Genetic and hypoxic control of dormancy in barley (*Hordeum vulgare*) is linked to alanine aminotransferase at the SD1 locus

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Abstract

In malting barley, rapid germination is desirable and linked to end use quality. Modern malting varieties have been bred for low seed dormancy leading to issues with pre-harvest sprouting in wetter growing regions. To maintain malting capacity while minimizing germination on the maternal plant requires in-depth understanding of the genetic regulation of dormancy in malting barley. Currently, the major effect QTLs SD1 and SD2 have been shown to influence dormancy across multiple populations of barley, though the physiological mechanisms involved remain unclear. To search for novel genetic regions that influence primary dormancy, three mapping populations were assessed including two Canadian biparental populations (Synch and Legci) as well as a diversity panel sourced from multiple locations worldwide (ICARDA AM-14). The SD2 locus had a major effect in the Synch population while the SD1 locus had a major effect in the Legci population and neither SD1 nor SD2 were linked to dormancy in the diversity panel. Instead, 14 additional marker trait associations were identified in AM-14 suggesting that investigating a broader range of genetic regulation of dormancy outside of North American varieties may provide solutions to regulate this trait. Additional testing on SD1 revealed that variation at this locus did not affect ABA sensitivity during germination or GA or ABA-regulated gene expression during grain fill. Indeed, lines containing the non-dormant SD1 allele germinate at a similar rate as the dormant SD1 seeds when the glumella was removed from the embryo. This indicated that the effect of the alanine aminotransferase gene underlying the SD1 allele is dependent on physical restriction on the embryo or the hypoxic effects produced by the glumella. Imposing a hypoxic (5% oxygen) environment on exposed embryos revealed an association between non-dormancy at SD1 and reduced sensitivity to the suppressive effects of hypoxia on germination. This suggests that alanine aminotransferase regulates dormancy release during barley germination at least in part through regulation of the seed's response to hypoxia.

Dans l'orge de brasserie, une germination rapide est souhaitable et liée à la qualité de l'utilisation finale. Les variétés de maltage modernes ont été sélectionnées pour une faible dormance des graines, ce qui entraîne des problèmes de germination sur pied dans les régions de croissance plus humides. Maintenir la capacité de maltage tout en minimisant la germination sur pied nécessite une compréhension approfondie de la régulation génétique de la dormance de l'orge de brasserie. Présentement, il a été démontré que les locus de caractère quantitatifs (QTL) à effet majeur SD1 et SD2 influencent la dormance dans plusieurs populations d'orge, bien que les mécanismes physiologiques impliqués restent ambigus. Pour rechercher de nouvelles régions génétiques qui influencent la dormance primaire, trois populations de cartographie génétique ont été évaluées, dont deux populations biparentales canadiennes (Synch et Legci) ainsi qu'un panel de diversité provenant de plusieurs endroits dans le monde (ICARDA AM-14). Le locus SD2 avait un effet majeur dans la population Synch tandis que le locus SD1 avait un effet majeur dans la population Legci, et ni SD1 ni SD2 n'étaient liés à la dormance dans le panel de diversité. Au lieu, 14 associations de traits marqueurs supplémentaires ont été identifiées dans l'AM-14, ce qui suggère que l'étude d'un éventail plus large de régulation génétique de la dormance en dehors des variétés nord-américaines pourrait fournir des solutions pour réguler ce trait. Des tests supplémentaires sur SD1 ont révélé que la variation à ce locus n'affectait pas la sensibilité de l'ABA pendant la germination ou l'expression des gènes régulés par GA ou ABA pendant le remplissage du grain. En effet, les lignées possédant l'allèle SD1 non dormant germent à un rythme similaire à celui des graines SD1 dormantes lorsque la glumelle a été retirée de l'embryon. Cela indique que l'effet du gène de l'alanine aminotransférase sous-jacent à l'allèle SD1 dépend de la restriction physique sur l'embryon ou des effets hypoxiques produits par la glumelle. L'imposition d'un environnement hypoxique (5 % d'oxygène) aux embryons exposés a révélé une association entre la non-dormance à SD1 et une insensibilité aux effets suppresseurs de l'hypoxie sur la germination. Cela suggère que l'alanine aminotransférase régule la libération de la dormance pendant la germination de l'orge au moins en partie par la régulation de la réponse de la graine à l'hypoxie.

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Abbreviations

ABA	Abscisic Acid
ABI3-5	ABA insensitive 3-5
AlaAT	Alanine aminotransferase
D	Dormant allele
DAA	Days after anthesis
DAI	Days after imbibition
DAP	Days after pollination
GID1	Gibberellin insensitive dwarf 1
GWAS	Genome wide association study
ICARDA	International center for agricultural research in dry areas
KASP	Kompetitive allele specific PCR
MKK3	Mitogen activated kinase kinase 3
NCED	Nine-cis-epoxy carotenoid dioxygenase
ND	Non-dormant allele
PHS	Pre-harvest sprouting
QTL	Quantitative trait loci
SD1	Seed dormancy 1
SD2	Seed dormancy 2
Snrk2	Sucrose non-fermenting I related protein kinase2

1. Chapter 1 – Introduction

1.1 The role of seeds in the plant lifecycle

In plant biology, the “seed” is a complex structure that provides protection and nutrients for the mature embryo and its function is critical to the survival of the emerging seedling. Seeds confer a strong evolutionary advantage, with the majority of extant plant species belonging to the seed-producing or spermatophyte lineage consisting of the gymnosperms (~1000 species) and the angiosperms (~295,000 species) (Christenhusz and Byng, 2016; Magallón *et al.*, 2019). The angiosperms or “flowering plants” are the most diverse plant clade and include nearly all species produced for human or animal consumption (Milla *et al.*, 2018). The sexual reproductive life cycle of the angiosperms can be divided into three phases: embryogenesis, vegetative growth and reproductive development (Boesewinkel and Bouman, 1984). Following the fertilisation of the ovule, the ovule tissue develops into the “seed” which supports the embryo between embryogenesis and the initiation of vegetative growth (Endress, 2011). The seed has three basic components; the seed coat, the endosperm and the embryo. The seed coat, or testa, provides structure and defense against predation and forms after fertilisation from the outer cell layer of the ovary (Fig. 1.1). Enclosed within the seed coat, the endosperm is a store of energy and nutrients to fuel seedling establishment and develops via double fertilisation of the polar nuclei (Simpson, 2010). Finally, the embryo is an organised collection of cells developed during embryogenesis that will eventually grow into the plant (Agrawal and Rakwal, 2012). Angiosperms are further divided into two major clades with variation in internal seed structure, dicotyledonous plants consume most of the endosperm during embryogenesis while the endosperm of monocotyledonous species persists until germination (Zhao *et al.*, 2017). The nutrients for growth of the *Arabidopsis* embryo are stored within the cotyledons following consumption of the endosperm, while barley seeds maintain a large endosperm through the end of seed maturation (Yan *et al.*, 2014; Sreenivasulu *et al.*, 2010). Other plant structures such as the fruit which is a mature ovary that contains the seed, generally aids with processes such as dispersal (Ozga and Reinecke, 2003; Dardick and Callahan, 2014). While there is large variation in fruit structures between species, fruit of the dicot *Arabidopsis thaliana* is contained within a silique or seed pod that contains multiple seeds (Roeder and Yanofsky, 2006) while some dicot species such as sunflower have a single seeded fruit called an achene (Simpson, 2010). For many cereal crops the grain is a caryopsis which is a one-seed fruit where the ovary wall or testa is fused to the pericarp (Handa *et al.*, 2012)(Figure 1.1a). In barley, the majority of mass in the seed is comprised of the endosperm and pericarp which are made of non-living cells at maturity, while living cells remain in the embryo and a cell layer surrounding the endosperm called the aleurone which produces catalytic enzymes for nutrient mobilisation during sprouting (Sreenivasulu *et al.*, 2010). Most barley varieties also have a tight adhering glumella that surrounds the caryopsis and persists past maturity which provides physical protection to the embryo and has implications for seed physiology (Olkku *et al.*, 2018). One of the major advantages to the seed habit is timing of vegetative growth to occur under the most favourable conditions which can be influenced by the seed remaining dormant or progressing to germination (Willis *et al.*, 2014). Many seed forms and processes exist among the spermatophytes which allows species to fill countless ecological niches and has contributed to the large diversity in land plants (Linkies *et al.*, 2010).

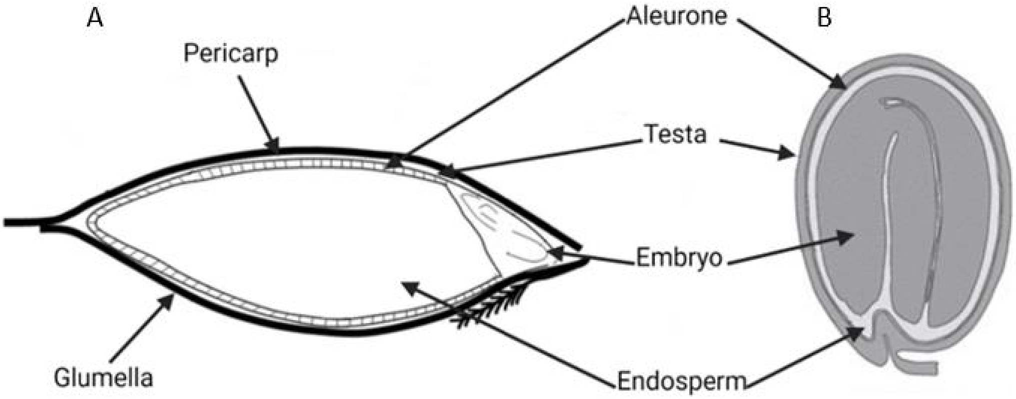

Figure 1.1. Common anatomical features of the seed in A) barley caryopsis or B) *Arabidopsis thaliana*. The figure was modified from Haughn and Chaudhury, 2005 and Filipowska et al., 2021.

1.2 Regulation of dormancy and germination

Since plants have limited motility, they are largely at the mercy of chance as to whether environmental conditions will permit growth for any given individual. One tool the plant has to improve the chances of success is temporal dispersion, where the embryonic plant will sense the environmental conditions that are most likely to enable survival in the vegetative stage of life which effects germination signalling (Willis *et al.*, 2014). This selectivity is dependent on two interrelated processes, germination and dormancy (Fig.1.2). During the final stages of seed maturation, orthodox seeds are desiccated and remain with minimal moisture content until water is imbibed from the external environment which is the primary signal to begin the germination process (Chin *et al.*, 1989). Germination is the process of initiating the growth of the quiescent embryo which begins with the uptake of water and ends with the emergence of the radical from the seed coat, initiating an irreversible progression to the vegetative stage (Bewley, 1997). Cellular signaling within the embryo can block germination if stressful environmental conditions are sensed such as unfavourable temperature or light conditions (Leymarie *et al.*, 2009; Barrero *et al.*, 2014; Hoang *et al.*, 2014). When imbibed in favourable conditions the seed does not necessarily germinate, "dormancy" is the resistance that a seed has to germination when conditions are suitable for germination to occur (Koornneef *et al.*, 2002). Two kinds of dormancy have been distinguished, primary and secondary dormancy, where primary dormancy is induced during grain maturation and prevents germination during the dry down phase and can persist into posit imbibition (Nadella *et al.*, 2003). Secondary dormancy occurs when a non-dormant seed is prevented from germinating because of a period of exposure to non-conducive environmental conditions, such as high temperature, from which the seed will delay germinate even when moved to environmental conditions that typically allow germination (Nadella *et al.*, 2003). Despite metabolism being predominantly halted in the desiccated seed, the dormancy level is not constant and will decrease over time in a process known as after-ripening (Holdsworth *et al.*, 2008). Germination, dormancy and after-ripening are three biological processes that actively improve the odds the plant will enter vegetative growth under tolerable conditions. Depending on the conditions in which seeds remain dormant, they will eventually senesce and lose viability (Nagel *et al.*, 2016). After-ripening reduces the seeds dormancy over time widening the scope of environmental conditions the seed will accept as suitable for germination (Carrera *et al.*, 2008). The interplay between dormancy, after-ripening and germination balances selectivity for favourable conditions with a change in priority to germination as the risk of loss of viability increases.

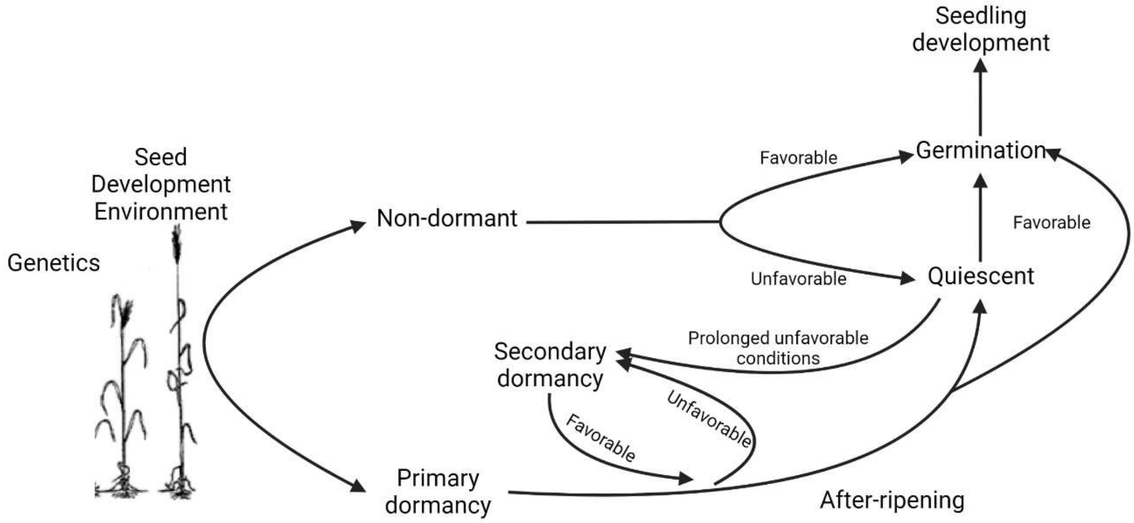

Figure 1.2. Interrelationship of fundamental seed processes between seed maturation and germination. Figure was recreated from Nadella et al., 2003 with elements from University of Minnesota spring barley growth and development guide (Anderson et al., 1995).

1.3 Establishing primary dormancy

Primary dormancy is induced during the later stages of seed maturation. Depth of dormancy within a species is determined by genetic effects, maternal environmental conditions and their interactions (Suriyasak *et al.*, 2020). Dormancy in cereal grains is a quantitative trait with large and small effect genes that contribute to the final phenotype (Tai *et al.*, 2021). Maternal environment can strongly influence the depth of dormancy induced during seed maturation, conditions such as light wavelength, light intensity and temperature can all influence the dormancy phenotype (Penfield and MacGregor, 2017). During seed maturation of the barley caryopsis, temperature influences the level of dormancy induced with cold temperatures increasing dormancy and warm temperatures decreasing dormancy (Rodríguez *et al.*, 2001). Primary dormancy is induced near the end of seed maturation and harvested immature seeds can germinate precociously. Arabidopsis seeds harvested 8 – 14 days after pollination (DAP) will germinate but become progressively more dormant when harvested 18 – 21 DAP coinciding with primary dormancy induction (Alboresi *et al.*, 2005).

In seeds, the two hormones that have been most commonly identified for the regulation of dormancy and germination are abscisic acid (ABA) which promotes dormancy and gibberellic acid (GA) which promotes germination (Finch-Savage and Leubner-Metzger, 2006). Whether the seed will progress to germination is more dependent on the ratio of ABA and GA rather than the absolute levels of either hormone (White *et al.*, 2000). During seed maturation and induction of dormancy, ABA is the primary hormone that promotes several aspects of seed maturation including, nutrient storage, desiccation tolerance and dormancy (Sreenivasulu *et al.*, 2010). ABA can be produced in the maternal vegetative tissue, typically earlier in maturation or in the embryo later in maturation, the latter of which is associated with dormancy (Tuan *et al.*, 2018). ABA biosynthesis is necessary for prevention of germination with developing maize kernels germinating precociously when treated with ABA biosynthesis inhibitor fluridon (Fong *et al.*, 1983). In developing wheat and barley seeds, ABA levels are low in the embryo but rapidly increase and peak at 35 DAP (Suzuki *et al.*, 2000; Chono *et al.*, 2006). Seeds harvested prior to 35 DAP germinate precociously while seeds harvested at 35 DAP do not germinate with dormancy decreasing in subsequent harvests (Suzuki *et al.*, 2000; Chono *et al.*, 2006). The effect of ABA concentration in barley grains on the depth of dormancy induced during grain maturation is controversial. Studies by Chono *et al.*, 2006 observed an association between the amount of ABA produced during dormancy induction and final resistance to germination. While studies by Romagosa *et al.*, 2001 found differences in germination percentage when plants were grown in different environments, but found limited differences in ABA content suggesting it is sensitivity to ABA rather than absolute value that is relevant for determining depth of dormancy. In maize plants, ABA deficient mutants germinate precociously but not if GA was inhibited, suggesting that the ratio between GA and ABA is still relevant during seed maturation, although many studies investigating hormonal induction of dormancy do not quantify GA levels (White *et al.*, 2000).

ABA is produced as a derivative of carotenoid biosynthesis for which the rate limiting step is cleavage of 9-cis-neoxanthin or 9-cis-violaxanthin to xanthoxin mediated by nine-cis-epoxycarotenoid dioxygenase (NCED) (Schwartz *et al.*, 2003). Catabolism of ABA to dihydrophasic acid (DPA) is mediate by *ABA-8'-OH* which catalyzes hydroxylation of (+)-ABA (Saito *et al.*, 2004). GA production is downstream of diterpene biosynthesis and is largely dependent on production of bioactive forms by *GA20ox* and *GA3ox* while conversion of bioactive GAs to inactive forms is mediated by *GA2ox* which supress the amount of GA signaling (Yamaguchi, 2008).

Hormonal signaling influences dormancy mainly through modification of gene expression. In the presence of ABA, ABA binds *PYR/PYL/RCAR* proteins causing inactivation of *protein phosphatase 2C* (*PP2C*) *abscisic acid insensitive 1* and *2* (*ABI1/2*) which alleviates inhibition of SUCROS NONFERMENTING 1 RELATED PROTEIN KINASE 2s (SnRK2s) (Ali *et al.*, 2022) (Fig. 1.3). Activated SnRK2s promote the transcription of ABA mediated *ABI4-5* which are bZIP transcription factors that promote transcription related to de-greening, desiccation tolerance, nutrient storage and seed dormancy (Tuan *et al.*, 2018).

GA signaling is repressed by the DELLA protein, when bioactive GA is present it binds GIBERALLIN INSENSITIVE DWORF 1 (GID1) and forms the GA-GID1-DELLA complex that is flagged for degradation (Murase *et al.*, 2008) (Fig. 1.3). Removal of DELLA stimulates the production of *GAMYB* transcription factor that promotes germination (Gubler *et al.*, 1995). An example of how GA promotes germination and seedling growth is its regulation of alpha amylase production during germination. Alpha amylase production can be turned on in plant tissue via a starvation signal induced by lack of sugar in the cell, GAMYB binds the promoter of an alpha amylase gene and locks the starvation signal on to maintain alpha amylase production despite an increase in sugar concentration in the cell which supports early seedling establishment (Hong *et al.*, 2012).

ABA and GA signaling acts antagonistically, with each hormone actively downregulating the others production and action (Finkelstein *et al.*, 2008). The way one hormone effects another is through changes in transcription related to hormone metabolism. For example, *PP2C* genes have been found to both negatively regulate ABA signaling and promote GA production such as inactivation of ABI5 which prevents promotion of *GA2ox* transcription thereby increasing the amount of bioactive GA (Cantoro *et al.*, 2013).

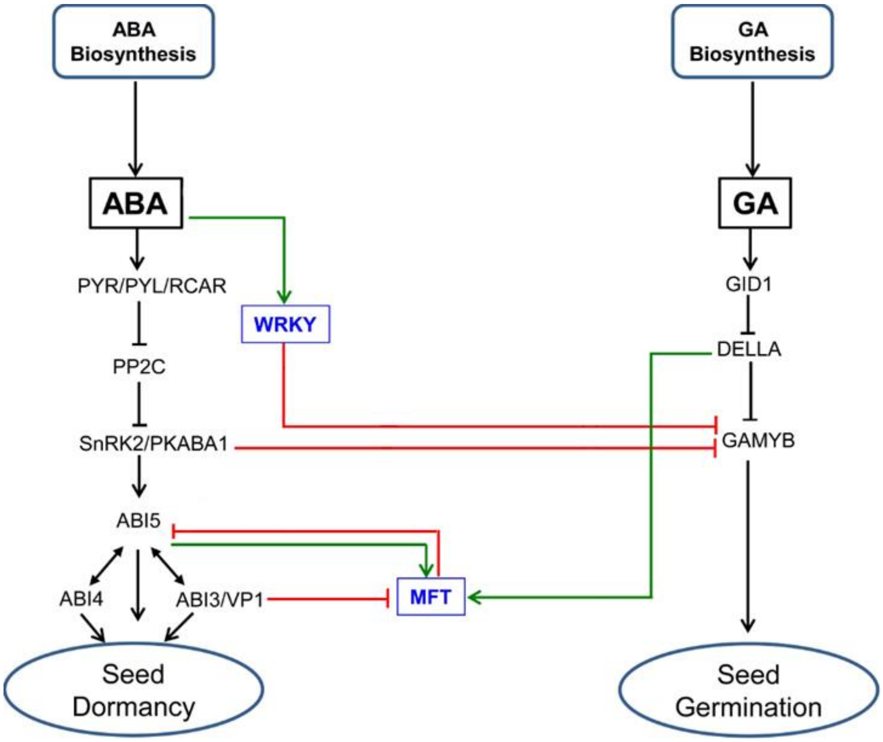

Figure 1.3. Crosstalk between ABA and GA regulating seed dormancy and germination. Figure was taken from Tuan et al., 2018.

1.4 Induction of secondary dormancy

Dormancy level is partially determined by environmental factors and seeds do not all experience exactly the same environment during imbibition. Several factors suppress germination aside from genetic components including temperature, light and osmotic stress (Leymarie *et al.*, 2009; Barrero *et al.*, 2014; Hoang *et al.*, 2014). Germinating barley seeds are sensitive to some environmental factors including temperature and light (Gubler *et al.*, 2005). Barley grains germinate optimally at 15˚C and will enter secondary dormancy above 30˚C (Leymarie *et al.*, 2009). Light can also increase dormancy post-imbibition in barley and potentially introduce additional environmental variation into a dormancy assessment (Barrero *et al.*, 2014). ABA is the predominant hormone signaling for dormancy in the seed, for induction of primary dormancy a spike in ABA content around physiological maturity produces a strong dormant signal that persists after ABA levels have declined (Chono *et al.*, 2006). When the seed imbibes water the metabolism in the embryo cells will resume and can sense the degree of stress in the environment related to common stress factors such as temperature and hypoxia (Hoang *et al.*, 2013). If environmental cues are stressful, ABA production will resume in the embryo through transcription of ABA biosynthesis genes such as *NCED1* which can re-impose a dormant state that prevents germination even if conditions change to more acceptable levels and may require further after-ripening to break (Leymarie *et al.*, 2008).

1.5 After-ripening

After-ripening is a process of dormancy reduction that occurs when desiccated seeds, typically with moisture contents of <0.1g H_2O g dry weight^{-1}, lose dormancy over time (Nagel *et al.*, 2015). In this desiccated state, internal solutions exist as a glassy matrix that severely limit or inhibit active metabolism but can allow movement of molecules (Ballesteros and Walters, 2011). After-ripening increases the seeds' predisposition for germination and increases the range of acceptable environmental conditions the seed will germinate in (Finch-Savage and Leubner-Metzger, 2006). For example, after-ripened barley seeds will lose their sensitivity to light-dependent inhibition of germination (Barrero *et al.*, 2014). The molecular basis for after-ripening is poorly understood, but protein oxidation caused by an increase in reactive oxygen species and lipid peroxidation has been linked with dormancy reduction in sunflower seeds (Oracz *et al.*, 2007). The rate of dormancy release during after-ripening is partially dependent on the temperature seeds are stored at higher temperatures have accelerating dormancy release and lower temperatures limit dormancy release (Rodríguez *et al.*, 2018). Storing seeds such as barley grains at -18˚C to -20˚C will prevent after-ripening and maintain the initial dormancy level upon storage (Nagel *et al.*, 2019).

1.6 Physical and hypoxic regulation of germination

In barley seeds two structures have been implicated in enforcing dormancy, the glumella and the coleorhiza. The glumella is made up of the lemma and palea which are bracts that tightly adhere to the barley seed at maturity and can limit oxygen diffusion to the embryo creating a partially isolated air environment (Lenoir et al., 1986). The coleorhiza is a tissue that surrounds the embryonic root or radical and physically restricts radical growth when the seed is in a dormant state. The ablation of coleorhiza tissue in wild oat induced germination in dormant seeds, while ablation of other seed structures did not (Holloway *et al.*, 2021). Furthermore, Holloway *et al.*, 2021 found the puncture force required to rupture the coleorhiza decreased over 24 to 48 hours after imbibition of after-ripened seeds but increased over the same time in dormant seeds. After-ripened seeds also had higher expression of cell wall degrading enzymes compared to dormant seeds which are typically repressed by ABA signaling (Barrero *et al.*, 2009).

Dormancy in barley is imposed at the physiological level by hypoxia in the embryo (Corbineau, 2022). Post-imbibition, limited oxygen availability in the embryo can prevent germination depending on dormancy status, with dormant seeds being more sensitive to hypoxia than non-dormant seeds through unknown mechanisms (Bradford *et al.*, 2008). Primary dormant barley grains have reduced germination below 10% oxygen and no germination bellow 5% (Hoang *et al.*, 2013). For barley, oxygen diffusion to the embryo is limited by the seed coverings or glumellae which reduces the level of available oxygen in the embryo even when seeds are germinated in air (Lenoir *et al*., 1986; Hoang *et al*., 2013). Barley seeds germinate best at lower temperatures (15˚C) and go into secondary dormancy at 30˚C (Leymarie *et al.*, 2009). One of the interactions the barley seed has with temperature is a decrease in oxygen diffusion to the embryo as temperature increases. Micro-oxygen sensors inserted into the embryo found 15% oxygen in the embryo at 15˚C and 0.3% at 30˚C when tested in air (21% oxygen) (Hoang *et al*., 2013). Hypoxia can also influence hormonal signalling related to dormancy, as ABA sensitivity of barley seeds increased in lower oxygen environments (Benech-Arnold *et al.*, 2006). Altogether, self-imposed oxygen limitation to the embryo is an important component of seed dormancy in barley.

The majority of work on hypoxic tolerance in plants has been done on root tissue, which has revealed how metabolism is adjusted to limit stress in reduced oxygen availability (Zabalza *et al.*, 2009; Rocha *et al.*, 2010). Glycolysis is the process of breaking down glucose which produces ATP and pyruvate. Under normoxic conditions, pyruvate is utilised in TCA cycle to produce ATP during aerobic respiration. However, under hypoxic conditions glycolysis must continue providing the cell with ATP while TCA activity is reduced (Gupta *et al.*, 2009). Hypoxic stress activates three fermentation pathways which utilize pyruvate: lactate fermentation, ethanolic fermentation and alanine production via alanine aminotransferase (AlaAT) (Bailey-Serres *et al.*, 2012). It has been suggested that one of the main functions of these pathways is to limit pyruvate accumulation rather than utilize it as an additional energy source (Rocha *et al*., 2010). Zabalza *et al*., 2009 found that when supplemental pyruvate was fed to internal root tissues with limited oxygen diffusion, the tissue was unable to limit respiration which decreased the oxygen status to anoxia. It is not well known how the embryo tolerates hypoxia, but given the importance of hypoxia in dormancy regulation, it may be important to understanding the physiology of seed dormancy.

Dormancy is affected by hypoxia in many species, where as hypoxia generally induces stronger dormancy, but in some species can break dormancy (Corbineau, 2022). The N-end rule is a pathway regulating protein stability based on N-terminal ubiquitination and is emerging as one of the main ways plant cells sense hypoxia (Licausi *et al.*, 2011). One of the pathways implicated in response to hypoxia is N-end rule degradation of group VII ethylene response factors (ERFs) where specific ERFs are degraded via the N-end rule under normoxia but are stabilised with reduced oxygen (Gibbs *et al.*, 2014). Gibbs *et al*., 2014 found that ERFs promoted dormancy through promotion of *ABI5* induction despite ethylene typically considered antagonistic to ABA (Corbineau *et al.*, 2014; Ahammed *et al.*, 2020). Studies investigating the effect of hypoxia on germinating seeds typically artificially impose hypoxia *in vitro* but *in vivo,* low oxygen levels can naturally occur around the embryo. It is unclear at what stage hypoxia occurs; during resumption of metabolism post-imbibition or during primary dormancy induction (Hoang *et al.*, 2013). While the effect hypoxia has on germination has been well documented, molecular signalling pathways interacting with oxygen level to regulate dormancy are poorly understood including how hypoxia around the embryo is initiated (Bradford *et al.*, 2008; Hoang *et al.*, 2013).

1.7 Hormonal and transcriptional regulation of germination

The germination process can be separated into three phases based on water uptake. In phase 1 the seed rapidly imbibes water which causes resumption of metabolism (Ma *et al.*, 2017) (Fig. 1.4a). In phase 2 there is little increase in the amount of water within the seed, during this time metabolism is active in both dormant and non-dormant seeds (Weitbrecht *et al.*, 2011). Dormant seeds will persist in phase 2 until dormancy is broken while non-dormant seeds will germinate. Phase 3 is timed with radical emergence and represents post-germination growth, at this time rapid water uptake resumes into the growing plant (Ma *et al.*, 2017). In dormant seeds, ABA production and sensitivity remain high enough to supress embryo growth while in non-dormant seeds, production of ABA8'-OH1 is stimulated which decreases ABA content (Rodríguez *et al.*, 2015). In cereals, GA signaling is increased upon imbibition and GA biosynthesis inhibitors will block germination when applied soon after imbibition. Later in the germination process GA production is no longer required since GA biosynthesis inhibitors can no longer prevent germination (Rodríguez *et al.*, 2012). An important part of GA signaling is release of mobile GA_3 that is moved to the aleurone layer and stimulates production of α-amylases to mobilise stored nutrients in the endosperm that is transported to the embryo through the scutellum providing nutrition to the growing parts of the embryo for germination and seedling establishment (Fig. 1.4b) (Gubler *et al.*, 1999; Gupta and Chakrabarty, 2013). Ethylene also increases following imbibition and sharply increases around the time of radical emergence (El-Maarouf-Bouteau *et al.*, 2015). During germination, ethylene supresses ABA as was demonstrated by ethylene insensitive mutants *etr1* and *ein2* in Arabidopsis having elevated ABA levels, elevated levels of *NCED* and reduced germination (Cheng *et al.*, 2009).

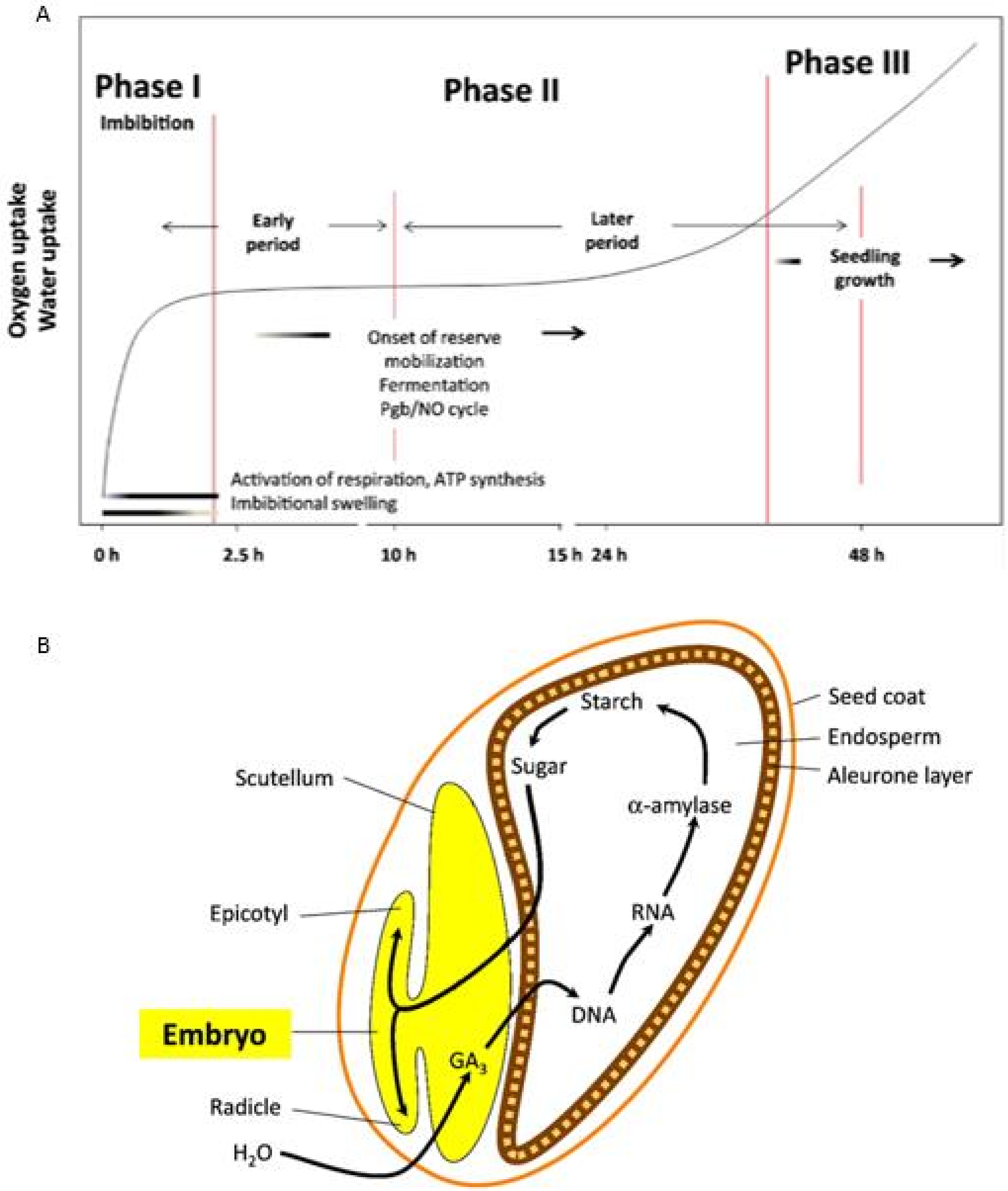

Figure 1.4. Processes important for seed germination post imbibition of water including **A)** the phases of germination in terms of water uptake and **B)** GA promotion of alpha amylase production. Both figures were taken from Ma et al., 2017 review of germination in barley.

ROS and nitric oxide (NO) also interact with GA and ABA to promote germination with both ROS and NO promoting GA and repressing ABA signaling (Kumar *et al.*, 2021). Upon imbibition, ROS including hydrogen peroxide and super oxide are produced enzymatically by NADPH oxidase and scavenged by catalase (Ishibashi *et al.*, 2010). In barley, *CAT2* is induced by ABA signaling to supress hydrogen peroxide while hydrogen peroxide inhibits the production of *GA2ox* which catabolises GA (Bahin *et al.*, 2011). Chemical inhibition of NADPH oxidase with diphenyleneiodonium supressed seed germination highlighting the importance of enzymatically produced ROS for the stimulation of germination (Bykova *et al.*, 2015). NO increases sharply upon imbibition in barley seeds and peaks in the early stages of seed germination (Ma *et al.*, 2017). Exogenous application of NO can stimulate germination in dormant Arabidopsis seeds (Bethke *et al.*, 2006). NO also interacts with VII ERFs and hypoxia to repress ABI5 which is a promanent enhancer of dormancy downstream of ABA signalling (Gibbs *et al.*, 2014).

1.8 Genetic regulation of dormancy in barley

Dormancy is an economically important trait in barley since malting barley requires low dormancy at maturity and as such many studies have previously investigated the genetic regulation of dormancy. QTL linkage mapping in the “Steptoe/Morex” double haploid population identified four major effect QTL termed seed dormancy 1-4 (SD1-4) (Hayes *et al.*, 1993; Oberthur *et al.*, 1995). Linkage mapping identified that SD1 and SD2 are located on chromosome 5H, SD3 is on chromosome 7H and SD4 is on chromosome 4H (Gao *et al.*, 2003). Since then, many more biparental QTL linkage maps have been performed mainly identifying the SD1 and SD2 loci as major effect QTL (Li *et al.*, 2003; Hori *et al.*, 2007; Ullrich *et al.*, 2009; Nakamura *et al.*, 2017). Several mapping populations featuring lines from the Canadian germplasm have been investigated including malting line Harrington and AC Metcalf both of which identified SD2 with non-dormant alleles coming from Canadian lines (Ullrich *et al.*, 2009; Zhang *et al.*, 2012). SD1 has not been identified as a major effect QTL in Canadian malting genotypes, possibly because it has little effect on dormancy when paired with a non-dormant SD2 (Sweeney *et al.*, 2022*b*).

Both SD1 and SD2 have been fine mapped and cloned to an alanine aminotransferase1 (*AlaAT1*) and mitogen activated kinase kinase 3 (*MKK3*) (Nakamura *et al.*, 2016; Sato *et al.*, 2016). The mechanism of how SD2 influences dormancy in barley has not been elucidated but studies of *MKKK62* in rice which results in loss of seed dormancy, identified an *MKKK62/MKK3/MAPK7/14* signalling pathway that influenced germination rate (Mao *et al.*, 2019). Knockout of *MKK3* upregulated *OsMFT* suggesting the kinase signaling cascade may regulate *MFT* expression, a gene known to influence seed germination in wheat (Nakamura *et al.*, 2011). Knockouts of *HvMKK3* in the barley variety “Golden Promise” produced a highly dormant but non-lethal phenotype which persisted when isolated embryos were assayed suggesting embryo growth was directly impaired (Hisano *et al.*, 2022). SD2 is associated with low primary dormancy and high germination soon after harvest (Nagel *et al.*, 2019; Vetch *et al.*, 2020).

Less is known about how SD1 influences dormancy compared to SD2. *HvAlaAT1* is an alanine aminotransferase that catalyses the reversibly conversion of pyruvate and glutamate to alanine and 2-oxogluterate (Duff *et al.*, 2012). *HvAlaAT3* has high sequence similarity to *HvAlaAT1* and is a homodimer with a pyridoxal 5-phosphate cofactor (Orzechowski *et al.*, 1999; Duff *et al.*, 2012). The effect the L214F polymorphism has on *HvAlaAT1* is not apparent since the residue is estimated to be outward facing on the external surface of the protein away from the active site, cofactor binding site and dimerization interface (Sato *et al.*, 2016). Knockouts of *HvAlaAT1* produced a highly dormant non-lethal phenotype similar to SD2, although *Hvalaat1* germinated no different from control plants when the embryos were isolated from the surrounding seed tissue (Hisano *et al.*, 2022). Mild differences in germination rate between dormant and non-dormant SD1 allele occurs at physiological maturity and differences increase with after-ripening (Vetch *et al.*, 2020). While it is unknown how HvAlaAT1/SD1 influences dormancy it has been suggested it may play a role in hypoxic response due to hypoxias' effect on dormancy and *AlaAT*s' induction during hypoxic stress in other contexts (Sato *et al.*, 2016; Monteverdi *et al.*, 2022). However, no study has previously tested an association between hypoxia and SD1 allele.

2. Chapter 2 - SD1 regulates dormancy in Canadian barley and influences hypoxia tolerance

2.1 Introduction

Roughly one third of the barley seed produced globally is used for malting, and growing malting barley is advantageous for farmers because it can bring in a higher return than feed barley (Baik and Ullrich, 2008). Malting is a post-harvest process of steeping and sprouting seeds to mobilise the stored sugars and nutrients by stimulating the natural catalytic enzymes the seed produces such as α-amylase. Germinated seeds are then kilned to stop metabolism and produce the malt extract (MacLeod and Evans, 2016). For the malting process seeds must germinate quickly and uniformly in the sprouting step to produce high quality malt and malt barley has a requirement of ≥95% germination in three days which is called germination energy (Gualano *et al.*, 2014). Malt barley can be stored which will reduce the dormancy level by after-ripening, but storing malt barley without loosing quality is costly and seeds stored for too long will lose viability (Reuss *et al.*, 2003). Seeds must be harvested at ~12% moisture or artificially dried down in seed drying machines to avoid damage from fungi or loss of germination capacity (Lin *et al.*, 2008). Due to the cost of storage for malt barley, it is desirable for malting varieties to be non-dormant at maturity so grains can be used for malt with minimal after-ripening (Rooney *et al.*, 2023). Since malting varieties have been developed to have low dormancy at maturity, they become vulnerable to pre-harvest sprouting (PHS) which is when seeds germinate in the field while still attached to the maternal tissue when the conditions are wet or humid around the time seeds reach maturity (Sweeney *et al.*, 2022*b*). PHS and malt quality are generally negatively correlated and no solution has been found for combining the highest quality malt with strong PHS resistance in the same variety, limiting the production of malt in wetter environments (Rooney *et al.*, 2023). Studies investigating the genetic regulation of dormancy can assist barley breeding programs produce new malting varieties with strong PHS resistance.

Dormancy and germination in barley is regulated by cross-talk between hormones their effect on transcription. The most influential hormones regulating dormancy are abscisic acid (ABA) which promotes dormancy and gibberellic acid (GA) which promotes germination (Tai *et al.*, 2021). ABA signalling activates transcription factors ABA INSESITIVE 3-5 (ABI3-5) which actively antagonises GA by promoting transcription of *GA2ox* an inhibitor of GA and can self regulate ABA production by increase of ABA biosynthesis gene *NCED1* (Cantoro *et al.*, 2013). Similarly GA promotes the degradation of PYL/RCARs which are important for ABA mediated signaling (Tuan *et al.*, 2018). During grain maturation there is a spike in ABA production around physiological maturity that induces primary dormancy depending on genetic and maternal environment (Chono *et al.*, 2006). Other signaling molecules such as reactive oxygen species (ROS) including hydrogen peroxide and reactive nitrogen species (RNS) such as nitrous oxide also have important roles in dormancy release during germination (Bahin *et al.*, 2011) and after-ripening (Bouteau *et al.*, 2007). Barley germination is suppressed by low oxygen, or hypoxia, which can occur naturally in the embryo with the glumella acting as a barrier to oxygen diffusion though how hypoxia suppresses germination is not clear (Lenoir *et al.*, 1986; Hoang *et al.*, 2013). Once primary dormancy is induced, around physiological maturity, dormancy decreases under dry storage during after-ripening which can cause additional variation in germination percentage (Veasey *et al.*, 2004).

Low dormancy at maturity is an important characteristic of high quality malting lines which also makes them prone to PHS susceptibility. In regions where wet and humid weather is common during the seed maturation period, growing high quality malt is risky and often leads to PHS damage making the seeds unviable to be used for malt. Germination percentage is a direct indication of dormancy and is strongly correlated with more direct PHS measurements when seeds are exposed to germination conditions still attached to the head structure (Lin *et al.*, 2009). Despite the close relationship, dormancy and PHS are district traits and QTL mapping of the two independently overlapped at some, but not all identified QTL (Ullrich *et al.*, 2009).

Two major QTL influencing dormancy in barley have been consistently identified which are designated SD1 and SD2, both of which are located on the long arm of chromosome 5H (Gao *et al.*, 2003). The SD2 locus is associated with low dormancy at maturity and favourable malt quality and high PHS susceptibility with several recent studies suggesting that pleiotropy rather than linkage is causing this association (Sweeney *et al.*, 2022*b*; Rooney *et al.*, 2023). The alanine aminotransferase1 (AlaAT1) in the SD1 locus is responsible for major variation in dormancy at maturity that further increases during after-ripening (Romagosa *et al.*, 1999; Vetch *et al.*, 2020). Multiple alleles associated with dormancy have been identified in the SD2 locus which can determine the magnitude of effect SD2 has on dormancy and the highly non-dormant SD2 will epistatically mask any dormancy effects of SD1 (Sweeney *et al.*, 2022*b*)

Identifying which markers are important for dormancy regulation in Canadian germplasm can help breeders to produce new PHS resistant varieties through marker-assisted selection. While some investigation has been done on dormancy regulation in Canadian malting varieties, feed varieties have received less attention for study of dormancy regulation.

Furthermore, identification of novel alleles from diversity panels encompassing a broader genetic base can provide novel alleles which may provide new genetic resources to help find solutions to PHS susceptibility in malting genotypes.

To search for novel associations between genetic markers and seed dormancy in spring barley, we evaluated dormancy in three distinct populations Synch, Legci and ICARDAs AM-14 panel. The Synch biparental population includes recombinant inbred lines originating from a cross between 2-row CH1224-1 and 2-row elite Canadian malting variety AAC Synergy which has been well-studied for PHS resistance as a highly non-dormant genotype for the SD2 locus (Legge *et al.*, 2014; Vetch *et al.*, 2020; Sweeney *et al.*, 2022*a*). Legci is a biparental doubled haploid population derived from 6-row feed barley Leger and 2-row CI9831A (Jui *et al.*, 1997). Legci parental lines segregate for dormancy level but have not been previously investigated for marker-trait association. The International Center for Agricultural Research in Dry Areas (ICARDA) produced the association mapping 2014 panel (AM-14) which features genetically diverse 2 and 6-row genotypes which vary in abiotic stress resistance and disease resistance but has never been tested for dormancy regulation (Amezrou *et al.*, 2018).

The objectives of this work were:

1. Identify genetic regions that underlie regulation of germination in the Synch, Legci and AM-14 populations.
2. Assess the impact of the SD1 dormant allele on hormonal and hypoxic responses during germination.

2.2 Methods

2.2.1 Plant growth conditions

To investigate genetic control of dormancy in the Canadian germplasm two biparental populations, Legci and Synch were tested. Legci is a doubled haploid population with 119 lines, derived from the Canadian 6-row feed variety Leger and 2-row CI9831A (Jui *et al.*, 1997). Synch is a 2-row recombinant inbred population with 88 lines that includes Canadian malting barley variety AAC Synergy and CH1224-1 (Legge *et al.*, 2014). For GWAS, 215 lines were tested from the ICARDA 2014 association mapping panel including variation for row type (88 2-row and 127 6-row) and input adaptation (76 high input, 118 low input and 21 land races) genotypes (Table S1). High input lines are adapted to favorable conditions and low input lines are adapted to abiotic stress including moisture (Amezrou *et al.*, 2018). All genotypes were grown in a greenhouse to physiological maturity defined as yellow awns, glumella and first internode region, in 6.5” fibre pots with Promix(75%):black earth (24%):lime(1%). Plants were grown with a 16-hour photoperiod, 20˚C daytime and 15˚C night time temperatures. Plants were watered in the morning and afternoon as needed and were supplemented with NPK 20:20:20 once per week for three weeks when shoots reached approximately 6 inches. From week 3 until heading, the treatment was 35:5:10 followed by 15:15:30 after heading. Heads were harvested at physiological maturity, dried for 48 hours at 37℃ and stored at -20℃ to preserve dormancy. To monitor gene expression plants were grown under the same conditions and heads were frozen in liquid nitrogen and stored at -80℃, 15, 25 or 35 days after anthesis when the awns are first visible.

2.2.2 Quantification of Dormancy

To quantify dormancy, seed germination was monitored in petri dishes following a brief sterilization by submerging seeds in 30% bleach for 20 minutes, washing four times in sterile Milli-Q water, and submerging seeds in 0.24% Vitaflow-280 fungicide (UPL AgroSolutions Canada, 11423) for 20 minutes (Figure S1-3). To simultaneously surface sterilize all lines within a population, seeds from each genotype were placed in 15 mL falcon tubes perforated on all sides placed in a custom tube rack within a plastic container containing solutions (Clarity 9L Shoe Box, 20726138_EA) (Figure S4). After fungicide treatment, 20 seeds were immediately placed embryo (dorsal) side up in 100 mm x 15 mm petri dishes on one circle of 8.5 cm diameter blue blotter paper (Whatman, 3644) with 11 mL of autoclaved Milli-Q water and the plates were sealed with parafilm and incubated at 20°C in darkness. Each genotype was planted in triplicate petri dishes and each population was planted on separate days due to space constraints. The ICARDA AM-14 panel was also split into two different days to accommodate the number of lines. The AM-14 lines 12, 59 and 104 were included in both experiments to control day to day variability. Germination was determined every 24 hours for the first seven days, day 10 and day 14 after imbibition by scoring the number of seeds with 1 mm coleorhiza emergence as germinated.

To evaluate seed responses to hormones, the same sterilization and imbibition methods were used as described above but 50 µM ABA or fluridone were added to the water applied to the filter paper. For ABA sensitivity testing seeds were considered germinated if any detectible radicle growth was observed to separate the effects of ABA on germination from effects on post-germination elongation. To test oxygen sensitivity, seeds were treated with either 21%

and 5% oxygen environments using a “Hands-In-Bag” (Spilfyter, WWG3NPA3) disposable atmospheric chamber attached to a cylinder of compressed N_2 gas where gas was distributed into the bag until the desired level of oxygen was attained. The O_2 percentage was continuously monitored throughout the experiment using the MO-200 oxygen probe (Apogee instruments). Seeds were either intact or dehulled by inserting fine tipped tweezers behind the rachilla and peeling back the section of the hull covering the embryo. For this experiment dehulled seeds were used to ensure the oxygen environment around the embryo was known, due to the glumellas interference with oxygen flow. Unless otherwise noted all experiments contained 3 replicate plates containing 20 seeds for each genotype and treatment combination and the experiments were replicated in triplicate.

2.2.3 Kompetitive Allele Specific PCR

To evaluate the SD1 and SD2 alleles within genotypes of interest, DNA was first extracted from three replicate plants from Leger, CI9831A, AAC Synergy, CH1224-1, H106-311 and H106-374 along with validation genotypes Esma, AAC Connect and AC Metcalf from Sweeney et al., 2022*b*. Leaf sections (2.5 cm) were ground by hand with a pestle in 1.5 mL tubes and incubated at 65°C for 30 minutes with 500 µL of extraction buffer (0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 8.0, 1.25% SDS). Samples were mixed with 250 µL 6M ammonium acetate before centrifuging for 10 minutes at 10,000 rpm and transferring the supernatant to tubes containing 360 µL isopropanol. Sample isopropanol solution was centrifuged for 10 minutes at 10,000 rpm to pellet DNA. The supernatant was discarded, the DNA was washed with 500 µL of 70% EtOH, dried and resuspended with 80 µL of sterile Milli-Q water. DNA concentration was determined by Nanodrop and diluted to 20 ng/µL for KASP analysis.

Next, to evaluate the alleles present in each plant, a KASP PCR was performed using Hex or FAM fluorescence labelling of gene-specific primers (Table S2). Primer mix was made by adding 12 µL of both forward primers, 30 µL of universal reverse primer and 46 µL water. KASP V4.0 2X Master mix (Lucigen, LGC, KBS-1050-102) was used for PCR reactions in FrameStar 96 well skirted PCR plate, low profile, white walls and black frame (4titude, 4ti-0961). KASP primers and thermocycling protocol was based on the methods of Sweeney et al., 2022*b*. KASP results were measured with Spark microplate reader (Tecan) and analysed using Kluster Caller software (LGC).

2.2.4 Statistical Analysis and Association Mapping

Comparisons of small-scale germination experiments was performed using ANOVA with post-hoc Tukey's HSD or using 2-sided student's t-tests ($p<0.05$) as indicated. For the population germination experiments, broad-sense heritability was calculated by taking the fraction of total phenotypic variation attributed to genotypic variation using a mixed linear model accounting for line, arrangement and shelf in chamber. To test for differences in germination percentage associated with ICARDA sub-populations, row type and adaptation, a nested ANOVA was used with trait nested within day and post-hoc Tukey's HSD.

Genome association was done using Tassel 5 software (Bradbury et al., 2007). Genotypic data was filtered to remove markers with minor allele frequency <5%. Association was done using a mixed linear model accounting for kinship matrix. Markers exceeding -log(p-value)= 3 were considered significant. 2-row and 6-row genotypes were also tested separately for association with germination percentage to account for potential differences in dormancy regulation across sub-populations.

In the Legci population, a linkage map was created using 15252 GBS markers and was placed in 1064 bins using MSTmap across seven major linkage groups (Abed *et al.*, 2022). To decrease computation time one marker was randomly selected from each bin to produce working linkage map with 963 markers and a length of 1896 cM (Figure S5). QTL linkage mapping for the Legci population was performed on 963 GBS markers using MSTMap (Wu *et al.*, 2008). Genotypic data was associated with phenotypes from 119 genotypes from the Legci population (Abed et al., 2022) using one- and two-dimensional QTL scans Haley-Knott regression and the multi-QTL model was performed using multiple imputation (Broman and Sen, 2009). A 95% significance threshold was determined using a permutation test with 1000 permutations. Proximity of Lod peaks to known dormancy QTLs SD1 and SD2 were done using the Morex V1 IBSCv2 reference genome (Mascher *et al.*, 2017). Synch linkage map was cleaned using MST map and constructed using IciMapping (Figure S6) (Meng *et al.*, 2015). QTL mapping for the Synch population was done using interval mapping with IciMapping software (Meng *et al.*, 2015). The significance threshold was determined by permutation test with 1000 permutations.

2.2.5 Expression analysis

Transcript levels from whole heads of developing spikes were tested for Leger, CI8831A, H106-311 and H106-374 genotypes. Four heads per genotype were harvested 15, 25 and 35 days after anthesis and flash frozen in liquid nitrogen then stored at -80°C. RNA was extracted from 100 µL of tissue ground in liquid nitrogen and placed in 750 µL of Trizol. Supernatant from a 15-minute spin at 12000xg and 4℃ was mixed with 200 µL of chloroform in phase lock tubes (Quantabio, 2302830). Phase lock tubes were spun at 12000xg for eight minutes and the aqueous phase was added to 400 µL isopropanol and placed at -20℃ for 24 hours. After the appropriate time interval 650 µL of sample was transferred to the pink column from the Qiagen Rneasy Plant Mini kit (Qiagen, 74904) and spun for 15 seconds at 8000xg. RNA samples were washed with 700 µL RW1 buffer and two washes with 500 µL RPE buffer. Finally, 50 µL of RNase-free water was added to each column and spun for one minute at 8000xg to harvest RNA. The RNA was then Dnase treated with 5 µL of TURBO Dnase buffer and 1 µL of TURBO Dnase enzyme per sample. Samples were incubated at 37℃ for 30 minutes followed by inactivation of the DNase enzyme by addition of 1 µL EDTA and incubation at 75℃ for 10 minutes. Quality and concentration of RNA was determined using a Nanodrop One (fisher scientific). cDNA was produced using High-Capacity cDNA Reverse Transcriptase Kit (Applied biosystems, 4368813) according to the manufacturer’s instructions.

Quantitative PCR was performed using Power up SYBR master mix in a 10 µL reaction including 1 µL of cDNA and analysed using a Quant studio 3 (Applied Biosystems). Thermocycler conditions for the qPCR were 2 minutes at 50℃, 2 minutes at 95℃, 40 x 1 second at 95℃ + 30 seconds at 60℃. qPCR primers were made using NCBI primer BLAST and ordered from IDT (Integrated DNA Technologies) (Table S3). Transcript abundance was normalised to expression of actin and cyclophilin and analysed relative to the Leger 15 days after anthesis (DAA) sample (Figure S7). Comparison of gene induction and difference in expression between lines was done using a two-sided student’s t-test. Experiments were repeated twice.

2.3 Results

2.3.1 Dormancy phenotypic variation

To elucidate genetic control of dormancy, QTL linkage mapping was performed on two biparental populations. These populations include lines from common subpopulations in barley, including the 2-row Synch population with elite Canadian malting variety AAC Synergy as a parent and the 2/6-row population Legci including 6-row Canadian feed variety Leger. In addition, association mapping was performed on the diverse ICARDA 2014 (AM-14) spring barley panel including both 2- and 6-row genotypes and lines adapted to high or low input environments (Amezrou *et al.*, 2018). Germination was monitored for the three populations *in vitro* over 14 days following imbibition by monitoring radicle emergence every 24 hours. The AM-14 panel had high variation in germination rate where about half of the genotypes germinated within 2 days (Fig. 2.1a, Table S4). By 14 days after imbibition (DAI), 214 genotypes had at least one seed germinated, the percentage of seeds germinated for each genotype was approaching a normal distribution with an average germination percentage of 54% and a standard deviation of 26% (Fig. 2.1b). Broad sense heritability was high for germination percentage ranging from 0.67 on 14 DAI to 0.84 on 2 DAI. No significant differences were found when comparing 2-row and 6-row genotypes though high input genotypes germinated more than low input genotypes from 3-5 DAI (Fig. 2.1cd).

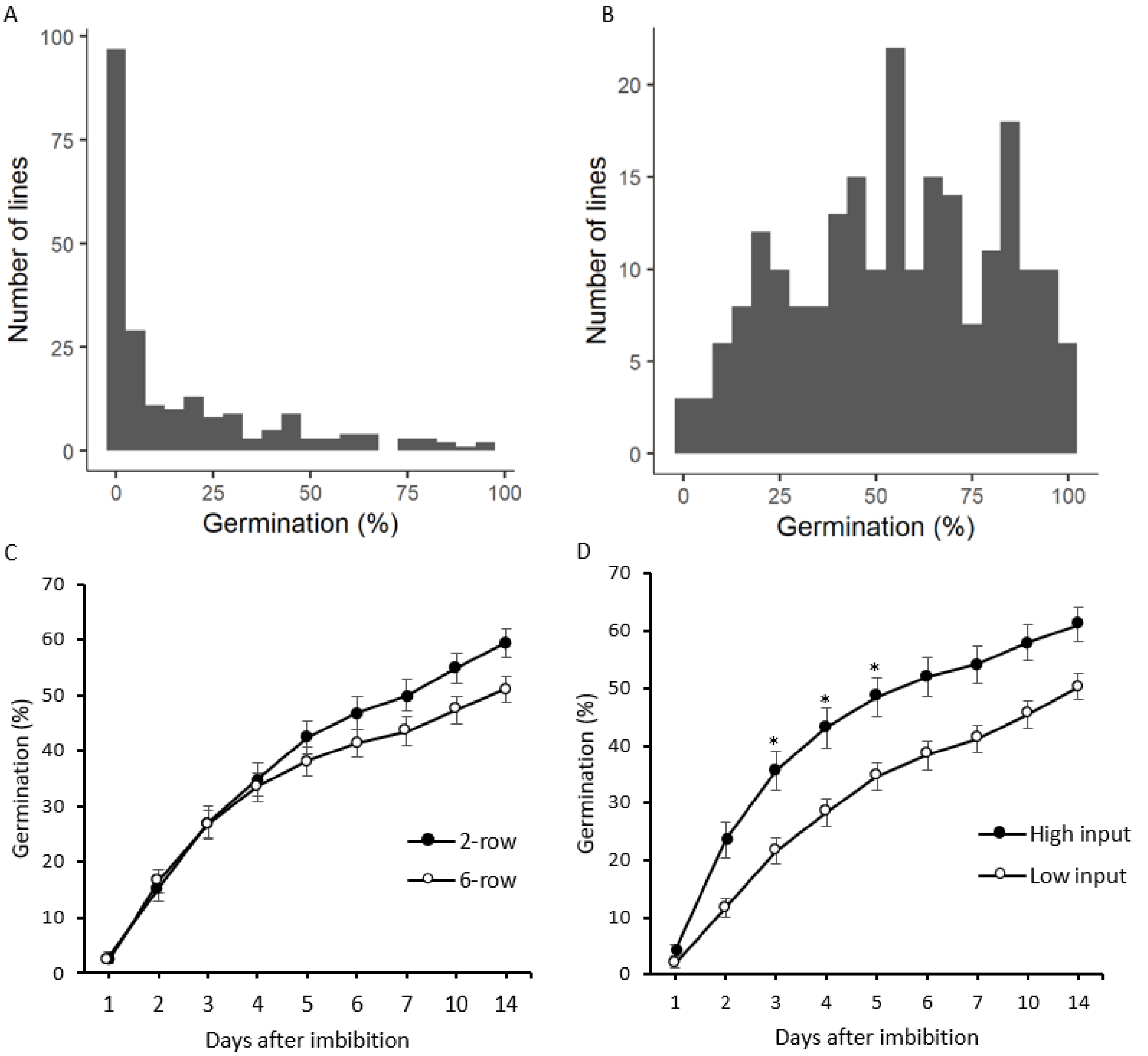

Figure 2.1. Germination percentage trends for the ICARDA AM-14 panel and relationship with phenotypic characteristics. For each genotype, 20 seeds were distributed in each of three replicate plates and germination was monitored at the indicated days after imbibition (DAI). The number of lines which display particular germination percentages at **A)** 2 DAI and **B)** 14 DAI is indicated. The average germination percentage ± standard error for all timepoints separated by **C)** row type (88 2-row, 127 6-row) or **D)** documented adaptation (118 low input, 21 landrace (not shown), 76 high input). The * indicates statistically significant difference ($p<0.05$) from nested ANOVA and the experiment was repeated once.

The 2-row Synch and 2/6-row Legci biparental populations displayed a faster or similar germination rate as compared to the AM-14 population. In the Synch population, the AAC Synergy parental line reached 96% germination while CH1224-1 reached 88% germination by 7 DAI (Fig. 2.2a). The Synch population was mainly non-dormant with 68 out of 90 genotypes requiring less than 24 hours for at least one seed to germinate (Table S5). At the population level, at 2 DAI there was bimodal distribution (excess mass = 0.148; $p<2\times10^{-16}$) in germination percentage with 28 genotypes clustering around CH1224-1 at less than 30% germination, while 54 genotypes clustered around AAC Synergy above 70% germination (Fig. 2.2b). Broad-sense heritability for germination percentage was high for the Synch population, ranging from 0.67 for 14 DAI to 0.94 for 2 DAI. In the Legci population, dormancy of the parental genotypes was stronger than the Synch population with Leger and CI9831A having 39% and 94% of seeds remaining ungerminated within the 14-day interval, respectively (Fig. 2.2c). Leger began to germinate 2 DAI, reaching 51% germination by 4 DAI (Fig. 2.2c). CI9831A remained ungerminated until 5 DAI and only reached 6% germination by 14 DAI (Fig. 2.2c). Leger germination was significantly higher than CI9831A after 3 DAI and remained significantly higher for the duration of the test (Fig. 2.2c). At the population level, out of 121 genotypes only 3 had at least one seed germinated within 24 hours of imbibition and by 2 DAI 59 genotypes had begun germination (Table S6). Legci lines were skewed towards higher dormancy similar to CI9831A at 5 DAI with 62 genotypes having ≤10% germination (Fig. 2.2d). Broad-sense heritability of germination percentage was high for the Legci population ranging from 0.82 for 14 DAI to 0.97 for 1 DAI. By 14 DAI 117 genotypes had at least one seed germinated and the greatest germination rate occurred on the interval between 2 and 3 DAI with an average increase of 8% of seeds germinated.

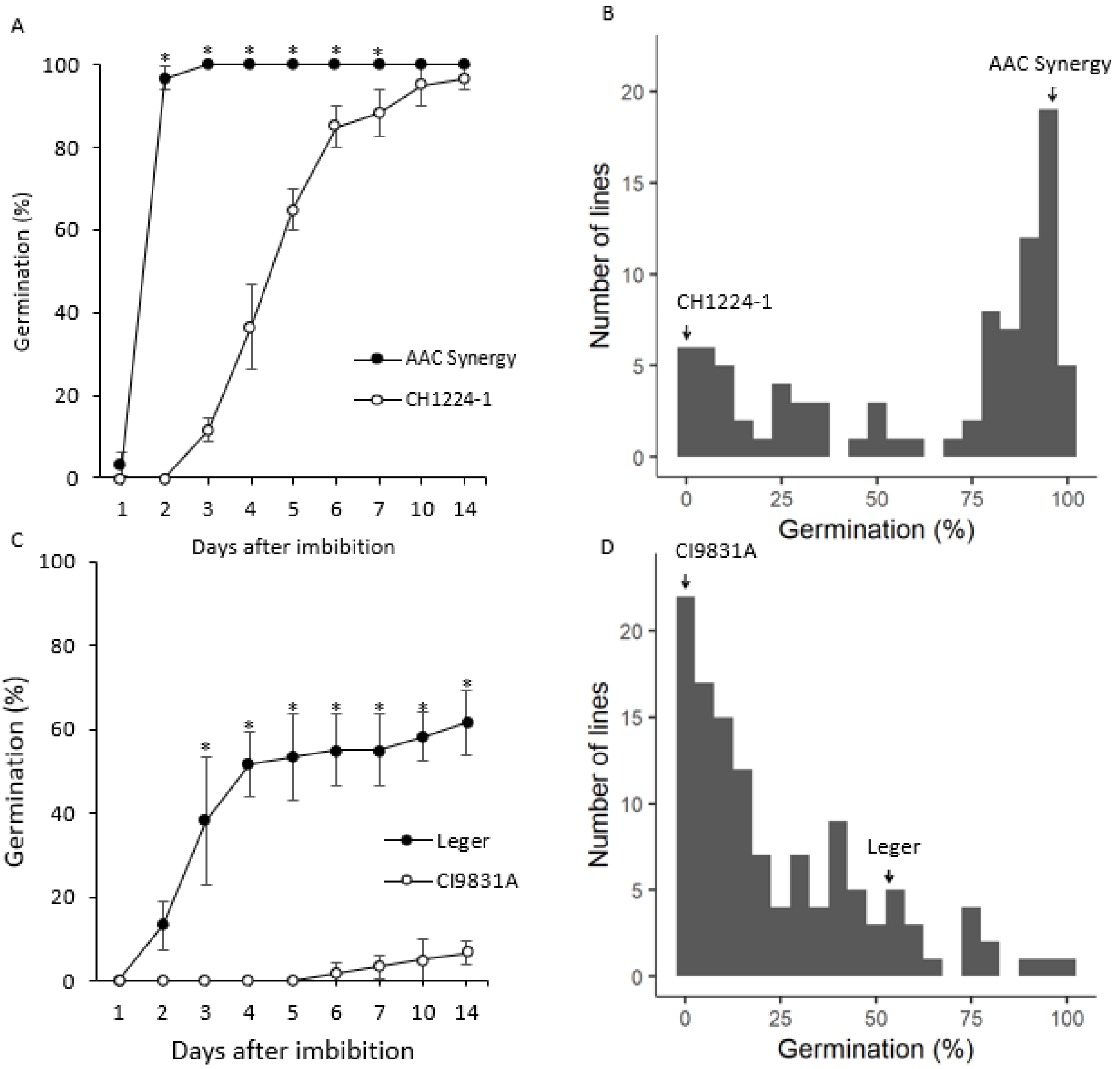

Figure 2.2. Germination percentage trends of the Synch and Legci biparental barley populations. For each genotype, 20 seeds were distributed in each of three replicate plates and germination was monitored at the indicated days after imbibition (DAI). Average germination percentage values ± standard deviation of parental lines **A)** AAC Synergy and CH1224-1 or **C)** Leger and CI9831A. **B)** and **D)** histograms display the number of lines population-wide that display specific average germination percentages for **B)** 2 DAI in Synch (88 lines) or **D)** 5 DAI in Legci (119 lines). The * indicates statistically significant difference ($p<0.05$) from ANOVA with genotype nested within day and the experiment was repeated three times with the parental lines and once at the population level.

2.3.2 Identity of major dormancy-associated loci in Canadian barley

Previous work has identified the SD1 and SD2 loci as major effect QTLs associated with seed dormancy in multiple barley populations highlighting the importance of the underlying alanine aminotransferase (AlaAT, SD1) and MAPK kinase 3 (MKK3, SD2) across multiple barley germplasms (Li *et al.*, 2003; Hori *et al.*, 2007; Ullrich *et al.*, 2009*b*; Nakamura *et al.*, 2017). To determine if the SD1 and SD2 loci could be contributing to the observed dormancy or lack thereof in our biparental populations, the KASP method was used to distinguish dormant and non-dormant associated SNPs (Sweeney *et al*., 2022*b*, appendix table for primers). One SNP was found to influence dormancy in SD1 (L214F) by Sato *et al*., 2016, and two in the SD2 locus (E165Q, R350G) (Vetch *et al.*, 2020). Sweeney *et al.*, 2022*b* did not directly test R350G but used a 50k matrix SNP highly correlated with R350G in their population, termed JHI-367342-KASP. As reported previously, AAC Synergy contains two non-dormant SD2 and a dormant SD1 allele (Table 2.1, Vetch *et al.*, 2020; Sweeney *et al.*, 2022*b*). The CH1224-1 is dormant for both SD2 alleles and non-dormant for SD1, contrasting the non-dormant parent AAC Synergy at every tested locus (Table 2.1). Leger had the dormant allele present at both SD2 loci but was non-dormant at SD1 (Table 2.1). CI9831A was dormant for the MKK3_E165Q allele the same as the non-dormant parent Leger but different at SD1 with the dormant allele. Out of six plants from this line, two were dormant and four were non-dormant at the JHI-367342-KASP SD2 locus, making it unclear what the true allele is in this genotype (Figure S8). Two recombinant lines H106-311 and H106-374 were selected from the Legci population for comparison with parental lines. The H106-311 genotype shared 78% of the 963 molecular markers with CI9831A but has the non-dormant allele at SD1 while the H106-374 shared 76% of its markers with Leger but had the dormant allele at the SD1 locus. Thus, these lines provide an opportunity to roughly compare the effects of the SD1 and surrounding regions and their effect on dormancy relative to their corresponding parents. Furthermore, both recombinant lines shared defining phenotypic traits with their respective parent, H106-311 was 2-row with purple glumella at maturity similar to CI9831A, while H106-374 was 6-row and had a yellow glumella at maturity similar to Leger (Figure S9). Interestingly recombinant lines from the Legci population clustered based on genetic background and SD1 allele, with 5 DAI germination rate was decreased for D H106-374 compared to ND Leger (ttest: p = 0.004, n=3) and ND H106-311 was increased compared to D CI9831A (ttest: p= 0.006,n=3) although germinating at similar levels to the more closely related parent over the course of 14 days (Table 2.1).

Table 2.1. SNP identity at known seed dormancy QTLs SD1 and SD2 in parental lines for Synch and Legci populations and recombinant lines H106-311 and H106-374. Three to six replicate plants were analyzed separately to determine consistency of the result. Germination results are average germination percentage ± standard deviation for 20 seeds germinated in three different plates from a single germination assay used to produce mapping phenotypes. Dormant (D)/ non-dormant (N) refers to G/C (MKK3_E165Q), A/G (JHI-367342-KASP) or G/C (AlaAT_L214F) respectively. Dormant allele is represented by D and non-dormant by N. For the SD2 allele N* indicates both SNPs have the non-dormant allele.

Genotype	Population	SD1	SD2	GP 3 DAI	GP 5 DAI	GP 14 DAI
AAC Synergy	Synch (parent)	D	N*	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH1224-1	Synch (parent)	N	D	12 ± 2.8	65 ± 5.0	97 ± 2.8
Leger	Legci (parent)	N	D	38 ± 15.2	53 ± 10.4	62 ± 7.6
CI9831A	Legci (parent)	D	N/D^{+}	0 ± 0.0	0 ± 0.0	7 ± 2.8
H106-311	Legci	N	D	12 ± 7.6	15 ± 5.0	25 ± 13.2
H106-374	Legci	D	D	7 ± 5.7	15 ± 5.0	57 ± 10.4

+ mixed results between replicate plants.

2.3.3 The SD1 & SD2 alleles regulate dormancy in Canadian populations but not in an international panel

To identify the genetic loci potentially regulating germination percentage in the 3 different populations, both GWAS and QTL linkage mapping approaches were used. For the AM-14 panel, GWAS was used for germination percentage at 2 DAI and 14 DAI to assess early and long-term germination trends. The 2 DAI data revealed two marker trait associations (MTAs) on chromosomes 4H (BOPA1_4919-1051) and 7H (BOPA1_4644-1363) with a third on 7H (BOPA2_12_11477) (Fig. 2.3a). The 14 DAI data did not reveal any significant MTAs (Fig. 2.3b). Since the genotypes of the AM-14 panel had different dormancy levels depending on adaptation and subpopulations related to row type, this may suggest different genetic control of dormancy within these groups (Fig. 2.1cd). Indeed, association mapping of 2-row and 6-row lines revealed significant marker associations on chromosome 3H and 7H for the 2-row lines and on chromosome 4H and 7H for 6-row lines (Figure S10cd). When high input-adapted lines were mapped independently one MTA was found on 7H at 2 DAI and another on 5H at 14 DAI (Figure S10ac). Low input-adapted lines had six MTAs found across chromosomes 2H, 3H, 6H and 7H (Figure S10a-c). Interestingly, the SD1 locus was not identified as significant in this population as associated with dormancy. One MTA was found on 5H closer to the SD2 locus when high input lines were mapped independently at 14 DAI, an unusual timepoint for SD2 association since non-dormant SD2 is typically highly non-dormant but no markers were mapped to 5H for the earlier timepoint (Sweeney *et al.*, 2022*b*).

Table 2.2. Marker trait associations (MTA) for the ICARDA AM-14 panel with germination percentage at 2 or 14 days after imbibition (DAI). Association mapping was performed independently on: all 215 lines, 88 2-row, 127 6-row, 76 high input or 118 low input genotypes. MTA with 2/6 or high/low were mapped with a collection of lines that contained genotypes with both types. MTA was considered significant at -log(p-value) ≥3. Information about markers was obtained from T3/barley and Morex v3 physical position was determine by BLAST using Grain Genes. The * indicates markers identified in multiple association maps.

Marker	DAI	Row type	Input Adaptation[1]	Chromosome	-log(p-value)	Position
BOPA1_4919-1051*	2	2/6	High / Low	4H	4.04	584593042
BOPA1_4644-1363*	2	2/6	High / Low	7H	4.03	2832220
BOPA2_12_11477	2	2/6	High / Low	7H	3.01	248325635
SCRI_RS_67208	2	2	High / Low	3H	3.3	2536489
BOPA2_12_30399	2	2	High / Low	3H	3.1	488628723
BOPA1_4919-1051*	2	6	High / Low	4H	3.4	584593042
SCRI_RS_4520	2	6	High / Low	7H	3.02	605413584
BOPA2_12_31173	14	2	High / Low	7H	3.5	9155301
BOPA1_4644-1363*	2	2/6	High	7H	3.1	2832220
SCRI_RS_234527	14	2/6	High	5H	3.4	571092964
SCRI_RS_736	2	2/6	Low	6H	3.8	521284380
SCRI_RS_13615	2	2/6	Low	7H	3.3	7360724
BOPA1_3965-353	2	2/6	Low	3H	3.1	564661364
BOPA1_3359-1118	2	2/6	Low	7H	3.1	6871605
SCRI_RS_136586	2	2/6	Low	7H	3.09	572855259
SCRI_RS_235860	2	2/6	Low	2H	3.07	567601498

1. Based on description of high input vs low input adapted line in (Amezrou *et al.*, 2018).

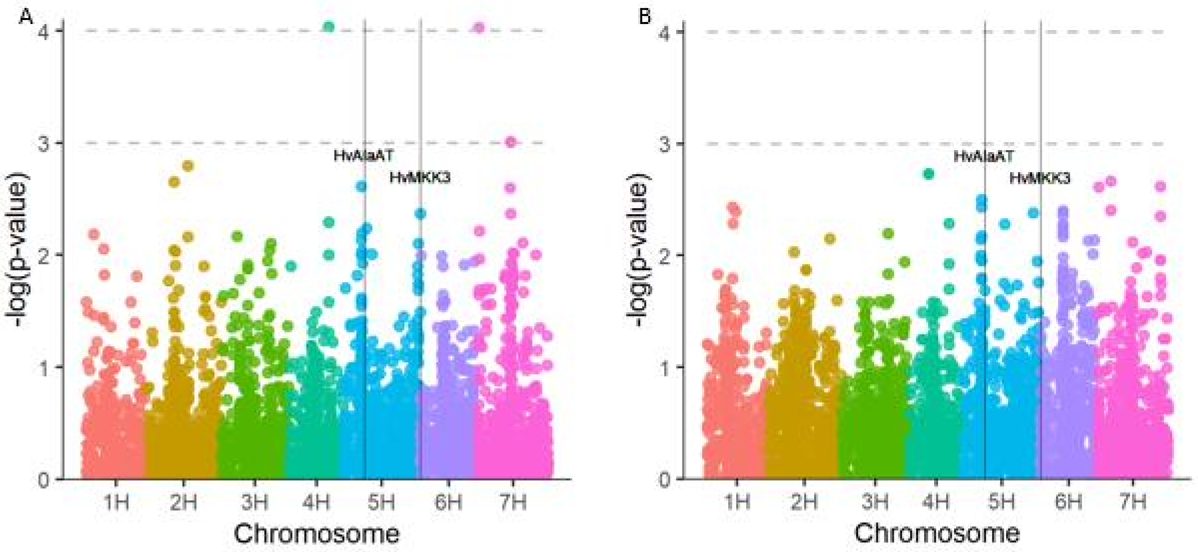

Figure 2.3. Association between **A)** 2 DAI or **B)** 14 DAI germination percentage and 4689 SNP-based markers for the AM-14 panel. Association was determined with a mixed linear model accounting for kinship structure using Tassel 5.0 software. Markers located with -log(p-value) ≥ 3 were considered significant. Approximate location of SD1 (HvAlaAT) and SD2 (HvMKK3) was done by locating the closest marker to each position respectively on the Morex v3 reference genome.

To determine if similar genetic regions underlie germination rate in the biparental populations, we conducted QTL mapping using the Synch and Legci populations. In the Synch population, QTL mapping was done on germination percentage at 2 DAI since this phenotype had the greatest difference between parental lines (Fig. 2.2b). Germination percentage at 2 DAI (H= 0.94) significantly associated with markers in the telomeric region of 5H (Fig. 2.4a). One of the MTA for 2 DAI in Synch was at marker chr5H_656653406 which explained 51% of the variation in 2 DAI germination percentage (Table 2.3). The maker chr5H_656653406 is located 11.6 Mbp away from *MKK3* (SD2) based on the Morex V1 IBSC v2 reference genome (Mascher *et al.*, 2017). There were 174 genetic markers that could not be assigned to a particular linkage group for the Synch population and were mapped as a separate linkage group. Two markers from this group strongly associated with 2 DAI germination percentage both explaining 86% of variation (Table 2.3; Fig. 2.4b). In the Legci population, QTL mapping of germination percentage from all timepoints revealed consistent MTA trends with the strongest association occurring 5 DAI (H=0.86) which was used for the following QTL analysis. Multiple imputation based multi-QTL model explained 46% of variation in germination percentage 5 DAI and indicated two significant loci for association with germination percentage on 2H and 5H respectively (Fig. 2.5a). The 2H loci had a minor effect on germination percentage explaining 3.3% of variation while the 5H loci had a major effect on germination percentage explaining 39.6% of variation. The QTL on 5H was located around marker chr5H:490954074 which has an allele frequency of roughly 54% Leger and 45% CI9831A identity respectively.

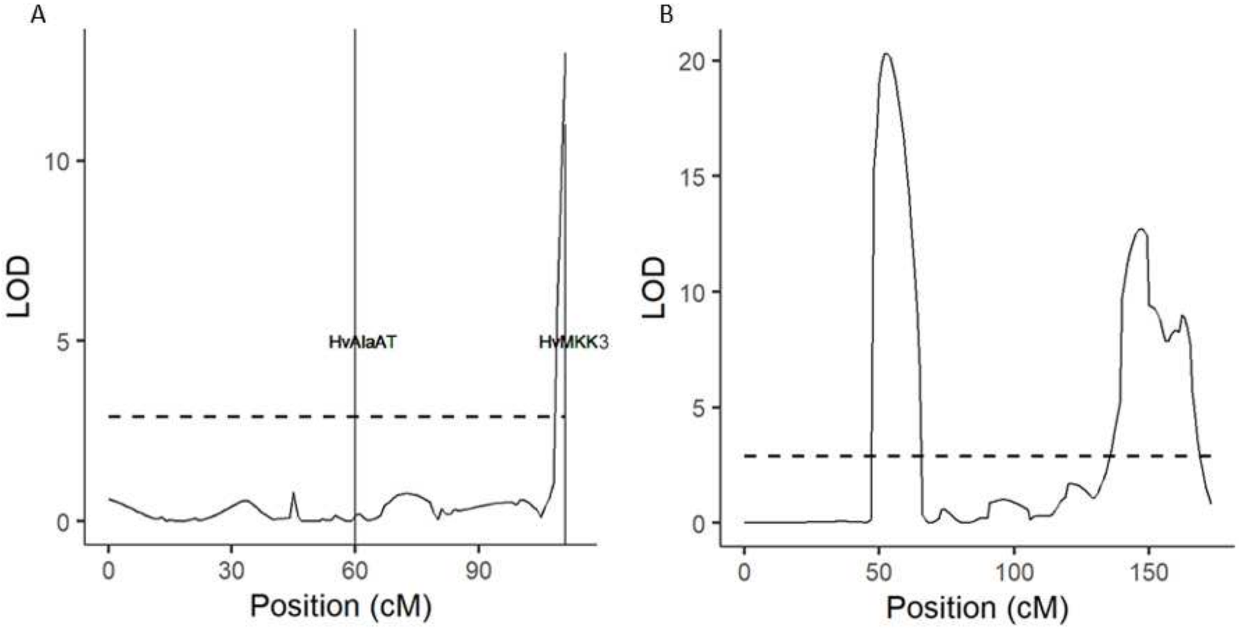

Figure 2.4. Association of germination percentage 2 DAI with 2827 markers from the Synch population. **A)** Chromosome 5H, **B)** Unassigned markers linkage group 8. Logarithm of odds was calculated using interval mapping with the IciMapping software (Meng et al., 2015). Significance threshold is 2.9 and was calculated via 1000 permutations. Approximate SD1 and SD2 locations were based on the Morex v1 IBSC v2 reference genome using grain genes (Mascher et al., 2017).

When comparing the germination percentage of genotypes with the dormant versus non-dormant SNP at chr5H:490954074 which is the closest marker to SD1 1.88 Mbp away, the dormant genotypes predominantly clustered under 20% germination at 5 DAI while non-dormant genotypes are more evenly distributed between 0-80% germination categories (Fig. 2.5c). This distribution suggests the dormant allele may be maintaining dormancy rather than the non-dormant allele breaking dormancy since non-dormant lines are not necessarily pushed to high germination. This result is in agreement with our KASP analysis demonstrating variation in dormancy-associated SNPs in SD1 between the Legci parental lines but not in the SD2 locus though results for the JHI-367342-KASP locus were unconclusive (Table 2.1).

Overall, we identified 14 unique genetic regions underlying dormancy from the AM-14 population, a single locus that is proximal to SD2 in the Synch population, and two loci in the Legci population, one of which is proximal to SD1. Interestingly, we did not identify strong control by SD1 and SD2 in the AM-14 population indicating that genetic regulation of germination is different in genetic backgrounds from the ICARDA collection compared to the Canadian genotypes. In the Canadian germplasm, SD1 and SD2 were identified as major effect QTL controlling seed dormancy with SD1 in the feed variety and SD2 in the malt variety.

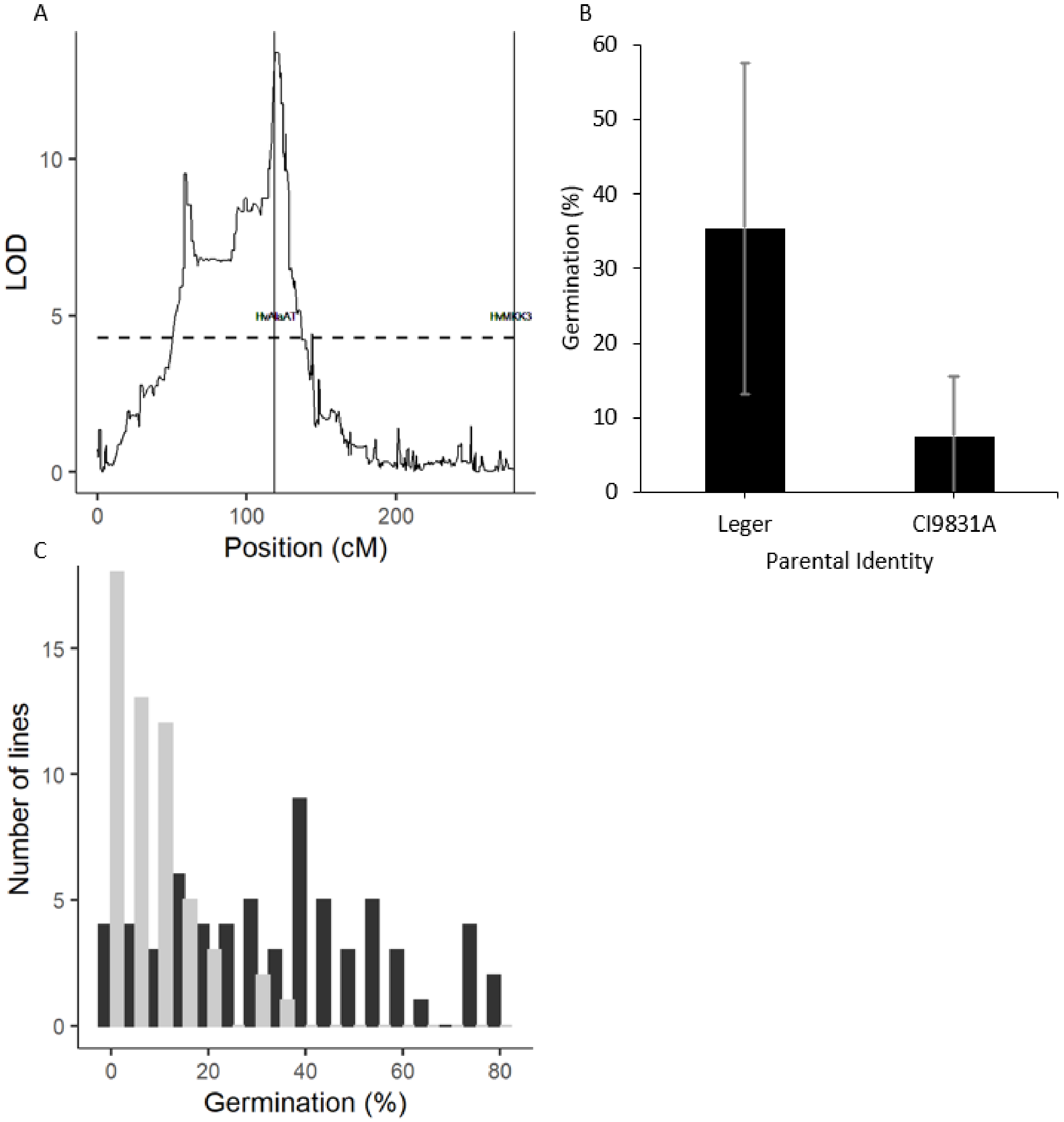

Figure 2.5. Association of the Legci population (119 lines) with germination percentage 5 DAI. **A)** Association of 963 genetic markers with germination percentage 5 DAI for the 5H chromosome estimated with Haley-Knott regression where the significance threshold is 4.28 determined with 1000 permutations. Approximate location of SD1 (HvAlaAT1) and SD2 (HvMKK3) was determined by identifying the marker with the closest physical position using the Morex v1 IBSCv2 reference genome. **B)** Germination percentage averaged (± SD) across all lines by identity at marker chr5H:490954074 where 65 lines have the Leger allele and 54 lines have the CI9831A allele. **C)** Distribution of germination percentage 5 days after imbibition separated by identity at marker chr5H:490954074 to either Leger (non-dormant: black) or CI9831A (dormant: grey).

Table 2.3. Marker trait associations identified in the Legci and Synch populations. Association was estimated in the Legci population using Haley-Knott regression (r/qtl; Broman and Sen, 2009) and in the Synch population using standard interval mapping (IciMapping; Meng et al., 2015). Association of genotypic markers was performed with germination percentage (GP) from multiple days with the strongest association for each population presented.

Population	Trait	Chromosome	Marker	Percent variation explained (%)
Legci	5 DAI GP	5H	chr2H_632650121	3.3
Legci	5 DAI GP	5H	chr5H_490954074	39
Synch	2 DAI GP	5H	chr5H_659541058	51
Synch	2 DAI GP	Unassigned	SUN_92613782	86
Synch	2 DAI GP	Unassigned	SUN_247107242	86

2.3.4 Physiological traits associated with the SD1 locus

The Legci population identified SD1 as the only major effect QTL controlling dormancy in this population and provides an opportunity to investigate if known signaling pathways are associated with dormant or non-dormant alleles for this locus. Our analysis of the Legci population revealed that SD1 and the underlying *HvAlaAT1* had a significant impact on germination in the absence of segregation at the SD2 (MKK3) locus. While previous work suggests that *HvAlaAT1* may influence dormancy through ABA metabolism or hypoxic recovery, the physiological mechanisms underlying this association are not well understood (Sato *et al.*, 2016). Barley primary dormancy is established during the grain maturation phase between anthesis and physiological maturity and previous work has shown that *HvAlaAT1* expression is induced during this time (Chono *et al.*, 2006; Sato *et al.*, 2016). To compare the expression of *HvAlaAT1* to the other five known alanine aminotransferases we tested gene expression during grain fill. We also tested marker genes for ABA metabolism, GA metabolism and hypoxia in genotypes carrying the dormant versus non-dormant *HvAlaAT1* allele, at 15, 25 and 35 days after anthesis (DAA). We used both the Leger and CI9831A parental lines for comparisons as well as two progeny lines H106-311 and H106-374 which shared a majority of molecular markers with one parent but had the opposite *HvAlaAT1* allele from that parent (Fig. 2.6, Table 2.1).

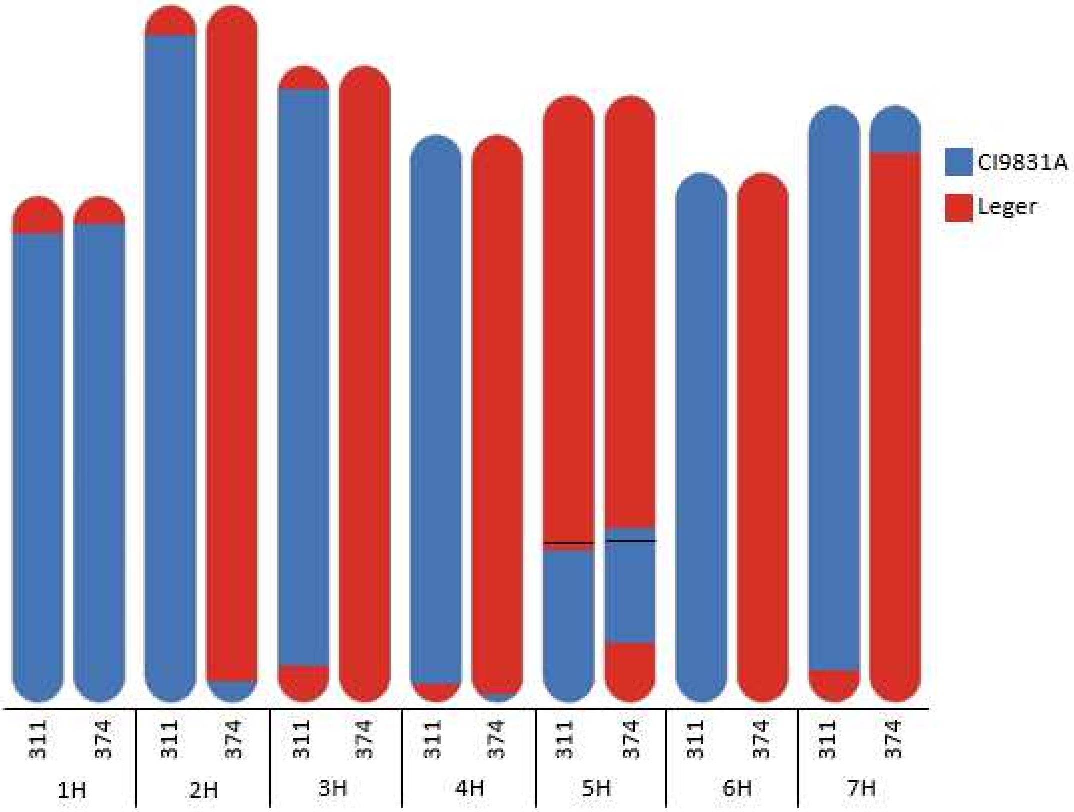

Figure 2.6. Alignment of shared molecular markers between doubled haploid recombinant lines H106-311 and H106-374 and their parental lines CI9831A (blue) or Leger (red). Relationship to parental lines was determined by 963 GBS markers. The solid black line on chromosome 5H indicates the approximate location of the HvAlaAT1/SD1 locus based on the physical location from the Morex v1 IBSCv2 reference genome (Mascher et al., 2017).

At 15 DAA, *HvAlaAT1* relative expression was similar across all lines and increased by 25 DAA remaining high through 35 DAA in three of the four lines (Fig. 2.6, Table S7). *HvAlaAT1* expression was significantly lower in (ND) H106-311 compared to (D) CI9831A (p= 0.006) at 25 DAA but not at 15 DAA, 35 DAA or at any timepoint between Leger and H106-374 suggesting that expression of the gene does not consistently associate with dormancy (Fig. 2.6). The expression of *HvAlaAT1* orthologues *HvAlaAT2*, *HvAlaAT3* and *HvAlaAT4* remained consistent or decreased throughout grain maturation with no induction across all lines (Table S7). Decreased relative expression of *HvAlaAT1* orthologues occurred in CI9831A and H106-311 but not Leger or H106-374 suggesting it may not be due to the dormancy allele.

When comparing hormonal response genes, the ABA responsive genes *NCED1*, *ABA8H-1, ABI5* were consistently expressed at most timepoints and were not significantly different between dormant and non-dormant lines within the timeframe of *HvAlaAT1* induction (Table S7). The exception was *ABI5* expression being higher at 25 DAA in (D) CI9831A compared to (ND) H106-311 (Table S7). This trend corresponded with a similar level of germination inhibition response to ABA application across the lines (Fig. S6). Despite lack of association with the *HvAlaAT1* allele, ABA metabolism gene expression was similar to the findings by Chono et al., 2006. There was also limited induction or difference between lines at the timepoints tested of *GA2ox3* which converts GA to an inactive form (Table S7). *GA3ox2* was significantly induced at 35 DAA in CI9831A and H106-311 genotypes, while average expression increased in Leger and H106-374 results were highly variable and not significant, expression was not significantly different between dormant and non-dormant *HvAlaAT1* alleles (Table S7).

Expression analysis of hypoxia-induced genes including lactate dehydrogenase *(LDH)*, alcohol dehydrogenase *(ADH)* and pyruvate decarboxylase *(PDC)* were also tested during grain maturation because hypoxia is likely imposed during this period due to the programmed cell death of the photosynthetic external cell layers and restriction of oxygen diffusion by seed coverings (Lenoir *et al.*, 1986). *LDH*, *ADH2* and *PDC* genes were modestly induced during grain maturation at 35 DAA, after the induction of *HvAlaAT1* though few differences between genotypes were observed (Table S7). Induction of *LDH* was only significant in Leger and H106-311, although average relative of expression increased for *ADH* in CI9831A and H106-311, it was highly variable and not significant (Table S7). *PDC* was significantly more expressed in (D) CI9831A compared to (ND) H106-311 at 25 DAA, but was not significant significantly different between Leger and H106-374 (Table S7). While hypoxia inducible genes were somewhat induced during seed maturation, induction was not seen in all lines and in most cases were not significantly different between dormant and non-dormant genotypes suggesting similar levels of hypoxia between dormant and non-dormant genotypes during grain fill.

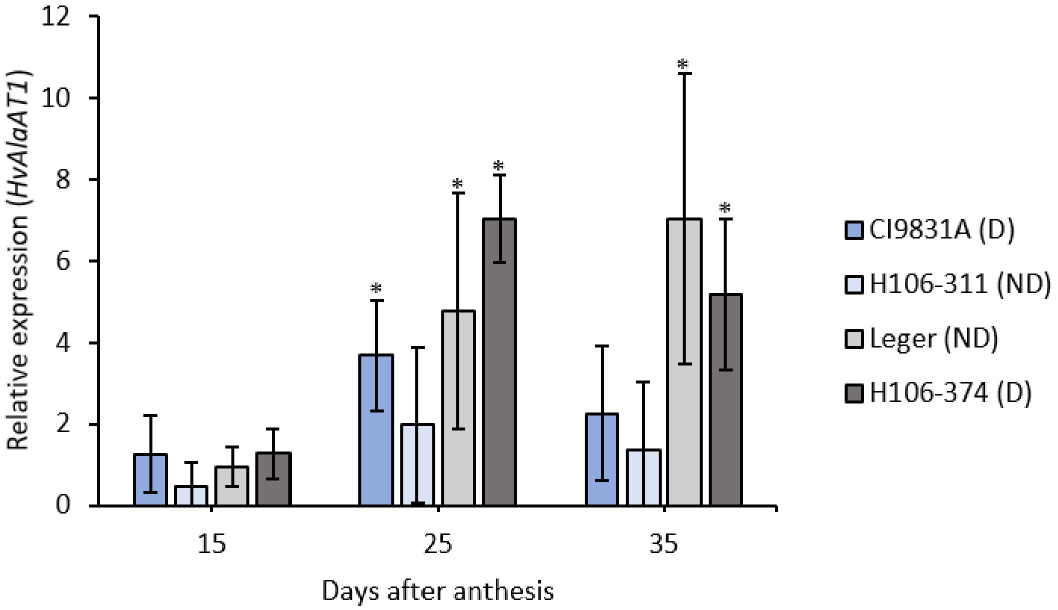

Figure 2.7. Relative expression of HvAlaAT1 throughout grain fill in barley. Barley spikes were collected from 4 biological replicate plants at the indicated days after anthesis and tested for gene expression in genotypes carrying the non-dormant (ND) or dormant (D) allele of HvAlaAT1. Transcript abundance for HvAlaAT1 was tested by qPCR and expressed relative to the average expression of two housekeeping genes, *Hvactin* and *Hvcyclophilin*. Significant differences were determined using students t-test (*: $p \leq 0.05$) and the experiment was repeated twice with similar results.

Hypoxic stress in barley seeds can limit germination below 10% oxygen and severely inhibits germination bellow 5% (Hoang *et al.*, 2013). Barley embryos can be exposed to 5% oxygen levels within mature seeds due to the tight adhering glumella and thus both levels of hypoxia and hypoxia sensitivity can vary between genotypes (Lenoir *et al.*, 1986). In our parental lines of interest, germination was strongly suppressed by a 5% oxygen environment representing hypoxic conditions in all genotypes as compared to a 21% oxygen environment representative of non-hypoxic conditions (Fig. 2.8ab). This further supports the notion that hypoxia suppresses germination in barley. To distinguish whether dormant or non-dormant *HvAlaAT1* might be associated with hypoxia sensitivity, germination was monitored in dehulled seeds where the glumella has been manipulated to expose the embryo to the environmental oxygen levels. In dehulled seeds, all genotypes germinated rapidly in 21% oxygen with visible signs of radicle growth within 1 DAI and approximately 100% germination by 2 DAI (Fig. 2.8cd). In the 5% oxygen environment, all dehulled genotypes had suppressed germination for the first 3 DAI compared to the 21% oxygen environment (Fig. 2.8ab; Fig. 2.9). Dehulled seeds from both genotypes featuring the non-dormant allele of SD1 (Leger and H106-311) were less sensitive to the inhibitory effect of 5% oxygen on germination compared the two lines featuring the dormant allele (CI9831A and H106-374) and had significantly higher germination percentage starting at 2 DAI (Fig. 2.8ab; Fig. 2.9). This suggests that the non-dormant *HvAlaAT1* is associated with reduced hypoxia sensitivity in both 2-row and 6-row barley. Our results suggest a role for *HvAlaAT1* in responses to hypoxic stress within the barley seed in the initial stages of germination, and that this effect is independent of ABA sensitivity.

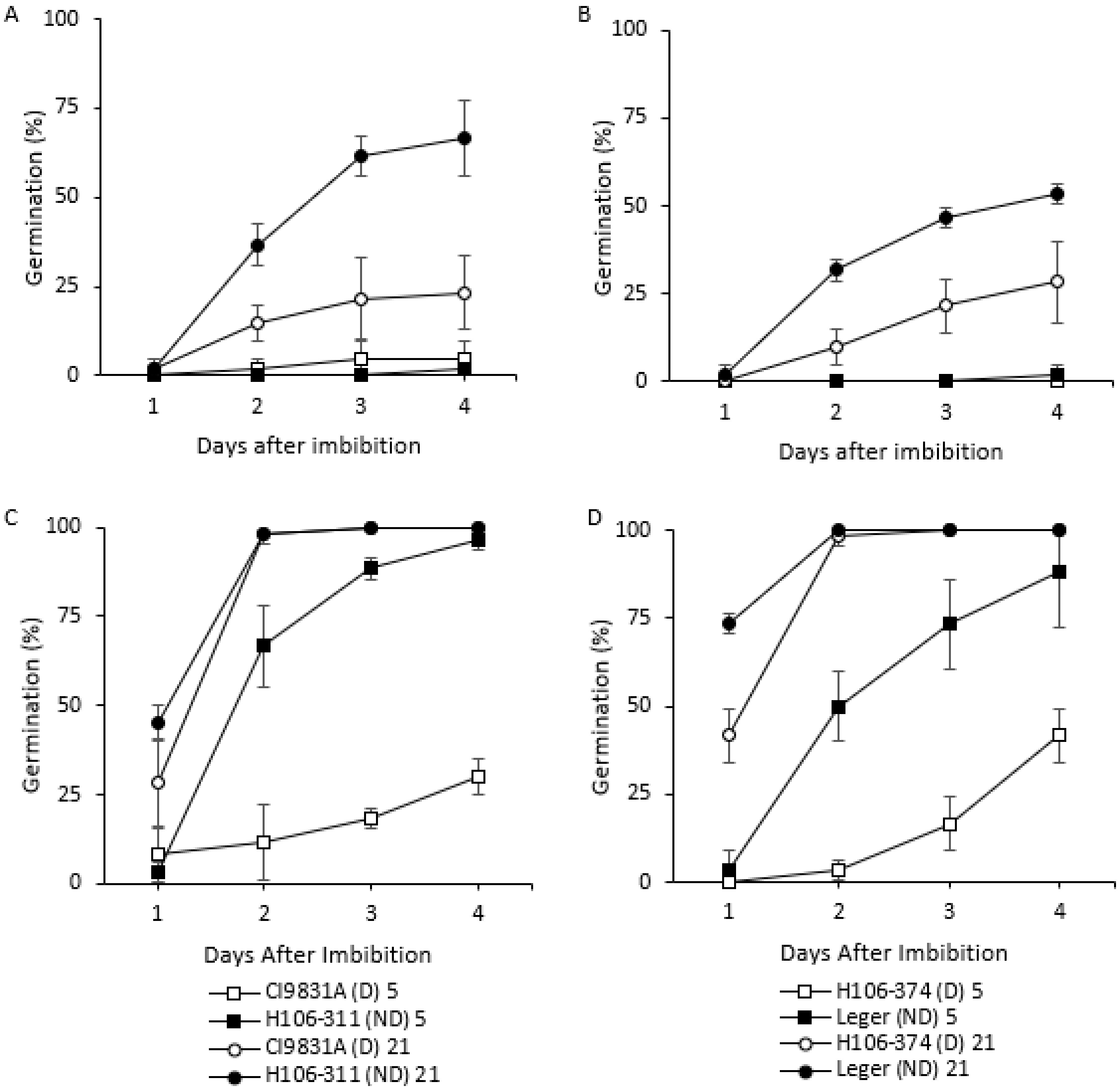

Figure 2.8. Association between SD1 and Hypoxic tolerance during germination of hulled (**A**, **B**) and dehulled (**C**, **D**) barley caryopsis. Tested lines contain either the non-dormant (ND; closed) or dormant (D; open) allele of HvAlaAT1 with a genetic background similar to CI9831A for **A)** and **C)** or Leger **B)** and **D)**. 20 seeds germinated at room temperature in air (~21% O_2; circle) or 5% O_2 (square) environments. Values represent average germination percentage ±standard deviation (n=3). Experiment was replicated three times for each genotypic pair with consistent findings.

Figure 2.9. Representative photo of hypoxia sensitivity during germination in the (D) CI9831A and (ND) H106-311 comparison. Photo was taken three days after imbibition. All seeds are dehulled.

2.4 Discussion

Genetic selection of malting-amenable lines of barley has released the natural dormancy that has evolved within this species leading to issues with germination on maternal plants. Ideally, malting barley would have high dormancy prior to harvest and fast, consistent release of dormancy during malting (Nakamura, 2018). Here, we identified novel and known regions of the genome associated with dormancy through association mapping in the AM-14 panel and biparental linkage mapping in the Synch and Legci populations of spring barley. Further, we characterized the SD1-associated dormancy as potentially involved in hypoxic sensitivity during the seed germination.

To identify which regions of the genome associated with variation of seed dormancy in spring barley, three populations were assessed and we identified 17 regions including some novel regions. The majority of associations were identified with germination percentage 2 DAI indicating the strongest genetic control of germination occurs soon after imbibition. Synch and Legci populations each identified strong regulation by SD2 and SD1 respectively, though the SD1 and SD2 alleles were not associated with dormancy in AM-14. This is consistent with previous work demonstrating that the SD2 allele explained 36% of the variation in dormancy in the Canadian malting variety AC Metcalf which was included in the production of AAC Synergy (Zhang *et al.*, 2012; Legge *et al.*, 2014). AAC Synergy was also recently included as the common parent in a connected half-sib population that identified SD2 linked MTA for regulation in PHS resistance further implicating SD2 as the major controlling factor of dormancy in this genotype (Sweeney *et al.*, 2020). In the Legci population, a major effect QTL at SD1 explained 39% of the variation in dormancy with Leger contributing the non-dormant allele. The QTL was located over an extended region of chromosome 5H similar to previous findings in Australian genotypes and could indicate multiple genes in this region influencing germination (Hickey *et al.*, 2012). Distribution of lines suggested the dormant SD1 allele is maintaining dormancy rather than the non-dormant allele being involved in promoting germination which agrees with previously published trends (Nagel *et al.*, 2019). Indeed, the effect of SD1 on dormancy in barley has been found to increase with after-ripening time but still has an effect on primary dormant seeds harvested at physiological maturity (Vetch *et al.*, 2020). Timing of harvesting for this study was done using visual indicators such as de-greening of the awns and barley head which is less precise than a direct moisture measurement at predicting physiological maturity. It is therefore likely that Legci seeds were partially after-ripened which may have increased the effect SD1 had on germination in this study.

The AM-14 panel was sourced from the ICARDA center which has a high input breeding program in Mexico that focused on development of feed and malt varieties and a low input breeding program in Syria focused on developing feed and food varieties (Amezrou *et al.*, 2018). Therefore, the reduced average dormancy in high input lines may be due to the prevalence of malting-related genetic backgrounds which are produced to be non-dormant at maturity. This suggests that in malt-focused germplasms minor associations influencing dormancy may be selected out making low input germplasms a potential source of novel dormancy regulation genes. For example, the AM-14 panel revealed an association with the 24-methylenesterol C-methyltransferase 2 (*SMT2*) gene on chromosome 4H that was also found in American germplasm (Correa-Morales, 2013). SMT2 is a key enzyme in the production of the sterols sitosterol and stigmasterol (Rogowska and Szakiel, 2020). Sterols have been observed to influence seed germination in *Arabidopsis* and could also play a role in cell membrane recovery from desiccation by modulating membrane fluidity (Rogowska and Szakiel, 2020; Shimada *et al.*, 2021). In addition, two of the MTAs located on chromosome 7H were identified were also identified for root length 12 days after imbibition in drought stressed barley seeds (Thabet *et al.*, 2018). Additional investigations into the minor effect alleles within the AM-14 would help to clarify the prevalence of dormant and non-dormant alleles in malting varieties and their distribution in North American populations.

While SD1 has been linked to dormancy regulation in multiple populations of barley, the role of the underlying alanine aminotransferase in regulating dormancy is not well understood. Our work indicates that the expression of *HvAlaAT1* is induced during grain fill but the amplitude of its expression is not different between lines carrying dormant versus non-dormant SD1 alleles similar to findings by Sato *et al.*, 2016. Limited expression of *HvAlaAT2-4* was found at the grain maturation stage for all genotypes suggesting *HvAlaAT1* is the main alanine aminotransferase produced during this developmental stage. If *HvAlaAT1*'s main role was in establishing dormancy, we would expect to see differences in gene expression in the primary pathways that regulate dormancy including ABA and GA signaling, but we observed no such effect, nor did we observe any differences in ABA sensitivity. Previous work on *Hvalaat1* barley knockouts indicated that mutants had significantly higher ABA content compared to controls suggesting there may have been altered ABA metabolism (Hisano *et al.*, 2022). Thus, the connection between *HvAlaAT1* and hormonal regulation remains unclear.

Alanine aminotransferases are linked to hypoxic response in plants. *HvAlaAT1* is induced at a similar time as the embryo of barley seeds likely becomes hypoxic following the programmed cell death of the photosynthetic pericarp cells during the later stages of barley maturation (Muench *et al.*, 1998; Rolletschek *et al.*, 2004; Rocha *et al.*, 2010; Hoang *et al.*, 2013; Sato *et al.*, 2016). However, hypoxia in the embryo of barley seeds during maturation has not been experimentally determined. Therefore, to investigate hypoxia and any differences between SD1 related alleles, gene expression of three marker genes associated with plants response to hypoxia were tested, pyruvate decarboxylase (*PDC*), alcohol dehydrogenase (*ADH*) and lactate dehydrogenase (*LDH*) (Hondred and Hanson, 1990; Macnicol and Jacobsen, 1992; Luan *et al.*, 2018). Results from this study indicate hypoxia genes are not strongly induced at later stages of grain maturation, with all three genes being upregulated in some lines but not all relative to 15 DAA and that no consistent difference was observed between SD1 alleles (Table S7). It has been established in several studies that localized hypoxic zones develop during grain maturation in the barley endosperm and that LDH and ADH genes are expressed in the aleurone layers of developing barley (Hanson and Jacobsen, 1984; Macnicol and Jacobsen, 1992; Rolletschek *et al.*, 2004, 2011).

Hypoxia has been demonstrated as an important factor controlling germination in barley grains, with seeds germinated in low O_2 environments (5% oxygen) having limited germination and eventually entering secondary dormancy (Bradford *et al.*, 2008; Hoang *et al.*, 2013). For all four genotypes selected for testing from the Legci population seeds were rendered non-dormant by removing the section of the glumella that covers the embryo which maintains dormancy by limiting oxygen diffusion (Lenoir *et al.*, 1986). Findings in this study identified association between *HvAlaAT1* allele with sensitivity of the dehulled germinating barley seed in a 5% oxygen environment. In genotypes with predominantly the Leger or

CI9831A background the line featuring the dormant allele (L) at the L214F SNP germinated significantly slower than the line featuring the non-dormant allele (F) beginning 48 hours after imbibition. This result supports hypoxia as the link between *HvAlaAT1* and its effect on dormancy in barley seeds (Fig. 2.10). How and when *HvAlaAT1* influences the speed of germination is still unclear. Post-imbibition some level of oxygen absorption is likely necessary for germination since dormant and non-dormant seeds generally did not germinate in the first 24 hours at 5% oxygen while dehulled seeds imbibed in air (21% oxygen) did, presumably because oxygen diffuses more slowly at lower oxygen levels. The difference between SD1 alleles likely results in either increased rate of absorption or decreased requirement for oxygen uptake in seeds with the non-dormant relative to seeds with the dormant allele which could result from altered metabolic process post imbibition or from the state of the seed prior to imbibition. Studies by Vetch *et al.*, 2020 found that the influence of SD1 on dormancy increases with after-ripening time suggesting the state of the seed prior to imbibition may be most important.

The association of *HvAlaAT1* allele with sensitivity to 5% oxygen during germination suggests that SD1 may influence dormancy by modifying the plant response to low oxygen. Alanine aminotransferase is an enzyme that reversibly converts pyruvate and glutamate to alanine and 2-oxogluterate, and is transcriptionally induced in response to hypoxia (Muench *et al.*, 1998; Duff *et al.*, 2012). Studies on oxygen status in pea roots found that the plant's control of pyruvate levels is critical to limiting oxygen consumption, and therefore limiting hypoxia on internal tissues (Zabalza *et al.*, 2009). Studies on waterlogging in *Lotus japonicus* found an increase in AlaAT activity and accumulation of alanine under waterlogging conditions, implicating AlaAT for a role in controlling pyruvate levels during hypoxia (Rocha *et al.*, 2010). Since alanine accumulates under hypoxia, greater activity of alanine aminotransferase could result in less pyruvate for respiration and greater oxygen retained in the tissue.

This raises the question, how does *HvAlaAT1* and the L214F polymorphism influence hypoxia response to alter germination rate? It is not clear how after-ripening occurs at the molecular level but the process is associated with lipid oxidation and ROS accumulation non-enzymatically in the desiccated seed (Oracz *et al.*, 2007; El-Maarouf-Bouteau and Bailly, 2008). Lipid oxidation is a self accelerating reaction where the partial pressure of oxygen can greatly effect the eventual rate of reaction (Schaich, 2005; Sun *et al.*, 2011). Treatment of barley seeds with H_2O_2 promotes transcription of GA biosynthesis genes and ABA catabolism genes (Ishibashi *et al.*, 2017). The rate of O_2^- produced by the mitochondria is proportional to the rate of oxygen consumption and local oxygen concentration in the tissue (Skulachev, 1996; Møller, 2003; Murphy, 2009). If HvAlaAT1 results in a relatively higher oxygen percentage in the embryo of non-dormant seeds during seed maturation, greater ROS accumulation due to autooxidation may lower the amount of oxygen consumption required to induce germination, resulting in a faster germination rate at 5% oxygen for non-dormant relative to dormant seeds (Fig. 2.11). Due to the importance of hypoxia for barley germination and its relationship with SD1, future studies testing hypoxic sensitivity of barley seeds may uncover other dormancy related genes that influence germination (Gómez-Álvarez *et al.*, 2023).

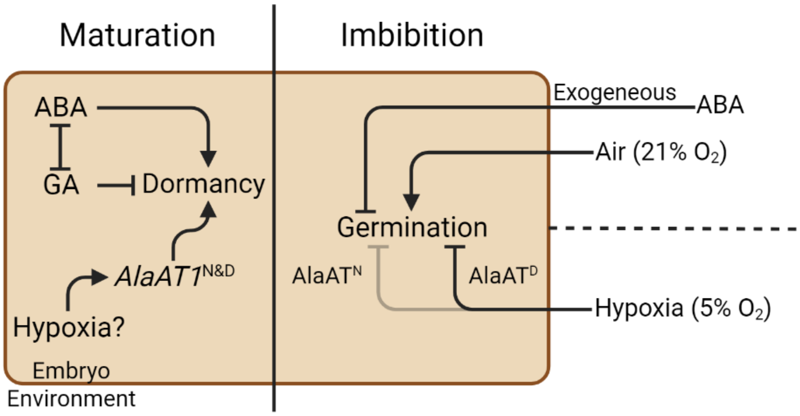

Figure 2.10 Model for findings related to hypoxia and its interaction with dormancy in relation to the dormancy status of the *HvAlaAT1* (SD1) allele. *HvAlaAT1* expression is induced during grain maturation possibly stimulated by hypoxic response. HvAlaAT1 affect on dormancy is likely unrelated to hormonal signalling (Table S7). Germination is delayed by hypoxia more in the dormant version of AlaAT1 (D) compared to the non-dormant AlaAT1 (N) with no difference in air. *HvAlaAT1* influence on germination was not related to ABA sensitivity since exogeneous application of 20 µM ABA significantly inhibited germination compared to control but was not associated with *HvAlaAT1* allele (Figure S11).

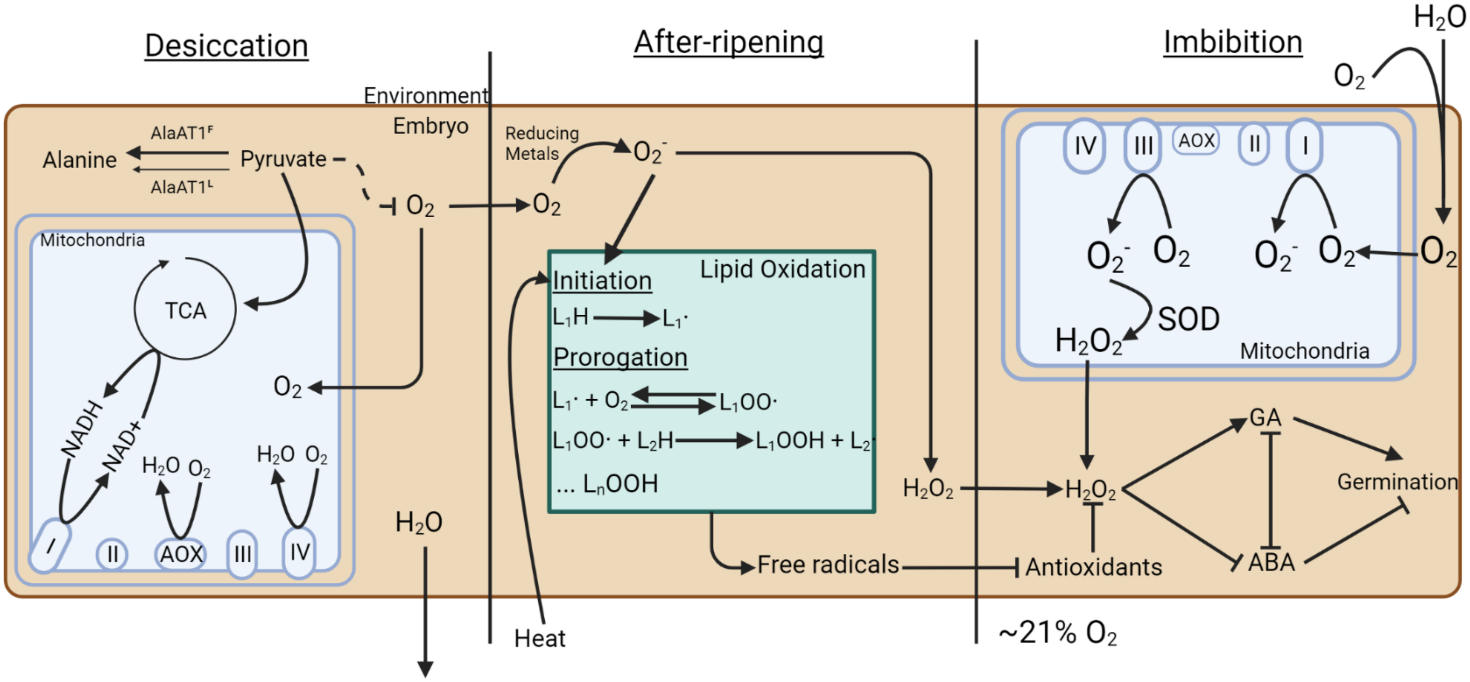

Figure 2.11 Hypothetical model for how changes in internal oxygen concentration could arise during desiccation and influence after-ripening rate and germination. Model components were adapted from (Møller, 2003; Schaich, 2005; Oracz et al., 2007; Zabalza et al., 2009; Schertl and Braun, 2014) and a similar model of after-ripening was proposed by (Bailly, 2004).

3. Chapter 3 Conclusions

In this study, 14 markers were identified for association with dormancy in the ICARDA AM-14 international panel and SD1 or SD2 in the Legci or Synch populations from the Canadian germplasm respectively. These findings provide the first assessment on which genetic elements are associated with dormancy in the ICARDA AM-14 panel and expanded on the understanding of genetic control of dormancy in the Canadian germplasm. This study also found novel associations between dormancy allele at the SD1 locus and germination rate in a low O_2 environment providing the first specific physiological evidence for how SD1 locus influences dormancy.

Despite being distinct traits, PHS sensitivity is often largely controlled by dormancy-related factors (Ullrich *et al.*, 2009). This study did not test PHS but still contributed to the body of work suggesting dormancy is controlling PHS susceptibility in AAC Synergy, since the same SD2 loci was associated with germination percentage under controlled conditions in our study and for PHS susceptibility tested by Sweeney *et al.*, 2020. Recent studies also on the AAC Synergy connected half sib population have found malting quality traits alpha amylase (AA) and free amino nitrogen (FAN) are correlated with the dormancy status of SD2 suggesting pleiotropy rather than tight linkage is responsible for the correlation between dormancy and malt quality (Rooney *et al.*, 2023). Therefore, SD2 is not a good candidate for improving PHS resistance without expecting a drop in malting quality characteristics.

SD1 and SD2 were the only notable QTL found in the Legci and Synch populations respectively. This indicates the prevalence for these well-established major effect QTL in both malting (SD2) and feed (SD2) genotypes. Due to the diversity limitations of biparental QTL mapping these results do not definitively assess dormancy regulation in Canadian spring barley. The importance of non-dormant SD2 locus for malting traits important for adjunct brewing such as alpha amylase and free amino nitrogen has been well documented and therefore, the Synch population likely does not provide a useful resource for improvement of PHS resistance in adjunct brewing varieties (Rooney *et al.*, 2023). Craft brewing tends to not use adjunct starch sources and tend to use only barley termed "all-malt" and therefore a non-dormant SD2 is not necessary (Brouwer *et al.*, 2016). The dormancy levels of CH1224-1 were only slightly reduced compared to AAC Synergy and may therefore have better PHS resistance. Depending on other malting quality characteristics CH1224-1 might be a good candidate for adjustment of dormancy and PHS resistance for production of malting varieties for craft brewers. The SD1 in the Legci population is likely not useful for modulation of PHS resistance in malting lines since other lines with better malting characteristics, including CH1224-1, would be better sources of the non-dormant SD1 allele.

Finally, this study investigated associations between SD1 dormant and non-dormant alleles with dormancy related physiology. Limited association was found with ABA, GA or hypoxia related genes however future studies should investigate gene expression at later time points in seed maturation to include post primary dormancy induction or during germination under hypoxic conditions. Differences in germination of dehulled seeds under hypoxic stress provides the first physiological link between SD1 dormancy and known dormancy regulation. While much previous work has been done establishing the effect hypoxia has on germination few previous studies have found pathways that may regulate this response *in planta*. The SD1 locus is associated with variation in after-ripening an important trait for dormancy release in malt barley and poorly understood physiological process. Elucidating how SD1 influences dormancy and after-ripening in barley may have applications for storage procedures as well as evidence as to the molecular process behind after-ripening. Future studies on the SD1 L214F allele should investigate ROS levels and oxygen levels in the after-ripening embryo and protein levels.

Seeds of some species such as barley and wheat germinate poorly in low oxygen environments which could effect seedling vigour in waterlogged fields (Monteverdi *et al.*, 2022). Modulating germination sensitivity to hypoxia may be important component to producing new flooding resistant crop varieties. Furthermore, germination of seeds from both monocot and dicot species are affected by hypoxia during germination (Corbineau, 2022). Seed processes clearly have an intimate relationship with oxygen status since altering the oxygen environment can alter seed germination, after-ripening and seed senescence (Bradford *et al.*, 2008; Gerna *et al.*, 2022; Bailly, 2023). The identification of genetic loci such as SD1 that influence hypoxic response of seeds indicates that pathways influencing dormancy may be related to hypoxic sensitivity on a larger scale. Altogether hypoxia may be an under-appreciated factor influencing seed processes and how a species responds to hypoxia may be relevant to any investigation into seed germination physiology.

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Appendix A: Supplemental plots

Table S0.1 Characteristics of the 215 lines tested from the ICARDA AM-14 population (Amezrou et al., 2018).

AM-14 ID	Source	Adaptation	Germplasm	Row type
AM-1	ICARDA	Low Input	Breeding lines	6
AM-2	ICARDA	Low Input	Breeding lines	6
AM-3	ICARDA	Low Input	Breeding lines	6
AM-4	ICARDA	Low Input	Breeding lines	6
AM-5	ICARDA	Low Input	Breeding lines	6
AM-6	ICARDA	Low Input	Breeding lines	6
AM-7	ICARDA	Low Input	Breeding lines	2
AM-8	ICARDA	Low Input	Breeding lines	6
AM-9	ICARDA	Low Input	Breeding lines	6
AM-10	ICARDA	Low Input	Breeding lines	6
AM-11	ICARDA	Low Input	Variety	2
AM-12	ICARDA	Low Input	Breeding lines	2
AM-13	ICARDA	Low Input	Breeding lines	6
AM-15	ICARDA	Low Input	Breeding lines	6
AM-16	ICARDA	Low Input	Breeding lines	6
AM-18	ICARDA	Low Input	Breeding lines	6
AM-19	ICARDA	Low Input	Breeding lines	6
AM-20	ICARDA	Low Input	Breeding lines	6
AM-21	ICARDA	Low Input	Breeding lines	6
AM-22	ICARDA	Low Input	Breeding lines	6
AM-23	ICARDA	Low Input	Breeding lines	6
AM-24	ICARDA	Low Input	Breeding lines	6
AM-25	ICARDA	Low Input	Breeding lines	6
AM-26	ICARDA	Low Input	Breeding lines	6
AM-27	ICARDA	High Input	Breeding lines	2
AM-28	ICARDA	High Input	Breeding lines	6
AM-29	ICARDA	High Input	Breeding lines	6
AM-30	ICARDA	High Input	Breeding lines	2
AM-31	ICARDA	High Input	Breeding lines	6
AM-32	ICARDA	High Input	Breeding lines	2
AM-33	ICARDA	High Input	Breeding lines	6
AM-34	ICARDA	High Input	Breeding lines	6
AM-35	ICARDA	High Input	Breeding lines	6
AM-36	ICARDA	High Input	Breeding lines	6
AM-37	ICARDA	High Input	Breeding lines	6
AM-39	ICARDA	High Input	Variety	6
AM-40	ICARDA	High Input	Breeding lines	2
AM-41	ICARDA	High Input	Variety	6
AM-42	ICARDA	Low Input	Breeding lines	6
AM-43	ICARDA	Low Input	Breeding lines	2
AM-44	ICARDA	Low Input	Breeding lines	2
AM-45	ICARDA	Low Input	Breeding lines	6
AM-46	ICARDA	Low Input	Breeding lines	2
AM-47	ICARDA	Low Input	Breeding lines	2
AM-48	ICARDA	Low Input	Breeding lines	2
AM-49	ICARDA	Low Input	Breeding lines	6
AM-50	ICARDA	Low Input	Breeding lines	2
AM-51	ICARDA	Low Input	Breeding lines	2
AM-52	ICARDA	Low Input	Breeding lines	2
AM-53	ICARDA	Low Input	Breeding lines	6
AM-54	ICARDA	High input	Breeding lines	2
AM-55	ICARDA	Low Input	Breeding lines	2

AM-56	ICARDA	Low Input	Breeding lines	2
AM-57	ICARDA	High Input	Breeding lines	2
AM-58	ICARDA	Low Input	Breeding lines	2
AM-59	ICARDA	High Input	Breeding lines	2
AM-60	ICARDA	Low Input	Breeding lines	6
AM-62	ICARDA	Low Input	Breeding lines	6
AM-63	ICARDA	High Input	Breeding lines	2
AM-64	ICARDA	Low Input	Breeding lines	2
AM-65	ICARDA	Low Input	Breeding lines	6
AM-66	ICARDA	High Input	Breeding lines	6
AM-67	India	High Input	Variety	6
AM-68	Syria	Low Input	Variety	6
AM-70	ICARDA	High Input	Collection	2
AM-71	ICARDA	Low Input	Breeding lines	2
AM-72	ICARDA	Low Input	Breeding lines	2
AM-73	ICARDA	Low Input	Breeding lines	6
AM-74	ICARDA	Low Input	Breeding lines	2
AM-75	ICARDA	Low Input	Breeding lines	6
AM-76	ICARDA	Low Input	Breeding lines	2
AM-77	ICARDA	Low Input	Breeding lines	2
AM-79	ICARDA	Low Input	Breeding lines	2
AM-80	ICARDA	Low Input	Breeding lines	2
AM-81	ICARDA	Low Input	Breeding lines	2
AM-82	ICARDA	Low Input	Breeding lines	2
AM-83	ICARDA	Low Input	Breeding lines	6
AM-84	ICARDA	Low Input	Breeding lines	2
AM-85	ICARDA	Low Input	Breeding lines	2
AM-86	ICARDA	Low Input	Breeding lines	2
AM-87	ICARDA	Low Input	Breeding lines	6
AM-88	ICARDA	Low Input	Breeding lines	6
AM-89	ICARDA	Low Input	Breeding lines	2
AM-90	ICARDA	Low Input	Breeding lines	2
AM-91	ICARDA	Low Input	Variety	6
AM-92	Morocco	Low Input	Variety	6
AM-93	ICARDA	Low Input	Breeding lines	6
AM-94	ICARDA	Low Input	Breeding lines	2
AM-95	ICARDA	Low Input	Breeding lines	6
AM-96	ICARDA	Low Input	Breeding lines	6
AM-97	ICARDA	Low Input	Breeding lines	6
AM-98	ICARDA	Low Input	Breeding lines	2
AM-99	ICARDA	Low Input	Breeding lines	2
AM-100	ICARDA	Low Input	Breeding lines	6
AM-101	ICARDA	Low Input	Breeding lines	2
AM-102	ICARDA	Low Input	Breeding lines	2
AM-103	ICARDA	Low Input	Breeding lines	2
AM-104	ICARDA	Low Input	Breeding lines	2
AM-105	ICARDA	High Input	Breeding lines	6
AM-106	ICARDA	Low Input	Breeding lines	2
AM-107	ICARDA	Low Input	Breeding lines	2
AM-108	ICARDA	High Input	Breeding lines	6
AM-109	ICARDA	Low Input	Breeding lines	2
AM-110	ICARDA	High input	Variety	6
AM-221	ICARDA	High Input	Breeding lines	6
AM-222	ICARDA	Low Input	Breeding lines	6
AM-223	ICARDA	Low Input	Breeding lines	6
AM-224	ICARDA	High Input	Breeding lines	6
AM-225	ICARDA	Low Input	Breeding lines	2
AM-226	ICARDA	Low Input	Breeding lines	6
AM-227	ICARDA	High Input	Breeding lines	6
AM-228	ICARDA	High Input	Breeding lines	2
AM-229	ICARDA	High Input	Breeding lines	2
AM-230	ICARDA	Low Input	Variety	6
AM-231	Australia	Low Input	Variety	2

AM-232	ICARDA	Low Input	Variety	2
AM-233	ICARDA	Low Input	Variety	2
AM-234	ICARDA	Low Input	Variety	6
AM-235	ICARDA	Low Input	Variety	6
AM-236	ICARDA	High Input	Breeding lines	6
AM-237	ICARDA	High Input	Breeding lines	6
AM-238	ICARDA	High Input	Breeding lines	6
AM-239	USA	High Input	Variety	6
AM-240	ICARDA	High Input	Breeding lines	6
AM-241	Brazil	High Input	Variety	6
AM-242	ICARDA	High Input	Variety	6
AM-243	ICARDA	High Input	Breeding lines	6
AM-244	ICARDA	High Input	Breeding lines	6
AM-245	ICARDA	High Input	Breeding lines	6
AM-246	ICARDA	High Input	Breeding lines	6
AM-247	ICARDA	High Input	Breeding lines	6
AM-248	ICARDA	High Input	Breeding lines	6
AM-249	ICARDA	High Input	Breeding lines	6
AM-250	ICARDA	High Input	Breeding lines	6
AM-252	ICARDA	High Input	Breeding lines	6
AM-253	India	Landrace	Variety	6
AM-254	India	Landrace	Variety	6
AM-255	India	Landrace	Variety	6
AM-256	India	Landrace	Variety	6
AM-257	India	High Input	Variety	6
AM-258	India	Low Input	Variety	6
AM-261	India	High Input	Variety	6
AM-262	India	Low Input	Variety	6
AM-263	India	High Input	Variety	6
AM-264	India	High Input	Variety	6
AM-265	India	High Input	Variety	6
AM-266	India	High Input	Variety	6
AM-267	India	Low Input	Variety	6
AM-268	India	High Input	Variety	2
AM-269	India	High Input	Variety	2
AM-270	India	High Input	Variety	2
AM-271	ICARDA	Low Input	Breeding lines	2
AM-272	ICARDA	Low Input	Breeding lines	2
AM-273	ICARDA	Low Input	Breeding lines	2
AM-274	ICARDA	Low Input	Breeding lines	2
AM-277	ICARDA	Low Input	Breeding lines	2
AM-278	ICARDA	Low Input	Breeding lines	2
AM-279	ICARDA	Low Input	Breeding lines	2
AM-280	ICARDA	Low Input	Breeding lines	2
AM-281	ICARDA	Low Input	Breeding lines	2
AM-282	ICARDA	Low Input	Breeding lines	2
AM-283	ICARDA	Low Input	Breeding lines	6
AM-284	ICARDA	Landrace	Collection	6
AM-285	ICARDA	Landrace	Collection	6
AM-286	ICARDA	Landrace	Collection	6
AM-287	ICARDA	Landrace	Collection	6
AM-288	ICARDA	Landrace	Collection	2
AM-289	ICARDA	Landrace	Collection	6
AM-290	ICARDA	Landrace	Collection	6
AM-291	ICARDA	Landrace	Collection	6
AM-292	ICARDA	Landrace	Collection	6
AM-293	ICARDA	Landrace	Collection	6
AM-294	ICARDA	Landrace	Collection	6
AM-295	ICARDA	Landrace	Collection	6
AM-296	Australia	High input	Collection	2
AM-297	Australia	High input	Variety	2
AM-298	USA	Landrace	Collection	2
AM-299	Australia	Low input	Variety	2

AM-300	Australia	High input	Variety	6
AM-301	Australia	High input	Variety	6
AM-302	Australia	High input	Collection	2
AM-303	Australia	High Input	Variety	2
AM-304	USA	Landrace	Collection	2
AM-305	USA	Landrace	Collection	2
AM-306	USA	High Input	Variety	6
AM-307	Australia	High input	Variety	6
AM-308	Australia	High input	Variety	6
AM-309	Australia	Low Input	Collection	2
AM-310	USA	Landrace	Collection	6
AM-311	Australia	High input	Variety	2
AM-312	Australia	High input	Variety	6
AM-313	Australia	High Input	Variety	2
AM-314	China	High input	Variety	2
AM-315	Australia	High Input	Variety	2
AM-316	Australia	High Input	Variety	2
AM-317	Australia	High Input	Variety	2
AM-318	Australia	High Input	Variety	2
AM-319	Australia	High input	Variety	2
AM-320	Australia	High input	Collection	2
AM-321	ICARDA	Low Input	Collection	6
AM-322	USA	Landrace	Collection	6
AM-323	Australia	High input	Collection	6
AM-324	Australia	Landrace	Collection	6
AM-325	Japan	Landrace	Variety	6
AM-326	ICARDA	Landrace	Collection	6
AM-327	Australia	High input	Variety	2
AM-328	Australia	High input	Variety	6
AM-329	ICARDA	Landrace	Collection	6
AM-330	ICARDA	Low Input	Breeding lines	6
AM-331	ICARDA	Low Input	Breeding lines	6
AM-332	ICARDA	Low Input	Breeding lines	6
AM-333	ICARDA	Low Input	Breeding lines	6
AM-334	ICARDA	Low Input	Breeding lines	6
AM-335	ICARDA	Low Input	Breeding lines	6
AM-336	ICARDA	Low Input	Breeding lines	2

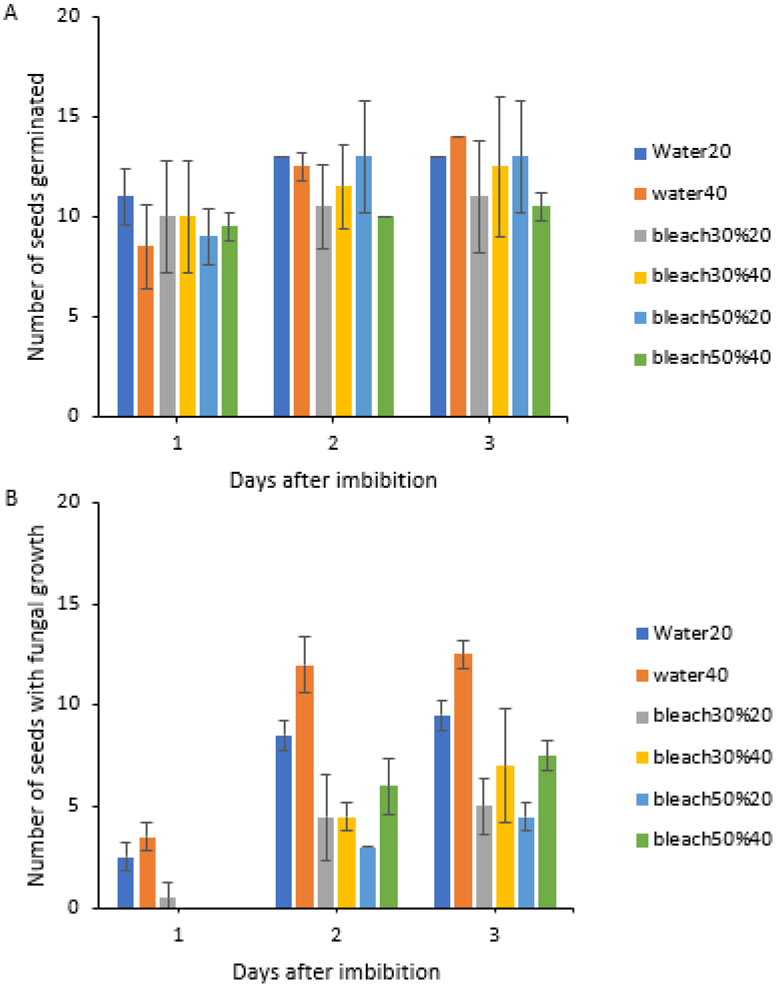

Figure S0.1 Optimisation of duration and concentration of bleach treatment. A) Number of seeds germinated. B) Number of seeds with visible hyphal growth. Fully after-ripened AAC Synergy was used for germination tests.

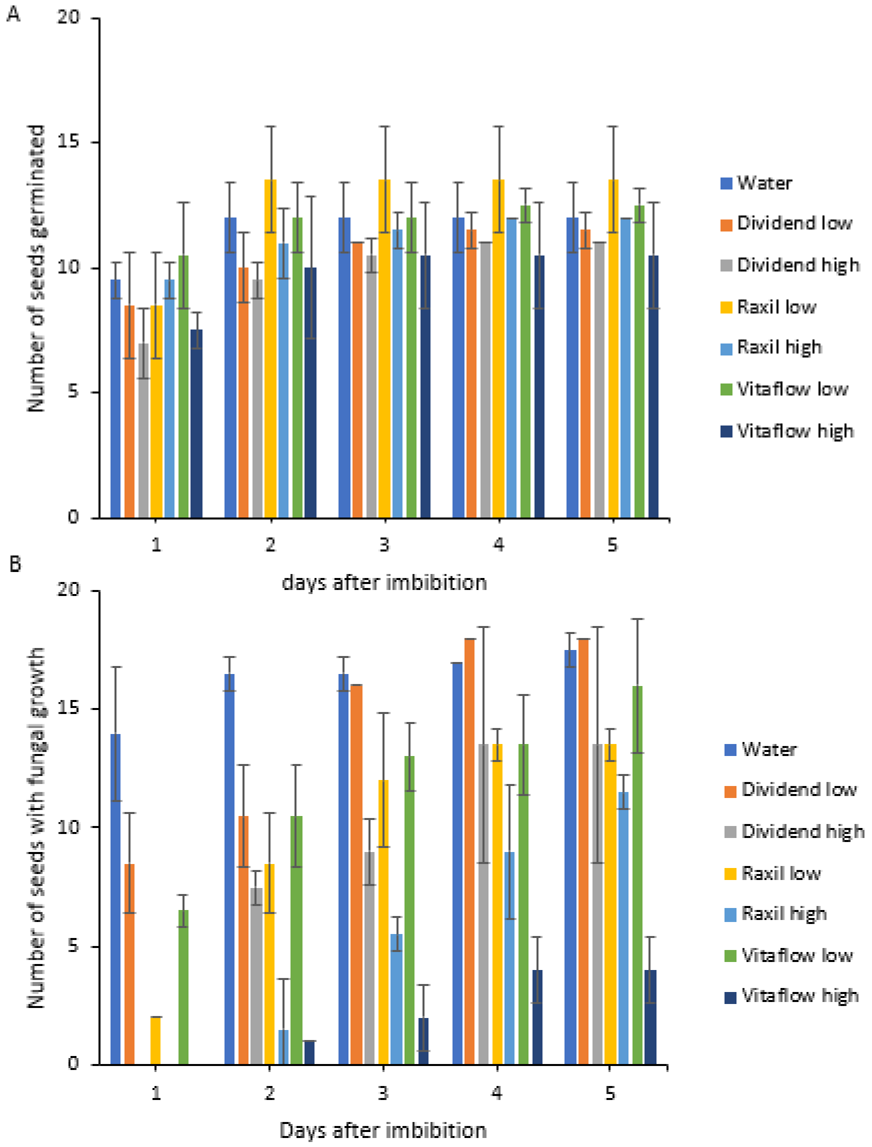

Figure S0.2 Optimisation of fungicide treatment and concentration. A) number of seeds germinated, B) number of seeds with visible hyphal growth. Fully after-ripened AAC Synergy was used for germination tests. Low treatments are 0.024% solution and high treatments are 0.24% solution.

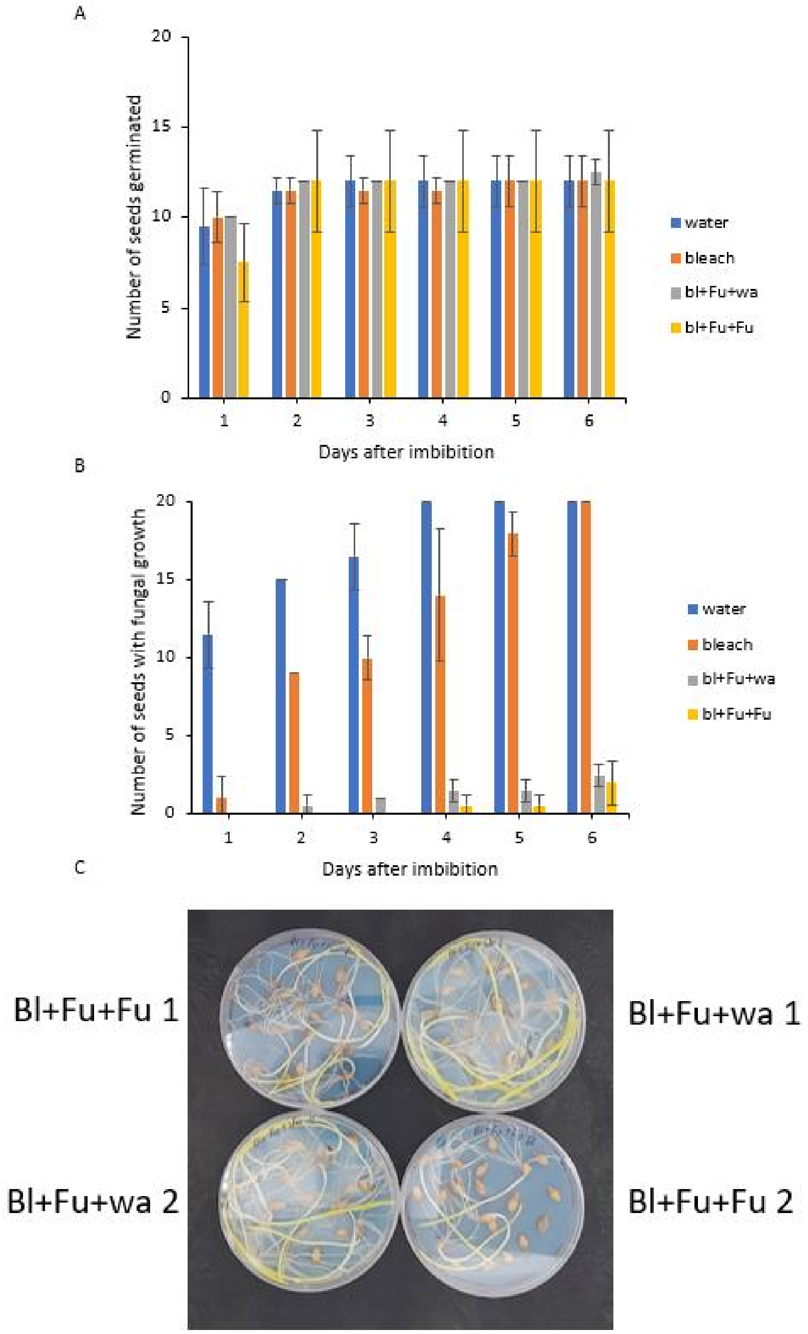

Figure S0.3 Optimisation of bleach + fungicide sterilisation methods. A) Number of seeds germinated, B) number of seeds with visible hyphal growth and C) image of post germination growth for treatments with seeds plated in water (wa) or plated in 0.24% Vitaflow-280 fungicide solution (Fu). Test was done using fully after-ripened AAC Synergy.

Figure S0.4 Seed sterilization rack developed for simultaneous large scale sterilisation.

Table S0.2. KASP primers used for determination of SD1 and SD2 alleles (Sweeney *et al.*, 2022*b*)

Marker	Chr	Gene/ QTL	Forward 1	Forward 2	Universal reverse	ND /D
AlaAT_L214F	5H	HvAlaAT/ SD1	GATTTTCGAAGTAAAGAGGTGCTT**G**	GATTTTCGAAGTAAAGAGGTGCTT**C**	CACGAACAGTCAAACCTGCG	G/C
MKK3_E165Q	5H	HvMKK3/ SD2	GGACAAATAAGCATTGCCCTT**G**	GGACAAATAAGCATTGCCCTT**C**	GCAAGAACCGGCTCTGGTAT	G/C
JHI-367342-KASP	5H	HvMKK3/ SD2	GTGATTCCTCGCTGCTTGGT**A**	GTGATTCCTCGCTGCTTGGT**G**	AGTGAGTAATAATGAGCCCAGCC	A/G

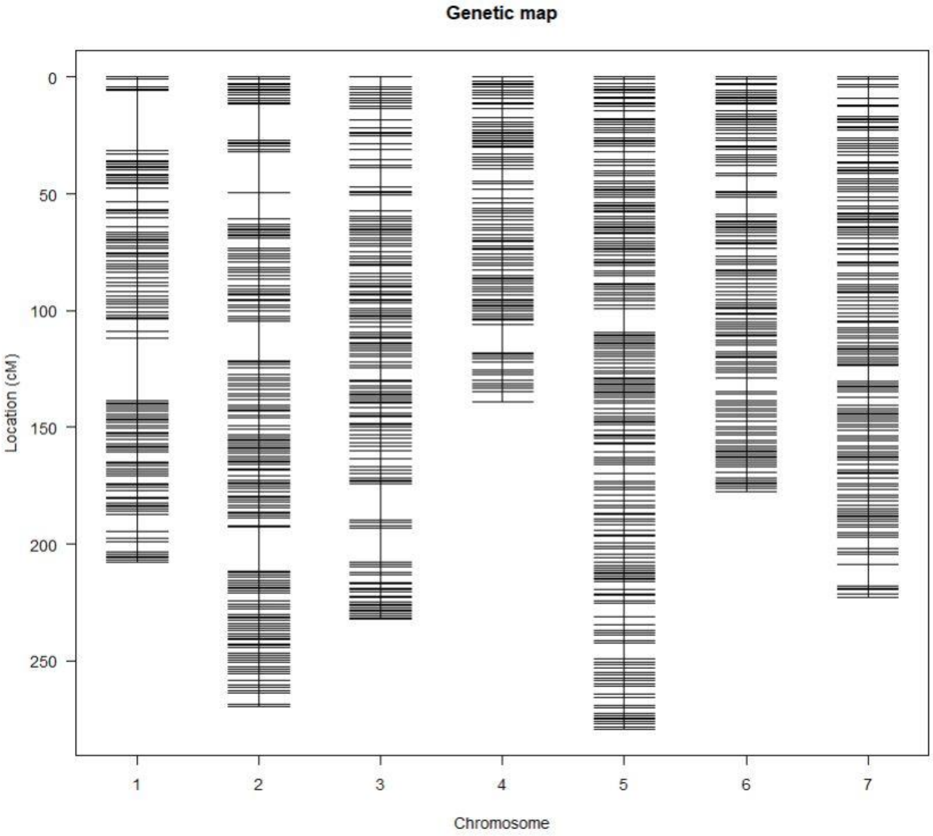

Figure S0.5. Linkage map for the Legci double haploid population based on 963 GBS markers (Abed et al., 2022). Linkage map was constructed using MSTmap (Wu et al., 2008).

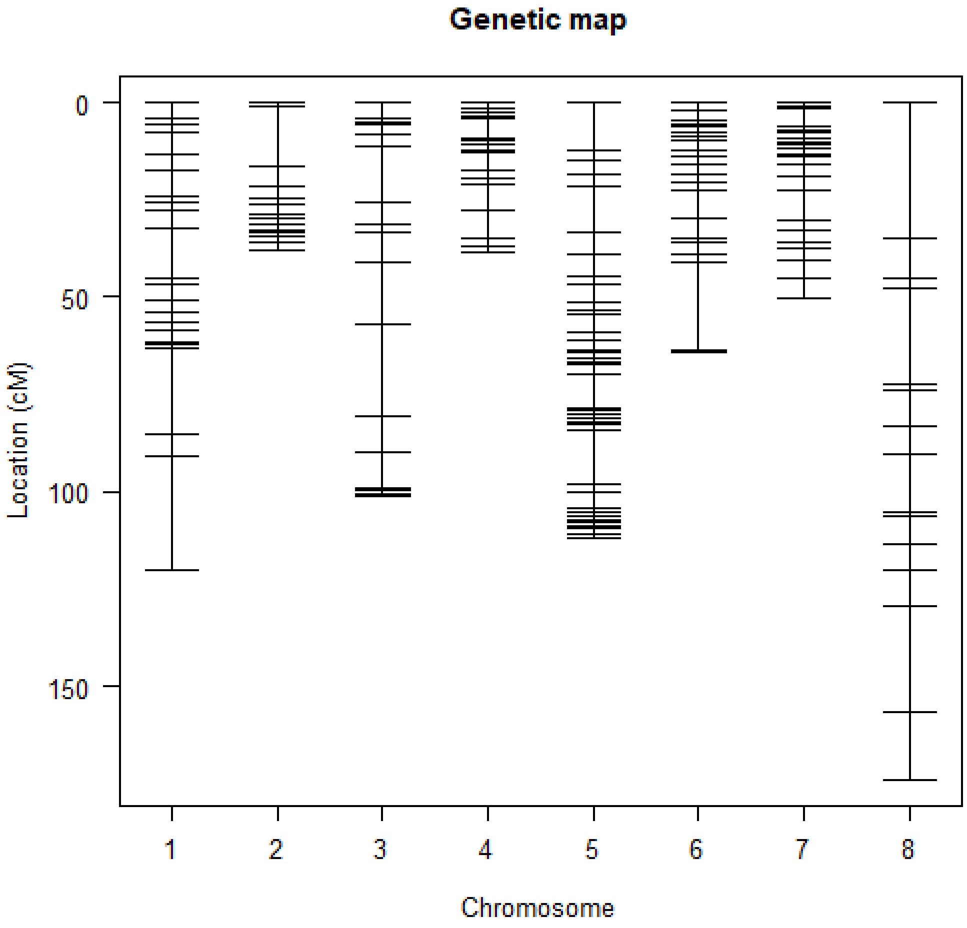

Figure S0.6. Synch recombinant inbred line population linkage map based on 2827 markers. Linkage map constructed using IciMapping (Meng et al., 2015).

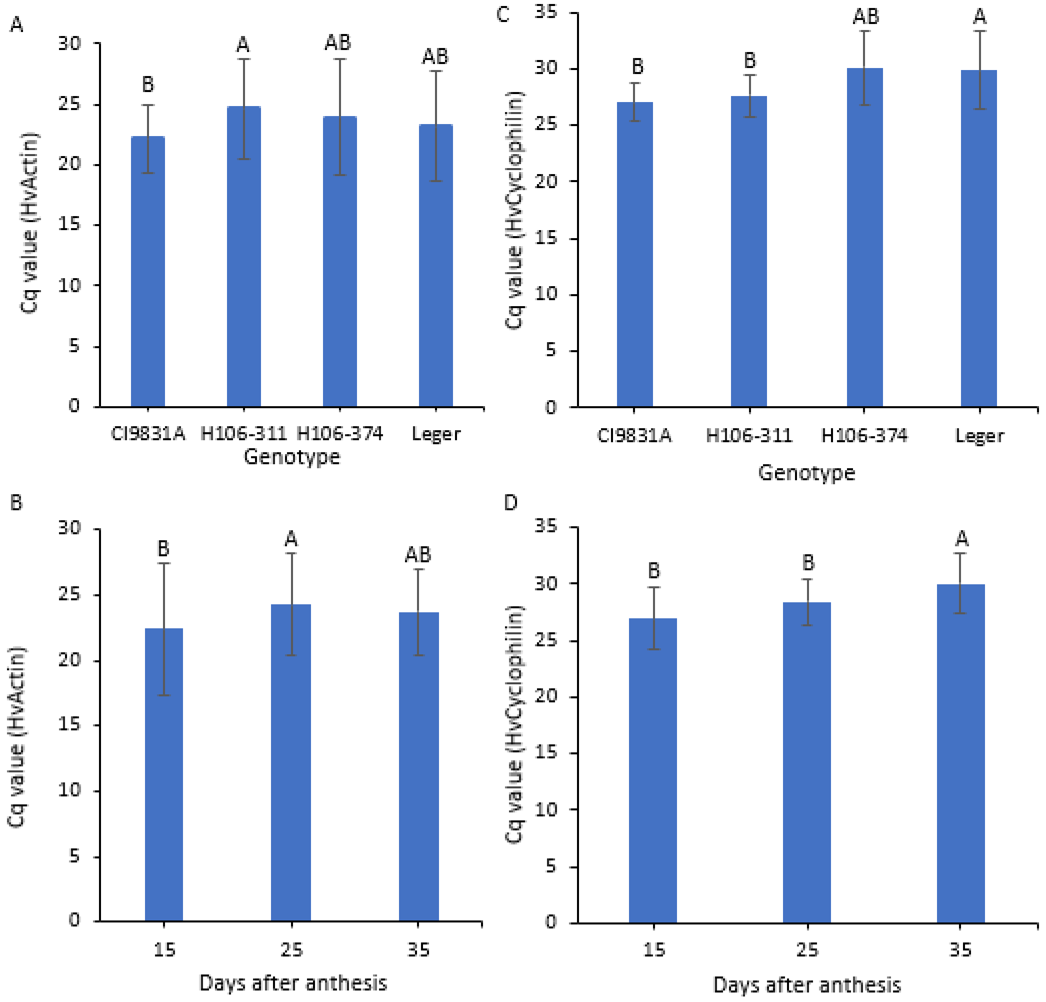

Figure S0.7 Cq values for qPCR normalization genes actin (A,B) and cyclophilin (C,D). Cq values were pooled across 12 samples per genotype and 16 samples per time point. Stability of normalization gene Cq values was determined using an ANOVA with post-hoc Tukey's HSD. Categories sharing a letter are not significantly different. RNA was extracted from maturing barley heads of four different genotypes at three different timepoints.

Table S0.3. Primers for qPCR during grain maturation of barley heads from the Legci population.

Gene	NCBI ID	Forward	Reverse	Marker	Reference
HvActin	AY145451.1	GATCAAGGTCGTCGCTCCAC	TGGAAAGTGCTGAGTGAGGC	Control	Ishibashi et al 2017
HvCyclophilin		CTCTGTTTCACAGGGAGGTCT	CGCACGATGCACAACAAAGA	Control	
HvAlaAT1	AK372829.1	CGCAGGTTTGACTGTTCGTG	CCTCCTGGTTGGTGACAGAC	SD1	Sato et al 2016
HvAlaAT2	AK376585.1	TATTGCTGCCCGTGATGGTT	TCATCATGTGGACTGCTGGG	SD1 paralog	Sato et al 2016
HvAlaAT3	AK252381.1	GAGGCGTCGGAAGATACTCG	TTGGGGTTCAGGTTGTCCAC	SD1 paralog	Sato et al 2016
HvAlaAT4	AK374436.1	ATGTGTTCTACGCTCTCCGC	GTGCCAGGTACTTGTCCGAA	SD1 paralog	Sato et al 2016
HvNCED1	AB239297	TCCCAAAGTACGCCAAGGAC	TCCTCCCACGAGTTCCAGAG	ABA biosynthesis	Ishibashi et al 2017
HvABA8OH-1	XP_044954164.1	TGTGGCATTGCTGTCCATCT	CTTCTCCAGCGTCAGGTAGC	ABA catabolism	Millar et al 2006
HvABI5	AY150676	TTGTGCAAACTGCGGTCAAG	GAGTACGGCATTGGTTCGGA	ABA response	Ishibashi et al 2017
HvGA3ox2	AY551431.1	TTCCACATCCTCACCAACGG	TACCCCAGCGATATCCGGTC	GA biosynthesis	Bahin et al 2011
HvGA2ox3	MN887597.1	GACTCCTTGCAGGTATGCCG	GACGCCGCACGTCATTTTAG	GA catabolism	Bahin et al 2011
HvPDC (pyruvate decarboxylase)	XM_045125934.1	GGACATGCTTACGGGTGACA	AATTCATACCCGCAGCCCTC	Ethanol fermentation (hypoxia)	Luan et al 2018
HvADH2 (alcohol dehydrogenase)	AK354611.1	TCCCTAGGATCTTGGGCCAT	ACAGGGGAAAGAGAGTGGGA	Ethanol fermentation (hypoxia)	Macnicol et al 1992
HvLDH A (lactate dehydrogenase)	AK370553.1	CGAGGAACGCCAGTTGATCT	GTGTGGGAGCAGGAACAGAG	Lactate fermentation (hypoxia)	Hondred et al 1990

Table S0.4. Average germination percentage ± standard deviation of lines in the ICARDA AM-14 population. Germination assessment was done using three replicate plates of 20 seeds germinated in darkness at 20˚C.

Line	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 10	Day 14
2014-AM-1	0 ± 0.0	2 ± 2.9	8 ± 2.9	23 ± 10.4	27 ± 10.4	28 ± 7.6	33 ± 2.9	38 ± 5.8	45 ± 5.0
2014-AM-2	0 ± 0.0	0 ± 0.0	12 ± 7.6	13 ± 5.8	22 ± 10.4	23 ± 7.6	27 ± 5.8	27 ± 5.8	27 ± 5.8
2014-AM-3	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
2014-AM-4	2 ± 2.9	12 ± 7.6	35 ± 5.0	47 ± 7.6	65 ± 13.2	70 ± 8.7	73 ± 10.4	75 ± 10.0	75 ± 10.0
2014-AM-5	0 ± 0.0	3 ± 5.8	10 ± 8.7	13 ± 11.5	18 ± 16.1	23 ± 20.8	25 ± 21.8	37 ± 23.6	42 ± 23.1
2014-AM-6	0 ± 0.0	2 ± 2.9	7 ± 7.6	12 ± 5.8	17 ± 2.9	30 ± 5.0	35 ± 8.7	40 ± 10.0	45 ± 8.7
2014-AM-7	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	7 ± 2.9	8 ± 5.8	13 ± 2.9	15 ± 5.0	20 ± 5.0
2014-AM-8	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	5 ± 0.0	8 ± 2.9
2014-AM-9	2 ± 2.9	10 ± 10.0	22 ± 15.3	23 ± 16.1	27 ± 18.9	35 ± 18.0	37 ± 15.3	42 ± 17.6	43 ± 17.6
2014-AM-10	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	7 ± 7.6	8 ± 7.6
2014-AM-11	0 ± 0.0	0 ± 0.0	0 ± 0.0	7 ± 5.8	13 ± 7.6	17 ± 10.4	18 ± 7.6	23 ± 10.4	33 ± 12.6
2014-AM-12	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 2.9	17 ± 5.8	27 ± 2.9	32 ± 2.9	45 ± 13.2	52 ± 7.6
2014-AM-13	1 ± 2.9	3 ± 2.9	13 ± 7.6	21 ± 5.8	29 ± 12.6	34 ± 11.5	38 ± 5.0	46 ± 10.4	50 ± 10.4
2014-AM-15	0 ± 0.0	0 ± 0.0	10 ± 0.0	15 ± 0.0	22 ± 2.9	22 ± 2.9	23 ± 2.9	28 ± 2.9	37 ± 5.8
2014-AM-16	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9
2014-AM-18	0 ± 0.0	0 ± 0.0	3 ± 5.8	8 ± 7.6	10 ± 10	12 ± 10.4	12 ± 10.4	13 ± 11.5	17 ± 15.3
2014-AM-19	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	17 ± 7.6	28 ± 5.8	32 ± 2.9	38 ± 5.8	40 ± 5.0
2014-AM-20	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	12 ± 7.6	17 ± 12.6	18 ± 12.6	20 ± 15.0	22 ± 17.6
2014-AM-21	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 7.6	10 ± 5.0	13 ± 7.6	20 ± 13.2	20 ± 13.2
2014-AM-22	0 ± 0.0	2 ± 2.9	17 ± 10.4	33 ± 12.6	47 ± 18.9	50 ± 17.3	53 ± 11.5	57 ± 15.3	57 ± 15.3
2014-AM-23	0 ± 0.0	45 ± 5.0	70 ± 5.0	72 ± 5.8	77 ± 10.4	80 ± 10.0	85 ± 13.2	87 ± 12.6	87 ± 12.6
2014-AM-24	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9	5 ± 5.0
2014-AM-25	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	3 ± 5.8	7 ± 7.6	8 ± 5.8
2014-AM-26	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	5 ± 0.0	10 ± 0.0	18 ± 2.9	22 ± 2.9
2014-AM-27	0 ± 0.0	7 ± 2.9	23 ± 10.4	38 ± 12.6	47 ± 11.5	50 ± 13.2	60 ± 13.2	65 ± 13.2	68 ± 12.6
2014-AM-28	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	5 ± 5.0	8 ± 7.6	12 ± 12.6	15 ± 10.0
2014-AM-29	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 5.8	12 ± 10.4	15 ± 8.7	15 ± 8.7	20 ± 0.0	22 ± 2.9
2014-AM-30	0 ± 0.0	0 ± 0.0	2 ± 2.9	10 ± 5.0	20 ± 10.0	27 ± 7.6	28 ± 10.4	35 ± 5.0	37 ± 5.8
2014-AM-31	12 ± 2.9	32 ± 5.8	65 ± 8.7	72 ± 2.9	77 ± 7.6	77 ± 7.6	77 ± 7.6	78 ± 5.8	78 ± 5.8
2014-AM-32	35 ± 10.0	73 ± 2.9	85 ± 5.0	87 ± 5.8	92 ± 5.8	93 ± 7.6	93 ± 7.6	95 ± 5.0	95 ± 5.0
2014-AM-33	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	8 ± 5.8	12 ± 5.8	12 ± 5.8	17 ± 7.6	23 ± 12.6
2014-AM-34	12 ± 10.4	65 ± 13.2	78 ± 10.4	82 ± 12.6	87 ± 14.4	87 ± 14.4	87 ± 14.4	87 ± 14.4	87 ± 14.4
2014-AM-35	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 5.0	5 ± 5.0	5 ± 5.0	8 ± 7.6
2014-AM-36	37 ± 5.8	63 ± 10.4	68 ± 10.4	70 ± 13.2	70 ± 13.2	73 ± 14.4	75 ± 13.2	75 ± 13.2	75 ± 13.2
2014-AM-37	18 ± 7.6	83 ± 2.9	88 ± 2.9	92 ± 2.9	92 ± 2.9	92 ± 2.9	92 ± 2.9	93 ± 2.9	93 ± 2.9
2014-AM-39	0 ± 0.0	2 ± 2.9	5 ± 5.0	8 ± 7.6	17 ± 10.4	20 ± 13.2	22 ± 14.4	27 ± 15.3	30 ± 13.2
2014-AM-40	0 ± 0.0	18 ± 15.3	48 ± 15.3	63 ± 12.6	72 ± 14.4	73 ± 11.5	75 ± 8.7	78 ± 2.9	82 ± 7.6
2014-AM-41	0 ± 0.0	30 ± 20.0	70 ± 21.8	75 ± 21.8	77 ± 23.6	83 ± 12.6	83 ± 12.6	83 ± 12.6	83 ± 12.6
2014-AM-42	2 ± 2.9	13 ± 2.9	55 ± 18.0	78 ± 5.8	83 ± 2.9	90 ± 5.0	90 ± 5.0	90 ± 5.0	90 ± 5.0
2014-AM-43	32 ± 7.6	73 ± 5.8	83 ± 7.6	85 ± 8.7	88 ± 7.6	88 ± 7.6	88 ± 7.6	88 ± 7.6	88 ± 7.6
2014-AM-44	0 ± 0.0	0 ± 0.0	7 ± 5.8	13 ± 11.5	30 ± 5.0	40 ± 13.2	48 ± 11.5	58 ± 12.6	63 ± 20.2
2014-AM-45	2 ± 2.9	2 ± 2.9	5 ± 5.0	5 ± 5.0	8 ± 5.8	12 ± 2.9	13 ± 2.9	15 ± 5.0	17 ± 5.8
2014-AM-46	8 ± 2.9	48 ± 11.5	63 ± 7.6	65 ± 5.0	65 ± 5.0	67 ± 2.9	67 ± 2.9	67 ± 2.9	67 ± 2.9
2014-AM-47	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 7.6	12 ± 11.5	20 ± 18.0	23 ± 15.3	38 ± 25.7	43 ± 24.7
2014-AM-48	3 ± 2.9	22 ± 12.6	70 ± 8.7	80 ± 10.0	83 ± 7.6	85 ± 8.7	85 ± 8.7	85 ± 8.7	85 ± 8.7
2014-AM-49	3 ± 2.9	47 ± 7.6	62 ± 7.6	67 ± 7.6	68 ± 7.6	70 ± 5.0	70 ± 5.0	70 ± 5.0	70 ± 5.0
2014-AM-50	0 ± 0.0	0 ± 0.0	3 ± 2.9	8 ± 2.9	10 ± 5.0	10 ± 5.0	10 ± 5.0	13 ± 2.9	17 ± 7.6
2014-AM-51	0 ± 0.0	7 ± 7.6	22 ± 10.4	42 ± 5.8	62 ± 2.9	68 ± 2.9	72 ± 2.9	72 ± 2.9	72 ± 2.9
2014-AM-52	0 ± 0.0	3 ± 2.9	7 ± 5.8	7 ± 5.8	20 ± 8.7	23 ± 2.9	25 ± 0.0	37 ± 20.2	43 ± 15.3
2014-AM-53	8 ± 2.9	28 ± 7.6	60 ± 13.2	72 ± 10.4	80 ± 13.2	82 ± 14.4	82 ± 14.4	83 ± 11.5	83 ± 11.5
2014-AM-54	0 ± 0.0	3 ± 5.8	33 ± 14.4	70 ± 21.8	82 ± 16.1	83 ± 14.4	87 ± 12.6	87 ± 12.6	87 ± 12.6
2014-AM-55	0 ± 0.0	2 ± 2.9	20 ± 5.0	23 ± 5.8	33 ± 2.9	37 ± 7.6	40 ± 5.0	43 ± 2.9	55 ± 13.2
2014-AM-56	22 ± 11.5	63 ± 7.6	78 ± 12.6	83 ± 17.6	85 ± 18.0	85 ± 18.0	85 ± 18.0	85 ± 18.0	85 ± 18.0
2014-AM-57	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 5.0	7 ± 7.6	10 ± 5.0	12 ± 7.6	13 ± 7.6	18 ± 12.6
2014-AM-58	2 ± 2.9	10 ± 8.7	17 ± 5.8	22 ± 7.6	25 ± 8.7	30 ± 8.7	30 ± 8.7	37 ± 10.4	40 ± 8.7
2014-AM-59	2 ± 2.9	28 ± 10.4	67 ± 11.5	78 ± 7.6	92 ± 5.8	93 ± 2.9	95 ± 0.0	95 ± 0.0	95 ± 0.0

2014-AM-60	8 ± 5.8	32 ± 15.3	45 ± 18.0	48 ± 16.1	50 ± 13.2	53 ± 7.6	55 ± 8.7	55 ± 8.7	57 ± 5.8
2014-AM-62	18 ± 2.9	60 ± 13.2	87 ± 7.6	92 ± 7.6	92 ± 7.6	92 ± 7.6	93 ± 7.6	93 ± 7.6	93 ± 7.6
2014-AM-63	0 ± 0.0	0 ± 0.0	2 ± 2.9	12 ± 2.9	18 ± 7.6	23 ± 11.5	28 ± 12.6	30 ± 10.0	32 ± 10.4
2014-AM-64	7 ± 5.8	40 ± 13.2	72 ± 7.6	75 ± 5.0	77 ± 5.8	87 ± 2.9	87 ± 2.9	87 ± 2.9	87 ± 2.9
2014-AM-65	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	10 ± 5.0	12 ± 2.9	12 ± 2.9	17 ± 11.5	22 ± 16.1
2014-AM-66	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	8 ± 7.6	8 ± 7.6	8 ± 7.6	13 ± 11.5	18 ± 11.5
2014-AM-67	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	10 ± 5.0	13 ± 2.9	18 ± 7.6	23 ± 2.9
2014-AM-68	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	8 ± 2.9	10 ± 5.0	15 ± 10.0
2014-AM-70	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	18 ± 10.4	32 ± 11.5	37 ± 16.1	38 ± 18.9	52 ± 20.8
2014-AM-71	0 ± 0.0	18 ± 7.6	55 ± 13.2	68 ± 15.3	83 ± 12.6	88 ± 5.8	93 ± 2.9	97 ± 2.9	97 ± 2.9
2014-AM-72	0 ± 0.0	0 ± 0.0	5 ± 5.0	12 ± 11.5	22 ± 7.6	30 ± 10.0	37 ± 15.3	43 ± 11.5	47 ± 10.4
2014-AM-73	0 ± 0.0	0 ± 0.0	15 ± 10.0	33 ± 12.6	40 ± 10.0	43 ± 7.6	52 ± 10.4	62 ± 7.6	63 ± 5.8
2014-AM-74	0 ± 0.0	2 ± 2.9	3 ± 2.9	7 ± 5.8	13 ± 2.9	20 ± 5.0	23 ± 2.9	23 ± 2.9	42 ± 12.6
2014-AM-75	0 ± 0.0	2 ± 2.9	10 ± 5.0	13 ± 7.6	25 ± 10.0	28 ± 5.8	32 ± 2.9	35 ± 8.7	42 ± 5.8
2014-AM-76	0 ± 0.0	5 ± 5.0	8 ± 7.6	17 ± 10.4	23 ± 5.8	28 ± 10.4	30 ± 10.0	42 ± 7.6	50 ± 0.0
2014-AM-77	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 5.8	10 ± 10.0	15 ± 10.0	15 ± 10.0
2014-AM-79	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	7 ± 2.9	10 ± 0.0	12 ± 2.9	15 ± 5.0	28 ± 7.6
2014-AM-80	0 ± 0.0	2 ± 2.9	15 ± 8.7	32 ± 10.4	37 ± 14.4	43 ± 16.1	43 ± 16.1	47 ± 18.9	48 ± 16.1
2014-AM-81	0 ± 0.0	2 ± 2.9	7 ± 5.8	10 ± 8.7	15 ± 5.0	18 ± 5.8	18 ± 5.8	22 ± 5.8	32 ± 7.6
2014-AM-82	0 ± 0.0	0 ± 0.0	23 ± 5.8	30 ± 5.0	43 ± 14.4	48 ± 14.4	55 ± 13.2	67 ± 7.6	72 ± 5.8
2014-AM-83	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	12 ± 7.6	13 ± 7.6	18 ± 2.9	20 ± 5.0	23 ± 5.8
2014-AM-84	0 ± 0.0	3 ± 5.8	10 ± 0.0	13 ± 2.9	27 ± 2.9	32 ± 2.9	42 ± 2.9	60 ± 13.2	67 ± 7.6
2014-AM-85	0 ± 0.0	0 ± 0.0	5 ± 0.0	18 ± 12.6	37 ± 23.6	42 ± 28.4	43 ± 30.6	50 ± 32.8	57 ± 28.4
2014-AM-86	0 ± 0.0	0 ± 0.0	8 ± 5.8	18 ± 14.4	22 ± 11.5	30 ± 5.0	33 ± 2.9	37 ± 2.9	48 ± 7.6
2014-AM-87	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 5.8	5 ± 8.7	7 ± 7.6	10 ± 5.0	13 ± 2.9	22 ± 7.6
2014-AM-88	0 ± 0.0	17 ± 7.6	22 ± 11.5	32 ± 5.8	40 ± 5.0	40 ± 5.0	48 ± 12.6	55 ± 18.0	55 ± 18.0
2014-AM-89	0 ± 0.0	12 ± 7.6	28 ± 5.8	47 ± 5.8	63 ± 20.2	72 ± 24.7	77 ± 20.8	80 ± 17.3	80 ± 17.3
2014-AM-90	0 ± 0.0	13 ± 2.9	42 ± 22.5	55 ± 21.8	63 ± 16.1	68 ± 20.8	73 ± 24.7	82 ± 18.9	88 ± 7.6
2014-AM-91	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	7 ± 5.8
2014-AM-92	0 ± 0.0	5 ± 5.0	18 ± 15.3	23 ± 18.9	25 ± 18	25 ± 18	25 ± 18	27 ± 20.8	27 ± 20.8
2014-AM-93	0 ± 0.0	2 ± 2.9	3 ± 5.8	15 ± 5.0	23 ± 7.6	23 ± 7.6	27 ± 10.4	30 ± 15.0	35 ± 20.0
2014-AM-94	0 ± 0.0	0 ± 0.0	7 ± 2.9	18 ± 5.8	28 ± 7.6	35 ± 5.0	38 ± 2.9	42 ± 2.9	53 ± 11.5
2014-AM-95	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 2.9	7 ± 2.9	8 ± 2.9	13 ± 2.9	17 ± 5.8	27 ± 12.6
2014-AM-96	0 ± 0.0	0 ± 0.0	8 ± 2.9	17 ± 2.9	25 ± 5.0	28 ± 2.9	30 ± 0.0	35 ± 5.0	40 ± 10.0
2014-AM-97	3 ± 5.8	30 ± 5.0	53 ± 7.6	58 ± 12.6	73 ± 7.6	78 ± 10.4	82 ± 7.6	83 ± 10.4	85 ± 8.7
2014-AM-98	0 ± 0.0	0 ± 0.0	3 ± 5.8	10 ± 10.0	18 ± 15.3	22 ± 15.3	30 ± 22.9	42 ± 22.5	42 ± 22.5
2014-AM-99	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	5 ± 0.0	8 ± 2.9	8 ± 2.9	13 ± 2.9	17 ± 7.6
2014-AM-100	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	3 ± 2.9	7 ± 5.8	15 ± 5.0	27 ± 20.2
2014-AM-101	0 ± 0.0	0 ± 0.0	3 ± 2.9	10 ± 13.2	18 ± 15.3	23 ± 10.4	30 ± 8.7	42 ± 7.6	52 ± 7.6
2014-AM-102	0 ± 0.0	0 ± 0.0	5 ± 0.0	12 ± 2.9	18 ± 2.9	28 ± 5.8	32 ± 7.6	40 ± 5.0	45 ± 8.7
2014-AM-103	0 ± 0.0	5 ± 5.0	15 ± 5.0	22 ± 16.1	32 ± 22.5	40 ± 15.0	48 ± 12.6	60 ± 5.0	70 ± 5.0
2014-AM-104	0 ± 0.0	0 ± 0.0	12 ± 11.5	23 ± 10.4	47 ± 5.8	55 ± 0.0	55 ± 0.0	60 ± 5.0	62 ± 5.8
2014-AM-105	0 ± 0.0	0 ± 0.0	12 ± 2.9	17 ± 5.8	25 ± 0.0	30 ± 5.0	37 ± 2.9	38 ± 2.9	40 ± 0.0
2014-AM-106	0 ± 0.0	0 ± 0.0	8 ± 7.6	13 ± 7.6	22 ± 15.3	30 ± 22.9	32 ± 25.2	35 ± 22.9	43 ± 29.3
2014-AM-107	0 ± 0.0	0 ± 0.0	7 ± 7.6	15 ± 13.2	32 ± 5.8	37 ± 5.8	43 ± 12.6	50 ± 18.0	53 ± 16.1
2014-AM-108	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	8 ± 5.8	13 ± 10.4	13 ± 10.4	20 ± 13.2	23 ± 10.4
2014-AM-109	0 ± 0.0	2 ± 2.9	12 ± 10.4	22 ± 10.4	42 ± 2.9	43 ± 2.9	45 ± 5.0	50 ± 8.7	57 ± 2.9
2014-AM-110	3 ± 2.9	17 ± 2.9	43 ± 5.0	54 ± 12.6	61 ± 7.6	63 ± 10.0	65 ± 7.6	65 ± 7.6	65 ± 7.6
2014-AM-221	0 ± 0.0	3 ± 2.9	8 ± 7.6	12 ± 2.9	17 ± 2.9	18 ± 5.8	18 ± 5.8	25 ± 8.7	33 ± 10.4
2014-AM-222	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	5 ± 0.0	8 ± 2.9	8 ± 2.9	13 ± 2.9
2014-AM-223	0 ± 0.0	7 ± 5.8	17 ± 2.9	32 ± 7.6	43 ± 10.4	47 ± 7.6	47 ± 7.6	57 ± 12.6	57 ± 12.6
AAC Synergy	8 ± 14.4	95 ± 8.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
12 exp2	0 ± 0.0	10 ± 0.0	37 ± 12.6	48 ± 7.6	52 ± 5.8	55 ± 0.0	60 ± 5.0	63 ± 2.9	73 ± 7.6
59 exp2	0 ± 0.0	23 ± 10.4	47 ± 16.1	67 ± 2.9	73 ± 2.9	75 ± 0.0	78 ± 2.9	83 ± 5.8	87 ± 7.6
104 exp2	0 ± 0.0	3 ± 2.9	23 ± 7.6	50 ± 10.0	57 ± 5.8	72 ± 15.3	75 ± 20.0	82 ± 11.5	83 ± 14.4
2014-AM-224	5 ± 5.0	25 ± 5.0	33 ± 10.4	40 ± 13.2	43 ± 12.6	50 ± 8.7	53 ± 5.8	62 ± 12.6	65 ± 10.0
2014-AM-225	7 ± 2.9	45 ± 20.0	75 ± 10.0	83 ± 10.4	92 ± 7.6	95 ± 8.7	98 ± 2.9	98 ± 2.9	98 ± 2.9
2014-AM-226	5 ± 5.0	43 ± 7.6	60 ± 8.7	73 ± 7.6	75 ± 5.0	75 ± 5.0	75 ± 5.0	75 ± 5.0	75 ± 5.0
2014-AM-227	10 ± 0.0	78 ± 7.6	82 ± 2.9	87 ± 5.8	87 ± 5.8	88 ± 2.9	88 ± 2.9	88 ± 2.9	88 ± 2.9
2014-AM-228	3 ± 2.9	27 ± 12.6	33 ± 12.6	38 ± 15.3	47 ± 15.3	47 ± 15.3	50 ± 18.0	58 ± 20.2	58 ± 20.2
2014-AM-229	0 ± 0.0	23 ± 11.5	62 ± 22.5	75 ± 26.5	80 ± 18	80 ± 18.0	82 ± 20.2	83 ± 20.8	83 ± 20.8
2014-AM-230	0 ± 0.0	0 ± 0.0	8 ± 2.9	22 ± 15.3	32 ± 15.3	40 ± 15.0	47 ± 17.6	50 ± 13.2	63 ± 7.6

2014-AM-231	0 ± 0.0	0 ± 0.0	8 ± 5.8	25 ± 5.0	43 ± 14.4	55 ± 21.8	58 ± 20.2	60 ± 18.0	60 ± 18.0
2014-AM-232	0 ± 0.0	20 ± 15.0	27 ± 12.6	38 ± 5.8	43 ± 5.8	45 ± 8.7	45 ± 8.7	48 ± 5.8	53 ± 5.8
2014-AM-233	2 ± 2.9	28 ± 2.9	58 ± 10.4	65 ± 5.0	73 ± 5.8	80 ± 13.2	83 ± 15.3	85 ± 13.2	87 ± 12.6
2014-AM-234	2 ± 2.9	20 ± 0.0	45 ± 17.3	50 ± 13.2	53 ± 18.9	55 ± 21.8	55 ± 21.8	57 ± 20.2	62 ± 17.6
2014-AM-235	0 ± 0.0	13 ± 7.6	23 ± 5.8	35 ± 5.0	38 ± 2.9	42 ± 2.9	42 ± 2.9	45 ± 5.0	53 ± 12.6
2014-AM-236	3 ± 5.8	3 ± 5.8	7 ± 7.6	22 ± 2.9	37 ± 2.9	45 ± 8.7	52 ± 12.6	57 ± 16.1	60 ± 18.0
2014-AM-237	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9
2014-AM-238	12 ± 5.8	82 ± 14.4	83 ± 11.5	83 ± 11.5	85 ± 8.7	85 ± 8.7	85 ± 8.7	85 ± 8.7	87 ± 5.8
2014-AM-239	0 ± 0.0	13 ± 11.5	28 ± 27.8	35 ± 25.2	39 ± 27.5	43 ± 27.5	43 ± 27.8	56 ± 28.4	60 ± 26.0
2014-AM-240	8 ± 5.8	45 ± 13.2	58 ± 20.8	62 ± 20.2	68 ± 24.7	72 ± 28.4	75 ± 22.9	77 ± 20.2	80 ± 15.0
2014-AM-241	0 ± 0.0	8 ± 10.4	25 ± 10.0	42 ± 11.5	47 ± 16.1	48 ± 18.9	50 ± 21.8	53 ± 18.9	55 ± 17.3
2014-AM-242	10 ± 13.2	58 ± 22.5	78 ± 20.8	85 ± 17.3	90 ± 17.3	92 ± 14.4	93 ± 11.5	93 ± 11.5	93 ± 11.5
2014-AM-243	3 ± 2.9	55 ± 8.7	70 ± 5.0	77 ± 5.8	78 ± 2.9	80 ± 5.0	82 ± 7.6	85 ± 5.0	92 ± 2.9
2014-AM-244	3 ± 2.9	58 ± 7.6	70 ± 8.7	80 ± 10.0	85 ± 10.0	90 ± 10.0	90 ± 10.0	90 ± 10.0	90 ± 10.0
2014-AM-245	5 ± 5.0	38 ± 7.6	55 ± 5.0	65 ± 5.0	70 ± 5.0	72 ± 7.6	73 ± 10.4	80 ± 13.2	82 ± 12.6
2014-AM-246	0 ± 0.0	20 ± 5.0	45 ± 10.0	53 ± 7.6	67 ± 5.8	75 ± 5.0	75 ± 5.0	75 ± 5.0	77 ± 5.8
2014-AM-247	0 ± 0.0	7 ± 2.9	28 ± 7.6	43 ± 12.6	43 ± 12.6	48 ± 11.5	52 ± 14.4	58 ± 7.6	68 ± 16.1
2014-AM-248	7 ± 2.9	50 ± 17.3	62 ± 20.8	68 ± 20.2	68 ± 20.2	68 ± 20.2	70 ± 18.0	70 ± 18.0	70 ± 18.0
2014-AM-249	0 ± 0.0	15 ± 8.7	38 ± 16.1	55 ± 13.2	58 ± 7.6	67 ± 7.6	72 ± 5.8	72 ± 5.8	72 ± 5.8
2014-AM-250	7 ± 2.9	47 ± 14.4	60 ± 5.0	68 ± 7.6	72 ± 10.4	73 ± 7.6	73 ± 7.6	73 ± 7.6	78 ± 12.6
2014-AM-252	3 ± 2.9	82 ± 17.6	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
2014-AM-253	0 ± 0.0	2 ± 2.9	5 ± 0.0	8 ± 2.9	15 ± 5.0	20 ± 13.2	25 ± 13.2	32 ± 7.6	35 ± 5.0
2014-AM-254	2 ± 2.9	38 ± 7.6	48 ± 16.1	58 ± 11.5	62 ± 5.8	62 ± 5.8	62 ± 5.8	63 ± 2.9	67 ± 7.6
2014-AM-255	2 ± 2.9	22 ± 5.8	35 ± 5.0	43 ± 7.6	53 ± 12.6	58 ± 15.3	58 ± 15.3	58 ± 15.3	70 ± 13.2
2014-AM-256	2 ± 2.9	2 ± 2.9	3 ± 2.9	5 ± 5.0	10 ± 5.0	15 ± 5.0	15 ± 5.0	20 ± 10.0	40 ± 18.0
2014-AM-257	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 2.9	7 ± 2.9	10 ± 5.0	15 ± 10.0	18 ± 15.3
2014-AM-258	0 ± 0.0	2 ± 2.9	7 ± 7.6	23 ± 7.6	30 ± 5.0	35 ± 8.7	38 ± 7.6	52 ± 7.6	60 ± 10.0
2014-AM-261	0 ± 0.0	27 ± 12.6	50 ± 20.0	63 ± 15.3	65 ± 18.0	68 ± 23.6	68 ± 23.6	82 ± 11.5	82 ± 11.5
2014-AM-262	2 ± 2.9	45 ± 10.0	58 ± 10.4	65 ± 10.0	65 ± 10.0	72 ± 11.5	73 ± 14.4	73 ± 14.4	80 ± 18.0
2014-AM-263	2 ± 2.9	2 ± 2.9	7 ± 2.9	17 ± 2.9	27 ± 12.6	32 ± 17.6	33 ± 17.6	40 ± 21.8	45 ± 21.8
2014-AM-264	8 ± 5.8	40 ± 15.0	47 ± 10.4	53 ± 2.9	55 ± 5.0	57 ± 2.9	60 ± 0.0	63 ± 5.8	63 ± 5.8
2014-AM-265	3 ± 2.9	25 ± 10.0	43 ± 7.6	52 ± 12.6	55 ± 13.2	55 ± 13.2	58 ± 7.6	60 ± 10.0	62 ± 7.6
2014-AM-266	2 ± 2.9	22 ± 2.9	37 ± 5.8	45 ± 8.7	50 ± 5.0	50 ± 5.0	55 ± 5.0	55 ± 5.0	58 ± 2.9
2014-AM-267	3 ± 2.9	62 ± 17.6	85 ± 8.7	87 ± 11.5	87 ± 11.5	92 ± 7.6	93 ± 5.8	98 ± 2.9	98 ± 2.9
2014-AM-268	2 ± 2.9	30 ± 5.0	42 ± 16.1	50 ± 21.8	65 ± 21.8	80 ± 13.2	83 ± 16.1	85 ± 13.2	85 ± 13.2
2014-AM-269	0 ± 0.0	15 ± 8.7	32 ± 7.6	42 ± 12.6	52 ± 10.4	53 ± 12.6	55 ± 10.0	70 ± 8.7	78 ± 12.6
2014-AM-270	0 ± 0.0	32 ± 20.8	52 ± 16.1	67 ± 16.1	68 ± 18.9	68 ± 18.9	68 ± 18.9	70 ± 21.8	72 ± 20.8
2014-AM-271	0 ± 0.0	2 ± 2.9	7 ± 7.6	8 ± 5.8	12 ± 2.9	12 ± 2.9	15 ± 5.0	18 ± 2.9	28 ± 5.8
2014-AM-272	0 ± 0.0	27 ± 17.6	52 ± 20.2	62 ± 12.6	65 ± 10.0	67 ± 10.4	75 ± 8.7	75 ± 8.7	77 ± 10.4
2014-AM-273	13 ± 18.9	20 ± 21.8	20 ± 21.8	22 ± 20.8	23 ± 20.2	25 ± 18.0	27 ± 16.1	37 ± 16.1	53 ± 16.1
2014-AM-274	0 ± 0.0	0 ± 0.0	3 ± 5.8	5 ± 5.0	5 ± 5.0	5 ± 5.0	5 ± 5.0	5 ± 5.0	5 ± 5.0
2014-AM-277	10 ± 5.0	83 ± 7.6	93 ± 5.8	93 ± 5.8	95 ± 5.0	95 ± 5.0	97 ± 5.8	97 ± 5.8	97 ± 5.8
2014-AM-278	0 ± 0.0	0 ± 0.0	2 ± 2.9	8 ± 2.9	23 ± 18.9	30 ± 21.8	38 ± 18.9	42 ± 17.6	47 ± 18.9
2014-AM-279	0 ± 0.0	33 ± 5.8	57 ± 7.6	62 ± 10.4	63 ± 11.5	65 ± 13.2	68 ± 7.6	68 ± 7.6	72 ± 5.8
2014-AM-280	2 ± 2.9	10 ± 5.0	13 ± 7.6	15 ± 10.0	18 ± 10.4	20 ± 10.0	23 ± 10.4	35 ± 13.2	43 ± 7.6
2014-AM-281	0 ± 0.0	5 ± 0.0	12 ± 7.6	18 ± 5.8	30 ± 5.0	40 ± 8.7	45 ± 15.0	55 ± 8.7	68 ± 16.1
2014-AM-282	3 ± 2.9	43 ± 10.4	55 ± 8.7	55 ± 8.7	57 ± 10.4	57 ± 10.4	58 ± 12.6	60 ± 13.2	60 ± 13.2
2014-AM-283	18 ± 5.8	55 ± 13.2	73 ± 10.4	83 ± 7.6	87 ± 10.4	90 ± 13.2	92 ± 14.4	92 ± 14.4	92 ± 14.4
2014-AM-284	3 ± 5.8	57 ± 5.8	68 ± 2.9	68 ± 2.9	73 ± 2.9	77 ± 2.9	77 ± 2.9	83 ± 7.6	90 ± 10.0
2014-AM-285	0 ± 0.0	2 ± 2.9	8 ± 2.9	15 ± 5.0	22 ± 10.4	28 ± 17.6	32 ± 15.3	32 ± 15.3	32 ± 15.3
2014-AM-286	0 ± 0.0	2 ± 2.9	7 ± 11.5	25 ± 13.2	33 ± 20.2	38 ± 14.4	38 ± 14.4	45 ± 13.2	45 ± 13.2
2014-AM-287	0 ± 0.0	3 ± 5.8	17 ± 11.5	32 ± 7.6	38 ± 10.4	50 ± 21.8	52 ± 20.8	60 ± 18.0	65 ± 18.0
2014-AM-288	0 ± 0.0	3 ± 5.8	5 ± 5.0	12 ± 10.4	18 ± 12.6	23 ± 10.4	32 ± 5.8	38 ± 15.3	42 ± 15.3
2014-AM-289	2 ± 2.9	8 ± 5.8	17 ± 10.4	27 ± 15.3	30 ± 18.0	32 ± 18.9	33 ± 20.8	33 ± 20.8	38 ± 16.1
2014-AM-290	2 ± 2.9	10 ± 5.0	37 ± 17.6	43 ± 12.6	48 ± 12.6	48 ± 12.6	48 ± 12.6	60 ± 22.9	68 ± 15.3
2014-AM-291	0 ± 0.0	0 ± 0.0	5 ± 5.0	32 ± 16.1	50 ± 13.2	58 ± 14.4	62 ± 11.5	65 ± 10.0	67 ± 12.6
2014-AM-292	5 ± 0.0	37 ± 17.6	60 ± 25.0	70 ± 20.0	72 ± 20.2	75 ± 15.0	80 ± 13.2	83 ± 10.4	85 ± 10.0
2014-AM-293	2 ± 2.9	5 ± 5.0	12 ± 5.8	18 ± 7.6	25 ± 8.7	37 ± 5.8	38 ± 7.6	45 ± 5.0	57 ± 15.3
2014-AM-294	2 ± 2.9	5 ± 5.0	20 ± 5.0	33 ± 10.4	37 ± 16.1	43 ± 18.9	43 ± 18.9	53 ± 18.9	63 ± 20.2
2014-AM-295	3 ± 2.9	63 ± 2.9	83 ± 7.6	85 ± 8.7	85 ± 8.7	87 ± 10.4	88 ± 12.6	92 ± 7.6	93 ± 7.6
2014-AM-296	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	3 ± 2.9	5 ± 0.0	5 ± 0.0	8 ± 5.8	8 ± 5.8
2014-AM-297	0 ± 0.0	2 ± 2.9	3 ± 5.8	8 ± 2.9	13 ± 2.9	22 ± 11.5	30 ± 15.0	42 ± 23.6	48 ± 29.3
2014-AM-298	0 ± 0.0	25 ± 10.0	47 ± 10.4	60 ± 0.0	65 ± 5.0	72 ± 2.9	73 ± 2.9	80 ± 13.2	82 ± 12.6

2014-AM-299	0 ± 0.0	3 ± 2.9	15 ± 8.7	37 ± 7.6	42 ± 5.8	45 ± 5.0	45 ± 5.0	47 ± 7.6	47 ± 7.6
2014-AM-300	0 ± 0.0	8 ± 2.9	22 ± 5.8	30 ± 10.0	37 ± 2.9	43 ± 2.9	45 ± 5.0	50 ± 8.7	55 ± 17.3
2014-AM-301	0 ± 0.0	3 ± 2.9	13 ± 2.9	25 ± 8.7	37 ± 17.6	43 ± 15.3	45 ± 18.0	52 ± 12.6	57 ± 11.5
2014-AM-302	53 ± 12.6	93 ± 5.8	95 ± 8.7	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
2014-AM-303	3 ± 5.8	40 ± 8.7	45 ± 13.2	50 ± 13.2	52 ± 10.4	53 ± 11.5	60 ± 20.0	77 ± 11.5	83 ± 7.6
2014-AM-304	0 ± 0.0	2 ± 2.9	2 ± 2.9	5 ± 5.0	13 ± 7.6	20 ± 18.0	23 ± 20.2	27 ± 22.5	32 ± 27.5
2014-AM-305	0 ± 0.0	15 ± 8.7	32 ± 16.1	45 ± 15	58 ± 12.6	58 ± 12.6	60 ± 15.0	63 ± 20.2	65 ± 20.0
2014-AM-306	0 ± 0.0	2 ± 2.9	2 ± 2.9	3 ± 2.9	12 ± 2.9	15 ± 0.0	18 ± 5.8	20 ± 5.0	23 ± 10.4
2014-AM-307	2 ± 2.9	3 ± 2.9	7 ± 2.9	20 ± 8.7	25 ± 10.0	32 ± 15.3	37 ± 15.3	47 ± 23.6	53 ± 20.2
2014-AM-308	0 ± 0.0	15 ± 10.0	28 ± 18.9	38 ± 18.9	43 ± 15.3	43 ± 15.3	45 ± 15.0	45 ± 15.0	53 ± 12.6
2014-AM-309	0 ± 0.0	0 ± 0.0	10 ± 5.0	18 ± 10.4	18 ± 10.4	20 ± 10.0	20 ± 10.0	25 ± 5.0	37 ± 12.6
2014-AM-310	0 ± 0.0	2 ± 2.9	7 ± 2.9	10 ± 5.0	17 ± 5.8	18 ± 7.6	20 ± 8.7	28 ± 2.9	32 ± 2.9
2014-AM-311	0 ± 0.0	20 ± 8.7	33 ± 2.9	50 ± 5.0	63 ± 5.8	73 ± 2.9	75 ± 0.0	77 ± 2.9	78 ± 2.9
2014-AM-312	0 ± 0.0	0 ± 0.0	2 ± 2.9	12 ± 2.9	15 ± 5.0	20 ± 5.0	20 ± 5.0	22 ± 7.6	23 ± 10.4
2014-AM-313	3 ± 5.8	35 ± 5.0	63 ± 12.6	70 ± 8.7	70 ± 8.7	73 ± 12.6	75 ± 15.0	82 ± 14.4	88 ± 7.6
2014-AM-314	0 ± 0.0	22 ± 7.6	62 ± 11.5	70 ± 13.2	75 ± 17.3	75 ± 17.3	77 ± 20.2	80 ± 18	87 ± 11.5
2014-AM-315	0 ± 0.0	8 ± 5.8	23 ± 7.6	35 ± 8.7	40 ± 10.0	40 ± 10.0	42 ± 12.6	48 ± 5.8	53 ± 14.4
2014-AM-316	8 ± 2.9	75 ± 10.0	93 ± 7.6	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
2014-AM-317	0 ± 0.0	2 ± 2.9	7 ± 7.6	12 ± 12.6	15 ± 15.0	17 ± 17.6	18 ± 15.3	25 ± 15	37 ± 16.1
2014-AM-318	2 ± 2.9	5 ± 5.0	28 ± 16.1	40 ± 17.3	67 ± 18.9	75 ± 8.7	85 ± 5.0	92 ± 2.9	95 ± 5.0
2014-AM-319	0 ± 0.0	3 ± 2.9	15 ± 5.0	23 ± 7.6	35 ± 13.2	42 ± 15.3	43 ± 17.6	50 ± 22.9	57 ± 27.5
2014-AM-320	33 ± 25.7	88 ± 20.2	93 ± 11.5	95 ± 8.7	95 ± 8.7	95 ± 8.7	95 ± 8.7	95 ± 8.7	95 ± 8.7
2014-AM-321	0 ± 0.0	20 ± 10.0	25 ± 13.2	32 ± 14.4	32 ± 14.4	33 ± 16.1	38 ± 16.1	42 ± 18.9	47 ± 23.6
2014-AM-322	2 ± 2.9	5 ± 5.0	12 ± 2.9	15 ± 0.0	15 ± 0.0	15 ± 0.0	15 ± 0.0	18 ± 5.8	22 ± 7.6
2014-AM-323	0 ± 0.0	7 ± 11.5	27 ± 11.5	28 ± 10.4	28 ± 10.4	28 ± 10.4	30 ± 8.7	33 ± 10.4	40 ± 17.3
2014-AM-324	0 ± 0.0	2 ± 2.9	13 ± 10.4	25 ± 15.0	25 ± 15.0	28 ± 17.6	30 ± 18.0	37 ± 18.9	47 ± 27.5
2014-AM-325	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 5.8	7 ± 7.6	8 ± 7.6	10 ± 8.7	12 ± 10.4
2014-AM-326	2 ± 2.9	52 ± 14.4	73 ± 12.6	85 ± 8.7	87 ± 10.4	90 ± 13.2	90 ± 13.2	90 ± 13.2	90 ± 13.2
2014-AM-327	0 ± 0.0	0 ± 0.0	3 ± 5.8	15 ± 5.0	30 ± 10.0	35 ± 10.0	45 ± 13.2	55 ± 18.0	55 ± 18.0
2014-AM-328	0 ± 0.0	0 ± 0.0	3 ± 5.8	13 ± 2.9	20 ± 5.0	28 ± 10.4	37 ± 16.1	45 ± 18.0	50 ± 22.9
2014-AM-329	0 ± 0.0	2 ± 2.9	8 ± 10.4	8 ± 10.4	12 ± 11.5	13 ± 10.4	13 ± 10.4	17 ± 12.6	18 ± 12.6
2014-AM-330	0 ± 0.0	2 ± 2.9	5 ± 5.0	8 ± 7.6	10 ± 5.0	20 ± 5.0	28 ± 7.6	35 ± 8.7	65 ± 5.0
2014-AM-331	0 ± 0.0	3 ± 5.8	13 ± 10.4	30 ± 10.0	37 ± 10.4	45 ± 17.3	47 ± 18.9	60 ± 25.0	70 ± 13.2
2014-AM-332	2 ± 2.9	5 ± 0.0	12 ± 11.5	27 ± 7.6	37 ± 5.8	40 ± 8.7	43 ± 12.6	53 ± 10.4	57 ± 7.6
2014-AM-333	8 ± 5.8	47 ± 15.3	53 ± 16.1	58 ± 11.5	63 ± 11.5	63 ± 11.5	63 ± 11.5	65 ± 13.2	73 ± 20.8
2014-AM-334	2 ± 2.9	20 ± 5.0	37 ± 2.9	45 ± 5.0	55 ± 15.0	57 ± 15.3	60 ± 10.0	65 ± 8.7	65 ± 8.7
2014-AM-335	0 ± 0.0	5 ± 0.0	18 ± 5.8	30 ± 8.7	33 ± 7.6	35 ± 8.7	40 ± 8.7	45 ± 5.0	48 ± 2.9
2014-AM-336	0 ± 0.0	0 ± 0.0	13 ± 7.6	20 ± 13.2	23 ± 16.1	30 ± 18.0	32 ± 15.3	33 ± 16.1	38 ± 22.5

Table S0.5. Average germination percentage ± standard deviation of lines in the Synch recombinant inbred population. Germination assessment was done using three replicate plates of 20 seeds germinated in darkness at 20˚C.

Line	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 10	Day 14
CH2019-SB-1	38 ± 12.6	92 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-3	2 ± 2.9	20 ± 13.2	60 ± 21.8	73 ± 20.2	75 ± 21.8	83 ± 24.7	85 ± 21.8	88 ± 20.2	88 ± 20.2
CH2019-SB-4	0 ± 0.0	15 ± 8.7	50 ± 10.0	85 ± 5	93 ± 2.9	93 ± 2.9	95 ± 0.0	98 ± 2.9	100 ± 0.0
CH2019-SB-5	0 ± 0.0	2 ± 2.9	12 ± 5.8	23 ± 5.8	38 ± 7.6	48 ± 16.1	53 ± 16.1	62 ± 22.5	73 ± 20.8
CH2019-SB-6	0 ± 0.0	3 ± 5.8	23 ± 5.8	40 ± 5	52 ± 16.1	60 ± 26.5	63 ± 32.1	75 ± 21.8	78 ± 20.2
CH2019-SB-7	0 ± 0.0	8 ± 14.4	32 ± 11.5	43 ± 15.3	55 ± 13.2	58 ± 14.4	65 ± 10.0	82 ± 7.6	88 ± 7.6
CH2019-SB-8	33 ± 11.5	97 ± 11.5	103 ± 5.8	103 ± 5.8	103 ± 5.8	103 ± 5.8	103 ± 5.8	103 ± 5.8	70 ± 52
CH2019-SB-9	10 ± 5.0	90 ± 0.0	93 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	98 ± 2.9
CH2019-SB-10	15 ± 5.0	78 ± 10.4	93 ± 7.6	93 ± 7.6	93 ± 7.6	95 ± 5	95 ± 5.0	97 ± 5.8	97 ± 5.8
CH2019-SB-11	42 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-12	13 ± 7.6	83 ± 2.9	93 ± 5.8	93 ± 5.8	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-13	48 ± 5.8	93 ± 2.9	95 ± 5.0	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9
CH2019-SB-14	50 ± 10.0	95 ± 5.0	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-15	33 ± 14.4	97 ± 5.8	98 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-16	0 ± 0.0	5 ± 0.0	23 ± 7.6	48 ± 7.6	65 ± 5.0	68 ± 2.9	72 ± 2.9	82 ± 10.4	80 ± 18.0
CH2019-SB-17	18 ± 10.4	90 ± 5.0	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	100 ± 0.0
CH2019-SB-18	3 ± 5.8	85 ± 5.0	97 ± 2.9	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0
CH2019-SB-19	5 ± 5.0	50 ± 8.7	68 ± 2.9	75 ± 0	82 ± 5.8	90 ± 5	90 ± 5.0	90 ± 5.0	90 ± 5.0
CH2019-SB-20	0 ± 0.0	33 ± 31.8	70 ± 5.0	82 ± 5.8	85 ± 5.0	93 ± 5.8	98 ± 2.9	100 ± 0.0	100 ± 0.0
CH2019-SB-21	0 ± 0.0	10 ± 5.0	52 ± 18.9	68 ± 16.1	73 ± 11.5	77 ± 10.4	78 ± 12.6	83 ± 10.4	85 ± 8.7
CH2019-SB-22	65 ± 5.0	93 ± 7.6	98 ± 2.9	98 ± 2.9	98 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-23	5 ± 8.7	25 ± 8.7	72 ± 14.4	87 ± 14.4	92 ± 10.4	95 ± 5	97 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-24	5 ± 5.0	62 ± 5.8	87 ± 5.8	93 ± 7.6	98 ± 2.9	98 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0
CH2019-SB-25	45 ± 5.0	98 ± 2.9	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-26	23 ± 7.6	43 ± 5.8	52 ± 2.9	57 ± 2.9	62 ± 5.8	65 ± 5	68 ± 2.9	82 ± 2.9	85 ± 5.0
CH2019-SB-27	15 ± 5.0	78 ± 7.6	97 ± 2.9	97 ± 2.9	97 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-28	27 ± 2.9	93 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 2.9	97 ± 2.9
CH2019-SB-29	0 ± 0.0	83 ± 5.8	90 ± 5.0	92 ± 2.9	93 ± 5.8	93 ± 5.8	93 ± 5.8	97 ± 2.9	97 ± 2.9
CH2019-SB-30	20 ± 10.0	87 ± 10.4	93 ± 7.6	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-32	32 ± 11.5	97 ± 2.9	97 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-33	2 ± 2.9	35 ± 5.0	55 ± 13.2	58 ± 7.6	65 ± 0.0	72 ± 7.6	72 ± 7.6	75 ± 13.2	78 ± 14.4
CH2019-SB-34	10 ± 5.0	50 ± 22.9	90 ± 8.7	93 ± 7.6	95 ± 5.0	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-35	18 ± 2.9	33 ± 7.6	47 ± 14.4	57 ± 18.9	57 ± 18.9	62 ± 18.9	62 ± 18.9	73 ± 20.8	80 ± 20.0
CH2019-SB-36	0 ± 0.0	5 ± 0.0	33 ± 12.6	50 ± 13.2	57 ± 17.6	67 ± 7.6	68 ± 10.4	82 ± 7.6	87 ± 7.6
CH2019-SB-37	17 ± 11.5	95 ± 8.7	98 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-38	5 ± 5.0	78 ± 2.9	90 ± 8.7	93 ± 7.6	95 ± 5.0	95 ± 5	95 ± 5.0	97 ± 2.9	97 ± 2.9
CH2019-SB-39	22 ± 5.8	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9
CH2019-SB-40	0 ± 0.0	48 ± 11.5	95 ± 5.0	97 ± 5.8	98 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-41	2 ± 2.9	2 ± 2.9	7 ± 11.5	17 ± 15.3	30 ± 8.7	38 ± 2.9	43 ± 2.9	58 ± 7.6	70 ± 5.0
CH2019-SB-42	0 ± 0.0	77 ± 7.6	92 ± 7.6	95 ± 5	97 ± 5.8	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-43	15 ± 10.0	88 ± 2.9	95 ± 5.0	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-44	8 ± 2.9	80 ± 5.0	92 ± 7.6	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8	97 ± 5.8
CH2019-SB-45	38 ± 20.8	95 ± 5.0	100 ± 5.0	102 ± 2.9	102 ± 2.9	102 ± 2.9	102 ± 2.9	102 ± 2.9	102 ± 2.9
CH2019-SB-46	32 ± 20.2	90 ± 5.0	95 ± 5.0	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-47	38 ± 5.8	93 ± 5.8	97 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-48	0 ± 0.0	12 ± 5.8	38 ± 16.1	52 ± 23.1	62 ± 32.1	70 ± 26.5	73 ± 29.3	77 ± 28.4	83 ± 17.6
CH2019-SB-49	22 ± 7.6	93 ± 2.9	97 ± 5.8	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-50	30 ± 8.7	87 ± 10.4	93 ± 7.6	95 ± 5	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9
CH2019-SB-51	8 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-52	2 ± 2.9	32 ± 7.6	53 ± 14.4	67 ± 10.4	73 ± 7.6	87 ± 10.4	88 ± 7.6	92 ± 2.9	95 ± 0.0
CH2019-SB-53	23 ± 5.8	90 ± 10.0	90 ± 10.0	92 ± 7.6	93 ± 5.8	97 ± 2.9	97 ± 2.9	97 ± 2.9	97 ± 2.9
CH2019-SB-54	0 ± 0.0	0 ± 0.0	8 ± 7.6	10 ± 8.7	13 ± 12.6	17 ± 12.6	17 ± 12.6	25 ± 8.7	42 ± 10.4
CH2019-SB-55	17 ± 20.8	88 ± 5.8	92 ± 7.6	93 ± 5.8	95 ± 5.0	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-56	20 ± 18.0	93 ± 2.9	97 ± 5.8	97 ± 5.8	98 ± 2.9	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-57	0 ± 0.0	8 ± 2.9	48 ± 17.6	67 ± 15.3	77 ± 15.3	85 ± 10	85 ± 10.0	92 ± 5.8	95 ± 0.0
CH2019-SB-58	50 ± 17.3	92 ± 2.9	97 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0	100 ± 0.0	100 ± 0.0	100 ± 0.0

CH2019-SB-59	10 ± 0	92 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-60	7 ± 2.9	70 ± 13.2	80 ± 10.0	88 ± 2.9	90 ± 5.0	97 ± 5.8	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-61	10 ± 5.0	78 ± 7.6	83 ± 5.8	90 ± 5	95 ± 5.0	95 ± 5	97 ± 2.9	97 ± 2.9	98 ± 2.9
CH2019-SB-62	0 ± 0.0	7 ± 2.9	17 ± 7.6	37 ± 20.2	42 ± 16.1	45 ± 13.2	48 ± 12.6	62 ± 7.6	77 ± 12.6
CH2019-SB-63	37 ± 16.1	82 ± 2.9	85 ± 5.0	90 ± 5.0	92 ± 5.8	95 ± 0.0	95 ± 0.0	95 ± 0.0	97 ± 2.9
CH2019-SB-64	5 ± 5.0	97 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-65	22 ± 7.6	80 ± 0.0	90 ± 5.0	93 ± 2.9	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-66	2 ± 2.9	8 ± 2.9	37 ± 10.4	45 ± 10.0	52 ± 14.4	62 ± 10.4	63 ± 12.6	83 ± 7.6	95 ± 5.0
CH2019-SB-67	32 ± 11.5	90 ± 8.7	97 ± 5.8	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-68	15 ± 5.0	78 ± 11.5	90 ± 8.7	93 ± 2.9	93 ± 2.9	93 ± 2.9	95 ± 5.0	95 ± 5.0	95 ± 5.0
CH2019-SB-69	0 ± 0.0	3 ± 2.9	13 ± 5.8	30 ± 5.0	45 ± 8.7	55 ± 5.0	57 ± 7.6	72 ± 12.6	78 ± 12.6
CH2019-SB-70	0 ± 0.0	2 ± 2.9	18 ± 10.4	28 ± 12.6	43 ± 5.8	53 ± 7.6	55 ± 8.7	63 ± 10.4	68 ± 7.6
CH2019-SB-71	7 ± 7.6	95 ± 5.0	97 ± 2.9	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-72	2 ± 2.9	27 ± 10.4	67 ± 11.5	82 ± 7.6	87 ± 2.9	92 ± 5.8	95 ± 0.0	97 ± 2.9	97 ± 2.9
CH2019-SB-73	40 ± 10.0	95 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-74	0 ± 0.0	3 ± 2.9	15 ± 5.0	32 ± 2.9	42 ± 2.9	45 ± 5.0	48 ± 10.4	57 ± 11.5	60 ± 13.2
CH2019-SB-75	18 ± 5.8	77 ± 2.9	83 ± 7.6	88 ± 7.6	90 ± 5.0	93 ± 2.9	95 ± 0.0	98 ± 5.8	100 ± 5.0
CH2019-SB-76	8 ± 2.9	83 ± 12.6	95 ± 5.0	98 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-77	3 ± 2.9	53 ± 16.1	85 ± 8.7	95 ± 5.0	95 ± 5.0	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-78	0 ± 0.0	28 ± 14.4	70 ± 5.0	80 ± 5.0	85 ± 5.0	90 ± 5.0	92 ± 5.8	93 ± 2.9	95 ± 5.0
CH2019-SB-79	0 ± 0.0	0 ± 0.0	3 ± 2.9	12 ± 11.5	28 ± 18.9	40 ± 17.3	50 ± 20.0	62 ± 20.2	68 ± 22.5
CH2019-SB-80	18 ± 2.9	92 ± 2.9	95 ± 5.0	97 ± 2.9	97 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-81	37 ± 15.3	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-82	23 ± 15.3	93 ± 7.6	98 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-83	22 ± 10.4	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-84	5 ± 0.0	28 ± 5.8	32 ± 2.9	40 ± 0.0	43 ± 2.9	48 ± 2.9	53 ± 2.9	65 ± 5.0	70 ± 5.0
CH2019-SB-85	17 ± 7.6	87 ± 12.6	93 ± 7.6	95 ± 8.7	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9	98 ± 2.9
CH2019-SB-86	2 ± 2.9	23 ± 17.6	37 ± 20.8	48 ± 18.9	60 ± 26.0	65 ± 30.4	72 ± 25.7	92 ± 7.6	93 ± 7.6
CH2019-SB-87	25 ± 8.7	95 ± 5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-88	2 ± 2.9	13 ± 11.5	32 ± 15.3	58 ± 20.2	65 ± 13.2	68 ± 12.6	77 ± 18.9	88 ± 11.5	90 ± 8.7
CH2019-SB-89	13 ± 2.9	88 ± 2.9	93 ± 2.9	98 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH2019-SB-90	0 ± 0.0	23 ± 16.1	63 ± 10.4	78 ± 10.4	87 ± 7.6	95 ± 8.7	95 ± 8.7	95 ± 8.7	95 ± 8.7
AAC Synergy	3 ± 2.9	97 ± 2.9	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
CH1224-1	0 ± 0.0	0 ± 0.0	12 ± 2.9	37 ± 10.4	65 ± 5.0	85 ± 5.0	88 ± 5.8	95 ± 5.0	97 ± 2.9

Table S0.6. Average germination percentage ± standard deviation of lines in the Legci double haploid population. Germination assessment was done using three replicate plates of 20 seeds germinated in darkness at 20˚C.

Line	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 10	Day 14
H106-4	0 ± 0.0	2 ± 2.9	8 ± 5.8	13 ± 2.9	13 ± 2.9	17 ± 2.9	20 ± 5.0	32 ± 14.4	45 ± 13.2
H106-6	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	3 ± 2.9	10 ± 5.0	15 ± 8.7
H106-10	0 ± 0.0	0 ± 0.0	7 ± 7.6	15 ± 8.7	28 ± 12.6	35 ± 13.2	47 ± 18.9	50 ± 13.2	52 ± 14.4
H106-11	0 ± 0.0	0 ± 0.0	8 ± 7.6	13 ± 7.6	20 ± 5.0	25 ± 10.0	27 ± 12.6	30 ± 13.2	33 ± 14.4
H106-12	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	13 ± 2.9	23 ± 2.9	30 ± 5.0	38 ± 2.9	47 ± 5.8
H106-13	0 ± 0.0	35 ± 8.7	68 ± 10.4	73 ± 7.6	77 ± 5.8	78 ± 7.6	88 ± 10.4	93 ± 5.8	93 ± 5.8
H106-14	0 ± 0.0	12 ± 7.6	32 ± 11.5	42 ± 5.8	47 ± 5.8	50 ± 8.7	52 ± 5.8	57 ± 12.6	57 ± 12.6
H106-15	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	8 ± 10.4	12 ± 16.1	12 ± 16.1	18 ± 15.3	23 ± 20.2
H106-16	0 ± 0.0	0 ± 0.0	3 ± 5.8	8 ± 10.4	13 ± 14.4	15 ± 17.3	15 ± 17.3	22 ± 16.1	30 ± 26.0
H106-19	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9
H106-20	0 ± 0.0	13 ± 12.6	23 ± 16.1	40 ± 13.2	47 ± 2.9	52 ± 7.6	55 ± 8.7	62 ± 5.8	62 ± 5.8
H106-22	0 ± 0.0	35 ± 5.0	58 ± 5.8	72 ± 11.5	75 ± 10.0	82 ± 15.3	88 ± 11.5	92 ± 10.4	92 ± 10.4
H106-25	0 ± 0.0	2 ± 2.9	5 ± 5.0	15 ± 5.0	18 ± 7.6	20 ± 5.0	20 ± 5.0	22 ± 7.6	25 ± 5.0
H106-28	0 ± 0.0	0 ± 0.0	5 ± 5.0	7 ± 5.8	10 ± 5.0	10 ± 5.0	12 ± 5.8	18 ± 5.8	25 ± 8.7
H106-29	0 ± 0.0	3 ± 2.9	10 ± 13.2	20 ± 15.0	28 ± 16.1	32 ± 15.3	40 ± 13.2	48 ± 16.1	57 ± 10.4
H106-30	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
H106-31	0 ± 0.0	10 ± 5.0	28 ± 12.6	42 ± 10.4	60 ± 10.0	72 ± 7.6	73 ± 10.4	80 ± 17.3	80 ± 17.3
H106-32	0 ± 0.0	8 ± 7.6	40 ± 13.2	53 ± 7.6	57 ± 10.4	60 ± 13.2	60 ± 13.2	62 ± 14.4	62 ± 14.4
H106-33	0 ± 0.0	5 ± 5.0	27 ± 2.9	37 ± 5.8	47 ± 7.6	58 ± 11.5	72 ± 5.8	82 ± 5.8	82 ± 5.8
H106-39	0 ± 0.0	25 ± 5.0	35 ± 0.0	37 ± 2.9	42 ± 5.8	45 ± 10.0	53 ± 16.1	57 ± 10.4	57 ± 10.4
H106-41	0 ± 0.0	37 ± 7.6	73 ± 7.6	78 ± 2.9	80 ± 5.0	80 ± 5.0	80 ± 5.0	83 ± 7.6	83 ± 7.6
H106-44	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	10 ± 5.0	13 ± 5.8
H106-45	0 ± 0.0	3 ± 2.9	12 ± 5.8	28 ± 7.6	37 ± 2.9	47 ± 2.9	57 ± 12.6	65 ± 8.7	67 ± 7.6
H106-46	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
H106-51	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	3 ± 2.9	7 ± 5.8	12 ± 7.6	18 ± 5.8	22 ± 7.6
H106-54	0 ± 0.0	37 ± 10.4	62 ± 15.3	75 ± 20.0	75 ± 20.0	80 ± 13.2	88 ± 12.6	93 ± 7.6	97 ± 2.9
H106-56	0 ± 0.0	30 ± 17.3	60 ± 8.7	72 ± 7.6	78 ± 12.6	85 ± 13.2	93 ± 5.8	97 ± 2.9	97 ± 2.9
H106-57	0 ± 0.0	0 ± 0.0	5 ± 0.0	17 ± 10.4	25 ± 10.0	32 ± 5.8	37 ± 10.4	43 ± 16.1	48 ± 16.1
H106-61	0 ± 0.0	0 ± 0.0	7 ± 2.9	8 ± 2.9	13 ± 2.9	18 ± 5.8	18 ± 5.8	23 ± 5.8	32 ± 20.2
H106-62	0 ± 0.0	3 ± 2.9	12 ± 7.6	23 ± 12.6	30 ± 15.0	33 ± 10.4	35 ± 8.7	47 ± 11.5	62 ± 7.6
H106-67	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9
H106-68	0 ± 0.0	12 ± 7.6	40 ± 13.2	58 ± 15.3	60 ± 13.2	60 ± 13.2	60 ± 13.2	65 ± 18.0	65 ± 18.0
H106-69	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	7 ± 5.8	7 ± 5.8	7 ± 5.8	10 ± 10.0	23 ± 20.8
H106-71	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 7.6	12 ± 11.5	13 ± 14.4	17 ± 11.5	17 ± 11.5
H106-74	0 ± 0.0	2 ± 2.9	10 ± 0.0	18 ± 2.9	32 ± 5.8	42 ± 2.9	48 ± 5.8	63 ± 2.9	65 ± 5.0
H106-75	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9	2 ± 2.9	3 ± 5.8
H106-76	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	5 ± 8.7	5 ± 8.7	5 ± 8.7	5 ± 8.7
H106-77	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	10 ± 5.0	13 ± 7.6	18 ± 11.5	18 ± 11.5	22 ± 15.3
H106-79	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9
H106-81	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
H106-83	0 ± 0.0	0 ± 0.0	3 ± 2.9	13 ± 7.6	13 ± 7.6	17 ± 7.6	17 ± 7.6	23 ± 5.8	28 ± 14.4
H106-84	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	5 ± 5.0	5 ± 5.0
H106-264	0 ± 0.0	3 ± 2.9	3 ± 2.9	17 ± 12.6	22 ± 12.6	28 ± 20.2	32 ± 16.1	35 ± 18.0	38 ± 20.2
H106-268	0 ± 0.0	0 ± 0.0	0 ± 0.0	7 ± 2.9	8 ± 2.9	12 ± 2.9	15 ± 5.0	27 ± 11.5	35 ± 15.0
H106-269	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	3 ± 5.8	10 ± 5.0	10 ± 5.0
H106-271	0 ± 0.0	3 ± 2.9	10 ± 8.7	23 ± 10.4	37 ± 12.6	40 ± 18.0	42 ± 17.6	48 ± 20.2	48 ± 20.2
H106-274	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9
H106-282	0 ± 0.0	7 ± 5.8	35 ± 5.0	40 ± 10.0	42 ± 10.4	45 ± 10	48 ± 15.3	57 ± 22.5	57 ± 22.5
H106-311	0 ± 0.0	5 ± 0.0	12 ± 7.6	13 ± 5.8	15 ± 5.0	17 ± 2.9	18 ± 5.8	25 ± 13.2	25 ± 13.2
H106-313	10 ± 5.0	30 ± 8.7	37 ± 14.4	47 ± 18.9	55 ± 22.9	57 ± 23.6	67 ± 32.1	75 ± 21.8	77 ± 18.9
H106-320	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	8 ± 2.9	10 ± 0.0	10 ± 0.0	12 ± 2.9	13 ± 5.8
H106-321	0 ± 0.0	0 ± 0.0	10 ± 8.7	20 ± 13.2	25 ± 18.0	32 ± 20.8	32 ± 20.8	47 ± 16.1	60 ± 15.0
H106-328	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	5 ± 0.0	7 ± 2.9	10 ± 5.0	13 ± 2.9
H106-330	0 ± 0.0	2 ± 2.9	13 ± 7.6	27 ± 14.4	35 ± 8.7	40 ± 8.7	43 ± 7.6	52 ± 14.4	62 ± 7.6
H106-348	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 5.8	3 ± 5.8	3 ± 5.8	3 ± 5.8	5 ± 5.0	5 ± 5.0
H106-349	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	7 ± 2.9	15 ± 5.0	17 ± 7.6	25 ± 10.0	30 ± 15.0

H106-353	0 ± 0.0	5 ± 5.0	10 ± 13.2	10 ± 13.2	10 ± 13.2	10 ± 13.2	12 ± 16.1	18 ± 23.6	18 ± 23.6
H106-357	0 ± 0.0	2 ± 2.9	3 ± 5.8	7 ± 7.6	7 ± 7.6	7 ± 7.6	7 ± 7.6	8 ± 5.8	13 ± 14.4
H106-360	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 5.8
H106-367	0 ± 0.0	0 ± 0.0	12 ± 12.6	17 ± 12.6	20 ± 13.2	25 ± 21.8	35 ± 22.9	38 ± 18.9	50 ± 18.0
H106-368	0 ± 0.0	3 ± 5.8	20 ± 5.0	28 ± 7.6	32 ± 7.6	33 ± 7.6	35 ± 8.7	35 ± 8.7	35 ± 8.7
H106-374	0 ± 0.0	2 ± 2.9	7 ± 5.8	10 ± 5.0	15 ± 5.0	20 ± 5.0	23 ± 7.6	50 ± 10.0	57 ± 10.4
H106-376	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 5.8	3 ± 5.8	5 ± 5.0	8 ± 5.8	10 ± 5.0
H106-380	0 ± 0.0	3 ± 5.8	20 ± 8.7	35 ± 13.2	45 ± 8.7	53 ± 7.6	67 ± 11.5	82 ± 2.9	82 ± 2.9
H106-386	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	3 ± 2.9	5 ± 5.0	7 ± 7.6	12 ± 10.4	12 ± 10.4
H106-388	0 ± 0.0	0 ± 0.0	8 ± 7.6	12 ± 10.4	15 ± 13.2	15 ± 13.2	20 ± 13.2	35 ± 22.9	43 ± 12.6
H106-389	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	3 ± 2.9	7 ± 7.6	10 ± 13.2	10 ± 13.2
H106-393	0 ± 0.0	0 ± 0.0	17 ± 7.6	45 ± 20.0	60 ± 20.0	72 ± 20.2	77 ± 15.3	82 ± 15.3	82 ± 15.3
H106-397	0 ± 0.0	2 ± 2.9	7 ± 5.8	15 ± 13.2	18 ± 16.1	18 ± 16.1	18 ± 16.1	27 ± 20.2	32 ± 25.2
H106-403	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 2.9	7 ± 2.9	10 ± 0.0	12 ± 2.9	13 ± 5.8	15 ± 8.7
H106-404	0 ± 0.0	8 ± 2.9	28 ± 7.6	33 ± 7.6	42 ± 12.6	42 ± 12.6	43 ± 15.3	47 ± 15.3	47 ± 15.3
H106-405	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	8 ± 2.9	12 ± 2.9	20 ± 0.0	20 ± 0.0
H106-407	0 ± 0.0	7 ± 2.9	20 ± 18.0	28 ± 17.6	40 ± 13.2	48 ± 18.9	53 ± 17.6	58 ± 16.1	63 ± 7.6
H106-408	0 ± 0.0	0 ± 0.0	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9	3 ± 2.9
H106-410	0 ± 0.0	8 ± 10.4	28 ± 7.6	33 ± 7.6	33 ± 7.6	33 ± 7.6	35 ± 10.0	35 ± 10.0	35 ± 10.0
H106-411	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	10 ± 8.7	22 ± 11.5	35 ± 26.0	38 ± 23.6	42 ± 22.5
H106-413	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 5.8	5 ± 5.0	5 ± 5.0	12 ± 5.8	25 ± 8.7
H106-418	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9
H106-420	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	3 ± 2.9	3 ± 2.9
H106-426	0 ± 0.0	15 ± 8.7	33 ± 10.4	42 ± 16.1	43 ± 18.9	55 ± 31.2	65 ± 31.2	73 ± 25.7	77 ± 23.6
H106-427	0 ± 0.0	2 ± 2.9	12 ± 5.8	18 ± 10.4	27 ± 5.8	30 ± 0.0	30 ± 0.0	35 ± 5.0	45 ± 13.2
H106-428	0 ± 0.0	2 ± 2.9	8 ± 5.8	20 ± 10.0	30 ± 8.7	35 ± 8.7	47 ± 7.6	57 ± 12.6	60 ± 15.0
H106-431	0 ± 0.0	5 ± 5.0	32 ± 20.2	45 ± 20.0	50 ± 18.0	58 ± 18.9	62 ± 20.2	67 ± 17.6	70 ± 18.0
H106-434	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	3 ± 2.9	5 ± 5.0	5 ± 5.0	5 ± 5.0
H106-439	0 ± 0.0	0 ± 0.0	5 ± 5.0	7 ± 5.8	10 ± 0.0	13 ± 2.9	15 ± 5.0	22 ± 2.9	38 ± 7.6
H106-443	0 ± 0.0	2 ± 2.9	8 ± 7.6	22 ± 2.9	25 ± 5.0	30 ± 5.0	43 ± 11.5	48 ± 17.6	50 ± 15.0
H106-448	0 ± 0.0	7 ± 7.6	7 ± 7.6	13 ± 10.4	13 ± 10.4	17 ± 7.6	22 ± 5.8	27 ± 12.6	37 ± 2.9
H106-454	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
H106-457	0 ± 0.0	8 ± 2.9	27 ± 14.4	38 ± 17.6	42 ± 18.9	43 ± 16.1	45 ± 17.3	53 ± 16.1	57 ± 10.4
H106-460	0 ± 0.0	0 ± 0.0	3 ± 2.9	7 ± 5.8	12 ± 5.8	17 ± 7.6	22 ± 11.5	28 ± 10.4	37 ± 7.6
H106-462	0 ± 0.0	3 ± 2.9	12 ± 7.6	23 ± 2.9	38 ± 10.4	45 ± 17.3	48 ± 15.3	48 ± 15.3	53 ± 16.1
H106-470	2 ± 2.9	27 ± 2.9	35 ± 5.0	40 ± 5.0	50 ± 10.0	57 ± 5.8	63 ± 7.6	77 ± 10.4	77 ± 10.4
H106-481	0 ± 0.0	2 ± 2.9	10 ± 5.0	12 ± 7.6	15 ± 5.0	18 ± 2.9	27 ± 2.9	33 ± 7.6	38 ± 11.5
H106-482	0 ± 0.0	0 ± 0.0	5 ± 5.0	15 ± 5.0	20 ± 5.0	25 ± 5.0	28 ± 7.6	42 ± 15.3	62 ± 17.6
H106-487	0 ± 0.0	2 ± 2.9	2 ± 2.9	7 ± 7.6	8 ± 5.8	13 ± 7.6	15 ± 10.0	23 ± 10.4	25 ± 13.2
H106-492	0 ± 0.0	0 ± 0.0	3 ± 5.8	3 ± 5.8	7 ± 2.9	12 ± 11.5	15 ± 13.2	23 ± 18.9	25 ± 17.3
H106-493	0 ± 0.0	2 ± 2.9	3 ± 5.8	12 ± 2.9	20 ± 13.2	25 ± 10.0	33 ± 7.6	37 ± 5.8	38 ± 7.6
H106-495	0 ± 0.0	7 ± 2.9	30 ± 21.8	37 ± 24.7	42 ± 24.7	50 ± 35.0	57 ± 34.0	73 ± 22.5	73 ± 22.5
H106-499	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	7 ± 5.8	10 ± 5.0	15 ± 5.0	18 ± 5.8	28 ± 11.5
H106-500	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	7 ± 2.9	7 ± 2.9
H106-502	0 ± 0.0	13 ± 7.6	33 ± 5.8	45 ± 5.0	48 ± 5.8	48 ± 5.8	57 ± 2.9	63 ± 10.4	67 ± 16.1
H106-503	0 ± 0.0	0 ± 0.0	2 ± 2.9	10 ± 5.0	12 ± 2.9	15 ± 0.0	17 ± 2.9	22 ± 5.8	25 ± 5.0
H106-505	0 ± 0.0	18 ± 10.4	58 ± 7.6	63 ± 5.8	67 ± 2.9	73 ± 7.6	77 ± 5.8	78 ± 7.6	80 ± 10.0
H106-506	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 0.0	8 ± 2.9	10 ± 0.0	13 ± 2.9	22 ± 7.6	33 ± 10.4
H106-509	0 ± 0.0	2 ± 2.9	2 ± 2.9	5 ± 5.0	8 ± 10.4	10 ± 8.7	10 ± 8.7	18 ± 15.3	23 ± 12.6
H106-515	2 ± 2.9	28 ± 23.6	52 ± 17.6	55 ± 13.2	57 ± 12.6	60 ± 18.0	60 ± 18.0	68 ± 16.1	68 ± 16.1
H106-517	0 ± 0.0	3 ± 5.8	35 ± 13.2	43 ± 17.6	53 ± 25.7	60 ± 27.8	63 ± 27.5	68 ± 27.5	70 ± 30.0
H106-523	0 ± 0.0	5 ± 5.0	25 ± 5.0	28 ± 7.6	28 ± 7.6	32 ± 7.6	37 ± 7.6	40 ± 5.0	42 ± 2.9
H106-529	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	7 ± 7.6	7 ± 7.6	10 ± 5.0	18 ± 2.9
H106-532	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	3 ± 2.9	3 ± 2.9
H106-545	0 ± 0.0	8 ± 5.8	28 ± 10.4	35 ± 10.0	38 ± 7.6	40 ± 5.0	50 ± 8.7	57 ± 5.8	63 ± 5.8
H106-552	0 ± 0.0	3 ± 5.8	20 ± 8.7	35 ± 21.8	42 ± 20.2	45 ± 21.8	45 ± 21.8	48 ± 23.1	48 ± 23.1
H106-553	0 ± 0.0	45 ± 8.7	65 ± 5.0	75 ± 5.0	77 ± 2.9	85 ± 8.7	87 ± 10.4	93 ± 2.9	93 ± 2.9
H106-556	0 ± 0.0	0 ± 0.0	2 ± 2.9	5 ± 8.7	8 ± 5.8	10 ± 8.7	13 ± 14.4	20 ± 21.8	20 ± 21.8
H106-560	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	2 ± 2.9	2 ± 2.9	8 ± 2.9	8 ± 2.9
H106-585	0 ± 0.0	2 ± 2.9	2 ± 2.9	5 ± 0.0	13 ± 10.4	15 ± 13.2	17 ± 11.5	17 ± 11.5	17 ± 11.5
H106-598	0 ± 0.0	2 ± 2.9	2 ± 2.9	5 ± 5.0	8 ± 7.6	8 ± 7.6	12 ± 5.8	15 ± 10.0	15 ± 10.0
CI9831A	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 2.9	3 ± 2.9	5 ± 5.0	7 ± 2.9
LEGER	0 ± 0.0	13 ± 5.8	38 ± 15.3	52 ± 7.6	53 ± 10.4	55 ± 8.7	55 ± 8.7	58 ± 5.8	62 ± 7.6

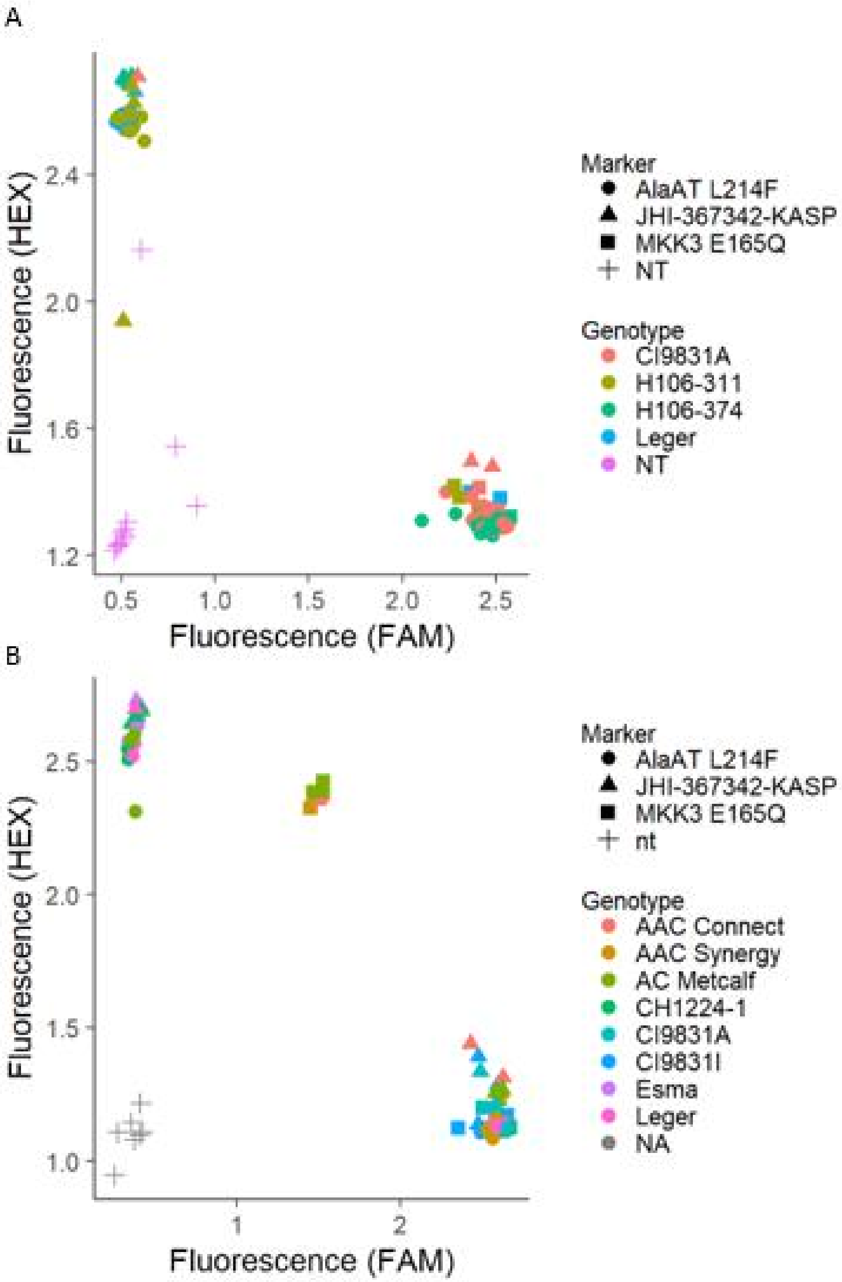

Figure S0.8. Amplification of KASP primers indicating identity at known SD1 and SD2 alleles influencing dormancy. **A)** Verification of SD1 and SD2 alleles in recombinant and parental lines used for physiology testing from the Legci population. **B)** Analysis of identity at important SNPs in parental lines AAC Synergy, CH1224-1, CI9831A, Leger and validation lines Esma, AAC Connect and AC Metcalf (Sweeney *et al.*, 2022*b*).

Leger H106-374 H106-311 CI9831A

Figure S0.9. Photos of head and seed colour of Legci parental lines and recombinant lines selected for physiological comparison. Photos were taken 25 days after anthesis.

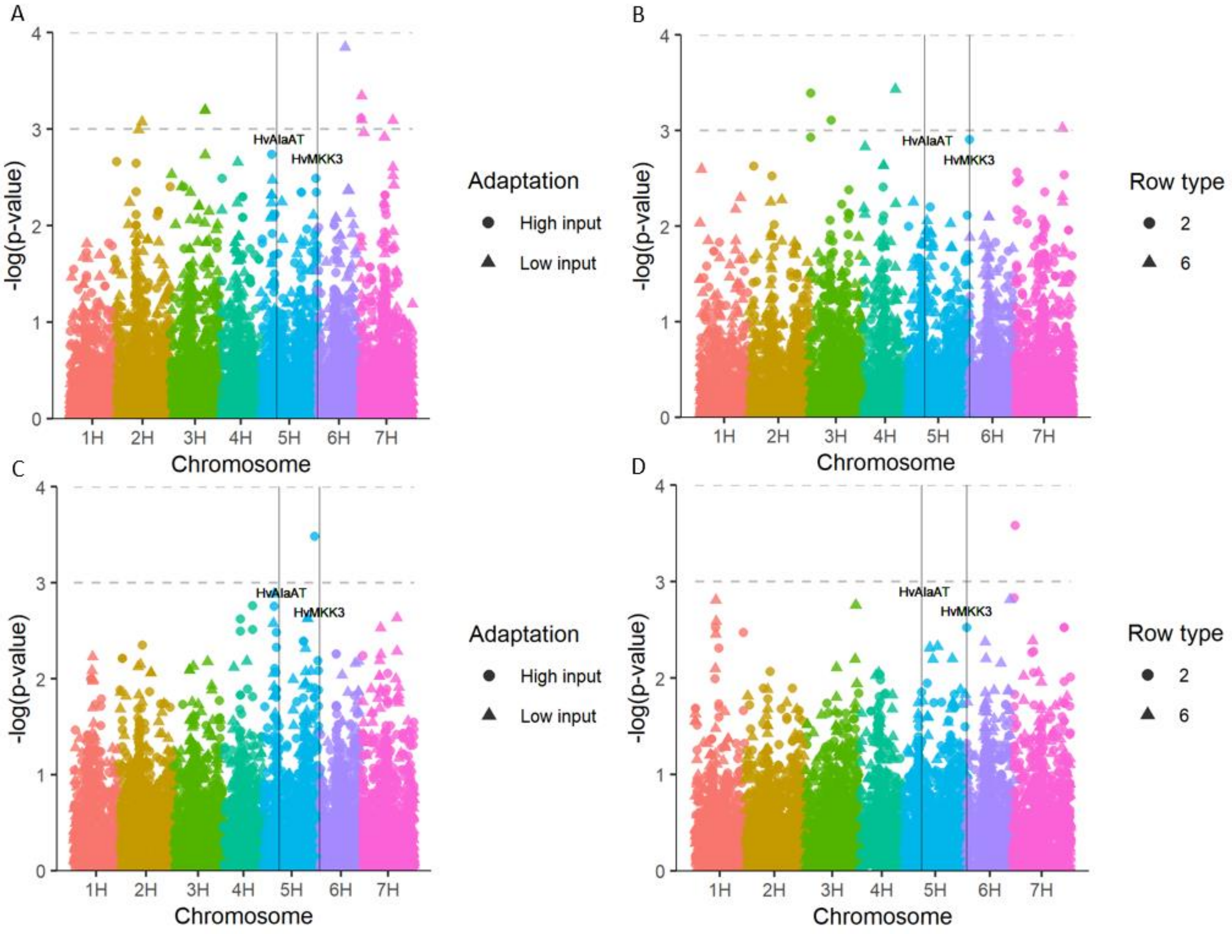

Figure S0.10. Association between (**A**, **B**) 2 DAI germination percentage or (**C**, **D**) 14 DAI with markers from AM-14 lines of (**A**, **C**) adaptation or (**B**, **D**) row type mapped independently. Approximate location of SD1 and SD2 was determined using the closest marker physical position to HvAlaAT1 and HVMKK3 in the Morex v3 refernce genome (Mascher et al., 2021).

Table S0.7. Induction of gene expression during grain maturation in the Legci population. Values are average ± SD of gene expression relative to 15 DAA Leger and are averaged over two experiments with four biological replicates each. Significant induction was done using a students t-test in comparison with 15 DAA value. Significantly induced genes are denoted by *, values confirmed by FDR are indicated with +.

Genotype	Gene	15 DAA	25 DAA	35 DAA
CI9831A	HvAlaAT1	1.3 ± 1.0	**3.7 ± 1.4***$^{+}$	2.3 ± 1.6
H106-311	HvAlaAT1	0.5 ± 0.6	2.0 ± 1.9	1.4 ± 1.7
Leger	HvAlaAT1	1.0 ± 0.5	**4.8 ± 2.9***	**7.0 ± 3.6***$^{+}$
H106-374	HvAlaAT1	1.3 ± 0.6	**7 ± 1.1***$^{+}$	**5.2 ± 1.9***$^{+}$
CI9831A	HvAlaAT2	1.9 ± 0.9	1.8 ± 0.5	0.7 ± 0.7
H106-311	HvAlaAT2	1.1 ± 0.6	0.8 ± 0.6	**0.1 ± 0.1***
Leger	HvAlaAT2	1.0 ± 0.1	1.3 ± 0.7	1.5 ± 0.5
H106-374	HvAlaAT2	-	1.0 ± 0.4	0.6 ± 0.3
CI9831A	HvAlaAT3	2.2 ± 0.8	1.8 ± 0.4	0.8 ± 0.9
H106-311	HvAlaAT3	1.7 ± 0.9	1.0 ± 1.0	**0.1 ± 0.1***
Leger	HvAlaAT3	0.8 ± 0.4	0.9 ± 0.5	0.7 ± 0.4
H106-374	HvAlaAT3	0.4 ± 0.6	0.4 ± 0.4	0.7 ± 0.8
CI9831A	HvAlaAT4	1.7 ± 0.9	**0.4 ± 0.1***	**0.4 ± 0.4***
H106-311	HvAlaAT4	1.4 ± 1.0	**0.1 ± 0.1***	0.0 ± 0.0
Leger	HvAlaAT4	1.1 ± 0.3	0.4 ± 0.3	0.5 ± 0.1
H106-374	HvAlaAT4	-	0.3 ± 0.0	0.5 ± 0.3
CI9831A	HvNCED1	2.3 ± 2.5	2.5 ± 3.9	1.9 ± 1.9
H106-311	HvNCED1	0.4 ± 0.4	1.9 ± 2.5	0.9 ± 1.0
Leger	HvNCED1	1.0 ± 0.3	**2.2 ± 1.3***	1.3 ± 0.7
H106-374	HvNCED1	0.9 ± 0.4	3.4 ± 5.9	1.2 ± 0.6
CI9831A	HvABA8OH-1	1.0 ± 0.6	0.6 ± 0.3	3.0 ± 3.8
H106-311	HvABA8OH-1	1.1 ± 1.5	4.1 ± 6.6	2.4 ± 2.4
Leger	HvABA8OH-1	1.2 ± 0.8	2.9 ± 3.4	1.5 ± 0.7
H106-374	HvABA8OH-1	1.0 ± 0.6	1.2 ± 1.5	1.9 ± 1.0
CI9831A	HvABI5	1.1 ± 0.6	1.1 ± 0.8	1.1 ± 0.6
H106-311	HvABI5	0.6 ± 0.6	2.0 ± 2.1	1.1 ± 0.9
Leger	HvABI5	1.0 ± 0.1	1.8 ± 1.1	1.2 ± 0.4
H106-374	HvABI5	2.0 ± 2.0	2.6 ± 2.8	1.4 ± 0.7
CI9831A	HvGA3ox2	1.2 ± 0.4	**0.4 ± 0.3***	**10.3 ± 5.9***
H106-311	HvGA3ox2	1.0 ± 0.7	4.4 ± 4.5	**6.6 ± 1.4***$^{+}$
Leger	HvGA3ox2	1.9 ± 0.8	1.0 ± 0.6	13.3 ± 11.3
H106-374	HvGA3ox2	-	1.8 ± 1.7	12.5 ± 16.6
CI9831A	HvGA2ox3	1.7 ± 0.9	1.1 ± 0.7	0.8 ± 0.2
H106-311	HvGA2ox3	0.4 ± 0.2	0.6 ± 0.4	**1 ± 0.2***
Leger	HvGA2ox3	1.1 ± 0.6	1.6 ± 1.0	0.5 ± 0.0
H106-374	HvGA2ox3	-	1.0 ± 0.4	1.1 ± 0.7
CI9831A	HvPDC	0.7 ± 0.4	2.0 ± 1.7	1.4 ± 1.9
H106-311	HvPDC	0.5 ± 0.5	1.3 ± 2.1	2.6 ± 4.2
Leger	HvPDC	1.1 ± 0.5	1.6 ± 0.9	2.3 ± 2.0
H106-374	HvPDC	1.3 ± 0.6	2.4 ± 1.1	**4.1 ± 2.2***
CI9831A	HvADH2	8.2 ± 7.1	5.7 ± 6.7	14.2 ± 15.1
H106-311	HvADH2	3.5 ± 5.7	8.5 ± 7.7	11.3 ± 11.0
Leger	HvADH2	1.2 ± 0.7	**5.4 ± 4.7***	20.1 ± 28.1
H106-374	HvADH2	4.3 ± 2.3	6.5 ± 2.4	5.0 ± 5.7
CI9831A	HvLDH A	1.3 ± 0.7	1.2 ± 0.5	1.1 ± 0.8
H106-311	HvLDH A	5.4 ± 10.7	1.1 ± 0.4	1.5 ± 2.0
Leger	HvLDH A	1.0 ± 0.4	**3.4 ± 1.5***	**4.6 ± 3.7***
H106-374	HvLDH A	1.9 ± 0.9	2.4 ± 1.5	**4.4 ± 2.2***

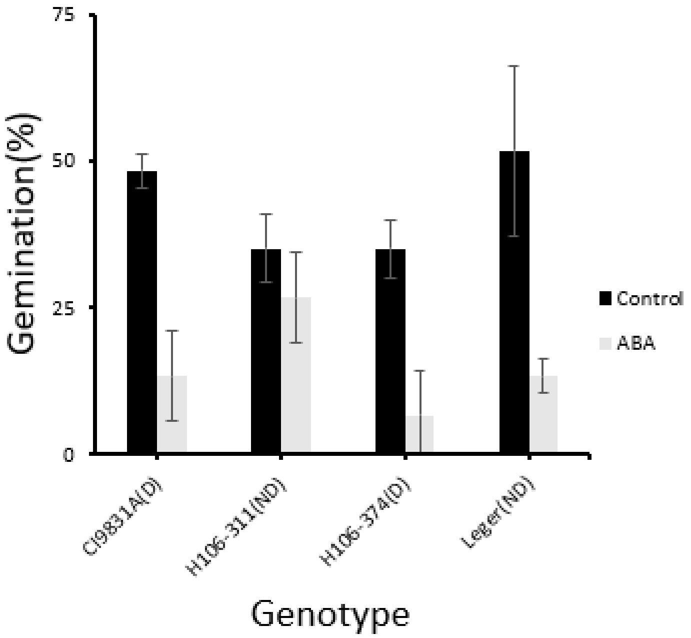

Figure S0.11. Effect of 20 µM ABA treatment on Legci genotypes with either (D) dormant or (ND) non-dormant alleles at the HvAlaAT_L214F locus. 20 seeds germinated in darkness at 20°C sampled once every 24 hours 1-6 days after imbibition. Values represent average germination percentage ± standard deviation 5 days after imbibition (n=3). Seeds were scored as germinated at any sign of radical emergence to separate the influence ABA has on germination from its effect on post-germination growth.