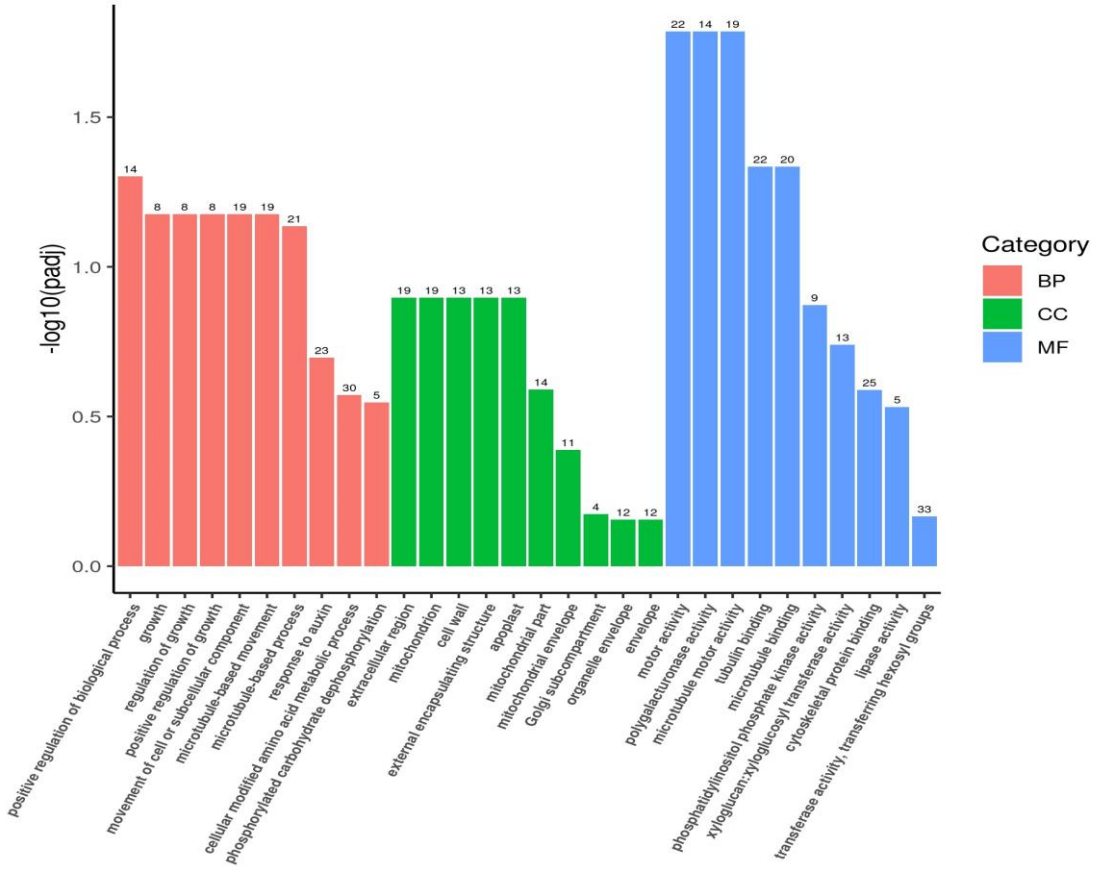


Figure 3.7. Gene ontologies enriched with genes downregulated in dormant as compared to after-ripened seeds at 24 HAI. Graph descriptions are as shown in Figure 3.3.

A total of 170 DEGs between D_24 and AR_24 seeds are enriched in the GO terms under the molecular function domain, which include the broad categories of catalytic activity, enzyme regulator activity, and transporter activity. with the top enriched GO terms specifically are the cytochrome-c oxidase activity, oxidoreductase activity, metalloendopeptidase activity, and microtubule motor activity GO terms (Figure 3.6). The genes downregulated in D_24 relative to AR_24 seeds are notably enriched with GO terms related to motor activity, polygalacturonase activity, microtubule motor activity, and tubulin binding (Figure 3.7). While the genes upregulated in the same seed sample are primarily related to the heme-copper terminal oxidase activity, cytochrome-c oxidase activity, oxidoreductase activity, endopeptidase inhibitor activity, and metalloendopeptidase activity GO terms (Figure 3.8).

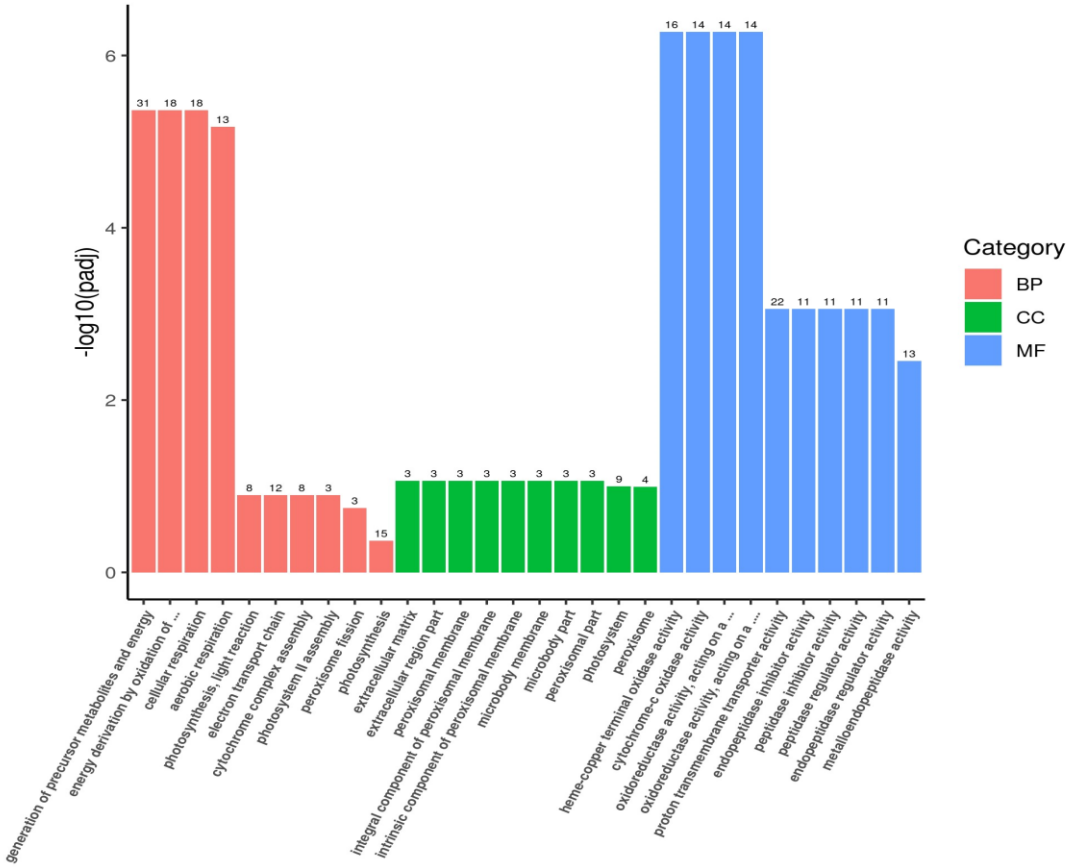


Figure 3.8. Gene ontologies enriched with genes upregulated in dormant as compared to after-ripened seeds at 24 HAI. Graph descriptions are as shown in Figure 3.3.

3.3.3.4 Genes differentially expressed in dormant seeds during imbibition

A total of 328 genes differentially expressed between dry and dormant seeds imbibed for 12 h (D_0 vs D_12) are enriched with biological processes GO terms. with the top GO terms enriched with these DEGs are telomere maintenance, telomere organization, cell recognition, and anatomical haemostasis (Appendix 5). The same GO terms were found to be enriched with genes downregulated in D_0 as compared to D12 seeds (Appendix 6a). Whereas gene upregulated in D_0 relative to D12 seeds are enriched in GO terms were related to aerobic respiration, DNA integration, and cellular respiration (Appendix 6b). With respect to cellular component GO domain, a total of 159 genes differentially expressed between D_0 and D_12 seeds are enriched in photosystem, photosynthetic membrane, thylakoid part, and photosystem II (Appendix 5). Genes downregulated in D_0 as compared to D12 seeds are enriched in GO terms related to cell wall, external encapsulating structure, apoplast, and cell periphery (Appendix 6a) while the genes upregulated in the same samples are primarily related to photosystem, photosynthetic membrane, thylakoid part, and photosystem I GO terms (Appendix 6b). Under the molecular function Go domain, a total of 305 are enriched in double-stranded DNA binding, pattern binding, and polysaccharide binding GO terms, and the same GO terms are primarily enriched with genes downregulated in D_0 as compared to D12 seeds (Appendix 5, 6a). The genes upregulated in the same seed samples are related mainly to the RNA-DNA hybrid ribonuclease activity, cytochrome-c oxidase activity, and oxidoreductase activity GO terms (Appendix 6b).

A total of 98 genes differentially expressed between dormant seeds imbibed for 12 and 24 h (D_12 and D_24) are enriched in specific biological processes GO terms; the top top GO terms enriched with these DEGs include DNA replication, DNA-dependent DNA replication, fatty acid biosynthetic process, and fatty acid metabolic process (Appendix 7). Genes downregulated in D_12 relative to D_24 seeds are enriched with GO terms related to cell wall

organization, aminoglycan metabolic process, and aminoglycan catabolic process (Appendix 8a). The genes upregulated in the same seed samples are primarily related to DNA replication, DNA-dependent DNA replication, and fatty acid biosynthetic process GO terms (Appendix 8b). Concerning the cellular component GO domain, only 16 genes differentially expressed between D_12 and D_24 samples are found to be enriched in specific GO terms, which include chromosome, cytochrome complex, origin recognition complex, and extracellular region (Appendix 7). Genes downregulated in D_12 relative to D_24 seeds are enriched in GO terms related to extracellular region, chloroplast, and plastid (Appendix 8a) while those upregulated in D_12 seeds are primarily related to chromosome, cytochrome complex, and origin recognition complex GO terms (Appendix 8b). In regard to the molecular function GO domain, a total of 53 DEGs between D_12 and D_24 samples are enriched with specific GO terms that include 3-beta-D-glucan synthase complex, chitin binding, tubulin binding, and microtubule binding (Appendix 7). Genes downregulated in D_12 relative to D_24 seeds are enriched in GO terms related to chitin binding, enzyme inhibitor activity, chitinase activity, and pectinesterase activity (Appendix 8a) while those upregulated in D_12 seeds are primarily related to chromosome, tubulin binding, microtubule binding, and cytoskeletal protein binding GO terms (Appendix 8b).

3.3.3.5 Genes differentially expressed in after-ripened seeds during imbibition

In regard to comparison between dry and 12 h imbibed AR seeds (AR_0 vs AR_12), 366 DEGs were found to be enriched in the biological processes GO terms, which include response to auxin, cell recognition, pollination and pollen-pistil interaction, and the same GO terms are enriched with those downregulated in AR_0 as compared to AR_12 seeds (Appendix 9, 10a). Genes upregulated in AR_0 as compared to AR_12 seeds are enriched in functional GO terms related to cellular respiration, generation of precursor metabolites and energy, and aerobic

respiration (Appendix 10b). With respect to cellular component GO domain, 134 DEGs between AR_0 vs AR_12 seeds are attributed to photosystem, photosynthetic membrane, cytoskeletal part, and thylakoid part (Appendix 9). Genes downregulated in AR_0 as compared to AR_12 seeds are enriched in GO terms related to cell wall, external encapsulating structure, apoplast, and extracellular region (Appendix 10a) while those upregulated are enriched in photosystem, photosynthetic membrane, thylakoid part, and thylakoid GO terms (Appendix 10b). Concerning the molecular function GO domain, 191 DEGs between AR_0 vs AR_12 seeds are shown to be enriched in double-stranded DNA binding, phospholipase activity, and lipase activity GO terms (Appendix 9). Genes downregulated in AR_0 as compared to AR_12 seeds are enriched with double-stranded DNA binding, pattern binding, and polysaccharide binding GO terms (Appendix 10a) while those upregulated in AR_0 are primarily related to the RNA-DNA hybrid cytochrome-c oxidase activity, oxidoreductase activity, and heme-copper terminal oxidase activity GO terms (Appendix 10b).

Concerning comparison between 12 h and 24 h imbibed AR seeds (AR_12 vs AR_24), a total of 307 DEGs are enriched with specific GO terms related to biological processes, which include multicellular organismal process, metal ion transport, and reproduction (Appendix 11). Genes downregulated in AR_12 as compared to AR_24 seeds are enriched with GO terms related to response to oxidative stress, metal ion transport, and multicellular organismal process (Appendix 12a). The upregulated genes, however, were primarily related to response to water, response to acid chemical, and response to oxygen-containing compound GO terms (Appendix 12b). With respect to cellular component GO domain, a total of 134 DEGs were found to be enriched in photosystem, photosynthetic membrane, and cytoskeletal part GO terms (Appendix 11). Genes downregulated as well as upregulated in AR_12 as compared to AR_24 seeds are primarily enriched in GO terms related to photosystem II, extrinsic component of membrane, photosystem II oxygen evolving complex, and thylakoid membrane (Appendix 12a, b).

Concerning molecular function GO domain, 244 DEGs are enriched with flavin adenine dinucleotide binding, FAD binding, pattern binding, and N,N-dimethylalaniline monooxygenase activity GO terms (Appendix 11). Genes downregulated in AR_12 as compared to AR_24 seeds are enriched in GO terms related to flavin adenine dinucleotide binding, N,N-dimethylalaniline monooxygenase activity, and oxidoreductase activity (Appendix 12a) while those upregulated are primarily related to chromosome, kinase binding, protein kinase binding, and phospholipase A2 activity GO terms (Appendix 12b).

3.3.4 Differential expression of genes involved in ABA metabolism

3.3.4.1 Expression of ABA biosynthesis genes

Of the *ZEP* homeologs of wheat, the *TaZEP_2A* homeolog showed similar level of expression between the dormant and AR seeds except that it was downregulated (over 2-fold) in dormant seeds relative to AR seeds at 12 h imbibition (Figure 3.9; Appendix 13). In dormant seeds, *TaZEP_2A* was downregulated (over 2-fold) after 12 h imbibition and remained at similar level between 12 and 24 HAI (Figure 3.9; Appendix 13). The first 12 h imbibition did not affect *TaZEP_2A* expression level in AR seeds; however, a marked downregulation (over 3-fold) was observed during transition from 12 to 24 h imbibition (Figure 3.9; Appendix 13). For the *TaZEP_2B* homeolog, no difference in its expression level was evident between dry dormant and dry AR seeds (Figure 3.9; Appendix 13). However, the same gene showed downregulation (over 3-fold) in dormant seeds at 12 and 24 HAI, respectively (Figure 3.9; Appendix 13). In dormant seeds, *TaZEP_2B* homeolog exhibited similar level of expression during imbibition. In AR seeds, it exhibited upregulation (over 3-fold) after 12 h imbibition but a decrease (over 2-fold) during 12 to 24 HAI (Figure 3.9; Appendix 13). Furthermore, the *TaZEP_2D* homeolog showed no change in expression level between the dormant and AR seeds at 0 and 24 HAI but was downregulated (over 3.5-fold) in dormant seeds after 12 h imbibition (Figure 3.9; Appendix

13). In dormant seeds, *TaZEP_2D* exhibited downregulation during the first 12 h imbibition but remained at a similar level between 12 and 24 HAI (Figure 3.9; Appendix 13). In contrast, it exhibited similar expression at 12 HAI in AR seeds but exhibited downregulation (over-2 fold) during transition from 12 to 24 h imbibition (Figure 3.9; Appendix 13).

With respect to the *VDE* homeologs of wheat; *TaVDE1_2A* and *TaVDE1_2B* showed similar expression levels between dormant and AR seeds at 0 and 12 HAI but were downregulated in dormant seeds (over 3-fold) by 24 HAI (Figure 3.9; Appendix 13). In dormant seeds, no difference in *TaVDE1_2A* and *TaVDE1_2B* expression level was evident during imbibition while in AR seeds both homeologs exhibited similar expression at 12 HAI and upregulation (over 6-fold) during transition from 12 to 24 h imbibition (Figure 3.9; Appendix 13). The *TaVDE1_2D* homeolog showed similar expression level between dormant and AR seeds at any time points of imbibition (Figure 3.12, Appendix 6). In contrast, the same homeolog showed upregulation in both dormant and AR seeds at 12 HAI (over 3-fold) and no change in its expression level between 12 and 24 HAI (Figure 3.9; Appendix 13).

Concerning the wheat homeologs of the *NCED1* gene, *TaNCED1_6B* showed similar expression level between dormant and AR seeds at 0 and 24 HAI but was upregulated (over 4-fold) in dormant seeds at 12 HAI (Figure 3.9; Appendix 13). In dormant seeds, *TaNCED1_6B* was upregulated (4.8-fold) by 12 h imbibition and remained at similar level following 12 HAI (Figure 3.9; Appendix 13). In AR seeds, the first 12 h imbibition did not affect its expression level but a marked upregulation (over 10-fold) of this gene was observed at 24 HAI (Figure 3.9; Appendix 13). The *TaNCED1_6D* homeolog showed similar level of expression between dormant and AR seeds at 0 HAI and 12 HAI but was downregulated (over 2-fold) in dormant seeds as compared to AR seeds at 24 HAI (Figure 3.9; Appendix 13). The *TaNCED1_6D* homeolog showed upregulation (over 2-fold) during imbibition of both dormant and AR seeds

except there was no difference in its expression level between 12 and 24 HAI in dormant seeds (Figure 3.9; Appendix 13).

The homeologs of another member of the *TaNCED* gene family, *TaNCED3,* showed differential expression between the dormant and AR seeds (Figure 3.9; Appendix 13). The *TaNCED3_5A* homeolog showed upregulation (over 6-fold) at 12 HAI in dormant seeds relative to AR seeds with no difference in its expression between the two seed samples at 0 and 24 HAI (Figure 3.9; Appendix 13). In dormant seeds, no difference in the expression level of *TaNCED3_5A* occurred during imbibition; however, it exhibited downregulation (over 4.5-fold) and upregulation (over 10-fold) after 12 h and 24 imbibition of AR seeds, respectively (Figure 3.9; Appendix 13). The *TaNCED3_5B* homeolog was the only gene that exhibited differential expression before imbibition, with an over 8-fold upregulation observed in dormant relative to AR seeds (Figure 3.9; Appendix 13). This homeolog also showed upregulation (over 3-fold) in dormant as compared to AR seeds at 12 HAI but had similar expression between the two seed samples (Figure 3.9; Appendix 13). In dormant seeds, *TaNCED3_5B* showed upregulation (over 3-fold) after 12 h imbibition and remained at similar level by 24 HAI (Figure 3.9; Appendix 13). Moreover, the same homeolog showed upregulation (over 5-fold) in AR seeds during imbibition (Figure 3.9; Appendix 13). The *TaNCED3_5D* homeolog showed a similar differential expression pattern as *TaNCED3_5A* between the two seed samples (Figure 3.9; Appendix 13). It exhibited upregulation (over 15-fold) at 12 HAI but showed similar expression level at 0 and 24 HAI (Figure 3.9; Appendix 13). In dormant seeds, *TaNCED3_5D* was downregulated (over 3-fold) at 12 HAI with no change in its expression during 12 to 24 h imbibition (Figure 3.9; Appendix 13). This homeolog also showed downregulation (over 28-fold) in AR seeds after 12 h imbibition; however, its expression was upregulated (over 20-fold) during 12 to 24 h imbibition (Figure 3.9; Appendix 13).

The *NCED4* gene was identified to have only one homeolog, *TaNCED4_2D*, which showed no change in its expression level between dormant and AR seeds at 0, 12 and 24 HAI (Figure 3.9; Appendix 13). In dormant seeds, *TaNCED4_2D* showed no change in expression during the first 12 h imbibition but exhibited upregulation (over 40-fold) at 24 HAI (Figure 3.9; Appendix 13). In AR seeds, the same homeolog showed no change in expression during imbibition (Figure 3.9; Appendix 13). The *NCED5* gene was identified to have only two homeolog, *TaNCED5_5B* which exhibited similar expression between dormant and AR seeds at 0 and 12 HAI but was upregulated (over 5-fold) in dormant seeds at 24 HAI (Figure 3.9; Appendix 13). In both dormant and AR seeds, the same homeolog showed upregulation (over 9-fold) during the first 12 h imbibition (Figure 3.9; Appendix 13). During 12 to 24 h imbibition, the same gene showed no change in expression in dormant seeds but exhibited downregulation (over 4-fold) in AR seeds (Figure 3.9; Appendix 13). The *TaNCED5_5D* homeolog showed similar expression between dormant and AR seeds before and after imbibition (Figure 3.9; Appendix 13). The same homeolog showed upregulation (over 4-fold) in both dormant and AR seeds during the first 12 h imbibition but exhibited similar expression and downregulation (over 3-fold) in dormant and AR seeds, respectively during 12 to 24 h imbibition (Figure 3.9; Appendix 13).

Expression of the homeologs another ABA biosynthesis gene, AAO, was examined. Homeolog *TaAAO_7A* showed similar expression level in dry dormant and dry AR seeds (Figure 3.9; Appendix 13). However, the same gene was upregulated (over 2-fold) between the two seed samples at 12 HAI and 24 HAI (Figure 3.9; Appendix 13). No change in the expression level of *TaAAO_7A* occurred during imbibition of the dormant seeds; however, in AR seeds it showed downregulation and upregulation (over 2-fold) during the first 12 h imbibition and during the 12 to 24 h imbibition, respectively (Figure 3.9; Appendix 13). The *TaAAO_7B* homeolog showed a similar expression as *TaAAO_7A* between the two seed samples

and during imbibition of dormant seeds (Figure 3.9; Appendix 13). In AR seeds, this homeolog showed downregulation (over 4-fold) after 12 h imbibition (Figure 3.9; Appendix 13). However, it exhibited similar expression during 12 h to 24 h imbibition (Figure 3.9; Appendix 13). The *TaAAO_7D* homeolog exhibited upregulation (over 6-fold) in dormant as compared to AR seeds at 12 HAI with no difference in its expression level between the two seed samples at 0 and 24 HAI (Figure 3.9; Appendix 13). The *TaAAO_7D* homeolog showed no change in expression during imbibition of dormant seeds, like that observed for *TaAAO_7B* and *TaAAO_7A* (Figure 3.9; Appendix 13). In AR seeds, the same gene exhibited downregulation (over 4-fold) during the first 12 h imbibition and upregulation (over 3-fold) during 12 to 24 h imbibition (Figure 3.9; Appendix 13).

3.3.4.2 Expression of ABA catabolism genes

We also analysed the expression of genes involved in ABA catabolism. Two homeologs of *TaCYP707A1*, a member of the *TaCYP707A* gene family, were identified (Figure 3.9; Appendix 13). The *TaCYP707A1_6A* homeolog showed similar expression between dormant and AR seeds at 0 and 24 HAI but it was downregulated (over 2-fold) in dormant seeds at 12 HAI (Figure 3.9; Appendix 13). The same homeolog exhibited downregulation (over 2-fold) after 12 h imbibition but remained at similar level during 12 to 24 h imbibition in both dormant and AR seeds (Figure 3.9; Appendix 13). The *TaCYP707A1_6B* homeolog showed no difference in its expression level between dormant and AR seeds at any imbibition time points (Figure 3.9; Appendix 13). It showed downregulation (over 2-fold) during the first 12 h imbibition (Figure 3.9; Appendix 13).

Two homeologs of another member of the *TaCYP707A* gene, *TaCYP707A3*, were identified. The *TaCYP707A3_5B* homeolog showed similar expression level in dormant relative to AR seeds at 0 and 12 HAI but was downregulated (over 100-fold) in dormant seeds by 24

HAI (Figure 3.9 Appendix 13). The same homeolog showed a similar expression level during the first 12 h imbibition of dormant seeds but was downregulated (over 100-fold) during the 12 to 24 h imbibition (Figure 3.9; Appendix 13). In AR seeds, it showed similar expression during the whole duration of imbibition (Figure 3.9; Appendix 13). The *TaCYP707A3_5D* homeolog showed no difference in its expression between the two seed samples at 0, 12 and 24 HAI (Figure 3.9; Appendix 13). However, it showed downregulation (over 2-fold) in both dormant and AR seeds during the first 12 h imbibition but no change in its expression level during the 12 to 24 h imbibition period (Figure 3.9; Appendix 13).

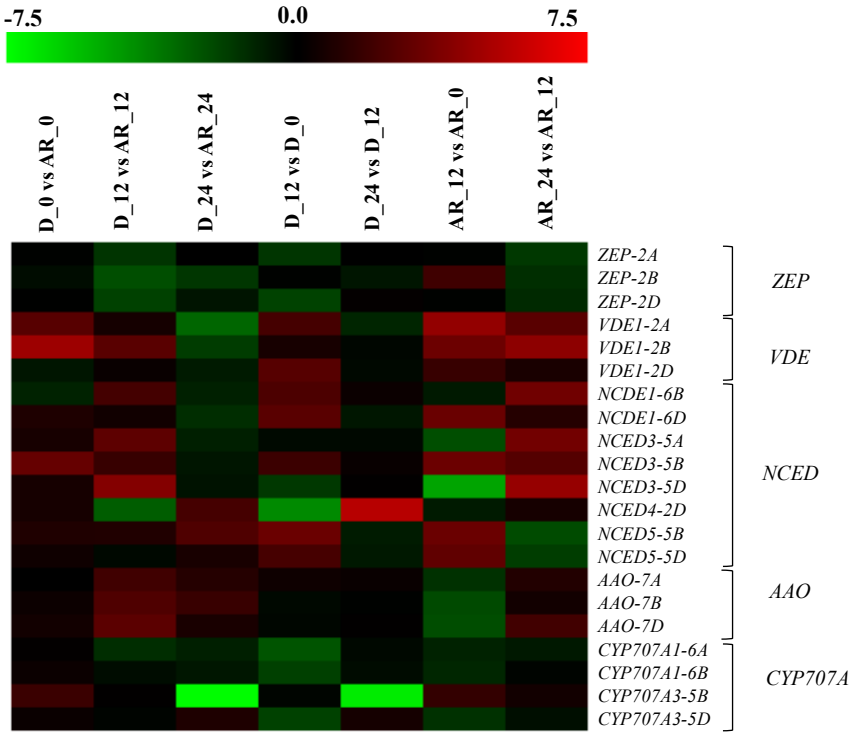


Figure 3.9. Heatmap depicting changes in the expression of genes involved in ABA metabolism. Log2-scale fold change in expression of ABA biosynthesis and catabolism genes between dormant and after-ripened seeds at different time points of imbibition (D_0 vs AR_0, D_12 vs AR_12 and D_24 vs AR_24) and during imbibition of dormant (D_12 vs D_0 and D_24 vs D_12) and after-ripened (AR_12 vs AR_0 and AR_24 vs AR_12) seeds. The negative and positive numbers on the bar show log2 fold change values. Red colour indicates upregulation and green colour indicated downregulation. ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NCED, 9-cis-epoxycarotenoid dioxygenase; AAO, abscisic aldehyde oxidase.

3.3.5. Differential expression of genes involved in ABA signaling

This study investigated the expression pattern of genes encoding PYL, the ABA receptors (Figure 3.10; Appendix 14). All the three homeologs *TaPYL4_2A*, *TaPYL4_4B*, and *TaPYL4_4D* homeologs showed similar expression level between dormant and AR seeds before imbibition (Figure 3.10; Appendix 14). All the three homeologs showed downregulation (over 2-fold) in dormant seeds relative to AR seeds during imbibition (Figure 3.10; Appendix 14). The *TaPYL4_2A* homeolog showed upregulation (over 4-fold) during the first 12 h imbibition in both dormant and AR seeds; however, it was either downregulated (over 2-fold) in dormant seeds and showed similar expression in AR seeds during the 12 to 24 h imbibition (Figure 3.10; Appendix 14). The expression level of *TaPYL4_4B* showed no change and upregulation (over 5-fold) during imbibition of dormant and AR seeds, respectively (Figure 3.10; Appendix 14). The *TaPYL4_4D* homeolog was upregulated (over 100-fold) after 12 h imbibition but remained at similar level during 12 to 24 h imbibition of the dormant seeds (Figure 3.10; Appendix 14). In AR seeds, it showed upregulation (over 2-fold) during imbibition (Figure 3.10; Appendix 14). A homeolog of another member of *TaPYL* gene family, *TaPYL9_7A*, showed upregulation (over 50-fold) between the dormant and AR seeds at 0 HAI with no difference in its expression level after 12 h imbibition (Figure 3.10; Appendix 14). By the 24 HAI, the same it was downregulated (over 2-fold) in dormant seeds as compared to AR seeds (Figure 3.10; Appendix 14). In both seed samples, *TaPYL9_7A* was upregulated (over 20-fold) after 12 h imbibition with no change in its expression level during the next 12 h imbibition (Figure 3.10; Appendix 14). For the *TaPYL10_1A* homeolog, no change in its expression was found at 0 and 12 HAI between the two seed samples (Figure 3.10; Appendix 14). The same gene showed downregulation (over 4-fold) in dormant relative to AR seeds after 24 h imbibition (Figure 3.10; Appendix 14). *TaPYL10_1A* homeolog exhibited similar expression during imbibition of both dormant and AR seeds (Figure 3.10; Appendix 14).

One of the homeologs of *TaPP2C*, *TaPP2C8_3A* exhibited no change in expression in dormant relative to AR seeds at 0 and 12 HAI. By 24 HAI, the same homeolog showed upregulation (over 3-fold) in dormant seeds relative to AR seeds (Figure 3.10; Appendix 14). The *TaPP2C8_3A* homeolog showed similar expression during imbibition of both dormant and AR seeds (Figure 3.10; Appendix 14). The *TaPP2C8_3B* homeolog showed upregulation (over 2-fold) in dormant as compared to AR seeds before and after imbibition; by 24 HAI, the level of its upregulation in dormant seeds was over 35-fold (Figure 3.10; Appendix 14). Within the dormant seeds, the same homeolog showed no change in its expression level during imbibition, whereas it showed a similar level of expression during the first 12 h imbibition but exhibited downregulation (over 12-fold) after 24 h imbibition in AR seeds (Figure 3.10; Appendix 14). For the *TaPP2C8_3D* homeolog, similar level of its expression was observed between dry dormant and AR seeds; however, it exhibited upregulation (over 2-fold) in dormant seeds relative to AR seeds during imbibition (Figure 3.10; Appendix 14). The same homeolog exhibited upregulation (over 2-fold) after 12 h imbibition of dormant seeds but remained at similar level from 12 to 12 HAI. In AR seeds, it showed similar expression during the first 12 h imbibition but showed downregulation (over 2-fold) during the 12 to 24 h imbibition (Figure 3.10, Appendix 14). Regarding the *TaPP2C30_4A* homeolog, it showed no difference in expression level between dormant and AR seeds at 0 and 24 HAI (Figure 3.10; Appendix 14). However, the same homeolog showed upregulation (over 12-fold) in dormant seeds relative to AR seeds after 12 h imbibition. In dormant seeds, it showed upregulation (over 2-fold) after 12 h imbibition but no change in its expression was found during the next 12 imbibition (Figure 3.10; Appendix 14). In contrast, it exhibited downregulation (over 6-fold) during the first 12 h imbibition and upregulation (over 7-fold) during the 12 to 24 h imbibition in AR seeds (Figure 3.10; Appendix 14).

The homeolog of another member of *TaPP2C* gene family, *TaPP2C37_2A*, showed no difference in its expression level between the dormant and AR samples at 0 HAI but showed upregulation (over 2-fold) in dormant seeds at 12 and 24 HAI (Figure 3.10; Appendix 14). The same homeolog showed downregulation (over 2-fold) during imbibition in both dormant and AR seeds, except there was no change in its expression level during 12 to 24 h imbibition in dormant seeds (Figure 3.10; Appendix 14). The *TaPP2C37_2B* homeolog showed no difference in its expression level between the two seed samples at 0 and 12 HAI. By 24 h imbibition, the same homeolog exhibited upregulation (over 7-fold) in dormant seeds relative to AR seeds (Figure 3.10; Appendix 14). It exhibited similar expression during imbibition of dormant seeds but downregulation (over 2-fold) in AR seeds (Figure 3.10; Appendix 14). The *TaPP2C50_1A* homeolog showed similar expression at 0 HAI but showed upregulation (over 5-fold) in dormant seeds relative to AR seeds at 12 and 24 HAI (Figure 3.10; Appendix 14). This homeolog showed similar expression level during imbibition of the dormant seeds (Figure 3.10; Appendix 14). In AR seeds, it showed downregulation (over 4-fold) during the first 12 h imbibition with no change in its expression level during the 12 h to 24 h imbibition (Figure 3.10; Appendix 14).

The homoeologous genes encoding another member of *TaPPP2Cs*, which includes *TaPP2C50* (*TaPP2C50_3A*, *TaPP2C50_3B*, and *TaPP2C50_3D*) and *TaPP2C51* (*TaPP2C51_1A*, *TaPP2C51_1B*, and *TaPP2C51_1D*) showed similar expression levels between dormant and AR seeds at 0 HAI and upregulation (over 2-fold) in dormant seeds during imbibition (Figure 3.10; Appendix 14). The homoeologous genes showed no change in their expression level during imbibition of dormant seeds but were downregulated (over 2-fold) during the first 12 h imbibition of AR seeds except the *TaPP2C51_1D* homeolog which showed no change in its expression level (Figure 3.10; Appendix 14). From 12 to 24 h imbibition, the gene sets showed no change in their expression level except that the *TaPP2C51_1D* homeolog

was downregulated (over 2-fold) in AR seeds (Figure 3.10; Appendix 14). This study also examined the expression pattern of the homeologs of another member of the *TaPP2C* gene family, which include *TaPP2C68_5A* and *TaPP2C68_5D.* The *TaPP2C68_5A* homeolog showed similar expression between the dormant and AR seeds at 0 HAI; however, it was upregulated (over 5-fold) in dormant seeds during imbibition (Figure 3.10; Appendix 14). In dormant seeds, no change in its expression level was found during imbibition while it showed downregulation (over 15-fold) in AR seeds during the first 12 h imbibition but remained at a similar level during the next 12 h imbibition (Figure 3.10; Appendix 14). The *TaPP2C68_5D* homeolog exhibited upregulation (over 2-fold) in dry and imbibed dormant seeds relative to the AR seeds (Figure 3.10; Appendix 14). The same homeolog showed no change in its expression level in both dormant and AR seeds during imbibition (Figure 3.10; Appendix 14).

Regarding *SnRK* genes, the *TaSnRK3_1A* and *TaSnRK3_1B* homeologs showed similar expression between dormant and AR genes at 0 HAI but exhibited upregulation (over 3-fold) in dormant seeds compared to AR seeds at both 12 and 24 HAI (Figure 3.10; Appendix 14). The two homeologs exhibited no change in expression level during imbibition of dormant seeds (Figure 3.10; Appendix 14). In AR seeds, *TaSnRK3_1A* showed downregulation (over 2-fold) and *TaSnRK3_1B* exhibited no change in expression during the first 12 h imbibition, but no change in expression level of both homeologs was observed during 12 to 24 h imbibition (Figure 3.10; Appendix 14). A homeolog of another member of the *TaSnRKs* gene family, *TaSnRK10_4A*, exhibited similar expression level between dormant and AR seeds before imbibition and 12 HAI. By 24 HAI, it exhibited strong upregulation (over 7-fold) in dormant relative to AR seeds (Figure 3.10; Appendix 14). The same homeolog showed downregulation (over 2-fold) in both dormant and AR seeds during imbibition except it had similar expression level from 12 to 24 h imbibition in dormant seeds (Figure 3.10; Appendix 14). Homeolog

TaSnRk10_4D also exhibited similar expression pattern as *TaSnRK10_4A* (Figure 3.10; Appendix 14).

Our study also investigated the expression pattern of genes encoding *TaABI5*, a transcription factor. The *TaABI5_3A*, *TaABI5_3B*, *TaABI5_3D*, *TaABI5_2A*, and *TaABI5_4A* homeologs of *TaABI5* showed similar expression patterns during imbibition (Figure 3.10; Appendix 14). These five homeologs showed no change in expression level between dormant and AR seeds at 0 and 12 h imbibition (Figure 3.10; Appendix 14). By 24 HAI, the *TaABI5_3A*, *TaABI5_3B*, and *TaABI5_3D* homeologs exhibited upregulation (over 5-fold) in dormant seeds compared to AR seeds except that the *TaABI5_2A* and *TaABI5_4A* homeologs exhibited downregulation (over 3-fold) in dormant seeds (Figure 3.10; Appendix 14). All the five homeologs showed no change in expression during imbibition of dormant seeds. They also showed no change in their expression level in AR seeds during the first 12 h imbibition except for *TaABI5_4A* homeolog which showed upregulation (over 2-fold) (Figure 3.10; Appendix 14). During 12 to 24 h imbibition, the *TaABI5_3A*, *TaABI5_3B*, and *TaABI5_3D* homeologs showed downregulation (over 5-fold) in AR seeds (Figure 3.10; Appendix 14). In contrast, the expression of *TaABI5_2A* homeolog increased (over 3-fold) while there was no change in the expression level of *TaABI5_4A* homeolog during 12 to 24 h imbibition in AR seeds (Figure 3.10; Appendix 14).

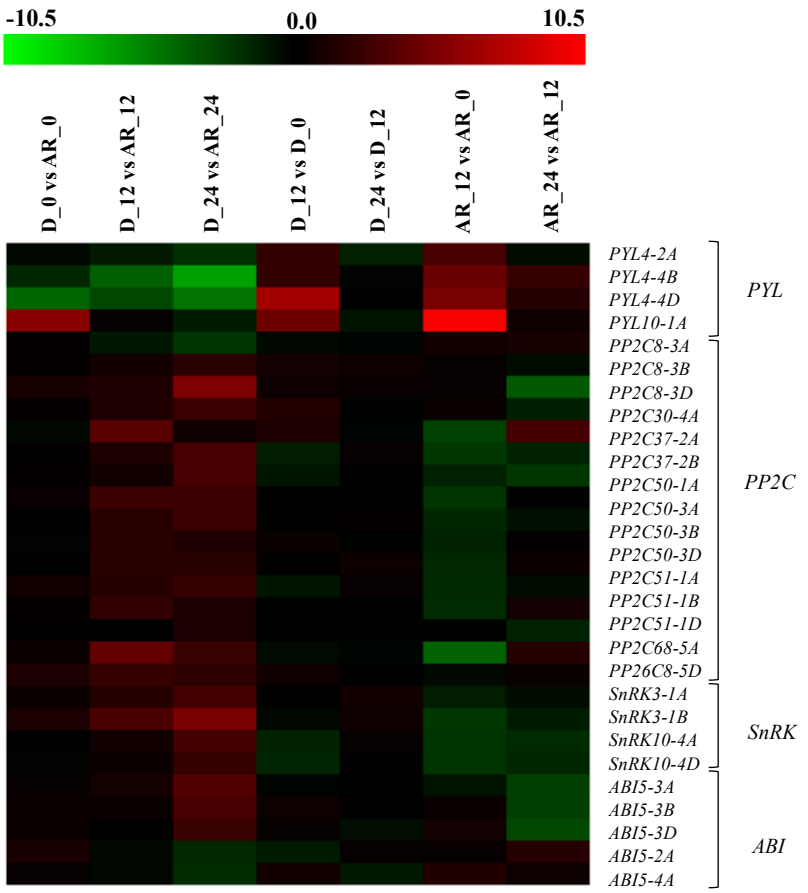


Figure 3.10. Heatmap depicting changes in the expression of genes involved in ABA signaling. Log2-scale fold change in expression of ABA signaling genes between dormant and after-ripened seeds at different time points of imbibition (D_0 vs AR_0, D_12 vs AR_12 and D_24 vs AR_24) and during imbibition of dormant (D_12 vs D_0 and D_24 vs D_12) and after-ripened (AR_12 vs AR_0 and AR_24 vs AR_12) seeds. The negative and positive numbers on the bar show log2 fold change values. Red colour indicates upregulation and green colour indicated downregulation. PYL, pyrabactin-like; PP2C, protein phosphatase 2C; SnRK, SNF1-related protein kinase; ABI, abscisic acid insensitive.

3.3.6. Differential expression of genes involved in GA metabolism

3.3.6.1 Expression of GA biosynthesis genes

This study also analysed the expression of genes involved in GA biosynthesis. The homeologs of *TaCPS1*, specifically *TaCPS1_1A*, *TaCPS1_7A*, and *TaCPS1_7B* showed no difference in expression level in dormant relative to AR seeds at all imbibition time points (Figure 3.11; Appendix 15). The only notable difference was the over 2-fold downregulation of *TaCPS1_1A*

homeolog after 12 h imbibition in dormant seeds (Figure 3.11; Appendix 15). The *TaCPS1_1A* homeolog showed similar expression level but upregulation (over 2-fold) during the initial 12 h imbibition in dormant and AR seeds, respectively (Figure 3.11; Appendix 15). In contrast, the *TaCPS1_7A* and *TaCPS1_7B* homeologs showed over 2-fold decrease in their expression levels in both seed samples after 12 h imbibition (Figure 3.11; Appendix 15). During 12 to 24 h imbibition, no change in the expression level of all the three homeologs was detected in both dormant and AR seeds (Figure 3.11; Appendix 15). For the *TaKO2_7B* homeolog, similar expression level was evident between the dormant and AR seeds at 0 and 12 h imbibition but by 24 h imbibition its expression level was over 2-fold higher in dormant relative to AR seeds (Figure 3.11; Appendix 15). The *TaKO2_7B* homeolog showed similar expression in both dormant and AR seeds during imbibition except it was downregulated (over 2-fold) during 12 to 24 h imbibition in AR seeds (Figure 3.11; Appendix 15).

The *TaKAO1* homeologs, which include *TaKAO1_4A*, *TaKAO1_5B*, and *TaKAO1_5D*, showed similar expression level in dormant seeds and AR seeds at 0 and 12 HAI (Figure 3.11; Appendix 15). However, these homeologs showed over 2-fold lower expression in dormant than AR seeds after 24 h imbibition (Figure 3.11; Appendix 15). The three homeologs showed no change in their expression level during the process of imbibition in dormant seeds except the *TaKAO1_5D* homeolog was downregulated (over 4-fold) after 12 h imbibition (Figure 3.11; Appendix 15). The same three homeologs showed similar expression level in AR seeds during the first 12 h imbibition (Figure 3.11; Appendix 15). During 12 to 24 h imbibition, the *TaKAO1_4A* and *TaKAO1_5D* homeologs were upregulated (over 2-fold) in AR seeds except there was no change in the expression of *TaKAO1_5B* homeolog (Figure 3.11; Appendix 15).

The expression patterns of homeologs of *TaGA20ox1*, (*TaGA20ox1A_4A*, *TaGA20ox1A_5B*, and *GA20ox1D_5D*) and *TaGA20ox2* (*TaGA20ox2_3A* and *TaGA20ox2_3B*) were also studied (Figure 3.11; Appendix 15). All the homeologs of *TaGA20ox1* and

TaGA20ox2 showed no difference in their expression level before imbibition between dormant and AR seeds (Figure 3.11; Appendix 15). The same five homeologs were downregulated (over 3-fold) during imbibition in dormant relative to AR seeds, except for the *TaGA20ox2_3A* and *TaGA20ox2_3B* homeologs which showed similar expression between the two seeds after 24 h imbibition (Figure 3.11; Appendix 15). Within dormant seeds, the *TaGA20ox1A_5B*, *GA20ox1D_5D*, and *TaGA20ox2_3B* homeologs showed upregulation (over 3-fold) during the first 12 h imbibition while the expression levels of *TaGA20ox1A_4A* and *TaGA20ox2_3A* showed no change in their expression level (Figure 3.11; Appendix 15). During 12 to 24 h imbibition, the expression level of all these homeologs remained unchanged except for *TaGA20ox2_3B* exhibited over 2-fold upregulation in dormant seeds (Figure 3.11; Appendix 15). All the homeologs of *TaGA20ox1* and *TaGA20ox2* showed upregulation (over 18-fold) in AR seeds after 12 h imbibition (Figure 3.11; Appendix 15). During 12 to 24 h imbibition, the *TaGA20ox1-A_4A*, *TaGA20ox1-A_5B*, and *TaGA20ox1-D_5D* homeologs showed no change in expression level but the *TaGA20ox2_3A* and *TaGA20ox2_3B* homeologs exhibited over 2-fold downregulation (Figure 3.11; Appendix 15).

This study also examined the expression of *TaGA3ox* homeologs, which include *TaGA3ox2-1_3A* and *TaGA3ox2-3_3B* (Figure 3.11; Appendix 15). The expression levels of the two homeologs in dormant compared to AR seeds were similar before imbibition but showed downregulation (over 19-fold) during imbibition in dormant seeds (Figure 3.11; Appendix 15). The same homeologs exhibited no change in expression during the process of imbibition in dormant seeds (Figure 3.11; Appendix 15). In AR seeds, both homeologs showed a marked upregulation (over 85-fold) after 12 h imbibition and over 3-fold upregulation during 12 to 24 h imbibition ((Figure 3.11; Appendix 15).

3.3.6.2 Expression of GA catabolism genes

The study also examined the expression of the homeologs of GA catabolism gene, *TaGA2ox*. The homeologs of *TaGA2ox3* (*TaGA2ox3_3A*, *TaGA2ox3_3B* and *TaGA2ox3_3D*) showed similar expression level in both dry dormant and dry AR seeds but were downregulated (over 2-fold) in dormant relative to AR seeds after 12 h imbibition (Figure 3.11; Appendix 15). By 24 HAI, the *TaGA2ox3_3A* homeolog exhibited over 2-fold downregulation in dormant relative to AR seeds while the *TaGA2ox3_3B* and *TaGA2ox3_3D* homeologs showed similar expression in dormant seeds (Figure 3.11; Appendix 15). In dormant seeds, the three homeologs showed over 10-fold upregulation after 12 h imbibition and showed no change in their expression level during 12 to 24 h imbibition (Figure 3.11; Appendix 15). The expression levels of the same homeologs increased significantly (over 90-fold) during the initial 12 h imbibition of AR seeds but exhibited over 2-fold downregulation during the 12 to 24 h imbibition period (Figure 3.11; Appendix 15).

Of the three homeologs of *TaGA2ox6* (*TaGA2ox6_2A*, *TaGA2ox6_2B*, and *TaGA2ox6_2D*), *TaGA2ox6_2A* showed similar expression in both dry dormant and AR seeds but exhibited over 5-fold downregulation during imbibition in dormant seeds compared to AR seeds (Figure 3.11; Appendix 15). The same homeolog was upregulated (over 3-fold) in both dormant and AR seeds after 12 h imbibition and there was no change in its expression level in both seed samples during 12 to 24 h imbibition (Figure 3.11; Appendix 15). The expression level of the *TaGA2ox6_2B* homeolog did not show difference between dormant and AR seeds at 0, 12 and 24 HAI. (Figure 3.11; Appendix 15). It exhibited a similar expression pattern as *TaGA2ox6_2A* during imbibition in both dormant and AR seeds. Homeolog *TaGA2ox6_2D* showed upregulation (over 2-fold) in dry dormant seeds relative to dry AR seeds but showed no change in expression between the two seed samples during imbibition. The same homeolog showed upregulation (over 6-fold) during the initial 12 h imbibition in both dormant and AR

seeds. During 12 to 24 h imbibition, it showed similar expression level in each seed samples (Figure 3.11; Appendix 15).

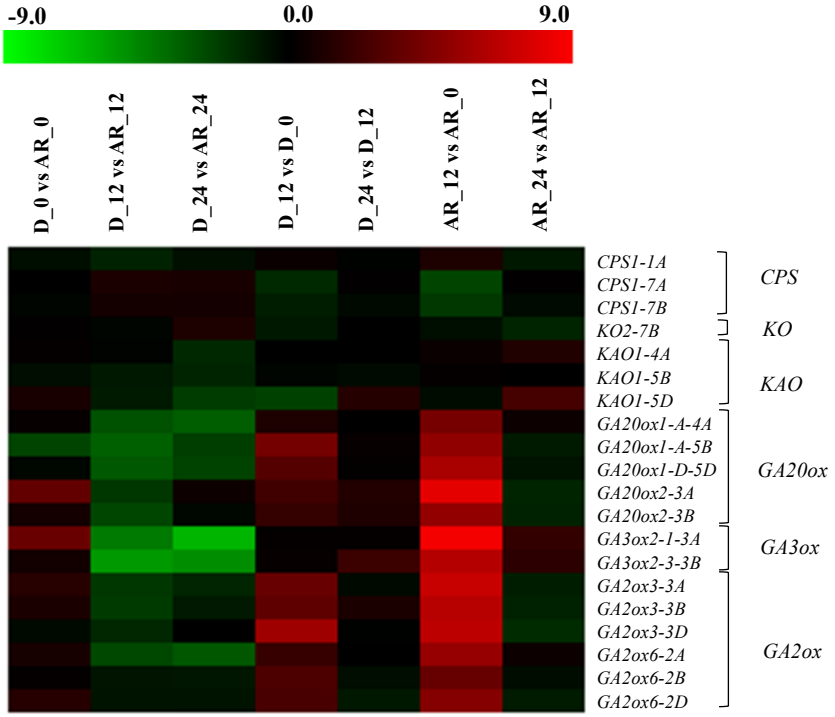


Figure 3.11. Heatmap depicting changes in the expression of genes involved in GA metabolism. Log2-scale fold change in expression of GA biosynthesis and catabolism genes between dormant and after-ripened seeds at different time points of imbibition (D_0 vs AR_0, D_12 vs AR_12 and D_24 vs AR_24) and during imbibition of dormant (D_12 vs D_0 and D_24 vs D_12) and after-ripened (AR_12 vs AR_0 and AR_24 vs AR_12) seeds. The negative and positive numbers on the bar show log2 fold change values. Red colour indicates upregulation and green colour indicated downregulation. CPS, ent-copalyl diphosphate synthase; KO, ent-kaurene oxidase; KAO, ent-kaurenoic acid oxidase; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA2ox, GA3ox, GA 3-oxidase and GA 2-oxidase.

3.3.7 Differential expression of genes involved in GA signaling genes

This study also examined the expression of genes related to GA signaling. The *TaGID1* homeologs, which include *TaGID1_1A*, *TaGID1_1B*, *TaGID1_1D*, and *TaGID1C_2D*, exhibited similar expression levels in dormant and AR seeds before and during imbibition (Figure 3.12; Appendix 16). The only noticeable exception was the upregulation of *TaGID1_1B*

and *TaGID1_1D* (over 2-fold) in dormant seeds relative to AR seeds at 24 HAI (Figure 3.12; Appendix 16). During the initial 12 h imbibition, the *TaGID1_1A*, *TaGID1_1D*, and *TaGID1C_2D* homeologs exhibited upregulation (over 2-fold) in dormant seeds and there was no change in the expression level of homeolog *TaGID1_1B* (Figure 3.12; Appendix 16). In AR seeds, only the *TaGID1_1A* and *TaGID1_1D* homeologs showed over 3-fold upregulation during the first 12 h imbibition while the expression level of the other two homeologs remained unchanged (Figure 3.12; Appendix 16). During 12 to 24 h imbibition period, the expression level of all the three homeologs of *TaGID1* remained unchanged in both dormant and AR seeds (Figure 3.12; Appendix 16).

The expression of an *TaRHT1* homeolog, *TaRHT1_4D*, had a similar expression level between dormant and AR seeds at 0, 12 and 24 HAI (Figure 3.12; Appendix 16). There was no change in its expression during imbibition of AR seeds, but it was upregulated (over 8-fold) during the initial 12 h imbibition of dormant seeds and remained at similar level from 12 to 24 h (Figure 3.12; Appendix 16). Our results indicated that the *TaGAMYB1_1A* homeolog of *TaGAMYB* showed no difference in its expression level between dormant and AR seeds at any point of imbibition. The same homeolog showed similar level of expression during imbibition in dormant seeds, but it was downregulated (over 2-fold) in AR seeds during the initial 12 h imbibition and there was no change in expression from 12 to 24 h imbibition (Figure 3.12; Appendix 16). The *TaGAMYB1_1B* homeolog showed similar level of expression in dormant relative to AR seeds at 0 and 12 h imbibition but it was upregulated (over 4-fold) in dormant seeds after 24 h imbibition (Figure 3.12; Appendix 16). The same homeolog showed similar expression level during imbibition in dormant seeds. In AR seeds, it showed 3-fold downregulation during the first 12 h imbibition and there was no change in its expression level from 12 to 24 h imbibition (Figure 3.12; Appendix 16). The *TaGAMYB1_1D* homeolog showed

similar expression patterns as *TaGAMYB1_1B* between dormant and AR seeds at any time point of imbibition as well as during imbibition of two seed samples (Figure 3.12; Appendix 16).

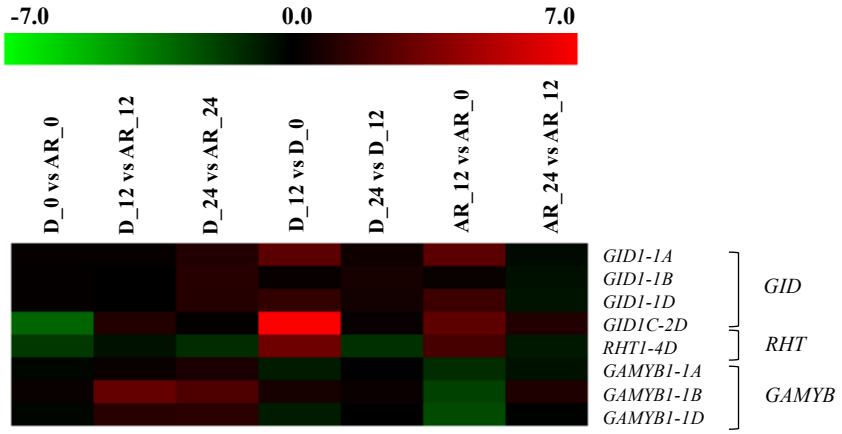


Figure 3.12. Heatmap depicting changes in the expression of genes involved in GA signaling. Log2-scale fold change in expression of GA signaling genes between dormant and after-ripened seeds at different time points of imbibition (D_0 vs AR_0, D_12 vs AR_12 and D_24 vs AR_24) and during imbibition of dormant (D_12 vs D_0 and D_24 vs D_12) and after-ripened (AR_12 vs AR_0 and AR_24 vs AR_12) seeds. The negative and positive numbers on the bar show log2 fold change values. Red colour indicates upregulation and green colour indicated downregulation. GID, gibberellin insensitive dwarf; GAMYB, GA regulated MYB transcriptional regulator and RHT, reduced height.

3.3.8 Validation of RNA-seq data

To validate the RNA-seq data, we conducted qPCR analysis of specific differentially expressed genes selected from the data. Our data showed strong positive correlation ($r \geq 0.9$) between the relative expression levels obtained from qPCR analysis and those determined from the RNA-seq data (Appendix 17).

3.4 Discussion

In this study, we employed RNA-seq analysis to identify biological processes and ABA and GA related candidate genes regulating seed dormancy. Comparative analysis of germination between dormant and AR seeds has shown that after-ripening is effective in breaking seed

dormancy in wheat (Carrera et al., 2008; Gao et al., 2012; Liu et al., 2013). We observed that AR seeds germinated within 24 h imbibition. This indicates that the 24 h mark is crucial for studying the mechanism of germination initiation (Liu et al.,2013; Li et al., 2022).

This study examined changes in gene expression within dormant and AR seeds as well as between the two seed samples at 0, 12 and 24 h imbibition. Analysis of the RNA-seq data showed differential expression of 33,905 genes between dormant and AR seeds at any stage imbibition. In addition, the data revealed that there were only 82 DEGs between dry dormant and dry AR seeds. This suggests that significant changes in gene expression mainly occur after seed imbibition. Previous studies have also reported the minimal changes in gene expression between dormant and non-dormant dry seeds, likely due to the absence of free water required for enzymatic reactions in a completely dry state (Carrera et al., 2008; Sano and Marion-Poll, 2021). Studies have suggested that the occurrence of oxidation and Amadori-Maillard reactions during after-ripening could be linked with the release of dormancy (El-Maarouf-Bouteau et al., 2013; Nee et al., 2017). In contrast, imbibed dormant and AR seeds are characterized by large sets of DEGs. Previous transcriptomic studies have identified a significant number of DEGs between dormant and non-dormant seeds during imbibition in several plant species, including Arabidopsis (Dekkers et al., 2016), barley (An and Lin, 2011), wheat (Gao et al., 2012), rice (Xie et al., 2019; Liu et al., 2022), and peanut (Xu et al., 2020).

In addition, this study showed that the number genes that showed differential expression during imbibition was higher in AR than the dormant seeds, suggesting that changes in physiological and biochemical processes are more activated in imbibed AR than dormant seeds. Our findings suggest that AR plays a significant role in regulating the expression of genes participating in biological processes linked with seed dormancy and germination during the process of imbibition, and the DEGs identified serve as reliable indicators of the specific biological processes, cellular components, and molecular functions involved in seed dormancy.

In general, the GO analysis indicated that energy production, hormonal responses, response to stimulus, and stress-related metabolic pathways as biological processes are enriched with the DEGs between dormant and AR seeds, suggesting that these biological processes play vital roles in regulating seed dormancy in wheat, as reported previously (Gao et al., 2012; Lee et al., 2021). In addition, GO terms related to membrane, cell, cell parts, and organelles are found to be the most common in the cellular component category enriched with the DEGs while GO terms related to catalytic activity, binding, and transporter activity are the most abundant in the molecular function category (Lee et al., 2021; Wang et al., 2021), indicating the importance of these GO terms in regulating seed dormancy in wheat.

Response to auxin, response to endogenous stimulus, response to hormone, response to oxidative stress, antioxidant activity, pentose, arabinose, and polysaccharide metabolic processes are among the GO terms enriched with DEGs between dormant and AR seeds at 12 HAI. Additionally, downregulation of genes associated with glutathione metabolic processes in dormant seeds compared to AR seeds at 12 HAI points toward the importance of these metabolic processes in dormancy decay. Glutathione (GSH) acts as a crucial component of antioxidant systems that help to eliminate excessive ROS molecules (Koramutla et al., 2021). Studies have indicated the role of GSH in breaking seed dormancy in barley and its significance as a signaling molecule in regulating ABA signaling (Kalemba and Ratajczak, 2018; Koramutla et al., 2021). Additionally, GO terms associated with cellular respiration, aerobic respiration, generation of precursor metabolites and energy, and electron transport chain are enriched with genes upregulated in dormant seeds at 24 HAI although dormant seeds did not germinate. The downregulation of genes related to endopeptidase and peptidase inhibitor activity in AR seeds at 24 HAI indicates enhanced mobilization of wheat storage proteins during and after germination is consistent with previous studies (Gao et al., 2012). Overall, these results

provided insights into the complex biological processes that controls dormancy and germination.

ABA plays a vital role in regulating seed dormancy during maturation and imbibition. Its biosynthesis is catalysed primarily by ZEP and NCED enzymes while its catabolism is catalysed mainly by ABA 8'-hydroxylase, which belongs to the cytochrome P450 (CYP) 707A family (Marin et al., 1996; Nambara et al., 2010). Previous research showed that the reduction of ABA levels during the imbibition process is the cause for seed dormancy decay and germination in cereals (Millar et al., 2006; Gubler et al., 2008). This reduction in seed ABA levels is primarily linked to the expression patterns of the *NCED* and *CYP707A* genes (Nambara et al., 2010; Tuan et al., 2018). The results of this study showed no differential expression of *TaZEP*, *TaVDE*, *TaNCED*, *TaAAO*, and *TaCYP707A* genes between the dry dormant seeds and dry AR seeds. Similarly, no difference in the amount of ABA was found between dry AR and dry dormant seeds (Millar et al., 2006; Liu et al., 2013; Gubler et al., 2008). This study also investigated the expression of ABA metabolic genes during imbibition. The results of this study showed downregulation of *TaZEP* in 12 h imbibed dormant seeds as compared to AR seeds. However, the loss of function mutation in *ZEP* causes decreased seed dormancy in Arabidopsis and *Nicotiana plumbaginifolia (*Koornneef et al., 1982; Marin et al., 1996) and overexpression of *ZEP* leads to an increased dormancy in tobacco seeds (Frey et al., 1999). Therefore, the lack of correlation between the expression pattern of *ZEP* and seed dormancy in this study might suggest that *ZEP* expression is subjected to post-transcriptional regulatory mechanisms.

Furthermore, this study found upregulation of *TaNCED* genes, including *TaNCED1* (*TaNCED1_6B*) and *TaNCED3* (*TaNCED3_5A*, *TaNCED3_5B*, *TaNCED3_5D*) in 12 h imbibed dormant seeds as compared to AR seeds. By 24 h imbibition, only *TaNCED5_5B* showed upregulation in dormant seeds relative to AR seeds. These results suggest the temporal differential roles of the *TaNCED* gene family members in regulating ABA levels and seed

dormancy. Similarly, upregulation of specific *NCED* genes such as *HvNCED1, TaNCED1* and *BdNCED1* were reported in imbibed dormant seeds of barley, wheat, and *Brachypodium*, respectively (Millar et al., 2006; Barrero et al., 2012; Jacobsen et al., 2013). Another study found higher expression of *TaNCED2* in both dry and imbibed seeds of a dormant wheat genotype than that found in a non-dormant genotype (Son et al., 2016). A study by Hwang et al. (2010) showed the role of *NCED3* of rice in regulating seed dormancy through its ectopic expression in Arabidopsis. Additionally, *TaAAO* (*TaAAO_7A*, *TaAAO_7B* and *TaAAO_7D*) genes showed upregulation in dormant seeds relative to AR seeds during imbibition, and this result is in support of the role of ABA biosynthesis in controlling seed dormancy. For instance, a study in Arabidopsis showed that mutation in *AAO* gene results in reduction in ABA content and level of seed dormancy (Seo et al., 2000).

ABA catabolism plays a crucial function in the regulation of ABA levels (Nambara and Marion-Poll, 2005; Tuan et al., 2018). The *TaCYP707A1_6A* gene showed downregulation at 12 HAI dormant seeds relative to AR seeds but exhibited no change in expression between the two samples at 24 HAI. On the other hand, *TaCYP707A3_5B* showed similar expression between dormant and AR seeds at 12 HAI but showed a strong downregulation in dormant seeds after 24 h imbibition. Thus, our findings suggest that genes related to ABA catabolism have specific temporal functions. Previous reports have also indicated a higher expression of *CYP707A1* in non-dormant barley seeds relative to dormant seeds, which results in the decrease of ABA levels (Millar et al., 2006) and repression of *CYP707A1* that causes higher ABA level and an increase in the degree of dormancy in barley (Gubler et al., 2008). It was also observed that a single mutation in *TaCYP707A1D* showed no effect on seed germination when compared to the wild type whereas double mutations in *TaCYP707A1A* and *TaCYP707A1D* homeologs caused an increase in ABA levels in the embryo and a decrease in the rate of germination, indicating the importance of the two *CYP707A1* homeologs in the regulation of ABA level and

germination (Chono et al., 2013). A study in Arabidopsis demonstrated that ectopic expression of the B genome copy of *TaCYP707A1* (*TaCYP707A1B*) is involved in regulating ABA level and seed dormancy (Son et al., 2016). Additionally, research in rice showed a correlation between reduced ABA levels and increased expression of *OsCYP707A* during imbibition of non-dormant rice seeds (Zhu et al., 2009; Du et al., 2015).

The ABA signaling pathway plays a crucial role in seed dormancy and germination. Our results showed the downregulation of the ABA receptor gene *TaPYL* (*TaPYL4_2A*, *TaPYL4_4B*, *TaPYL4_4D*, *TaPYL9_7A*, and *TaPYL10_1A*) in dormant seeds relative to AR seeds during imbibition, and the expression patterns of *TaPYL* genes did not show any correlation with dormancy level. A previous gene expression analysis in wheat also showed similar downregulation of *PYL* genes in dormant as compared to AR seeds following imbibition (Liu et al., 2013). Our results also showed upregulation of *TaPP2Cs* that regulate ABA signaling negatively in dormant seeds compared to AR seeds during imbibition, but there was no association between *TaPPP2C* expression and the dormancy level. Previous studies have also observed a lack of association between the expression of *PP2C* and dormancy phenotype in Arabidopsis and wheat seeds (Maia et al., 2014; Izydorczyk et al., 2018). Lack of correlation between expression patterns of *TaPYL* and *TaPP2C* genes might indicate that these two genes are regulated post-transcriptionally. With respect to the *TaSnRK* genes that regulate ABA signaling positively, this study showed upregulation of *TaSnRKs* in imbibed dormant seeds, suggesting the positive role of these genes regulating seed dormancy in wheat. The *TaSnRK3_1A* and *TaSnRK3_1B* gene showed upregulation in dormant seeds relative to AR seeds during the whole imbibition period. However, *TaSnRK10_4A* and *TaSnRK10_4D* exhibited similar expression between dormant and AR seeds at 12 HAI and showed upregulation in dormant seeds at 24 HAI. A previous study in wheat revealed that loss of ABA sensitivity with after-ripening is linked with the downregulation of specific *SnRK2* genes in

imbibed AR seeds (Liu et al., 2013). Another study showed that a triple mutant *snrk2.2 snrk2.3 snrk2.6* in Arabidopsis resulted in the loss of seed dormancy (Fujji et al., 2009). The transcription factors ABI3, ABI4 and ABI5 are recognised as the key regulators mediating the seed response to ABA (Holdsworth et al., 2008; Tuan et al. 2018; Ali et al. 2022). Results of this study showed upregulation of *TaABI5* (*TaABI5_3B and TaABI5_3D*) in 24 HAI dormant seeds compared to AR seeds, suggesting an increase in ABA sensitivity and dormancy and similar results have reported previously (Liu et al, 2013; Izydorczyk et al. 2018; Tuan et al., 2021). A study in sorghum also reported higher expression levels of *ABI5* and the ABI5 protein in seeds of a sorghum cultivar with high dormancy than a cultivar with low degree of dormancy (Rodríguez et al., 2009).

GA is a key plant hormone that plays a crucial role in the regulation of seed dormancy and germination (Tuan et al., 2018; Ali et al., 2019). The results of this study indicated downregulation of the GA biosynthesis genes *TaGA3ox and TaGA20ox* in imbibing dormant seeds as compared to AR seeds, suggesting a decrease in the amount of GA and maintenance of dormancy. Similarly, loss of dormancy in AR barley and wheat seeds was shown to be linked with an increase in the expression of the *GA20ox* and *GA3ox* genes, as well as a higher concentration of bioactive GA_1 (Gubler et al., 2008; Liu et al., 2013; Kashiwakura et al., 2016; Tuan et al., 2018). In addition, increased expression of *GA20ox* orthologs of sorghum has been reported to result in an elevated level of GA_4 in embryos of non-dormant sorghum seeds (Rodriguez et al., 2012). This study also investigated the expression pattern of *GA2ox* genes that encode a major GA catabolic enzyme in plants. The results showed repression of *TaGA2ox* genes including *TaGA2ox3_3A*, *TaGA2ox3_3B*, *TaGA2ox3_3D*, and *TaGA2ox6_2A* by 12 HAI and *TaGA2ox3_3A* and *TaGA2ox6_2A* by 24 HAI in imbibing dormant as compared to AR seeds. The observation of repression of the *GA2ox* genes could be due to the low level of GA in the dormant seeds since this gene is known to feedback regulated by GA. Previous studies

have shown no change in the expression level of *GA2ox* genes between dormant and non-dormant seeds of Arabidopsis and wheat during their imbibition, suggesting a minor effect of GA catabolism in controlling bioactive GA level and germination (Ogawa et al., 2003; Izydorczyk et al., 2018).

GA signaling also plays a crucial role in regulating germination. Our results showed upregulation of *TaGID1* (*TaGID1_1B* and *TaGID1_1D*) in 24 h imbibed dormant seeds as compared to AR seeds, suggesting regulation of *TaGID1* by post-transcriptional mechanism. A previous transcriptomic study in wheat also showed that *TaGID1* exhibited either similar expression or upregulation in imbibed dormant compared to AR seeds (Liu et al., 2013). Such lack of correlations between seed dormancy levels and expression profile of *GID1* have been documented in barley and wheat by other studies (Barrero et al., 2009; Izydorczyk et al., 2018; Tuan et al., 2018). In agreement with these results, mutations in *GID1* of rice have no effect on germination but result in the suppression of α-amylase activity (Sasaki et al., 2003; Ueguchi-Tanaka et al., 2005). This study also found upregulation of *TaGAMYB* (*TaGAMYB1_1B* and *TaGAMYB1_1D*) in imbibed dormant seeds compared to AR seeds, indicating lack of correlation between expression of this gene and dormancy phenotype. However, a loss of function mutation in *GAMYB* of rice, which inhibits the expression of *α-amylase*, did not affect seed germination (Kaneko et al., 2004).

4.0 GENERAL DISCUSSION AND CONCLUSION

Wheat is one of the most widely cultivated cereal crop and serves as a staple food for billions of people globally. However, wheat production faces various challenges, including preharvest sprouting (PHS). PHS is the germination of seeds on the mother plant due to humid and rainy weather conditions and significantly reduces the quality of wheat grains. Moreover, climate change is resulting in more frequent and intense extreme weather occurrences such as heavy precipitation and heatwaves, all of which can contribute to the conditions conducive to PHS and therefore worsening the problem. Seed dormancy is considered as the key genetic factor determining PHS resistance in which varieties with inadequate or low seed dormancy levels are susceptible to PHS. Therefore, researchers are keenly interested in understanding the molecular mechanisms of seed dormancy to improve PHS resistance in wheat. With the advent of NGS technologies, RNA sequencing (RNA-seq) has become the preferred method for genome-wide transcriptomic analysis of different crop traits, including seed dormancy. RNA-seq provides a comprehensive and accurate assessment of gene expression levels and does not rely on pre-existing genomic annotations.

This thesis project conducted a comparative genome-wide transcriptomic analysis between dormant and after-ripened (AR) or non-dormant seeds of wheat. It initially examined the effect of after-ripening on dormancy release, and our results indicated that after-ripening effectively breaks seed dormancy. RNA-seq analysis of the AR and dormant seeds revealed differential expression of 33,905 genes between the two seeds before and during their imbibition. Our data also showed that major changes in gene expression occur primarily during seed imbibition as only a few DEGs were detected between dry dormant and AR seeds. Gene ontology (GO) analysis was performed to annotate and categorize differentially expressed genes based on their molecular functions, biological processes, and cellular components. GO analysis showed that after-ripening upregulates the expression of genes involved in glutathione

metabolic process, response to hormone, response to organic substance, response to oxidative stress, growth, and oxidoreductase activity. While the genes downregulated in AR seeds were related to endopeptidase and peptidase inhibitor activity, electron transport chain, photosynthesis and pentose metabolic process GO terms. Overall, these results suggests that these processes may regulate seed transition from dormancy to germination.

This study also examined the expression pattern of genes involved in ABA and GA metabolism and signaling. Genes involved in ABA biosynthesis, specifically *TaNCED1* and *TaNCED3* exhibited upregulation in dormant seeds relative to AR seeds at 12 HAI, while only *TaNCED5* shows upregulation by 24 HAI in dormant seeds. These findings indicate temporally distinct roles of *TaNCED* gene family members in controlling ABA levels and seed dormancy. Furthermore, *TaAAO* exhibited higher expression in dormant seeds compared to AR seeds during imbibition. This finding provides further evidence for the involvement of ABA biosynthesis in regulating ABA level and seed dormancy. The study also indicated downregulation of *TaCYP707A1* in dormant relative to AR seeds at 12 HAI. In addition, downregulation of *TaCYP707A3* was observed in dormant seeds at 24 HAI, suggesting that genes related to ABA catabolism also have temporally distinct functions. A lack of correlation was observed between the expression patterns of specific genes involved in ABA signaling (*TaPYL* and *TaPP2C*) and the level of seed dormancy, suggesting post-transcriptional regulation of these genes. In contrast, our data showed upregulation of *TaSnRK*, a positive regulator of ABA signaling, and *TaABI5*, a downstream transcription factor, in dormant seeds indicating their involvement in maintaining seed dormancy. With respect to the expression pattern of genes involved in GA metabolism, *TaGA3ox* and *TaGA20ox* exhibited downregulation in dormant seeds relative to AR seeds during imbibition, suggesting reduction in GA level and maintenance of dormancy. The decreased expression of GA catabolism gene, *TaGA2ox*, in imbibing dormant seeds as compared to AR seeds may suggest feedback

regulation of this gene by GA. The results of this study also showed lack of correlation between the level of dormancy and expression patterns of genes involved in GA signaling (*GID1* and *GAMYB*), suggesting their regulation by post-transcriptional mechanisms.

In summary, the findings of this study provided important information about transcriptional regulations of wheat genes representing biological processes and molecular functions associated with seed dormancy as well as genes involved in the metabolism and signaling pathways of ABA and GA, which play crucial roles in controlling dormancy. However, functional characterization of these genes through the use of different methods such as CRISPR/Cas9-mediated gene knockout and gene overexpression study are required to confirm their roles in regulating seed dormancy.

LITERATURE CITED

Abbo S, Lev-Yadun S, Heun M, Gopher A (2013) On the 'lost'crops of the neolithic Near East. Journal of Experimental Botany 64:815-822

Abdulbaki AS, Alsamadany H, Alzahrani Y, Olayinka BU (2022) Rubisco and abiotic stresses in plants: Current assessment. Turkish Journal of Botany 46:541-552

Abe F, Haque E, Hisano H, Tanaka T, Kamiya Y, Mikami M, Kawaura K, Endo M, Onishi K, Hayashi T, Sato K (2019) Genome-edited triple-recessive mutation alters seed dormancy in wheat. Cell Reports 28:1362-1369

Akintunde O, Tucker T, Carabetta VJ (2023) The evolution of next-generation sequencing technologies. arXiv preprint arXiv:2305.08724

Ali A, Cao J, Jiang H, Chang C, Zhang HP, Sheikh SW, Shah L and Ma C (2019) Unraveling molecular and genetic studies of wheat (*Triticum aestivum* L.) resistance against factors causing pre-harvest sprouting. Agronomy 9:117

Ali F, Qanmber G, Li F, Wang Z (2022) Updated role of ABA in seed maturation, dormancy, and germination. Journal of Advanced Research 35:199-214

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, Jullien M (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. Planta 219:479-488

An YQ, Lin L (2011) Transcriptional regulatory programs underlying barley germination and regulatory functions of gibberellin and abscisic acid. BMC plant biology 11:1-24

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biology 11:10

Anders S, Pyl PT, Huber W (2014) HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166-169

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Appleford NE, Wilkinson MD, Ma Q, Evans DJ, Stone MC, Pearce SP, Powers SJ, Thomas SG, Jones HD, Phillips AL, Hedden P, Lenton JR (2007) Decreased shoot stature and grain α-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. Journal of Experimental Botany 58:3213-3226

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815

Ariizumi T, Hauvermale AL, Nelson SK, Hanada A, Yamaguchi S, Steber CM (2013) Lifting della repression of arabidopsis seed germination by nonproteolytic gibberellin signaling. Plant Physiology 162:2125-2139

Ariizumi T, Steber CM (2007) Seed germination of GA-insensitive sleepy1 mutants does not require RGL2 protein disappearance in Arabidopsis. The Plant Cell 19:791-804

Ashikawa I, Abe F, Nakamura S (2013) DOG1-like genes in cereals: Investigation of their function by means of ectopic expression in Arabidopsis. Plant Science 208:1-9

Ashikawa I, Mori M, Nakamura S, Abe F (2014) A transgenic approach to controlling wheat seed dormancy level by using Triticeae DOG1-like genes. Transgenic Research 23:621-629

Barnard A, van Deventer CS, Maartens H (2005) Comparison between methods for estimating sprout damage in wheat. South African Journal of Plant and Soil 22:44-48

Barrero JM, Jacobsen JV, Talbot MJ, White RG, Swain SM, Garvin DF, Gubler F (2012) Grain dormancy and light quality effects on germination in the model grass *Brachypodium distachyon*. New Phytologist 193:376-386

Barrero JM, Talbot MJ, White RG, Jacobsen JV, Gubler F (2009) Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue in regulating dormancy in barley. Plant Physiology 150:1006-1021

Bartlett JM (2002) Approaches to the analysis of gene expression using mRNA: a technical overview. Molecular Biotechnology 21:149-160

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Science Research 14:1-16

Bazin J, Langlade N, Vincourt P, Arribat S, Balzergue S, El-Maarouf-Bouteau H, Bailly C (2011) Targeted mRNA Oxidation Regulates Sunflower Seed Dormancy Alleviation during Dry After-Ripening. The Plant Cell 23:2196-2208

Benech-Arnold RL, Gualano N, Leymarie J, Côme D, Corbineau F (2006) Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains. Journal of Experimental Botany, 57:1423-1430

Bewley JD (1997) Seed Germination and Dormancy. The Plant Cell 9:1055-1066

Bewley JD, Black M (1994) Seeds: physiology of development and germination. 2nd edn New York: Plenum Press

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120

Bolser D, Staines DM, Pritchard E, Kersey P (2016) Ensembl plants: integrating tools for visualizing, mining, and analyzing plant genomics data. Plant bioinformatics: Methods and Protocols 1374:115-140

Bove J, Lucas P, Godin B, Ogé L, Jullien M, Grappin P (2005) Gene expression analysis by cDNA-AFLP highlights a set of new signaling networks and translational control during seed dormancy breaking in *Nicotiana plumbaginifolia*. Plant Molecular Biology 57:593-612

Brady SM, Long TA, Benfey PN (2006) Unraveling the dynamic transcriptome. The Plant Cell 18:2101-2111

Brown TA (2002) Genomes. 2nd edn Oxford: Wiley-Liss

Buijs G (2020) A perspective on secondary seed dormancy in *Arabidopsis thaliana*. Plants 9:749

Buller AHR (1919) Essays on wheat: including the discovery and introduction of marquis wheat, the early history of wheat-growing in Manitoba, wheat in western Canada, the origin of red bobs and kitchener, and the wild wheat of Palestine. Macmillan.

Bumgarner R (2013) Overview of DNA microarrays: types, applications, and their future. Current Protocols in Molecular Biology 101:22-1

Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW (2016) Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nature Reviews Genetics 17:257-271

Cabral AL, Jordan MC, McCartney CA, You FM, Humphreys DG, MacLachlan R, Pozniak C J (2014) Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.). BMC Plant Biology 14:1-12

Cadman CS, Toorop PE, Hilhorst HW, Finch-Savage WE (2006) Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. The Plant Journal 46:805-822

Campbell AB (2015) Wheat. The Canadian Encyclopedia

Carrera E, Holman T, Medhurst A, Dietrich D, Footitt S, Theodoulou FL, Holdsworth MJ (2008) Seed after-ripening is a discrete developmental pathway associated with specific gene networks in Arabidopsis. The Plant Journal 53:214-224

Chambers DC, Carew AM, Lukowski SW, Powell JE (2019) Transcriptomics and single-cell RNA-sequencing. Respirology 24:29-36

Cheng X, Tian B, Gao C, Gao W, Yan S, Yao H, Wang X, Jiang Y, Hu L, Pan X, Cao J, Lu J, Ma C, Chang C, Zhang H (2021) Identification and expression analysis of candidate genes related to seed dormancy and germination in the wheat GATA family. Plant Physiology and Biochemistry 169:343-359

Chitnis VR, Gao F, Yao Z, Jordan MC, Park S, Ayele BT (2014) After-ripening induced transcriptional changes of hormonal genes in wheat seeds: the cases of brassinosteroids, ethylene, cytokinin and salicylic acid. PloS One 9:e87543

Chono M, Matsunaka H, Seki M, Fujita M, Kiribuchi-Otobe C, Oda S, Kojima H, Kobayashi D, Kawakami N (2013) Isolation of a wheat (*Triticum aestivum* L.) mutant in ABA 8'-hydroxylase gene: effect of reduced ABA catabolism on germination inhibition under field conditions. Breeding Science 63:104-115

Clarke FR, Clarke JM, DePauw RM, Fernandez MR, Fox S, Gilbert J, Humphreys G, Knox RE, McCaig TN, Procunier D, Sissons M, Somers D (2005) Strategic approach to mitigating weather induced defects of wheat quality. Euphytica 143:285-290

Cutler A (2005) Understanding abscisic acid. Journal of Plant Growth Regulation 24:251-252

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annual Review of Plant Biology 61:651-679

Dai M, Thompson RC, Maher C, Contreras-Galindo R, Kaplan MH, Markovitz DM, Omenn G, Meng F (2010) NGSQC: cross-platform quality analysis pipeline for deep sequencing data. BMC Genomics 11:1-9

Dante RA, Larkins BA, Sabelli PA (2014) Cell cycle control and seed development. Frontiers in Plant Science 5:493

Das A, Kim DW, Khadka P, Rakwal R, Rohila JS (2017) Unraveling key metabolomic alterations in wheat embryos derived from freshly harvested and water-imbibed seeds of two wheat cultivars with contrasting dormancy status. Frontiers in Plant Science 8:1203

Davis MP, van Dongen S, Abreu-Goodger C, Bartonicek N, Enright AJ (2013) Kraken: A set of tools for quality control and analysis of high-throughput sequence data. Methods 63:41–49

de Sousa T, Ribeiro M, Sabença C, Igrejas G (2021) The 10,000-Year Success Story of Wheat! Foods 10:2124

de Wit M, Galvao VC, Fankhauser C (2016) Light-mediated hormonal regulation of plant growth and development. Annual Review of Plant Biology 67:513-537

Debeaujon I, Koornneef M (2000) Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiology 122:415-424

Dekkers BJ, Pearce SP, van Bolderen-Veldkamp RP, Holdsworth MJ, Bentsink L (2016) Dormant and after-ripened *Arabidopsis thaliana* seeds are distinguished by early transcriptional differences in the imbibed state. Frontiers in Plant Science 7:1323

Dekkers BJ, Willems L, Bassel GW, van Bolderen-Veldkamp RP, Ligterink W, Hilhorst HW, Bentsink L (2012) Identification of reference genes for RT–qPCR expression analysis in Arabidopsis and tomato seeds. Plant and Cell Physiology 53:28-37

DePauw RM, Clarke FR, Fofana B, Knox R, Humphreys G, Cloutier S (2009) RL4137 contributes preharvest sprouting resistance to Canadian wheats. Euphytica 168:347–361

DePauw RM, McCaig TN (1991) Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in Triticum spp. Euphytica 52:221-229

Derera NF (1990) A perspective of sprouting research. In: Fifth international symposium on pre-harvest sprouting in cereals 5:3-11

Derera NF, Bhatt GM, McMaster GJ (1977) On the problem of pre-harvest sprouting of wheat. Euphytica 26:299-308

Deshpande D, Chhugani K, Chang Y, Karlsberg A, Loeffler C, Zhang J, et al. (2023) RNA-seq data science: From raw data to effective interpretation. Frontiers in Genetics 14: 997383

Dhaka V, Khatkar B (2015) Effects of Gliadin/Glutenin and HMW-GS/LMW-GS Ratio on Dough Rheological Properties and Bread-Making Potential of Wheat Varieties. Journal of Food Quality 38:71-82

Dhariwal R, Hiebert CW, Sorrells ME, Spaner D, Graf RJ, Singh J, Randhawa HS (2021) Mapping pre-harvest sprouting resistance loci in AAC Innova× AAC Tenacious spring wheat population. BMC Genomics 22:1-20

Di Y (2015) Single-gene negative binomial regression models for RNA-Seq data with higher-order asymptotic inference. Statistics and Its Interface 8:405-418

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2012) STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21

Du W, Cheng J, Cheng Y, Wang L, He Y, Wang Z, Zhang H (2015) Physiological characteristics and related gene expression of after-ripening on seed dormancy release in rice. Plant Biology 17:1156-1164

Duclos DV, Ray DT, Johnson DJ, Taylor AG (2013) Investigating seed dormancy in switchgrass (*Panicum virgatum* L.): understanding the physiology and mechanisms of coat-imposed seed dormancy. Industrial Crops and Products 45:377-387

Dvorak J, Akhunov ED (2005) Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. Genetics 171:323-332

El-Maarouf-Bouteau H, Meimoun P, Job C, Job D, Bailly C (2013) Role of protein and mRNA oxidation in seed dormancy and germination. Frontiers in Plant Science 4:7

Endo A, Sawada Y, Takahashi H, Okamoto M, Ikegami K, Koiwai H, Seo M, Toyomasu T, Mitsuhashi W, Shinozaki K, Nakazono M, Kamiya Y, Koshiba T, Nambara E (2008) Drought induction of Arabidopsis 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. Plant Physiology 147:1984-1993

Enghiad A, Ufer D, Countryman AM, Thilmany DD (2017) An overview of global wheat market fundamentals in an era of climate concerns. International Journal of Agronomy 2017:1-15

Erenstein O, Jaleta M, Mottaleb KA, Sonder K, Donovan J, Braun HJ (2022) Global trends in wheat production, consumption and trade. In Wheat improvement: food security in a changing climate (pp. 47-66). Cham: Springer International Publishing

Eversole K, Feuillet C, Mayer KFX, Rogers J (2014) Slicing the wheat genome. Science 345:285-287

Faris, JD (2014) Wheat Domestication: Key to Agricultural Revolutions Past and Future. In Genomics of Plant Genetic Resources (pp. 439-464). Springer Netherlands

Feldman M, Kislev ME (2007) Domestication of emmer wheat and evolution of free-threshing tetraploid wheat. Israel Journal of Plant Sciences 55:207-221

Feldman M, Levy AA (2012) Genome evolution due to allopolyploidization in wheat. Genetics 192:763-774

Finch-Savage WE, Cadman CS, Toorop PE, Lynn JR, Hilhorst HW (2007) Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. The Plant Journal 51:60-78

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytologist 171:501-523

Finkelstein R (2013) Abscisic acid synthesis and response. Arabidopsis Book 11:e0166

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular Aspects of Seed Dormancy. Annual Review of Plant Biology 59:387-415

Flint-Garcia SA (2013) Genetics and consequences of crop domestication. Journal of Agricultural and Food Chemistry 61:8267-8276

Foley ME, Fennimore SA (1998) Genetic basis for seed dormancy. Seed Science Research 8:173-182

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. Proceedings of the National Academy of Sciences 108:20236-20241

Frey A, Audran C, Marin E, Sotta B, Marion-Poll A (1999) Engineering seed dormancy by the modification of zeaxanthin epoxidase gene expression. Plant Molecular Biology 39:1267-1274

Fujii H, Zhu JK (2009) Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. Proceedings of the National Academy of Sciences 106:8380-8385

Gagne JM, Downes BP, Shiu SH, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proceedings of the National Academy of Sciences 99:11519-11524

Gao F, Ayele BT (2014) Functional genomics of seed dormancy in wheat: advances and prospects. Frontiers in Plant Science 5:458

Gao F, Jordan MC, Ayele BT (2012) Transcriptional programs regulating seed dormancy and its release by after-ripening in common wheat (*Triticum aestivum* L.). Plant Biotechnology Journal 10:465-476

Gao F, Rampitsch C, Chitnis VR, Humphreys GD, Jordan MC, Ayele BT (2013) Integrated analysis of seed proteome and mRNA oxidation reveals distinct post-transcriptional features regulating dormancy in wheat (*Triticum aestivum* L.). Plant Biotechnology Journal 11:921-932

Gao S, Chu C (2020) Gibberellin metabolism and signaling: targets for improving agronomic performance of crops. Plant and Cell Physiology 61:1902-1911

Gatford KT, Eastwood RF, Halloran GM (2002) Germination inhibitors in bracts surrounding the grain of *Triticum tauschii*. Functional Plant Biology 29:881-890

Gene Ontology Consortium (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Research 32:D258-261

Gianinetti A, Vernieri P (2007) On the role of abscisic acid in seed dormancy of red rice. Journal of Experimental Botany 58:3449-3462

Giraldo P, Benavente E, Manzano-Agugliaro F, Gimenez E (2019) Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis. Agronomy 9:352

Glaus P, Honkela A, Rattray M (2012) Identifying differentially expressed transcripts from RNA-seq data with biological variation. Bioinformatics 28:1721-1728

Gong F, Qi T, Zhang T, Lu Y, Liu J, Zhong X, He J, Li Y, Zheng Y, Liu D, Huang L, Wu B (2022) Comparison of the Agronomic, Cytological, Grain Protein Characteristics, as Well as Transcriptomic Profile of Two Wheat Lines Derived From Wild Emmer. Frontiers in Genetics 12: 804481

Government of Manitoba (2021) Estimates of production 2021. https://www.gov.mb.ca/agriculture/markets-and-statistics/crop-statistics/pubs/estimates-of-production-2021.pdf (Accessed on 28 April 2022)

Govindarajan R, Duraiyan J, Kaliyappan K, Palanisamy M (2012) Microarray and its applications. Journal of Pharmacy and Bioallied Sciences 4:S310

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29:644-652

Grahl A (1965) Induction of secondary dormancy in wheat by means of high temperature and the effect of the humidity of the seed bed. Proceedings of the International Seed Testing Association 30:787-801

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, Gong F, Phillips AL, Hedden P, Sun TP, Thomas SG (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. The Plant Cell 18:3399-3414

Groos C, Gay G, Perretant M R, Gervais L, Bernard M, Dedryver F, Charmet G (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci

analysis in a white× red grain bread-wheat cross. Theoretical and Applied Genetics 104:39-47

Grote U, Fasse A, Nguyen TT, Erenstein O (2021) Food security and the dynamics of wheat and maize value chains in Africa and Asia. Frontiers in Sustainable Food Systems 4:617009

Gualano NA, Benech-Arnold RL (2009) Predicting pre-harvest sprouting susceptibility in barley: Looking for "sensitivity windows" to temperature throughout grain filling in various commercial cultivars. Field Crops Research 114:35-44

Guan J, Garcia DF, Zhou Y, Appels R, Li A, Mao L (2020) The battle to sequence the bread wheat genome: a tale of the three kingdoms. Genomics, Proteomics and Bioinformatics 18:221-229

Gubler F, Hughes T, Waterhouse P, Jacobsen J (2008) Regulation of dormancy in barley by blue light and after-ripening: effects on abscisic acid and gibberellin metabolism. Plant Physiology 147:886-896

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Current Opinion in Plant Biology 8:183-187

Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen JV (1999) Target genes and regulatory domains of the GAMYB transcriptional activator in cereal aleurone. The Plant Journal 17:1-9

Gupta R, Chakrabarty SK (2013) Gibberellic acid in plant: still a mystery unresolved. Plant Signaling and Behavior 8:e25504

Han F, Zhu B (2011) Evolutionary analysis of three gibberellin oxidase genesin rice, Arabidopsis, and soybean. Gene 473:23-35

Hannon GJ (2010) FASTX-toolkit: FASTQ/A short-reads pre-processing tools. http://hannonlab.cshl.edu/fastx_toolkit/

Hardcastle TJ, Kelly KA (2010) baySeq: empirical Bayesian methods for identifying differential expression in sequence count data. BMC Bioinformatics 11:1-14

Hauvermale AL, Ariizumi T, Steber CM (2012) Gibberellin signaling: a theme and variations on DELLA repression. Plant Physiology 160:83-92

Hauvermale AL, Tuttle KM, Takebayashi Y, Seo M, Steber CM (2015) Loss of *Arabidopsis thaliana* seed dormancy is associated with increased accumulation of the GID1 GA hormone receptors. Plant Cell Physiology 56:1773-1785

Hedden P, Thomas SG (2012) Gibberellin biosynthesis and its regulation. Biochemical Journal 444:11-25

Helliwell CA, Poole A, Peacock WJ, Dennis ES (1999) Arabidopsis ent-kaurene oxidase catalyzes three steps of gibberellin biosynthesis. Plant Physiology 119(2):507-10

Heun M, Schäfer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F (1997) Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting. Science 278:1312-1314

Hilhorst HWM (1995) A critical update on seed dormancy. I. Primary dormancy. Seed Science Research 5:61-73

Hirano K, Asano K, Tsuji H, Kawamura M, Mori H, Kitano H, Ueguchi-Tanaka M, Matsuoka M (2010) Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. Plant Cell 22:2680-2696

Hoang HH, Sechet J, Bailly C, Leymarie J, Corbineau F (2014) Inhibition of germination of dormant barley (*Hordeum vulgare* L.) grains by blue light as related to oxygen and hormonal regulation. Plant Cell Environment 37:1393-1403

Holdsworth MJ, Bentsink L, Soppe WJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist 179:33-54

Hopper WC (1923) Agriculture in Eastern Canada. The Annals of the American Academy of Political and Social Science 107:67-73

Hussain A, Peng J (2003) DELLA proteins and GA signalling in Arabidopsis. Journal of Plant Growth Regulation 22:134-140

Hwang SG, Chen HC, Huang WY, Chu YC, Shii CT, Cheng WH (2010) Ectopic expression of rice OsNCED3 in Arabidopsis increases ABA level and alters leaf morphology. Plant Science 178:12-22

Iehisa JCM, Matsuura T, Mori IC, Takumi S (2014) Identification of quantitative trait locus for abscisic acid responsiveness on chromosome 5A and association with dehydration tolerance in common wheat seedlings. Journal of Plant Physiology 171:25-34

Iglesias-Fernández R, del Carmen Rodriguez-Gacio M, Matilla AJ (2011) Progress in research on dry afterripening. Seed Science Research 21:69-80

Iquebal MA, Sharma P, Jasrotia RS, Jaiswal S, Kaur A, Saroha M, Angadi UB, Sheoran S, Singh R, Singh GP, Rai A, Tiwari R, Kumar D (2019) RNAseq analysis reveals drought-responsive molecular pathways with candidate genes and putative molecular markers in root tissue of wheat. Scientific Reports 9:13917

Iuchi S, Suzuki H, Kim YC, Iuchi A, Kuromori T, Ueguchi-Tanaka M, Asami T, Yamaguchi I, Matsuoka M, Kobayashi M, Nakajima M (2007) Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. The Plant Journal 50:958-966

Izydorczyk C, Nguyen TN, Jo S, Son S, Tuan PA, Ayele BT (2018) Spatiotemporal modulation of abscisic acid and gibberellin metabolism and signalling mediates the effects of suboptimal and supraoptimal temperatures on seed germination in wheat (*Triticum aestivum* L.). Plant, Cell and Environment 41:1022-1037

Jacobsen JV, Barrero JM, Hughes T, Julkowska M, Taylor JM, Xu Q, Gubler F (2013) Roles for blue light, jasmonate and nitric oxide in the regulation of dormancy and germination in wheat grain (*Triticum aestivum L.*). Planta 238:121-138

Jacobsen JV, Pearce DW, Poole AT, Pharis RP, Mander LN (2002) Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. Physiologia Plantarum 115:428-441

Jensen S, Munck L, Kruger J (1984) A rapid fluorescence method for assessment of pre-harvest sprouting of cereal grains. Journal of Cereal Science 2:187-201

Jiang H, Lei R, Ding SW, Zhu S (2014) Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15:1-12

Johnson RR, Wagner RL, Verhey SD, Walker-Simmons MK (2002) The abscisic acid-responsive kinase PKABA1 interacts with a seed-specific abscisic acid response element-binding factor, TaABF, and phosphorylates TaABF peptide sequences. Plant Physiology 130:837-846

Joshi NA, Fass J (2011) Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33). https://github.com/najoshi/sickle

Kalemba EM, Ratajczak E (2018) The effect of a doubled glutathione level on parameters affecting the germinability of recalcitrant *Acer saccharinum* seeds during drying. Journal of Plant Physiology 223:72-83

Kanehisa M (2002) The KEGG database. Novartis Foundation Symposium 247:91-101

Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research 28:27-30

Kaneko M, Inukai Y, Ueguchi-Tanaka M, Itoh H, Izawa T, Kobayashi Y, Hattori T, Miyao A, Hirochika H, Ashikari M, Matsuoka M (2004) Loss-of-function mutations of the rice *GAMYB* gene impair *α-amylase* expression in aleurone and flower development. The Plant Cell 16:33-44

Karssen CM, Laçka E (1986) A Revision of the Hormone Balance Theory of Seed Dormancy: Studies on Gibberellin and/or Abscisic Acid-Deficient Mutants of *Arabidopsis thaliana*. In M. Bopp (Ed.), Plant Growth Substances 1985. Proceedings in Life Sciences (pp. 315-323). Springer Berlin

Kashiwakura YI, Kobayashi D, Jikumaru Y, Takebayashi Y, Nambara E, Seo M, Kamiya Y, Kushiro T, Kawakami N (2016) Highly sprouting-tolerant wheat grain exhibits extreme dormancy and cold imbibition-resistant accumulation of abscisic acid. Plant and Cell Physiology 57:715-732

Kato T, Saito N, Kashimura K, Shinohara M, Kurahashi T, Taniguchi K (2002) Germination and growth inhibitors from wheat (*Triticum aestivum* L.) husks. Journal of Agricultural and Food Chemistry 50:6307-6312

Kawakami N, Miyake Y, Noda K (1997) ABA insensitivity and low ABA levels during seed development of non-dormant wheat mutants. Journal of Experimental Botany 48:1415-1421

Keller EF (2011) Genes, genomes, and genomics. Biological Theory 6:132-140

Khalid A, Hameed A, Tahir MF (2023) Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. Frontiers in Nutrition 10:1053196

Kim D, Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nature Biotechnology 37:907-915

King RW, von Wettstein-Knowles P (2000) Epicuticular waxes and regulation of ear wetting and pre-harvest sprouting in barley and wheat. Euphytica 112:157-166

Kogenaru S, Yan Q, Guo Y, Wang N (2012) RNA-seq and microarray complement each other in transcriptome profiling. BMC Genomics 13:1-16

Koornneef M, Jorna M. L., Brinkhorst-Van der Swan, D. L. C., Karssen, C. M. (1982). The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) heynh. Theoretical and Applied Genetics, 61, 385-393.

Koramutla MK, Negi M, Ayele BT (2021) Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. Genes 12:1620

Kovaka S, Zimin AV, Pertea GM, Razaghi R, Salzberg SL, Pertea M (2019) Transcriptome assembly from long-read RNA-seq alignments with StringTie2. Genome Biology 20:1-13

Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Science Research 15:281-307

Kukurba KR, Montgomery SB (2015) RNA sequencing and analysis. Cold Spring Harbor Protocols 2015:951-969

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology 10:1-10

Lassmann T, Hayashizaki Y, Daub CO (2010) SAMStat: monitoring biases in next generation sequencing data. Bioinformatics 27:130–131

Lee YJ, Park SY, Kim DY, Kim JY (2021) Differential Expression Analysis of Phytohormone-Related Genes of Korean Wheat (*Triticum aestivum*) in Response to Preharvest Sprouting and Abscisic Acid (ABA). Applied Sciences 11:3562

Leubner-Metzger G (2005) β-1, 3-Glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. The Plant Journal 41:133-145

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754-1760

Li H, Li X, Wang G, Zhang J, Wang G (2022) Analysis of gene expression in early seed germination of rice: landscape and genetic regulation. BMC Plant Biology 22:1-14

Li X, Qiao H, Wang Z, Han B, Xing Y, Yang Y (2021) A Comparative Transcriptome Analysis Reveals New Insights into Pre-Harvest Sprouting (PHS) in Wheat. Research Square

Lin M, Cai S, Wang S, Liu S, Zhang G, Bai G (2015) Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. Theoretical and Applied Genetics 128:1385-1395

Liu A, Gao F, Kanno Y, Jordan MC, Kamiya Y, Seo M, Ayele BT (2013) Regulation of wheat seed dormancy by after-ripening is mediated by specific transcriptional switches that induce changes in seed hormone metabolism and signaling. PloS One 8:e56570

Liu D, Zeng M, Wu Y, Du Y, Liu J, Luo S, Zeng Y (2022) Comparative transcriptomic analysis provides insights into the molecular basis underlying pre-harvest sprouting in rice. BMC Genomics 23:771

Liu J, Yu T, Jiang T, Li G (2016) TransComb: genome-guided transcriptome assembly via combing junctions in splicing graphs. Genome Biology 17:1-9

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. Methods 25:402-408

Locascio A, Blázquez MA, Alabadí D (2013) Genomic analysis of DELLA protein activity. Plant and Cell Physiology 54:1229-1237

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:1-21

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C Phosphatase Activity Function as Abscisic Acid Sensors. Science 324:1064-1068.

MacMillan J (2001) Occurrence of gibberellins in vascular plants, fungi, and bacteria. Journal of Plant Growth Regulation, 20:387-442

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957-2963

Maia J, Dekkers BJ, Dolle MJ, Ligterink, W, Hilhorst, HW (2014) Abscisic acid (ABA) sensitivity regulates desiccation tolerance in germinated Arabidopsis seeds. New Phytologist 203:81-93

Maleki F, Ovens K, Hogan DJ, Kusalik AJ (2020) Gene Set Analysis: Challenges, Opportunities, and Future Research. Frontiers in Genetics 11:654

Mamrutha HM, Rinki, Singh SK, Gopalareddy K, Tiwari R, Venkatesh K, Kumar Y, Singh G, Singh GP (2022) Abiotic Stress Tolerance in Wheat: Physiological Interventions. In New Horizons in Wheat and Barley Research: Global Trends, Breeding and Quality Enhancement (pp. 507-530). Springer Singapore

Maretty L, Sibbesen JA, Krogh A (2014) Bayesian transcriptome assembly. Genome Biology 15:1-11

Marin E, Nussaume L, Quesada A, Gonneau M, Sotta B, Hugueney P, Frey A, Marion-Poll A (1996) Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of *Arabidopsis thaliana*. The EMBO journal 15:2331-2342

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: An assessment of technical reproducibility and comparison with gene expression arrays. Genome Research 18:1509-1517

Martinez SA, Tuttle KM, Takebayashi Y, Seo M, Campbell KG, Steber CM (2016) The wheat ABA hypersensitive ERA8 mutant is associated with increased preharvest sprouting tolerance and altered hormone accumulation. Euphytica 212:229-245

Matsuoka Y, Nasuda S (2004) Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F1 hybrid with Aegilops tauschii Coss. Theoretical and Applied Genetics 109:1710-1717

Mayer KFX, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Kubaláková M, Číhalíková J, Dubská Z, Vrána J, Šperková R, Šimková H, Febrer M, Clissold L, et al. (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:251788

Mbi TK, Ntsefong NG, Lenzemo TE (2022) Seed dormancy: induction, maintenance and seed technology approaches to break dormancy. In Seed Biology Updates. IntechOpen, London, UK.

McCallum BD, DePauw RM (2008) A review of wheat cultivars grown in the Canadian prairies. Canadian Journal of Plant Science 88:649-677

Meimoun P, Mordret E, Langlade NB, Balzergue S, Arribat S, Bailly C, El-Maarouf-Bouteau H (2014) Is Gene Transcription Involved in Seed Dry After-Ripening? PloS One 9:e86442

Miao C, Wang Z, Zhang L, Yao J, Hua K, Liu X, Shi H, Zhu JK (2019) The grain yield modulator miR156 regulates seed dormancy through the gibberellin pathway in rice. Nature Communications 10:3822

Miao XL, Zhang YJ, Xia XC, He ZH, Zhang Y, Yan J, Chen XM (2013) Mapping quantitative trait loci for pre-harvest sprouting resistance in white-grained winter wheat line CA 0431. Crop and Pasture Science 64:573-579

Millar AA, Jacobsen JV, Ross JJ, Helliwell CA, Poole AT, Scofield G, Reid JB, Gubler F (2006) Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8′-hydroxylase. The Plant Journal 45:942-954

Mo Y, Wan R, Zhang Q (2012) Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. Methods in Molecular Biology 926:99-112

Morrison JW (1960) Marquis wheat-a triumph of scientific endeavor. Agricultural History 34:182

Morrison MJ (2008) Sir Charles Edward Saunders, Dominion Cerealist. Genome 51:465–469

Mukherjee S, Stasolla C, Ayele BT (2015) RNA In situ Hybridization. Plant Microtechniques and Protocols (pp. 351-370). Cham: Springer

Naguleswaran S, Li J, Vasanthan T, Bressler D, Hoover R (2012) Amylolysis of large and small granules of native triticale, wheat and corn starches using a mixture of α-amylase and glucoamylase. Carbohydrate Polymers 88:864-874

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H (2011) A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. The Plant Cell 23:3215-3229

Nambara E, Marion-Poll A (2005) ABSCISIC ACID BIOSYNTHESIS AND CATABOLISM. Annual Review of Plant Biology 56:165-185

Nambara E, Okamoto M, Tatematsu K, Yano R, Seo M, Kamiya Y (2010) Abscisic acid and the control of seed dormancy and germination. Seed Science Research 20:55-67

Nautiyal PC, Sivasubramaniam K, Dadlani M (2023) Seed dormancy and regulation of germination. Seed Science and Technology (pp. 39-66) Singapore: Springer

Née G, Kramer K, Nakabayashi K, Yuan B, Xiang Y, Miatton E, Finkemeier I, Soppe WJJ (2017) DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. Nature Communications 8:72

Nelson DR, Schuler MA, Paquette SM, Werck-Reichhart D, Bak S (2004) Comparative genomics of rice and Arabidopsis. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. Plant Physiology 135:756-772

Nelson SK, Ariizumi T, Steber CM (2017) Biology in the dry seed: Transcriptome changes associated with dry seed dormancy and dormancy loss in the Arabidopsis GA-insensitive sleepy1-2 mutant. Frontiers in Plant Science 8:2158

Nelson SK, Steber CM (2017) Transcriptional mechanisms associated with seed dormancy and dormancy loss in the gibberellin-insensitive sly1-2 mutant of *Arabidopsis thaliana*. PloS One 12:e0179143

Newberry M, Zwart AB, Whan A, Mieog JC, Sun M, Leyne E, Pritchard J, Daneri-Castro SN, Ibrahim K, Diepeveen D, Howitt CA, Ral JF (2018) Does late maturity alpha-amylase impact wheat baking quality? Frontiers in Plant Science 9:1356

Newman LH (1928) The history and present status of wheat production in Canada. Dominion of Canada, Department of Agriculture, Ottawa, ON. Pamphlet No. 89

Nonogaki H, Bassel GW, Bewley JD (2010) Germination—Still a mystery. Plant Science 179:574-581

Nyachiro JM, Clarke FR, DePauw RM, Knox RE, Armstrong KC (2002) Temperature effects on seed germination and expression of seed dormancy in wheat. Euphytica 126:123-127

O'brien MA, Costin BN, Miles MF (2012) Using genome-wide expression profiling to define gene networks relevant to the study of complex traits: from RNA integrity to network topology. International Review of Neurobiology 104:91-133

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell 15:1591-1604

Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N, Kamiya Y, Koshiba T, Nambara E (2006) CYP707A1 and CYP707A2, Which Encode Abscisic Acid 8′-Hydroxylases, Are Indispensable for Proper Control of Seed Dormancy and Germination in Arabidopsis. Plant Physiology 141:97-107

Olaerts H, Roye C, Derde LJ, Sinnaeve G, Meza WR, Bodson B, Courtin CM (2016) Impact of preharvest sprouting of wheat (*Triticum aestivum*) in the field on starch, protein, and arabinoxylan properties. Journal of Agricultural and Food Chemistry 64:8324-8332

Oshlack A, Wakefield MJ (2009) Transcript length bias in RNA-seq data confounds systems biology. Biology Direct 4:1-10

Peña RJ (2002) Wheat for Bread and Other Foods. http://www.fao.org/3/y4011e/y4011e0w.htm (Accessed on 7 March 2022)

Peng JH, Sun D, Nevo E (2011) Domestication evolution, genetics and genomics in wheat. Molecular Breeding 28:281-301

Pereira MA, Imada EL, Guedes RLM (2017) RNA-seq: applications and best practices. Applications of RNA-Seq and Omics Strategies-From Microorganisms to Human Health (pp. 3-36). InTechOpen London

Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nature Protocols 11:1650-1667

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nature Biotechnology 33:290-295

Pfeifer SP (2016) From next-generation resequencing reads to a high-quality variant data set. Heredity 118:111-124

Prakash C, Haeseler AV (2017) An Enumerative Combinatorics Model for Fragmentation Patterns in RNA Sequencing Provides Insights into Nonuniformity of the Expected Fragment Starting-Point and Coverage Profile. Journal of Computational Biology 24:200-212

Qin X, Zeevaart JA (1999) The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. Proceedings of the National Academy of Sciences 96:15354-15361

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends in Plant Science 15:395-401

Rahman S, Islam S, Yu Z, She M, Nevo E, Ma W (2020) Current Progress in Understanding and Recovering the Wheat Genes Lost in Evolution and Domestication. International Journal of Molecular Sciences 21:5836

Rajjou L, Gallardo K, Debeaujon I, Vandekerckhove J, Job C, Job D (2004) The Effect of α-Amanitin on the Arabidopsis Seed Proteome Highlights the Distinct Roles of Stored and Neosynthesized mRNAs during Germination. Plant Physiology 134:1598-1613

Ral JP, Whan A, Larroque O, Leyne E, Pritchard J, Dielen AS, Howitt CA, Morell MK, Newberry M (2016) Engineering high α-amylase levels in wheat grain lowers Falling Number but improves baking properties. Plant Biotechnology Journal 14:364-376

Ravindran P, Verma V, Stamm P, Kumar PP (2017) A novel RGL2–DOF6 complex contributes to primary seed dormancy in *Arabidopsis thaliana* by regulating a GATA transcription factor. Molecular Plant 10:1307-1320

Regnault T, Davière JM, Heintz D, Lange T, Achard P (2014) The gibberellin biosynthetic genesAtKAO1andAtKAO2have overlapping roles throughout Arabidopsis development. The Plant Journal 80:462-474

Rehal PK, Tuan PA, Nguyen TN, Cattani DJ, Humphreys DG, Ayele BT (2022) Genetic variation of seed dormancy in wheat (*Triticum aestivum* L.) is mediated by transcriptional regulation of abscisic acid metabolism and signaling. Plant Science 324:111432

Rikiishi K, Maekawa M (2010) Characterization of a novel wheat (*Triticum aestivum* L.) mutant with reduced seed dormancy. Journal of Cereal Science 51:292-298

Ritchie M E, Phipson B, Wu D, Hu Y, Law C W, Shi W, Smyth G K (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research 43:e47

Roberts A, Trapnell C, Donaghey J, Rinn J L, Pachter L (2011) Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biology 12:1-14

Robinson M D, McCarthy D J, Smyth G K (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139-140

Robinson MD, Oshlack A (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biology 11:1-9

Rodríguez MV, Barrero JM, Corbineau F, Gubler F, Benech-Arnold RL (2015) Dormancy in cereals (not too much, not so little): about the mechanisms behind this trait. Seed Science Research 25:99-119

Rodríguez MV, Mendiondo GM, Cantoro R, Auge GA, Luna V, Masciarelli O, Benech-Arnold RL (2012) Expression of seed dormancy in grain sorghum lines with contrasting pre-harvest sprouting behavior involves differential regulation of gibberellin metabolism genes. Plant and Cell Physiology 53:64-80

Rodríguez MV, Mendiondo GM, Maskin L, Gudesblat GE, Iusem ND, Benech-Arnold RL (2009) Expression of ABA signalling genes and ABI5 protein levels in imbibed Sorghum

bicolor caryopses with contrasting dormancy and at different developmental stages. Annals of Botany 104:975-985

Rodríguez-Gacio MDC, Matilla-Vázquez MA, Matilla AJ (2009) Seed dormancy and ABA signaling: the breakthrough goes on. Plant Signaling and Behavior 4:1035-1048

Sabelli PA (2012) Seed development: a comparative overview on biology of morphology, physiology, and biochemistry between monocot and dicot plants. Seed Development: OMICS Technologies toward Improvement of Seed Quality and Crop Yield: OMICS in Seed Biology:3-25

Sabelli PA, Larkins BA (2009) The development of endosperm in grasses. Plant physiology 149:14-26

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M (2004) Arabidopsis CYP707A s encode (+)-abscisic acid 8′-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant physiology 134:1439-1449

Sano N, Marion-Poll A (2021) ABA metabolism and homeostasis in seed dormancy and germination. International Journal of Molecular Sciences 22:5069

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, Matsuoka M (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299:1896-1898

Sawada Y, Aoki M, Nakaminami K, Mitsuhashi W, Tatematsu K, Kushiro T, Koshiba T, Kamiya Y, Inoue Y, Nambara E, Toyomasu T (2008) Phytochrome-and gibberellin-mediated regulation of abscisic acid metabolism during germination of photoblastic lettuce seeds. Plant physiology 146:1386-1396

Schramm EC, Nelson SK, Steber CM (2012) Wheat ABA-insensitive mutants result in reduced grain dormancy. Euphytica 188:35-49

Schulz MH, Zerbino DR, Vingron M, Birney E (2012) Oases: robust de novo RNA-seq assembly across the dynamic range of expression levels. Bioinformatics 28:1086-1092

Schurch NJ, Schofield P, Gierliński M, Cole C, Sherstnev A, Singh V, Wrobel N, Gharbi K, Simpson GG, Owen-Hughes T, Blaxter M (2016) How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? Rna 22:839-851

Schwartz SH, Qin X, Zeevaart JA (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. Plant Physiology 131:1591-1601

Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science. 276:1872-1874

Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JA, Koornneef M, Kamiya Y, Koshiba T (2000) The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proceedings of the National Academy of Sciences 97:12908-12913

Seo M., Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. Trends in Plant Science 7:41-48

Shao M, Kingsford C (2017) Accurate assembly of transcripts through phase-preserving graph decomposition. Nature Biotechnology 35:1167-1169

Shavanov MV, Shigapov II (2020) Wheat industry compared and contrasted between Russia and the USA. IOP Conference Series: Earth and Environmental Science 548:022099

Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W (2022) DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research 50:W216-W221

Shi H, Zhou Y, Jia E, Pan M, Bai Y, Ge Q (2021) Bias in RNA-seq library preparation: current challenges and solutions. BioMed Research International 2021:6647597

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317

Simpson GM (1990) Seed dormancy in grasses. Cambridge, UK: Cambridge University Press

Simsek S, Ohm JB, Lu H, Rugg M, Berzonsky W, Alamri MS, Mergoum M (2014) Effect of pre-harvest sprouting on physicochemical properties of starch in wheat. Foods 3: 194-207

Smith S, Li C, Li J (2017) Hormone function in plants. Academic Press

Sohn SI, Pandian S, Kumar TS, Zoclanclounon YAB, Muthuramalingam P, Shilpha J, Satish L, Ramesh M (2021) Seed dormancy and pre-harvest sprouting in rice-an updated overview. International Journal of Molecular Sciences 22:11804

Son S, Chitnis VR, Liu A, Gao F, Nguyen TN, Ayele BT (2016) Abscisic acid metabolic genes of wheat (*Triticum aestivum* L.): identification and insights into their functionality in seed dormancy and dehydration tolerance. Planta 244:429-447

Statistics Canada (2021) https://www150.statcan.gc.ca/n1/daily-quotidien/201203/dq201203b-eng.htm (Accessed on 5 october, 2021)

Statistics Canada (2022a) https://www150.statcan.gc.ca/n1/daily-quotidien/221202/dq221202b-eng.htm (Accessed on 26 December, 2022)

Statistics Canada (2022b) https://www150.statcan.gc.ca/n1/daily-quotidien/220705/dq220705b-eng.htm (Accessed on 17 December 2022)

Steber CM, Cooney SE, McCourt P (1998) Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in *Arabidopsis thaliana*. Genetics 149:509-521

Steinbach HS, Benech-Arnold RL, Kristof G, Sanchez RA, Marcucci-Poltri S (1995) Physiological basis of pre-harvest sprouting resistance in Sorghum bicolor (L.) Moench. ABA levels and sensitivity in developing embryos of sprouting resistant and susceptible varieties. Journal of Experimental Botany 45:701-709

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences 102:15545-15550

Sun J, Okada M, Tameshige T, Shimizu-Inatsugi R, Akiyama R, Nagano A, Sese J, Shimizu K (2023) A low-coverage 3′ RNA-seq to detect homeolog expression in polyploid wheat. NAR Genomics and Bioinformatics 5:lqad067

Sun TP (2008) Gibberellin metabolism, perception and signaling pathways in Arabidopsis. The Arabidopsis Book/American Society of Plant Biologists 6:e0103

Sun TP, Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. Annual Review Plant Biology 55:197-223

Suzuki T, Matsuura T, Kawakami N, Noda K (2000) Accumulation and leakage of abscisic acid during embryo development and seed dormancy in wheat. Plant Growth Regulation 30:253-260

Symko S (1999) From a single seed: Tracing the Marquis wheat success story in Canada to its roots in the Ukraine. A web publication of Research Branch, Agriculture and Agri-Food Canada. http://careerchem.com/NAMED/Patents/SSymko-wheat.pdf

Tai L, Wang HJ, Xu XJ, Sun WH, Ju L, Liu WT, Li WQ, Sun J, Chen KM (2021) Pre-harvest sprouting in cereals: Genetic and biochemical mechanisms. Journal of Experimental Botany 72:2857-2876

Thompson AJ, Jackson AC, Symonds RC, Mulholland BJ, Dadswell AR, Blake PS, Burbidge A, Taylor IB (2000) Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. The Plant Journal 23:363-374

Tognacca RS, Botto JF (2021) Post-transcriptional regulation of seed dormancy and germination: current understanding and future directions. Plant Communications 2:100169

Toh S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y, Hanada A, Aso Y, Ishiyama K, Tamura N, Iuchi S, Kobayashi M, Yamaguchi S, Kamiya Y, Nambara E, Kawakami N (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant physiology 146:1368-1385

Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25:1105-1111

Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature Biotechnology, 28:511-515

Tuan PA, Kumar R, Rehal PK, Toora PK, Ayele BT (2018) Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. Frontiers in Plant Science 9:668

Tuan PA, Nguyen TN, Jordan MC, Ayele BT (2021) A shift in abscisic acid/gibberellin balance underlies retention of dormancy induced by seed development temperature. Plant, Cell and Environment 44:2230-2244

Tuttle KM, Martinez SA, Schramm EC, Takebayashi Y, Seo M, Steber CM (2015) Grain dormancy loss is associated with changes in ABA and GA sensitivity and hormone accumulation in bread wheat, *Triticum aestivum* (L.). Seed Science Research 25:179-193

Uauy C (2017) Wheat genomics comes of age. Current Opinion in Plant Biology 36:142-148

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YIC, Kitano H, Yamaguchi I, Matsuoka M (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437:693-698

Ueguchi-Tanaka M, Hirano K, Hasegawa Y, Kitano H, Matsuoka M. (2008). Release of the repressive activity of rice DELLA protein SLR1 by gibberellin does not require SLR1 degradation in the gid2 mutant. The Plant Cell 20:2437-2446

VanGuilder HD, Vrana KE, Freeman WM (2008) Twenty-five years of quantitative PCR for gene expression analysis. Biotechniques 44:619-626

Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. BMC Plant Biology 16:1-10

Walker-Simmons M (1987) ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiology 84:61-66

Wang K, Singh D, Zeng Z, Coleman SJ, Huang Y, Savich GL, He X, Mieczkowski P, Grimm SA, Perou CM, MacLeod JN, Chiang DY, Prins JF, Liu J (2010) MapSplice: Accurate mapping of RNA-seq reads for splice junction discovery. Nucleic Acids Research, 38:e178

Wang L, Wang S, Li W (2012) RSeQC: quality control of RNA-seq experiments. Bioinformatics 28: 2184-2185

Wang X (2021) Genetic analysis of seed dormancy for pre-harvest sprouting resistance in wheat [Doctoral dissertation, University of Western Australia]. Research Repository UWA. https://api.research-repository.uwa.edu.au/ws/portalfiles/portal/111493010/THESIS_DOCTOR_OF_PHILOSOPHY_WANG_Xingyi_2021.pdf

Wang X, Liu H, Siddique KHM, Yan G. (2021). Transcriptomic profiling of wheat near-isogenic lines reveals candidate genes on chromosome 3A for pre-harvest sprouting resistance. BMC Plant Biology, 21:1-14

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10:57-63

Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. The Crop Journal 4:162-176

Wei Y, Wang S, Yu D (2023) The Role of light quality in regulating early seedling development. Plants 12:2746

Westra RN, Loomis WE (1966) Seed dormancy in *Uniola paniculata*. American Journal of Botany 53:407-411

Wheat grain quality - falling number and pre harvest sprouting resistance (2022) https://www.agric.wa.gov.au/wheat/wheat-grain-quality-falling-number-and-pre-harvest-sprouting-resistance (Accessed on 5 April 2022)

Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G (2021) clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation 2:100141

Wu TD, Nacu S (2010) Fast and SNP-tolerant detection of complex variants and splicing in short reads. Bioinformatics 26:873-881

Xie K, Bai J, Yang YY, Duan N, Ma Y, Guo T, Yao F, Ding H (2019) The RNA-seq transcriptome analysis identified genes related to rice seed dormancy. Biologia Plantaraum 63:308-313

Xu P, Tang G, Cui W, Chen G, Ma CL, Zhu J, Li P, Shan L, Liu Z, Wan S (2020) Transcriptional Differences in Peanut (Arachis hypogaea L.) Seeds at the Freshly Harvested, After-ripening and Newly Germinated Seed Stages: Insights into the Regulatory Networks of Seed Dormancy Release and Germination. PloS One 15: e0219413

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annual Review of Plant Biology 59:225-251

Yan L, Li G, Yu M, Fang T, Cao S, Carver BF. (2015) Genetic mechanisms of vernalization requirement duration in winter wheat cultivars. In Advances in Wheat Genetics: From Genome to Field: Proceedings of the 12th International Wheat Genetics Symposium (pp. 117-125). Springer Japan.

Yazdanpanah F, Hanson J, Hilhorst HW, Bentsink L (2017) Differentially expressed genes during the imbibition of dormant and after-ripened seeds – a reverse genetics approach. BMC Plant Biology 17:1-12

Ye H, Feng J, Zhang L, Zhang J, Mispan MS, Cao Z, Beighley DH, Yang J, Gu XY (2015) Map-based cloning of seed dormancy1-2 identified a gibberellin synthesis gene regulating the development of endosperm-imposed dormancy in rice. Plant Physiology 169:2152-2165

Zapalska-Sozoniuk M, Chrobak L, Kowalczyk K, Kankofer M (2019) Is it useful to use several "omics" for obtaining valuable results? Molecular biology Biology reports Reports 46:3597-3606

Zhang XQ, Li C, Tay A, Lance R, Mares D, Cheong J, Cakir M, Ma J, Appels R (2008) A new PCR-based marker on chromosome 4AL for resistance to pre-harvest sprouting in wheat (*Triticum aestivum* L.). Molecular Breeding 22:227-236

Zhang Y, Miao X, Xia X, He Z (2014) Cloning of seed dormancy genes (TaSdr) associated with tolerance to pre-harvest sprouting in common wheat and development of a functional marker. Theoretical and Applied Genetics 127:855-866

Zhao Y, Zhang Z, Gao J, Wang P, Hu T, Wang Z, Hou YJ, Wan Y, Liu W, Xie S, Lu T (2018) Arabidopsis Duodecuple Mutant of PYL ABA Receptors Reveals PYL Repression of ABA-Independent SnRK2 Activity. Cell Reports 23:3340-3351.e5

Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature Communications 10:1523

Zhu G, Ye N, Zhang J (2009) Glucose-induced delay of seed germination in rice is mediated by the suppression of ABA catabolism rather than an enhancement of ABA biosynthesis. Plant and Cell Physiology 50:644-651

Zhu T, Wang L, Rimbert H, Rodriguez JC, Deal KR, De Oliveira R, Choulet F, Keeble-Gagnère G, Tibbits J, Rogers J, Eversole K, Appels R, Gu YQ, Mascher M, Dvorak J, Luo MC (2021) Optical maps refine the bread wheat *Triticum aestivum* cv Chinese Spring genome assembly. The Plant Journal 107:303-314

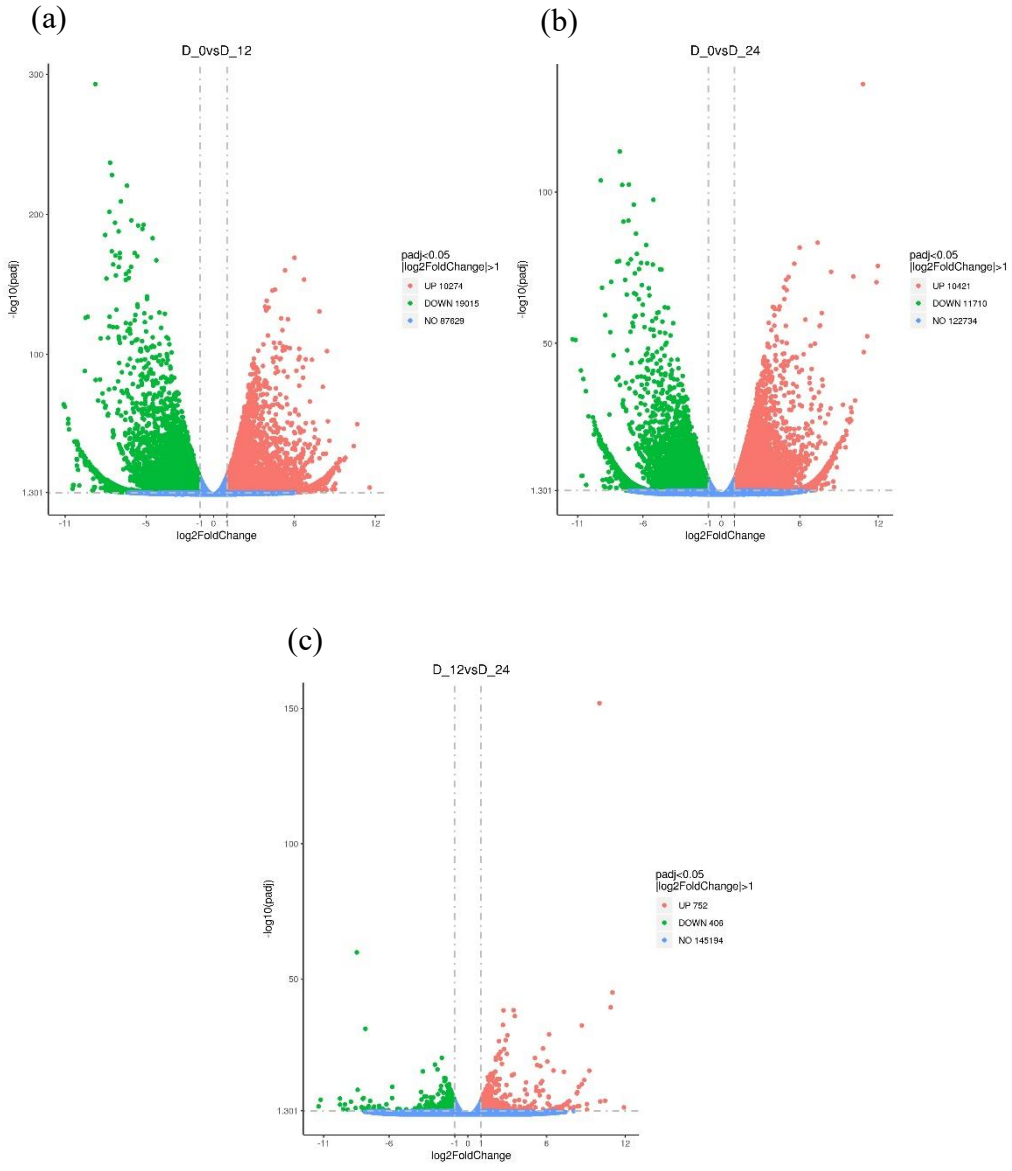
Zohary D, Hopf M, Weiss E (2012) Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. 4th edn Oxford University Press

APPENDIX

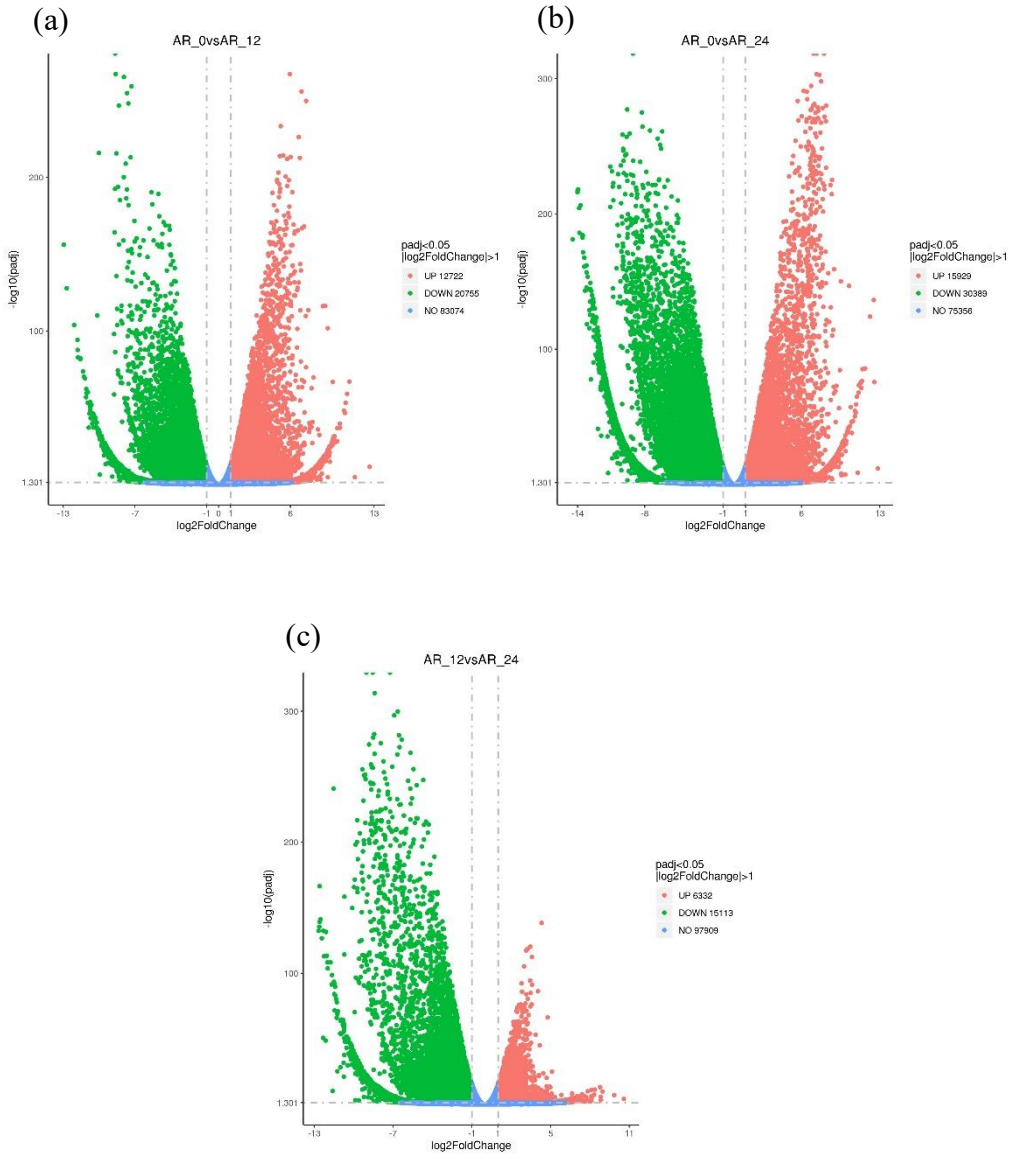
Appendix 1. Primer sequences used for qPCR.

Gene	Type	Primer sequence (5' to 3')
TaEAR1	FP	GAGGTGGTGGACAGAGGAAC
TaEAR1	RP	CTGCCGACGAACTTGTTGAT
TaNCED3	FP	GTATGACTCGGACGATTCCC
TaNCED3	RP	CATGCAGGAGCAAATGATGAC
TaPGAM	FP	GAGATGATCGACGACCAGATG
TaPGAM	RP	TAGCTGAAATGGGTGAAGAAGG
TaGA2OX3	FP	CGGCTGTCGGTGATCTACTT
TaGA2OX3	RP	TTGTAGGCGGCCTTCTTGTA
TaTIP1-2	FP	CATCGCCATCGGCTTCATC
TaTIP1-2	RP	CCCAGTGGTTCTCCCAGAC

Appendix 2. Volcano plot showing the number of DEGs between dormant seeds at 0 and 12 HAI (a) 0 and 24 HAI (b) 12 and 24 HAI (c). The x-axis represents the log2 fold change, while the y-axis represents the P-values. The upregulated genes are depicted in red, while the downregulated genes are depicted in green. Genes exhibiting no significant differential expression between the respective comparison are represented in blue.

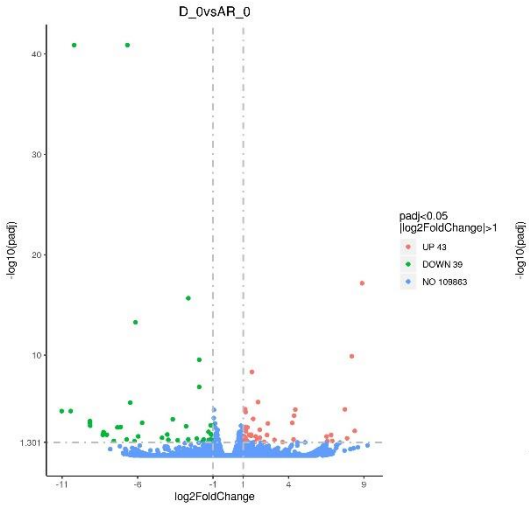


Appendix 3. Volcano plot showing the number of DEGs between after-ripened seeds at 0 and 12 HAI (a) 0 and 24 HAI (b) 12 and 24 HAI (c). Graph descriptions are as shown in Appendix 2.

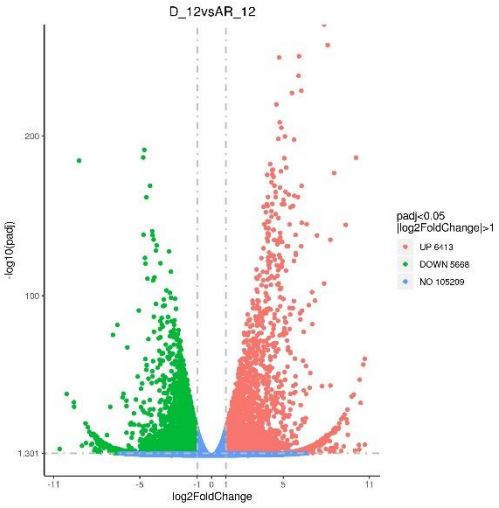


Appendix 4. Volcano plot showing the number of DEGs between dormant and after-ripened seeds at 0 and 12 HAI (a) 0 and 24 HAI (b) 12 and 24 HAI (c). Graph descriptions are as shown in Appendix 2.

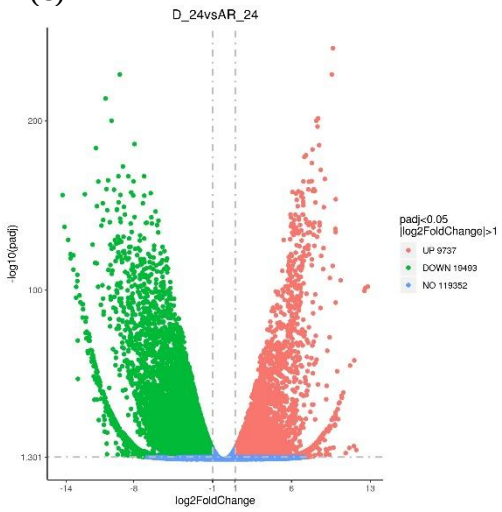
(a)



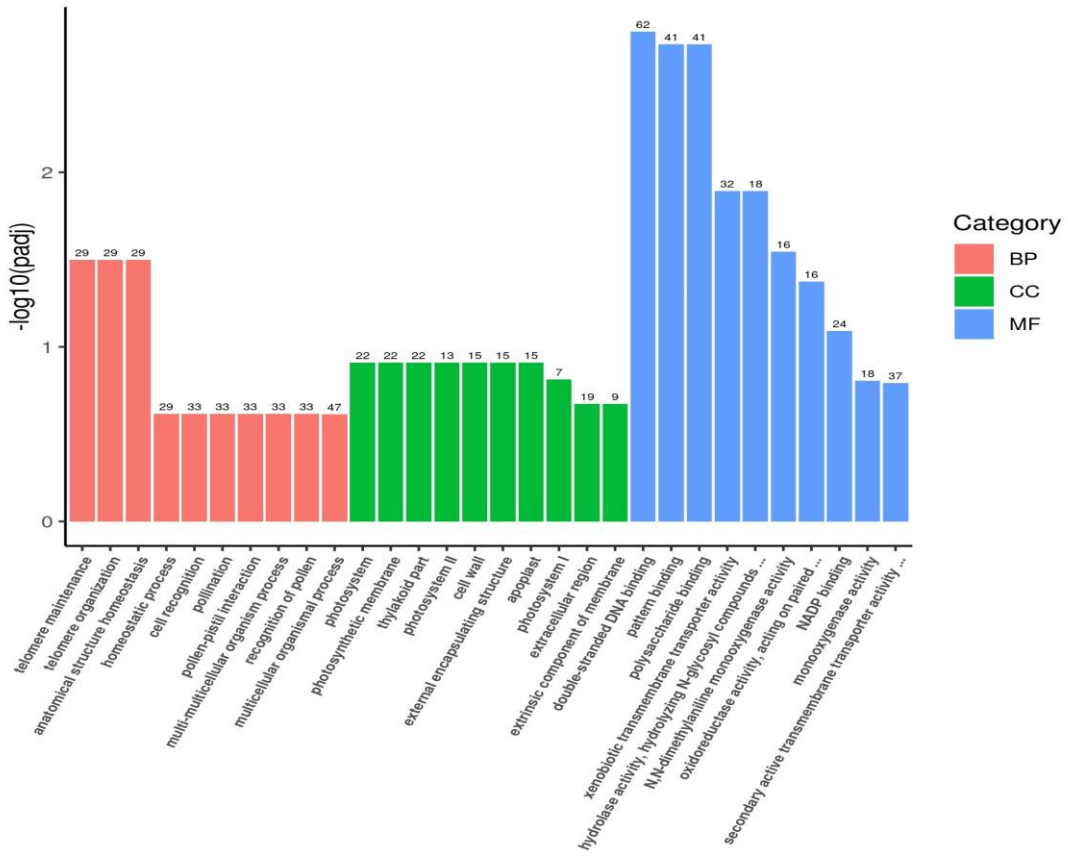
(b)



(c)

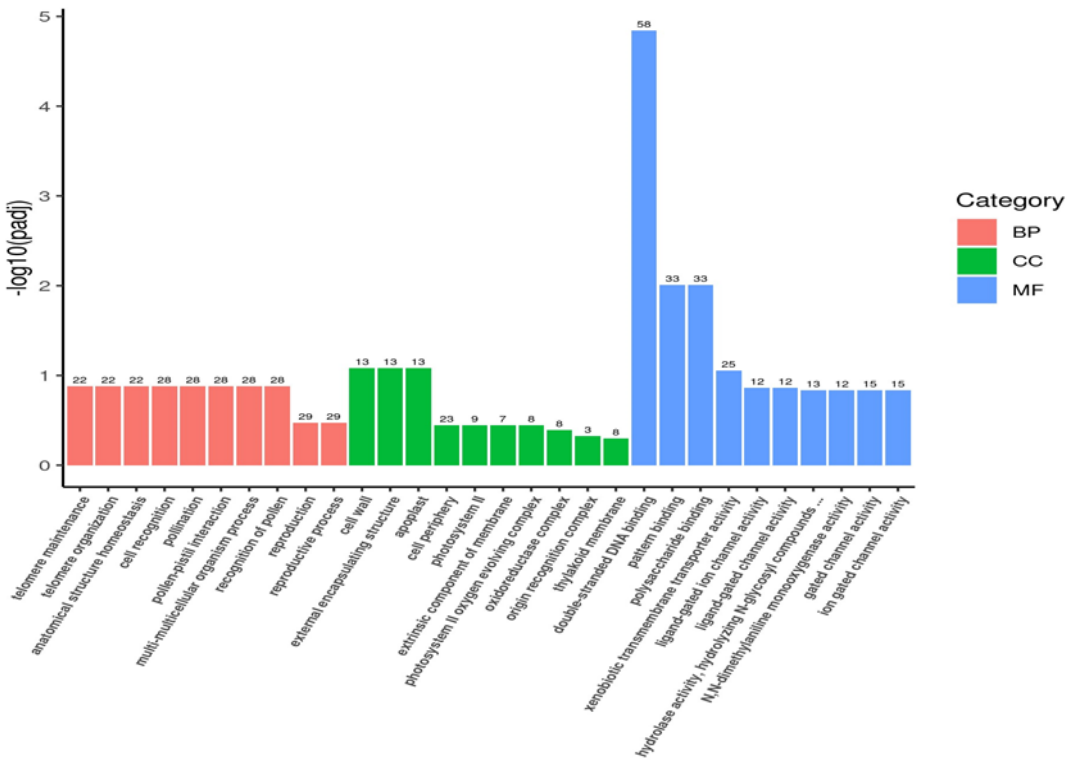


Appendix 5. Gene ontologies enriched with genes differentially expressed between 0 and 12 HAI dormant seeds. Different colours of the bar graphs represent the three main gene ontology domains: biological process (BP), cellular component (CC) and molecular function (MF).

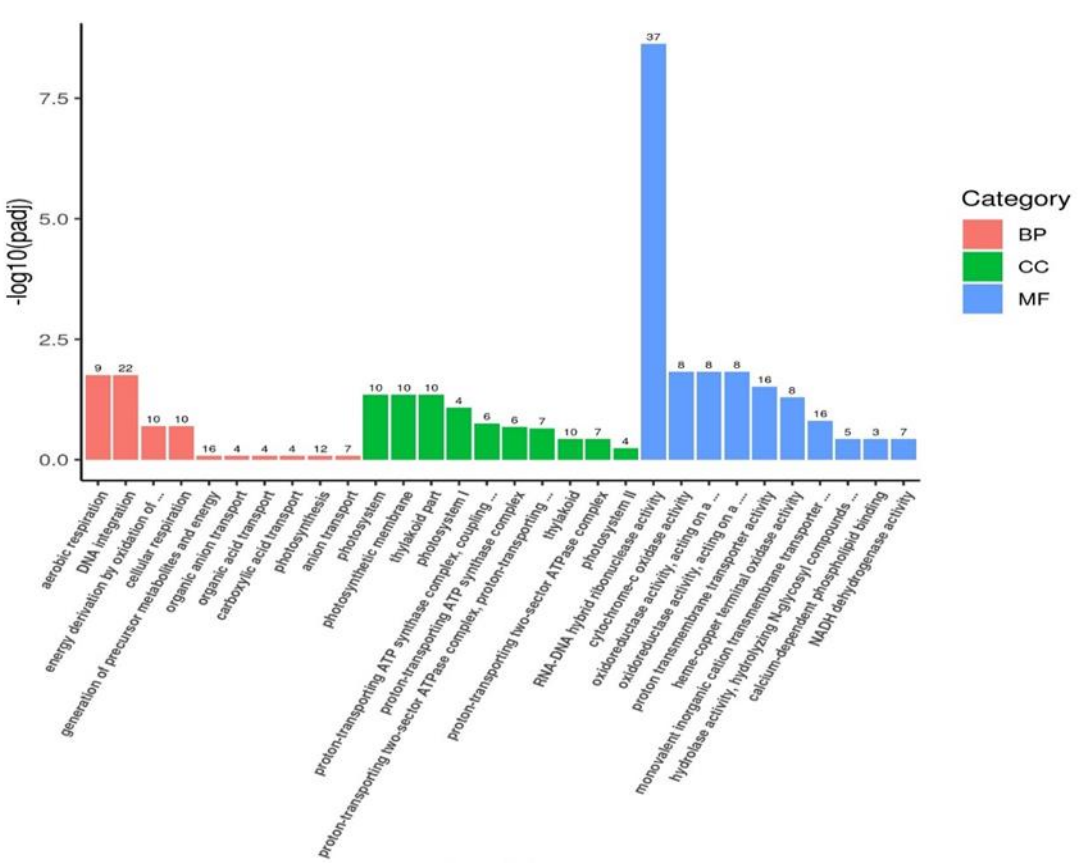


Appendix 6. Gene ontologies enriched with genes downregulated (a) and upregulated (b) in 0 HAI as compared to 12 HAI dormant seeds. Graph descripitions are as shown in Appendix 5.

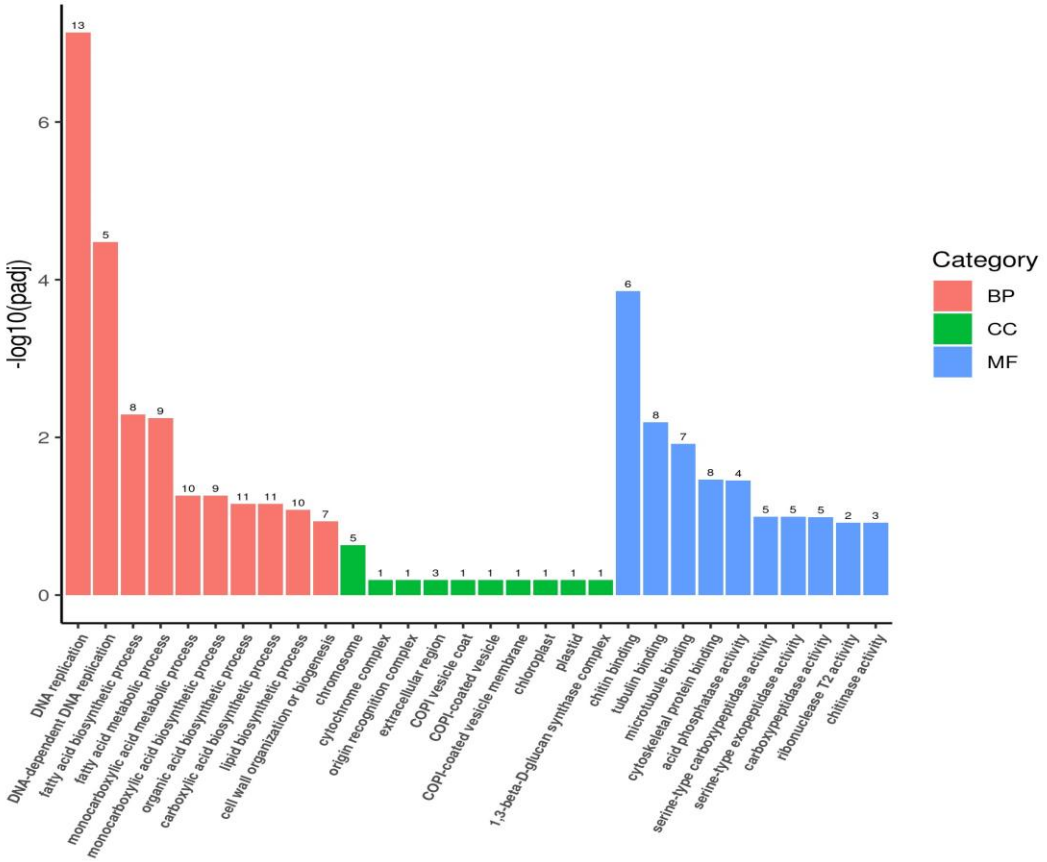
(a)



(b)

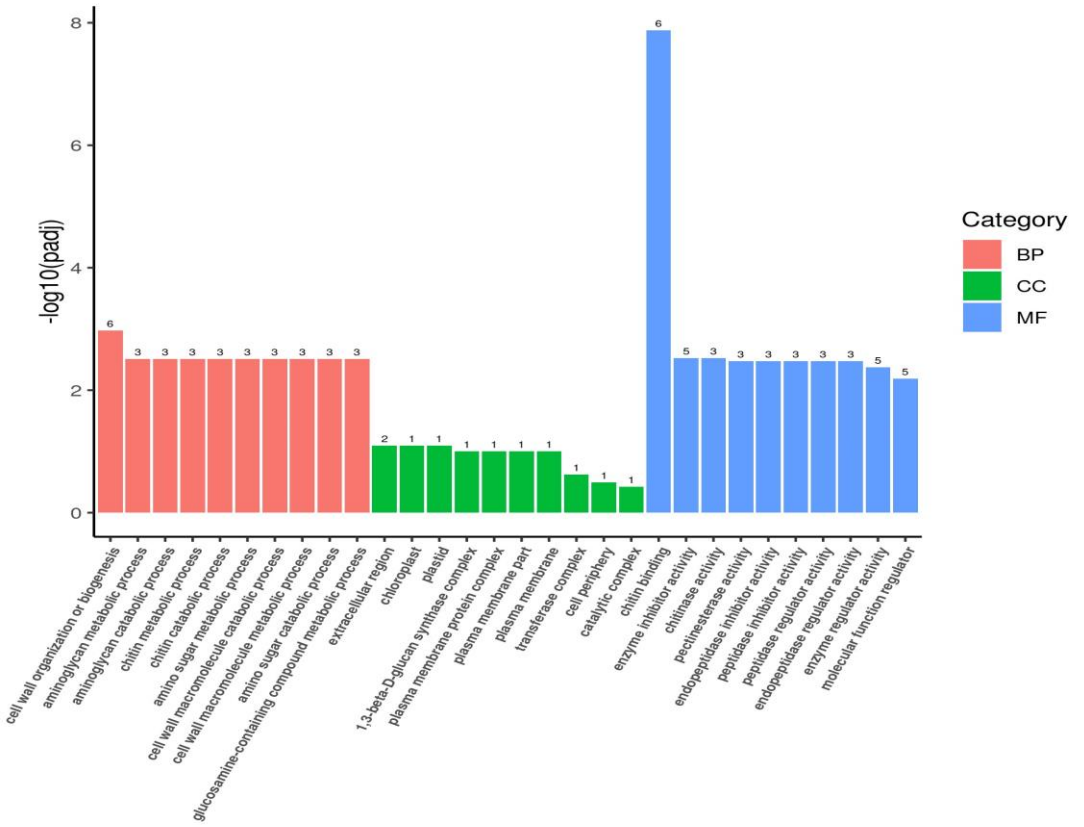


Appendix 7. Gene ontologies enriched with genes differentially expressed between 12 and 24 HAI dormant seeds. Graph descriptions are as shown in Appendix 5.

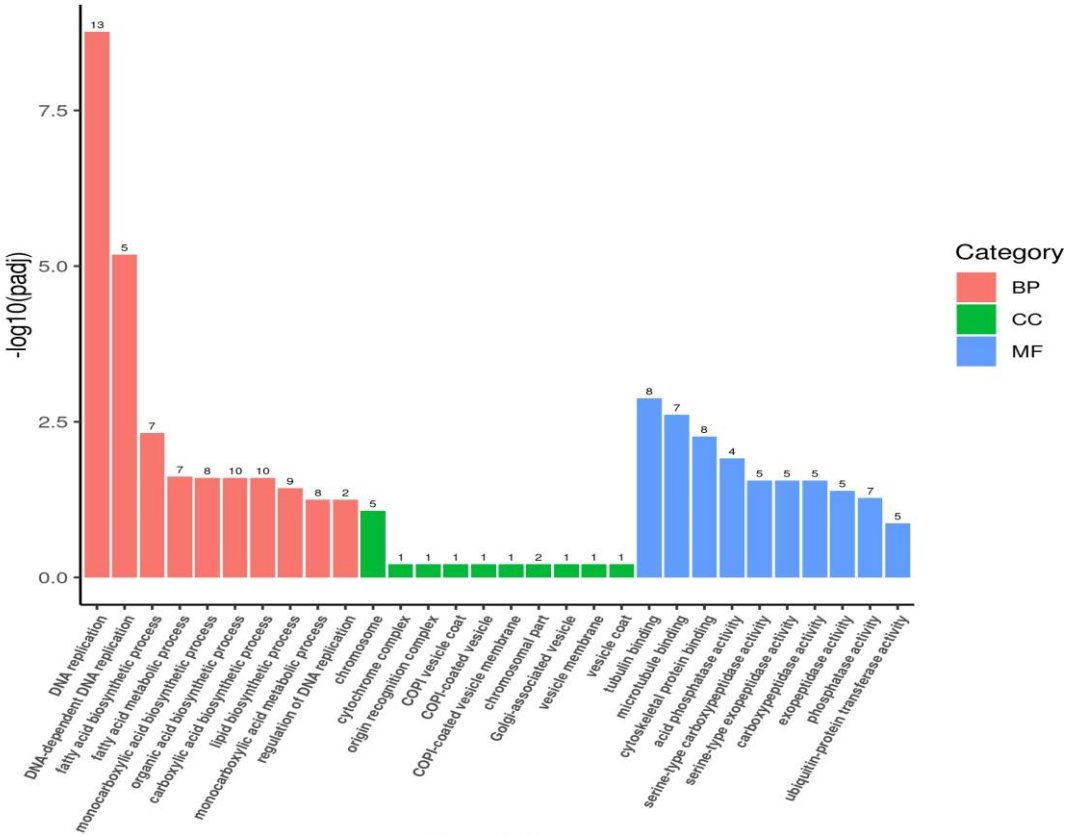


Appendix 8. Gene ontologies enriched with genes downregulated (a) and upregulated (b) in 12 HAI as compared to 24 HAI dormant seeds. Graph descriptions are as shown in Appendix 5.

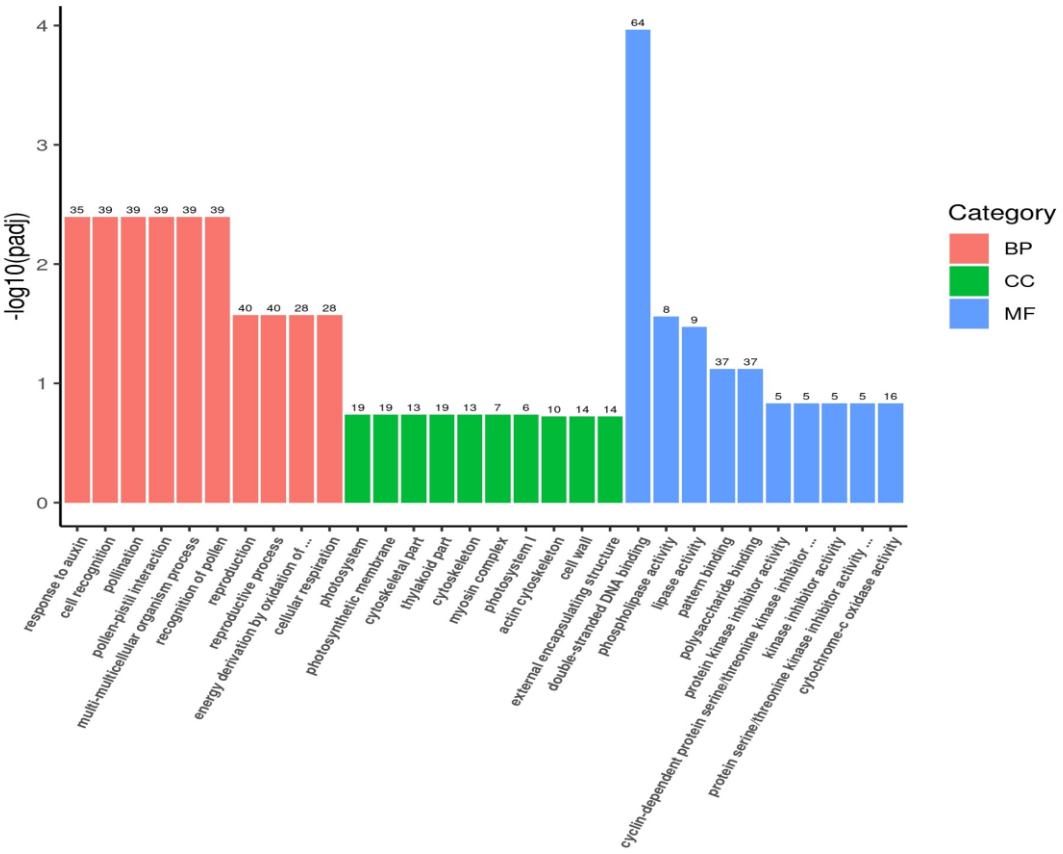
(a)



(b)

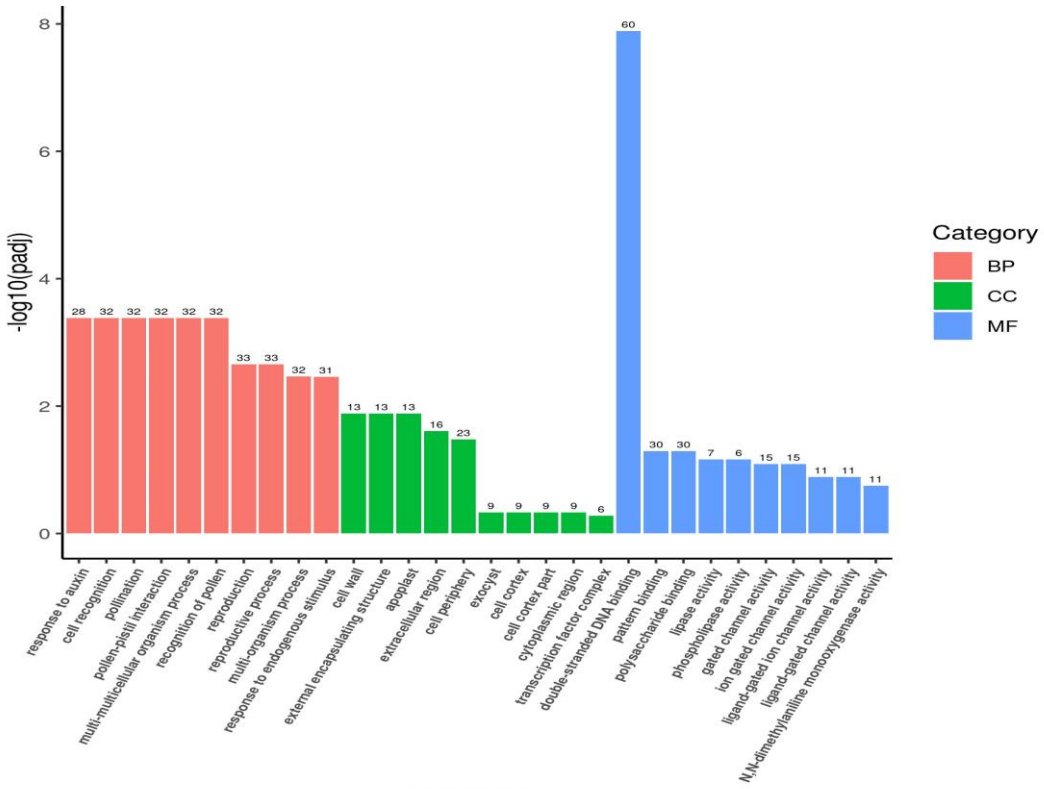


Appendix 9. Gene ontologies enriched with genes differentially expressed between 0 and 12 HAI after-ripened seeds. Graph descriptions are as shown in Appendix 5.

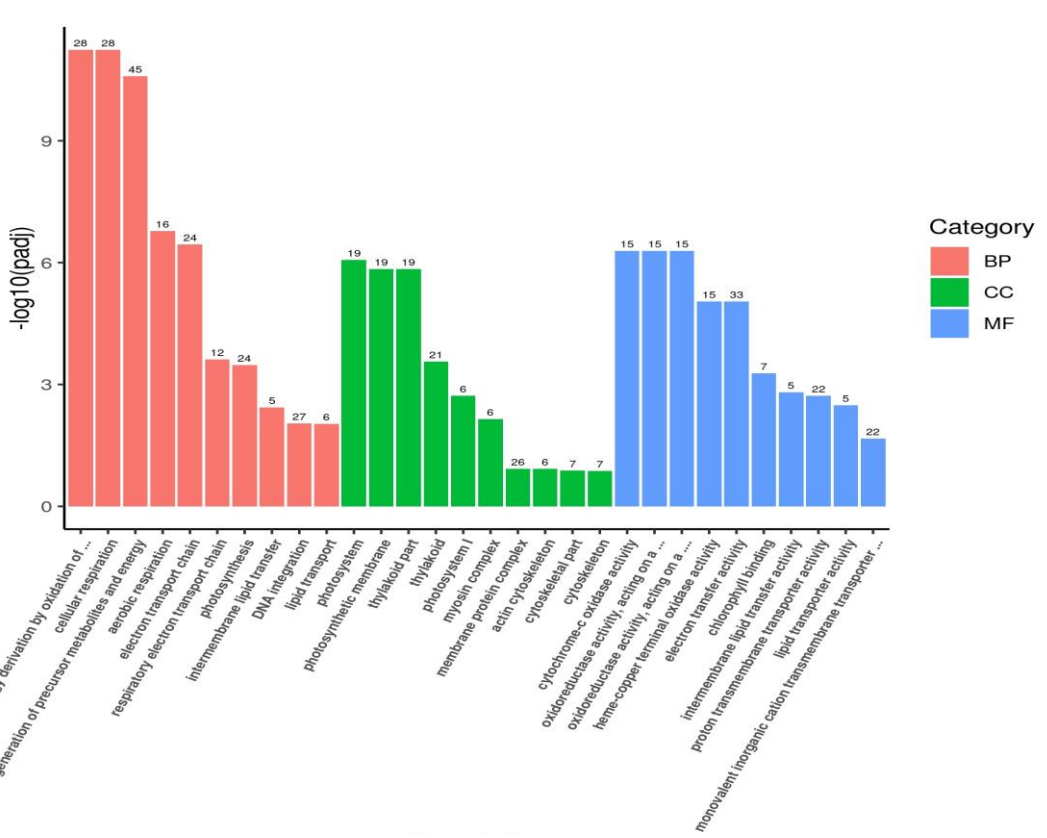


Appendix 10. Gene ontologies enriched with genes downregulated (a) and upregulated (b) in 0 HAI as compared to 12 HAI after-ripened seeds. Graph descriptions are as shown in Appendix 5.

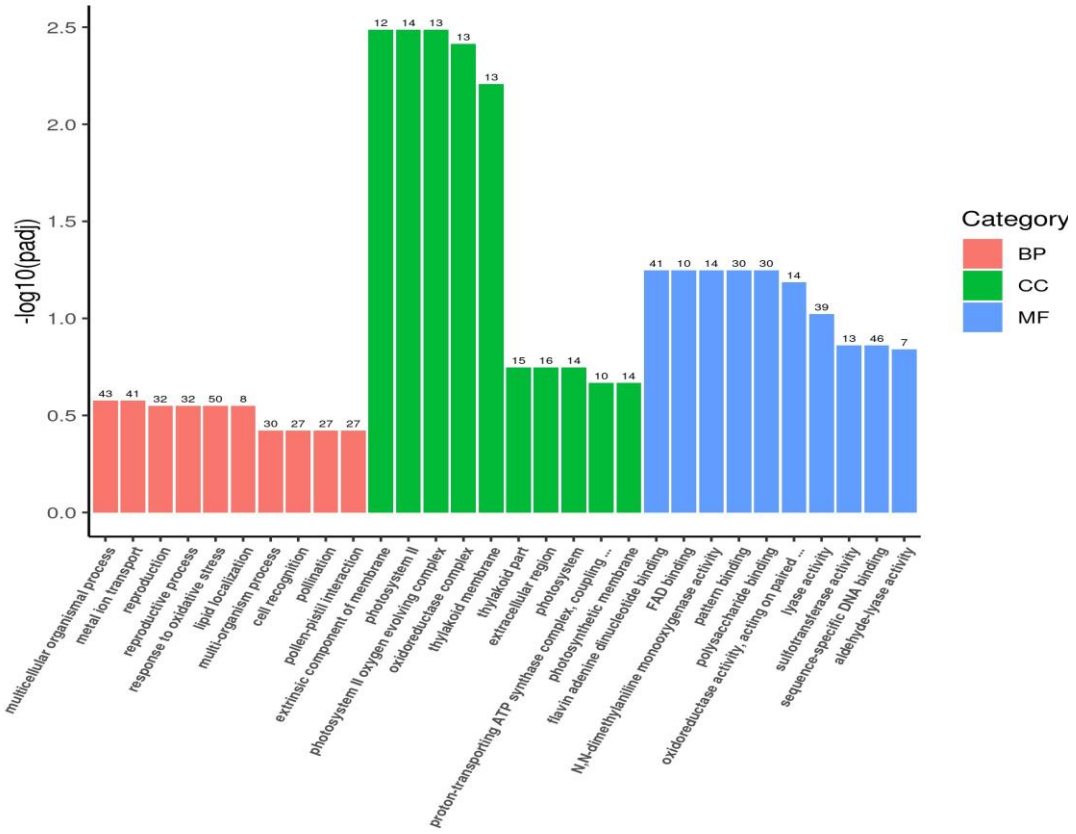
(a)



(b)

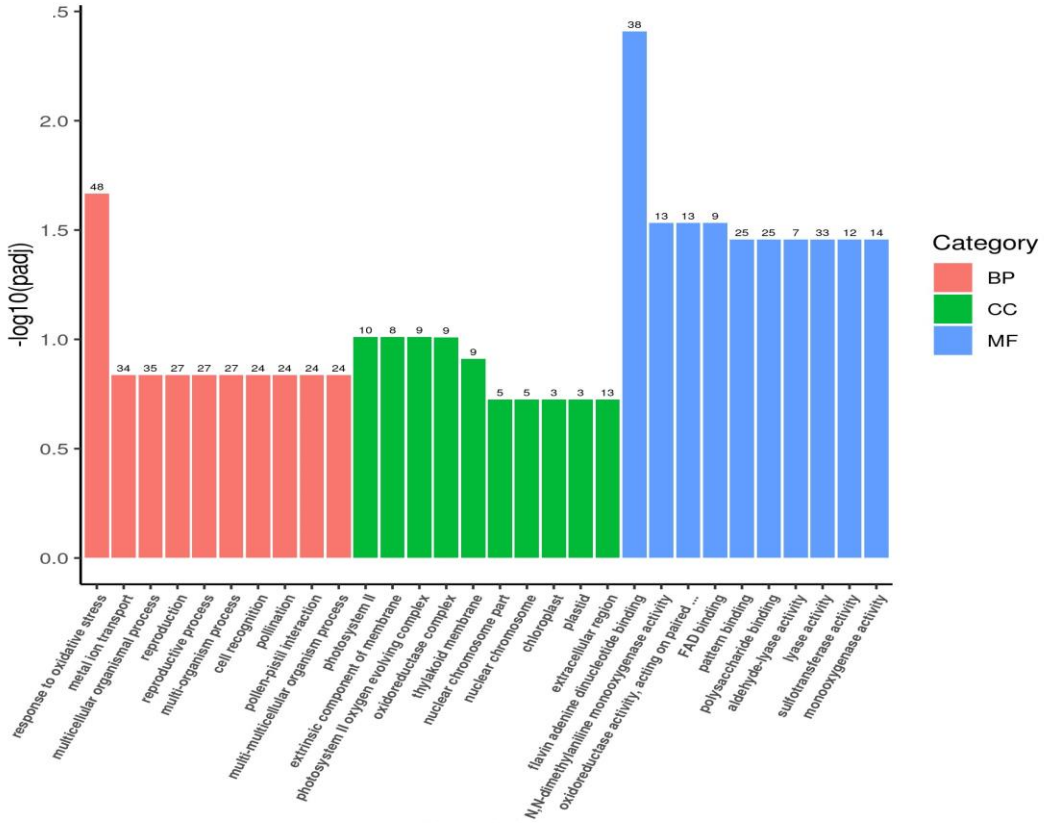


Appendix 11. Gene ontologies enriched with genes differentially expressed between 12 and 24 HAI after-ripened seeds. Graph descriptions are as shown in Appendix 5.

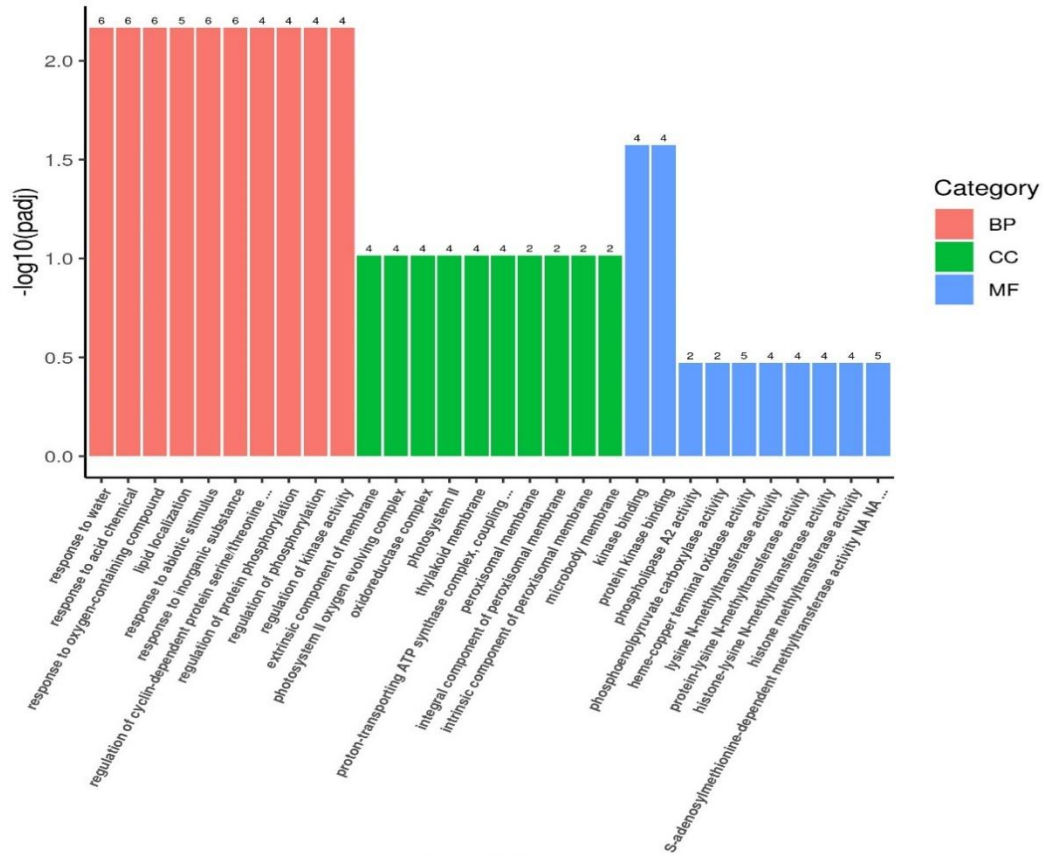


Appendix 12. Gene ontologies enriched with genes downregulated (a) and upregulated (b) in 12 HAI as compared to 24 HAI after-ripened seeds. Graph descriptions are as shown in Appendix 5.

(a)



(b)



Appendix 13. DEGs involved in ABA metabolism

		D_0 vs AR_0			D_12 vs AR_12			D_24 vs AR_24			D_12 v D_0			D_24 vs D_12			AR_12 vs AR_0			AR_24 vs AR_12		
IWGSC gene ID	Gene	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value
TraesCS2A03G0784800	*ZEP_2A*	-0.08	-1.06	0.59	-1.55	-2.93	0.00	0.05	1.04	0.81	-1.56	-2.95	0.00	0.01	1.01	0.97	-0.07	-1.05	0.61	-1.66	-3.15	0.00
TraesCS2B03G0871900	*ZEP_2B*	-0.38	-1.30	0.02	-2.30	-4.94	0.00	-1.61	-3.05	0.00	-0.06	-1.04	0.70	-0.62	-1.53	0.00	1.89	3.71	0.00	-1.38	-2.60	0.00
TraesCS2D03G0723400	*ZEP_2D*	-0.05	-1.04	0.74	-1.92	-3.78	0.00	-0.61	-1.53	0.00	-1.97	-3.91	0.00	0.16	1.12	0.43	-0.08	-1.06	0.58	-1.21	-2.32	0.00
TraesCS2A03G0699100	*VDE1_2A*	2.59	6.02	0.75	0.67	1.59	0.73	-3.02	-8.10	0.00	2.08	4.23	0.24	-1.09	-2.13	0.52	4.36	20.52	0.23	2.65	6.27	0.01
TraesCS2B03G0779000	*VDE1_2B*	4.67	25.39	0.26	2.65	6.28	0.08	-1.79	-3.46	0.01	0.73	1.66	0.65	-0.20	-1.15	0.91	3.22	9.32	0.52	4.20	18.44	0.00
TraesCS2D03G0648100	*VDE1_2D*	-0.63	-1.54	0.70	0.30	1.23	0.39	-0.79	-1.73	0.06	2.58	5.98	0.00	-0.25	-1.19	0.00	1.66	3.17	0.04	0.78	1.71	0.01
TraesCS6B03G0859400	*NCED1_6B*	-1.00	-2.01	0.78	2.03	4.08	0.03	-0.97	-1.96	0.07	2.29	4.90	0.04	0.38	1.30	0.61	-0.74	-1.66	0.75	3.33	10.06	0.00
TraesCS6D03G0608400	*NCED1_6D*	0.94	1.92	0.70	0.54	1.46	0.14	-1.33	-2.52	0.00	2.66	6.34	0.00	-0.65	-1.57	0.22	3.12	8.70	0.00	1.14	2.21	0.00
TraesCS5A03G0898000	*NCED3_5A*	0.72	1.65	0.11	2.78	6.87	0.00	-0.93	-1.90	0.03	-0.28	-1.21	0.42	-0.26	-1.20	0.53	-2.31	-4.95	0.00	3.39	10.48	0.00
TraesCS5B03G0943700	*NCED3_5B*	3.01	8.03	0.03	1.67	3.17	0.00	-0.62	-1.53	0.09	1.79	3.47	0.00	0.28	1.22	0.51	3.20	9.21	0.01	2.50	5.66	0.00
TraesCS5D03G0859900	*NCED3_5D*	0.68	1.60	0.23	3.92	15.11	0.00	-0.53	-1.44	0.36	-1.67	-3.19	0.00	0.09	1.07	0.87	-4.84	-28.55	0.00	4.47	22.09	0.00
TraesCS2D03G0580300	*NCED4_2D*	0.68	1.60	0.89	-2.75	-6.72	0.66	2.08	4.23	0.22	-4.07	-16.81	0.27	5.42	42.75	0.03	-0.79	-1.73	0.79	0.70	1.62	0.85
TraesCS5B03G0068800	*NCED5_5B*	0.96	1.94	0.04	0.98	1.97	0.00	2.36	5.12	0.00	3.17	9.00	0.00	-0.80	-1.74	0.00	3.17	9.01	0.00	-2.23	-4.71	0.00
TraesCS5D03G0099600	*NCED5_5D*	0.49	1.40	0.06	-0.26	-1.19	0.06	0.76	1.69	0.00	2.10	4.29	0.00	-0.72	-1.65	0.00	2.87	7.31	0.00	-1.80	-3.47	0.00
TraesCS7A03G1236900	*AAO_7A*	-0.01	-1.01	0.98	1.94	3.83	0.00	1.13	2.19	0.00	0.47	1.38	0.01	0.30	1.23	0.15	-1.46	-2.74	0.00	1.04	2.06	0.00
TraesCS7B03G1123600	*AAO_7B*	0.41	1.33	0.02	2.38	5.19	0.00	1.69	3.23	0.00	-0.26	-1.20	0.12	-0.07	-1.05	0.72	-2.20	-4.59	0.00	0.56	1.47	0.00
TraesCS7D03G1177500	*AAO_7D*	0.58	1.50	0.00	2.71	6.53	0.00	0.79	1.73	0.00	-0.18	-1.13	0.27	0.10	1.08	0.57	-2.28	-4.85	0.00	1.96	3.88	0.00
TraesCS6A03G0725000	*CYP707A1_6A*	0.12	1.09	0.45	-1.34	-2.53	0.00	-0.92	-1.89	0.00	-2.49	-5.63	0.00	-0.24	-1.18	0.26	-1.01	-2.02	0.00	-0.72	-1.65	0.00
TraesCS6B03G0858400	*CYP707A1_6B*	0.37	1.29	0.01	-0.38	-1.30	0.01	-0.65	-1.57	0.00	-1.91	-3.77	0.00	-0.35	-1.28	0.08	-1.14	-2.20	0.00	-0.14	-1.11	0.34
TraesCS5B03G0619900	*CYP707A3_5B*	1.75	3.37	0.13	0.09	1.06	0.94	-7.46	-175.5	0.00	-0.15	-1.11	0.90	-6.97	-125.1	0.00	1.55	2.93	0.13	0.57	1.48	0.41
TraesCS5D03G0566800	*CYP707A3_5D*	0.34	1.26	0.49	-0.12	-1.09	0.86	0.93	1.90	0.16	-1.94	-3.83	0.00	0.67	1.59	0.22	-1.46	-2.75	0.01	-0.44	-1.36	0.50

Appendix 14. DEGs involved in ABA signaling

		D_0 vs AR_0			D_12 vs AR_12			D_24 vs AR_24			D_12 vs D_0			D_24 vs D_12			AR_12 vs AR_0			AR_24 vs AR_12		
IWGSC gene ID	Gene	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value
TraesCS2A03G0179800	*PYL4_2A*	-0.27	-1.20	0.50	-1.05	-2.07	0.00	-1.96	-3.90	0.00	2.16	4.47	0.00	-1.36	-2.56	0.00	2.96	7.80	0.00	-0.51	-1.42	0.00
TraesCS4B03G0534100	*PYL4_4B*	-1.57	-2.97	0.58	-3.94	-15.33	0.00	-6.62	-98.10	0.00	2.07	4.20	0.14	-0.13	-1.10	1.00	4.39	20.93	0.00	2.33	5.04	0.00
TraesCS4D03G0474100	*PYL4_4D*	-4.18	-18.07	0.26	-3.00	-7.99	0.00	-4.74	-26.74	0.00	6.78	110.26	0.00	0.06	1.04	0.95	4.98	31.51	0.00	1.57	2.98	0.00
TraesCS7A03G0875800	*PYL9_7A*	5.71	52.51	0.03	0.35	1.28	0.05	-1.15	-2.22	0.00	4.55	23.41	0.00	-0.79	-1.73	0.00	10.31	1270.60	0.00	0.65	1.57	0.00
TraesCS1A03G0514200	*PYL10_1A*	0.21	1.16	0.29	-0.92	-1.90	0.00	-2.15	-4.44	0.00	-0.36	-1.28	0.05	-0.18	-1.14	0.45	0.80	1.74	0.00	0.99	1.98	0.00
TraesCS3A03G0611800	*PP2C8_3A*	0.25	1.19	0.67	0.80	1.74	0.02	1.85	3.60	0.00	0.85	1.80	0.03	0.63	1.55	0.04	0.33	1.26	0.49	-0.48	-1.40	0.22
TraesCS3B03G0691500	*PP2C8_3B*	1.03	2.05	0.02	1.34	2.53	0.00	5.31	39.61	0.00	0.60	1.52	0.05	0.39	1.31	0.22	0.33	1.25	0.53	-3.66	-12.63	0.00
TraesCS3D03G0560900	*PP2C8_3D*	0.27	1.21	0.55	1.35	2.55	0.00	2.45	5.48	0.00	1.48	2.78	0.00	-0.10	-1.07	0.68	0.42	1.34	0.31	-1.27	-2.41	0.00
TraesCS4A03G0193400	*PP2C30_4A*	-0.31	-1.24	0.61	3.70	12.99	0.00	0.59	1.50	0.17	1.30	2.47	0.00	-0.19	-1.14	0.65	-2.67	-6.36	0.00	2.87	7.29	0.00
TraesCS2A03G0000700	*PP2C37_2A*	0.13	1.09	0.57	1.22	2.33	0.00	2.95	7.74	0.00	-1.23	-2.35	0.00	0.36	1.29	0.15	-2.31	-4.95	0.00	-1.43	-2.69	0.00
TraesCS2B03G0000400	*PP2C37_2B*	0.22	1.17	0.32	0.78	1.72	0.00	2.94	7.68	0.00	-0.83	-1.78	0.00	0.00	-1.00	1.00	-1.36	-2.57	0.00	-2.22	-4.67	0.00
TraesCS1A03G0875600	*PP2C50_1A*	0.39	1.31	0.27	2.53	5.79	0.00	2.59	6.02	0.00	-0.03	-1.02	0.93	0.04	1.03	0.89	-2.16	-4.46	0.00	-0.07	-1.05	0.93
TraesCS3A03G0639700	*PP2C50_3A*	-0.08	-1.06	0.73	1.69	3.23	0.00	2.56	5.88	0.00	0.18	1.13	0.35	0.24	1.18	0.31	-1.57	-2.97	0.00	-0.69	-1.61	0.02
TraesCS3B03G0718700	*PP2C50_3B*	-0.14	-1.10	0.58	1.75	3.35	0.00	1.32	2.50	0.00	0.45	1.37	0.03	-0.11	-1.08	0.62	-1.41	-2.65	0.00	0.25	1.19	0.24
TraesCS3D03G0584600	*PP2C50_3D*	-0.05	-1.04	0.81	1.70	3.24	0.00	1.72	3.29	0.00	0.10	1.07	0.65	0.57	1.48	0.02	-1.63	-3.09	0.00	0.48	1.40	0.17
TraesCS1A03G1005000	*PP2C51_1A*	0.76	1.69	0.13	1.58	2.99	0.00	2.29	4.90	0.00	-0.90	-1.87	0.04	0.33	1.26	0.49	-1.70	-3.25	0.00	-0.47	-1.38	0.33
TraesCS1B03G1186600	*PP2C51_1B*	0.22	1.17	0.74	2.09	4.24	0.00	1.16	2.23	0.02	0.07	1.05	0.90	-0.02	-1.01	1.00	-1.75	-3.37	0.04	0.84	1.79	0.25
TraesCS1D03G0967400	*PP2C51_1D*	0.20	1.15	0.63	0.03	1.02	0.94	1.23	2.35	0.01	-0.01	-1.00	1.00	-0.10	-1.07	0.80	0.19	1.14	0.67	-1.36	-2.57	0.00
TraesCS5A03G0486100	*PP2C68_5A*	0.48	1.40	0.22	4.16	17.88	0.00	2.35	5.10	0.00	-0.41	-1.33	0.24	-0.18	-1.14	0.71	-4.04	-16.48	0.00	1.56	2.94	0.06
TraesCS5D03G0449300	*PP2C68_5D*	1.24	2.36	0.01	2.29	4.90	0.00	1.86	3.63	0.00	0.67	1.60	0.02	0.09	1.06	0.79	-0.36	-1.28	0.59	0.46	1.38	0.36
TraesCS1A03G0569000	*SnRK3_1A*	0.47	1.38	0.04	1.62	3.08	0.00	2.83	7.11	0.00	-0.08	-1.06	0.68	0.73	1.66	0.00	-1.21	-2.32	0.00	-0.53	-1.45	0.02
TraesCS1B03G0660500	*SnRK3_1B*	1.15	2.22	0.16	3.17	8.99	0.00	4.99	31.74	0.00	-0.26	-1.20	0.23	0.70	1.62	0.01	-2.25	-4.77	0.14	-1.18	-2.26	0.53
TraesCS4A03G0621500	*SnRK10_4A*	0.00	-1.00	0.98	0.77	1.71	0.00	2.82	7.07	0.00	-1.44	-2.71	0.00	0.35	1.27	0.07	-2.19	-4.57	0.00	-1.76	-3.39	0.00
TraesCS4D03G0149100	*SnRK10_4D*	-0.16	-1.12	0.26	0.50	1.41	0.00	2.19	4.57	0.00	-1.55	-2.93	0.00	0.16	1.11	0.42	-2.19	-4.57	0.00	-1.60	-3.02	0.00
TraesCS3A03G0880400	*ABI5_3A*	0.30	1.23	0.08	0.93	1.91	0.00	3.36	10.30	0.00	-0.17	-1.12	0.25	-0.11	-1.08	0.57	-0.78	-1.71	0.00	-2.61	-6.09	0.00
TraesCS3B03G1007500	*ABI5_3B*	0.44	1.35	0.01	0.53	1.44	0.00	3.01	8.05	0.00	0.60	1.51	0.00	-0.02	-1.01	0.92	0.53	1.44	0.00	-2.56	-5.90	0.00
TraesCS3D03G0808000	*ABI5_3D*	0.53	1.44	0.00	0.01	1.01	0.94	2.38	5.22	0.00	0.32	1.24	0.02	-0.56	-1.47	0.00	0.86	1.81	0.00	-2.99	-7.96	0.00
TraesCS2A03G0203300	*ABI5_2A*	1.06	2.08	0.24	-0.36	-1.28	0.59	-1.64	-3.13	0.02	-1.11	-2.15	0.09	0.36	1.28	0.72	0.33	1.25	0.73	1.63	3.10	0.00
TraesCS4A03G0272300	*ABI5_4A*	0.32	1.25	0.72	-0.31	-1.24	0.45	-1.89	-3.70	0.01	0.75	1.69	0.16	-0.96	-1.95	0.23	1.41	2.66	0.01	0.59	1.51	0.07

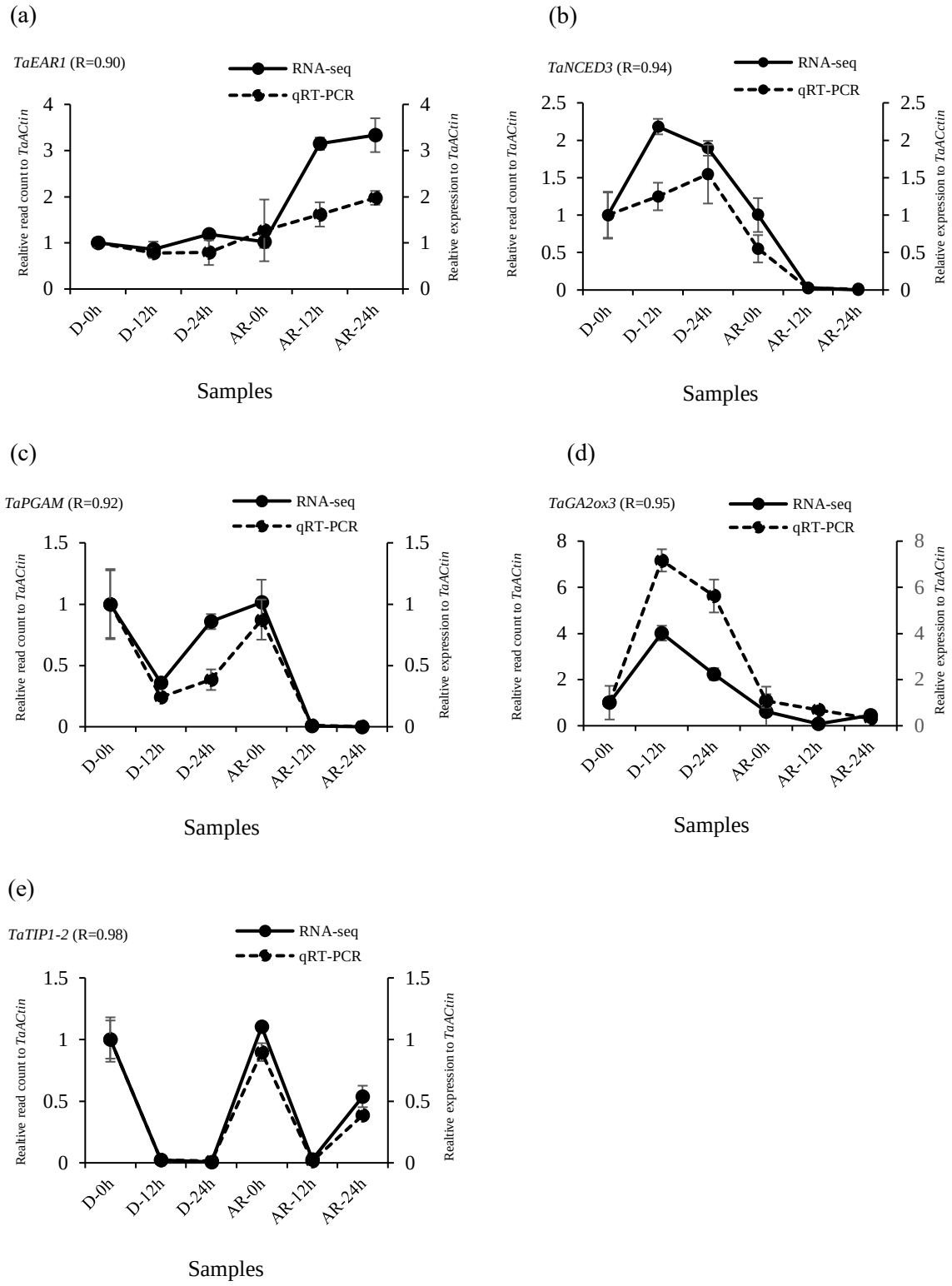
Appendix 15. DEGs involved in GA metabolism

		D_0 vs AR_0			D_12 vs AR_12			D_24 vs AR_24			D_12 vs D_0			D_24 vs D_12			AR_12 vs AR_0			AR_24 vs AR_12		
IWGSC gene ID	Gene	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value
TraesCS1A03G0294800	*CPS1_1A*	-0.52	-1.44	0.41	-1.20	-2.30	0.00	-0.55	-1.46	0.28	0.40	1.32	0.49	-0.10	-1.07	0.88	1.09	2.13	0.00	-0.82	-1.77	0.02
TraesCS7A03G1343500	*CPS1_7A*	0.02	1.02	0.98	1.02	2.02	0.06	0.95	1.94	0.16	-1.46	-2.75	0.00	0.13	1.09	0.82	-2.42	-5.37	0.00	0.13	1.10	0.90
TraesCS7B03G1281000	*CPS1_7B*	-0.18	-1.13	0.42	0.80	1.74	0.00	0.76	1.69	0.03	-1.03	-2.04	0.00	-0.35	-1.28	0.27	-1.98	-3.94	0.00	-0.38	-1.30	0.20
TraesCS7B03G0729700	*K02_7B*	0.11	1.08	0.58	-0.19	-1.14	0.24	1.06	2.09	0.00	-0.83	-1.77	0.00	0.07	1.05	0.76	-0.50	-1.41	0.01	-1.25	-2.38	0.00
TraesCS4A03G1158000	*KAO1_4A*	0.23	1.18	0.29	-0.13	-1.09	0.48	-1.44	-2.71	0.00	0.05	1.04	0.82	-0.03	-1.02	0.91	0.43	1.35	0.02	1.22	2.32	0.00
TraesCS5B03G0103000	*KAO1_5B*	-0.46	-1.38	0.40	-0.83	-1.78	0.03	-1.29	-2.44	0.01	-0.18	-1.14	0.72	-0.36	-1.29	0.52	0.20	1.15	0.66	0.05	1.03	0.91
TraesCS5D03G0113400	*KAO1_5D*	0.94	1.92	0.35	-0.94	-1.91	0.51	-2.12	-4.34	0.00	-2.31	-4.96	0.00	1.31	2.48	0.18	-0.40	-1.32	0.84	2.48	5.59	0.01
TraesCS4A03G0796400	*GA20ox1-A_4A*	0.26	1.20	0.93	-2.86	-7.27	0.00	-3.29	-9.76	0.00	1.09	2.12	0.24	0.10	1.07	0.96	4.20	18.39	0.00	0.50	1.41	0.03
TraesCS5B03G1356500	*GA20ox1-A_5B*	-2.40	-5.26	0.06	-3.39	-10.47	0.00	-2.22	-4.66	0.00	4.18	18.10	0.00	0.29	1.23	0.50	5.15	35.59	0.00	-0.92	-1.89	0.00
TraesCS5D03G1210400	*GA20ox1-D_5D*	-0.22	-1.16	0.94	-3.12	-8.72	0.00	-2.36	-5.13	0.00	3.06	8.31	0.00	0.16	1.12	0.70	6.01	64.28	0.00	-0.65	-1.57	0.00
TraesCS3A03G0950800	*GA20ox2_3A*	3.50	11.32	0.54	-1.94	-3.83	0.00	0.51	1.42	0.46	2.33	5.04	0.06	1.25	2.38	0.11	8.14	281.45	0.00	-1.28	-2.43	0.01
TraesCS3B03G1087000	*GA20ox2_3B*	0.77	1.71	0.72	-2.50	-5.65	0.00	-0.22	-1.16	0.59	1.91	3.76	0.01	1.11	2.16	0.01	5.21	37.02	0.00	-1.23	-2.35	0.00
TraesCS3A03G0268400	*GA3ox2-1_3A*	3.75	13.49	0.31	-4.32	-19.95	0.00	-6.40	-84.30	0.00	0.21	1.16	1.00	0.19	1.14	1.00	8.68	410.96	0.00	1.87	3.65	0.00
TraesCS3B03G0336700	*GA3ox2-3_3B*	0.68	1.60	0.89	-5.40	-42.29	0.00	-5.04	-33.01	0.00	0.30	1.23	1.00	2.12	4.34	0.11	6.43	86.02	0.00	1.60	3.03	0.00
TraesCS3A03G0732000	*GA2ox3_3A*	1.43	2.70	0.46	-1.97	-3.92	0.00	-1.32	-2.50	0.00	3.70	12.99	0.00	-0.34	-1.27	0.28	7.07	134.34	0.00	-1.04	-2.06	0.00
TraesCS3B03G0838600	*GA2ox3_3B*	1.01	2.01	0.39	-2.06	-4.17	0.00	-0.91	-1.87	0.00	3.41	10.62	0.00	0.00	1.00	0.98	6.51	91.12	0.00	-1.22	-2.34	0.00
TraesCS3D03G0675100	*GA2ox3_3D*	-0.33	-1.26	1.00	-1.39	-2.63	0.00	0.03	1.02	0.97	5.63	49.48	0.00	-0.08	-1.06	0.82	6.68	102.56	0.00	-1.55	-2.94	0.00
TraesCS2A03G0918900	*GA2ox6_2A*	0.86	1.82	0.39	-2.52	-5.73	0.00	-3.08	-8.43	0.00	1.95	3.86	0.00	0.03	1.02	0.94	5.35	40.75	0.00	0.49	1.41	0.03
TraesCS2B03G1008100	*GA2ox6_2B*	0.19	1.14	0.71	-0.72	-1.65	0.00	-0.81	-1.75	0.00	2.73	6.65	0.00	-0.46	-1.37	0.09	3.67	12.72	0.00	-0.43	-1.35	0.01
TraesCS2D03G0858900	*GA2ox6_2D*	1.41	2.65	0.03	-0.70	-1.62	0.00	-0.65	-1.57	0.03	2.60	6.05	0.00	-0.85	-1.80	0.00	4.73	26.61	0.00	-0.96	-1.94	0.00

Appendix 16. DEGs involved in GA signaling

		D_0 vs AR_0			D_12 vs AR_12			D_24 vs AR_24			D_12 vs D_0			D_24 vs D_12			AR_12 vs AR_0			AR_24 vs AR_12		
IWGSC gene ID	Gene	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value	Log2 FC	Linear FC	P-value
TraesCS1B03G0743300	*GID1_1B*	0.14	1.10	0.51	0.08	1.06	0.60	1.09	2.13	0.00	0.20	1.15	0.28	0.64	1.55	0.00	0.29	1.22	0.13	-0.45	-1.36	0.00
TraesCS1D03G0613500	*GID1_1D*	0.19	1.14	0.27	-0.02	-1.01	0.87	1.02	2.03	0.00	1.44	2.71	0.00	0.57	1.48	0.00	1.68	3.20	0.00	-0.54	-1.45	0.00
TraesCS2D03G0307200	*GID1C_2D*	-2.75	-6.74	0.74	0.95	1.94	0.22	0.16	1.12	0.77	6.96	124.20	0.00	0.21	1.15	0.74	2.65	6.27	0.13	0.92	1.90	0.24
TraesCS1A03G0764700	*GAMYB1_1A*	-0.15	-1.11	0.43	0.35	1.28	0.02	0.75	1.68	0.00	-0.75	-1.69	0.00	-0.03	-1.02	0.88	-1.23	-2.35	0.00	-0.48	-1.40	0.00
TraesCS1B03G0874000	*GAMYB1_1B*	0.30	1.23	0.62	2.79	6.92	0.00	2.18	4.53	0.00	0.67	1.60	0.14	0.31	1.24	0.52	-1.79	-3.45	0.01	0.86	1.81	0.16
TraesCS1D03G0731600	*GAMYB1_1D*	-0.14	-1.10	0.44	1.17	2.26	0.00	1.21	2.31	0.00	-0.79	-1.73	0.00	0.03	1.02	0.93	-2.08	-4.23	0.00	-0.07	-1.05	0.79
TraesCS4D03G0067000LC	*RHT1_4D*	-1.55	-2.92	0.71	-0.48	-1.40	0.36	-1.21	-2.32	0.47	3.08	8.46	0.00	-1.32	-2.49	0.42	1.96	3.89	0.07	-0.68	-1.60	0.24

Appendix 17. Validation of the RNA-seq data with qPCR. Comparison of relative expression values of selected differentially expressed genes between the RNA-seq and qPCR data. *TaEAR1* (a) *TaNCED3* (b) *TaPGAM* (c) *TaGA2ox3* (d) *TaTIP1-2* (e).



Abbreviations

AAO3	abscisic aldehyde oxidase 3
ABA	abscisic acid
ABA2	ABA deficient 2
ABF	ABA-responsive element binding factor
ABF	ABRE binding factor
ABI	abscisic acid insensitive
AFLP	amplified fragment length polymorphism
AR	after-ripened
AREB	ABA-responsive element binding protein
ASG5	altered seed germination 5
BP	before present
BR	brassinosteroids
BWA	burrows-Wheeler Aligner
BWT	burrows-Wheeler transform
cDNA	complementary DNA
CK	cytokinins
CPS	copalyl diphosphate synthase
CWRS	Canada Western Red Spring
DAP	days after pollination
DEGs	differentially expressed genes
DNA	deoxyribonucleic acid
DOG1	Delay of Germination 1
DPA	days post anthesis

ERA8	enhanced response to ABA8
ESR	embryo-surrounding region
FM	ferragina-Manzini
FPKM	fragments per kilobase per million mapped fragments
GA	gibberellin
GA13ox	*GA13-oxidase*
GA20ox	GA 20-oxidase
GA2ox	GA 2-oxidase
GA3ox	GA 3-oxidase
GAI	*GA-INSENSITIVE*
GAMYB	GA regulated MYB transcriptional regulator
GARE	GA-responsive elements
GGDP	geranyl-geranyl diphosphate
CDP	copalyl diphosphate
GGPP	geranylgeranyl pyrophosphate
GID1	gibberellin insensitive dwarf 1
GID2	gibberellin insensitive dwarf 2
GO	gene Ontology
GSH	glutathione
GTF	gene transfer format
HAI	hours after imbibition
HDA6	*histone deacetylase 6*
IDP	isopentenyl diphosphate
IWGSC	International Wheat Genome Sequencing Consortium
JA	jasmonates

KAO	kaurenoic acid oxidase
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	kaurene oxidase
KS	kaurene synthase
LMA	late-maturity α-amylase
MD	morphological dormancy
MPD	morphophysiological dormancy
mRNA	messenger ribonucleic acid
NB	negative binomial
NCED	9-*cis*-epoxycarotenoid dioxygenase
NGS	next generation sequencing
NSY	neoxanthin synthase
ODDs	oxoglutarate-dependent dioxygenases
PA	phaseic acid
PD	physiological dormancy
PD-D	deep physiological dormancy
PD-I	intermediate physiological dormancy
PD-N	non-deep physiological dormancy
PHS	preharvest sprouting
phy	phytochrome
PIF1	phytochrome-interacting factor 1
PIF6	phytochrome-interacting factor 6
PP2Cs	protein phosphatase 2Cs
PY	physical dormancy
PYL	pyrabactin-like

PYR	pyrabactin resistance
qPCR	quantitative polymerase chain reaction
RCAR	regulatory components of ABA receptors
RHT	reduced height
RNA	ribonucleic acid
RNA-seq	RNA sequencing
RPKM	reads per kilobase of transcript per million mapped
rRNA	ribosomal RNA
RT	reverse transcription
SA	salicylic acid
SCF	S-phase-kinase-associated protein 1, Cullin-1/Cdc53, F-Box protein
SIL1	*modifiers of silencing 1*
SnRK2s	SNF1-related protein kinase2s
TPM	transcripts per kilobase million
TPSs	terpene synthases
VDE	violaxanthin de-epoxidase
ZEP	zeaxanthin epoxidase