REGULATION OF WHEAT SEED GERMINATION AND SEEDLING GROWTH BY BRASSINOSTEROID

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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

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor Dr. Belay T. Ayele, all due to whom I got this opportunity which serves as a milestone of my dream to do research in this field. I am thankful for his way of guidance with so much simplification and clarity that helped me in completing my project. I respect his dedication, hard work and unceasing efforts towards research. Furthermore, I would like to thank him, University of Manitoba, Manitoba grain innovation hub and NSERC for financial support that helped me in the smooth functioning of my research without worrying about study expenses. Besides my advisor, I would also like to extend my gratitude to Dr. Dana Schroeder and Dr. Mark Jordan for providing their valuable feedback while serving as members of my advisory committee. I would also like to pay heartfelt gratitude to the postdoctoral fellow Dr. Anh Tuan Pham for his immense guidance throughout the research process. I can remember many a times when I had a discussion with him on research work and thesis preparation.

I also thank and appreciate Dr. Belay Ayele's lab members who have helped me out with their abilities in many aspects due to which I became capable of completing my thesis. I thank lab technician Dr. Aihua Liu, postdoctoral fellows Dr. Murali, postgraduate students Tran-Nguyen, Ujjal and especially former and current graduate students for their immense care, encouragement and support namely Parneet Kaur, Pawanpuneet Kaur, Menghan, Ankita and Gurkamal.

I am deeply grateful to my grandmother Sr. Pritam Kaur, my parents Sh. Kewal Singh and Sr. Labhdeep Kaur, my brother Jatinder, Harwinder, Sehajdeep, Arsh, Shubhdeep (sister) and friend Sukhman for always supporting me. Especially, my sister Handeep Kaur without whom this degree would have not been possible for me. Most of all, I would like to thank our Almighty God for giving His endless blessings among which I will always be grateful for offering this great learning experience.

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ABBREVIATIONS

AAO3	aldehyde oxidase
ABA	abscisic acid
ABA8'OH	ABA 8'-hydroxylase
ABI1	ABA insensitive1
ABI5	ABA insensitive5
BAK1	BRI1 associated kinase1
BRI1	BR insensitive 1
BKI1	BRI1 kinase inhibitor 1
BES1	BRI1-EMS-suppressor 1
BIN2	brassinosteroid-insensitive2
BL	brassinolide
BRs	brassinosteroids
BR6ox	BR-6-oxidase
BSK1	BR signaling kinase 1
BSU1	BRI1 suppressor 1
BZR1	Brz resistant 1
CD	combinational dormancy
CDG1	constitutive differential growth1
CN	campestanol
CPD	constitutive photomorphogenesis and dwarfism
CPS	*ent*-copalyl diphosphate synthase
CR	campesterol
CS	castasterone
CT	cathasterone
CYP450s	cytochrome P450
CYP707A	cytochrome P450 monooxygenase
DAI	days after imbibition
DET2	deetiolated2
DPA	dihydro phaseic acid
DWF4	dwarf4
GA	gibberellin
GGDP	geranyl diphosphate
GGPP	geranylgeranyl pyrophosphate
GA20ox	GA 20-oxidase
GA2ox	GA 2-oxidase
GA3ox	GA 3-oxidase
KAO	*ent*-kaurenoic acid oxidase
KO	*ent*-kaurene oxidase
KS	*ent*-kaurene synthase

MD	morphological dormancy
MPD	morphophysiological dormancy
NCED	nine-cis-epoxycarotenoid dioxygenase
PA	phaseic acid
Pcz	propiconazole
PD	physiological dormancy
PGRs	plant growth regulators
PHS	preharvest sprouting
PP2A	protein phosphatase 2A
PRE	paclobutrazol resistance
PY	physical dormancy
QTLs	quantitative trait loci
TE	teasterone
TY	typhasterol
VDE	violaxanthin de-epoxidase
ZEP	zeaxanthin epoxidase

ABSTRACT

Brassinosteroids (BRs) are plant hormones known to play a role in regulation of seed germination and seedling growth, and these roles of BR involve its interaction with other phytohormones such as abscisic acid (ABA) and gibberellin (GA). This study analyzed the effects of inhibition of BR biosynthesis on seed germination and seedling growth, and on expression of genes involved in the metabolism of two other phytohormones: ABA, a germination inhibitor and GA, germination enhancers. Treatment with BR biosynthesis inhibitor has no effect on seed germination, but it reduced seedling growth. Co-application of the BR biosynthesis inhibitor and exogenous BR rescued the coleoptile growth but caused reduction of root growth in later phases of imbibition as compared to the control or treatment with BR biosynthesis inhibitor alone. Treatment with BR biosynthesis inhibitor affected the expression of ABA metabolic *NCED2*, *CYP707A1*, *CYP707A2* and GA biosynthetic *GA20ox1* and *GA3ox2* genes in different tissues such as endosperm, embryo, coleoptile, and root. These results suggest that BR plays a role in regulating the expression of ABA and GA metabolic genes during and after seed germination.

1.0 INTRODUCTION

Common wheat (*Triticum aestivum* L.), which belongs to the *Poaceae* family, is a major cereal crop (Gilissen *et al.,* 2008). Canada produces over 25 million tonnes and exports approximately 15 million tonnes of wheat annually (Campbell, 2015). Wheat is used as the major dietary intake for humans and feed for animals, and for other industrial applications (Liu *et al.*, 2013). This extensive use necessitates the high yield and quality of wheat seeds. Yield and quality of wheat is partially determined by seed germination and vigor, and growth of the seedlings (Finch-Savage and Bassel, 2015; Wolny, 2018).

Seeds of angiosperm plants act as important units for plant propagation (Gehring *et al.,* 2004). Seed germination initiates when the seed uptakes water followed by growth of the radicle (Bewley, 1997). Seed germination involves three phases of water uptake, the first phase (imbibition), the second phase (synthesis of mitochondria and proteins from newly produced mRNAs and radicle emergence), and the third phase (storage reserve mobilization and seedling development) (Bewley, 1997; Nonogaki *et al*., 2007). However, seeds also possess an adaptive trait called dormancy that inhibits their germination under favourable environmental conditions (Gao and Ayele, 2014). Seedling establishment directly or indirectly depends on the seed germination and environmental conditions (Ayalew *et al.,* 2018). Improved seedling establishment leads to enhanced seedling vigour that in turn improves the seed yield (Elliott *et al*., 2008). Thus, seed germination, vigor and seedling growth affect crop productivity tremendously (Ellis, 1992).

Previous studies have shown that plant hormones play important roles in the regulation of seed germination (Kucera *et al.,* 2005; Liu *et al*., 2013), and treatments, including application of exogenous hormones and hormone antagonists (Kauschmann *et al.,* 1996), that advance or inhibit seed germination induce changes in seed hormone content and sensitivity (Finch-Savage *et al*.,

2006). Among these plant hormones, ABA and GA are the major regulators of seed germination and plant growth (Kucera *et al*., 2005). It is well documented that gibberellin (GA) promotes seed germination, while abscisic acid (ABA) induces seed dormancy (Kermode, 2005; Tuan *et al*., 2018). In addition to ABA and GA, other phytohormones such as brassinosteroids (BRs) regulate many plant growth and developmental processes including seed germination and seedling growth in different species such as *Arabidopsis*, maize, rice and pea seeds (Anuradha and Rao, 2001; Özdemir *et al*., 2004; Kagale *et al*., 2007; Arora *et al*., 2008; Villedieu-Percheron *et al*., 2014). For instance, in *Arabidopsis*, application of BR biosynthesis inhibitor reduces seedling growth (Asami *et al.,* 2001; Hartwig *et al.,* 2012). On the other hand, exogenous application of a BR results in concentration dependent increase of seedling growth (Müssig *et al.,* 2003). Seed germination in wheat was found to be associated with changes in the expression levels of genes involved in BR biosynthesis (*DET2* and *DWF4*) and signaling (*BSK* and *BIN2*) (Chitnis *et al.,* 2014). However, BR biosynthesis inhibitor was found to have no effect on seed germination (Asami *et al.,* 2001).

Earlier studies have reported crosstalk between BR, ABA and GA in the regulation of seed germination and other seed development processes (Peres *et al.,* 2019). Previous studies have shown that seed treatment with BR decreases the expression levels of ABA biosynthetic genes *NCEDs* and *AAO3* in *Arabidopsis* (Zhang *et al*., 2010; Ha *et al*., 2018). BR also inhibits ABA signaling during germination and this results in decay of seed dormancy and enhancement of seed germination (Zhang *et al*., 2009; Hu and Yu, 2014). Moreover, BR biosynthetic mutants of *Arabidopsis* exhibit more inhibition of germination by ABA, as the BR signal is required to resist ABA mediated inhibition of seed germination (Steber and McCourt, 2001). Reports on BIN2 in *Arabidopsis*, which acts a negative regulator of BR signaling, showed its synergistic interaction

with ABA and inhibit seed germination. In contrast, a mutation in the *BIN2* (*bin2-3*) antagonizes ABA response and therefore enhances seed germination (Hu and Yu, 2014).

Since both BR and GA are growth promoting plant hormones, they often show synergistic interaction and promote seed germination (Depuydt and Hardtke, 2011; Bai *et al*., 2012; Li *et al*., 2012; Li and He, 2013). Gibberellin can promote BR biosynthesis by upregulating the BR biosynthetic enzyme, DWF1 (Wang *et al*., 2009). Moreover, exogenous GA treatment rescues the hypocotyl growth of BR deficient and insensitive mutants of *Arabidopsis* (Hofmann *et al*., 2015; Unterholzner *et al*., 2015). On the other hand, BR treatment causes an increase in the expression of GA biosynthetic gene *GA20ox* while BR biosynthesis inhibitor decreases the expression of *GA20ox* and *GA3ox* (Stewart Lilley *et al*., 2013; Unterholzner *et al*., 2015). Likewise, exogenous BR treatment was found to recover the germination and hypocotyl growth of GA biosynthetic and insensitive mutants (Steber and McCourt, 2001). Although these and other studies reported the interaction between BR, ABA and GA in regulating seed germination and seedling growth, how the interaction between these hormones takes place at the molecular level has not been studied sufficiently, especially in cereals species such as wheat. The objectives of this thesis project therefore are: 1) to investigate the effect of BR biosynthesis inhibitor on germination and seedling growth in wheat, and 2) to examine the effect of BR biosynthesis inhibitor on the expression of wheat ABA and GA metabolic genes during germination and seedling growth.

2.0 LITERATURE REVIEW

2.1 Wheat

2.1.1 Wheat origin and history of its domestication

Common wheat (*Triticum aestivum* L.) is a major cereal crop produced for edible components of its grain. It belongs to the genus *Triticum* in the *Poaceae* family. Hexaploid wheat is the result of a hybridized cross between a tetraploid wheat, which arose from hybridization between *T. urartu* (the A genome donor) and *T. speltoides* (the B genome donor), and a diploid species, *T. tauschii*, that donated the D genome (Gilissen *et al.,* 2008; Bennett and Leitch, 2010).

To date, wheat has around 25,000 unique cultivars. Domestication of wheat dates to 12,000 years ago from a yet alive precursor plant known as Emmer (Hirst, 2012). Emmer wheat originated from the area of the Karacadag mountains, which are in what is presently known as southeastern Turkey. Furthermore, knowledge about the utilization of emmer was found 23,000 years back by the people who lived at the archeological site, Ohalo II in Israel (Hirst, 2012). Wild type emmer is a self-pollinating grass and a winter annual in nature. It is known as *T. dicocoides* and *T. araraticum*. It is distributed throughout the Fertile Crescent, which includes countries such as northern Iraq, western Iran, Lebanon, Jordan, Israel, eastern Turkey, and Syria. It grows mostly in areas with long, dry hot summers followed by short mild, wet winter conditions with fluctuating rainfall (Lev-Yadun *et al.,* 2000; Hirst, 2012; Cooper, 2015). Moreover, emmer wheat is tetraploid derived from *T. urartu* (the A genome donor) and *T. speltoides* (the B genome donor) (Nevo, 2011). Apart from emmer wheat, einkorn (*T. monococcum*) is another type of a diploid early wheat that was domesticated from its wild precursor *T. boeoticum*, approximately 10,400 years ago and both wild and cultivated precursors of *T. monococcum* are derived from the A genome donor, *T. urartu* (Haldorsen *et al.,* 2011; Nevo, 2011; Brandolini *et al.,* 2016).

The majority of the 25,000 unique types of current wheat are varieties of two general categories, known as common wheat or bread wheat and durum wheat (*T. turgidum* ssp. *durum*). Timopheev's (*T. timopheevii*) and spelt (*T. spelta*) wheat were also domesticated from wild emmer wheat, but these are not commonly available in the market like common and durum wheat, (Hirst, 2012). Overall, common wheat represents approximately 90%, while durum wheat represents 4 to 5% of all wheat consumed in the world (Peng *et al.*, 2011).

Wheat was found in North America from central Alberta (Canada) to central Texas (U.S.) in the late 15th and 16th century. In Canada, wheat was initially grown in around 1605 while the first exports were made in 1654. Although wheat cultivation in Western Canada during the early years were difficult, workers at the Hudson's Bay Company and the pioneers of the Red River Colony conducted experiments on wheat cultivation and achieved some successes in 1815. After many trials and failures using spring and winter wheat, in 1870 cultivar Red Fife became very famous due to its high yield and milling qualities. However, during extreme low temperature conditions, this cultivar also failed to cope with frost conditions. Later, in 1903 Sir Charles Saunders developed the cultivar Marquis from the cross of Hard Red Calcutta and Red Fife, which was observed to possess excellent yield and milling qualities in 1904. In 1909, this cultivar became widespread all over Canada, which lead to an increase in wheat production from 2 million tonnes in 1904 to 7.7 million in 1913. Subsequently, hard red spring wheat became popular in Canada (Charmet, 2011; Campbell, 2015).

2.1.2 Wheat production and its use

Wheat ranks third in global cereal crop production behind maize (*Zea mays*) and rice (*Oryza sativa*) and Canada is the sixth-largest wheat producer. Statistics Canada reported that up to 24.7 million acres were seeded with wheat in the year 2018. In the Prairie provinces Manitoba, Saskatchewan, and Alberta, an increase of 14.6, 6.6, and 7.5 percent in seeded area was reported respectively from 2017 to 2018. Canada produced 30.0 and 31.8 million tonnes of wheat in the year 2017 and 2018, respectively. Wheat is mainly used a major dietary intake for humans due to its high content of carbohydrates. Furthermore, it serves as a rich source of proteins, minerals, and vitamins. Therefore, over 40% of the world's population uses wheat as their staple food and wheat contribute to approximately 20% of the calories consumed by humans. Wheat bran and auxiliary flours from the processing procedures are frequently marketed as feed for animals and poultry. Furthermore, wheat is also used in the preparation of beer, vodka, and biofuel. Wheat flour is utilized for the generation of baked goods and grain products. Wheat gluten is mainly utilized as a protein source to enhance baking qualities of flour (Shewry, 2009).

Out of many wheat species, common wheat (*T. aestivum*) is utilized to make bread while pasta and semolina products (spaghetti and macaroni) are prepared by utilizing durum wheat (*T. turgidum* ssp. *durum*) (Ficco *et al.*, 2014). Moreover, club wheat (*T. compactum*) which is a softer type, is utilized in preparation of crackers, cookies, cake, pastries and it represents 1 to 2 % of total wheat (Atwell and Finnie, 2016).

2.1.3 Factors affecting wheat production

Abiotic factors such as drought, salinity, high and low temperature and biotic factors such as diseases caused by microbial pathogens affect wheat production worldwide (Kajla *et al.*, 2015;

Abhinandan *et al.,* 2018). Another major factor affecting the production of wheat is preharvest sprouting (PHS), which is defined as the germination of grains in the spike before harvest when there is continuous rain after the seed maturity. This problem is associated with low degree of dormancy in the seeds. Therefore, it is of utmost importance to have a balance between seed germination and dormancy since too much seed dormancy could lead to failure of germination or non-uniform germination and poor seedling establishment (Derera,1989; Simpson, 2007; Gao and Ayele, 2014). In many cereal crops, especially in wheat (*T. aestivum* L.), PHS causes reduction in grain yield as well as quality. For instance, in Canada, there is an annual loss of 100 million dollars due to PHS (DePauw *et al.,* 2012). This marks a dire need to identify sources of PHS resistance in wheat. In addition, PHS is a complex genetic trait as it is governed by the interaction between genetic and environmental factors (Arif *et al.,* 2012).

2.2 Seed

2.2.1 Seed development

Seed is an important unit of propagation and good seed quality is a vital factor for crop production. The life cycle of a plant begins as a seed, which germinates to produce a juvenile plant called a seedling. Then the seedling will develop to maturity and grow into a complete plant. The growing plant will produce flowers and produce new seeds, which serve as materials to start the next life cycle.

A seed is comprised of three hereditary components, which include the endosperm, embryo and seed coat. During the reproductive life cycle of a plant, the male and female gametophyte are formed by meiosis followed by mitosis, to maintain the haploid phase. The site of fertilization is the female gametophyte. The most predominant form of the developed female gametophyte is a

seven-celled stage comprising of three antipodal cells, two synergid cells, an egg cell, and a diploid central cell. Synergid cells at the micropylar end attract the pollen tube from the male gametophyte to the female gametophyte (Higashiyama *et al.,* 2001). Adjacent to the synergid cells, lies the egg cell which acts as the progenitor of embryo formation. Likewise, the diploid central cell with two polar nuclei acts as the progenitor for the formation of endosperm. The male gametophyte with two haploid sperm cells is formed from microspores in anther (Ohto *et al.,* 2007).

Seed development starts upon double fertilization. The pollen tube formed from the vegetative cell of the male gametophyte enters the female gametophyte through the micropyle and discharges two sperm cells into the synergid cell. Out of these two sperm cells, one sperm fertilizes with the egg cell and gives rise to the diploid embryo whereas the other sperm undergoes fertilization with the central cell to form triploid endosperm. The ovary matures to form fruit and differentiation of the ovule integuments results in the formation of seed coat (Gehring *et al.*, 2004). Following double fertilization, the morphogenesis phase begins in which the endosperm undergoes multiple divisions during syncytium formation followed by the cellularization and differentiation phases. Endosperm is described as a syncytium during the initial stages of seed development. During the cellularization phase, division of the syncytium into individual cells occurs followed by differentiation phase which involves the formation of different cells: transfer, aleurone, starchy endosperm, and embryo-surrounding region cells (Sabelli and Larkins, 2009). In the maturation phase, the seed stores nutrients in the endosperm to ensure survival in the upcoming germination stage. The completion of seed maturation phase is associated with desiccation tolerance and developmental arrest of the embryo growth (Finkelstein, 2010).

2.2.2 Seed vigor

Seed vigor is characterized as the sum of all those attributes of the seed which decides the potential for fast uniform seed germination and seedling emergence under various environmental conditions. Seed storage conditions, physiological and genetic potential of a seed determines the life span of a seed which plays a critical role in determining seed vigor (Rajjou *et al.,* 2012). Improved crop productivity depends on the success of seed germination success and establishment of seedlings (Ayalew *et al.,* 2018). Early vigor is one of the necessary traits for improved crop establishment, which ultimately results in enhanced crop yield through efficient resource utilization (Mahender *et al.,* 2015; Wilson *et al.,* 2015). High seed vigor depends on many factors such as high leaf space, high biomass production and deep root system (Rebetzke and Richards, 1999; Atkinson *et al.,* 2015; Rebolledo *et al.,* 2015). Moreover, larger seed size and higher seed weight contribute directly to high seed vigor by accumulating additional reserve food (Ellis, 1992; Khan *et al.,* 2012). However, suboptimal environmental conditions such as high temperature before harvest could also lead to loss of seed vigor. During seed storage, progressive loss of seed vigor usually occurs, and this also causes non-uniform and slow germination (Rajjou *et al.,* 2008; Powell and Matthews, 2012). It has been reported that approximately 25% of harvested crop undergoes annual seed quality loss, which is indirectly related to poor seed vigor (Khan *et al.,* 2016). Detection, mapping, and cloning of quantitative trait loci (QTLs) that control seed germination under various environmental stresses could help to improve seed vigor (Rajjou *et al.,* 2012). In brief, rapid and uniform seed germination under various environmental stresses, seedling establishment, seed longevity are the main attributes for determination of seed vigor.

2.2.3 Seed dormancy

Seed dormancy is defined as the failure of intact viable seeds to germinate even under favorable environmental conditions (Bewley, 1997; Kucera *et al.,* 2005; Gao and Ayele, 2014). Seeds with low level of dormancy can cause the problem of PHS, which results in low crop yield and quality. In contrast, high degree of dormancy causes non-uniform germination and seedling establishment (Tuan *et al.,* 2018). Thus, an intermediate level of dormancy is required that prevents PHS and lead to uniform seed germination and thereby overall crop development (Gao and Ayele, 2014).

Dormancy can be of two types: primary or secondary, based on whether the seed released from the plant is already in a dormant state or became dormant after being released due to unfavorable environmental conditions (Kermode, 2005; Gao and Ayele, 2014). Throughout seed maturation, seed undergoes primary dormancy (Karssen *et al.,* 1983). Primary dormancy can be further classified into five types including physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and combinational dormancy (CD) as outlined by Baskin and Baskin (1998, 2004). In brief, PD is the most common type of dormancy which is further classified into three types which are deep, intermediate and non-deep (Baskin and Baskin, 2004). In the case of deep PD, embryo extracted from seeds will not develop or form abnormal seedlings while seeds with non-deep PD are able to produce normal seedlings (Cadman *et al.,* 2006; Finch-Savage *et al.,* 2006). Morphological dormancy is characterized by physiologically non-dormant embryo that only require time (couple of days to 1-2 weeks) to develop into full size, and germination occurs afterwards. Contrary to MD, embryo with MPD are physiologically dormant and they require an extensively longer period to break the dormancy (Baskin and Baskin, 2004). Physical dormancy occurs due to the presence of at least one water impermeable layer of palisade cells in the seed coat (Baskin *et al.,* 2000; Baskin, 2003;

Baskin and Baskin, 2004) while CD is a combination of PD and PY, which is characterized by the presence of a physiologically dormant embryo and water impermeable seed coat (Finch-Savage and Leubner-Metzger, 2006).

Seed dormancy is regulated by several environmental factors (light, temperature, moisture, oxygen), a period of seed dry storage, and intrinsic factors including plant hormones, of which gibberellin (GA) and abscisic acid (ABA) are considered as the major regulators (Hilhorst *et al.*, 1995; Cadman *et al.*, 2006). Seed dormancy is induced by ABA while released by GA (Leubner and Metzger, 2001; Kermode, 2005; Kucera *et al.*, 2005). In this manner, alterations in ABA and GA level and their sensitivity play predominant roles in the regulation of the induction and decay of seed dormancy (Shu *et al.*, 2016; Finch-Savage and Footitt, 2017). After ripening, which refers to storage of dry seeds from a few weeks to several months, stratification (warm and cold), and treatment with nitrate lead to breakage of seed dormancy (Karssen *et al.*, 1983; Bewley and Black, 1994; Bailly, 2004; Kucera, 2005; Bethke, 2006). During dormancy loss, physiological alterations have been observed in seeds of several plant species including changes in the expression of genes and proteins, and oxidative and epigenetic modifications of genes and proteins (Gao *et al.*, 2012, 2013; Gao and Ayele, 2014).

2.2.4 Seed germination

Seed germination is a vital process for establishment and development of the next-generation of plants, and determination of crop yield and quality (Ohto *et al.*, 2007; Chitnis *et al.*, 2014). It is a process that starts with seed imbibition, which refers to an uptake of water, and terminates with protrusion of the radicle, through the seed coat. Seed germination is characterized by three phases of water uptake. During the first phase, the dry seed uptakes water rapidly, and this induces repair

of DNA and mitochondria, protein synthesis from extant mRNAs, along with changes in the size and shape of the seed (Bewley, 1997). Moreover, uptake of water by a dry seed causes a change from a dry to a hydrated state, and this forces significant stresses upon cellular parts of the seed. For instance, leakage of solutes occurs upon imbibition, which is a characteristic of transitory damage to the membrane. The second phase or plateau phase is identified by a decrease in the uptake of water and is characterized by the synthesis of proteins from newly produced mRNAs and synthesis of new mitochondria (Bewley, 1997). Germination is completed at the end of the second phase, which marks the start of the third phase, and this phase involves another increase in water uptake, and mobilization of storage reserves as well as radicle elongation (Nonogaki *et al.*, 2007). Radicle elongation occurs during the third phase with slight increment in water uptake, which is followed by a considerably bigger uptake that leads to the overall development of the seedling due to increase in mitotic cell divisions and cell extension (Nonogaki *et al.*, 2010). For completion of germination, there is a need to overcome the obstruction conferred on the expanding embryo by its encompassing structures. Of these encompassing structures, the endosperm is the significant restricting tissue in some dicot species. The resistance of these tissues is overcome by enzymatic weakening of the cell wall surrounding these tissues (Nonogaki *et al.*, 2010).

2.2.5 Plant hormones in the regulation of germination

Previous studies have shown that plant hormones play an important role in the regulation of plant developmental processes (Evans, 1984; Kucera *et al.*, 2005; Liu *et al.*, 2013), and plant development relies upon controlled cellular events, for example, division, elongation, and differentiation (Evans, 1984). Application of exogenous hormone and hormone antagonists (Kauschmann *et al.*, 1996), which lead to changes in endogenous hormone levels, have been used

to study the roles of plant hormones in regulating seed germination (Klee and Estelle, 1991; Preston *et al.,* 2009; Liu *et al.,* 2013). Although, GA and ABA are considered as the major phytohormones involved in seed germination regulation, other hormones such as BR are also reported to participate in the regulation of the seed germination (Kucera *et al.,* 2005; Finkelstein *et al.,* 2008; Linkies and Leubner-Metzger, 2012).

2.3 Role of brassinosteroids in the regulation of seed germination and seedling growth

2.3.1 Brassinosteroid

Brassinosteroids (BRs) are polyhydroxylated steroidal plant hormones involved in the regulation of plant growth and developmental processes such as seed germination, stem elongation, inhibition of root growth, and vascular differentiation (Mandava 1988; Li and Chory, 1999; Steber and McCourt, 2001; Zhang *et al.,* 2014). The isolation of the most biologically active form of BR, brassinolide (BL), from oilseed rape (*Brassica napus*) led to the discovery of BR (Grove *et al.,* 1979; Mandava, 1988; Wang *et al.,* 2001). BR is present in every tissue with the maximum amount in seeds and pollen (Arteca, 1995; Schmidt *et al.,* 1997), and very low concentrations of BR have been shown to affect plant developmental processes (Kucera *et al.,* 2005).

In *Arabidopsis* and pea, exogenous BR application has been demonstrated to rescue growth defects of BR-biosynthetic/sensitive mutants (Yokota, 1997; Hayat *et al.,* 2003), while BR signalling mutants showed insensitivity to BR (Clouse *et al.,* 1996; Li *et al.,* 2001). In addition, BRs have been found to play important roles in seed germination and seedling development by interacting with other hormones such as GA and ABA (Steber and McCourt, 2001; Krishna, 2003; Zhang *et al.,* 2009; Divi and Krishna, 2010; Oh *et al.,* 2016). Furthermore, BRs are essential signals considering environmental stimuli as they help plants by protecting them from various

environmental stresses such as high and low temperatures, drought, salinity, and pathogen attack (Divi and Krishna 2009; Oh *et al.,* 2016).

2.3.2 Brassinosteroid biosynthesis pathway

The BR biosynthetic pathway involves the formation of BL from campesterol (CR) (Fig. 2.1). Brassinosteroid biosynthesis proceeds either via the campestanol (CN) independent or CN dependent pathway in which formation of CN occurs from CR (Suzuki *et al.,* 1995; Fujioka *et al.,* 1997). Furthermore, the conversion of CN to BL involves two distinctive pathways, known as the early C-6 oxidation and late C-6 oxidation pathway and involves the stepwise metabolism of CN (Choi *et al.,* 1996; Yokota, 1997; Choe *et al.,* 1998). In the early C-6 oxidation pathway, CN is converted sequentially to cathasterone (CT), teasterone (TE), typhasterol (TY), and castasterone (CS) whereas, in the late C-6 oxidation pathway, CN gets converted sequentially to 6-deoxoCT, 6-deoxoTE, 6-deoxoTY and 6-deoxoCS, and CS (Fujioka and Sakurai, 1997; Noguchi *et al.,* 2000). Then, the CS produced by both pathways undergoes oxidation reaction to mark the last step in the BR biosynthetic pathway, which marks the formation of BL (Yokota *et al.,* 1991; Suzuki *et al.,* 1995).

The BR biosynthetic pathway involves hydroxylation, reduction, epimerization, and oxidation reactions (Yokota, 1997) and are catalyzed by cytochrome P450 (CYP450s) such as dwarf4 (DWF4/CYP90B1), constitutive photomorphogenesis and dwarfism (CPD/CYP90A), deetiolated2 (DET2/DWF6) and BR-6-oxidase (BR6ox) (Choe *et al.*,1998; Noguchi *et al.,* 1999; Ohnishi *et al.,* 2006). Briefly, the BR biosynthetic enzyme DET2 catalyzes the reduction reaction that converts CR to CN, which in turn forms 6-deoxoCS by the action of DWF4 in the late C-6 oxidation pathway (Suzuki *et al.,* 1995; Fujioka *et al.,* 1997). CPD/CYP90A catalyzes the

conversion of 6-deoxo CS to 6-deoxo TE, which is finally converted to 6-deoxo TY that yields 6-deoxo CS in the last step. In the early C-6 oxidation pathway, CS is formed from 6-oxo CN by the action of DWF4, which in turn is converted to TE by CPD. Another biosynthetic enzyme, BR6ox1/2 (CYP85A1 and CYP85A2) catalyses the conversion of CN, 6-deoxo CT, 6-deoxo TE, 6-deoxo TY and 6-deoxo CS of the late oxidation pathway to 6-oxo CN, CT, TE, TY and CS of the early oxidation pathway, respectively. The last step marks the formation of the final product of this pathway, BL, and this step is believed to be catalyzed by BR6ox2 (Gruszka *et al.,* 2011; Oh *et al.,* 2016).

To date, more than 40 naturally occurring BRs have been found. The difference in their structure occurs due to the presence of different substituents in the A or B ring and side chain of BR. During the biosynthesis of BR, hydroxylation of CR and TE occurs at C-22 and C-23 position of the side chain, respectively (Yokota, 1997; Oh *et al.,* 2016). These hydroxylation reactions are catalysed by CYP90B1 at C-22, CYP90C1 and D1 at C-23 positions (Oh *et al.,* 2016).

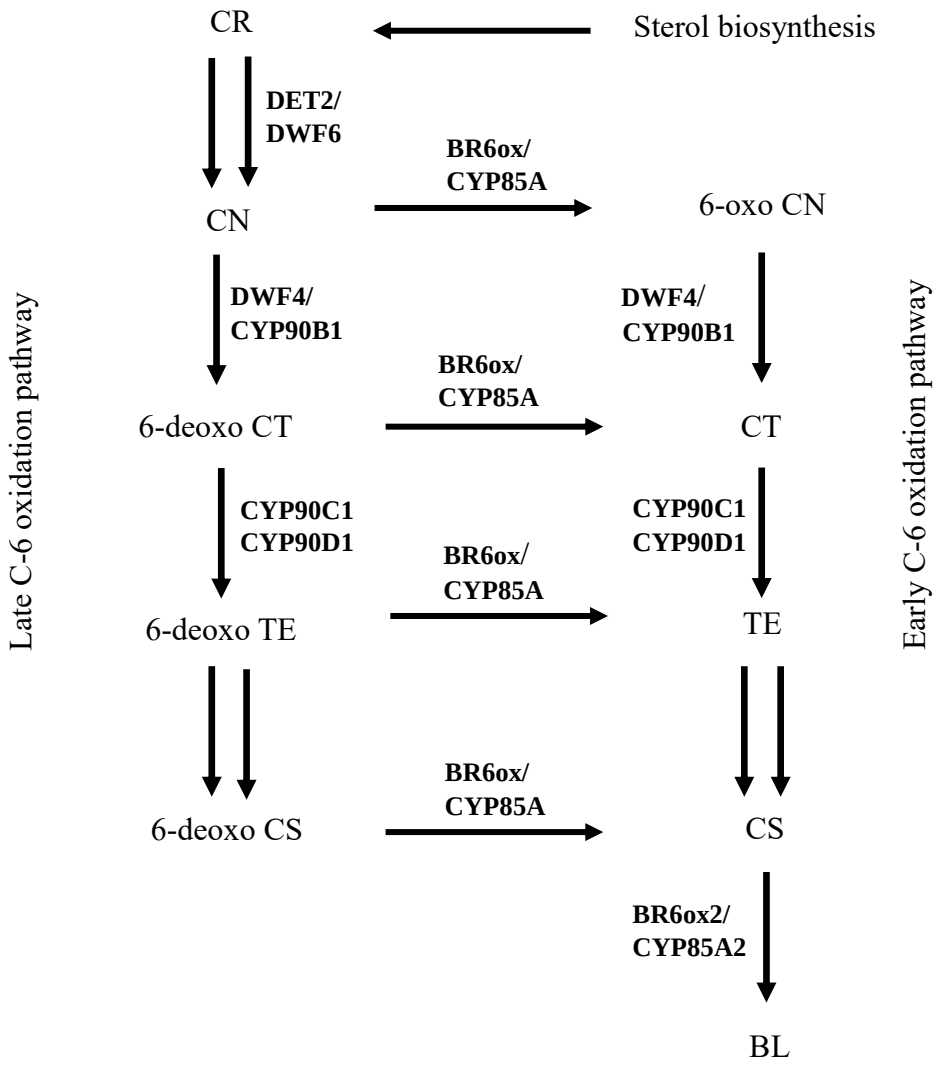

Figure 2.1 A simplified schematic diagram of brassinosteroid biosynthesis pathway which involves CR, campesterol; CN, campestanol; 6-oxo CN, 6-oxocampestanol; 6-deoxo CT, 6-deoxocathasterone; CT, cathasterone; 6-deoxo TE, 6-deoxoteasterone; TE, teasterone; 6-deoxo CS, 6-deoxocastasterone; CS, castasterone; BL, brassinolide; DWF6/DET2, dwarf6/de-etiolated2; CYP90B1/DWF4, cytochrome P450 90B1/dwarf4; CYP90C1 and CYP90D1, cytochrome P450 90C1 and, cytochrome P450 90D1; CYP85A/BR6ox, cytochrome P450 85A/BR-6-oxidase. (Adapted from Oh *et al.*, 2015).

2.3.3 Phytohormone biosynthesis inhibitors

2.3.3.1 Triazole-type plant growth regulators as BR biosynthesis inhibitors

Plant growth regulators (PGRs) are naturally occurring or synthetic chemicals involved in the regulation of many plant growth and developmental processes. Plant hormones such as auxin, cytokinin, ethylene, ABA and GA are naturally occurring PGRs (Jaleel *et al.,* 2007; Rademacher, 2015). Apart from these naturally occurring compounds there are synthetic chemicals that act as PGRs and significantly modulate plant physiological processes (Jaleel *et al.,* 2007; Kavina *et al.,* 2011). Triazoles are synthetic compounds that act as inhibitors of ergosterol biosynthesis, and can function as PGRs (Jung *et al.,*1985; Jaleel *et al.,* 2007). The effectiveness of triazoles as PGRs can be determined from their role in inhibition of the biosynthesis of sterols, BRs, and therefore growth elongation, in many crop species such as rice, barley and oilseed rape (Jung *et al.,*1985; Rademacher, 2000). However, triazoles have been demonstrated to enhance the production of cytokinin and ABA (Gopi *et al.,* 2009). Understanding of the BR functions using triazole PGRs help in studying BR mediated plant growth processes such as cell elongation, cell division, and stress tolerance (Asami *et al.,* 2001).

Paclobutrazol, uniconazole, brassinazole and propiconazole (Pcz) are examples of triazole PGRs. In pea (*Pisum sativum*), uniconazole has been found to inhibit BR-induced tracheary element differentiation by binding to CYP90D1 (Iwasaki and Shibaoka, 1991; Yokota *et al.,* 1991). Furthermore, brassinazole has been reported to bind to DWF4/CYP90B1 and inhibit hydroxylation of BR intermediates, leading to inhibition of BR synthesis and dwarfism (Asami *et al.,* 2001). In addition to serving to elucidate the role of BR in plants, brassinazole can be used to examine the role of BR signaling genes (Min *et al.,* 1999; Sekimata *et al.,* 2001; Vert *et al.,* 2005; Gomes, 2011). The practical utilization of brassinazole in agricultural crops is very limited due to their low

availability and high cost. As a result, Pcz replaces brassinazole as effective, specific and low-cost BR inhibitor in plants (Hartwig *et al.,* 2012).

2.3.3.2 Propiconazole as BR biosynthesis inhibitor

Propiconazole acts as an inhibitor of BR biosynthesis (Hartwig *et al.,* 2012). In cress plant (*Lepidium sativum*), inhibition of hypocotyl growth by Pcz has demonstrated its function as a BR biosynthetic inhibitor (Sekimata *et al.,* 2002). Inhibition of hypocotyl growth by Pcz can be rescued by treatment of Pcz in combination with BL. In *Arabidopsis*, mutation in BR biosynthesis genes and application of Pcz results in reduction of growth of seedlings (Hartwig *et al.,* 2012), and maize seedlings treated with Pcz have been found to exhibit reduction in mesocotyl, coleoptile and true leaf elongation, (Hartwig *et al.,* 2012; Cheon *et al.,* 2013). Moreover, Pcz suppresses root growth and Pcz-mediated repression of genes involved in BR biosynthesis (*DWF4*, *BR6ox2*, and *CPD*) has been shown to be reversed by a BR, 24-epi-brassinolide (EBL) (Hartwig *et al.,* 2012). Considering such outcomes, Pcz can be presumed an effective inhibitor of BR biosynthesis, outweighing limitations associated with the use of brassinazole. The lower expense and easy accessibility of Pcz further help to extensively explore the BR applications in plants (Hartwig *et al.,* 2012).

The reactions involved in the conversion of CN to TE in the BR biosynthetic pathway (Fig. 2.1), which are catalyzed by CYP90B1, CYP90C1, and CYP90D1, are affected by Pcz. Previous study has shown the binding affinity of Pcz towards CYP90D1, a protein involved in the BR biosynthetic pathway, and the binding occurs at C23 position of CYP90D1, providing decisive proof that Pcz affects the hydroxylation reaction at the side chain in the BR biosynthetic pathway (Oh *et al.,* 2016).

2.3.4 Brassinosteroid signaling pathway

The vast majority of information about BR signaling was obtained from studies in *Arabidopsis* (Yang *et al.,* 2011). A complete BR signaling pathway (Fig. 2.2) has been built up including various essential components including BR insensitive 1 (BRI1), BRI1 associated kinase 1 (BAK1), BRI1 kinase inhibitor 1 (BKI1), constitutive differential growth1 (CDG1), BR signaling kinase 1 (BSK1), BRI1 suppressor 1 (BSU1), protein phosphatase 2A (PP2A), BR insensitive 2 (BIN2), BRI1 EMS suppressor 1 (BES1), and Brz resistant 1 (BZR1) (Wang *et al.,* 2003; Mora-García *et al.*, 2004; Wang *et al.,* 2012).

Brassinosteroid signaling starts with the binding of BL to the island domain of BRI1 (Karlova *et al.,* 2006; Oh *et al.*, 2009; Wang *et al.,* 2008). The association of BL with BRI1/BKI1 complex will initiate the trans-phosphorylation of multiple regions and changes in confirmation of the cytosolic domain (Wang *et al.,* 2006). Auto and transphosphorylation events between BRI1 and BAK1 cause the inhibitor, BKI, to disassociate from BRI1 (Wang *et al.,* 2008; Oh *et al.,* 2009). Then, BRI1 forms a heterodimer with BAK1, and BRI1 kinase phosphorylates BSK1 and this results in its interaction with BSU1. Then BSU1 in turn causes rapid dephosphorylation of a negative regulator of BR signaling, BIN2 (Tang *et al.,* 2008; Kim *et al.,* 2009; Kim *et al.,* 2011). As a consequence, BIN2 undergoes 26S proteasome-mediated degradation, which results in activation of the substrates of BIN2, BES1 and BZR1 (Wang *et al.,* 2011). Activation of BZR1 will repress BR biosynthesis as BIN2 dephosphorylation leads to the nuclear localization of BES1 and BZR1 transcription factors that interact with the promoter of numerous genes involved in BR biosynthesis and repress their expression (Wang *et al.,* 2002; Yin *et al.,* 2002; He *et al.,* 2005). Moreover, BZR1 can regulate BR biosynthesis via inhibition and activation of the BR biosynthetic genes including *CPD*, *DWF4* and *BR6ox* (He *et al.,* 2005).

However, when BR signaling is not activated, phosphorylation of BZR1 and BES1 occurs, as a result they will be trapped by 14-3-3 proteins while remaining outside of the nuclear region (Gampala *et al.*, 2007; Ryu *et al.*, 2007). Alternatively, nuclear localized BZR1 after phosphorylation shows inefficient interaction with the promoter regions of target genes (He *et al.*, 2002; Yin *et al.*, 2002).

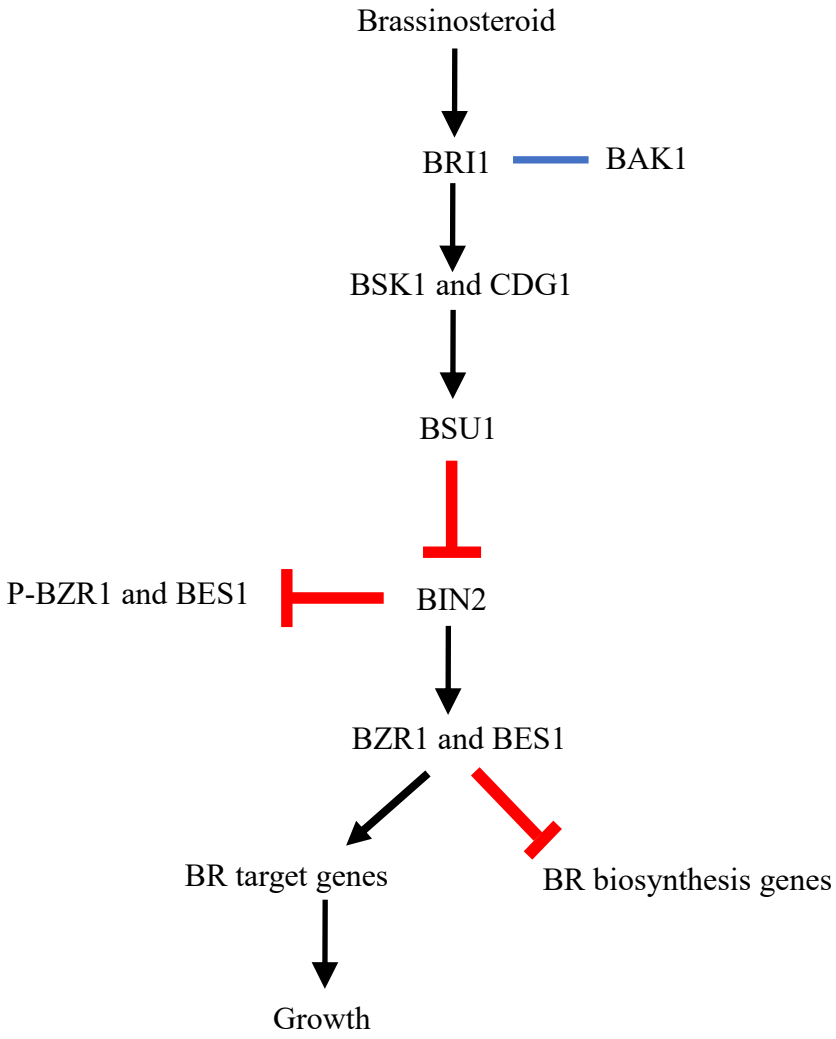

Figure 2.2 A simplified diagram of BR signaling pathway. BRI1, BR insensitive 1; BAK1, BRI1 associated receptor kinase 1; BSK1, BRI1 suppressor 1; CDG1, constitutive differential growth 1; BSU1, BRI1 suppressor 1; BIN2, BR insensitive 2; P-BZR1, phosphorylated brassinazole resistant 1; BES1, BRI1 EMS suppressor 1. Arrows represent progress activities and bar ends represent inhibitory activities. Solid blue line shows direct regulation. (Adapted from Tong and Chu 2012).

2.3.5 Brassinosteroid regulates seed germination and seedling growth

Brassinosteroids are found to influence seed germination and seedling growth processes (Deng *et al.,* 2007). Previous studies have examined the role of BR in the regulation of germination and seedling growth in maize, rice, cucumber, *Arabidopsis*, oilseed rape and sorghum under salt, cold, heat, drought and water stressed conditions (Anuradha *et al.,* 2003; Özdemir *et al.,* 2004; Vardhini and Rao, 2005; Kagale *et al.,* 2007; Arora *et al.,* 2008; Mahesh *et al.,* 2013). In *Arabidopsis*, overexpression of the BR biosynthetic gene *DWF4* brought about a 59% increase in seed yield, indicating the role of BR in regulating plant growth and yield (Choe *et al.,* 2001). Both BR metabolism and signaling has been suggested to play a significant role in regulating seed germination and dormancy. For example, in wheat seeds, after-ripening treatment that breaks seed dormancy, caused significant changes in the expression of genes involved in BR biosynthesis, such as *DET2* and *DWF4*, and signaling, such as *BSK* and *BIN2* (Chitnis *et al.*, 2014). Furthermore, differences in the expression level of these BR biosynthetic and signalling genes upon imbibition of dormant and non-dormant seeds appear to be correlated with differences in the expression levels of BR responsive genes such as *BR enhanced expression* and *paclobutrazol resistance* genes that are considered to play significant roles in cell elongation (Zhang *et al.*, 2009).

In *Arabidopsis*, exogenous application of BR biosynthesis inhibitor was found to bring dwarfism due to impaired biosynthesis and signaling of BR (Nakashita *et al.,* 2003), while exogenous BR application was shown to rescue the growth defects of BR deficient mutants (Clouse, 2002). Furthermore, BR treatment in sorghum has been reported to enhance seed germination and seedling growth under drought stress through increasing proline and protein contents (Vardhini and Rao, 2003). In cotton, overexpression of the BR biosynthetic gene *DET2*, which encodes for steroid 5α-reductase, caused a significant increase in the length and number of

fibers (Luo *et al.*, 2007), while BR deficient mutants of rice exhibit a dwarf phenotype (Sakamoto *et al.,* 2006). Moreover, treatment of rice seedlings with the BR biosynthesis inhibitor, yucaizol reduces seedling growth (stem length) and induces a dwarf phenotype (Matusmoto *et al.*, 2016), while BR improves rice seedling growth under saline conditions (Anuradha and Rao, 2001). BR treatment has been also shown to promote seedling growth in wheat under salinity stress through promoting antioxidant enzymes and defensive proteins (El-Feky and Abo-Hamad, 2014). The results of all these studies indicate the positive role of BRs in the seed, regulating germination and seedling growth, which in turn leads to improvement in crop yield (Divi and Krishna, 2010; Oh *et al.,* 2016).

2.4 Abscisic acid and its cross-talk with brassinosteroid in relation to seed germination

2.4.1 Abscisic acid

Abscisic acid is a phytohormone that is involved in the regulation of several plant growth and developmental processes. ABA is involved in storage reserve accumulation (proteins and lipids), enhancement in seed desiccation tolerance and suppression of the change from embryo development to germination and from vegetative to reproductive development (Giraudat, 1998; Rock, 2000; Kermode, 2005; Kucera *et al.,* 2005). ABA is also known as a ‘stress hormone’, as it significantly regulates plant adaptation to various stress factors, such as induction of stomata closure during drought and water stress, promotion of seed dormancy to ensure seed germination when favourable environmental conditions occur, and tolerance to salt, cold and pathogens (Leung and Giraudat, 1998; Rock, 2000; Kermode and Finch-Savage, 2002; Zeng and Kermode, 2004; Miyakawa *et al.,* 2012).

2.4.2 Abscisic acid metabolism

The level of ABA in plants depends on its biosynthetic and catabolic rates (Nambara and Marion-Poll, 2005). Plant carotenoids act as precursors for ABA biosynthesis. Carotenoids are derived by the condensation of two C-20, geranylgeranyl pyrophosphate (GGPP) molecules, catalyzed by phytoene synthase that leads to the formation of a C-40 carotenoid, phytoene (Hirschberg, 2001). Phytoene is then converted to lycopene, and this reaction is catalyzed by phytoene desaturase and zeta-carotene desaturase that mediate four desaturation reactions. Lycopene then undergoes a cyclization reaction to form β-carotene by the action of the enzyme lycopene β-cyclase. The β-carotene forms zeaxanthin through a hydroxylation reaction mediated by β-carotene hydroxylase (Cunningham and Gantt, 1998; Hirschberg, 2001). Zeaxanthin synthesis contributes to the initiation of the ABA biosynthesis pathway, which mainly takes place in the chloroplast and cytoplasm in three steps (Fig. 2.3) (Cunningham and Gantt, 1998; Hirschberg, 2001; Liu and Hou, 2018).

The first step involves epoxy-carotenoid synthesis (Nambara and Marion-Poll, 2005). In this step, epoxidation of zeaxanthin into violaxanthin occurs, and this reaction is catalyzed by zeaxanthin epoxidase (ZEP) involving antheraxanthin as an intermediate. The violaxanthin produced through this reaction can be reversed to zeaxanthin by violaxanthin de-epoxidase (VDE). The violaxanthin can be isomerized to form cis-violaxanthin and cis-neoxanthin by the action of isomerase. In the second step, xanthoxin is formed by nine-cis-epoxycarotenoid dioxygenase (NCED) mediated cleavage of violaxanthin and neoxanthin and then export of xanthoxin from chloroplast to cytoplasm occurs (Nambara and Marion-Poll, 2005; Liu and Hou, 2018; Tuan *et al.*, 2018). The NCED enzyme appears to be the major regulator involved in the biosynthesis of ABA (Nambara and Marion-Poll, 2005). In *Arabidopsis*, nine *NCED* genes have been isolated, however,

only five of them play important roles in ABA biosynthesis (*NCED2*, *NCED3*, *NCED5*, *NCED6* and *NCED9*) (Iuchi *et al.,* 2001; Lefebvre *et al.,* 2006). In cereals such as wheat and barley, two *NCED* genes have been identified to date, *NCED1* and *NCED2* (Chono *et al.,* 2006; Zhang *et al.,* 2014; Son *et al.,* 2016). In barley, *NCED2* is reported to have a prominent role in the regulation of ABA level during early to mid-phase of seed development (Chono *et al.,* 2006). In wheat a high level of ABA synthesis is found to occur during the late phase of seed maturation (Tuan *et al.,* 2018). In rice seeds, an increase in *NCED1* expression and ABA accumulation appear to occur at the early stages of seed development (Liu *et al.,* 2014). During the third step, which occurs in the cytosol, xanthoxin is converted to ABA aldehyde by short-chain dehydrogenase reductase. Oxidation of the ABA aldehyde by ABA aldehyde oxidase (AAO3) forms ABA (Fig. 2.3). Naturally, ABA in plants occurs in a *cis* form which is the biologically active form of ABA.

The catabolism of bioactive ABA is categorized into two reactions: hydroxylation and conjugation. In detail, the hydroxylation pathway results in catabolism in three different pathways that oxidize the carbon atoms at 7′, 8′ and 9′ positions of ABA to produce 7′, 8′ and 9′-hydroxy ABA, respectively. These hydroxylated ABA forms are relatively unstable as compared to ABA. Among these three hydroxylated ABA forms, 8′-hydroxy ABA is prominently involved in the catabolism of ABA (Cutler and Krochko, 1999; Nambara and Marion-Poll, 2005, 2010; Gao and Ayele, 2014). Subsequently, the formation of phaseic acid (PA) will occur after the isomerization of 8′-hydroxy ABA (Cutler and Krochko, 1999). PA is then catabolized in the presence of a soluble enzyme to form dihydro phaseic acid. The enzyme ABA 8'-hydroxylase (ABA8'OH) catalyzes the ABA catabolic reactions that convert the bioactive form of ABA into hydroxy ABA (Nambara and Marion-Poll, 2005). In *Arabidopsis*, ABA8'OH is encoded by four members of the *CYP707A* gene family, which are designated as *CYP707A1*, *CYP707A2*, *CYP707A3* and *CYP707A4* (Kushiro *et*

al., 2004; Saito *et al.*, 2004). In both embryo and endosperm tissues of *Arabidopsis*, ABA level is found to be regulated by both *CYP707A1* and *CYP707A2*. However, *CYP707A2* showed prominent expression during later stages of seed development, indicating stage-specific role of these two genes in the regulation of seed development (Okamoto *et al.,* 2006; Son *et al.,* 2016). In wheat and barley, two *CYP707A* genes have been identified to date namely, *CYP707A1* and *CYP707A2*. In barley, *CYP707A1* is found to play a prominent role in the catabolism of ABA after the mid-phase of seed development (Chono *et al.,* 2006), while its role in wheat appear to be predominant during the mid to late phases of seed maturation (Chono *et al.,* 2013; Tuan *et al.,* 2018).

In addition to the hydroxylation reactions, ABA catabolism also occurs through ABA conjugation, which involves the conjugation of ABA and its hydroxylated catabolites with aldohexose glucose (Nambara and Marion-Poll, 2005). The most dominant form of biologically inactive ABA conjugate is ABA glucosyl ester (ABA-GE), and it is widely spread within the plant kingdom (Hartung *et al.,* 2002). Overall, NCED plays a predominant role in ABA biosynthesis while ABA8'OH has a significant role in the catabolism of ABA (Nambara and Marion-Poll, 2005).

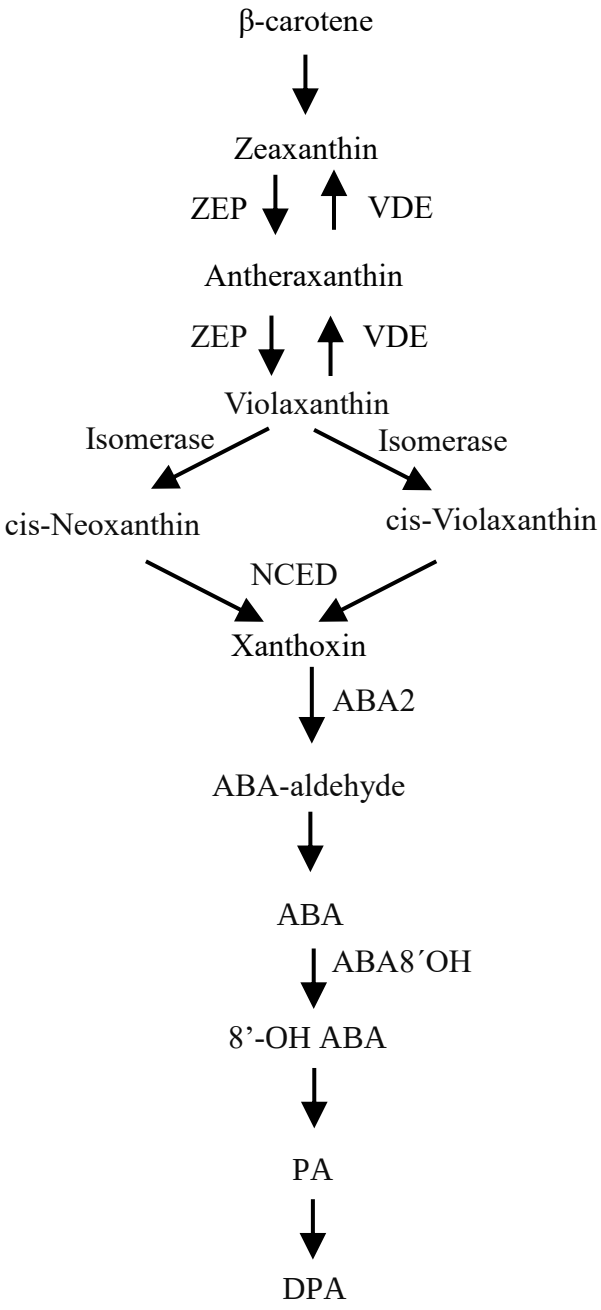

Figure 2.3 A simplified outlining of ABA biosynthesis pathway. β-carotene, beta carotene; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NCED, nine-cis-epoxycarotenoid dioxygenase; ABA, abscisic acid; ABA-aldehyde, abscisic aldehyde; ABA2, ABA deficient 2; ABA8´OH, ABA 8'-hydroxylase; 8'-OH ABA, 8'-hydroxy abscisic acid; PA, phaseic acid; DPA, dihydrophaseic acid. (Adapted from Nambara and Marion-Poll, 2005; Hirschberg, 2001).

2.4.3 Abscisic acid regulates seed germination and seedling growth

Abscisic acid has been found to regulate plant developmental processes including seed germination and seedling growth (Finkelstein and Gibson, 2002). In *Arabidopsis* and tobacco, seeds overexpressing *NCED* genes exhibit delay in germination or enhanced dormancy (Qin and Zeevaart, 2002; Martínez-Andújar *et al.*, 2011). Studies with barley and *Brachypodium distachyon* seeds also revealed the significance of ABA synthesis in regulating seed germination (Tuan *et al.*, 2018). For example, in after ripened *Brachypodium* seeds, reduction in the expression level of *NCED1* is observed to be correlated with a decrease in ABA level and induction of seed germination (Barrero *et al.*, 2012), while an increase in the expression level of *NCED1* is reported to induce ABA level and prevent seed germination in barley seeds under white light conditions (Gubler *et al.*, 2008). Moreover, overexpression of wheat *NCED* genes in *Arabidopsis* seeds showed a delay in seed germination (Son *et al.*, 2016; Tong *et al.*, 2017).

Seed germination and dormancy have also been found to be regulated by *CYP707A* genes that are involved in ABA catabolism (Kushiro *et al.*, 2004; Millar *et al.*, 2006; Okamoto *et al.*, 2006). In rice, ABA catabolism inhibitor has been found to prevent seed germination suggesting the significance of ABA deactivation in seed germination regulation (Zhu *et al.*, 2009). In *Arabidopsis*, after-ripening treatment of dormant seeds results in a significant reduction of ABA level during imbibition that correlates with increased expression of *CYP707As* (*CYP707A2*) (Millar *et al.*, 2006). On the other hand, ABA application to *Arabidopsis* seeds is found to inhibit weakening of the endosperm, suppress radicle emergence through the micopyle or seed germination (Muller *et al.*, 2006). Moreover, after-ripening treatment in rice resulted in a reduction in ABA level by upregulating the expression level of *CYP707A5*, resulting in increased seed germination (Du *et al.*, 2015). Likewise, in barley, ABA level decreases in after-ripened seeds

following imbibition, and this is correlated with increased expression level of *CYP707A11* and decay of dormancy or induction of germination (Baskin and Baskin, 1998; Gubler *et al.,* 2008). In wheat, embryos derived from double mutants of *CYP707A1* exhibited high ABA content and inhibition of germination as compared to their single mutants (Chono *et al.*, 2013), whereas overexpression of the wheat *CYP707A1* gene in *Arabidopsis* caused enhanced seed germination or a decrease in dormancy (Son *et al.,* 2016).

Regarding seedling growth, ABA deficient mutants of barley exhibit reduction in shoot growth whereas high ABA concentration has been found to positively regulate shoot growth in comparison to the control (Mulholland *et al.,* 1996*a, b*; Roberts *et al.,* 2002). In maize, treatment of root seedlings with high concentrations of ABA under optimum water conditions was demonstrated to inhibit root growth. Additionally, reduction of ABA content using its inhibitor or mutational analysis also resulted in inhibition of root growth (Spollen *et al.,* 2000). In wheat, ABA is observed to inhibit seed germination and seedling growth through its inhibitory effect on storage reserves and growth of the embryo axis (Liu *et al.,* 2013).

2.4.4 Cross-talk between Abscisic acid and Brassinosteroids

Brassinosteroids shows interaction with ABA in numerous plant growth processes including seed germination (Zhang *et al.,* 2009). In general, BRs and ABA show antagonistic effects to each other. However, numerous genes are regulated by BRs and ABA in common, with either synergistic or antagonistic effects (Nemhauser *et al.,* 2006; Chung *et al.,* 2014). Based on tissue and species specificity, BRs are found to increase as well as decrease ABA biosynthesis (Tan *et al.,* 2003; Zhang *et al.,* 2010). In *Arabidopsis*, BR and ABA are known to antagonistically regulate each other's level, as BR is found to decrease ABA level, preventing ABA-mediated closure of stomata

(Ha *et al.,* 2018). Overexpression of a BR biosynthetic gene (*DWF4*) was found to antagonizes ABA-mediated inhibition of seed germination (Divi and Krishna, 2010), while ABA treatment inhibits BR signaling (Nemhauser *et al.,* 2006). However, in maize leaves, the level of ABA biosynthesis has been shown to increase upon BR treatment but decreased by treatment with the BR biosynthesis inhibitor, Brz (Zhang *et al.,* 2010). During embryo development, BRs show antagonism to ABA-mediated inhibition of seed germination. It has been demonstrated that in *Arabidopsis*, BR biosynthesis and insensitive mutants (*det2-1* and *bri1-1*) exhibit significant inhibition of seed germination (Steber and McCourt, 2001). The protective function of BR to abiotic stresses also depend on endogenous ABA level, which increased to a higher level under unfavorable conditions (Janeczko and Swaczynova, 2010). For instance, in *Arabidopsis*, BRs and ABA were found to regulate specific target genes and improve its growth under stress conditions (Fujita *et al.*, 2004).

Previous studies in *Arabidopsis* showed that crosstalk of ABA with BR occurs through its signalling pathway, as negative regulators of ABA signaling, ABA insensitive1 (ABI1) and (ABI2), dephosphorylate the negative regulator of BR signaling, BIN2 (Merlot *et al.,* 2001; Wang *et al.,* 2018). In another study, inhibition of BR signaling by BIN2 was found to increase ABA signaling through its interaction with ABI5 (Cai *et al.,* 2014; Hu and Yu, 2014). Furthermore, BRs were found to antagonize ABA's inhibitory action by restraining the action of BIN2. Thus, BIN2 serves as a bridge to coordinate signals of BR and ABA in the regulation of seed germination and post-germinative development (Hu and Yu, 2014). Moreover, BIN2 regulates genes, particularly those that are upregulated by ABA and downregulated by BR, through controlling the activities of BZR1 and BES1 (Fujita *et al.,* 2004). Thus, the transcription factors BES1and BZR1 mediate the interaction between BR and ABA (Zhang *et al.,* 2009). Microarray analysis has shown that ABA

inhibits the BR signaling pathway and many of the BR-responsive genes are under the regulation of ABA (Zhang *et al.,* 2009).

2.5 Gibberellin and its cross-talk with Brassinosteroids in relation to seed germination

2.5.1 Gibberellin

Gibberellin is a diterpene phytohormone that plays a significant role in plant development and growth (Yamaguchi, 2008). Gibberellins were discovered in Japan while conducting research on bakanae disease in rice, which was known to be caused by fungus *Gibberella fujikuroi*. This disease resulted in stem elongation and yield reduction due to the presence of compound that was later termed as gibberellin (Hedden and Sponsel, 2015). In the 1930s, Japanese researchers isolated impurified crystals from *Gibberella* fungal filtrate, which was named gibberellin A. Later in the 1950s, 'gibberellin A' was found to be a mixture of three different gibberellins, GA_1, GA_2, and GA_3, with GA_3 being considered as the prominent component by the scientists from USA, Britain and Japan. GA_3 is available commercially on a large scale for various applications. In plant growth and development, GA imparts its role by regulating seed germination and dormancy, promotion of flowering, induction of cell elongation and fruit ripening (Ullah *et al.,* 2002; Finch-Savage and Leubner-Metzger, 2006; Gupta and Chakrabarty, 2013).

2.5.2 Gibberellin metabolism

Gibberellin biosynthesis occurs in three phases (Fig. 2.4). During the first phase, geranyl diphosphate (GGDP) forms *ent*-kaurene in two steps, which are mediated by CPS (*ent*-copalyl diphosphate synthase) and *ent*-kaurene synthase (KS), respectively (Yamaguchi, 2006). During the second phase, *ent*-kaurene oxidation results in the formation of GA_{12} through consecutive

reactions catalyzed by KO *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). In the third phase, GA_{12} is hydroxylated to form GA_{53}. Both GA_{53} and GA_{12} produce bioactive GAs, GA_1 and GA_4, respectively, through a series of 13-hydroxylation and non-hydroxylation reactions followed by oxidative reactions mediated by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox). The reaction catalyzed by GA3ox marks the final phase of GA biosynthesis (Yamaguchi, 2008). Genes encoding these two enzymes have been identified from numerous plants and they form gene families. For example, in *Arabidopsis*, *GA20ox* has five gene family members including *GA20ox1*, *GA20ox2*, *GA20ox3*, *GA20ox4*, and *GA20ox5* (Plackett *et al.*, 2012), whereas *GA3ox* consists of four gene family members namely *GA3ox1*, *GA3ox2*, *GA3ox3* and *GA3ox4*. In rice, the *GA20ox* family is found to consist of four genes namely, *GA20ox1*, *GA20ox2*, *GA20ox3* and *GA20ox4* (Sakamoto *et al.*, 2004) and *GA3ox* family consists of two genes, *GA3ox1* and *GA3ox2* (Itoh *et al.*, 2001; Sakamoto *et al.*, 2004).

The level of GA in tissues of plants is also determined by its catabolic rate. The catabolism of GA is catalyzed mainly by GA 2-oxidase (GA2ox). Genes encoding the GA2ox enzyme of *Arabidopsis* include *GA2ox1*, *GA2ox2*, *GA2ox3*, *GA2ox4*, *GA2ox6*, *GA2ox7* and *GA2ox8*, while four GA2ox genes have been identified from rice (Schomburg *et al.*, 2003; Sakamoto *et al.*, 2004). Other reactions involved in GA inactivation include epoxidation of non-13-hydroxylated GAs, catalyzed by elongated uppermost internode (EUI), a member of the P450 monooxygenase class of enzymes (Zhu *et al.*, 2006), and methylation of GAs, which is catalyzed by GA methyl transferases (Varbanova *et al.*, 2007). Moreover, GA catabolism can also occur by a conjugation reaction involving glucose (Yamaguchi, 2008).

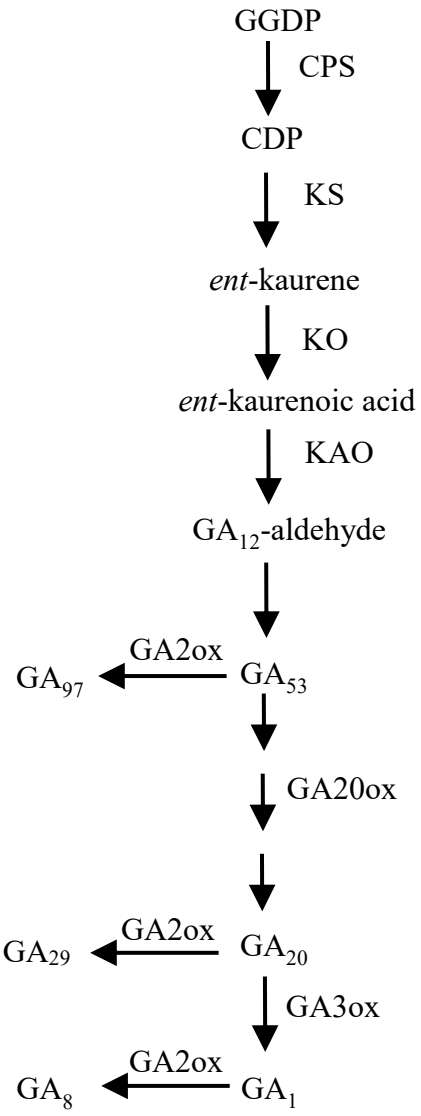

Figure 2.4 A simplified outlining of the GA biosynthesis pathway. GGDP, geranyl diphosphate; CDP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA12-aldehyde, a precursor for other GA's; GA53, GA97, GA20, GA29, GA1 and GA8 are different gibberellins; GA20ox, GA20 oxidase; GA3ox, GA3 oxidase; GA2ox, GA2 oxidase. (Adapted from Yamaguchi, 2008).

2.5.3 Gibberellin signaling

Gibberellin signaling is initiated upon perception of bioactive GA by its receptor GA insensitive dwarf1 (GID1). Binding of GA to GID1 induces formation of a complex involving DELLA protein, which acts as a negative regulator of GA signalling (Eckardt, 2007), ultimately leading to the degradation of DELLAs by ubiquitin-26S proteasome pathway. The degradation of DELLA activates GAMYB which in turn facilitate GA response (Gubler, 1995). In *Arabidopsis* and rice, it has been demonstrated that GA positively regulates plant growth and development by inhibiting the action of DELLA proteins. DELLA proteins do not have DNA binding domain; therefore, they interact with other transcription factors that have the potential to bind to the DNA sequences of GA regulated genes. In *Arabidopsis*, five DELLA proteins are identified namely GA insensitive (GAI), repressor of GA1-3 (RGA), RGA-like1 (RGL1), RGL2 and RGL3 while wheat has so far single DELLA protein, designated as reduced height (RHT) (Gao and Ayele, 2014). In *Arabidopsis*, *RGL2* was found to play an important role during seed germination (Tyler *et al.*, 2018).

2.5.4 Gibberellin regulates seed germination and seedling growth

Gibberellin is one of the plant hormones that positively regulates seed germination and seedling growth. Seeds of GA deficient mutants of *Arabidopsis* exhibit no or delayed germination, and additional exogenous GA is required for the completion of their germination (Koornneef and Van der Veen 1980; Groot and Karssen 1987). In agreement, germinating *Arabidopsis* seeds show increased GA biosynthesis (Ogawa *et al.,* 2003), and this is correlated with increased expression levels of the GA biosynthetic *GA3ox1* and *GA3ox2* genes and decreased expression level of the GA catabolic gene *GA2ox2* (Yamaguchi *et al.*, 1998). Studies in lettuce seeds also showed that an

increase in the expression of *GA3ox1* is associated with seed germination (Toyomasu *et al.,* 1993) and GA treatment induces germination in maize seeds (Tian *et al.,* 2014). In addition, upregulation of the GA biosynthetic genes, including *GA20ox1*, *GA20ox2*, *GA20ox3* and *GA3ox2*, is evident in germinating rice seeds (Kaneko *et al.,* 2002; Du *et al.,* 2015), while mutation in *GA20ox2* results in inhibition of seed germination (Ye *et al.,* 2015; Magwa *et al.,* 2016). Similarly, the role of after-ripening treatment in the dormancy decay and induction of germination has been shown to be associated with increased expression level of the GA biosynthetic genes *GA20ox* and *GA3ox* in wheat and barley (Gubler *et al.,* 2008, Liu *et al.,* 2013; Du *et al.,* 2015). The role of GA in regulating wheat and barley seed germination is related with its effect in inducing the expression of enzymes involved in the mobilization of starch stored in the endosperm to provide nutrients to the embryo. Moreover, GA is directly involved regulating the growth of the embryo itself (Jacobsen *et al.,* 1995).

With respect to seedling growth, exogenous GA application was reported to rescue seeding growth suppressed by DELLA proteins (Achard and Genschik, 2009). Moreover, mutations in the *GA20ox1*, *GA20ox2* and *GA20ox3* genes lead to an extreme dwarf phenotype in *Arabidopsis* plants (Yamaguchi *et al.,* 1998; Hu *et al.,* 2008; Rieu *et al.,* 2008b; Plackett *et al.,* 2012). In addition, GA treatment causes stem elongation in GA deficient mutants of pea in the presence of light (Yang *et al.,* 1996). Furthermore, GA also positively regulates root growth in seedlings. For example, inhibition of GA biosynthesis results in concentration-dependent inhibition and abnormal growth of the root (Ubeda-Tomás *et al.,* 2009). Moreover, in pea the role of GA in the induction of root growth has been demonstrated by using a high concentration of GA inhibitor (ancymidol) that inhibits GA biosynthesis and consequently root growth. However, inhibition of root growth could be rescued by application of a low dose (1 nM) of GA_3 whereas inhibition of shoot growth could

be reversed using a comparatively higher concentration (10 µM) of GA (Tanimoto, 1988), suggesting that roots are more sensitive to GA than the shoot tissues. All these results show that GA has a positive role in seedling growth mainly by increasing the number and length of cells (Yang *et al.,* 1996).

2.5.5 Cross-talk between Gibberellin and Brassinosteroids

Brassinosteroids play important roles in the regulation of GA biosynthesis and GA-mediated phenotypes in *Arabidopsis* (Unterholzner *et al.,* 2015). For instance, exogenous BR application causes seed germination in GA deficient and insensitive mutants (Leubner-Metzger, 2001; Steber and McCourt, 2001). On the other hand, exogenous GA treatment rescues plant developmental processes regulated by BR in BR signaling-defective mutants (*bri1-301*). For example, exogenous application of GA rescues germination and re-establishes hypocotyl elongation in BR mutants of *Arabidopsis* (Hofmann *et al.,* 2015; Unterholzner *et al.,* 2015). In addition, BR and GA have been reported to regulate each other's biosynthesis in *Arabidopsis*. Inhibition of BR production in BR biosynthetic mutants leads to a severe decrease in GA level of *Arabidopsis* through a decrease in the expression levels of *GA20ox* and *GA3ox* genes (Unterholzner *et al.,* 2015), whereas BR treatment causes upregulation of the *GA20ox* expression (Stewart Lilley *et al.,* 2013). Crosstalk between BR and GA is observed to undergo feedback regulation, as low levels of BR induced the biosynthesis of GA, while high BR level reduced GA biosynthesis and elevated GA catabolism (Tong *et al.,* 2014). However, in rice roots BR application was found to repress the expression of the *GA20ox* gene while enhancing that of *GA2ox* gene, suggesting the occurrence of antagonistic interaction between BR and GA in a tissue specific manner (De Vleesschauwer *et al.,* 2012; Tong *et al.,* 2014).

Crosstalk between BR and GA is also reported to occur at the signalling level (Unterholzner *et al.,* 2015) (Fig. 2.5). In *Arabidopsis*, interaction between BR and GA signaling has been shown to have a role in cell elongation (Gallego-Bartolomé *et al.,* 2012). For instance, hypocotyl elongation in *Arabidopsis* is synergistically regulated by BL and GA_3 at least at higher concentrations (Tanaka *et al.,* 2003). Root growth in *Arabidopsis* is also reported to be additively regulated by the interaction between BR and GA signaling (Müssig *et al.,* 2003). Moreover, crosstalk of BR and GA signaling on the basis of their concentrations is also observed in the regulation of flowering time in *Arabidopsis*. For instance, GA and BR deficient single mutants display a delay in the flowering time while double mutants of GA and BR showed further delay in flowering. A line expressing both BR and GA biosynthetic genes (*DWF4* and *GA5*), on the other hand, showed early flowering (Domagalska *et al.,* 2010). Seed germination may be regulated by the interaction between BZR1, a BR signaling component that regulates BR signaling positively, and RGA, a GA signaling component representing DELLA protein, a negative regulator of GA signaling (Li *et al.,* 2012). BRs also stimulate GA biosynthesis to enhance breakdown of the GA signaling negative regulator DELLA, thus releasing the repressed transcriptional factors BES1 and BZR1 (Ye *et al.,* 2011; Li *et al.,* 2012). The release of BES1 and BZR1 will lead to transcription of many downstream target genes required for plant developmental processes including seed germination. Thus, the interaction between transcription regulators BZR1/BES1 and DELLAs mediates the crosstalk between BR and GA (Li *et al.,* 2012).

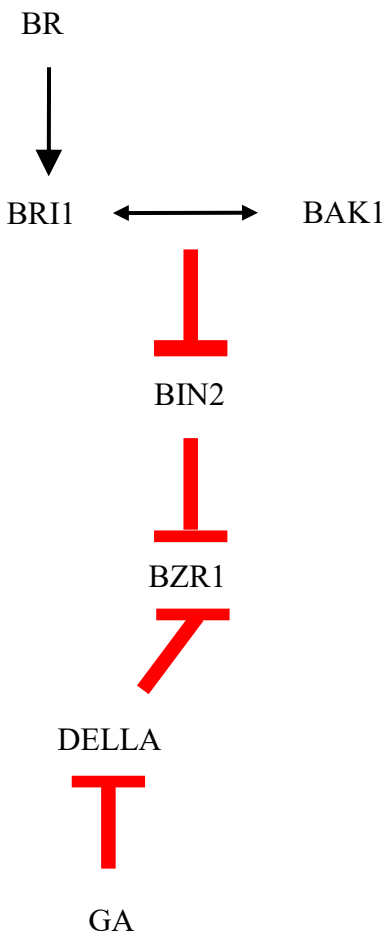

Figure 2.5 Schematic model outlining crosstalk of BR and GA signaling through BZR1 and DELLA proteins interaction. BZR1 and DELLAs show antagonistic effects in controlling transcription of BRs and GAs to regulate plant growth and development. Ultimately, it shows that GA is required for BR-intervened inhibition of seedling development in the dark. (Adapted from Lozano-Durán *et al.*, 2013; Li and He, 2013).

3.0 MATERIALS AND METHODS

3.1 Plant materials and growth conditions

Wheat (*T. aestivum* L.) genotype RL4452, which is derived from a backcross between wheat cultivar Glenlea*6 with Kitt, was used for this research. RL4452 produces non-dormant seeds at maturity. Mature seeds were collected from plants grown at 22/20°C (day and night) with 16/8 hours photoperiod.

3.2 Analysis of seed germination and seedling growth

Mature seeds of RL4452 were sterilized by treatment with 70% ethanol for 1 min, followed by surface sterilization with a 5% sodium hypochlorite solution for 20 min. Afterward, sterilized seeds were washed five times using sterile water. The sterilized seeds were placed between two layers of sterile Whatman #1 filter paper (GE Healthcare, Little Chalfont, UK) in the Petri-plate (20 seeds per plate per replicate, three replicates) and moistened with 7 ml of sterile water. To examine the effects of BR on seed germination and seedling growth, seeds were imbibed 0.5 mM of propiconazole (Pcz), BR biosynthesis inhibitor (Sigma-Aldrich, St. Louis, MO, USA) or 0.01 mM of 24-Epi-Brassinolide (EBL) (Sigma-Aldrich) and 0.5 mM Pcz. The Petri plates were then placed in a growth cabinet at 22°C in darkness. Seeds were marked as germinated when protrusion of coleorhiza through the seed coat was visible (Gao *et al.,* 2012). For seedling growth analysis, lengths of the coleoptile, and primary root and seminal root were measured for seven days using a millimeter scale.

3.3 Tissue harvesting and sample preparation

The endosperm (with aleurone layer), embryo, coleoptile, and root tissues were harvested from Pcz treated and control seeds for expression analysis of ABA metabolic and GA biosynthetic genes. The endosperm was harvested at 1, 3, 5 and 7 days after imbibition (DAI), and the embryo was harvested at 1 DAI while the coleoptile and root were harvested at 3, 5 and 7 DAI. The tissue samples after harvest were immediately frozen in liquid nitrogen and then stored at -80°C until further use.

3.4 RNA extraction

Total RNA was extracted from endosperm, embryo, coleoptile and root tissues. For endosperm tissue, RNA was isolated using method reported in Li and Trick (2005) with minor modifications. Approximately 50-100 mg tissue samples were ground into a fine powder using a pre-chilled mortar and pestle with liquid nitrogen, and the fine powder of the ground tissue was mixed well with 800 µl extraction buffer I (100mM Tris, 150mM LiCl, 50mM EDTA, 1.5% SDS, 1.5% β-mercaptoethanol). After complete mixing, 500 µl phenol-chloroform was added and mixed by inversion followed by centrifugation at 13000*g* for 15 min at 4°C. The upper aqueous phase was then transferred to a tube containing 500 µl extraction buffer II (4.2 M guanidinium sulphate, 25 mM sodium citrate, 0.5% lauryl sarcosine, 1 M sodium acetate) and mixed by gentle inversion. This mixture was then incubated at room temperature for 10 min followed by addition of 400 µl chloroform-isoamyl alcohol (24:1). Following centrifugation at 13000*g* for 15 min at 4°C, the supernatant was mixed with 600 µl isopropanol and 500 µl 1.2 M sodium chloride followed by incubation on ice for 15 min. This mixture was then centrifuged at 13000*g* for 15 min at 4°C and supernatant was discarded. Then, the RNA pellet was washed with 800 µl of 70% ethanol and then

air dried and resuspended in 50 µl RNase free water. Extracted total RNA was stored at -80°C for further use.

Total RNA from embryo tissue was extracted using a method reported in Mornkham (2013) with minor modifications. Approximately 50 mg embryo tissues were ground into a fine powder in liquid nitrogen using pestle and mortar. The fine powder was mixed with pre-chilled polyvinylpyrrolidone, and then 1 ml buffer I (8 M LiCl, 2% PVP, 5% β-mercaptoethanol) was added. The mixture was incubated at room temperature for 5 min and then mixed with 150 µl chloroform followed by centrifugation at 2400*g* for 5 min. Afterwards, supernatant was discarded, and the pellet was resuspended by adding 600 µl buffer II (1.4% SDS, 0.075 M NaCl, 0.025 M EDTA, 2% β-mercaptoethanol) followed by gentle mixing with 600 µl chloroform, and then centrifuged at 2400*g* for 5 min at 4°C. After centrifugation, the supernatant was transferred into a new tube and then mixed with the same volume of isopropanol. This mixture was incubated at -20°C for 15 min and then centrifuged at 12,000*g* for 10 min at 4°C. After discarding the supernatant, 1 mL of 75% ethanol was added and centrifuged at 7500*g* for 5 min at 4°C. The pellet in the tube was air dried and then 50 µl of RNase free water was added to dissolve the pellet. Following incubation at room temperature for 5 min, 700 µl Trizol was added to the dissolved RNA pellet and vortexed briefly. Then 140 µl chloroform was added and mixed well followed by incubation for 3 min at room temperature and centrifuged for 15 min at 12000*g*. After centrifugation, the supernatant was again transferred into a new tube and mixed with same volume of isopropanol followed by incubation and centrifugation at the same conditions as described earlier. Supernatant was again discarded. The RNA pellet was then washed again with 1 ml of 75% ethanol solution and centrifuged for 5 min at 7500*g*. The total RNA sample was dissolved by

adding 30-50 µl RNase free water. The extracted total RNA sample was immediately stored at -80°C for further use.

Total RNA from coleoptile and root was isolated using TRIzol® reagent following the manufacturer's protocol (Life Technologies, Carlsbad, CA, USA).

3.5 DNase treatment

The extracted total RNA was digested with DNase (DNA-free kit, Ambion, TX, USA) to get rid of any genomic DNA contamination. Briefly, 5 µL 10X DNase buffer and 1 µl DNase were added to 10 µg of total RNA to a total reaction volume of 50 µL followed by incubation at 37°C for 30 min. A 5 µL DNase inactivation reagent was subsequently mixed, and the mixture was incubated again for 5 min at room temperature. Following centrifugation at 10000*g* for 2 min, purified RNA was transferred to a fresh tube for future use.

3.6 cDNA synthesis

The purified total RNA from endosperm, embryo, coleoptile, and root was used for cDNA synthesis using the iScript cDNA synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's guidelines. In brief, 1 µg of total RNA was mixed with 4 µl of 5X iScript reverse transcription supermix and nuclease-free water to get a total reaction volume 20 µl. The complete reaction mixture was then incubated for 5 min at 25℃, reverse transcription for 20 min at 46℃ and reverse transcription inactivation for 1 min at 95℃. The resulting cDNA was diluted 20X using sterile water and the stored at -20℃ until further use for RT-qPCR analysis.

3.7 Primers

Primers for wheat ABA biosynthetic genes, *NCED1* and *NCED2*, ABA catabolic genes, *CYP707A1* and *CYP707A2*, GA biosynthetic genes, *GA20ox1*, *GA20ox2* and *GA3ox2*, and *β-actin* as a reference gene (Table 3.1) were designed for real time qRT-PCR as described previously (Liu *et al.*, 2013; Pearce *et al.*, 2015; Son *et al.*, 2016; Izydorczyk *et al.*, 2018).

Table 3.1 Primers used for expression analysis of ABA metabolic and GA biosynthetic genes.

Gene	Type	Sequence (5’ to 3’)
NCED1	FP	ACCTCTGGAACTCGTGGGA
	RP	CGTCCGTGTCGTTGAAGAT
NCED2	FP	GCTTCTGCTTCCACCTCTG
	RP	GCACTCGTCCGACTCGTT
CYP707A1	FP	CCATGACCTTCACCCGCAAG
	RP	GGACACTGACGGATGGAGAAC
CYP707A2	FP	TGACGCACAGGGTGATTT
	RP	CCCTTGGGGATAAGAAACC
GA20ox1	FP	CCCTGGAAGGAGACCCTCT
	RP	GGCTCATCTCCGAGCAGTAG
GA20ox2	FP	AAGCTTCCCTGGAAGGAGAC
	RP	TCCCCTAGGTGCATGAAGTC
GA3ox2	FP	GCCCATCTCCTCCTTCTTCT
	RP	CCCTGTGGAACTCCTCCAT
β-Actin	FP	GCTGGAAGGTGCTGAGGGA
	RP	GCATCGCCGACAGGATGAG

FP, forward primer; RP, reverse primer

3.8 Real-time quantitative (q) RT-PCR

Real-time qRT-PCR was carried out with 5 µl diluted cDNA as template, 1.2 µl forward and reverse primers (5 µM; final concentration 300 nM), 10 µL SsoFast Eva Green Supermix (Bio-Rad) and 2.6 µl water in a total reaction volume of 20 µL on CFX96 real-time system (Bio-Rad). qRT-PCR assay was performed in three biological replicates for both control and Pcz treated samples and each replicate in duplicate. The following thermal cycling conditions were applied: initial denaturation and DNA polymerase activation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for the 30s, annealing at 60°C for 30s and extension at 72°C for 30s. Relative transcript level was determined by the $2^{-\Delta\Delta Ct}$ method reported in Livak and Schmittgen (2001). The expression level was analyzed using β-actin gene (*Taβactin*) as the housekeeping gene to normalize transcript levels of target genes. The transcript levels for the target genes were determined relative to their expression level in the controls at 1 DAI for endosperm and embryo, and at 3 DAI for coleoptile and root tissues, which were set to 1.

3.9 Statistical analysis

Student's t-test ($P<0.05$) was used to identify statistically significant difference in germination, seedling growth and gene expression data between the control and treated samples.

4.0 RESULTS

4.1 Seed germination in response to inhibition of BR biosynthesis

Inhibition of BR biosynthesis with Pcz did not affect seed germination (Fig. 4.1). In both control and treated seeds, emergence of coleorhiza through the seed coat was visible in ~98% of the seeds at 1 day after imbibition, and 100% of the seeds completed their germination after 2-days of imbibition.

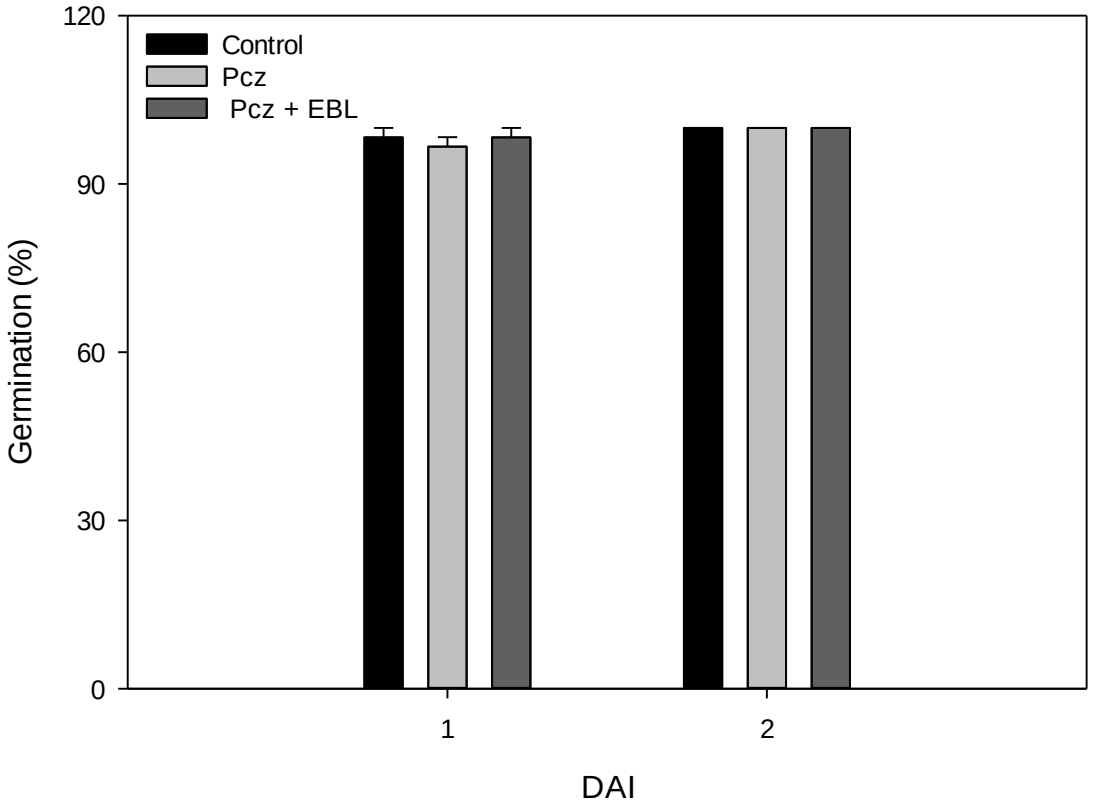

Figure 4.1 Effect of treatment with BR biosynthesis inhibitor, propiconazole (0.5 mM) or propiconazole (0.5 mM) + 24-Epi-Brassinolide (0.01 mM) on seed germination. Data are means of three independent biological replicates ± standard error. DAI: days after imbibition.

4.2 Seedling growth in response to inhibition of BR biosynthesis

The effect of seed treatment with Pcz on seedling growth was evaluated by measuring length of the three tissues including coleoptile, and primary and seminal root (Fig. 4.2). Inhibition of BR biosynthesis with Pcz treatment significantly inhibited coleoptile, and primary and seminal root growth as compared to the control tissues throughout 7 DAI; the largest reduction in length was observed in the coleoptile.

To test whether the reduction in growth of coleoptile, and primary and seminal root treated with Pcz is due to inhibition of BR biosynthesis, seeds were imbibed with a combination of Pcz and EBL. The inhibitory effect of Pcz on coleoptile growth was partially reversed by treatment with EBL (Fig. 4.2A). Treatment of Pcz in combination with EBL significantly reduced primary and seminal root lengths during later phases of imbibition (4, 5, 6 and 7 DAI) as compared to that observed in the controls or those treated with Pcz alone (Fig. 4.2B, C).

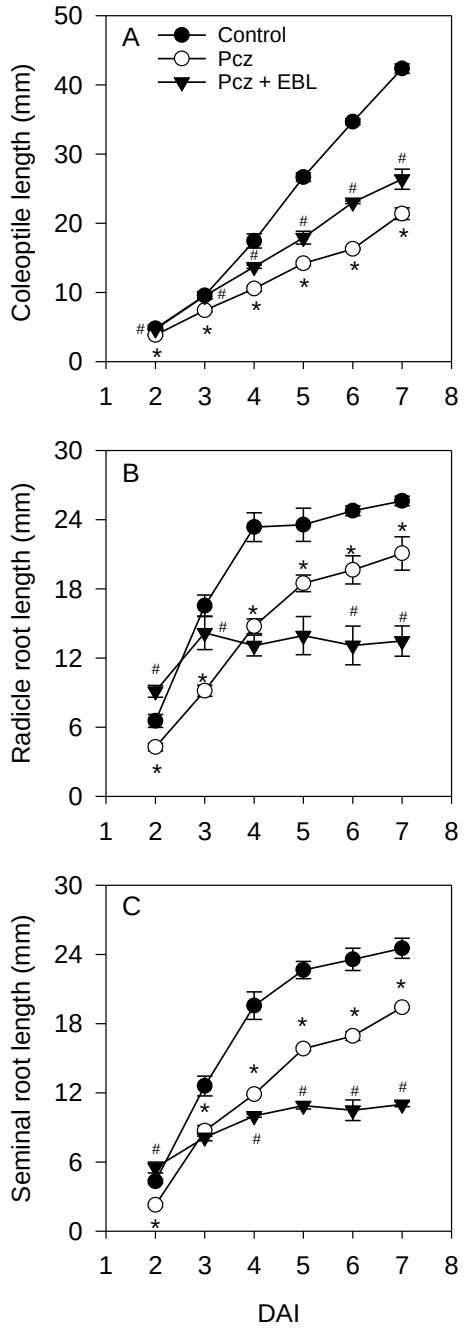

Figure 4.2 Effect of Pcz or Pcz in combination with EBL on the growth of coleoptile (A), primary root (B), and seminal root (C) tissues. Data are means of three replicates ± standard error. The * and # symbols indicates a statistically significant difference in length between the control and Pcz and between Pcz or Pcz + EBL treated samples, respectively.

4.3 Expression of ABA metabolism genes during seed germination and seedling growth

4.3.1 Expression of ABA metabolic genes in endosperm

This study revealed that *NCED2* was expressed in the endosperm during seed imbibition and germination but no expression of *NCED1* was detected. The expression of *NCED2* was similar from 1 to 5 DAI, then increased substantially at 7 DAI in both control and treated samples. Treatment of Pcz did not affect its expression (Fig. 4.3A).

The *CYP707A1* and *CYP707A2* genes were detected in the endosperm throughout imbibition. The expression of *CYP707A1* in control samples was maintained at a similar level from 1 to 7 DAI (Fig. 4.3B). Treatment with Pcz significantly upregulated the expression levels of this gene at 1, 3 and 7 DAI by 2- to 9-fold. The expression of *CYP707A2* in control samples strongly increased from 1 to 3 DAI and then decreased to lower levels at 5 and 7 DAI. As a result of Pcz treatment, the expression of *CYP707A2* was significantly upregulated by 6-fold at 1 DAI but downregulated by over 2-fold at 3 and 5 DAI (Fig. 4.3C).

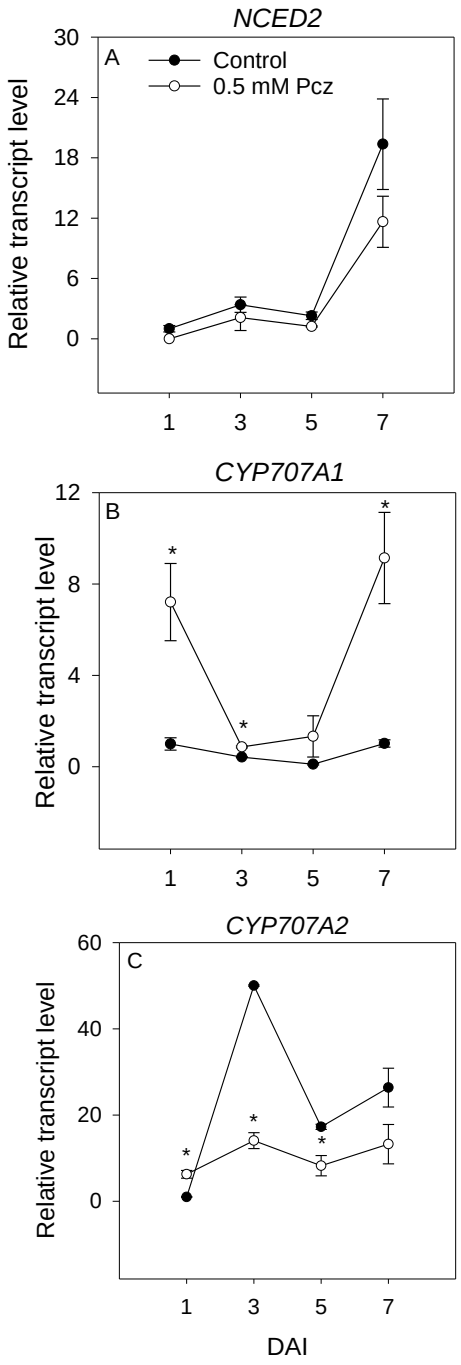

Figure 4.3 Effect of Pcz treatment on the transcript levels of *NCED1* (A), *CYP707A1* (B) and *CYP707A2* (C) in the endosperm tissues during seed imbibition and germination. Transcript levels of were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control endosperm at 1 day after imbibition (DAI), which was to 1. Data are means of three replicates and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated endosperm tissues. Transcript of *NCED1* were not detected.

4.3.2 Expression of ABA metabolic genes in the embryo

The expression of the two *NCED* genes was studied in embryo tissue at 1 day after imbibition. Only *NCED2* was expressed in the embryo at 1 DAI. However, Pcz treatment did not have significant effect on the expression of this gene (Fig. 4.4A). This study also analyzed the expression of the two *CYP707A* genes. The expression of *CYP707A1* was observed in the embryo at 1 DAI. The Pcz treatment caused 6-fold reduction in the expression of *CYP707A1* as compared to the control embryo at 1 DAI (Fig. 4.4B). The expression of the other *CYP707A* gene (*CYP707A2*) was not detected.

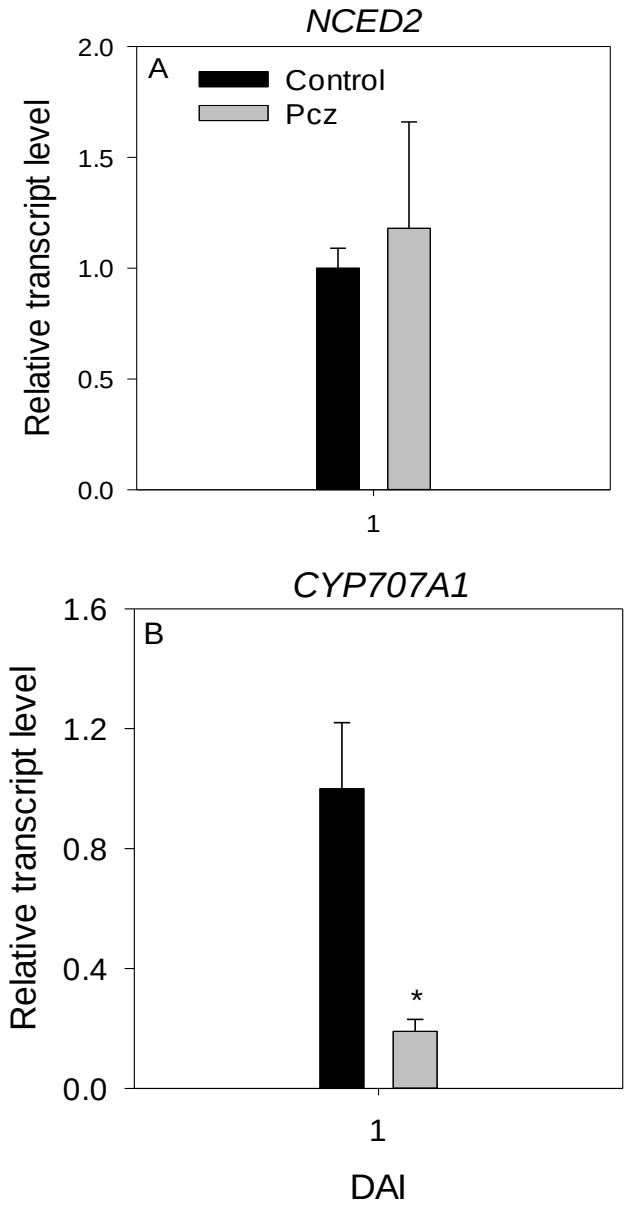

Figure 4.4 Effect of Pcz treatment on the transcript levels of *NCED1* (A) and *CYP707A1* (B) in the embryo tissues at 1 DAI. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control embryo at 1 days after imbibition (DAI), which was set to 1. Data are means of three replicates except *CYP707A1* with 2 replicates and error bars represent standard errors. The asterisks indicate statistically significant difference between control and Pcz treated embryo tissues. Transcripts of *NCED1* and *CYP707A2* were not detected.

4.3.3 Expression of ABA metabolic genes in the coleoptile

The study also analyzed the expression of the two NCED genes in the coleoptile of the seedlings studied. The expression of *NCED1* was not detected. According to the data shown in Fig. 4.5A *NCED2* was expressed in the coleoptile from 3 to 7 DAI. The expression of *NCED2* increased from 3 to 5 DAI and maintained at almost similar level from 5 to 7 DAI in control samples. The expression of *NCED2* in 5 DAI coleoptiles was significantly upregulated (2-fold) by the Pcz treatment.

Both *CYP707A1* and *CYP707A2* genes were expressed in the coleoptile (Figs. 4.5B, C). The expression of *CYP707A1* in the control coleoptile first increased from 3 to 5 DAI and then decreased from 5 to 7 DAI (Fig. 4.5B). The expression of *CYP707A2* continually increased with imbibition (Fig. 4.5C). Pcz treatment downregulated the expression of *CYP707A1* at 5 DAI by 1.7-fold and *CYP707A2* at 7 DAI by 5-fold. During early stage (3 DAI), the expression of both *CYP707A1* and *CYP707A2* genes was not be affected by Pcz treatment.

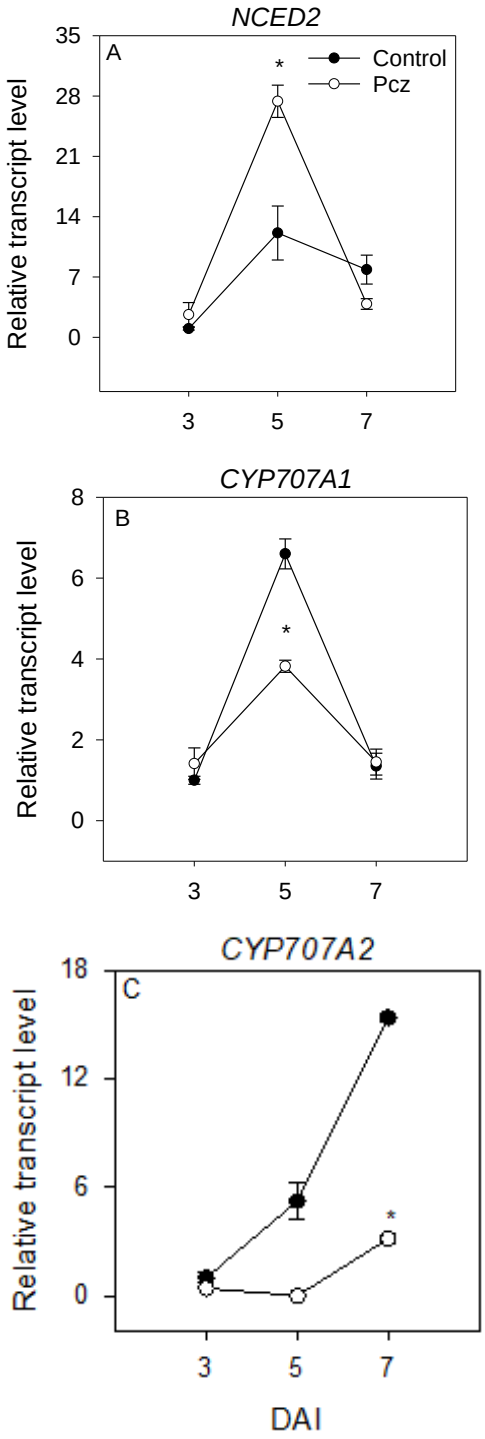

Figure 4.5 Effect of Pcz treatment on the transcript levels of *NCED1* (A), *CYP707A1* (B) and *CYP707A2* (C) in the coleoptile tissues. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control coleoptile at 3 day after imbibition (DAI), which was set to 1. Data are means of three replicates except 5 DAI and 7 DAI samples are with two to three replicates and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated coleoptile samples. Transcripts of *NCED1* were not detected.

4.3.4 Expression of ABA metabolic genes in the root

The study also examined the expression of ABA metabolic genes in the root tissues. The *NCED1* gene was not expressed in the root tissue at any stage. In contrast, the expression of *NCED2* was detected in the root at all stages (Fig. 4.6A). Its expression peaked at 5 DAI in the control samples, and Pcz treatment caused an over 3-fold decrease in the expression levels of *NCED2* at 3, 5 and 7 DAI by 3.4-, 4.9- and 3.6-fold. The expression of *CYP707A1* was detected in the root from 3 to 7 DAI (Fig. 4.6B). In control roots, the expression of *CYP707A1* continuously increased with growth, but its expression showed a 3-fold down regulation by Pcz treatment at 7 DAI (Fig. 4.6B). The expression of *CYP707A2* was not detected.

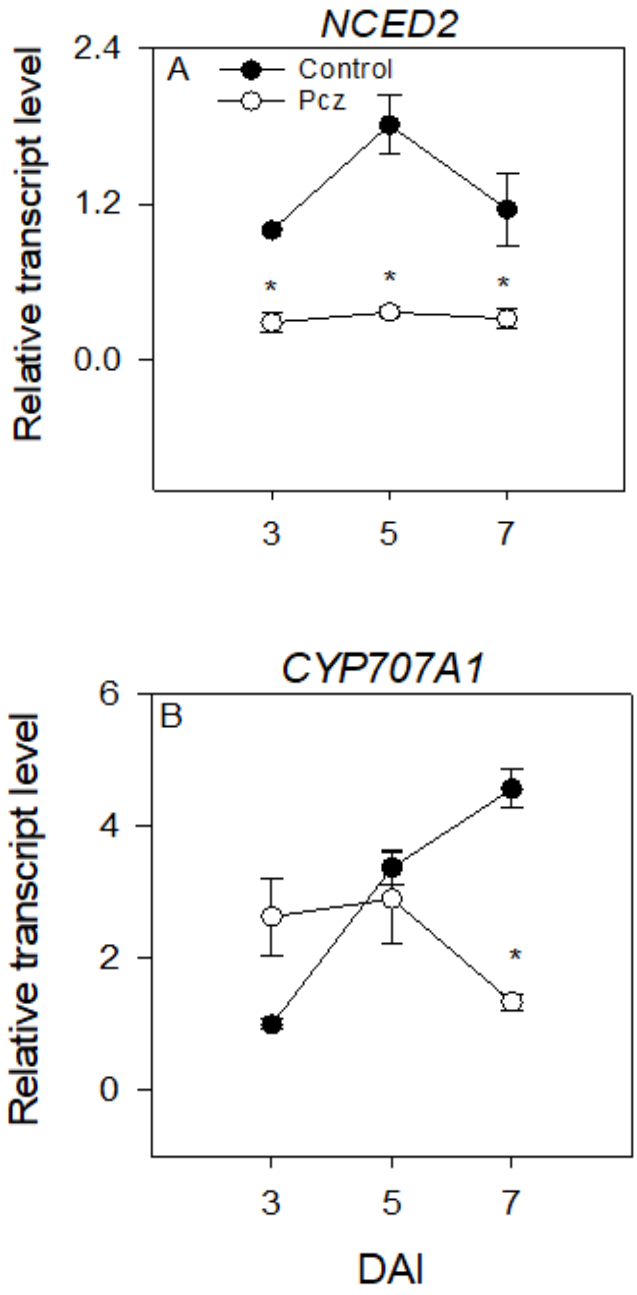

Figure 4.6 Effect of Pcz treatment on the transcript levels of *NCED1* (A) and *CYP707A1* (B) in the root tissues. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control root at 1 day after imbibition (DAI), which was set to 1. Data are means of three replicates except data for *NCED2* and *CYP707A1* are with two to three replicates and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated roots. Transcripts of *NCED1* and *CYP707A2* were not detected.

4.4 Expression of GA metabolism genes during seed germination and seedling growth

4.4.1 Expression of GA biosynthetic genes in the endosperm

The transcripts of *GA20ox1* and *GA3ox2* were present in the endosperm from 1 through 7 DAI (Fig. 4.7). While the expression of *GA20ox2* was not detected. The expression of *GA20ox1* in both control and treated samples was low from 1 to 5 DAI but increased at 7 DAI (Fig. 4.7A). The expressions of *GA20ox1* was upregulated by 4- and 2.4-fold by the Pcz treatment at 1 and 5 DAI, respectively. In contrast, Pcz treatment reduced the expression of *GA20ox1* at 7 DAI by 3-fold. The expression of *GA3ox2* in the endosperm was also low from 1 to 5 DAI (Fig. 4.7B). Its expression, similar to that of *GA20ox1*, increased by 7 DAI in both control and treated endosperm tissues. The Pcz treatment upregulated the expression of *GA3ox2* at 1 DAI but caused a decrease in the expression of this gene at 3 and 5 DAI.

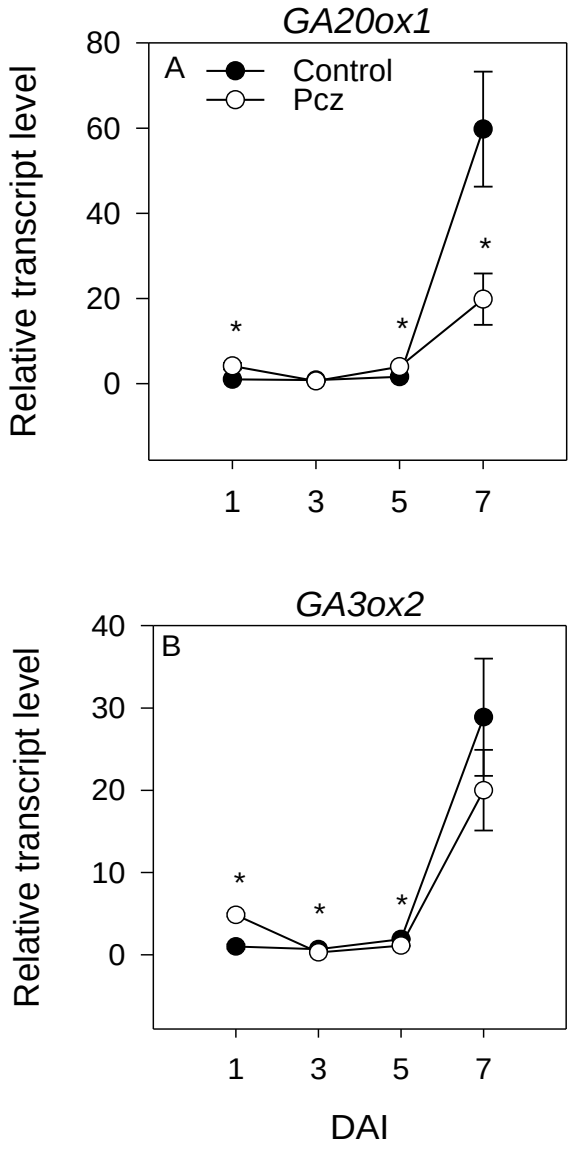

Figure 4.7 Effect of Pcz treatment on the transcript levels of *GA20ox1* (A) and *GA3ox2* (B) in the endosperm tissues. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control endosperm at 1 day after imbibition (DAI), which was set to 1. Data are means of three replicates except data for *GA20ox1* (Pcz at 1 DAI) and *GA3ox2* (Pcz at 1 DAI and 5 DAI) with two replicates each and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated endosperm tissues. Transcripts of *GA20ox2* were not detected.

4.4.2 Expression of GA biosynthetic genes in the embryo

The transcripts of *GA20ox1* and *GA20ox2*, and *GA3ox2* were present in the embryo at 1 DAI (Fig. 4.8). The Pcz treatment did not show any effect on the expression of *GA20ox1* and *GA20ox2* genes (Fig. 4.8A, B). In contrast, the treatment caused a marked decrease (3-fold) in the expression level of *GA3ox2* (Fig. 4.8C).

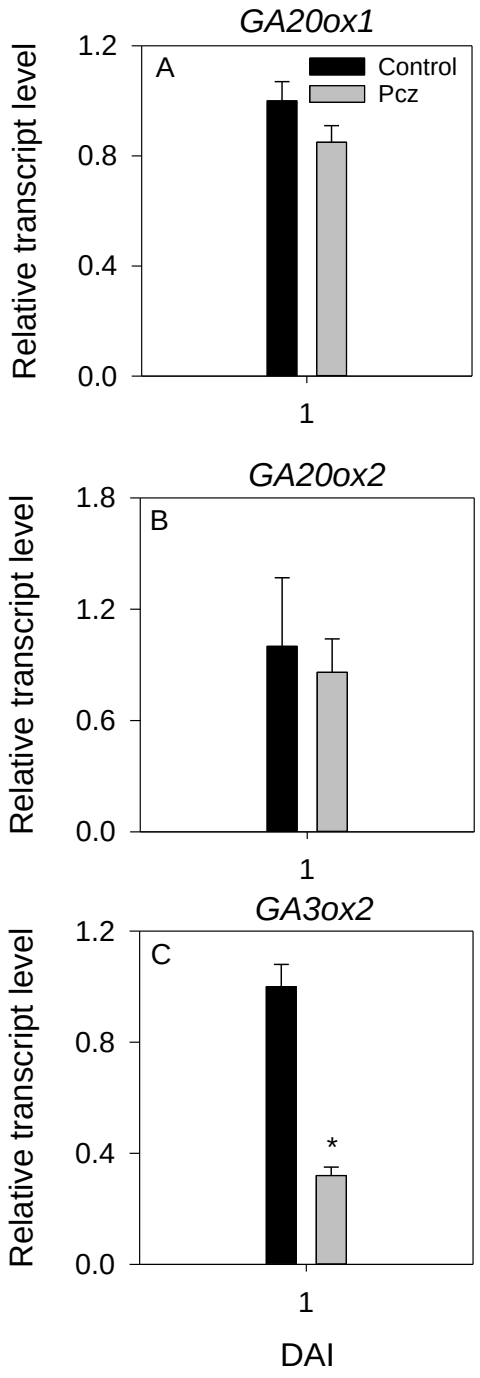

Figure 4.8 Effect of Pcz treatment on the transcript levels of *GA20ox1* (A), *GA20ox2* (B) and *GA3ox2* (C) in the embryo tissues at 1 day after imbibition (DAI). Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control embryo, which was set to 1. Data are means of three replicates except data for *GA20ox1* (control at 1 DAI) and *GA3ox2* (1 DAI) with two replicates each and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated embryos.

4.4.3 Expression of GA biosynthetic genes in the coleoptile

Transcripts of *GA20ox1*, *GA20ox2* and *GA3ox2* were detected in the coleoptiles at all stages (Fig. 4.9). The expression levels of *GA20ox1* and *GA20ox2* increased from 3 to 5 DAI, when they showed the maximum level in both control and treated coleoptiles (Fig. 4.9A, B). The expression of *GA20ox1* showed no significant difference between the control and treated samples (Fig. 4.9A). In contrast, the expression of *GA20ox2* was increased more than two-fold by Pcz treatment at 5 DAI and approximately two-fold at 7 DAI (Fig. 4.9B). The expression level of *GA3ox2* in control increased from 3 to 5 DAI but decreased from 5 to 7 DAI (Fig. 4.9C). The expression of *GA3ox2* at 3 DAI was upregulated about 5-fold by Pcz treatment.

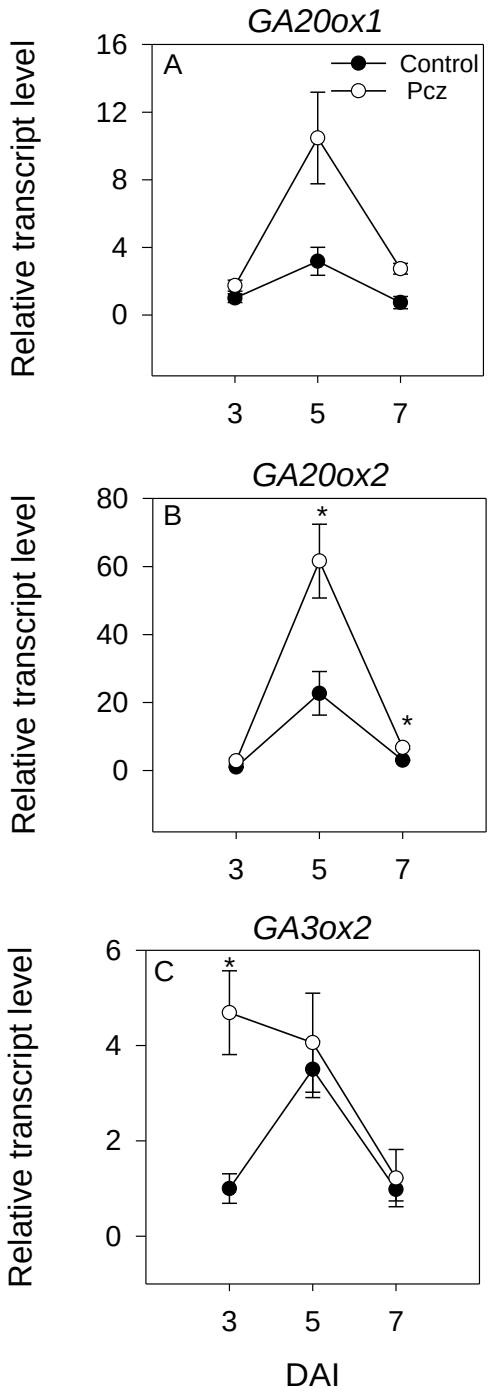

Figure 4.9 Effect of Pcz treatment on the transcript levels of *GA20ox1* (A), *GA20ox2* (B) and *GA3ox2* (C) in the coleoptile tissues. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control coleoptile at 3 days after imbibition (DAI), which was set to 1. Data are means of three replicates except 3 DAI Pcz (*GA20ox1*, *GA20ox2*, *GA3ox2*) and 7 DAI (*GA20ox1*, *GA20ox2*) samples, which are with two replicates and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated coleoptiles.

4.4.4 Expression GA biosynthetic genes in the root

Of three analyzed GA biosynthetic genes, the expressions of *GA20ox1* and *GA3ox2* were detected in the root tissues at all stages (Fig. 4.10). The *GA20ox2* gene was not expressed in the root tissue. The expression of *GA20ox1* and *GA3ox2* in the control root increased from 3 to 5 DAI (Fig. 4.10A, B). The expression observed at 5 DAI was the highest level of expression for both genes. A decline in expression level of *GA20ox1* and *GA3ox2* was observed from 5 to 7 DAI. Pcz treatment increased the expression level of *GA20ox1* by 2.8-fold at 3 DAI as compared to the control (Fig. 4.10A). However, the treatment downregulated the expression of this gene at 5 DAI. The effect of Pcz on expression of *GA3ox2* was observed at 7 DAI, when it caused a 5.6-fold decrease in expression (Fig. 4.10B).

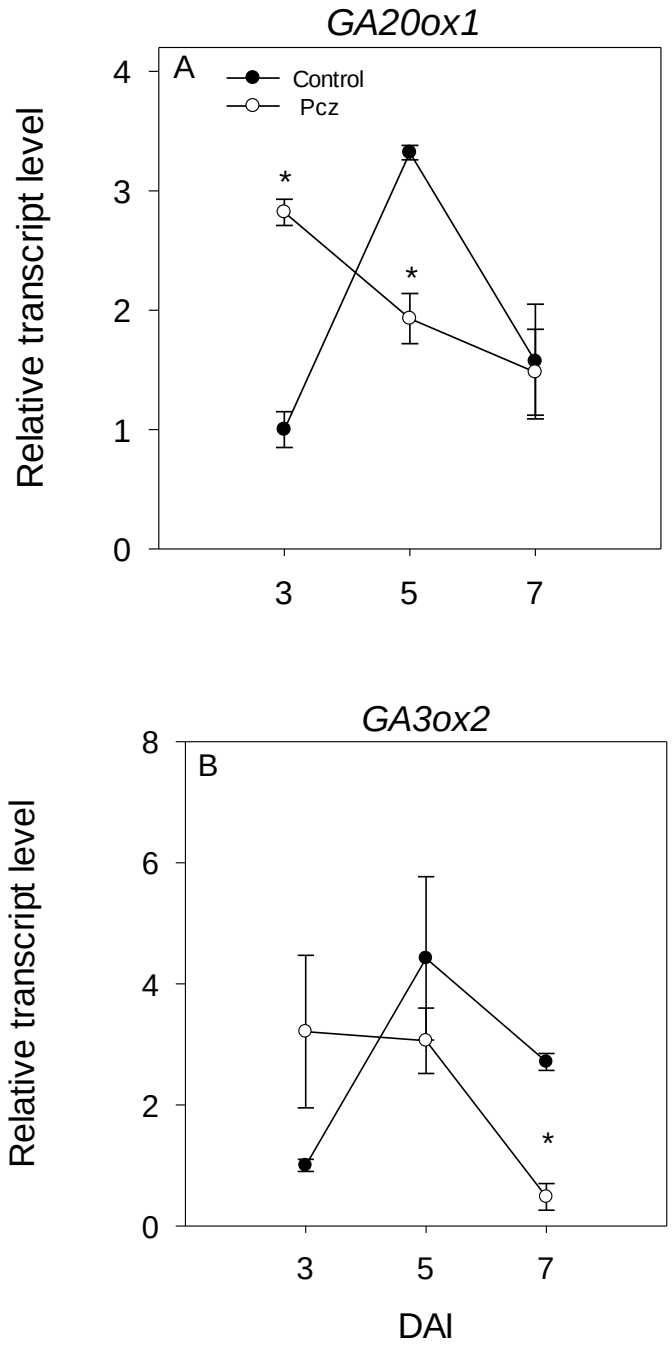

Figure 4.10 Effect of Pcz treatment on the transcript levels of *GA20ox1* (A) and *GA3ox2* (B) in the root tissues. Transcript levels of the genes were determined using *β-actin* as a reference gene and the transcript levels of each gene was expressed relative to that found in the control root at 3 days after imbibition (DAI), which was set to 1. Data are means of three replicates except 3 DAI and 5 DAI of *GA20ox1* and *GA3ox2* with two to three replicates and error bars represent standard errors. The asterisks indicate significant difference between control and Pcz treated roots. Transcripts of *GA20ox2* were not detected.

5.0 DISCUSSION

This thesis investigated the regulation of seed germination and seedling growth, and expression of ABA and GA metabolic genes in wheat induced by change in BR level. Germination tests showed that inhibiting BR biosynthesis by Pcz did not affect germination (Fig 4.1). This could be due to the presence of endogenous BR that is adequate for the completion of the germination process. A previous study also showed treatment with BR biosynthesis inhibitor Brz has no effect on the germination of *Arabidopsis* seeds (Asami *et al.*, 2000).

In contrast, treatment with Pcz significantly reduced the growth of different parts of seedlings including coleoptile, and primary and seminal root (Fig. 4.2). Similarly, Pcz negatively affected root length in *Arabidopsis* (Hartwig *et al.,* 2012) and hypocotyl length in cress plants (*Lepidium sativum*) (Sekimata *et al.,* 2002). When comparing its effect between coleoptile length, and primary and seminal root length, the Pcz effect was strongest on coleoptile growth (Fig. 4.2). The reduced effect of Pcz on primary and seminal root growth may suggest that different tissues have different levels of sensitivity to BR (Fig. 4.2A, B, C). Studies in *Arabidopsis* and maize also reported the tissue-specific reduction of mesocotyl, coleoptile, and true leaf elongation by Pcz treatment (Hartwig *et al.,* 2012, Ling *et al.*, 2017). Exogenous application of 24-epi-brassinolide (EBL) rescued the decrease of coleoptile growth by Pcz, indicating the suppression of coleoptile growth by Pcz is the result of decrease in BR level in the tissue (Fig. 4.2A). This suggests the direct physiological role of BR in promoting coleoptile growth in wheat seedlings (Sasse, 1985). EBL was also found to rescue primary and seminal root growth at 2 DAI but inhibited the elongation in later timepoints (Fig. 4.2B, C). This decrease in root elongation suggests that root growth has its own optimal amount of BR, and high EBL level (0.01 mM) or excessive BR causes negative effect. In support of this, Müssig *et al.* (2003) showed in previous study that low

concentration of EBL (0.05 nM) stimulate root growth but higher concentration of EBL (5 nM) inhibit root growth in *Arabidopsis*.

Brassinosteroids and ABA phytohormones control various plant growth and developmental processes including seed germination and seedling growth by interacting antagonistically (Hu and Yu, 2014; Wang *et al.,* 2018). Studies showed that ABA inhibits seed germination in BR deficient and BR response mutants (*det2-1* and *bri1-1*) (Steber and McCourt, 2001), and BL application overcome germination inhibitory effect of ABA (Zhang *et al.,* 2009). ABA level in plants is regulated by its biosynthesis and catabolism (Nambara and Marion-Poll, 2005). Pcz treatment that inhibited coleoptile growth reduced the expression of *CYP707A1* in embryo at 1 DAI (Fig. 4.4B) and increased the expression of *NCED2* but decreased expressions of both *CYP707A1* and *CYP707A2* in the coleoptile at 5 DAI (Fig. 4.5A-C). These results suggest that inhibition of BR biosynthesis by Pcz induces accumulation of ABA by inducing biosynthetic while repressing catabolic genes, and the accumulated ABA inhibited coleoptile growth. Furthermore, these results can be further validated by measuring the endogenous ABA content. In agreement, a previous study in *Arabidopsis* showed that EBL treatment causes a decrease in ABA accumulation by downregulating expression of ABA biosynthetic genes *NCEDs* and *AAO3* (Ha *et al.,* 2018). In addition, exogenous BR treatment was found to inhibit the interaction of BR signalling inhibitor, BIN2, with ABI5, a positive regulator of ABA signaling, to antagonize the effect of ABA in inhibiting seed germination (Hu and Yu, 2014).

In contrast, Pcz treatment caused downregulation of *NCED2* in root at 3, 5 and 7 DAI and of *CYP707A1* at 7 DAI (Fig. 4.6A), proposing role of BR in altering the expression levels of ABA metabolic genes. Moreover, BR treatment was found to increase the expression of ABA biosynthetic gene *NCED1* and ABA accumulation while BR biosynthesis inhibitor, Brz, decreased

ABA biosynthesis in maize leaves (Zhang *et al.,* 2010). Moreover, BR mutants were found to show hypersensitivity to ABA (Chung *et al.,* 2014). In addition, the upregulation of *NCED2* in coleoptile and its downregulation in the root by Pcz treatment suggests the tissue-specific regulation of this gene by BR. In view of this hypothesis, five members of the *NCED* gene family of *Arabidopsis* are reported to differentially express and localize in specific tissues to control the level of ABA (Tan *et al.,* 2003). In the endosperm, Pcz increased the expression of *CYP707A1* but decreased the expression of *CYP707A2* at the late stage of imbibition (Fig. 4.3B, C), suggesting gene specific regulation of wheat *CYP707A* family by BR. A study in *Arabidopsis* also showed that the expression of only *CYP707A3* but not the other three *CYP707A* genes is regulated by BR (Saito *et al.,* 2004).

Plant growth and development processes including seed germination, cell elongation, vascular differentiation, stomatal formation and flowering are regulated by interaction between BR and GA (Steber and McCourt, 2001; Bouquin *et al.,* 2001; Weiss and Ori, 2007; Stewart Lilley *et al.,* 2013; Saini *et al.,* 2015). Brassinosteroid and GA can have synergistic interaction through the participation of BR transcription factors and DELLA proteins (Gregory and Mandava, 1982; Tanaka *et al.,* 2003; Depuydt *et al.,* 2011; Hofmann, 2015).

In the endosperm and root, the expression of *GA20ox1* and *GA3ox2* increased at the early stage but decreased at later stages of seed imbibition with Pcz treatment, suggesting that a decrease in BR level alters GA biosynthesis in a stage specific manner (Figs. 4.7A, B and 4.10A, B). A previous study in *Arabidopsis* also reported stage-specific action of DELLA proteins in the promotion or inhibition of BR mediated response towards growth (Stewart Lilley *et al.,* 2013). In maize seedlings, expression of *GA20ox1* was found to be upregulated by Pcz at the earlier stage but downregulated at the later stage (Ling *et al.,* 2017). Reduction in expression of a GA

biosynthetic gene was reported in BL treated roots of rice (De Vleesschauwer *et al.,* 2012). In rice, reduction of GA biosynthetic gene expression by BR was associated with accumulation of DELLA proteins (De Vleesschauwer *et al.,* 2012), and BR was seen to stabilize the level of DELLA proteins that resulted in no change in the expression of GA biosynthetic genes (*GA20ox* and *GA3ox*) in *Arabidopsis* (Unterholzner *et al.,* 2015). Decrease in the expression of *GA3ox2* in the embryo at 1 DAI (Fig. 4.8C), and *GA20ox1* and *GA3ox2* in the root at later stages (Fig. 4.10A, B) by Pcz treatment suggests the decrease in GA biosynthesis with decrease in BR and therefore synergistic interaction between BR and GA in wheat. Increases in the expression of *GA20ox1*, *GA20ox2* and *GA20ox5* were reported in *Arabidopsis* seedling grown from BR treated seeds (Steber and McCourt, 2001; Stewart Lilley *et al.,* 2013). In addition, expressions of *GA20ox1* and *GA3ox1* were reported to be reduced in the BR deficient mutants of *Arabidopsis* and rice (Bouquin *et al.,* 2001; Gallego-Bartolome *et al.,* 2012; Tong *et al.,* 2014; Unterholzner *et al.,* 2016).

Because BR and GA show synergistic cross-talk, upregulation of *GA20ox2* expression at 5 and 7 DAI and *GA3ox2* expression at 3 DAI in the coleoptile by Pcz (Fig. 4.9B, C) may indicate the negative feedback regulation of the two genes. Moreover, the upregulation of *GA20ox2* and *GA3ox2* expression in the coleoptile by Pcz suggest antagonistic interaction between the BR and GA. This type of antagonistic interaction of BR with GA was suggested by previous studies in *Arabidopsis* that reported higher mRNA level of a GA-responsive gene (*GASA1*) and lower mRNA level of a GA repressor gene (*GA5*) in the BR insensitive mutant (*bri1-201*) than the control (Bouquin *et al.,* 2001). Degradation and activation of DELLA proteins by BR signaling transcription factors play a crucial role in the regulation of BR and GA interaction. These results suggest that BR plays role in regulating the expression of ABA metabolic and GA biosynthetic genes during and after seed germination based on tissue, stage and gene specificity. Overall, the

data from this thesis project indicates that changes in the expression of ABA metabolic and GA biosynthetic genes are coordinated with effects of BR on seedling growth.

6.0 GENERAL DISCUSSION AND CONCLUSION

Seed germination plays an important role in the plant's life cycle. It starts with water uptake and terminates with radicle protrusion through the seed coat. The first phase involves repair of DNA and mitochondria, protein synthesis from extant mRNAs, change in the shape and size of the seed; the second phase is characterized by the synthesis of proteins from newly produced mRNAs and mitochondria; and the third phase leads to mobilization of stored reserves and radicle elongation. Seed germination is regulated by plant hormones in which ABA and GA are the major regulators. Brassinosteroids are among the plant hormones that are involved in the regulation of a wide range of plant growth and developmental processes including germination and seedling growth. BR interaction with ABA and GA and its role in the regulation of seed germination and seedling growth has been studied in different species. Nonetheless, the exact molecular mechanism involved in regulation of seed germination and seedling growth by BR still needs to be studied in cereals such as wheat.

The use of hormone biosynthesis or signaling inhibitors has played an important role in studying and evaluating the function of phytohormones in different plant growth and development processes. This study used a BR biosynthesis inhibitor referred to as propiconazole (Pcz), which inhibits the function of P450s and thereby BR biosynthesis, for elucidation of the different roles of BR in wheat seed germination and seedling growth. This study conducted a detailed analysis of the effects of Pcz and therefore inhibition of BR synthesis on seed germination, seedling growth and expression pattern of the ABA metabolic and GA biosynthetic genes to gain understanding of BR interaction with ABA and GA in wheat and elucidate its roles in these developmental processes. The results of this study showed no effect of inhibiting BR synthesis by Pcz on germination percentage and rate, but the treatment reduced seedling growth. Co-application of a

BR with the inhibitor rescued growth of the coleoptile, but the primary and seminal roots growths were rescued only at early days of their growth as compared to seeds treated with and without BR biosynthesis inhibitor.

Further, the study analyzed the effect of suppressing the biosynthesis of BR by Pcz on the expression of ABA metabolic genes including family members of the ABA biosynthetic gene *NCED* and ABA catabolic gene *CYP707A* in different tissues over several days after imbibition. Propiconazole treatment increased the expression of *NCED2* in coleoptile but reduced the expression of *CYP707A2* in the endosperm, *CYP707A1* in embryo, *CYP707A1* and *CYP707A2* in coleoptile and *CYP707A2* in root tissue, indicating that BR plays role in the regulation of ABA metabolic genes in different tissues.

In addition to the ABA metabolism genes, the study also examined the effects of suppressing the biosynthesis of BR by Pcz on the expression of GA metabolism gens including family members of the GA biosynthetic genes, *GA20ox* and *GA3ox*. The expression of GA biosynthetic genes, *GA20ox1* and *GA3ox2* showed upregulation at earlier and downregulation at later stages of imbibition in endosperm suggesting the stage-specific regulation of these genes by BR. While decreases in expression of *GA3ox2* in embryo and root of Pcz treated seeds suggest the synergistic interaction between GA and BR. Increased expression of *GA20ox2* and *GA3ox2* by Pcz in the coleoptile suggests reduction of GA level that causes negative feedback regulation of GA biosynthesis.

In conclusion, these findings give some information about the interaction of BR with GA and ABA in the regulation of seed germination and seedling growth in wheat. However, further functional studies with mutants is needed to gain detailed knowledge on this topic.

LITERATURE CITED

Abhinandan K., Skori L., Stanic M., Hickerson N. M., Jamshed M., Samuel M. A. (2018). Abiotic stress signaling in wheat–an inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Front. Plant Sci.* 9, 734.

Achard P., Genschik P. (2009). Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. *J. Exp. Bot.* 60, 1085–1092.

Ahammed G. J., Zhang S., Shi K., Zhou Y. H., Yu, J. Q. (2012). Brassinosteroid improves seed germination and early development of tomato seedling under phenanthrene stress. *Plant Growth Regul.* 68, 87–96.

Anuradha S., Rao S. S. R. (2001). Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). *Plant Growth Regul.* 33, 151–153.

Appleford N. E. J., Evans D. J., Lenton J. R., Gaskin P., Croker S. J., Devos K. M., Phillips A. L., Hedden P. (2006). Function and transcript analysis of gibberellin-biosynthetic enzymes in wheat. *Planta* 223, 568–582.

Arif M. R., Neumann K., Nagel M., Kobiljski B., Lohwasser U., Börner A. (2012). An association mapping analysis of dormancy and pre-harvest sprouting in wheat. *Euphytica* 188, 409-417.

Arora N., Bhardwaj R., Sharma P., Arora H. K. (2008). Effects of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. *Acta Physiol. Plant.* 30, 833.

Arteca R. N. (1995). "Brassinosteroids," in *Plant Hormones*: *Physiology, Biochemistry, and Molecular Biology,* ed Davies P. J., editor. (Dordrecht, NL: Kluwer Academic Publishers;) 206–213.

Asami T., Min Y. K., Nagata N., Yamagishi K., Takatsuto S., Fujioka S., Yoshida S. (2000). Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. *Plant Physiol.* 123, 93–100.

Asami T., Yoshida S. (1999). Brassinosteroid biosynthesis inhibitors. *Trends Plant Sci.* 4, 348–353.

Asami T., Min Y. K., Nagata N., Yamagishi K., Takatsuto S., Fujioka S., Murofushi N., Yamaguchi I., Bentsink L., Koornneef M. (2008). Seed dormancy and germination. *The Arabidopsis Book* 6:e0119.

Asami T., Mizutani M., Fujioka S., Goda H., Min Y. K., Shimada Y., Sakata K. (2001). Selective interaction of triazole derivatives with DWF4, a cytochrome P450 monooxygenase of the brassinosteroid biosynthetic pathway, correlates with brassinosteroid deficiency in planta. *J. Biol. Chem.* 276, 25687–25691.

Atkinson J. A., Wingen L. U., Griffiths M., Pound M. P., Gaju O., Foulkes M. J., Wells D. M. (2015). Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *J. Exp. Bot.* 66, 2283–2292.

Atwell W. A., Finnie S. (2016). *Wheat flour* 2nd Edn. Minnesota, U.S.A: Elsevier.

Awan S. Z., Chandler J. O., Harrison P. J., Sergeant M. J., Bugg T. D.,Thompson A. J. (2017). Promotion of germination using hydroxamic acid inhibitors of 9-cis-epoxycarotenoid dioxygenase. *Front. Plant Sci.* 8, 357.

Ayalew H., Dessalegn T., Liu H., Yan G. (2016). Performance of Ethiopian bread wheat (*Tritium aestivum* L.) genotypes under contrasting water regimes: potential sources of variability for drought resistance breeding. *Aust. J. Crop Sci.* 10, 370–376.

Ayalew H., Ma X., Yan G. (2015). Screening wheat (*Triticum* spp.) genotypes for root length under contrasting water regimes: potential sources of variability for drought resistance breeding. *J. Agron. Crop Sci.* 201, 189–194.

Ayalew H., Liu H., Liu C. Yan G. (2018). Identification of Early Vigor QTLs and QTL by Environment Interactions in Wheat (*Triticum eastivum* L.). *Plant Mol*. *Biol*. *Rep*. 36, 399-405.

Bai M. Y., Shang J. X., Oh E., Fan M., Bai Y., Zentella R., Wang Z. Y. (2012). Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in *Arabidopsis*. *Nat. Cell Biol.* 14, 810–817.

Barrero J. M., Talbot M. J., White R. G., Jacobsen J. V., Gubler F. (2009). Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue in regulating dormancy in barley. *Plant Physiol*. 150, 1006–1021.

Barrero J. M., Jacobsen J. V., Talbot M. J., White R. G., Swain S. M., Garvin D. F. (2012). Grain dormancy and light quality effects on germination in the model grass *Brachypodium distachyon*. *New Phytol*. 193, 376–386.

Baskin C. C. (2003). Breaking physical dormancy in seeds-focussing on the lens. *New Phytol.* 158, 229–232.

Baskin C. C., Baskin J. M. (1998*). Seeds: ecology, biogeography, and, evolution of dormancy and germination*. Kentucky, U.S.A: Elsevier

Baskin J. M., Baskin C. C. (2000). Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. *Seed Sci. Res.* 10, 409–413.

Baskin J. M., Baskin C. C. (2004). A classification system for seed dormancy. *Seed Sci. Res.* 14, 1–16.

Bentsink L., Koornneef M. (2008). Seed dormancy and germination. *The Arabidopsis Book* 6:e0119.

Bennett M. D., Leitch I. J. (2005). Plant genome size research: a field in focus. *Ann. Bot.* 95, 1-6.

Bethke P. C., Libourel I. G., Reinöhl V., Jones R. L. (2006). Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. *Planta* 223, 805-812.

Bewley J. (1997). Seed germination and dormancy. *Plant Cell* 9, 1055–1066.

Bewley J. D., Black M. (1994). *Seeds: Physiology of Development and Germination* 2nd Edn. New York, NY: Plenum Press

Bewley J. D., Bradford K. J., Hilhorst H. W., Nonogaki H. (2013). "Development and maturation," in *Seeds* eds Baskin C. C., Baskin J. M., editors. (New York, NY: Springer;) 27–83

Blum A. (2005). Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* 56, 1159–1168.

Bishop G. J., Yokota T. (2001). Plants steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. *Plant Cell Physiol.* 42, 114–120.

Bouquin T., Meier C., Foster R., Nielsen M. E., Mundy J. (2001). Control of specific gene expression by gibberellin and brassinosteroid. *Plant Physiol.* 127, 450–458.

Bradford K. J., Nonogaki H. (2007). *Seed development, dormancy and germination*. Iowa, USA: Blackwell Publishing; 392.

Brandolini A., Volante A., Heun M. (2016). Geographic differentiation of domesticated einkorn wheat and possible Neolithic migration routes. *Heredity* 117, 135.

Cadman C. S., Toorop P. E., Hilhorst H. W., Finch-Savage W. E. (2006). Gene expression profiles of *Arabidopsis* Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* 46, 805–822.

Cai Z., Liu J., Wang H., Yang C., Chen Y., Li Y., Fujii H. (2014). GSK3-like kinases positively modulate abscisic acid signaling through phosphorylating subgroup III SnRK2s in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 111, 9651–9656.

Campbell A. (2015). Wheat. In *the Canadian Encyclopedia*. Retrieved from https://www.thecanadianencyclopedia.ca/en/article/wheat

Cantliffe D. J., Shuler K. D., Guedes A. C. (1981). Overcoming seed thermo dormancy in a heat sensitive romaine lettuce by seed priming. *HortScience* 16, 196–198.

Capelle V., Remoué C., Moreau L., Reyss A., Mahé A., Massonneau A., Coursol S. (2010). QTLs and candidate genes for desiccation and abscisic acid content in maize kernels. *BMC Plant Biol.* 10, 2.

Charmet G. (2011). Wheat domestication: lessons for the future. *C. R. Biol*. 334, 212-220.

Chen J. G., Pandey S., Huang J., Alonso J. M., Ecker J. R., Assmann S. M., Jones A. M. (2004). GCR1 can act independently of heterotrimeric G-protein in response to brassinosteroids and gibberellins in *Arabidopsis* seed germination. *Plant Physiol.* 135, 907–915.

Cheon J., Fujioka S., Dilkes B. P., Choe S. (2013). Brassinosteroids regulate plant growth through distinct signaling pathways in Selaginella and *Arabidopsis*. *PLoS ONE* 8:e81938.

Cheon J., Park S. Y., Schulz B., Choe S. (2010). *Arabidopsis* brassinosteroid biosynthetic mutant dwarf7-1 exhibits slower rates of cell division and shoot induction. *BMC Plant Biol.* 10, 270.

Chitnis V. R., Gao F., Yao Z., Jordan M. C., Park S., Ayele B. T. (2014). After-ripening induced transcriptional changes of hormonal genes in wheat seeds: the cases of brassinosteroids, ethylene, cytokinin and salicylic acid. *PLoS ONE* 9:e8754.

Choe S., Fujioka S., Noguchi T., Takatsuto S., Yoshida S., Feldmann K. A. (2001). Overexpression of DWARF4 in the brassinosteroid biosynthetic pathway results in increased vegetative growth and seed yield in *Arabidopsis*. *Plant J.* 26, 573–582.

Choe S., Schmitz R. J., Fujioka S., Takatsuto S., Lee M. O., Yoshida S., Feldmann K. A., Tax F. E. (2002). *Arabidopsis* brassinosteroid-insensitive dwarf12 mutants are semidominant and defective in a glycogen synthase kinase 3beta-like kinase. *Plant Physiol.* 130, 1506–1515.

Choe S., Dilkes B. P., Fujioka S., Takatsuto S., Sakurai A., Feldmann K. A. (1998). The DWF4 gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22a-hydroxylation steps in brassinosteroid biosynthesis. *Plant Cell* 10, 231–243.

Choi Y. H., Fujioka S., Harada A., Yokota T., Takatsuto S., Sakurai A. (1996). A brassinolide biosynthetic pathway via 6-deoxocastasterone. *Phytochemistry* 43, 593–596.

Chono M., Honda I., Shinoda S., Kushiro T., Kamiya Y., Nambara E., Watanabe Y. (2006). Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. *J. Exp. Bot.* 57, 2421–2434.

Chono M., Matsunaka H., Seki M., Fujita M., Kiribuchi-Otobe C., Oda S., 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 condition. *Breed Sci.* 63, 104–115.

Chung Y., Choe S. (2013). The regulation of brassinosteroid biosynthesis in *Arabidopsis*. *Crit. Rev. Plant Sci.* 32, 396–410.

Chung Y., Kwon S. I., Choe S. (2014). Antagonistic regulation of *Arabidopsis* growth by brassinosteroids and abiotic stresses. *Mol. Cells* 37, 795–803.

Chung Y., Maharjan P. M., Lee O., Fujioka S., Jang S., Kim B., Takatsuto S., Tsujimoto M., Kim H., Cho S., Park T., Cho H., Hwang I., Choe S. (2011). Auxin stimulates DWARF4 expression and brassinosteroid biosynthesis in *Arabidopsis*. *Plant J.* 66, 564–578.

Clouse S. D. (2002). Brassinosteroid signal transduction: clarifying the pathway from ligand perception to gene expression. *Mol. Cells* 10, 973–982.

Clouse S. D. (2011). Brassinosteroids. *The Arabidopsis Book* 9:e0151.

Clouse S. D., Hall A. F., Langford M., McMorris T. C., Baker M. E. (1993). Physiological and molecular effects of brassinosteroids on *Arabidopsis thaliana*. *J. Plant Growth Regul.* 12, 61–66.

Clouse S. D., Langford M., McMorris T. C. (1996). A brassinosteroid-insensitive mutant in *Arabidopsis* thaliana exhibits multiple defects in growth and development. *Plant Physiol.* 111, 671–678.

Clouse S. D., Sasse J. M. (1998). Brassinosteroids: essential regulators of plant growth and development. *Annu*. *Rev*. *Plant Biol*. 49, 427-451.

Cooper R. (2015). Re-discovering ancient wheat varieties as functional foods. *J. Tradit*. *Complement Med*. 5, 138–143.

Cunningham F. X., Gantt E. (1998). Genes and enzymes of carotenoid biosynthesis in plants. *Annu*. *Rev*. *Plant Physiol*. *Plant Mol*. *Biol*. 49, 557–583.

Cutler A. J., Krochko J. E. (1999). Formation and breakdown of ABA. *Trends Plant Sci*. 4, 472–478.

Deng Z., Zhang X., Tang W., Oses-Prieto J. A., Suzuki N., Gendron J. M., Burlingame A. L. (2007). A proteomics study of brassinosteroid response in *Arabidopsis*. *Mol Cell Proteomics* 6, 2058–2071.

DePauw R. M., Knox R. E., Singh A. K., Fox S. L., Humphreys D. G., Hucl P. (2012). Developing standardized methods for breeding preharvest sprouting resistant wheat, challenges and successes in Canadian wheat. *Euphytica* 188, 7–14.

Depuydt S., Hardtke C. S. (2011). Hormone signalling crosstalk in plant growth regulation. *Curr. Biol.* 21, R365–R373.

De Vleesschauwer D., Buyten E. V., Satoh K., Balidion J., Mauleon R., Choi I. R., Vera-Cruz C., Kikuchi S., Höfte M. (2012). Brassinosteroids antagonize gibberellin- and salicylate-mediated root immunity in rice. *Plant Physiol.* 158, 1833–1846.

Divi U. K., Krishna P. (2009). Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *New Biotechnol*. 26, 131–136.

Divi U. K., Krishna P. (2010). Overexpression of the brassinosteroid biosynthetic gene *AtDWF4* in *Arabidopsis* seeds overcomes abscisic acid-induced inhibition of germination and increases cold tolerance in transgenic seedlings. *J. Plant Growth Regul.* 29, 385–393.

Domagalska M. A., Sarnowska E., Nagy F., Davis S. J. (2010). Genetic analyses of interactions among gibberellin, abscisic acid, and brassinosteroids in the control of flowering time in *Arabidopsis thaliana*. *PLoS ONE* 5:e14012.

Dubreucq B., Baud S., Debeaujon I., Dubos C., Marion-Poll A., Miquel M., North H., Rochat C., Routaboul J. M., Lepiniec L. (2010). “Seed development,” in *Plant Developmental Biology*

Biotechnological Perspectives eds Pua E. C., Davey, M. R., editors. (Heidelberg, NY: Springer;) 341–359.

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 Biol.* 17, 1156–1164.

Duran M., González C., Acosta A., Olea A., Díaz K., Espinoza L. (2017). Synthesis of five known brassinosteroid analogs from hyodeoxycholic acid and their activities as plant-growth regulators. *Int. J. Mol. Sci* 18, 516.

Dvorak J. (2009). "Triticeae genome structure and evolution," in *Genetics and genomics*: *crops and models* eds Feuiller C., Muehlbauer G. J., editors. (Berlin, DE: Springer;) 685–711.

Eckardt N. A. (2007). GA perception and signal transduction: molecular interactions of the GA receptor GID1 with GA and the DELLA protein SLR1 in rice. *Am. Soc. Plant Biol.* 19, 2095–2097.

Eisenreich W., Bacher A., Arigoni D., Rohdich F. (2004). Biosynthesis of isoprenoids via the non-mevalonate pathway. *Cell. Mol. Life Sci*. 61, 1401-1426.

El-Feky S. S., Abo-Hamad S. A. (2014). Effect of exogenous application of brassinolide on growth and metabolic activity of wheat seedlings under normal and salt stress conditions. *Annu. Res. Rev. Biol.* 4, 3687–3698.

Elliott R. H., Franke C., Rakow G. F. W. (2008). Effects of seed size and seed weight on seedling establishment, vigour and tolerance of Argentine canola (*Brassica napus*) to flea beetles, Phyllotreta spp. *Can. J. Plant Sci.* 88, 207–217.

Ellis R. H. (1992). Seed and seedling vigour in relation to crop growth and yield. *Plant Growth Regul.* 11, 249–255.

Evans M. L. (1984). “Functions of hormones at the cellular level of organization,” in *Hormonal regulation of development II* ed Scott T. K., editor. (Berlin, DE: Springer;) 23–79.

Fàbregas N., Caño-Delgado A. I. (2014). Turning on the microscope turret: a new view for the study of brassinosteroid signaling in plant development. *Physiol. Plant*. 151, 172–183.

Ficco D. B., Mastrangelo A. M., Trono D., Borrelli G. M., De Vita P., Fares C., Beleggia R., Platani C., Papa R. (2014). The colours of durum wheat: a review. *Crop Pasture Sci*. 65, 1–15.

Finch-Savage W. E., Bassel G. W. (2015). Seed vigour and crop establishment: extending performance beyond adaptation. *J. Exp. Bot*. 67, 567–591.

Finch-Savage W. E., Footitt S. (2017). Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *J. Exp. Bot*. 68, 843–856.

Finch-Savage W. E., Leubner-Metzger G. (2006). Seed dormancy and the control of germination. *Phytol.* 171, 501–523.

Finkelstein R. R. (2010). “The Role of Hormones during Seed Development and Germination,” in Plant Hormones ed Davies P.J., editor. (Dordrecht, NL: Springer;) 549–573.

Finkelstein R. R., Gampala S. S., Rock C. D. (2002). Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14, S15–S45.

Finkelstein R. R., Gibson S. I. (2002). ABA and sugar interactions regulating development: cross-talk or voices in a crowd? *Curr Opin Plant Biol*. 5, 26–32.

Finkelstein R. R., Lynch T. J. (2000). The *Arabidopsis* abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell* 12, 599–609.

Finkelstein R., Reeves W., Ariizumi T., Steber C. (2008). Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* 59, 387–415.

Friedrichsen D. M., Nemhauser J., Muramitsu T., Maloof J. N., Alonso J., Ecker J. R., Furuya M., Chory J. (2002). Three redundant brassinosteroid early response genes encode putative bHLH transcription factors required for normal growth. *Genetics* 162, 1445–1456.

Fujioka S., Sakurai A. (1997). Biosynthesis and metabolism of brassinosteroids. *Physiol. Plant.* 100, 710–715.

Fujita M., Fujita Y., Maruyama K., Seki M., Hiratsu K., Ohme-Takagi M., Shinozaki K. (2004). A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *Plant J.* 39, 863–876.

Gallego-Bartolome J., Minguet E. G., Grau-Enguix F., Abbas M., Locascio A., Thomas S. G., Alabadi´ D., and Bla´zquez M. A. (2012). Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 109, 13446–13451.

Gampala S. S., Kim T. W., He J. X., Tang W., Deng Z., Bai M. Y., Chen H. (2007). An essential role for 14-3-3 proteins in brassinosteroid signal transduction in *Arabidopsis*. *Dev. Cell* 13, 177–189.

Gao F., Ayele B. T. (2014). Functional genomics of seed dormancy in wheat: advances and prospects. *Front. Plant Sci.* 5, 458.

Gao F., Jordan M. C., Ayele B. T. (2012). Transcriptional programs regulating seed dormancy and its release by after-ripening in common wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* 10, 465-476.

Gao X., Hu,C. H., Li H. Z., Yao Y. J., Meng M., Dong J., Li X. Y. (2013). Factors affecting pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.): a review. *J. Anim. Plant Sci*. 23, 556-565.

Garciarrubio A., Legaria J. P., Covarrubias A. A. (1997). Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. *Planta* 203, 182–187.

Gehring M., Choi Y., Fischer R. L. (2004). Imprinting and seed development. *Plant Cell* 16, S203–S213.

Gilissen L. J. van der Meer I. M., Smulders M. J. (2008). "Beyond coeliac disease toxicity," in *Frontiers in Celiac Disease* eds Fasano A., Troncone R., Branski D., editors. (Basel, CH: Karger Publishers;)139–147.

Gomes M. M. A. (2011). "Physiological effects related to brassinosteroid application in plants," in *Brassinosteroids*: *A Class of Plant Hormone* eds Hayat S., Ahmad A., editors. (Dordrecht, NL: Springer Press) 193–242.

Gopi R., Jaleel C. A., Divyanair V., Azooz M. M., Panneerselvam R. (2009). Effect of paclobutrazol and ABA on total phenol contents in different parts of holy basil (*Ocimum sanctum*). *Academic Journal of Plant Sciences* 2, 97–101.

Grappin P., Bouinot D., Sotta B., Miginiac E., Jullien M. (2000). Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* 210, 279–285.

Gregory L. E., Mandava N. B. (1982). The activity and interaction of brassinolide and gibberellic acid in mung bean epicotyls. *Physiol. Plant.* 54, 239–243.

Groot S. P. C., Karssen C. M. (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. *Planta* 171, 525–531.

Grove M. D., Spencer G. F., Rohwedder W. K., Mandava N., Worley J. F., Warthen Jr J. D., Cook Jr J. C. (1979). Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature 281, 216.

Gruszka D., Szarejko I., Maluszynski M. (2011). Identification of barley DWARF gene involved in brassinosteroid synthesis. Plant Growth Regul. 65, 343.

Gubler F., Kalla R., Roberts J. K., Jacobsen J. V. (1995). Gibberellin regulated expression of a myb gene in barley aleurone cells: evidence for Myb transactivation of a high-pI alpha-amylase gene promoter. Plant Cell. 7, 1879–1891.

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 Physiol. 147, 886–896.

Gubler F., Millar A. A., Jacobsen J. V. (2005). Dormancy release, ABA and pre-harvest sprouting. *Curr. Opin, Plant Biol.* 8, 183–187.

Guo R., Qian H., Shen W., Liu L., Zhang M., Cai C., Zhao Y., Qiao J., Wang Q. (2013). BZR1 and BES1 participate in regulation of glucosinolate biosynthesis by brassinosteroids in *Arabidopsis*. *J. Exp. Bot.* 64 2401–2412.

Gupta R., Chakrabarty S. K. (2013). Gibberellic acid in plant: still a mystery unresolved. *Plant Signal Behav.* 8:e25504.

Gustafson P., Raskina O., Ma X., Nevo E. (2009). "Wheat evolution, domestication, and improvement," in *Wheat*: *science and trade* ed Carver B. F., editor. (Iowa, USA: Blackwell;) 5–30.

Haldorsen S., Akan H., Çelik B., Heun M. (2011). The climate of the Younger Dryas as a boundary for Einkorn domestication. *Veg*. *Hist*. *Archaeobot*. 20, 305.

Han Z., Ku L., Zhang Z., Zhang J., Guo S., Liu H., Dong L. (2014). QTLs for seed vigor-related traits identified in maize seeds germinated under artificial aging conditions. *PLoS ONE* 9: e92535.

Hartung W., Sauter A., Hose E. (2002). Abscisic acid in the xylem: where does it come from, where does it go to? *J. Exp. Bot.* 53, 27–32.

Hartwig T., Corvalan C., Best N. B., Budka J. S., Zhu J. Y., Choe S., Schulz B. (2012). Propiconazole is a specific and accessible brassinosteroid (BR) biosynthesis inhibitor for *Arabidopsis* and maize. *PLoS ONE* 7: e36625.

Hashimoto T. (1987). Gibberellin structure-dependent interaction between gibberellins and deoxygibberellin C in the growth of dwarf maize seedlings. *Plant Physiol.* 83, 910–914.

Haubrick L. L., Assmann S. M. (2006). Brassinosteroids and plant function: some clues, more puzzles. *Plant Cell Environ.* 29, 446–457.

Ha Y. M., Shang Y., Yang D., Nam K. H. (2018). Brassinosteroid reduces ABA accumulation leading to the inhibition of ABA-induced stomatal closure. *Biochem. Biophys. Res. Commun.* 504, 143–148.

Hayat S., Ahmad A., Ahmad A. (2003). *Brassinosteroids: bioactivity and crop productivity*. Boston, UK: Springer

Hedden P., Phillips A. L. (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci*. 5, 523–530.

Hedden P., Sponsel V. (2015). A century of gibberellin research. *J. Plant Growth Regul*. 34, 740-760.

He J. X., Gendron J. M., Sun Y., Gampala S. S., Gendron N., Sun C. Q., Wang Z. Y. (2005). BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. *Sci.* 307, 1634–1638.

He J. X., Joshua M. G., Yanli Y., Jianming L., Zhi-Yong W. (2002). The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 10185–10190.

He Z., Wang Z. Y., Li J., Zhu Q., Lamb C., Ronald P., Chory J. (2000). Perception of brassinosteroids by the extracellular domain of the receptor kinase BRI1. *Sci.* 288, 2360–2363.

Higashiyama T., Yabe S., Sasaki N., Nishimura Y., Miyagishima S. Y., Kuroiwa H., Kuroiwa T. (2001). Pollen tube attraction by the synergid cell. *Sci.* 293, 1480–1483.

Hilhorst H. W. M. (1995). A critical update on seed dormancy. I. Primary dormancy. *Seed Sci. Res.* 5, 61–73.

Hirschberg J. (2001). Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.*, 4, 210–218.

Hirst K. K. (2012). Wheat Domestication. *The History and Origin of floury grain*. Retrieved from https://www.thoughtco.com/wheat-domestication-the-history-170669

Hofmann N. R. (2015). Taking hormone crosstalk to a new level: brassinosteroids regulate gibberellin biosynthesis. *Plant Cell* 27, 2081–2081.

Hu J., Mitchum M. G., Barnaby N., Ayele B. T., Ogawa M., Nam E., Swain S. M. (2008). Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. *Plant Cell* 20, 320–336.

Hu Y., Yu D. (2014). Brassinosteroid insensitive 2 interacts with abscisic acid insensitive 5 to mediate the antagonism of brassinosteroids to abscisic acid during seed germination in *Arabidopsis*. *Plant Cell 26,* 4394–4408.

Iehisa J. C. M., Matsuura T., Mori I. C., Takumi S. (2014). Identification of quantitative trait locus for abscisic acid responsiveness on chromosome 5A and association with dehydration tolerance in common wheat seedlings. *J. Plant Physiol.* 171, 25–34.

Itoh H., Ueguchi-Tanaka M., Sentoku N., Kitano H., Matsuoka M., Kobayashi M. (2001). Cloning and functional analysis of two gibberellin 3β-hydroxylase genes that are differently expressed during the growth of rice. *Proc. Natl. Acad. Sci.* 98, 8909–8914.

Iwasaki T., Shibaoka H. (1991). Brassinosteroids act as regulators of tracheary-element differentiation in isolated Zinnia mesophyll cells. *Plant Cell Physiol.* 32, 1007–1014.

Izydorczyk C., Nguyen T. N., Jo S., Son S., Tuan P. A., Ayele B. T. (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 Environ.* 41, 1022–1037.

Jacobsen J. V., Gubler F., Chandler P. M. (1995). "Gibberellin action in germinated cereals," in *Plant Hormones* ed Davies P. J., editor. (Ithaca, USA: Springer) 246–271.

Jacobsen J. V., Pearce D. W., Poole A. T., Pharis R. P., Mander L. N. (2002). Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. *Physiol. Plant.* 115, 428–441.

Jager C. E., Symons G. M., Ross J. J., Smith J. J., Reid J. B. (2005). The brassinosteroid growth response in pea is not mediated by changes in gibberellin content. *Planta* 221, 141–148.

Janeczko A., Swaczynová J. (2010). Endogenous brassinosteroids in wheat treated with 24-epibrassinolide. *Biol. Plant.* 54, 477–482.

Ji X., Dong B., Shiran B., Talbot M. J., Edlington J. E., Hughes T., Dolferus R. (2011). Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant physiol.* 156, 647–662.

Jiroutova P., Oklestkova J., Strnad M. (2018). Crosstalk between brassinosteroids and ethylene during plant growth and under abiotic stress conditions. *Int. J. Mol. Sci.* 19, 3283.

Kagale S., Divi U. K., Krochko J. E., Keller W. A., Krishna P. (2007). Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. *Planta* 225, 353–364.

Kajla M., Yadav V. K., Khokhar J., Singh S., Chhokar R. S., Meena R. P., Sharma R. K. (2015). Increase in wheat production through management of abiotic stresses: a review. *J. Appl. Nat. Sci.* 7, 1070–1080.

Kaneko M., Itoh H., Ueguchi-Tanaka M., Ashikari M., Matsuoka M. (2002). The α-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. *Plant Physiol.* 128, 1264–1270.

Kang J. Y., Choi H. I., Im M. Y., Kim S. Y. (2002). *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell* 14, 343–357.

Karlova R., Boeren S., Russinova E., Aker J., Vervoort J., de Vries S. (2006). The *Arabidopsis* somatic embryogenesis receptor-like kinase1 protein complex includes brassinosteroid-insensitive1. *Plant Cell* 18, 626–638.

Karssen C. M., Brinkhorst-Van der Swan D. L. C., Breekland A. E., Koornneef M. (1983). Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* 157, 158–165.

Karssen C., Zagorski S., Kepczynski J., Groot S. P. C. (1989). Key role for endogenous gibberellins in the control of seed germination. *Ann. Bot.* 63, 71–80.

Kauschmann A., Jessop A., Koncz C., Szekeres M., Willmitzer L., Altmann T. (1996). Genetic evidence for an essential role of brassinosteroids in plant development. *Plant J.* 9, 701–713.

Kavina J., Gopi R., Panneerselvam R. (2011). Traditional and non-traditional plant growth regulators alter the growth and photosynthetic pigments in *Mentha piperita* Linn. *Int. J. Environ. Sci.* 2, 124.

Kermode A. R. (2005). Role of abscisic acid in seed dormancy. *J. Plant Growth Regul*. 24, 319–344.

Kermode A. R., Finch-Savage B. E. (2002). "Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development," in *Desiccation and survival in plants*: *Drying without dying* eds Black M., Pritchard H. W., editors. (Wallingford, UK: CAB1;) 149–184.

Khan F. A., Maqbool R., Narayan S., Bhat S. A., Narayan R., Khan F. U. (2016). Reversal of age induced seed deterioration through priming in vegetable crops-a review. *Advances in Plant and Agriculture Research* 4, 00159.

Khan N., Kazmi R. H., Willems L. A., Van Heusden A. W., Ligterink W., Hilhorst H. W. (2012). Exploring the natural variation for seedling traits and their link with seed dimensions in tomato. *PLoS ONE* 7: e43991.

Kim B., Fujioka S., Kwon M., Jeon J., Choe S. (2013). *Arabidopsis* brassinosteroid-overproducing gulliver3-D/dwarf4-D mutants exhibit altered responses to jasmonic acid and pathogen. *Plant Cell Rep.* 32, 1139–1149.

Kim H., Park P. J., Hwang H. J., Lee S. Y., Oh M. H., Kim S. G. (2006). Brassinosteroid signals control expression of the AXR3/IAA17 gene in the cross-talk point with auxin in root development. *Biosci. Biotech. Biochem.* 70, 768–773.

Kim T. W., Guan S., Burlingame A. L., Wang Z. Y. (2011). The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. *Mol. Cell* 43, 561–571.

Kim T. W., Guan S., Sun Y., Deng Z., Tang W., Shang J. X., Sun Y., Burlingame A. L., Wang Z. Y. (2009). Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. *Nat. Cell Biol.* 11, 1254–1260.

Kim T. W., Wang Z. Y. (2010). Brassinosteroid signal transduction from receptor kinases to transcription factors. *Annu. Rev. Plant Biol.* 61, 681–704.

Kinoshita T., Cano-Delgado A., Seto H., Hiranuma S., Fujioka S., Yoshida S., Chory J. (2005). Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. *Nature* 433, 167.

Klee H., Estelle M. (1991). Molecular genetic approaches to plant hormone biology. *Annu. Rev. Plant Biol.* 42, 529–551.

Koornneef M., Bentsink L., Hilhorst H. (2002). Seed dormancy and germination. *Curr. Opin. Plant Biol.* 5, 33–36.

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. *Theor. Appl. Genet.* 61, 385–393.

Koornneef M., Van der Veen J. H. (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theor. Appl. Genet.* 58, 257–263.

Krishna P. (2003). Brassinosteroid-mediated stress responses. *J. Plant Growth Regul*. 22, 289-297.

Kucera B., Cohn M. A., Leubner-Metzger G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* 15, 281–307.

Kushiro T., Okamoto M., Nakabayashi K., Yamagishi K., Kitamura S., Asami T., Nambara E. (2004). The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO J.* 23, 1647–1656.

Landjeva S., Lohwasser U., Börner A. (2010). Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth. *Euphytica* 171, 129.

Lee S. A., Jang S., Yoon E. K., Heo J. O., Chang K. S., Choi J. W., Kwon C. (2016). Interplay between ABA and GA modulates the timing of asymmetric cell divisions in the *Arabidopsis* root ground tissue. *Mol. Plant* 9, 870–884.

Lefebvre V., North H., Frey A., Sotta B., Seo M., Okamoto M., Marion-Poll A. (2006). Functional analysis of *Arabidopsis NCED6* and *NCED9* genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. *Plant J.* 45, 309–319.

Leubner-Metzger G. (2001). Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. *Planta* 213, 758–763.

Leung J., Giraudat J. (1998). Abscisic acid signal transduction. *Annu. Rev. Plant Biol.* 49, 199–222.

Lev-Yadun S., Gopher A., Abbo S. (2000). The cradle of agriculture. *Sci.* 288, 1602–1603.

Li J., Biswas M. G., Chao A., Russell D. W., Chory J. (1997). Conservation of function between mammalian and plant steroid 5α-reductases. *Proc. Natl. Acad. Sci.* 94, 3554–3559.

Li J., Chory J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* 90, 929–938.

Li J., Chory J. (1999). Brassinosteroid actions in plants. *J. Exp. Bot.* 50, 275–282.

Li J., Jin H. (2007). Regulation of brassinosteroid signaling. *Trends Plant Sci.* 12, 37–41.

Li J., Nagpal P., Vitart V., McMorris T. C., Chory J. (1996). A role for brassinosteroids in light-dependent development of Arabidopsis. *Sci*. 272, 398–401.

Li J., Nam K. H. (2002). Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. *Sci.* 295, 1299–1301.

Li J., Nam K. H., Vafeados D., Chory J. (2001). BIN2, a new brassinosteroid-insensitive locus in *Arabidopsis*. *Plant Physiol.* 127, 14–22.

Li Q. F., He J. X. (2013). Mechanisms of signaling crosstalk between brassinosteroids and gibberellins. *Plant Signal Behav.* 8:e24686.

Li Q. F., Wang C., Jiang L., Li S., Sun S. S., He J. X. (2012). An interaction between BZR1 and DELLAs mediates direct signaling crosstalk between brassinosteroids and gibberellins in *Arabidopsis. Sci. Signal* 5, ra72–ra72.

Li Z., Trick H. N. (2005). Rapid method for high-quality RNA isolation from seed endosperm containing high levels of starch. *Biotechniques* 38, 872–876.

Ling H., Jia-Peng X., Liu-Sheng D., Ming-Cai Z., Zhao-Hu L. (2017). Growth Regulation and the mechanism of propiconazole in maize seedlings. *Acta Agron. Sin.* 43, 1603–1610.

Linkies A., Leubner-Metzger G. (2012). Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. *Plant Cell Rep.* 31, 253–270.

Liu A., Gao F., Kanno Y., Jordan M. C., Kamiya Y., Seo M., Ayele B. T. (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 Y., Fang J., Xu F., Chu J., Yan C., Schläppi M. R. (2014). Expression patterns of ABA and GA metabolism genes and hormone levels during rice seed development and imbibition: a comparison of dormant and non-dormant rice cultivars. *J. Genet. Genomics* 41, 327–338.

Liu X., Hou X. (2018). Antagonistic regulation of ABA and GA in metabolism and signaling pathways. *Front. Plant Sci.* 9, 251.

Livak K. J., Schmittgen T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2- ??CT method. *Methods* 25, 402–408.

Lopez-Molina L., Mongrand S., Chua N. H. (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 98, 4782–4787.

Lozano-Durán R., Macho A. P., Boutrot F., Segonzac C., Somssich I. E., Zipfel C. (2013). The transcriptional regulator BZR1 mediates trade-off between plant innate immunity and growth. *elife* 2: e00983.

Luo M., Xiao Y., Li X., Lu X., Deng W., Li D., Pei Y. (2007). *GhDET2*, a steroid 5a-reductase, plays an important role in cotton fiber cell initiation and elongation. *Plant J.* 51, 419–430.

Magwa R. A., Zhao H., Xing Y. (2016). Genome-wide association mapping revealed a diverse genetic basis of seed dormancy across subpopulations in rice (*Oryza sativa* L.). *BMC Genet.* 17, 28.

Mahender A., Anandan A., Pradhan S. K. (2015). Early seedling vigour, an imperative trait for direct-seeded rice: an overview on physio-morphological parameters and molecular markers. *Planta* 241, 1027–1050.

Mandava N. B. (1988). Plant growth-promoting brassinosteroids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39, 23–52.

Martínez-Andújar C., Ordiz M. I., Huang Z., Nonogaki M., Beachy R. N., Nonogaki H. (2011). Induction of 9-*cis*-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. *Proc. Natl. Acad. Sci.* 108, 17225–17229.

Matusmoto T., Yamada K., Yoshizawa Y., Oh K. (2016). Comparison of effect of brassinosteroid and gibberellin biosynthesis inhibitors on growth of rice seedlings. *Rice Sci.* 23, 51–55.

Merlot S., Gosti F., Guerrier D., Vavasseur A., Giraudat J. (2001). The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. *Plant J.* 25, 295–303.

Millar A. A., Jacobsen J. V., Ross J. J., Helliwell C. A., Poole A. T., Scofield G., Reid J. B., Gubler F. (2006). Seed dormancy and ABA metabolism in *Arabidopsis* and barley: the role of ABA 8'-hydroxylase. *Plant J.* 45, 942–954.

Mimura M., Itoh J. I. (2014). Genetic interaction between rice PLASTOCHRON genes and the gibberellin pathway in leaf development. *Rice* 7, 25.

Min Y. K., Asami T., Fujioka S., Murofushi N., Yamaguchi I., Yoshida S. (1999). New lead compounds for brassinosteroid biosynthesis inhibitors. *Bioorg. Med. Chem. Lett.* 9, 425–430.

Miyakawa T., Fujita Y., Yamaguchi-Shinozaki K., Tanokura M. (2012). Structure and function of abscisic acid receptors. *Trends Plant Sci.* 18, 259–266.

Mizutani M., Ohta D. (2010). Diversification of P450 genes during land plant evolution. *Annu. Rev. Plant Biol.* 61, 291–315.

Mora-García S., Vert G., Yin Y., Caño-Delgado A., Cheong H., Chory J. (2004). Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in *Arabidopsis*. *Genes Dev.* 18, 448–460.

Mornkham T., Wangsomnuk P., Fu Y. B., Wangsomnuk P., Jogloy S., Patanothai A. (2013). Extractions of high-quality RNA from the seeds of Jerusalem artichoke and other plant species with high levels of starch and lipid. *Plants* 2, 302–316.

Mulholland B. J., Black C. R., Taylor I. B., Roberts J. A., Lenton J. R. (1996a). Effect of soil compaction on barley (*Hordeum vulgare* L.) growth: I. Possible role for ABA as a root-sourced chemical signal. *J. Exp. Bot.* 47, 539–549.

Mulholland B. J., Taylor I. B., Black C. R., Roberts J. A. (1996b). Effect of soil compaction on barley (*Hordeum vulgare* L.) growth: II Are increased xylem sap ABA concentrations involved in maintaining leaf expansion in compacted soils? *J. Exp. Bot.* 47, 551–556.

Müller K., Carstens A. C., Linkies A., Torres M. A., Leubner-Metzger G. (2009). The NADPH-oxidase AtrbohB plays a role in *Arabidopsis* seed after-ripening. *New Phytol.* 184, 885–897.

Müller K., Tintelnot S., Leubner-Metzger G. (2006). Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. *Plant Cell Physiol.* 47, 864–877.

Müssig C. (2005). Brassinosteroid-promoted growth. *Plant Biol.* 7, 110–117.

Müssig C., Shin G. H., Altmann T. (2003). Brassinosteroids promote root growth in *Arabidopsis*. *Plant Physiol.* 133, 1261–1271.

Nagata N., Asami T., Yoshida S. (2001). Brassinazole, an inhibitor of brassinosteroid biosynthesis, inhibits development of secondary xylem in cress plants (*Lepidium sativum*). *Plant Cell Physiol.* 42, 1006–1011.

Nakashita H., Yasuda M., Nitta T., Asami T., Fujioka S., Arai Y., Yoshida S. (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant J.* 33, 887–898.

Nambara E., Marion-Poll A. (2005). Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 56, 165–185.

Nambara E., McCourt P., Naito S. (1995). A regulatory role for the ABI3 gene in the establishment of embryo maturation in *Arabidopsis thaliana*. *Development* 121, 629–636.

Nambara E., Okamoto M., Tatematsu K., Yano R., Seo M., Kamiya Y. (2010). Abscisic acid and the control of seed dormancy and germination. *Seed Sci. Res*. 20, 55–67.

Nemhauser J. L., Hong F., Chory J. (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell 126, 467–475.

Nevo E. (2011) "Triticum", in *Wild crop relatives: genomic and breeding resources, cereals* ed. Kole C. editor. (Berlin, DE: Springer;) 407–456.

Noguchi T., Fujioka S., Choe S., Takatsuto S., Tax F. E., Yoshida S., Feldmann K. A. (2000). Biosynthetic pathways of brassinolide in *Arabidopsis*. *Plant Physiol*. 124, 201–210.

Noguchi T., Fujioka S., Takatsuto S., Sakurai A., Yoshida S., Li J., Chory J. (1999). *Arabidopsis* det2 is defective in the conversion of (24R)-24-methylcholest-4-En-3-one to (24R)-24-methyl-5alpha-cholestan-3-one in brassinosteroid biosynthesis. *Plant Physiol*. 120, 833–840.

Noguchi T., FujiokaS., Choe S., Takatsuto S., Yoshida S., Yuan H., Tax F. E. (1999). Brassinosteroid-insensitive dwarf mutants of *Arabidopsis* accumulate brassinosteroids. *Plant Physiol*. 121, 743–752.

Nomura T., Nakayama M., Reid J. B., Takeuchi Y., Yokota T. (1997). Blockage of brassinosteroid biosynthesis and sensitivity causes dwarfism in garden pea. *Plant Physiol.* 113, 31–37.

Nomura T., Ueno M., Yamada Y., Takatsuto S., Takeuchi Y., Yokota T. (2007). Roles of brassinosteroids and related mRNAs in pea seed growth and germination. *Plant Physiol.* 143, 1680–1688.

Nonogaki H., Bassel G. W., Bewley J. D. (2010). Germination-still a mystery. *Plant Sci.* 179, 574–581.

Nonogaki K., Chen F., Bradford K. J. (2007). "Mechanisms and genes involved in germination sensu stricto," in *Seed development, dormancy and germination* eds Bradford K. J., Nonogaki H., editors. (Oxford, UK: Blackwell Publishing;) 264–304.

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.

Oh K., Matsumoto T., Hoshi T., Yoshizawa Y. (2016). In vitro and in vivo evidence for the inhibition of brassinosteroid synthesis by propiconazole through interference with side chain hydroxylation. *Plant Signal Behav.* 11:e1158372.

Oh K., Matsumoto T., Yamagami A., Ogawa A., Yamada K., Suzuki R., Nakano T. (2015). YCZ-18 is a new brassinosteroid biosynthesis inhibitor. *PloS ONE* 10:e0120812.

Oh M. H., Wang X., Kota U., Goshe M. B., Clouse S. D., Huber S. C. (2009). Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 106, 658–663.

Ohto M., Stone S. L., Harada J. J. (2007). “Genetic control of seed development and seed mass,” in *Seed Development, Dormancy and Germination* eds Bradford K. J. and Nonogaki H., editors. (Oxford, UK: Blackwell Publishing;) 1–24.

Okamoto M., Kuwahara A., Seo M., Kushiro T., Asami T., Hirai N., Kamiya Y., Koshiba T., Nambara E. (2006). CYP707A1 and CYP707A2, which encode ABA 8’ hydroxylases, are indispensable for a proper control of seed dormancy and germination in *Arabidopsis*. *Plant Physiol.* 141, 97–107.

Okamoto M., Tatematsu K., Matsui A., Morosawa T., Ishida J., Tanaka M., Endo T. A., Mochizuki Y., Toyoda T., Kamiya Y., Shinozaki K. (2010). Genome-wide analysis of endogenous abscisic acid-mediated transcription in dry and imbibed seeds of *Arabidopsis* using tiling arrays. *Plant J.* 62, 39–51

Osborne D. J., Boubriak I. I. (1994). DNA and desiccation tolerance. *Seed Sci. Res.* 4, 175–185.

Ohnishi T., Szatmari A. M., Watanabe B., Fujita S., Bancos S., Koncz C., Szekeres M. (2006). C-23 hydroxylation by *Arabidopsis* CYP90C1 and CYP90D1 reveals a novel shortcut in brassinosteroid biosynthesis. *Plant Cell* 18, 3275–3288.

Özdemir F., Bor M., Demiral T., Türkan İ. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regul*. 42, 203–211.

Pacini E., Dolferus R. (2016) “The trials and tribulations of the plant male gametophyte—Understanding reproductive stage stress tolerance”, in *Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives*. (London, UK; IntechOpen;) 703–754.

Palta J. A., Chen X., Milroy S. P., Rebetzke G. J., Dreccer M. F., Watt M. (2011). Large root systems: are they useful in adapting wheat to dry environments? *Funct. Plant Biol.* 38, 347–354.

Pearce S., Huttly A., Prosser I. M., Li Y., Vaughan S., Gallova B., Phillis A. L. (2015). Heterologous expression and transcript analysis of gibberellin biosynthetic genes of grasses reveals novel functionality in the *GA3ox* family. *BMC Plant Biol.* 15, 130.

Peleg Z., Fahima T., Korol A. B., Abbo S., Saranga Y. (2011). Genetic analysis of wheat domestication and evolution under domestication. *J. Exp. Bot.* 62, 5051–5061.

Peng J. H., Sun D., Nevo E. (2011). Domestication evolution, genetics and genomics in wheat. *Mol. Breed.* 28, 281–301.

Peng P., Yan Z., Zhu Y., Li J. (2008). Regulation of the *Arabidopsis* GSK3-like kinase brassinosteroid-insensitive 2 through proteasome-mediated protein degradation. *Mol. Plant* 1, 338–346.

Peres A. L. G., Soares J. S., Tavares R. G., Righetto G., ZulloM A., Mandava N. B., Menossi M. (2019). Brassinosteroids, the sixth class of phytohormones: a molecular view from the discovery to hormonal interactions in plant development and stress adaptation. *Int. J. Mol. Sci.* 20, 331.

Pérez-Pérez J. M., Ponce M. R., Micol J. L. (2002). The UCU1 *Arabidopsis* gene encodes a SHAGGY/GSK3-like kinase required for cell expansion along the proximodistal axis. *Dev. Biol.* 242, 161–173.

Perry D. A. (1980). Seed vigour and seedling establishment. *Advances in research and technology of seeds* 5, 25–40.

Plackett A. R., Powers S. J., Fernandez-Garcia N., Urbanova T., Takebayashi Y., Seo M., Phillips A. L. (2012). Analysis of the developmental roles of the *Arabidopsis* gibberellin 20-oxidases demonstrates that *GA20ox1, -2,* and-*3* are the dominant paralogs. *Plant Cell* 24, 941–960.

Plackett A. R., Wilson Z. A. (2018). Gibberellins and plant reproduction. *Annu. Rev. Plant Biol.* 49, 323–358.

Powell A. A., Matthews S. (2012). Seed aging/repair hypothesis leads to new testing methods. *Seed Technol.* 34, 15–25.

Preston J., Tatematsu K., Kanno Y., Hobo T., Kimura M., Jikumaru Y., Nambara E. (2009). Temporal expression patterns of hormone metabolism genes during imbibition of *Arabidopsis thaliana* seeds: a comparative study on dormant and non-dormant accessions. *Plant Cell Physiol*. 50, 1786–1800.

Qin X., Zeevaart J. A. (2002). Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiol*. 128, 544–551.

Rademacher W. (2000). Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annu. Rev. Plant Biol.* 51, 501–531.

Rademacher W. (2015). Plant growth regulators: backgrounds and uses in plant production. *J. Plant Growth Regul.* 34, 845–872.

Rajjou L., Duval M., Gallardo K., Catusse J., Bally J., Job C., Job D. (2012). Seed germination and vigor. *Annu. Rev. Plant Biol.* 63, 507–533.

Rajjou L., Gallardo K., Debeaujon I., Vandekerckhove J., Job C., Job D. (2004). The effect of a-amanitin on the *Arabidopsis* seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant Physiol.* 134, 1598–1613.

Rajjou L., Lovigny Y., Groot S. P., Belghazi M., Job C., Job D. (2008). Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. *Plant Physiol.* 148, 620–641.

Ram S., Mishra B. (2008). Biochemical basis and molecular genetics of processing and nutritional quality traits of wheat. *J. Plant Biochem. Biotechnol.* 17, 111–126.

Rebetzke G. J., Richards R. A. (1999). Genetic improvement of early vigour in wheat. *Aust. J. Agric. Res.* 50, 291–302.

Rebolledo M. C., Dingkuhn M., Courtois B., Gibon Y., Clément-Vidal A., Cruz D. F., Duitama J., Lorieux M., Luquet D. (2015). Phenotypic and genetic dissection of component traits for early vigour in rice using plant growth modelling, sugar content analyses and association mapping. *J. Exp. Bot.* 66, 5555–5566.

Reinecke D. M., Wickramarathna A. D., Ozga J. A., Kurepin L. V., Jin A. L., Good A. G., Pharis R. P. (2013). Gibberellin 3-oxidase gene expression patterns influence gibberellin biosynthesis, growth, and development in pea. *Plant Physiol.* 163, 929–945.

Rieu I., Eriksson S., Powers S. J., Gong F., Griffiths J., Woolley L., Phillips A. L. (2008). Genetic analysis reveals that C19-GA2-oxidation is a major gibberellin inactivation pathway in *Arabidopsis*. *Plant Cell* 20, 2420–2436.

Roberts M. R., Salinas J., Collinge D. B. (2002). 14-3-3 proteins and the response to abiotic and biotic stress. *Plant Mol. Biol.* 50, 1031–1039.

Rock C. D. (2000). Tansley Review No. 120: Pathways to abscisic acid-regulated gene expression. *New Phytol.* 148, 357–396.

Rodriguez P. L. (2016). Abscisic acid catabolism generates phaseic acid, a molecule able to activate a subset of ABA receptors. *Mol. Plant* 9, 1448–1450.

Rock C. D. (2000). Tansley Review No. 120: Pathways to abscisic acid-regulated gene expression. *New Phytol.* 148, 357–396.

Rock C. D., Quatrano R. S. (1995). "The role of hormones during seed development," in *Plant hormones* ed. Davies P. J. editor. (Dordrecht, NL: Springer;) 671–697.

Rodriguez P. L. (2016). Abscisic acid catabolism generates phaseic acid, a molecule able to activate a subset of ABA receptors. *Mol. Plant* 9, 1448–1450.

Ross J. J., Reid J. B., Dungey H. S. (1992). Ontogenetic variation in levels of gibberellin A, in Pisum. *Planta* 186, 166–171.

Ryu H., Kim K., Cho H., Hwang I. (2010). Predominant actions of cytosolic BSU1 and nuclear BIN2 regulate subcellular localization of BES1 in brassinosteroid signaling. *Mol. Cells* 29, 291–296.

Ryu H., Kim K., Cho H., Park J., Choe S., Hwang I. (2007). Nucleocytoplasmic shuttling of BZR1 mediated by phosphorylation is essential in *Arabidopsis* brassinosteroid signaling. *Plant Cell* 19, 2749–2762.

Sabelli P. A., Larkins B. A. (2009) Update on endosperm development in grasses. *Plant Physiol*. 149, 14–26.

Saini S., Sharma I., Pati P. K. (2015). Versatile roles of brassinosteroid in plants in the context of its homoeostasis, signaling and crosstalks. *Front. Plant Sci.* 6, 950.

Saito S., Hirai N., Matsumoto C., Ohigashi H., Ohta D., Sakata K., Mizutani M. (2004). *Arabidopsis* CYP707As encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiol.* 134, 1439–1449.

Sakamoto T., Miura K., Itoh H., Tatsumi T., Ueguchi-Tanaka M., Ishiyama K., Miyao A. (2004). An overview of gibberellin metabolism enzyme genes and their related mutants in rice. *Plant Physiol. 134,* 1642–1653.

Sakamoto T., Morinaka Y., Ohnishi T., Sunohara H., Fujioka S., Ueguchi-Tanaka M., Tanaka H. (2006). Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nature Biotechnol.* 24: 105.

Sasse J. M. (1985). The place of brassinolide in the sequential response to plant growth regulators in elongating tissue. *Physiol*. *Plant.* 63, 303–308.

Schmidt J., Altmann T., Adam G. (1997). Brassinosteroids from seeds of *Arabidopsis thaliana*. *Phytochemistry* 45, 1325–1327.

Schomburg F. M., Bizzell C. M., Lee D. J., Zeevaart J. A. D., Amasino R. M. (2003) Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. *Plant Cell* 15, 151–163.

Schwember A. R., Bradford K. J. (2010). Quantitative trait loci associated with longevity of lettuce seeds under conventional and controlled deterioration storage conditions. *J. Exp. Bot.* 61, 4423–4436.

Sekimata K., Han S. Y., Yoneyama K., Takeuchi Y., Yoshida S., Asami T. (2002). A specific and potent inhibitor of brassinosteroid biosynthesis possessing a dioxolane ring. *J. Agric. Food Chem.* 50, 3486–3490.

Seo M., Hanada A., Kuwahara A., Endo A., Okamoto M., Yamauchi Y., Kamiya Y. (2006). Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. *Plant J.* 48, 354–366.

Seo M., Nambara E., Choi G., Yamaguchi S. (2009). Interaction of light and hormone signals in germinating seeds. *Plant Mol. Biol.* 69, 463.

Sharp R. E., LeNoble M. E. (2002). ABA, ethylene and the control of shoot and root growth under water stress. *J. Exp. Bot.* 53, 33–37.

Shewry P. R. (2009). Wheat. *J. Exp. Bot.* 60, 1537–1553.

Shu K., Liu X. D., Xie Q., He Z. H. (2016). Two faces of one seed: hormonal regulation of dormancy and germination. *Mol. Plant*. 9, 34–45.

Silverstone A. L., Chang C. W., Krol E., Sun T. P. (1997). Developmental regulation of the gibberellin biosynthetic gene GA1 in *Arabidopsis thaliana*. *Plant J.* 12, 9–19.

Simpson G. M. (1990). *Seed Dormancy in Grasses.* Cambridge: Cambridge University Press

Skubacz A., Daszkowska-Golec A. (2017). "Seed dormancy: the complex process regulated by abscisic acid, gibberellins, and other phytohormones that makes seed germination work," in *Phytohormones-Signaling Mechanisms and Crosstalk in Plant Development and Stress Responses*. ed. El-Esawi M., editor. (Zagreb, HR: In Tech;) 77–100.

Song J., Guo B., Song F., Peng H., Yao Y., Zhang Y., Ni Z. (2011). Genome-wide identification of gibberellins metabolic enzyme genes and expression profiling analysis during seed germination in maize. *Gene* 482, 34–42.

Song L., Zhou X. Y., Li L., Xue L. J., Yang X., Xue H. W. (2009). Genome-wide analysis revealed the complex regulatory network of brassinosteroid effects in photomorphogenesis. *Mol. Plant* 2, 755–772.

Son S., Chitnis V. R., Liu A., Gao F., Nguyen T. N., Ayele B. T. (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.

Spollen W. G., LeNoble M. E., Samuels T. D., Bernstein N., Sharp R. E. (2000). Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiol*. 122, 967–976.

Steber C. M., McCourt P. (2001). A role for brassinosteroids in germination in *Arabidopsis*. *Plant Physiol.* 125, 763–769.

Stewart Lilley J. L., Gan Y., Graham I. A., Nemhauser J. L. (2013). The effects of DELLAs on growth change with developmental stage and brassinosteroid levels. *Plant J.* 76, 165–173.

Sun T. P. (2008). Gibberellin metabolism, perception and signaling pathways in *Arabidopsis*. *The Arabidopsis Book* 6, e0103.

Sun T. P., Gubler F. (2004). Molecular mechanism of gibberellin signaling in plants. *Annu. Rev. Plant Biol.* 55, 197–223.

Sun Y., Fan X. Y., Cao D. M., Tang W., He K., Zhu J. Y., Patil S. (2010). Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in *Arabidopsis*. *Dev. Cell* 19, 765–777.

Suzuki H., Inoue T., Fujioka S., Saito T., Takatsuto S., Yokota T., Sakurai A. (1995). Conversion of 24-methylcholesterol to 6-oxo-24-methylcholestanol, a putative intermediate of the biosynthesis of brassinosteroids, in cultured cells of *Catharanthus roseus*. *Phytochemistry* 40, 1391–1397.

Swain S. M., Singh D. P. (2005). Tall tales from sly dwarves: novel functions of gibberellins in plant development. *Trends Plant Sci.* 10, 123–129

Szekeres M., Németh K., Koncz-Kálmán Z., Mathur J., Kauschmann A., Altmann T., Koncz C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* 85, 171–182.

Szira F., Balint A. F., Börner A., Galiba G. (2008). Evaluation of drought-related traits and screening methods at different developmental stages in spring barley. *J. Agron. Crop Sci.* 194, 334–342.

Tan B. C., Joseph L. M., Deng W. T., Liu L., Li Q. B., Cline K., McCarty D. R. (2003). Molecular characterization of the *Arabidopsis* 9-cis epoxycarotenoid dioxygenase gene family. *Plant J.* 35, 44–56.

Tanaka K., Nakamura Y., Asami T., Yoshida S., Matsuo T., Okamoto S. (2003). Physiological roles of brassinosteroids in early growth of *Arabidopsis*: brassinosteroids have a synergistic relationship with gibberellin as well as auxin in light-grown hypocotyl elongation. *J. Plant Growth Regul.* 22, 259–271.

Tang W., Kim T. W., Oses-Prieto J. A., Sun Y., Deng Z., Zhu S., Wang R., Burlingame A. L., Wang Z. Y. (2008). BSKs mediate signal transduction from the receptor kinase BRI1 in *Arabidopsis*. *Sci.* 321, 557–560.

Tanimoto E. (1988). Gibberellin regulation of root growth with change in galactose content of cell walls in Pisum sativum. *Plant Cell Physiol.* 29, 269–280.

Tao Y., Zheng J., Xu Z., Zhang X., Zhang K., Wang G. (2004). Functional analysis of ZmDWF1, a maize homolog of the *Arabidopsis* brassinosteroids biosynthetic DWF1/DIM gene. *Plant Sci.* 167, 743–751.

Thomas S.G., Phillips A.L., Hedden P. (1999). Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation. *Proc. Natl. Acad. Sci*. 96, 4698–4703.

Tian Y., Guan B., Zhou D., Yu J., Li G., Lou Y. (2014). Responses of seed germination, seedling growth, and seed yield traits to seed pretreatment in maize (*Zea mays* L.). *Sci. World J.* 2014

Tong H., Chu C. (2012). Brassinosteroid signaling and application in rice. *J. Genet. Genome* 39, 3–9.

Tong H., Xiao Y., Liu D., Gao S., Liu L., Yin Y., Chu C. (2014). Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in rice. *Plant Cell* 26, 4376–4393.

Tong S. M., Xi H. X., Ai K. J., Hou H. S. (2017). Overexpression of wheat *TaNCED* gene in *Arabidopsis* enhances tolerance to drought stress and delays seed germination. *Biol*. *Plant* 61, 64–72.

Toyomasu T., Tsuji H., Yamane H., Nakayama M., Yamaguchi I., Murofushi N., Inoue Y. (1993). Light effects on endogenous levels of gibberellins in photoblastic lettuce seeds. *J. Plant Growth Regul.* 12, 85.

Tuan P. A., Kumar R., Rehal P. K., Toora P. K., Ayele B. T. (2018). Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. *Front*. *Plant Sci*. 9, 668.

Tyler L., Thomas S.G., Hu J., Dill A., Alonso J.M., Ecker J.R. & Sun T. P. (2004). DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. *Plant Physiol.* 135, 1008–1019.

Ubeda-Tomás S., Federici F., Casimiro I., Beemster G. T., Bhalerao R., Swarup R., Bennett M. J. (2009). Gibberellin signaling in the endodermis controls *Arabidopsis* root meristem size. *Curr*. *Biol*. 19, 1194–1199.

Ullah H., Chen J. G., Wang S., Jones A. M. (2002). Role of a heterotrimeric G protein in regulation of *Arabidopsis* seed germination. *Plant Physiol.* 129, 897–907.

Unterholzner S. J., Rozhon W., Papacek M., Ciomas J., Lange T., Kugler K. G., Poppenberger B. (2015). Brassinosteroids are master regulators of gibberellin biosynthesis in *Arabidopsis*. *Plant Cell* 27, 2261–2272.

Unterholzner S. J., Rozhon W., Poppenberger B. (2016). Reply: interaction between brassinosteroids and gibberellins: synthesis or signaling? In *Arabidopsis*, both! *Plant Cell* 28, 836–839.

Varbanova M., Yamaguchi S., Yang Y., McKelvey K., Hanada A., Borochov R., Ma C. J. (2007). Methylation of gibberellins by *Arabidopsis* GAMT1 and GAMT2. *Plant Cell* 19, 32–45.

Vardhini B. V., Rao S. S. R. (2003). Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul.* 41, 25–31.

Vert G., Nemhauser J. L., Geldner N., Hong F., Chory J. (2005). Molecular mechanisms of steroid hormone signaling in plants. *Annu. Rev. Cell Dev. Biol*. 21, 177–201.

Villedieu-Percheron E., Lachia M., Jung P. M., Screpanti C., Fonné-Pfister R., Wendeborn S., De Mesmaeker A. (2014). Chemicals inducing seed germination and early seedling development. *CHIMIA International Journal for Chemistry* 68, 654–663.

Wang H., Tang J., Liu J., Hu J., Liu J., Chen Y., Wang X. (2018). Abscisic acid signaling inhibits brassinosteroid signaling through dampening the dephosphorylation of BIN2 by ABI1 and ABI2. *Mol. Plant* 11, 315–325.

Wang H., Yang C., Zhang C., Wang N., Lu D., Wang J., Wang X. (2011). Dual role of BKI1 and 14-3-3 s in brassinosteroid signaling to link receptor with transcription factors. *Dev. Cell* 21, 825–834.

Wang L., Wang Z., Xu Y., Joo S. H., Kim S. K., Xue Z., Chong K. (2009). OsGSR1 is involved in crosstalk between gibberellins and brassinosteroids in rice. *Plant J.* 57, 498–510.

Wang X., Chory J. (2006). Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. *Sci.* 313, 1118–1122.

Wang X., Kota U., He K., Blackburn K., Li J., Goshe M. B., Huber S. C., Clouse S. D. (2008). Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Dev. Cell* 15, 220–235.

Wang X., Li X., Meisenhelder J., Hunter T., Yoshida S., Asami T., Chory J. (2005). Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. *Dev. Cell* 8, 855–865.

Wang Z. Y. (2012). Brassinosteroids modulate plant immunity at multiple levels. *Proc. Natl. Acad. Sci.* 109, 7–8.

Wang Z. Y., Bai M. Y., Oh E., Zhu J. V. (2012). Brassinosteroid signalling network and regulation of photomorphogenesis. *Annu. Rev. Genet.* 46, 701–724.

Wang Z. Y., Nakano T., Gendron J., He J., Chen M., Vafeados D., Chory J. (2002). Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. *Dev. Cell* 2, 505–513.

Wang Z. Y., Seto H., Fujioka S., Yoshida S., Chory J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* 410, 380–383.

Waterworth W. M., Footitt S., Bray C. M., Finch-Savage W. E., West, C. E. (2016). DNA damage checkpoint kinase ATM regulates germination and maintains genome stability in seeds. *Proc. Natl. Acad. Sci.* 113, 9647–9652.

Weiss D., Ori N. (2007). Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol.* 144, 1240–1246.

Wilson H., Miles A. F., Daane K. M., Altieri M. A. (2015). Landscape diversity and crop vigor influence biological control of the Western grape leafhopper (*E. elegantula* Osborn) in vineyards. *PLoS ONE* 10:e0141752.

Wolny E., Betekhtin A., Rojek M., Braszewska-Zalewska A., Lusinska J., Hasterok R. (2018). Germination and the early stages of seedling development in *Brachypodium distachyon*. *Int. J. Mol. Sci.* 19, 2916.

Yamaguchi S. (2006). Gibberellin biosynthesis in *Arabidopsis*. *Phytochem Rev*. 5, 39–47.

Yamaguchi S. (2008). Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* 59, 225–251.

Yamaguchi S., Smith M. W., Brown R. G., Kamiya Y., Sun T. P. (1998). Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating *Arabidopsis* seeds. *The Plant Cell* 10, 2115–2126.

Yang C. J., Zhang C., Lu Y. N., Jin J. Q., Wang X. L. (2011). The mechanism of brassinosteroids action: from signal transduction to plant development. *Mol. Plant* 4, 588–600.

Yang T., Davies P. J., Reid J. B. (1996). Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact light-grown peas. *Plant physiol.* 110, 1029–1034.

Yang X., Bai Y., Shang J., Xin R., Tang W. (2016). The antagonistic regulation of abscisic acid-inhibited root growth by brassinosteroids is partially mediated via direct suppression of ABSCISIC ACID INSENSITIVE 5 expression by BRASSINAZOLE RESISTANT 1. *Plant, Cell Environ.* 39, 1994–2003.

Ye H., Feng J., Zhang L., Zhang J., Mispan M. S., Cao Z., Gu X. Y. (2015). Map-based cloning of seed dormancy1-2 identified a gibberellin synthesis gene regulating the development of endosperm-imposed dormancy in rice. *Plant physiol.* 169, 2152–2165.

Ye H., Li L., Yin Y. (2011). Recent Advances in the Regulation of Brassinosteroid Signaling and Biosynthesis Pathways F. *J. Integr. Plant Biol*. 53, 455–468.

Yin Y., Wang Z. Y., Mora-Garcia S., Li J., Yoshida S., Asami T., Chory J. (2002). BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. *Cell* 109, 181–191.

Yokota T. (1997). The structure, biosynthesis and function of brassinosteroids. *Trends Plant Sci*. 2, 137–143.

Yokota T., Nakamura N., Takahadhi N., Nonaka M., Sekimoto H., Oshio H., Takatsuto S. (1991). "Inconsistency between growth and endogenous levels of gibberellins, brassinosteroids, and sterols in *Pisum sativum* treated with uniconazole antipodes," in *Gibberellins* eds Takahashi N., Phinney B. O., Macmillan J., editors. (New York, NY: Springer;) 339–349.

Yoshida S. (2000). Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. *Plant Physiol*. 123, 93–100.

Yu X., Li L., Zola J., Aluru M., Ye H., Foudree A., Rodermel S. (2011). A brassinosteroid transcriptional network revealed by genome-wide identification of BES1 target genes in *Arabidopsis thaliana*. *Plant J*. 65, 634–646.

Zhang A., Zhang J., Zhang J., Ye N., Zhang H., Tan M., Jiang M. (2010). Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves. *Plant Cell Physiol*. 52, 181–192.

Zhang S., Cai Z., and Wang X. (2009). The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling. *Proc. Natl. Acad. Sci*. 106, 4543–4548.

Zhang S. J., Song G. Q., Li Y. L., Gao J., Liu J. J., Fan Q. Q., Li G. Y. (2014). Cloning of 9-cis-epoxycarotenoid dioxygenase gene (*TaNCED1*) from wheat and its heterologous expression in tobacco. *Biol. Plant* 58, 88–98.

Zheng L., Gao C., Zhao C., Zhang L., Han M., An N., Ren X. (2019). Effects of brassinosteroid associated with auxin and gibberellin on apple tree growth and gene expression patterns. *Hort. Plant J.* 5, 93–108.

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 Cell Physiol.* 50, 644–651.

Zhu Y., Nomura T., Xu Y., Zhang Y., Peng Y., Mao B., Zhu X. (2006). Elongated uppermost internode encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. *Plant Cell* 18, 442–456.

Zeng Y., Kermode A. R. (2004). A gymnosperm ABI3 gene functions in a severe abscisic acid-insensitive mutant of *Arabidopsis* (abi3-6) to restore the wild-type phenotype and demonstrates a strong synergistic effect with sugar in the inhibition of post-germinative growth. *Plant Mol. Biol.* 56, 731–746.

APPENDIX

Appendix 1-1 Relative transcript abundance of *NCED*s between control and Pcz treated seeds at different days after imbibition in endosperm of wheat.

Gene	DAI	Sample	Relative transcript level	SE
NCED1	1	Control	n.d.	
		Pcz	n.d.	
	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	
NCED2	1	Control	1	0.32
		Pcz		
	3	Control	3.39	0.76
		Pcz	2.11	1.28
	5	Control	2.29	0.37
		Pcz	1.23	0.06
	7	Control	19.36	4.5
		Pcz	11.64	2.54

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 1-2 Relative abundance of *CYP707A*s transcripts between control and Pcz treated seeds at different days after imbibition in endosperm of wheat.

Gene	DAI	Sample	Relative transcript level	SE
CYP707A1	1	Control	1	0.27
		Pcz	7.21	1.69
	3	Control	0.42	0.02
		Pcz	0.87	0.04
	5	Control	0.11	0.03
		Pcz	1.33	0.9
	7	Control	1.02	0.16
		Pcz	9.14	2
CYP707A2	1	Control	1	0.07
		Pcz	6.26	0.89
	3	Control	50.02	0.16
		Pcz	14.07	1.84
	5	Control	17.27	0.59
		Pcz	8.26	2.35
	7	Control	26.38	4.5
		Pcz	13.26	4.56

DAI, Days after imbibition; SE, Standard error; n.d.: not detected

Appendix 1-3 Relative abundance of *GA20ox*s and *GA3ox* transcripts between control and Pcz treated seeds at different days after imbibition in endosperm of wheat.

Gene	DAI	Sample	Relative transcript level	SE
GA20ox1	1	Control	1	0.18
		Pcz	4.13	0.78
	3	Control	0.85	0.33
		Pcz	0.64	0.1
	5	Control	1.62	0.34
		Pcz	3.96	0.1
	7	Control	59.78	13.5
		Pcz	19.84	6.04
GA20ox2	1	Control	n.d.	
		Pcz	n.d.	
	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	
GA3ox2	1	Control	1	0.31
		Pcz	4.8499	0.23
	3	Control	0.6899	0.05
		Pcz	0.29	0.06
	5	Control	1.9	0.07
		Pcz	1.11	0.03
	7	Control	28.88	7.12
		Pcz	20	4.91

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 2-1 Relative transcript abundance of *NCED*s between control and Pcz treated seeds in embryo of wheat.

Gene	DAI	Sample	Relative transcript level	SE
NCED1	1	Control	n.d.	
		Pcz	n.d.	
NCED2	1	Control	1	0.09
		Pcz	1.18	0.48

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 2-2 Relative abundance of *CYP707A*s transcripts between control and Pcz treated seeds in embryo of wheat.

Gene	DAI	Sample	Relative transcript level	SE
CYP707A1	1	Control	1	0.20
		Pcz	0.16	0.04
CYP707A2	1	Control	n.d.	
		Pcz	n.d.	

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 2-3 Relative abundance of *GA20ox*s and *GA3ox* transcripts between control and Pcz treated seeds in embryo of wheat.

Gene	DAI	Sample	Relative transcript level	SE
GA20ox1	1	Control	1	0.07
		Pcz	0.9	0.04
GA20ox2	1	Control	1	0.37
		Pcz	0.79	0.29
GA3ox2	1	Control	1	0.10
		Pcz	0.32	0.04

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 3-1 Relative transcript abundance of *NCED*s between control and Pcz treated seeds at different days after imbibition in coleoptile of wheat.

Gene	DAI	Sample	Relative transcript level	SE
NCED1	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	
NCED2	3	Control	1	0.16
		Pcz	2.63	1.4
	5	Control	12.11	3.13
		Pcz	27.39	1.87
	7	Control	7.86	1.68
		Pcz	3.87	0.62

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 3-2 Relative abundance of *CYP707A*s transcripts between control and Pcz treated seeds at different days after imbibition in coleoptile of wheat.

Gene	DAI	Sample	Relative transcript level	SE
CYP707A1	3	Control	1	0.1
		Pcz	1.41	0.39
	5	Control	6.6	0.37
		Pcz	3.82	0.15
	7	Control	1.35	0.32
		Pcz	1.45	0.32
CYP707A2	3	Control	1	0.28
		Pcz		
	5	Control	5.24	1
		Pcz		
	7	Control	15.38	0.23
		Pcz	3.16	0.21

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 3-3 Relative abundance of *GA20ox*s and *GA3ox* transcripts between control and Pcz treated seeds at different days after imbibition in coleoptile of wheat.

Gene	DAI	Sample	Relative transcript level	SE
GA20ox1	3	Control	1	0.26
		Pcz	1.74	0.41
	5	Control	4.06	1.06
		Pcz	10.47	2.71
	7	Control	0.74	0.44
		Pcz	2.74	0.39
GA20ox2	3	Control	1	0.06
		Pcz	2.84	1.03
	5	Control	22.72	6.41
		Pcz	61.61	10.84
	7	Control	3.01	0.10
		Pcz	6.74	0.68
GA3ox2	3	Control	1	0.31
		Pcz	4.69	0.88
	5	Control	3.5	0.59
		Pcz	4.06	1.04
	7	Control	0.98	0.24
		Pcz	1.22	0.6

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 4-1 Relative transcript abundance of *NCED*s between control and Pcz treated seeds at different days after imbibition in root of wheat.

Gene	DAI	Sample	Relative transcript level	SE
NCED1	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	
NCED2	3	Control	1	
		Pcz	0.29	0.07
	5	Control	1.81	0.28
		Pcz	0.37	0.04
	7	Control	1.16	0.28
		Pcz	0.32	0.07

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 4-2 Relative abundance of *CYP707A*s transcripts between control and Pcz treated seeds at different days after imbibition in root of wheat.

Gene	DAI	Sample	Relative transcript level	SE
CYP707A1	3	Control	1	0.10
		Pcz	2.63	0.58
	5	Control	3.37	0.26
		Pcz	2.9	0.69
	7	Control	4.56	0.29
		Pcz	1.34	0.12
CYP707A2	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	

DAI, Days after imbibition; SE, Standard error; n.d., not detected

Appendix 4-3 Relative abundance of *GA20ox*s and *GA3ox* transcripts between control and Pcz treated seeds at different days after imbibition in root of wheat.

Gene	DAI	Sample	Relative transcript level	SE
GA20ox1	3	Control	1	0.18
		Pcz	2.82	0.14
	5	Control	3.32	0.06
		Pcz	1.93	0.21
	7	Control	1.57	0.48
		Pcz	1.48	0.36
GA20ox2	3	Control	n.d.	
		Pcz	n.d.	
	5	Control	n.d.	
		Pcz	n.d.	
	7	Control	n.d.	
		Pcz	n.d.	
GA3ox2	3	Control	1	0.1
		Pcz	3.21	1.26
	5	Control	5.64	1.02
		Pcz	3.06	0.54
	7	Control	2.71	0.14
		Pcz	0.48	0.22

DAI, Days after imbibition; SE, Standard error; n.d., not detected