AN INVESTIGATION OF SEED DORMANCY CYCLING MECHANISMS AND LOCAL ADAPTATIONS IN *ARABIDOPSIS THALIANA*

by

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As is often the case with many subjects, be it politics, philosophy or psychology, the truth lies somewhere among the middle of different perspectives. In the listed subjects, settling on a policy of truth usually requires compromises, a tough request from many opinionated consciences. I believe that in biological sciences finding a compromise rather requires carefully investigating the previous data, cleverly designing new experiments, precisely following the schedule, and trusting the process. The results of this study once again strengthened my belief in the wisdom of crowd, if one is willing to pay attention to it without any prejudice. The wisdom of crowd, however, can also be found present in journal articles, books, and contradictory datapoints that sometimes argued in researchers' head until the morning.

Ironically, I have to accept that this study has frequently been a lonely journey, not due to the physical or emotional absence of people but due to the insufficiencies in the inflow of useful tips and ideas. While I felt lonely at a strange disposition, it would not be possible to complete this study without the kind help of countless interns that circled in and out of the Seed Lab. Neither, would it be possible without the touching people who were a relief from my constant stress. It would definitely not have been accomplished without the patience and understanding of Dr. Steven Footitt and Prof. Dr. Fevziye Ç. Toprak. And most dearly, it would definitely not have been possible to undertake this journey without my always supporting family. Once again thank you very much to all...

ABSTRACT

AN INVESTIGATION OF SEED DORMANCY CYCLING MECHANISMS AND LOCAL ADAPTATIONS IN *ARABIDOPSIS THALIANA*

Seeds track environmental signals to avoid unfavorable conditions for germination and match the time of their germination to the growing season. Sensitivities to environmental signals that result in the release or the induction of dormancy differs between genetic backgrounds. In this study, a recombinant inbred lines (RILs) mapping population created between a winter and a summer annual ecotypes of *Arabidopsis thaliana* was subjected to a dormancy cycling treatment. Ten quantitative trait loci (QTLs) were identified for different aspects of dormancy cycling within the phenotypic variation of the population response. The results reveal that *DOG1* controls the germination potential at 25°C under the influence of primary dormancy (PD) and secondary dormancy (SD) after the cold incubation of seeds at 5°C. However, the germination potential at 10°C under the influence of SD after the warm incubation of seeds at 25°C is not controlled by *DOG1* (the warm incubation follows the cold incubation). A candidate gene within Peak 5, *ATHB-5*, is proposed to be in control of the germination potential after the warm incubation by a network analysis of publicly available transcriptome data. *ATHB-5* is a potential source of local adaptation in regulation of seed dormancy cycling. The identification of dormancy cycling QTLs, the analysis of a newly generated double mutant between *dog1-2* and *mft2*, and the results of the network analysis propose a new perspective on the regulation of dormancy cycling in *Arabidopsis thaliana*.

ÖZET

TOHUM UYUŞUKLUĞU DÖNGÜSÜ MEKANİZMALARININ VE LOKAL ADAPTASYONLARININ *ARABİDOPSİS THALİANA*'DA İNCELENMESİ

Tohumlar çevresel işaretleri takip ederek çimlenmek için elverişsiz koşullardan kaçınır ve büyüme sezonunun içerisinde çimlenirler. Uyuşukluğun indüklenmesine ve salınmasına yol açan çevresel sinyallere olan duyarlılık tohumların genetiğine göre farklılık göstermektedir. Bu çalışmada yazın ve kışın büyüme gösteren iki *Arabidopsis thaliana* ekotipi arasında üretilmiş rekombinant kendilenmiş hatlardan oluşan haritalama popülasyonu bir tohum uyuşukluğu döngüsüne maruz bırakılmıştır. Popülasyon tepkisindeki fenotipsel çeşitlilikten yararlanılarak, tohum uyuşukluğu döngüsünün farklı yönlerini niteleyen 10 lokus keşfedilmiştir. Sonuçlara göre, birincil uyuşukluk halinin ve tohumların 5°C'de soğuğa maruz bırakılması ile oluşan ikincil uyuşukluk halinin etkisindeyken *DOG1* 25°C'deki çimlenme potansiyelini kontrol etmektedir. Fakat tohumların soğuğu takriben 25°C'de sıcağa maruz bırakılmasıyla oluşan ikincil uyuşukluk halinin etkisindeyken, 10°C'deki çimlenme potansiyeli *DOG1* tarafından kontrol edilmemektedir. Bu koşullar altında çimlenmeyi kontrol ettiği tespit edilen Peak 5'in içerisindeki aday bir gen, *ATHB-5*, açık erişimdeki transkriptom verileri kullanılarak gerçekleştirilen bir ağ analizi ile tespit edilmiştir. *ATHB-5* tohum uyuşuklukluğu döngüsünü kontrol eden lokal adaptasyonların potansiyel bir kaynağını teşkil etmektedir. Tohum uyuşukluğunu kontrol eden lokusların tespiti, *dog1-2* ve *mft2* ile üretilmiş yeni bir çift mutantın analizi, ve ağ analizinin sonuçları, *Arabidopsis thaliana*'da tohum uyuşukluğu döngüsünün regülasyonuna dair yeni bir perspektif sunmaktadır.

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LIST OF SYMBOLS

cm	centimetre
cM	centiMorgan
gr	gram
H^2	Broad-sense Heritability
h	hour
M	Molar
min	minute
ml	millilitre
mM	millimolar
nm	nanometre
μM	micromolar
μl	microlitre
μ	micro

LIST OF ACRONYMS/ABBREVIATIONS

ABA	Abscisic Acid
AHG	ABA-Hypersensitive Germination
AUC	Area Under Curve
Bur	Burren
CI	Confidence Interval
Col-0	Columbia-0
Cvi	Cape Verdi Isle
DOG	Delay Of Germination
Exp.	Experiment
FLC	Flowering Locus C
FRI	FRIGIDA
GA	Gibberellic Acid
GO	Gene Ontology
HCl	Hydrochloric acid
Ler	Landsberg *erecta*
LED	Light Emitting Diode
LFR	Low Fluence Response
LOD	Logarithm of the odds
MFT	Mother of FT and TFL1
NIL	Near Isogenic Line
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PD	Primary Dormancy
PEG	Polyethylene Glycol-8000
Phy	Phytochrome
QTL	Quantitative Trait Locus/Loci
RDO	Reduced Dormancy

RILs	Recombinant Inbred Lines
ROS	Reactive Oxygen Species
RT	Room Temperature
SE	Standard Error
SET	Seedling Emergence Time
SD	Secondary Dormancy
VLFR	Very Low Fluence Response
WT	Wildtype

1. INTRODUCTION

1.1. Need for Seed

Plants are sessile organisms, unable to escape from unfavorable environments. Together with a decreased morphological redundancy which increases the regeneration ability, a plant instead has complex mechanisms to sense, adapt and respond to prevailing environmental conditions [1]. Apart from interacting with its' immediate environment, a plant keeps a "memory" of the seasonal trends [2-4] and stress events [5]. Depending on the robustness and plasticity of relevant traits, these determine a plant's subsequent interactions with its' environment.

An interesting aspect of these interactions is the ability of plants to time their development [6,7]. The most important developmental transitions in a flowering plants' life cycle are germination and flowering. The former takes place in seeds while the latter results in the generation of them. Seeds, therefore, represent an important part of the plant life-cycle.

Seeds emerged as the reproductive modules of seed-plants (spermatophytes) about 400 million years ago [8]. In the case of *Arabidopsis thaliana*, the seed structure is relatively simple on the physical scale. At maturity, a fully developed embryo fills the seed coat. The radicle and two cotyledons constitute the embryonic root and leaves, respectively. The hypocotyl is the embryonic stem which connects these tissues. Endosperm that surrounds embryo is reduced to a single cell layer after seed maturation, but endosperm cells are morphophysiologically specialized and environmentally sensitive. The seed coat represents a barrier to the external environment. Despite their specializations, all structures determine seed characteristics in concert.

The length of Arabidopsis seeds is under a millimeter and seeds turn to yellowish brown after obtaining maturity. The size and the colour of seeds are dependent on the

ecotype and the environment [9,10]. The variation in physical characteristics of seeds have been correlated with various behavioral aspects [11,12].

Seeds also have the ability to survive harsh conditions and tolerate desiccation. Desiccation tolerance is a common trait in the plant kingdom, but mostly reserved for seeds in angiosperms [13,14]. The ability to survive harsh conditions during unfavorable periods is improved ironically by the arrested development of embryo under favorable ones [14]. This phenomenon is known as seed dormancy.

1.2. Seed Dormancy

Unpredictable environments or predictable environments with competition result in a selective pressure on plant species to accurately time their development [1], [15]. This resulted in the evolution of seed dormancy that blocks embryonic development [15,16]. This block is removed upon receiving certain sequence of environmental signals with adequate information (Figure 1.1) [17,18]. Environmental tracking aims to time the development to the growing season [18]. Therefore, germination cueing by seed dormancy represents a form of developmental niche construction [19]. Timing of germination determines the environmental interactions of a plant in subsequent stages of its life cycle [19].

Seed dormancy can be divided into two types: primary dormancy (PD) and secondary dormancy (SD). These types are defined based on the timing and the context of dormancy. PD is established during seed maturation and is shaped by the environmental context of the maternal plant. PD, if it persists, prevents germination upon the first imbibition of seeds after seed dispersal [17].

After being imbibed, seeds can enter SD under prolonged unfavorable conditions. SD is regulated by the prevailing environment and the memory of past environments. SD, if it persists, prevents germination during the rest of the period spent in the soil seed bank [17].

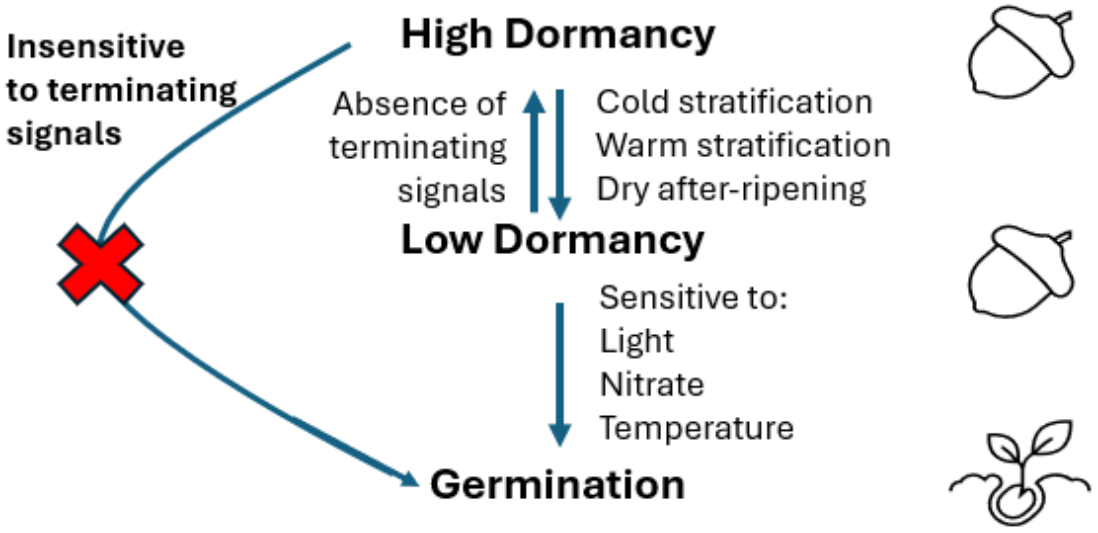

Figure 1.1. Environmental signals regulate seed dormancy. Dormancy levels can transition between high and low dormancy states. In the low dormancy state, seeds acquire sensitivity to the terminating signals. Upon detection of the terminating signals, seeds in the low dormancy state germinate. In the absence of terminating signals, high dormancy state is established.

There is well-documented genetic variation in both PD [20-22] and SD in *Arabidopsis thaliana* [23]. The level of PD is influenced by environmental experiences of the mother plant. This establishes an environmental memory in the seed that can also exert an effect on SD [24,25]. In other words, the initial state of PD dictates the later trajectory during dormancy cycling.

1.3. Dormancy Cycling

The constant reshaping of the mode, the depth and the dynamics of dormancy is referred to as dormancy cycling [18]. During cycling, increase (induction) and decrease (release) of dormancy can occur multiple times and a seed can skip multiple growing seasons (Figures 1.1 and 1.2). Dormancy cycling follows seasonal patterns in the environmental factors [18]. Soil temperature, light (photoperiod, intensity, and wavelength), osmotic potential, biotic factors, and soil nutrient status are among the

environmental factors that regulate dormancy cycling [18].

The effects of environmental factors are modulated by the genetic background [20]. Periods of seedling emergence and flowering times of plant species in the nature differ. This simple observation is sufficient to indicate that genetic background is a modulatory factor in environmental regulation of the plant life cycle.

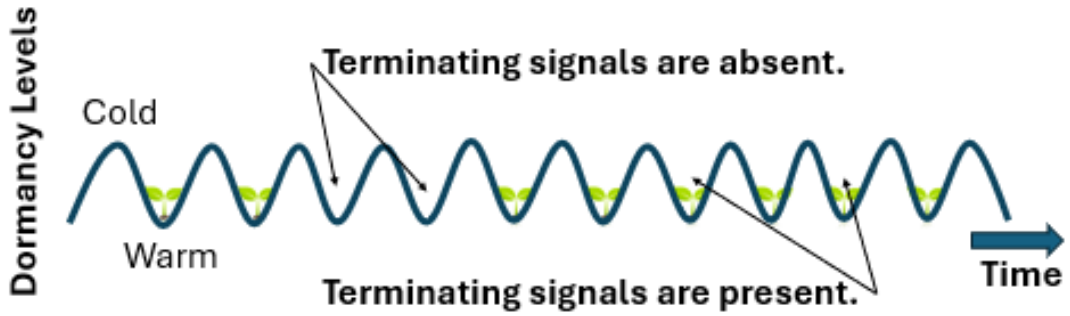

Figure 1.2. Seeds track seasonal patterns during dormancy cycling. The variation in dormancy is continous during the cycling and follows seasonal patterns in the environmental factors (i.e. Temperature). Seeds with lower dormancy levels germinate when terminating signals are present and skip the growing season when terminating signals are absent.

1.4. Individual and Populational Definitions of Seed Dormancy

An individualistic perspective of seed dormancy was assumed until this point. In this perspective, any seed that does not germinate under otherwise a permissible conditions (i.e optimum supply of water, light, temperature, nutrient, and matrix) is referred to as dormant [17]. When dormant, the seed is still physiologically active and responsive to the environment. Even in the absence of imbibition, in a physiologically inactive state, seeds interact with the environment through biochemical reactions [26-28].

The individualistic definition is inadequate in an ecological context that focuses on a population. Germination and dormancy behaviors vary within a population [29]. As a result, it becomes harder to define dormancy in a dualistic manner when some members of a population opt to germinate and some other opt not to. Therefore, at the populational level, dormancy is better defined by assaying germination under multiple conditions [17], [29]. For example, only a half of the seeds from a hypothetical population, X, may germinate in a hypothetical condition, A. Variation of germination within population X represents typical bet hedging [30]. The same population, X, may germinate to full extent in alternative condition, B.

Another hypothetical population, Y, with lower dormancy may display complete germination under both conditions, A and B. However, upon dormancy release germination may occur completely in both conditions, A and B, for both populations, X and Y. On the other hand, an induction of dormancy may result both conditions, A and B, not permiting germination in both populations, X and Y. Yet there could still be other conditions that permit germination to various extents. In this regard, when seed dormancy increases the range of conditions that permit germination are restricted and vice versa [17], [29].

1.5. The Importance of Seeds from an Anthropological Perspective

The variation of dormancy behavior in a population contributes to the formation of a persistent soil seed bank [31]. The soil seed bank ensures maintenance of genetic variation of a species and preserves the stability of their ecosystem by contributing to its regenerative potential [31]. Plants and their seeds provide valuable sources of nutrients in ecological systems [32].

The value of seeds cannot be underestimated from an anthropocentric perspective, either. Humans domesticated various plant species not only for consumption but also for medicinal and cultural uses [33]. The domestication of plants evolved into a co-existence, or rather a co-dependence [34]. With the advent of agriculture, most staple

food sources transitioned into seed-based products in diverse cultures [35]. Improving, spreading and preserving agricultural varieties depend on seeds [36,37].

As the hunter gatherers transitioned into an agrarian society, not only life in general, but also the crops significantly adapted to our agricultural practices [33]. Seed qualities of the common agricultural varieties have undergone an artificial selection process [38,39]. Most prominently, overall dormancy levels decreased in seeds of agricultural varieties as farmers favored the speed of germination, a key factor in improving yield [40]. This practice, however, selected against seed dormancy trait. The decrease in dormancy levels caused significant problems such as viviparous germination, which results in decreased yield and loss of grain quality [39]. Studying dormancy also contributes to the efforts for dealing with common agricultural weeds by modeling their dormancy cycling behavior [41].

The study of seeds will improve the odds of overcoming above-mentioned agricultural problems. Obtaining ecological insights into the regulation of dormancy cycling at the molecular and the physiological level is another important motive. This forms the primary perspective of the ideas, the hypotheses and the experiments presented in this study.

2. LITERATURE SURVEY

2.1. Molecular Control of Dormancy

2.1.1. Hormonal Control of Dormancy

The balance between two phytohormones, Abscisic acid (ABA) and Giberrellic acid (GA), play antagonistic roles during the regulation of dormancy [18]. The hormonal balance between the embryonic growth potential and its repression constitutes an important interface for the regulation of dormancy by the environmental signals [18].

2.1.1.1. Abscisic Acid. During dormancy induction, ABA is synthesized predominantly in the endosperm and transported to embryo [42,43]. Maternal ABA still regulates other aspects of seed development, but not dormancy [44]. ABA can prevent germination, even in seeds with low dormancy, in a dose dependent manner. During the seed development and maturation, ABA signaling has an important role in the accumulation of storage products, the acquirement of desiccation tolerance, and the establishment of PD [42], [45]. Mutants compromised in ABA biosynthesis [45,46], or ABA signaling [47,48] display abnormalities in these processes and vivipary [39]. The co-existence of altered phenotypes in ABA related mutants underlines an integration in the control of these traits.

Low levels of ABA are retained in seeds with low dormancy upon imbibition compared to seeds with high dormancy [50]. Fluctuations in ABA levels (in barley [51]), sensitivity (in *Polygonum aviculare* [52]) and modifications (*Fagus sylvatica* [53]) follow dormancy cycling. Contrasting accounts favor one mode of regulation over the others [54,55]. It is possible that the mode of regulation is specific to the combination of genetic background and type of dormancy [54]. Yet, it is unequivocal that ABA has a role in establishing and maintaining dormancy.

2.1.1.2. Giberrellic Acid. GA is the phytohormone that triggers the germination program [56]. In fact, mutants compromised in GA biosynthesis do not germinate without an external supply [54]. GA can trigger germination in seeds with high dormancy in a dose dependent manner. GA signaling is also regulated during dormancy cycling. For example, loss of GA sensitivity was shown to be dominant during entrance into SD [54].

The germination program is initiated by GA by repressing the action of DELLA proteins that repress GA signaling [56]. Indeed, the mutants of DELLA proteins display a non-dormant phenotype [54]. Interestingly, the expression of *RGL2* and *RGA*, two DELLAs, increase during low dormancy periods [55]. The interpretation is that DELLA proteins constitute the main block on germination during low dormancy periods [18]. This block is then removed by dormancy terminating factors, typically light [18].

2.1.2. Phytochromes

Temperature and light are the two main factors that control the transition between low dormancy and germination. The response to light depends on the fluence of light and the phytochrome profile [57-59]. At the range of low fluence response (LFR) mediated by Phytochrome B (PhyB), the exposure to red light (R) leads to an increase in GA signaling, and the exposure to far-red light (FR) leads to an increase in ABA signaling [60,61]. At the range of very low fluence response (VLFR) mediated by Phytochrome A (PhyA), FR light instead triggers germination. [61].

Ecologically, LFR mediated by PhyB is attributed to the detection of neighboring plants with an established canopy that would lead to a strong competition for resources [62]. VLFR mediated by *PhyA* becomes prominent for the detection of brief light exposures in the soil [59].

The light response is also controlled by temperature [63]. When seeds are exposed to red light at high temperatures outside of the permissible range for germination,

protein conformation of *phyB* reverts back to the inactive state [63,64]. The interaction of light and temperature responses is constantly regulated during dormancy cycling.

2.1.3. *Delay of Germination 1*

DELAY OF GERMİNATİON 1 (*DOG1*) is highly influential in determination of PD levels as highlighted in several QTL mapping studies [22], [65, 66]. At the molecular level, *DOG1* functions by increasing the sensitivity to ABA signals by blocking ABA HYPERSENSİTİVE GERMİNATİON 1 and 3 (AHG1-3) [67]. *AHG1* and *AHG3* encode type 2C protein phosphatases which negatively regulate ABA sensitivity [67]. DOG1 regulates the responsiveness of dormancy levels to cold temperatures during seed maturation and its role in the regulation of dormancy might be conditional on temperature [68].

Mutations in the genes that determine PD do not necessarily result in germination upon imbibition. For example *dog1-2*, retain the ability to be induced into SD [69]. This indicates that dormancy and germination are distinct processes, and that seed dormancy is regulated by multiple pathways.

2.1.4. *Mother of FT and FTL1*

MOTHER OF FT AND FTL1 (*MFT*) overexpression causes an increase in PD [70]. *MFT* expression level was higher during seed development of more dormant seed batches [70]. Strangely, it was also suggested that MFT is involved in the promotion seed germination by blocking ABA signalling [71]. This result is highly counterintuitive as in the same study it was shown that DELLA proteins promote the gene expression of *MFT* which would suggest a negative effect of GA on germination [71].

2.1.5. Other Factors in Control of Dormancy

There are other chemicals and phytohormones have been focused on to a lesser extent. These include nitrate [73], karrikins [74], brassinostreiods [75], ethylene [76], and reactive oxygen species (ROS) [77]. The environment is interfaced to the regulation of dormancy through the above-mentioned molecules. Temperature, nutrition and light are important during the establishment of PD levels at seed maturation [68], [78-81]. The regulation of dormancy by temperature and osmotic conditions become more prominent after seeds are shed [41], [55], [82].

Chromatin landscape, transcriptome, proteome and protein activity (by post-translational modifications) are shaped by the signaling through molecular factors [20], [24], [63], [68], [82-86]. These eventually alter the physical properties of seed tissues, i.e., cell wall structure and endosperm elasticity, determining the balance between growth potential and its repression [87,88].

In summary, environmental regulation of dormancy depends on the configuration of the molecular pathways (Section 2.3). During seed maturation and dormancy cycling, environmental conditions determine the range of conditions permissible for germination by determining the phytohormone content and the expression levels of key genes such as *DOG1*. The block on germination is removed upon sensing a set of signals within the permissible range for germination. The perspective on molecular control agrees with the proposed conception of dormancy cycling in the Introduction.

2.2. The Literature on the Life Cycle Phenology in Natural Populations of *Arabidopsis thaliana*

2.2.1. Types of Dormancy

Dormancy is classified into five types (physical, morphological, physiological, morphophysiological and combinational) according to the seed structures and the mecha-

nisms that impose it [89]. *A. thaliana* displays non-deep physiological dormancy and the work in the literature mainly focuses on this type.

Non-deep physiological dormancy is divided into sub-types according to the thermal sensitivity observed during germination [90]. For example, seeds of winter annuals typically germinate to higher extents at lower temperatures, displaying high temperature thermodormancy [89,90]. Seeds of summer annuals, on the other hand, typically germinate to higher extents at higher temperatures, displaying low temperature thermodormancy [89,90].

An important feature of physiological dormancy is that dormancy can be released both by cold (<10°C) and warm stratification (>15°C) [89]. Different sub-types are present in Arabidopsis ecotypes indicating a natural variation for dormancy trait [90].

2.2.2. Flowering Behaviors in *Arabidopsis thaliana*

A discussion of dormancy cycling cannot be made independently of flowering, the other major life cycle transition. The combination of flowering and dormancy cycling behaviors determine the life cycle phenology in plant species. There are four main life cycle phenologies associated with *A. thaliana*. To a certain extent, the geographical variation of observed phenologies is attributable to local climate patterns [91].

Some ecotypes possess a strict vernalization requirement in which plants do not transition into the reproductive stage without exposure to cold temperatures over a certain duration [92]. Another group of ecotypes does not have a strict vernalization requirement but vernalization significantly decreases flowering time [92]. A last group of ecotypes is unresponsive to vernalization treatment, such as Burren-0 (Bur) [92].

2.2.3. Facultative Winter Annual Behavior

Facultative winter annual behavior is a life cycle strategy that prevails in some Mediterranean ecotypes [91]. Seed germination occurs over three seasons during the fall, the winter and the spring. If a seed germinates in the fall or during the winter, the resulting plant spends the cold period in the vegetative phase as a rosette. Surviving plants transition into the reproductive phase during the spring. Seeds are not induced to high dormancy at cold temperatures during the winter and therefore can germinate in the early spring.

The best studied examples of facultative winter annual populations are in the Iberian Peninsula [93]. These populations display variations in life cycle phenology even within close geographical proximity [93, 94]. This suggests that populations can accumulate locally adaptive behaviors over small distances. Additionally, the altitude of origin determines the plasticity of life cycle behaviors in populations with facultative winter annual behavior [95].

2.2.4. Obligate Winter Annual Behavior

Another life cycle strategy corresponds to the obligate winter annual behavior [91]. Seeds strictly germinate in the fall while temperatures are decreasing. The resulting plants hibernate during the winter as a small rossette and then flower in the spring. Seeds that did not receive adequate germination cues are induced into high SD by cold winter temperature and cannot germinate in the following spring.

Ecotypes with obligate winter annual behavior usually display a strict vernalization requirement [91,92]. Therefore, flowering does not occur when plants do not experience the winter conditions. The best characterized examples of population with obligate winter annual behavior reside in Scandinavia and mountainous regions of Iberia.

2.2.5. Rapid Cycling Behavior

Rapid cycling is another life cycle strategy with best characterized examples originating from the Central Europe [91]. The growing season is wide due to abundant rainfall and mild temperatures in the Central Europe. Natural populations have low dormancy and can germinate over three seasons during the spring, the summer and the fall. Seeds are induced into SD under moist warm conditions in the dark, if adequate germination cues are not recieved [69].

Rapid cycling ecotypes display high plasticity in life cyle behavior according to the germination time [91], [96, 97]. Simulations predicted that rapid cycling ecotypes may be able to complete two generations in a single growing season [97]. Rapid cycling ecotypes include preferred laboratory strains, Columbia (Col-0) and Landsberg *erecta* (Ler), due to their low dormancy and short flowering time ($\approx$ 30 days compared to $\approx$ 50-60 days for Bur without vernalization) [98].

2.2.6. Summer Annual Behavior

In summer annuals, seedling emergence occurs during the spring and the summer season while temperatures are increasing [20], [99]. Seeds have low PD and are not induced into high SD in response to cold unlike obligate winter annuals. Moist incubation at warm temperatures can induce SD similar to rapid cycling ecotypes [90]. In some ecotypes, plants transition into the reproductive period quickly [99], while others require exceptionally long periods [20], [92]. An example of the latter is Bur ecotype isolated from The Burren region of Ireland [20].

While an obligate summer annual would be expected to emerge from soil only during increasing temperatures, Bur was observed to emerge from the soil both during increasing and decreasing temperatures [20]. The thermal sensitivity of fresh Bur seeds is low at intermediate temperatures ($\approx$15°C) and when maturated at 15°C, Bur seeds can display high temperature thermodormancy in the light [90]. Fresh seeds

display low temperature thermodormancy in the dark but during dormancy release by after-ripening, seeds acquire high temperature thermodormancy in the dark [90]. Therefore, if a conservative stance is taken, obligate summer annual behavior for Bur is contentious.

The environmental regulation of dormancy cycling depends on the genetic background, which alters the underlying mechanism to adjust behavioral responses (the next Section). Alterations in the mechanism represent adaptations to the local climate for a natural populations. Adaptations in PD levels, dormancy cycling and flowering behaviors to local climate determine the life-cycle phenology.

2.3. Sources of Natural Genetic Variation in Dormancy and Dormancy Cycling Behavior

2.3.1. Natural Variation in *DOG1*

The most investigated gene for dormancy cycling related natural variations is *DOG1*. Two haplotype groups of *DOG1* result in different dormancy cycling behaviors. Population with D-alleles (D-RY or D-SY), display reduced cold induced SD. This phenotype may allow germination during the spring [91], [100]. Populations with "functional" E-alleles (ECCY or ECSY), however, are induced into high SD with cold incubation. Seeds of these ecotypes would not be expected to germinate with increasing temperatures during the spring [91], [100]. E-alleles are mainly restricted to Iberian Peninsula and Scandinavia, where obligate winter annual life cycle behavior is observed [100]. D-alleles, with reduced cold induced SD are widespread in Central Europe, England and regions with low elevations in Iberia [100].

A strong SD induction in response to cold is important in the winter annual life cycle adapted to climates with hot and dry summers (Iberia) or long freezing winters (Scandinavia). In the former region, if seeds germinate after the winter, completing a reproductive period would not be possible. While in the latter region, germina-

tion in the mid-winter would be highly disadvantageous due to snow coverage. *DOG1* is the dominating genetic factor for the cold induced dormancy. Therefore, different haplotypes have been highly selected for in natural populations during their local adaptations.

If the effect of PD levels on SD induction rates are considered, it might be speculated that natural variation in *DOG1* gene regulates SD induction rate not directly, but instead by determining PD levels (*DOG1* determined PD primes the seed dormancy cycling dynamics) [24,25]. However, there is a transcriptomic evidence that *DOG1* maintains high levels of SD during dormancy cycling, under prolonged unfavorable periods [20]. In other words, the role of DOG1 might be restricted to the establishment of PD levels and maintenance of SD. During these events underlying biological mechanisms involving DOG1 might coincide.

2.3.2. Natural Variation in *DOG6*

DOG6 has the highest effect on PD after DOG1 according to QTL mapping studies [22], [65, 66]. It encodes for a membrane bound NAC family transcription factor, ANAC060 [101]. Nuclear localization of the protein differs between natural variants and is higher in the ecotypes with lower dormancy [101]. Deletion of the transmembrane domain in the Ler background leads to an increased nuclear localization [101].

Mutants of *ANAC060* display higher dormancy and are hypersensitive to ABA [101]. The promoters of genes that encode type 2C protein phosphatases are among the putative binding targets of ANAC060 [101]. This indicates a common contextual role for two major dormancy controlling QTL which are related to regulation of type 2C protein phosphatases [67], [101]. The effect of *DOG1* and *DOG6* is additive, therefore they might be in control of independent downstream elements [101].

2.3.3. Natural Variation in *Reduced Dormany 5*

Another dormancy controlling locus, *REDUCED DORMANCY 5* (*RDO5*, alternatively *DOG18* or *IBO*), encodes a pseudophosphatase [102,103]. Type 2C protein phosphatases dephosphorylate SNF1-related kinases to decrease ABA signaling [104]. Pseudophosphatase activity of RDO5 enhances seed dormancy by preventing the dephosphorylation [102]. Loss of function alleles were found in the northwestern Europe and England, and were suggested to represent local adaptations [102,103]. *RDO5* mutants were more plastic in response to variations in maturation temperature compared to *DOG1* mutants [102]. This emphasizes the reserved role of *DOG1* conditionally regulating seed dormancy according to maturation temperature.

2.3.4. Natural Variation in Loci Affecting Flowering and Dormancy

Natural variations in *FLOWERING LOCUS C* (*FLC*) have been associated with PD levels [105]. Interestingly, FLC mainly regulates the flowering time by its role in the vernalization sensitive flowering pathway [106]. FRIGIDA (FRI) upregulates the gene expression of *FLC* which is downregulated after cold vernalization. Loss of function alleles of both genes are associated with early flowering in natural populations. The effect of *FLC* on dormancy indicates that the regulation of two life cycle transitions is genetically linked [105]. Bur ecotype has an inactive *FLC* allele, but the late-flowering phenotype of Bur is governed by other loci [92].

In fact, two other genes *SPATULA* (*SPT*) and *MFT* play roles in both flowering and dormancy [70], [72], [107,108]. In addition to the regulating dormancy (Subsection 2.1.4), *MFT* and is a floral inducer that accelerates flowering time under long days when overexpressed [72]. *SPT* regulates floral transition and photomorphogenesis during seedling development through a *PhyB* dependent pathway [107]. *SPT* and *MFT* regulate light responsivity in seeds with low dormancy [108]. Variation in *SPT* between two ecotypes result in opposing responses to cold stratification and ABA treatment [108].

2.3.5. Natural Variation in Seedling Emergence Time

An alternative approach focused on seedling emergence timing (SET) and determined that SET was regulated independently of *DOG1* [20]. The QTL with the highest explained variance for SET, *SET1*, resided after *DOG1* towards the end of chromosome 5 [20]. Interestingly, *SET1* included *AHG1*. This indicates to a possible adaptation, that is independent of *DOG1*, important in SD release rather than SD induction. As the activity of *AHG1* is important in the release of dormancy, such an adaptation can be considered likely [67]. Together with their transcriptomics analysis, Footitt et.al. (2020) concluded that *DOG1* controlled initial dormancy levels and subsequent path of dormancy cycling but not SET [20].

2.3.6. Natural Variation in Control of Dormancy Cycling

The current state of literature on dormancy cycling reveals that, (except for a few studies [23], [109]) the research has focused on identifying the natural variation in control of life-time fitness via PD. Genetic factors in control of dormancy cycling might track different environmental signals compared to factors that control PD. This might have resulted in the failure appreciate the effect of dormancy cycling on life-time fitness [20], [23], [109,110].

Ecotypes that are locally adapted to different climates might respond differentially to certain environmental signals such as temperature and osmotic potential during dormancy cycling. A dormancy cycling treatment was applied to a recombinant inbred line (RIL) mapping population derived from parents with winter and summer annual behaviors. A temperature shift was performed to first induce then release SD in the winter annual ecotype (originating from a region with hot and arid summers) and first release then induce SD in the summer annual ecotype (originating from a region with mild and wet summers).

The study mainly aims to detect new sources of environmentally responsive genetic factors that regulate dormancy cycling behavior and to emphasize the local adaptive value of these genetic factors. In addition, dormancy cycling behaviors of lines with mutations in *DOG1* and *MFT* were characterized. Overall, the study highlighted a new perspective on the mechanisms of dormancy cycling.

3. METHODS

3.1. Plant Material

The *Arabidopsis thaliana* (L.) Heynh ecotypes Columbia (Col-0; CS70000), Cape Verde Islands (Cvi; N8580) and Burren (Bur-0; CS6643) were used in various physiological assays focusing on germination and dormancy cycling behaviors. Mutant seeds of *dog1-2* [111] and *mft2* [71] are in the Col-0 background. *dog1-2/mft2* double mutant line was produced as a part of this study (Subsubsection A.3.4). RIL mapping population was produced between Cvi and Bur ecotypes in another study [20].

The methods and the schedule of seed production, and the storage conditions of all seed batches are detailed in Subsection A.3. The seed batch codes are referred to within relevant sections.

3.2. Dormancy Cycling of Ecotypes

The practical work of dormancy cycling experiments, the materials, the setup of incubation boxes and germination boxes, and the germination scoring procedure are detailed in the Subsection A.4

Multiple dormancy cycling experiments were performed with Bur and Cvi ecotypes. Experiments were performed with different seed batches. However, within ecotypes, overall responses to the main experimental parameters were similar.

3.2.1. Determination of Interactions between Temperature and Water Potential in Ecotypes during Dormancy Cycling

Seed batches #1 and #6 were used for Cvi and Bur, respectively (Table A.1). Three samples of each ecotype were sown in boxes (the setup was detailed in Subsub-

section A.4.1).

Boxes were incubated in the dark at 5°C and 25°C at four different osmotic potentials; 0.0, -0.4, -0.8 and -1.2 MPa (Figure 3.1, see Subsection A.4.1 for the adjustment of osmotic potential). The setup results in eight combinations of temperature and osmotic potential. On days 2, 3, 4 (second experimental batch, see below), 5, 7, 14, 21 and 28 (first experimental batch, see below) samples were transferred to germination boxes (Subsubsection A.4.2). Germination tests were at 20°C/light with three solutions; the buffer control solution (1.7 mM Citric Acid and 3.3 mM Potassium Phosphate, pH = 5.0), 10 mM KNO_3 in buffer and 50 μM GA in buffer (Subsection A.2). Germination was scored for 21 days (Subsubsection A.4.3).

The experiment was set up in two batches, timepoints corresponding to 2-4 days were set at a later date. Incubations were performed at 4°C rather than 5°C due to lack of any incubator at the time. The latter batch (days 2-4) of experiments were used only in graphical representations. SD induction and release rates were calculated using the first batch (days 5-28).

3.2.2. Dormancy Cycling with Shifting Temperatures

Seed batches #41 and #55 were used for Cvi and Bur, respectively (Table A.1). The production timeline and conditions of these batches coincided with the production of the last two batches of RIL seeds.

Three samples of each ecotype were sown in boxes (the setup is detailed in Subsubsection A.4.1). Boxes were incubated at 5°C/dark, -1.0 MPa for 14 days (Figure 3.2). Boxes were then transferred to 25°C/dark and incubated for up to 51 days. Solutions were not refreshed during the transfer between two temperatures. Therefore, osmotic potential increased to $\approx$ -0.8 MPa.

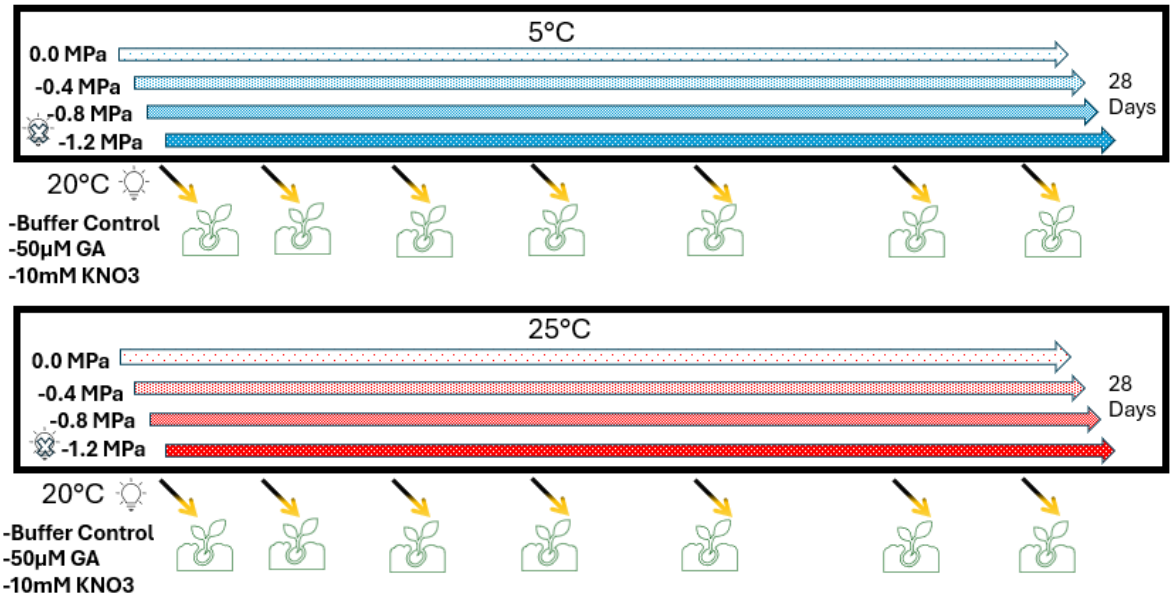

Figure 3.1. Scheme of interaction assay on temperature and osmotic response during dormancy cycling. Samples were incubated at 5°C (blue arrows) or 25°C (red arrows) with four osmotic potentials (color concentration) in the dark (black box) for up to 28 days. Germination tests (yellow-tipped arrows) were at 20°C/light.

Germination tests were performed (on days 5,10, 15, 17, 19, 21, 23, 31, 48, 65) at 10°C/light with three different solutions: buffer control, 10 μM in buffer. The choice of germination test solution depended on the observed level of dormancy. Samples were scored for 28 days.

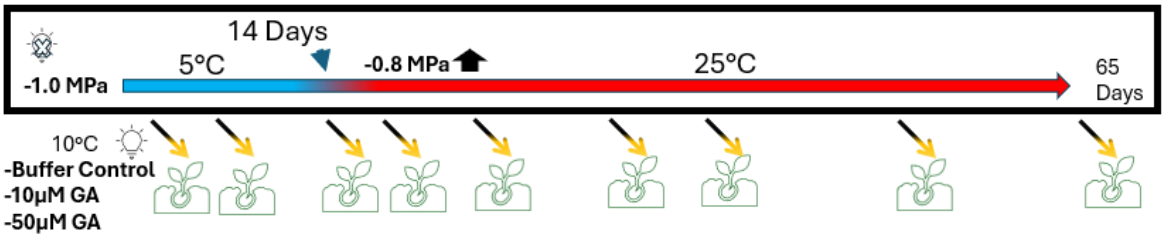

Figure 3.2. Determination of SD induction conditions for ecotypes. Samples were incubated at 5°C, -1.0 MPa in the dark (black box) for 14 days (the blue part of the arrow). Samples were then transferred to 25°C/dark and incubated for up to 51 days (the red part of the arrow). Germination tests (yellow-tipped arrows) were at 10°C/light.

3.2.3. Time Dependence of Temperature Responses

Seed batch #7 for Bur were used (Table A.1). Six samples were sown in boxes (the setup is detailed in Subsubsection A.4.1). Boxes were incubated at 5°C/dark, -1.0 MPa for up to 61 days (Figure 3.3). At different time points, four boxes were transferred to 25°C/dark for up to 4 days. Incubation solutions were not refreshed at this point. Therefore, the osmotic potential of boxes increased to ≈ -0.8 MPa as a result of the temperature increase.

Germination tests were at 20°C/light with two of three different solutions; ddH_2O, 1 mM or 5 mM KNO_3, depending observed level of dormancy. Samples were scored for 14 days.

3.3. Genetic Screening of Dormancy Cycling Behavior in RILs

Seeds of F9 generation of RILs that were collected from the same block were used in the experiments (Table A.3). If collected seeds for a plant from that block ran out, seeds collected from the next block were used instead.

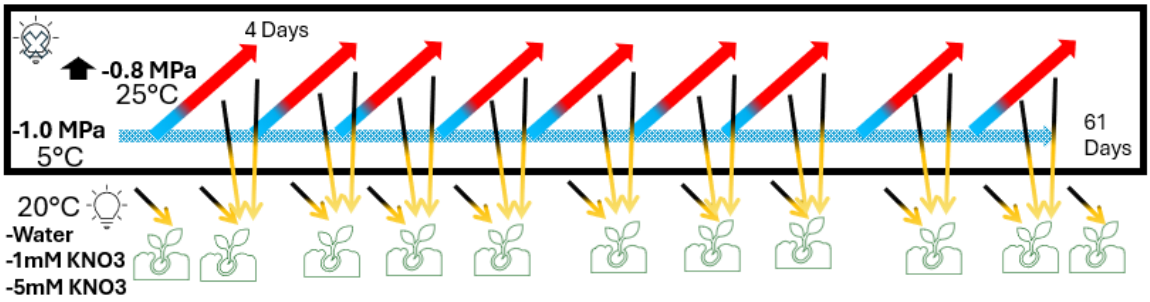

Figure 3.3. Scheme of time dependence of temperature responses. Samples were incubated at 5°C, -1.0 MPa for up to 61 days (blue horizontal arrow) in the dark (black box). Samples were transferred to 25°C/dark at multiple timepoints (red tipped diagonal arrows) and were incubated for up to 4 days. Germination tests (yellow-tipped arrows) were at 20°C/light.

Screening conditions were determined based on the behavior of the parental ecotypes in the dormancy cycling experiments. Samples experienced six different incubation conditions (Figure 3.4). Combined with germination tests in two solutions, twelve screens assayed the dormancy cycling behavior in the mapping population. This required 6400 observations. Due to the sheer number of observations and material constraints, each screen was divided into multiple batches.

Four PD screens were performed at 10°C/light and 25°C/light in either buffer control or 10 μM GA in buffer with three replicates (Sample distribution and box setup is detailed in the Subsubsection A.4.2).

During the incubation period for SD screens, samples for a RIL were incubated in the same containers. This configuration limits the randomization for incubator position. However, a safe and viable method of randomization of RILs between boxes was not discovered, as seeds move around in boxes at high osmotic potentials due to the large solution volumes. However, during the transfers for germination tests, samples for a RIL were randomly distributed between multiple germination containers (Sample distribution and box setup is detailed in the Subsubsection A.4.1).

Four SD screens were performed by incubating six samples of each RIL at 5°C/dark at 0.0 MPa or -1.0 MPa for 14 days. Then samples were transferred to assay germination at 25°C/light with a buffer control and 10 μM GA in buffer with triplicates.

Another set of SD experiments were performed by incubating six samples of each RIL at 5°C/dark in 0.0 MPa or -1.0 MPa for 14 days. Boxes were then transferred to a 25°C/dark for an additional 45 days. Osmotic solutions were not refreshed at this point. Therefore, the osmotic potential of boxes, which were originally at -1.0 MPa increased to ≈ -0.8 MPa. Germination tests were performed at 10°C/light with a buffer control and 10 μM GA in buffer with triplicates.

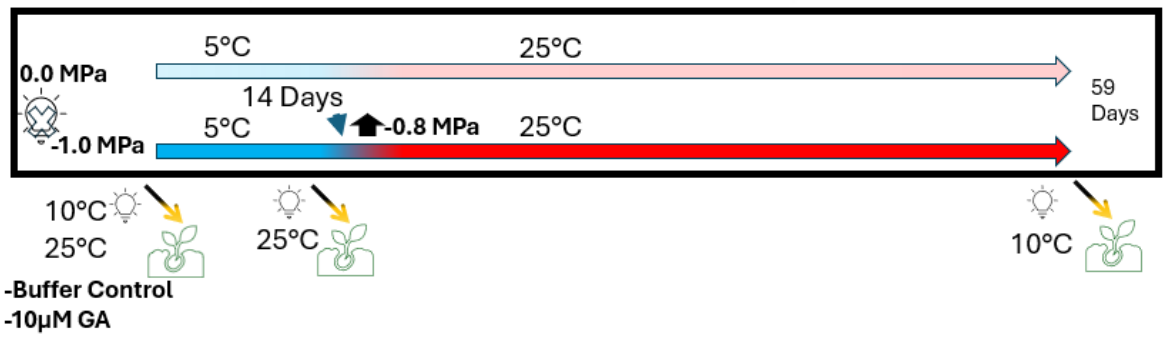

Figure 3.4. Scheme of genetic screening experiments. Samples were incubated in the dark (black box) at 5°C at 0.0 MPa or -1.0MPa (color density) phase for 14 days (blue parts of the arrows). After 14 days, samples were transferred to 25°C/dark (red parts of the arrows) for an additional 45 days. Germination tests (yellow tipped arrows) were at 10°C/light (on day 59) or 25°C/light (on day 14, both on day 0).

The design of the screens resembles a minimized dormancy cycling with only three timepoints (Figure 3.4). The sampling times and the experimental conditions were chosen to maximize the difference between germination potential of parental ecotypes. The first timepoint (day 0) aimed to determine the effect of genotypic variation in PD of RIL seeds produced in a common environment.

The first SD (day 14, after 5°C phase) timepoint favors Bur germination (a summer annual). Following the cold stratification period the winter annual Cvi is induced into SD more rapidly than the summer annual Bur (Figure 4.2). The genotypic variation in germination potential after cold incubation was determined at this timepoint.

The second SD (day 59, after 25°C phase) timepoint favors Cvi germination (a winter annual). The warm incubation initiates a rapid SD induction in the summer annual of Bur, while a gradual SD release occurs in the winter annual Cvi (Figure 4.5). The stacking of incubation temperatures determined the genotypic variation in germination potential after warm incubation at this timepoint.

3.4. Dormancy Cycling of Dormancy Mutants

Two dormancy cycling experiments were performed with mutant lines (Figure 3.5). Seeds from Col-0, *dog1-2*, *mft2* and the double mutant *dog1-2/mft2* generated in this study were used in these experiments.

In the M-Pilot experiment, seeds of F3 generation of mutants were used (Table A.1). In the R-experiment experiment, seeds of F4 generation of mutants were used (Table A.1). In the R-experiment, three different *dog1-2/mft2* lines were present.

Samples of each background were sown in the same box in the M-pilot (The setup was detailed in Subsubsection A.4.1). Boxes were kept at 5°C/dark, -1.0 MPa for 14 days and then were transferred to 25°C/dark and incubated for up to 16 days. The osmotic potential of boxes increased to ≈ -0.8 MPa as osmotic solutions were not refreshed during transfer. Germination tests (on days 2, 6, 10, 14, 17, 21, 24, 30) were at 20°C or 25°C in the light with buffer control. Germination was scored for 14 days.

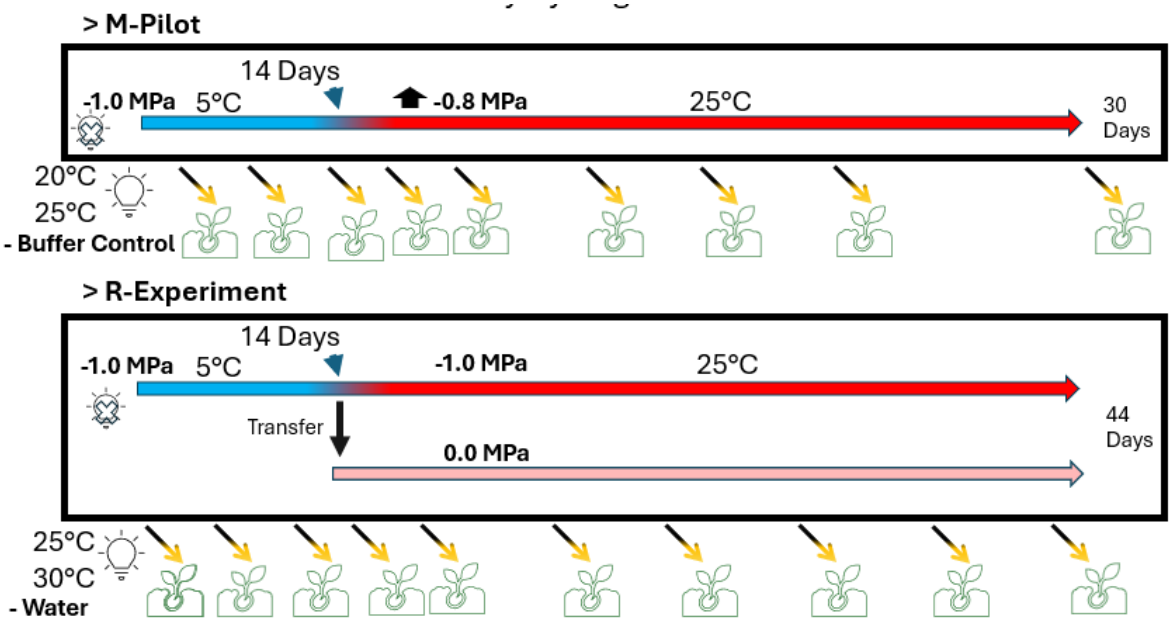

Figure 3.5. Scheme of mutant dormancy cycling experiments. Samples were incubated at 5°C in the dark (black box) for 14 days, -1.0 MPa (blue parts of the arrows). Then, samples were transferred to 25°C/dark and incubated for up to 16 or 30 days in M-pilot and R-experiment, respectively (red parts of the arrows). Germination tests (yellow-tipped arrows) were at 20°C, 25°C or 30°C in the light.

In the R-experiment, triplicate boxes for each time point contained a sample from each mutant line plus the Col-0 wildtype (Box setup was detailed in Subsubsection A.4.1). Boxes were incubated at 5°C/dark, -1.0 MPa for 14 days. Then, samples were transferred to new boxes under green safe light and incubated at 25°C/dark in either 0.0 MPa or -1.0 MPa for up to 30 days. Germination tests (on days 2, 6, 10, 14, 17, 21, 25, 29, 36, 44) were at 25°C and 30°C in the light with ddH_2O. Germination was scored for 10 days.

For future transcriptome analysis of the impact of the mutations on dormancy cycling, samples (4 x 20mg) were subjected to same dormancy cycling procedure in the R-experiment. Samples were recovered in the dark under a green safe light at Day 42 after 28 days at 25°C/dark. During sample collection, dark germinants were removed. Seeds were washed with ddH_2O three times and then flash frozen in liquid nitrogen.

Samples were stored at -80°C.

3.5. Germination Response to Wavelength

F4 generation of mutant seeds and Bur from seed batch #57 were used for the experiment (Table A.1). Fresh or after-ripened (57 days) seeds were assayed for germination with combinations of four temperatures (15°C, 20°C, 25°C, and 30°C) and five light treatments (Figure A.4). Light treatments included no light exposure (NL), constant white light (WL), 15min of 27 μmol/m^{2}s 660 nm Red light (R), 15min of 2-3 μmol/m^{2}s 730nm Far-red light (FR), and 15min Far-red light followed by 15 min of Red light (FR-R) (Subsection A.6). Samples were exposed to light after 4 hours of imbibition on ddH_2O at RT/dark. Total germination was scored after four days.

3.6. Data Analysis

All calculations and figures were made in R [112]. All out-sourced functions can be found in the referenced packages. Function names are signified by the empty parenthesis "()" at the end of their names. If an established protocol in the package manual was used, only the package name and the value of the input parameters are provided.

3.6.1. Quantitative Trait Loci Analysis

The analysis of germination data is detailed in the Subsection A.7. All germination data is visually summarized in the Figure 4.12. The heatmap was generated using the pheatmap package [113]. During the heatmap construction, germination rates (Rate) were calculated using the probit estimation and were scaled between 0 and 1 within an experiment. Total germination (Total Germ.) and dark germination (Dark Germ.) values were not scaled. In the rest of the analysis, the data was not scaled.

Calculated germination indices were imputed for a few missing experiments using imputePCA() in the missMDA package [114]. FactoMineR package was used to perform Principal Component Analysis (PCA) [115]. Coordinate figures (Figures 4.13 and 4.14) were created using fviz_pca_ind() from the factoextra package [116]. The correlation figure (Figure 4.15) was created using corrplot() from the corrplot package [117].

Coordinates of lines on the principal components were used as the input of Quantitative Trait Loci (QTL) analysis. Marker map for RILs consists of 347 markers covering the genetic map at an average of 1.4 cM. QTL analysis was performed using the qtl2 package [118]. Phenotypic data was permuted 1000 times to calculate logarithm of the odds (LOD) thresholds corresponding FDR values of 0.05 and 0.1. The center of a peak corresponds to the marker with the highest LOD score within a peak. Bayesian confidence intervals of $\approx 95\%$ were calculated with a drop size equal to 1.5 in LOD score. The peakdrop parameter was set to 1.8 in LOD score to differentiate between multiple peaks that are close in distance. Biologically interesting genes within the peaks were detected using the annotation in the org.At.tair.db package [119].

4. RESULTS

4.1. Determination of Interactions between Temperature and Water Potential in Ecotypes during Dormancy Cycling

Temperature has the central role in regulation of dormancy cycling [120]. The role of osmotic potential is less investigated compared to temperature. Yet its potential to modulate temperature responses have long been known [121]. Modulation of SD induction depends on the ecotype [25], [69]. In Cvi, decreasing osmotic potential accelerated the SD induction [69]. While in Col-0, SD induction was inhibited at lower osmotic potentials.The aim of the following experiment was to investigate the interaction between osmotic potential and temperature responses in dormancy cycling in Bur and Cvi ecotypes of *A. thaliana* which differ in their annual behavior.

Cumulative germination plots were investigated for the means of over 300 experiments. These indicated distinct general patterns in the temperature and osmotic potential responses of two ecotypes. These general patterns are summarized below.

4.1.1. Dark Germination Behavior

Dark germination in Cvi did not exceed 3% (Figure 4.1). However, dark germination occurs when Bur is incubated in water (0.0 MPa) at both temperature 5°C and 25°C. Dark germination at 5°C, 0.0 MPa increased progressively for Bur during the incubation. However, dark germination at 25°C, 0.0 MPa reached its' maximum by Day 6. Dark germination at its' maximum was not different between two temperatures ($P > 0.31$). Dark germination was reduced to almost zero at -0.4 MPa in both temperature, and it was totally blocked at lower water potentials.

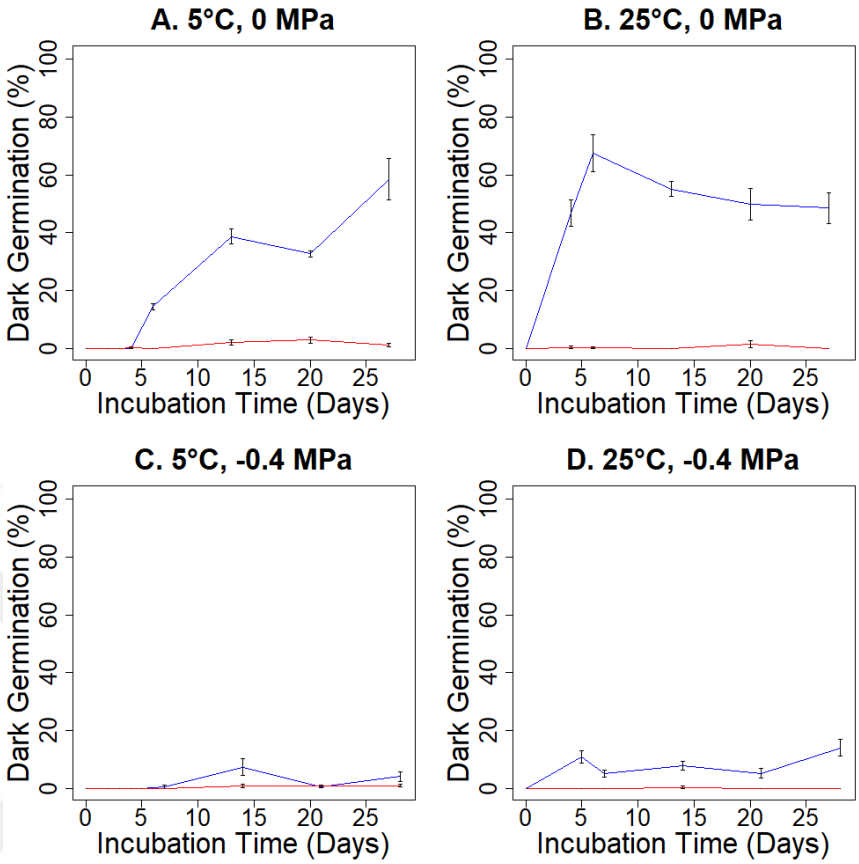

Figure 4.1. Ecotypic differences in dark germination during incubation. Incubation period was at (A) 5°C, 0.0 MPa, (B) 25°C, 0.0 MPa (C) 5°C, -0.4 MPa and (D) 25°C, -0.4 MPa. Dark germination is measured at the time of transfer for germination tests for Bur (blue lines) and Cvi (red lines). (Data represent the mean ± SE, n=9)

4.1.2. Response to Cold Incubation

In some experiments, total germination of samples was equal but significant differences were present in germination rates (i.e. Figure 4.3, see the Subsection A.7). In such cases, area under the curve (AUC) was preferred as a summary. AUC is an advantageous parameter that integrates the total germination and the germination rate.

PD was released at earlier timepoints during cold incubation. This effect is known as cold stratification commonly applied to break dormancy [25]. Bur had higher AUC

than Cvi except during the period where the effects of cold stratification persisted in both ecotypes. During the cold stratification period, both ecotypes had low dormancy (Figures 4.2 - 4.4).

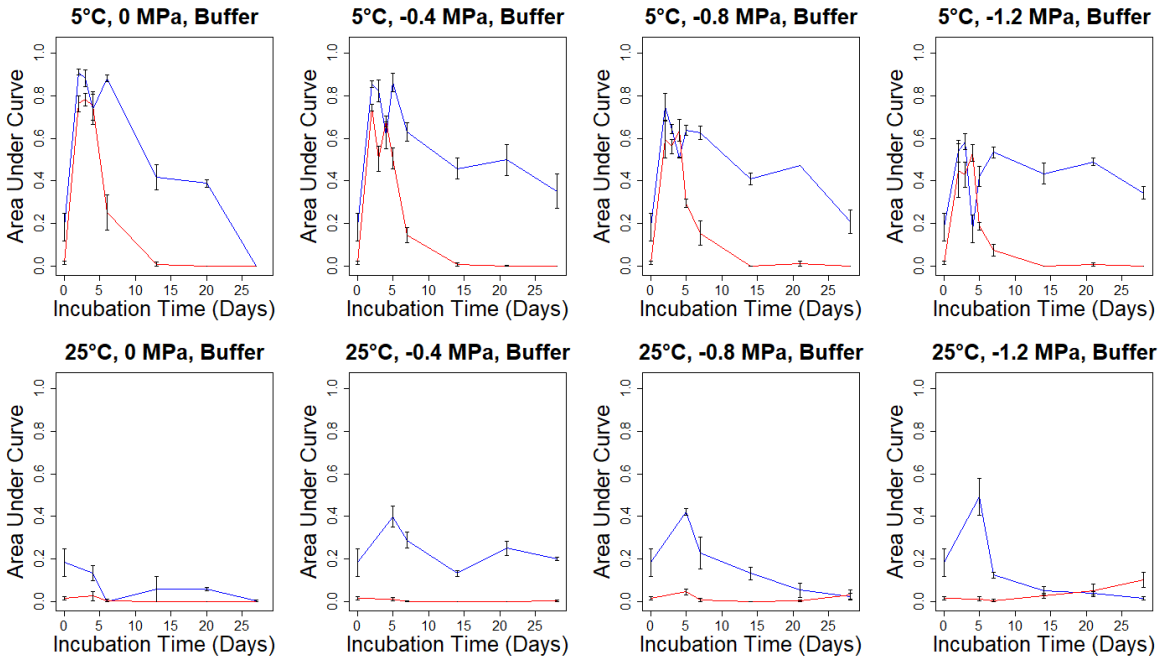

Figure 4.2. Dormancy cycling in response to temperature and osmotic potential. Incubation conditions are indicated above each plot. Germination tests were at 20°C/light on Citrate Phosphate Buffer (pH = 5.0). AUC values for Cvi (red line) and Bur (blue line) were calculated after the removal of dark germinants. (Data represent the mean ± SE, n=3)

PD release was followed by a SD induction (Figure 4.2). SD induction was more rapid in Cvi than in Bur at 5°C/dark. This might be due to prolonged effect of cold stratification in Bur. Alternatively, Bur might be less susceptible to SD induction at lower temperatures. During SD induction, GA and nitrate sensitivity remained high in Bur (Figures 4.3 and 4.4) (Figure 4.2). However, the sensitivity to both agents was lost in Cvi with SD induction (Figures 4.3 and 4.4).

Differences were seen between two experimental batches. The reason could be the lower incubation temperature which was at 4°C/dark rather than 5°C/dark in the latter experimental batch due to lack of incubator space (Figures 4.2 - 4.4). Regardless, results for the second batch were excluded during the statistical analysis of osmotic responses (see below).

The extent of PD release was limited at higher osmotic potentials (Figure 4.2, P <0.001) The modulation of cold stratification effect by osmotic potentials was similar between ecotypes (no interactions between Ecotype and Osmotic Potential).

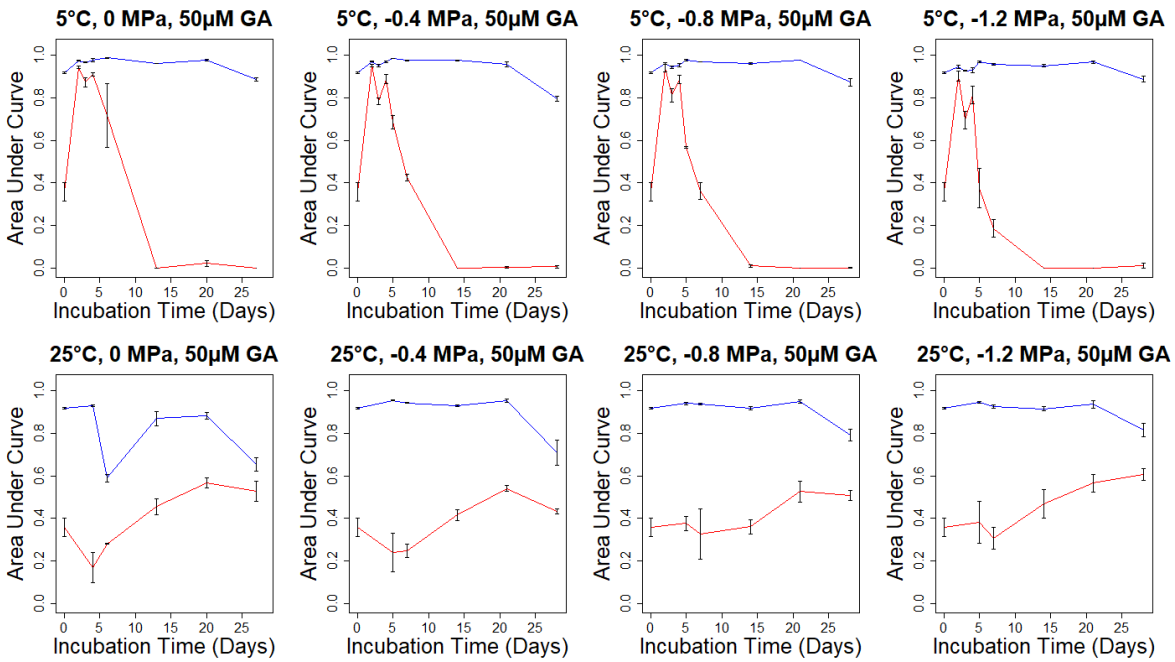

Figure 4.3. GA sensitivity during dormancy cycling in response to temperature and osmotic potential. Incubation conditions are indicated above each plot. Germination tests were at 20°C/light, 50 μM GA in buffer. AUC values for Cvi (red line) and Bur (blue line) were calculated after the removal of dark germinants. (Data represent the mean ± SE, n=3)

SD induction was accelerated at the lowest osmotic potential (-1,2 MPa) in Cvi at 5°C/dark (ANOVA of exponential decay models, P <0.001). Interestingly, SD induc-

tion in Bur was accelarated at higher osmotic potentials at 5°C/dark (validated by a simple linear model $R^2 = 0.77$, $P < 10^{-}6$). Therefore, the modulative effect of osmotic potential was opposite between ecotypes during SD induction at 5°C/dark (Figure 4.2, $P < 0.05$, two-way ANOVA).

4.1.3. Response to Warm Incubation

Bur was released from PD at 25°C/dark initially (Figure 4.2). This effect is known as warm stratification and used to break dormancy [25] [89]. The warm stratification effect was followed by a rapid SD induction. It is interpreted that PD release and SD release is too rapid at at 0.0 MPa. Accordingly, the peak corresponding to dormancy release is missing compared to other water potentials. Therefore, it SD release is slowed down by lower osmotic potentials at 25°C/dark.

Dormancy release at 25°C/dark, if present, was much slower and gradual in Cvi compared to Bur (Figures 4.2 - 4.4). However, a SD induction in Cvi was not observed at 25°C/dark compared to 5°C/dark. GA and nitrate sensitivity was also not lost in Cvi during 25°C/dark incubation. The higher AUC values were recorded for Bur in GA and KNO_3 sensitivity assays. This was due to higher germination rate rather than total germination (Figures 4.3 and 4.4).

During dark incubation at 25°C/dark, the osmotic modulation is less obvious. In Cvi, a slight dormancy release was only observed at -1.2 MPa after 28 days, when germination assays were performed in buffer control solution (Figure 4.2, 95% CI[0.055, 0.151] for -1,2 MPa and 95% CI[0.00,+0,047] for 0,0 MPa.) In Bur, dormancy induction was more rapid without any osmotic stress. This resulted in lower AUC values in buffer control solution on Day 5 (Figure 4.2, 95%CI[0.01,0.26] for 0,0 MPa, outside the confidence intervals of AUC values of the lower osmotic potentials). However as opposed to the incubation at 5°C/dark, there was no evidence that dormancy release or SD induction rates over incubation period are not modulated linearly by osmotic potential.

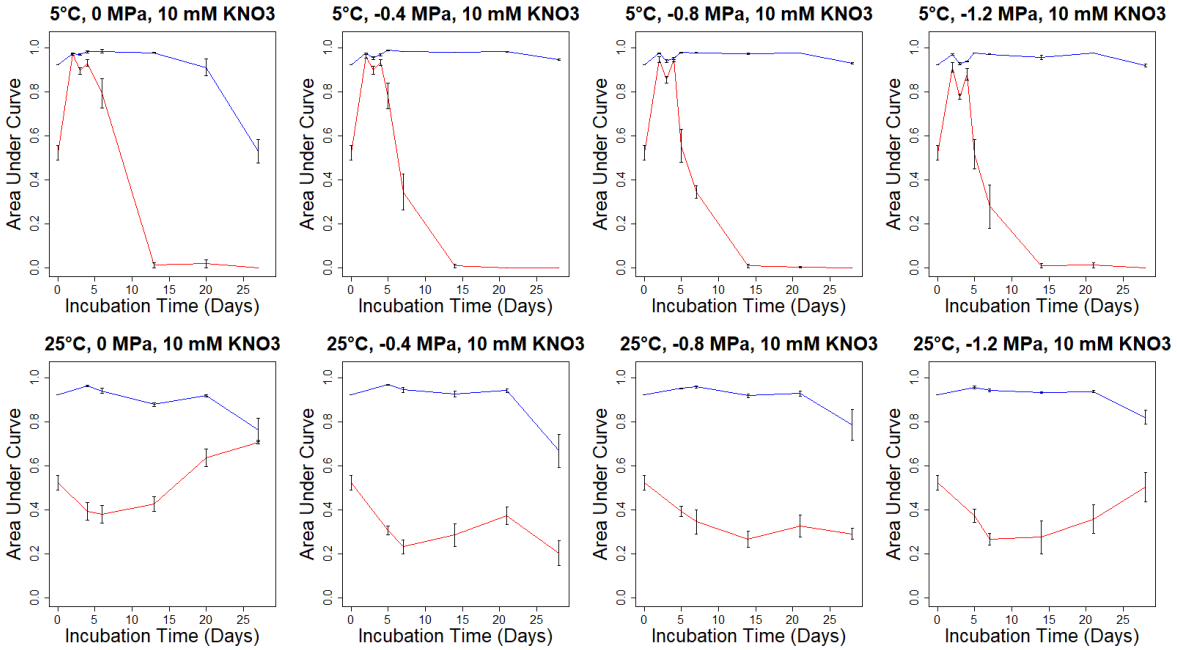

Figure 4.4. Nitrate sensitivity during dormancy cycling in response to temperature and osmotic potential. Incubation conditions are indicated above each plot. Germination tests were at 20°C/light, 10 mM KNO_3 in citrate phosphate buffer. AUC values for Cvi (red line) and Bur (blue line) were calculated after the removal of dark germinants. (Data represent the mean ± SE, n=3)

4.2. Dormancy Cycling with Shifting Temperatures

The range of conditions for SD induction in Bur is limited by the tendency to germinate in the dark (Figure 4.1). This tendency can be constrained by decreasing osmotic potentials of incubation. Bur has comparatively high GA sensitivity which prevents the use of GA concentrations above 10 μM GA to assay its dormancy state (Figure B.1). Lastly, Bur has a strong cold stratification response below 10°C, even in the presence of light which leads to continuous dormancy release. This limits the extent of temperatures to assay germination (Figure 4.1) [90].

Considering the above limitations, an experiment was designed to test dormancy cycling for the impact of transfer between two temperatures was evaluated. This involved incubation at 5°C/dark, -1.0 MPa for 14 days followed by transfer to 25°C/dark.

Osmotic potential increased to ≈ -0.8 MPa as the osmotic solution was not changed at transfer. The germination was tested at 10°C/light to favor Cvi germination which has a high temperature thermodormancy [90].

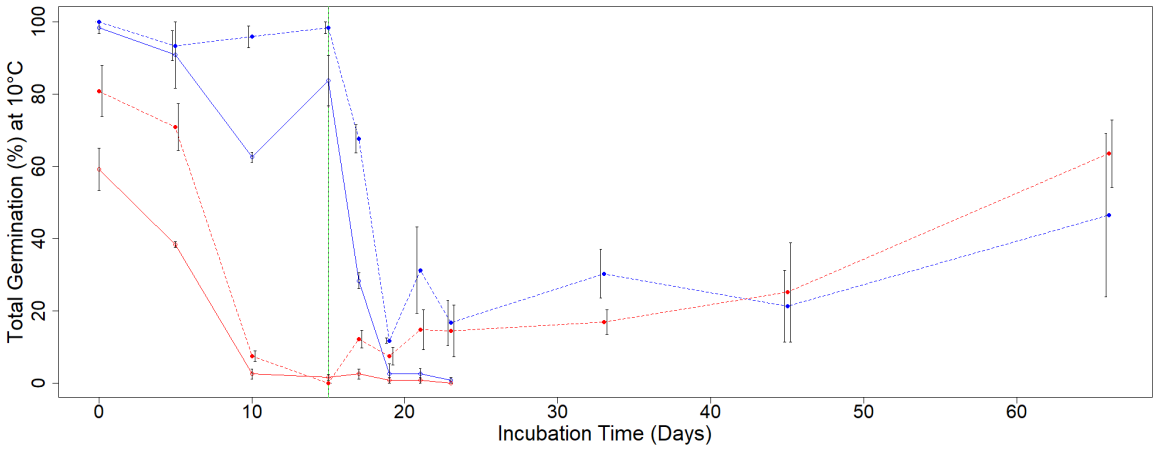

Figure 4.5. Dormancy cycling in shifting temperatures. Germination tests were at 10°C for Bur (blue points / lines) and Cvi (red points / lines) in either buffer control (solid lines, open symbols) or 10 μM GA in buffer (dashed lines, closed symbols). Samples were transferred to 25°C (green-black dashed line) after 14 days of incubation at 5°C. (Data are the mean ± SE, n=3)

In Bur, dormancy was low during the initial 5°C/dark phase due to cold stratification (Figure 4.5). At 5°C/dark, SD induction was initiated in Cvi and the sensitivity to 10 μM GA was lost. Following the transfer to the 25°C/dark phase, Bur was quickly induced into SD. Bur lost sensitivity to 10 μM GA during SD induction. However, the sensitivity to 10 μM GA increased upon prolonged incubation at 25°C/dark in both ecotypes.

The results indicate that Cvi is released from SD in the 25°C/dark phase if the period follows a 5°C/dark phase (Figure 4.5). Within the same treatment Bur is induced into SD rapidly and sensitivity to 10 μM GA is lost during the 25°C/dark phase. However, with prolonged incubation in the 25°C/dark phase GA sensitivity increase in both ecotypes. Nevertheless, compared to their past states at the end of

5°C/dark phase, Bur is induced into SD, while Cvi is gradually released from SD.

4.2.1. Time Dependence of Temperature Responses

The Bur ecotype has a summer annual characteristics, and seedling emergence in the field is known to occur in the spring [20]. Seeds of summer annuals are typically released from dormancy over the winter to germinate in the upcoming spring in response to increasing temperatures [89], [122]. However, under laboratory conditions, seeds are induced back into SD upon extended cold incubation (Figure 4.2) [90].

Upon transfer to a warm incubation phase at 25°C/dark, after a cold incubation phase in the dark, dormancy cycling of Bur was inconsistent between experiments. In some experiments, SD release was observed before the rapid dormancy induction. Apart from the use of different seed batches the main difference was the length of the cold incubation period. An experiment was designed to assay if there is dependency of temperature responses on time during seed dormancy cycling (Figure 3.3).

The ability to release dormancy at 25°C/dark progressively decreased as the time spent at 4°C/dark incubation increased (Figure 4.6). Seeds remained sensitive to 1 mM KNO_3 until 40 days during 4°C/dark incubation. SD induction was initiated by day seven at 4°C/dark, yet transferring seeds to 25°C/dark temporarily relieved SD induction.

However, SD dormancy induction was accelerated at later time points. After day 35, upon transfer to 25°C/dark, the relief was completely lost and instead SD induction was accelerated directly. At later time points direct SD induction was observable at higher concentrations of nitrate. This indicates that time spent at 4°C/dark which increases SD rather than germination levels are the driving factor for the dependency.

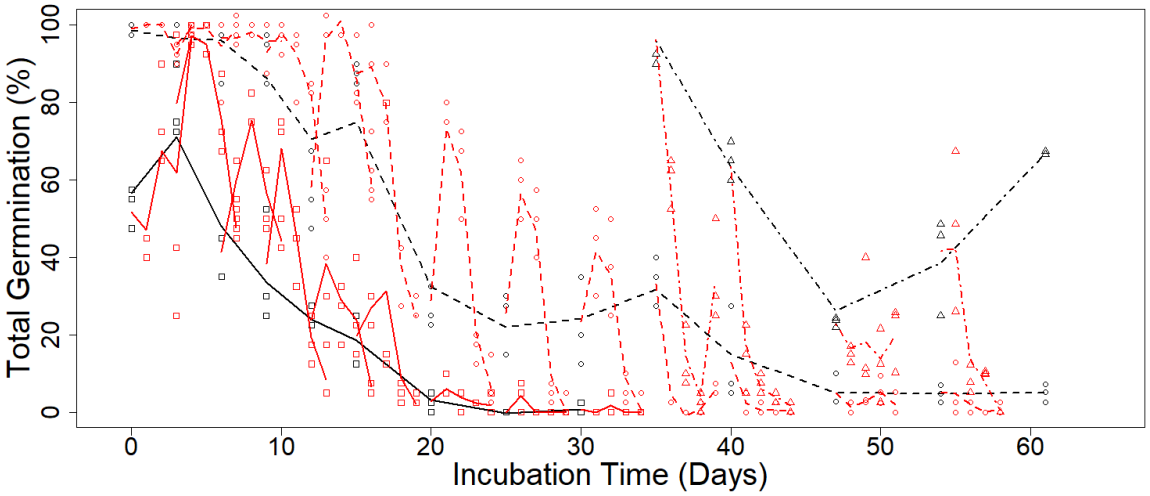

Figure 4.6. Progression of total germination in time dependence assay. Samples were incubated at 4°C/dark for up to 63 days (in black) or were transferred to 25°C/dark at various timepoints for up to 4 days (in red). Germination tests were at 20°C/light with ddH_2O (squares / solid lines), 1 mM (circles / dashed lines), or 5 mM KNO_3 (triangles / dotdashed lines). Lines were fit using natural splines.

4.3. Determination of Dormancy Cycling Screening Conditions for Cvi x Bur RIL Mapping Population

The screening conditions for Cvi x Bur RIL mapping population were selected according to the behavior of parental ecotypes in the previous experiments (Figure 3.4). SD was induced in Cvi after 7-10 days of cold incubation in the dark. SD induction in Cvi was more rapid than Bur at cold temperatures (Figures 4.2 and 4.5).

SD was induced more efficiently in Bur at warm temperatures in the dark compared to cold temperatures (Figure 4.5). However, SD induction rate depended on the time of transfer, and was more rapid after a cold temperature phase (Figure 4.6). Dormancy cycling curves of two ecotypes cross each other during warm incubation in the dark in these conditions (Figure 4.5). The switch in the tendency to germinate under equivalent conditions allows differential genetic screening of dormancy cycling behavior in the RIL mapping population under laboratory conditions.

The overall dormancy cycling behaviors of ecotypes were also corroborated by independent experiments performed by other members of the laboratory.

4.4. Detection of QTLs Regulating Dormancy Cycling in the Cvi x Bur RIL Mapping Population

4.4.1. Germination Behavior during Dormancy Cycling

Progression of cumulative germination in all experimental conditions represents the variation in dormancy cycling behavior of Cvi x Bur RIL mapping population (Figure 4.7). Three different modes of germination were seen when germination was tested at 25°C/light. Some lines, including Bur, germinated rapidly within 3 days of light exposure. Another group of lines germinated in slowly in a linear fashion. The rest of the lines, including Cvi, did not germinate (Figure 4.7).

Dark germination occurred mainly when samples were incubated at 25°C/dark, 0.0 MPa (Figure 4.7, J. and K.). Dark germination was abolished at -1.0 MPa (Figure 4.7, L. and M.). Except for the screens after the incubation at 25°C/dark, -1.0 MPa, mean total germination of Bur samples was higher than Cvi samples (Figure 4.7, L., M.).

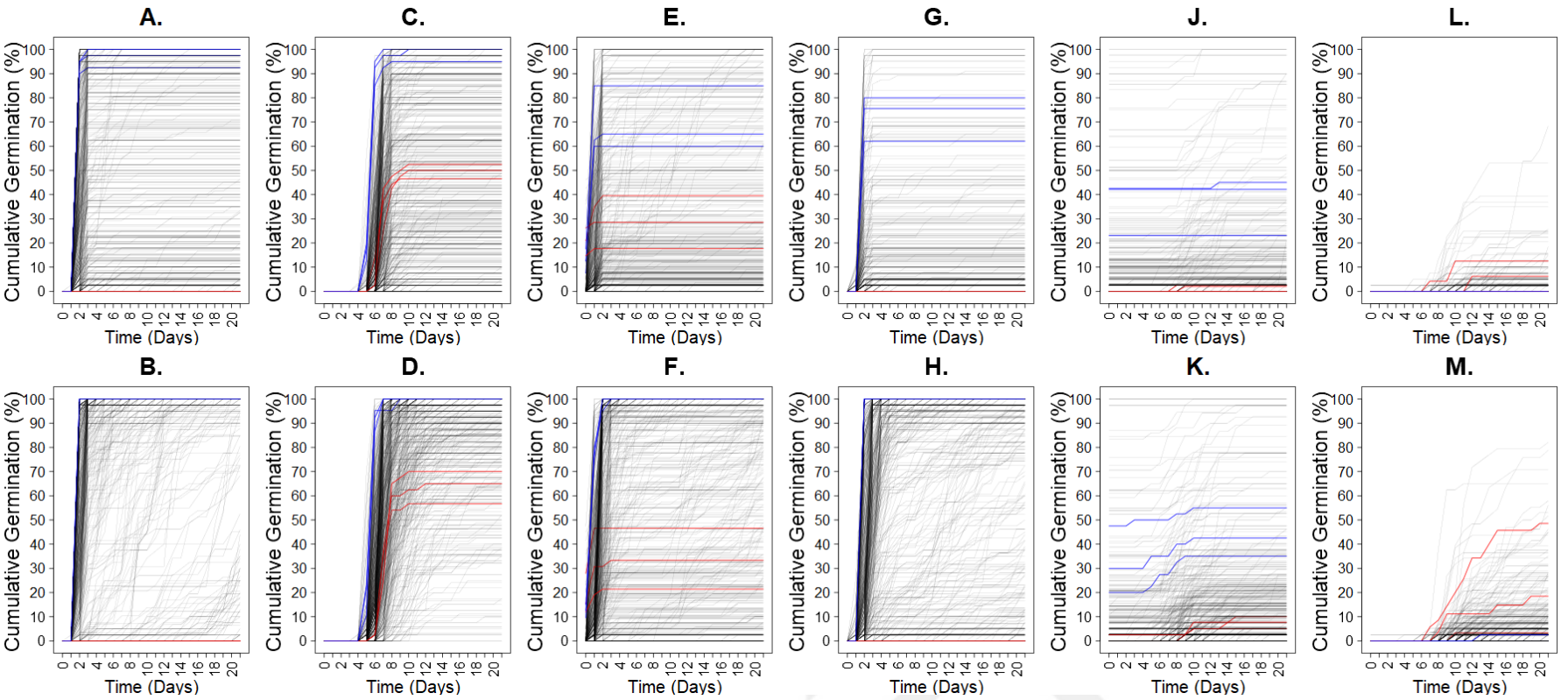

Figure 4.7. Germination behavior of RILs in dormancy cycling. Germination tests were (A, B) at 25°C or (C, D) at 10°C (A, C) in buffer control or (B, D) in 10 μM GA for PD screens. Germination tests after 5°C/dark for 14 days at (E, F) 0.0 MPa or at (G, H) -1.0 MPa were at 25°C (E, G) in buffer control or (F, H) in 10 μM GA. Germination tests after 25°C/dark for 45 days at (J, K) 0.0 MPa or at (L, M) -1.0 MPa were at 10°C (J, L) in buffer control or (K, M) in 10 μM GA.

4.4.2. Variation of Thermal Sensitivity at PD

Total germination of the population in buffer control was evenly scattered across two temperatures (Figure 4.8 A.). This indicates a strong variation in thermal sensitivity in the mapping population. Cvi displayed high temperature thermodormancy, as expected in a winter annual [89]. Bur germinated effectively to 100% at both temperatures, which prevents the assesment of the mode of thermodormancy for Bur grown with the population. However, there are many lines that displayed low temperature thermodormancy in both buffer control and 10 μM GA(Figure 4.8 A. and B.). Therefore, it can be argued that Bur would have displayed low temperature thermodormancy if higher PD levels had been established.

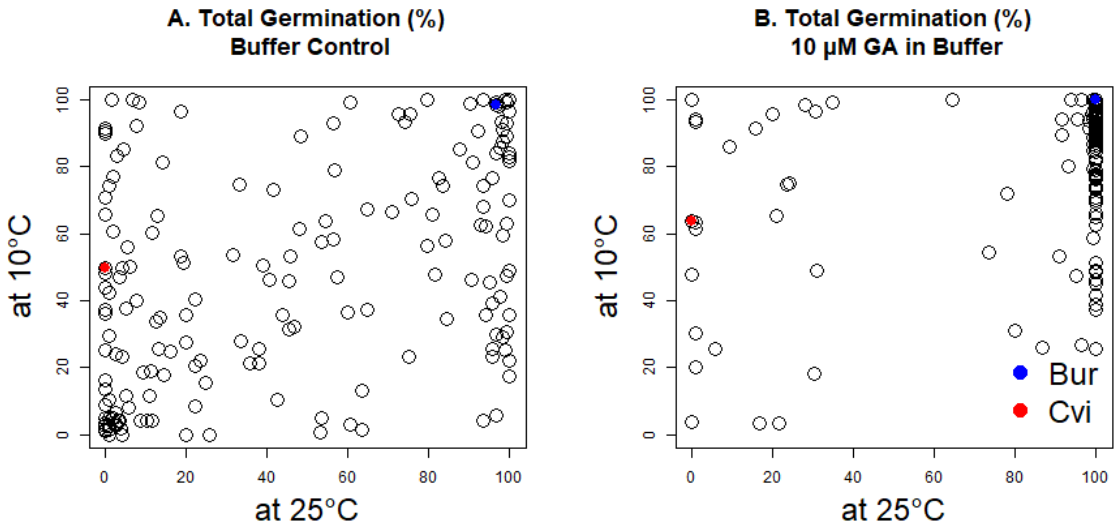

Figure 4.8. Primary dormancy of RILs. Total germination (A) at 10°C (y-axis) and at 25°C (x-axis) in buffer control in PD screens. (B) Total germination at 10°C (y-axis) and at 25°C (x-axis) in 10 μM GA. Parental lines, Cvi (red) and Bur (blue), are included as references. (Data represent the mean, n=3)

4.4.3. GA Sensitivity during Dormancy Cycling

Total germination in GA was higher than buffer control (Figure 4.9). At PD, most lines had high germination in 10 μM GA at 25°C/light (Figure 4.8). The variation in GA sensitivity at PD was mainly seen when assayed at 10°C/light, where some lines including Cvi did not have increased total germination in 10 μM GA. Bur, germinated to full extent in all PD screens (Figure 4.9, A. and B.).

After incubation at 5°C/dark, 0.0 MPa, GA sensitivity of the population decreased (Figure 4.9, C.). Lines including Bur preserved their GA sensitivity, similar to the behavior of Bur in cold phases of dormancy cycling experiments (Figures 4.3 and 4.9, C.). Other lines, including Cvi lost their sensitivity to 10 μM GA during the incubation at 5°C/dark.

However, after the incubation at 5°C/dark at -1.0 MPa, GA sensitivity of the population was higher compared to 0.0 MPa incubation (Figures 4.9, D. and 4.10, B.). This indicates that in a part of the population GA sensitivity increased with lower osmotic potential. Some lines including Cvi did not respond to 10 μM GA at lower osmotic potential either.

After the transfer and incubation at 25°C/dark, 0.0 MPa, GA sensitivity of the population decreased prominently (Figure 4.9, E. and F.). In Bur, GA sensitivity decreased after the incubation at 25°C/dark, similar to the behavior of Bur in warm phases of dormancy cycling experiments employing a temperature shift (Figure 4.5). There was residual GA sensitivity in Bur after 25°C/dark, 0.0 MPa (Figure 4.9 E. and F.). In Cvi, GA sensitivity at the end of the incubation at 25°C/dark decreased at 0.0 MPa and increased at -1.0 MPa compared to previous timepoint at the end of the incubation at 5°C/dark.

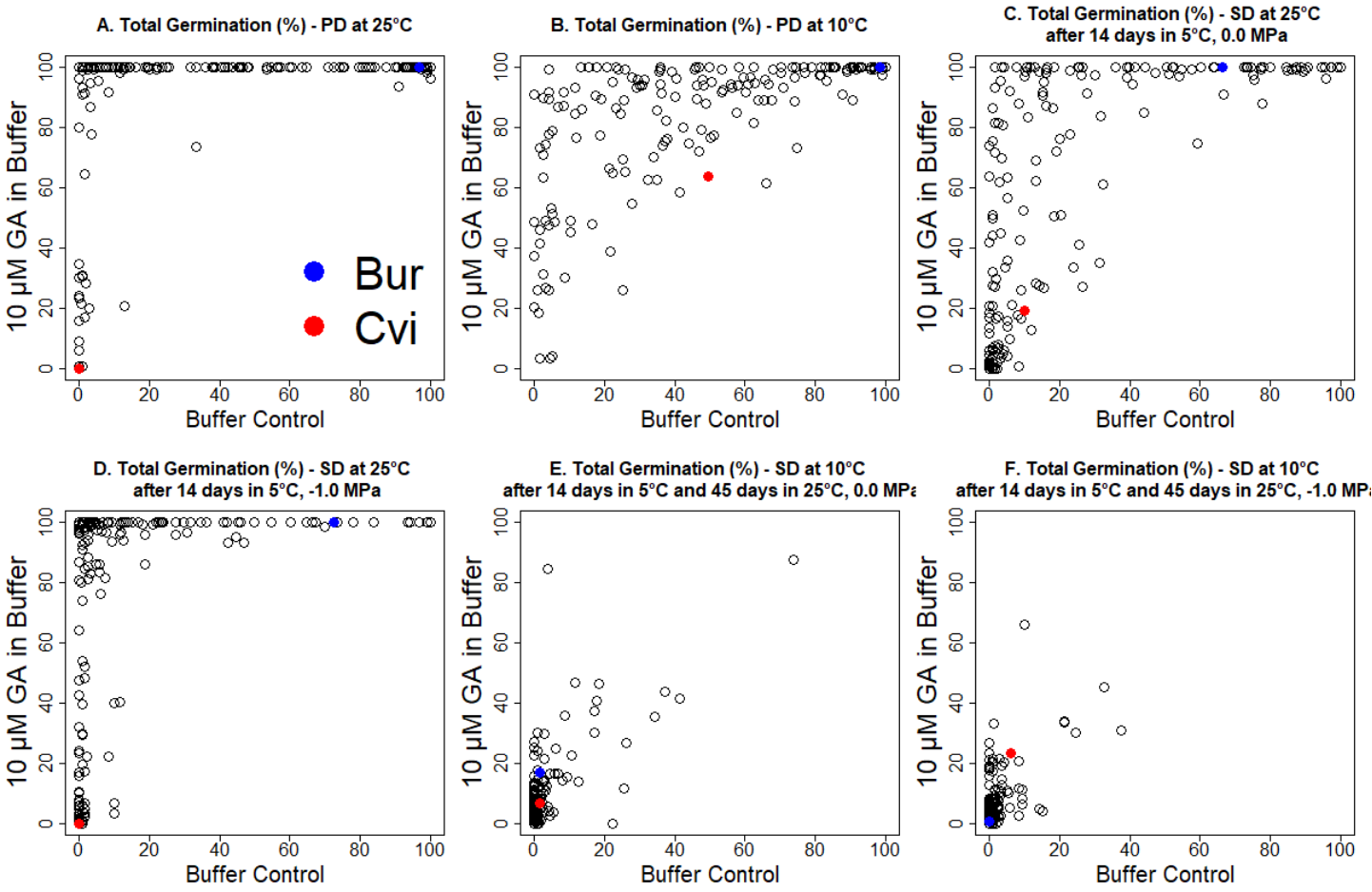

Figure 4.9. GA sensitivity of RILs in dormancy cycling. Total germination in buffer control (x-axis) and GA (y-axis). Germination tests were (A, C, D) at 25°C, or (B, E, F) at 10°C in the light. Dormancy was sampled (A, B) at PD, and (C, D) after 5°C (E, F) or 25°C dark phases with incubations at (C, E) 0.0 MPa or (D, F) at -1.0 MPa. (Data represent the mean, n=3)

4.4.4. Osmotic Modulation of Dormancy Cycling

When germinated in the buffer control solution, germination decreased in the population with increasing incubation time and decreasing osmotic potential (Figure 4.10, A. and C.). When assayed in GA, however, the response to osmotic potential varied between RILs (Figure 4.10, B. and D.).

This variation in the change of GA sensitivity with osmotic potential treatment indicates a complex relationship (Figure 4.10 B. and D.). It is intresting that GA sensitivity can increase with osmotic treatment while overall germination potential of the population in buffer control decreases (Figure 4.10 A.). For example, Cvi had higher GA sensitivity after incubation at 25°C/dark in -1.0 MPa compared to 0.0 MPa (Figure 4.10 D.). Germination was similar after 14 days of incubation at 5°C/dark in both osmotic treatments in Bur (Figure 4.10 A.). This indicates that osmotic potential did not affect the rate SD induction as opposed to behavior of Bur in dormancy cycling (Figure 4.2).

4.4.5. Comparison of Dormancy Cycling Timepoints

When two SD incubation phases are compared, variation in dormancy cycling behavior of the population is highlighted (Figure 4.11). Germination in GA centered at minimum (0%) and maximum (100%) after the incubation at 5°C/dark (Figure 4.11, B. and D.). This indicates that GA sensitivity was high in one part of the population while it was limited in the other part after the incubations at 5°C/dark.

RILs that germinated after the incubation at 25°C/dark were evenly spread among germinating and non-germinating parts of the population after the incubation at 5°C/dark (Figure 4.11). This reveals that the germination potential is governed by distinct loci for two timepoints. However, the effect of these dormancy regulating loci that possibly control SD release during 25°C/dark phase is percentagewise more limited.

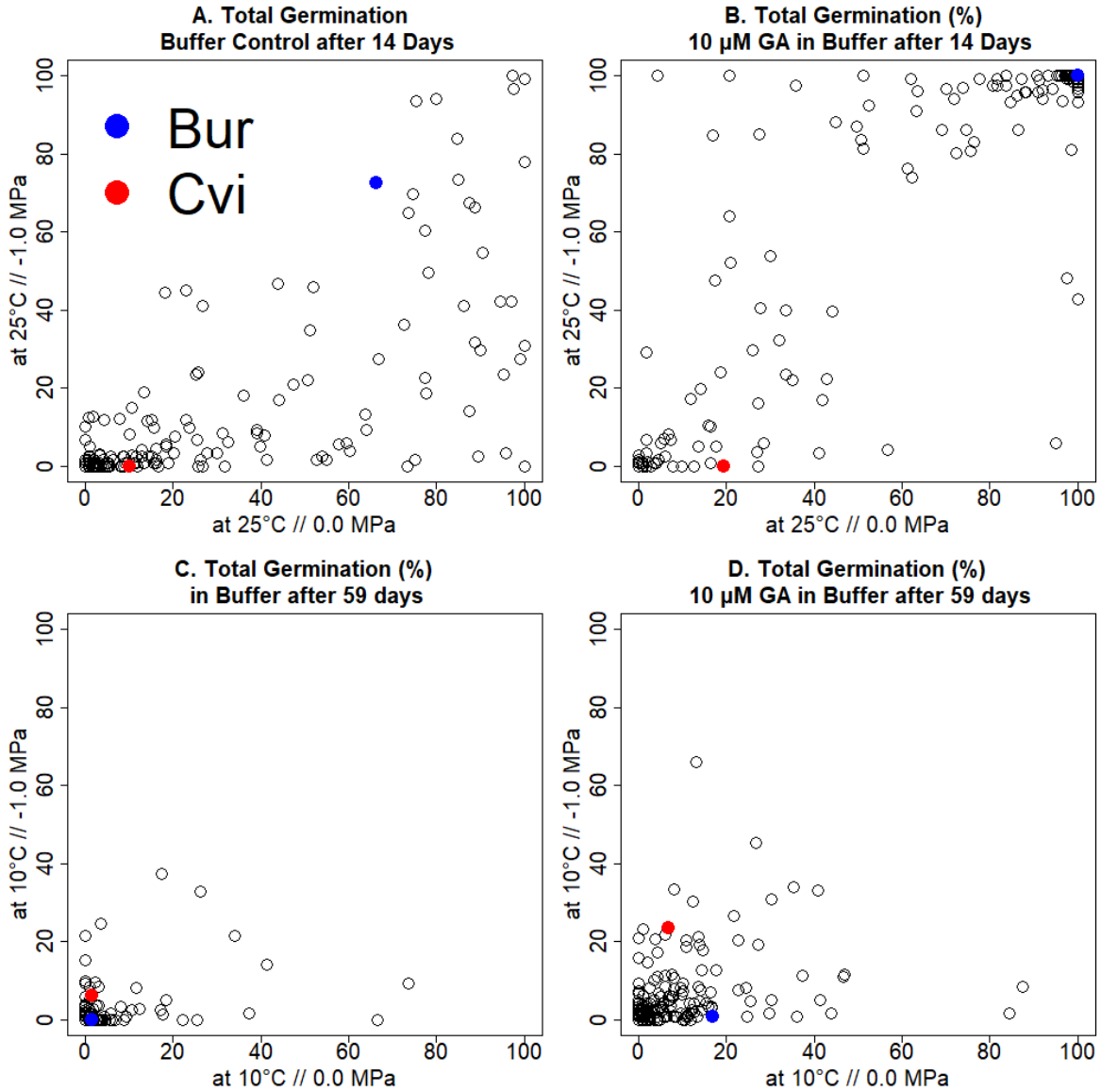

Figure 4.10. Osmotic modulation of dormancy cycling of RILs. Total germinations after incubations in 0.0 MPa (x-axis) and -1.0 MPa (y-axis). Germination tests were (A, B) at 25°C/light after 5°C/dark phase or (C, D) at 10°C/light after 25°C/dark phase (A, C) in buffer control or (B, D) in 10 μM GA. (Data represent the mean, n=3)

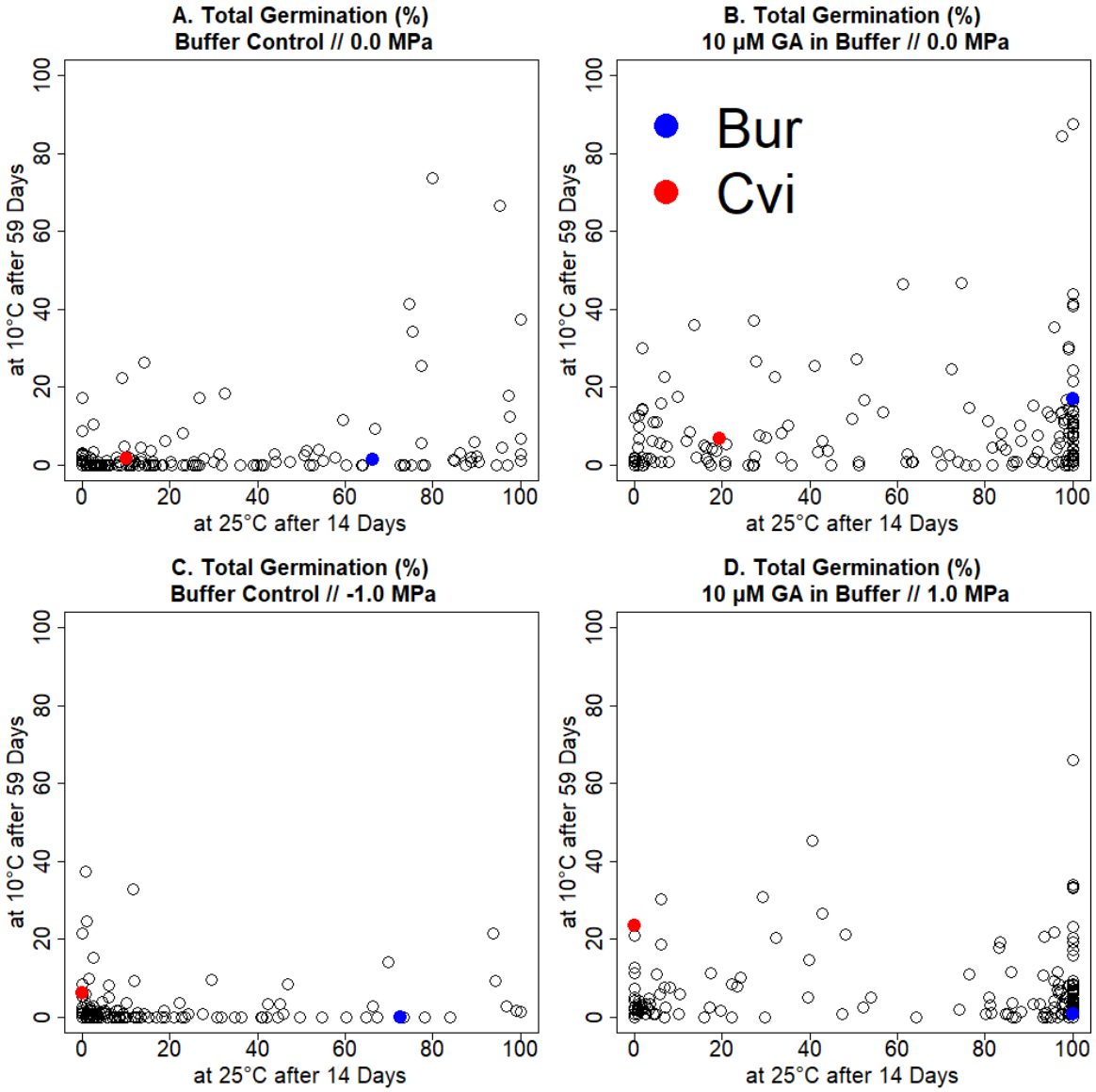

Figure 4.11. Comparison between dormancy cycling timepoints. Germination tests were at 25 °C/light after 5°C/dark (x-axis) or at 10°C/light after 25°C/dark (y-axis). Incubations were at (A, B) 0.0 MPa, or (C, D) -1.0 MPa. Germination was tested in (A, B) buffer control or (C, D) 10 μM GA. (Data represent the mean, n=3)

4.4.6. Summary of Dormancy Cycling Behavior in the Mapping Population

All calculated germination indices are summarized in a heatmap in the Figure 4.12. The heatmap also included germination rate and dark germination (see the Subsection A.7). According to the heatmap, dormancy cycling behavior of the mapping population can be clustered mainly into two branches (Figure 4.12 - Hierarchical clustering tree on the left). These main branches are further divided into smaller sub-branches.

Two main branches are separated according to response of the population to incubation at 5°C/dark. Lines on the top main branch did not germinate in these conditions (Figure 4.12 Exps. E-H), while lines on the bottom branch germinated either in all or some of these conditions. Cvi was in the top main branch, while Bur was in the bottom main branch. This indicates that the populational response followed the two parental ecotypes.

The top branch includes about 40% of the population and is divided into two further sub-branches. Lines that behaved similar to Cvi, only germinated to a high extent at 10°C/light in PD assays (Figure 4.12 Exps. C-D), The lines on the other sub-branch were also able to germinate at 25°C/light in PD assays, especially with 10 μM GA (Figure 4.12 Exps. A-B).

The bottom main branch includes about 60% of the population, and is divided into two sub-branches. Lines that behaved similar to Bur germinated in almost all assays except after the 25°C/dark phase (Figure 4.12 Exps. J-M). Some of these lines were even less responsive to SD induction than Bur in the 25°C/dark phase. These germinated in the dark or above 50% in the light when incubated at 0.0 MPa during this period (Figure 4.12 Exps. J-K.).

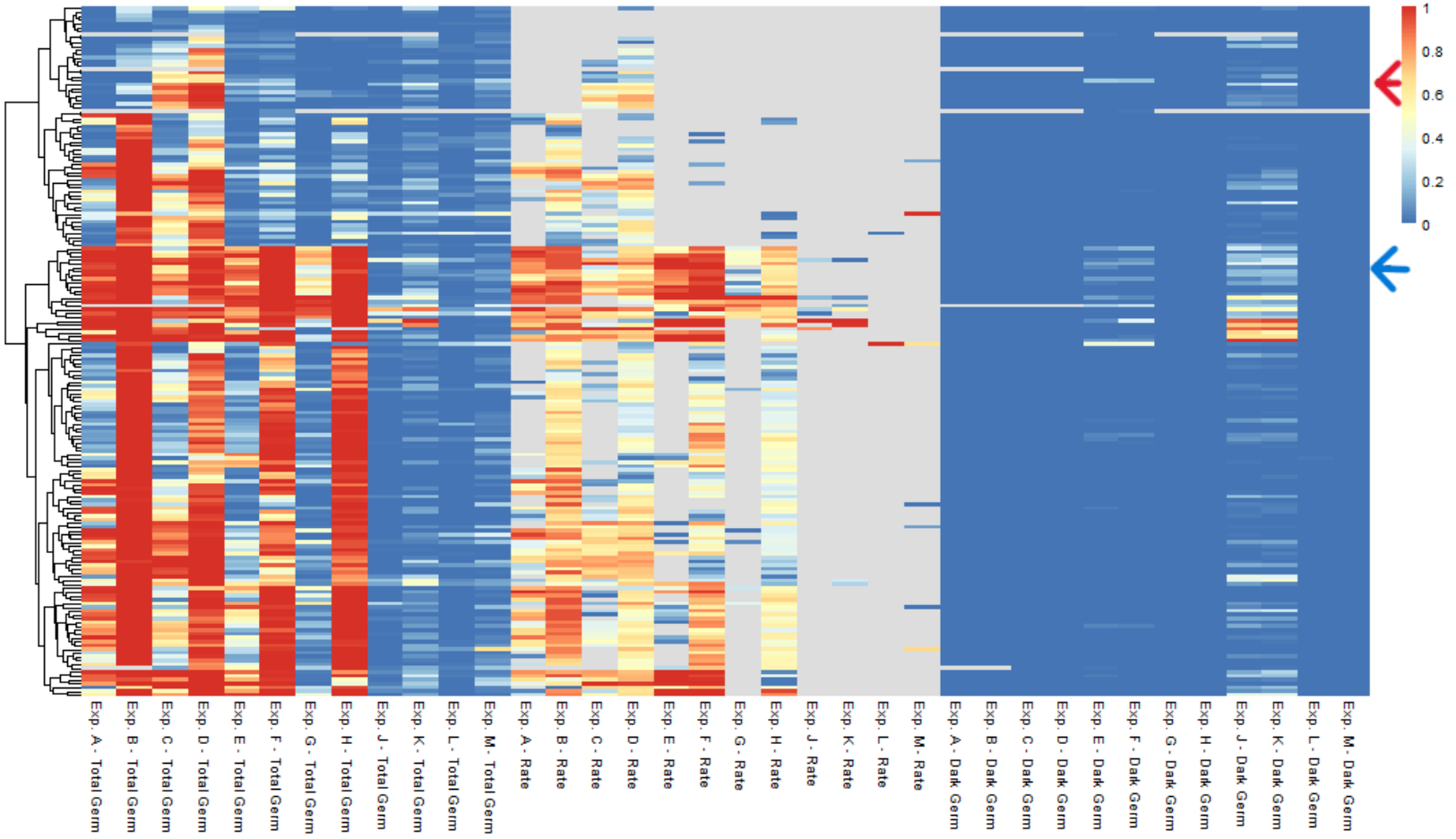

Figure 4.12. Heatmap of the screening results. Experimental conditions (columns) are explained in the Figure 4.7. Missing values (beige color), and Cvi (red arrow) and Bur (blue arrow) rows are included. (Data represent the mean, n=3)

Lines in the other sub-branch, below that including Bur, mainly germinated to high extent only when treated with 10 μM GA (Figure 4.12 Exps. B-D-F-H). They vary in their germination behavior according to germination rate and dark germination behavior. Therefore, they did not cluster very well.

Lines that germinated in the dark during the incubations at 25°C or in the light at 10°C did not cluster efficiently (Figure 4.12 Dark Germ Exps. J-K. and Exps. J-M.). Hierarchical clustering is inherently two dimensional. Therefore, the main axis that dominates understandably coincides with the greatest ecotypic differences (the germination after 5°C/dark phase) rather than the other dormancy cycling behaviors (with smaller effects but of importance).

The heatmap indicates that at the PD stage, lines that exhibited low temperature thermodormancy are more abundant in the population than the lines that exhibited high temperature thermodormancy (Figure 4.12 Exps. A-D). Additionally, the lines that were induced into high levels of SD during 5°C/dark incubation are also less abundant than the lines that were not (Figure 4.12 Exps. E-H). Similar to the scatterplots (Figure 4.11), the heatmap concludes that lines that germinated after the 25°C phase (Figure 4.12 Exps. J-M) are equally distributed within different parts (branches in this case) of the population.

4.4.7. Principal Component Analysis

A principal component analysis (PCA) identified separate aspects of germination behavior during dormancy cycling of the mapping population (Figure 4.12). Every principal component is orthogonal to each other as opposed to the input values. As a result, this decreases the amount of axis that describes the population behavior. PCA as a multidimensional analysis is better at determining smaller subpopulations but harder to visualize compared to hierarchical clustering (worse at determining smaller subpopulations but easier to visualize).

The first two PCs explained 48.4% of the variation in the data (34% and 14.4%, respectively - Figure 4.13). After the tenth PC, total variance explained exceeded 90%. Therefore, only first ten PCs were included in later analyses.

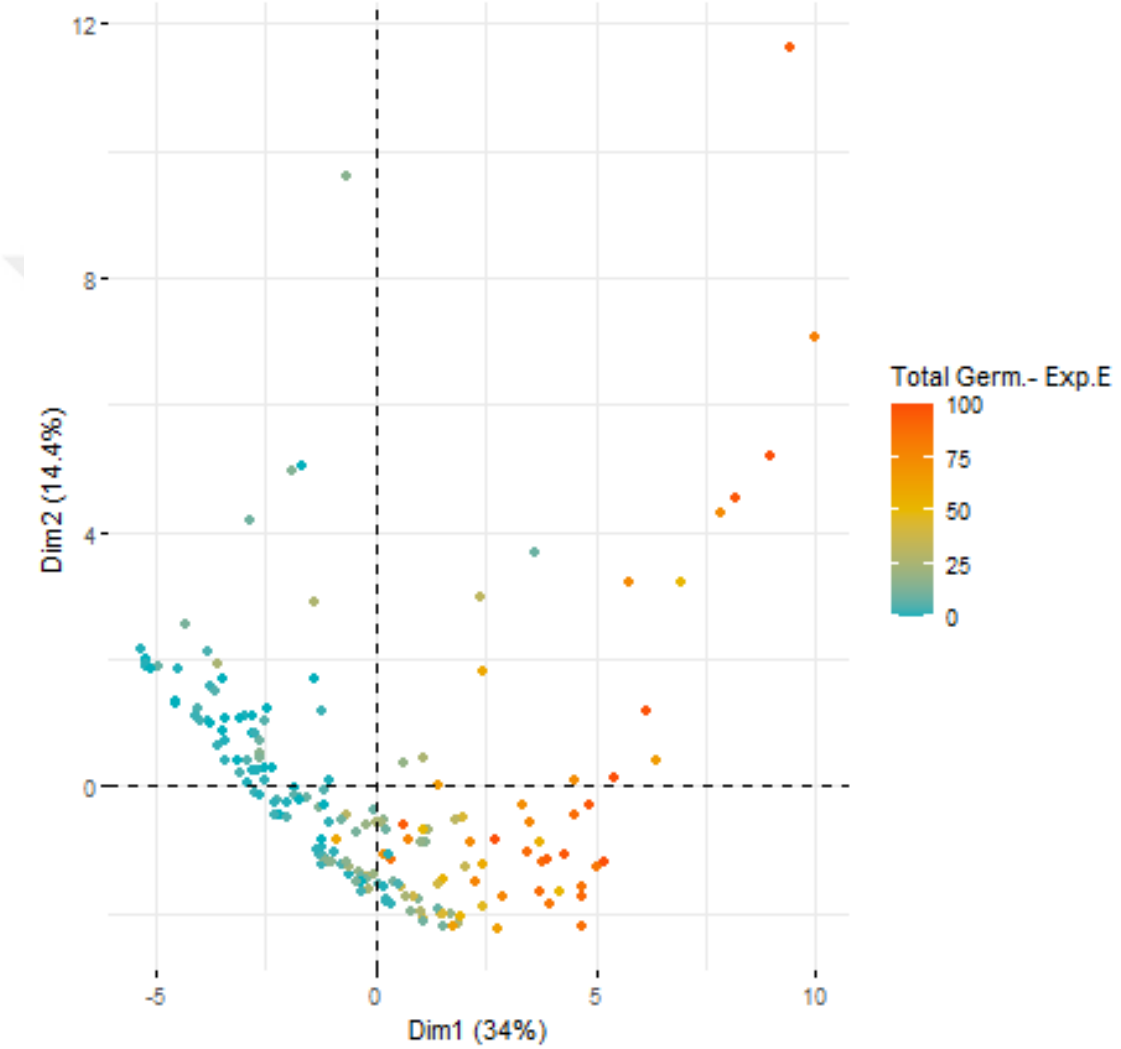

Figure 4.13. Scatter in first two PCs colored with total germination in the experiment E in Figure 4.7. Percent variance explained by the PC is given in parentheses within the axes titles. The color scale is on the right (n = 3).

The identified aspects can be determined by correlating principal components (PC) to the input phenotypic values. The first PC correlated positively with total germination and germination rate in all experiments except in Exps. L and M. (Figures 4.13 and 4.15). The second PC positively correlated with total germination in

Exps. J- K-M after 25°C/dark phase, while negatively correlating with almost all other experiments in previous timepoints (Figures 4.14 and 4.15).

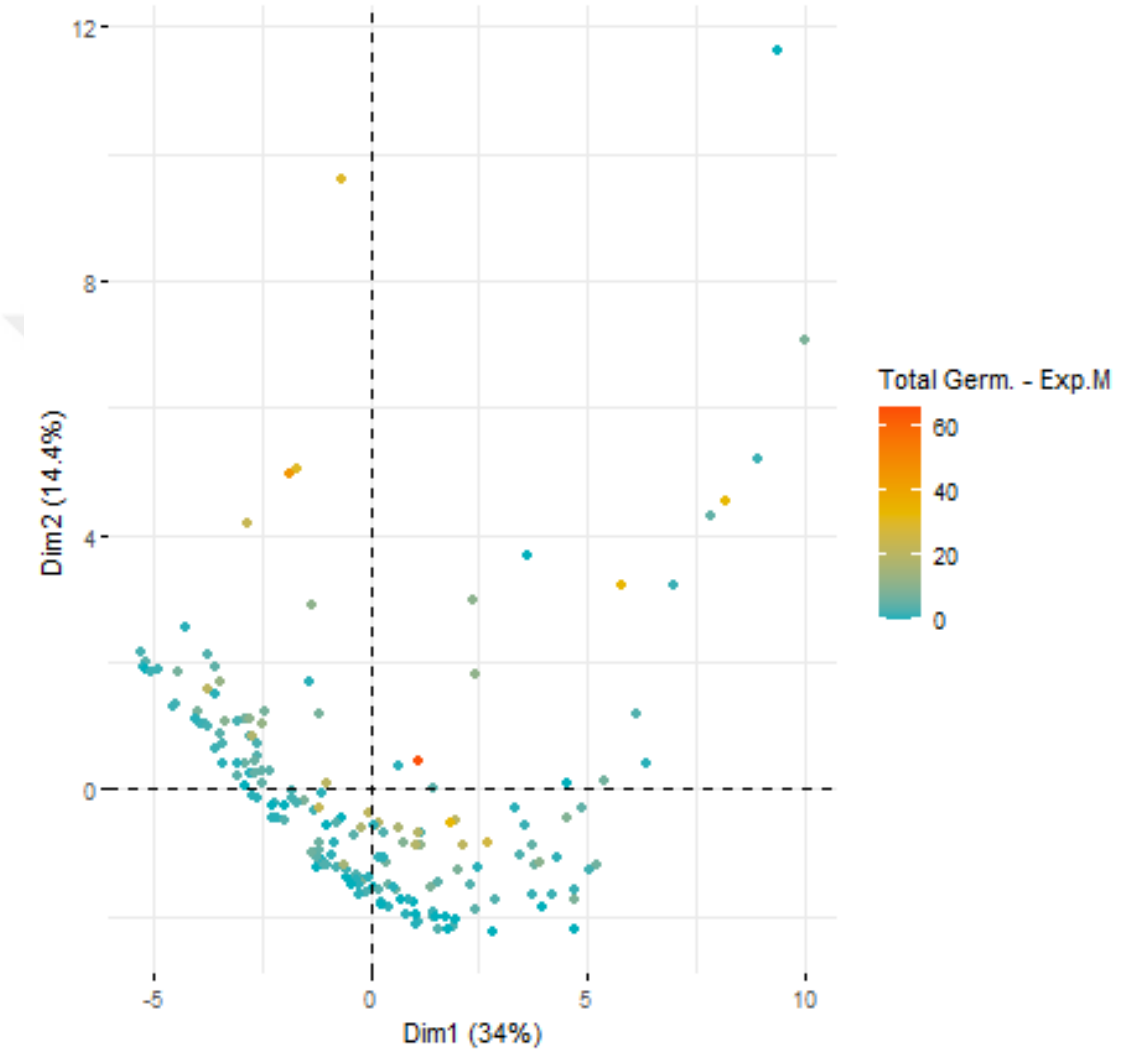

Figure 4.14. Scatter in first two PCs colored with total germination in the experiment M in Figure 4.7. Percent variance explained by the PC is given in parentheses within the axes titles. The color scale is on the right (n = 3).

The rest of the PCs correlated with other experimental parameters in dormancy cycling. PC-3 positively correlated with GA treatment at 25°C (Figure 4.15, Exps. B-F-H). PC-4 positively correlated with germination at PD at 10°C Exps. C-D, respectively (Figure 4.15). PC-7 positively correlated to Exps. A and B in total germination or germination rate (Figure 4.15)

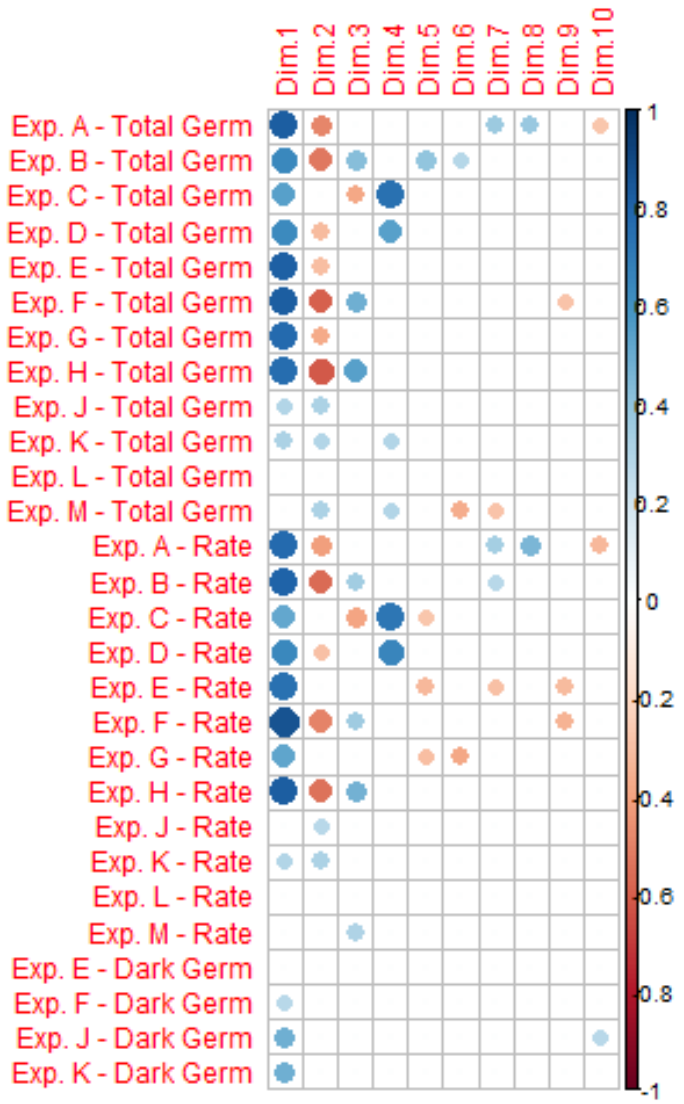

Figure 4.15. Correlations between germination indices and PC coordinates. Experimental conditions are detailed in Figure 4.7. Correlations were adjusted for multiple testing by the Holm's method. Correlations are colored and size adjusted according to absolute value. The color scale is on the right (Only significant correlations below <0.05 are shown, Spearman's method).

4.4.8. QTL Analysis

PC coordinates were used in the QTL analysis. It was reasoned that identifiying PCs would highlight overall dormancy cycling behavior in the population in a reduced number of dimensions. Therefore, the use of PCs avoids testing for highly correlating screens (i.e, between total germination in buffer control and 10 μM GA following the same dormancy treatments). If the results of two screens correlate, they are likely to detect the same QTLs.

Ten QTL peaks were identified at the LOD threshold corresponding to P <0.1 (Figure 4.16, green line and Table 4.1). Only one QTL dropped below the significance level if the threshold was increased to a LOD score corresponding to P <0.05 (Figure 4.16, red line).

Table 4.1. The results of QTL analysis (p <0.1). Positions are in genetic distance (cM).

Peak	Principal Component	Chromosome	Peak Center	LOD-score	CI. low	CI. high	Effect Allele	No. Genes in the Peak
1	PC-1	3	75.71	5.1	63.99	96.81	Bur	2000
2	PC-1	5	89.72	14.21	85.28	98.57	Bur	650
3	PC-2	2	62.57	2.92	58.61	71.7	Cvi	1038
4	PC-2	5	103.73	7.75	85.28	113.17	Cvi	1871
5	PC-2	5	115.8	8.21	113.17	118.71	Cvi	470
6	PC-3	3	75.71	2.72	31.52	89.17	Bur	1837
7	PC-4	5	91.03	2.4	71.35	103.73	Cvi	1667
8	PC-7	1	14.93	5.36	13.12	29.42	Bur	1022
9	PC-7	3	0	3.57	0	12.74	Bur	1336
10	PC-7	5	25.96	3.06	22.08	33.93	Bur	537

For PC-1, two peaks were identified both (Table 4.1, Peaks 1 and 2). Three peaks were identified for PC-2 (Table 4.1, Peaks 3, 4 and 5). Peaks 4 and 5 are merged together when the "peakdrop" parameter in was removed during the peak detection. In that case, the lower end of confidence interval for the merged peak increased to 98.57. The merged peak centered around 115.8, the same as peak 5. A peak was identified for PC-3 and PC-4, each (Table 4.1, Peaks 6 and 7, respectively). Three peaks were identified for PC-7 (Table 4.1, Peaks 8, 9 and 10).

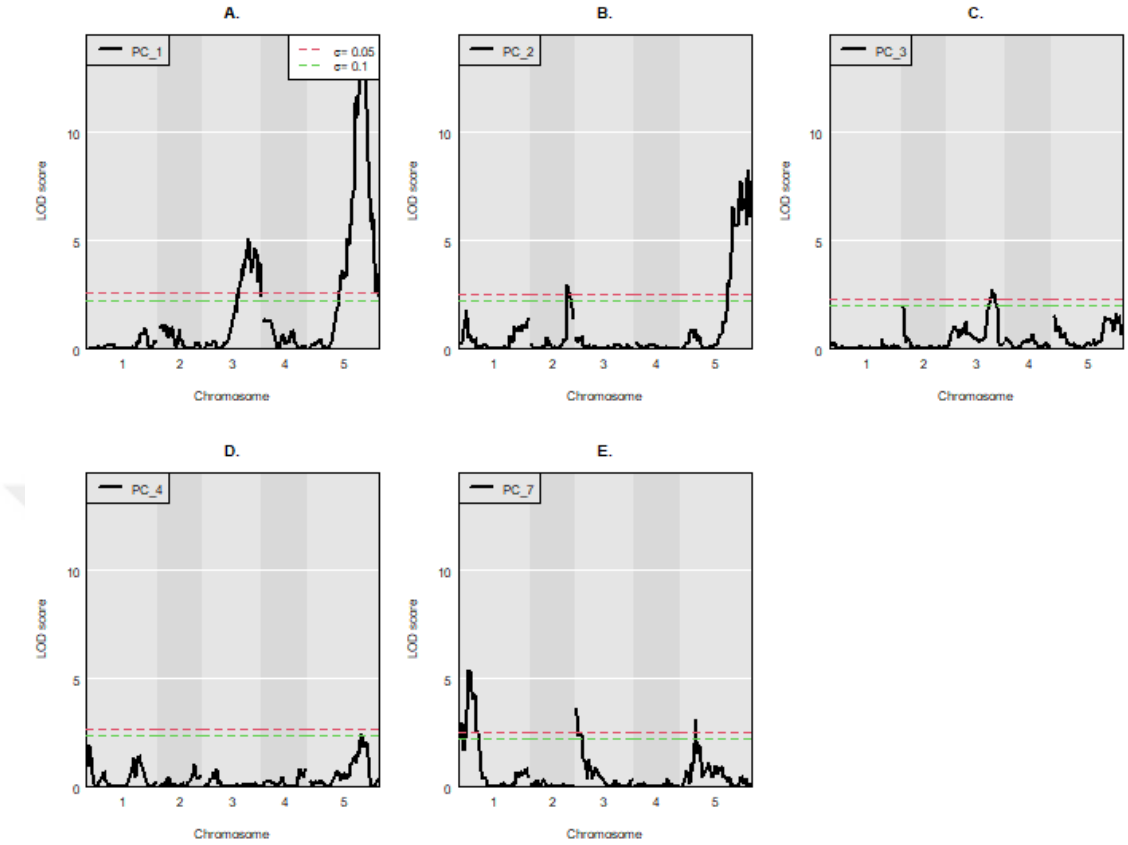

Figure 4.16. QTL analysis results. Progression of LOD scores (black lines) along the chromosomes. Only PC for which QTLs were detected are shown: (A) PC1, (B) PC2, (C) PC3, (D) PC4 and (E) PC7. Two LOD score thresholds are equivalent to p <0.1 (green dashed line) and p <0.05 (red dashed line).

Positive effects on coordinates (increased values of PCs) were driven by Bur alleles for QTLs on PC-1, PC-3 and PC-7. While positive effects were driven by Cvi alleles on PC-2 and PC-4 (Table 4.1). QTLs identified on chromosome 5 for PC-1 (Peak 2) and PC-2 (Peak 4 and 5) are close to each other in genetic distance. However, the effect alleles that drive the effects are different (Bur in the case of Peak 2 and Cvi in the case of Peak 4 and 5, Figure 4.17). The effect of Peak 5 on the total germination are lower compared to Peak 2 (Figure 4.11).

The QTL identified for PC-4 on chromosome 5 (Peak 7) is also centered in the same region with Peak 2. The center of peaks are separated by less than 2 cM. The

confidence intervals for Peaks 2, 4 and 7 overlapped each other and included *DOG1*. However, confidence interval of Peak 5 did not overlap with these peaks and did not contain *DOG1*. When the "peakdrop" parameter was removed *DOG1* was not included in the confidence interval of the merged QTL. Therefore, the genetic factor that drives the effect of Peak 5 is distinct from the others identified in the region. The effect of Peaks 2, 4 and 7 may be driven by *DOG1*. However, the *DOG1* gene is situated right next to the center of Peak 2. Therefore, it is possible that the effect of Peak 4 is independent of *DOG1*.

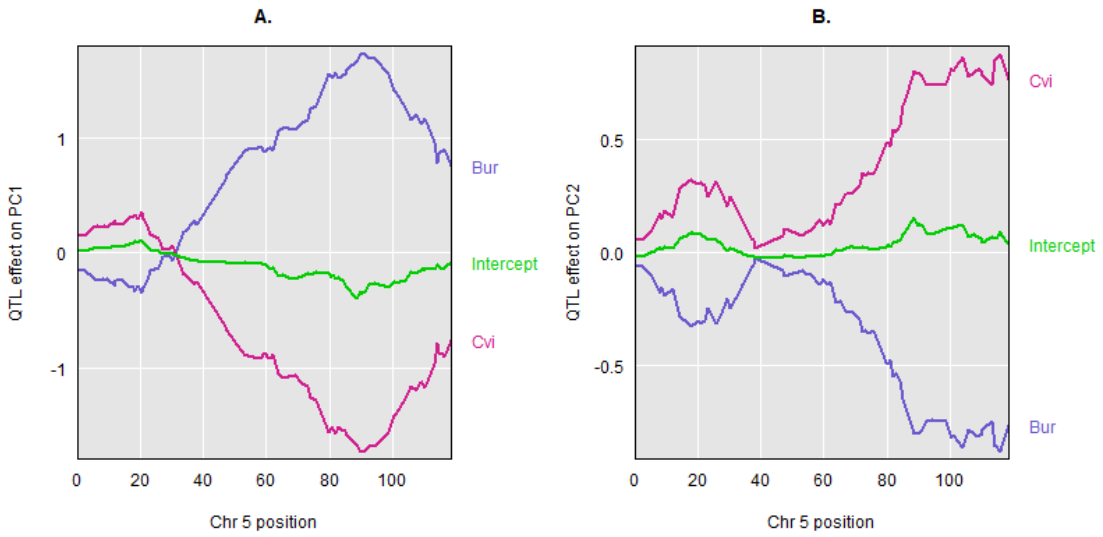

Figure 4.17. Comparison of the QTL effects for Peaks 2 and 5. The QTL effects of Bur allele (purple lines), and Cvi allele (pink lines) on PC-2 of (A) Peak 2 and (B) Peak 5 are displayed along the chromosome 5. Genetic distance is in cM from the start of the chromosome.

4.5. Dormancy Cycling of Mutants - R Experiment

DOG1 has been the most central gene in dormancy research. *DOG1* has been attributed with the central position in dormancy regulation [20], [67], [82], [100], [111]. Yet, there have been other genes identified together with *DOG1* [22], [65, 66]. The mutants of *DOG1* and *MFT* were shown to be compromised in establishing PD levels [68], [71], [111]. Yet, SD induction can still take place in the absence of *DOG1* and

MFT [69].

The main aim of the experiment was to investigate if dormancy induction still takes place in the absence of both *DOG1* and *MFT*. The effect of osmotic potential in SD induction and its interaction with the presence of these two genetic factors was also investigated. The dormancy cycling regime was similar to the one applied during the screening of the RIL mapping population.

Dark germination was marginal in the Col-0 (WT) and *dog1-2* compared to *mft2* and *dog1-2/mft2* (Figures 4.18 - Left and B.5 - Left). Dark germination varied according to incubation duration. However, dark germinants were alive (not withered) only at the first timepoint. Accordingly, the average of dark germination over 25°C period is more informative (Figure 4.18 - Right). Dark germination was significantly higher in *mft2* compared to double mutants #4 and #23 but not #7 ($P < 0.05$). Independent lines #4 (circles), #7 (squares), and #23 (triangles) slightly differ in dark germination.

Dark germination during 5°C/dark period (prior to dashed green line in the Figure 4.18 - Right) was negligible (<5%).

At the beginning of dormancy cycling, *mft2* seeds had a lower germination potential at 25°C/light than other lines including the WT (Figure 4.19). During the incubation at 5°C/dark, -1.0 MPa, PD was released by cold stratification in all lines. Upon transfer from 5°C/dark to 25°C/dark, SD induction was induced in all lines.

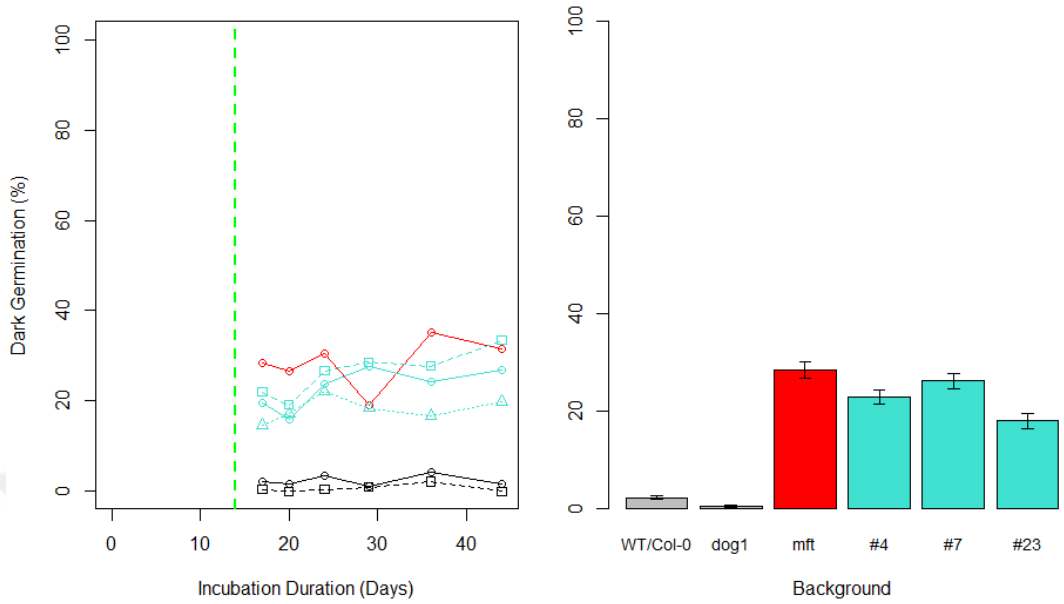

Figure 4.18. Dark germination in the R-Experiment. Dark germination after the transfer to 25°C/dark, 0.0MPa. Col-0 (WT) (black circle / solid line), *dog1-2* (black squares / dashed line), *mft2* (red circles / solid line)
, *dog1-2/mft2* (dashed blue lines). (Data represent the mean, n = 6) Timepoints were averaged within lines on the right. (Data represent the mean ± SE, n = 36)

When incubated at 0.0 MPa, SD induction was more rapid in *dog1-2* and *mft2* compared to WT (Figure B.8). *dog1-2/mft2* had the lowest SD induction rate among all lines. When incubated at -1.0 MPa, SD induction was more rapid in *dog1-2* and *mft2* compared to WT according to germination behaviors at 30°C but not at 25°C (Figure B.8). SD induction was more rapid in *dog1-2* than *mft2* in most experiments (Figure B.5 - Right).

However, notice that AUC values stabilized at higher values in *dog1-2* and *dog1-2/mft2* compared to *mft2* and WT. This was related to slow germination after 3 days occurring to low extents (<30-40%) in the case of *dog1-2* compared to earlier timepoints where germination occurs typically within 2 days after the light exposure. *dog1-2/mft2* germination rate also decreased resulting in progressively lower AUC values but total germination was higher than *dog1-2*.

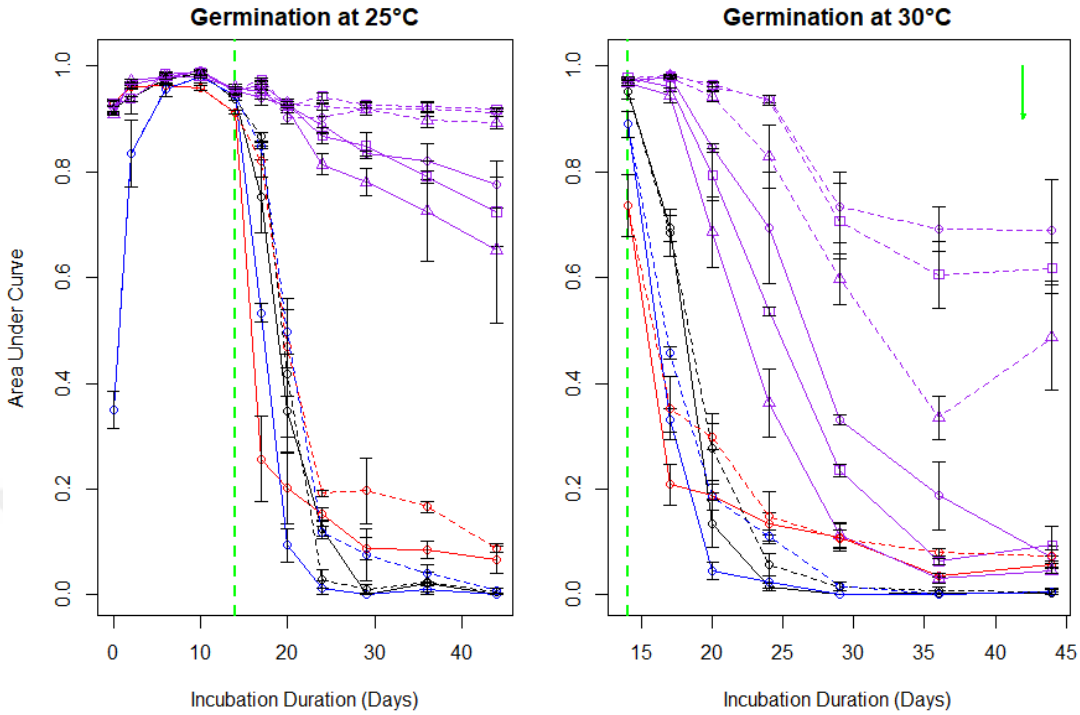

Figure 4.19. Dormancy cycling of the mutants in the R-Experiment. Col-0/WT (black), *dog1-2* (red), *mft2* (blue), and three *dog1-2/mft2* lines: (purple) #4 (circles), #7 (squares) and #23 (triangles) were germinated at 25°C/light (on the left) or 30°C (on the right). Transfer to 25°C/dark was after day 14 (green dashed lines) to -1.0 MPa (dashed lines) or 0.0 MPa (solid lines). (Data represent the mean ±SE, n=3)

It is possible that assaying the germination behavior at a higher temperature (25°C and 30°C in R-Experiment compared to 20°C in M-Pilot) allowed the detection of SD induction at earlier timepoints. SD induction may be initiated as soon as temperature transfer occurs in Col-0 background as opposed to Bur (Figure 4.6). This SD is apparent earlier when germination behavior is assayed at higher temperatures as Col-0 displays high temperature thermodormancy (Figure 4.20).

Interestingly, SD induction was slower in all backgrounds at -1.0 MPa compared to 0.0 MPa. This was also apparent in *dog1-2/mft2* indicating that the effect of osmotic potential is independent of two genetic factors (Figure B.8). This reveals that the osmotic modulation of dormancy cycling is similar between Col-0 and Bur.

MFT prevents dark germination and maintains of low levels of dormancy (lower SD induction rate). However, *DOG1* is more prominent in PD establishment and maintaining high levels of dormancy. SD is still induced in the absence of both genes. This reveals that SD induction is independently regulated of both genes. However, neither low or high dormancy state can be maintained in the absence of both genes. In that case, germination is triggered after light exposure. In single mutants, high or low dormancy state can be maintained in the absence of one of these factors (Figure 5.1). Only when germinated at 30°C/light after incubations at 0.0 MPa, total germination rather than germination rate was the driving factor in decreasing AUC values. Therefore, it is possible there could still be other factors able to maintain dormancy.

4.6. Germination Response to Wavelength

In addition to assaying germination during constant light exposure, germination can also be assessed during the dark incubation after short light treatments. Such experiments provide information about components involved in the phytochrome mediated light signaling [107, 108]. In this study, dark germination was observed in in the absence of *MFT* and in the Bur ecotype. Phytochrome mediated light signaling is regulated by both the temperature and the wavelength [107,108]. Considering these, the response to wavelength and temperature was investigated for both fresh and after-ripened (AR) seeds of different lines (Figure A.4).

4.6.1. Fresh Seeds

Fresh Bur seeds completely germinated in all combinations of the temperatures and the light treatments (Figure 4.20). In contrast, fresh WT and mutant seeds in the Col-0 background (grown with mutants F4 generation not the seed batch #101) displayed high temperature thermodormancy. Overall, dormancy was highest in *dog1-2* and lowest in *dog1-2/mft2*. Col-0 (WT) and *mft2* had similar germination profiles under the assayed conditions.

FR treatment was not effective for the fresh Bur seeds (Figures 4.20 and B.6). In Col-0, FR treatment blocked germination compared to R treatment at 20°C ($p<0.01$) and 25°C ($p<0.05$), but not at 15°C. Germination was restored back in the FR-R treatment at 20°C in responsive backgrounds indicating that it was possible to cycle phytochrome without any AR in Col-0 ($p<0.01$).

The inhibitory effect of FR was only observed in *mft2* at 20°C ($p<0.01$) (Figure 4.20). In *dog1-2/mft2*, FR only inhibited germination at 30°C compared to R ($p<0.01$), but phytochrome was not observed to cycle back following FR-R treatment. At 15°C, FR instead promoted germination without any AR, and dark germination was higher compared to other temperatures in the lines in Col-0 background (Figure 4.20).

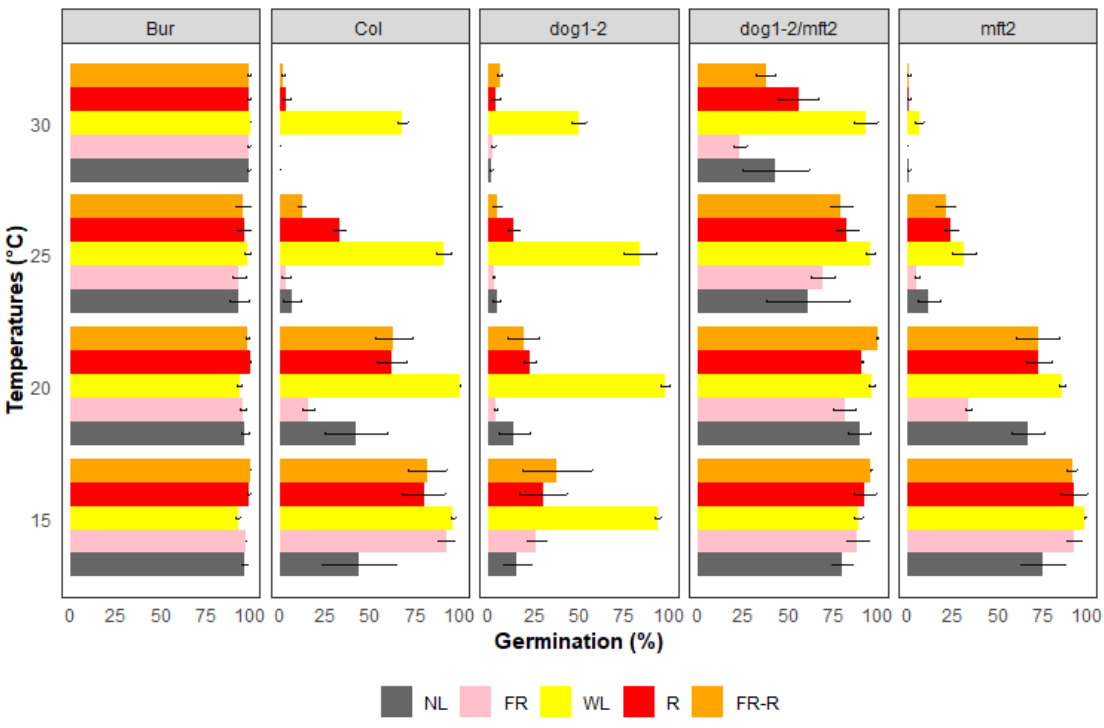

Figure 4.20. Germination response to wavelength in fresh seeds. Total germination in backgrounds (in columns) at different light treatments (colors in the figure legend) and temperatures (°C, in rows). (Data represent the mean ±SE, n=3)

4.6.2. After-ripened Seeds

A previous experiment revealed that phytochrome obtains the ability to cycle between the active and the inactive states after AR treatment (Figure B.6). AR is also known to release dormancy [26]. After dormancy release, the inhibitory effect of FR may become more clear. Therefore, the experiment was repeated following dry afterripening of seeds for 57 days.

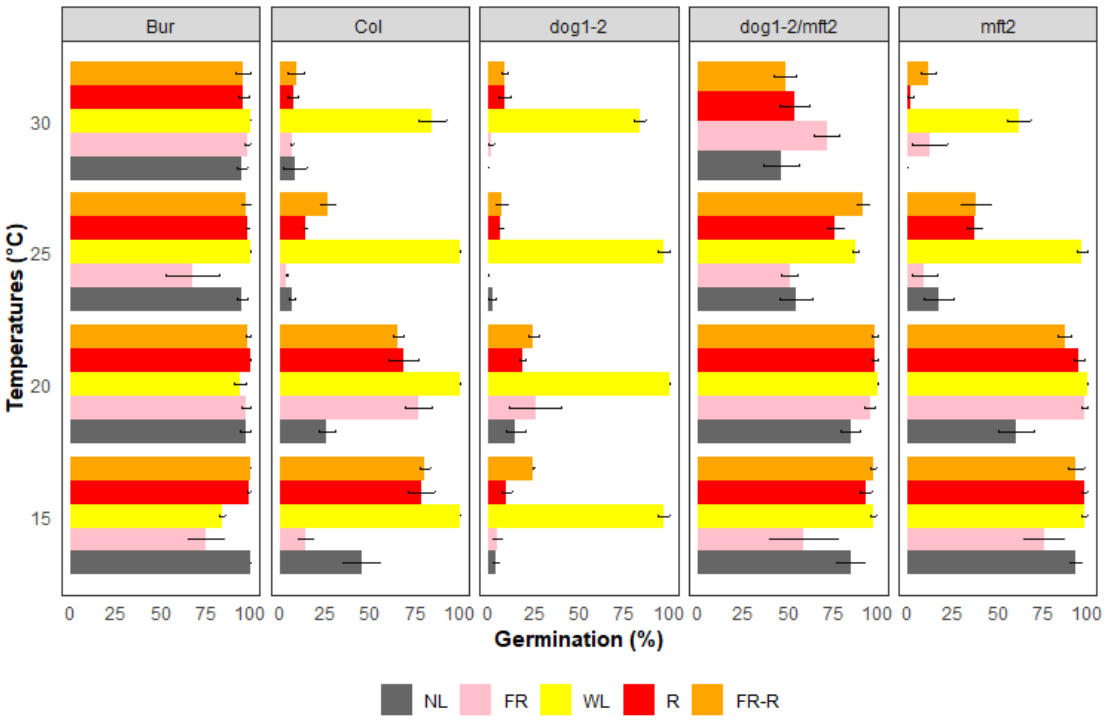

Figure 4.21. Germination response to wavelength in the after-ripened seeds. Total germination in backgrounds (in columns) at different light treatments (colors in the figure legend) and temperatures (°C, in rows). (Data represent the mean ±SE, n=3)

All lines in the Col-0 background displayed a reduced thermodormancy after AR treatment (Figure 4.21, WL treatments). After AR in Col-0, FR treatment led to increased germination at 20°C (compared to NL, $p<0.01$) decreased germination at 15°C (compared to R and FR-R, $p<0.01$) and 25°C (compared to FR-R but not R, $p<0.01$) (Figure 4.21). Similar results were obtained in mutant bakcgrounds including

dog1-2/mft2, where phytochrome was possible to cycle only after AR. It was also possible to cycle phytochrome at 15°C and 25°C in Bur but not at 20°C and 30°C.

5. DISCUSSION

The experimental results were synthesized (Figure 5.1). The figure will be helpful in understanding the ideas put forward in the following sections.

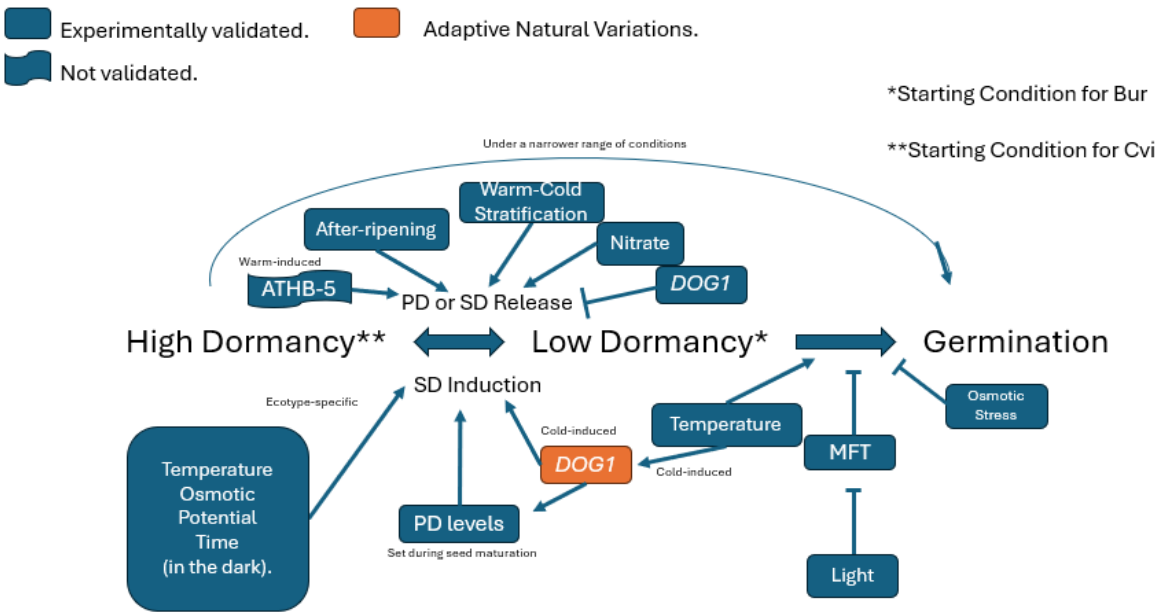

Figure 5.1. A summary of the mechanisms in control of dormancy cycling. Three hypothetical states are determined within dormancy cycling states: High Dormancy, Low Dormancy, and Germination. Arrows indicate regulations of possible transitions between these states, or between regulatory factors themselves. Sharp and blunt arrows indicate positive and negative regulations, respectively.

5.1. A Note on the Setup of Dormancy Cycling Experiments

5.1.1. The Problem Associated with the Setup of Dormancy Cycling Experiments

Dormancy cycling experiments in this study were always performed by assaying germination with multiple replicates (multiple replicates approach). This approach does not provide a direct estimation for the behavior between timepoints. As a result, dormancy cycling curves are punctuated and connected linearly (Figure 4.2).

However, the environmetal responses in organisms seldomly progress in a linear fashion and are usually sigmoidal (Figures 3.3 and B.4). Also, the dormancy release and induction periods overlap as members of the population behave individually during dormancy cycling. This would theoretically smoothen the dormancy cycling curves instead of the sudden drops and sharp peaks that were obtained in the absence frequent sampling.

Moreover, having no estimation of the behavior between assayed timepoints limits the interpretation (Figure 4.2, 25 °C experiments). Performing additional timepoints afterwards to fill in between timepoints is even more problematic. Small differences between experimental setups can change the dynamics of dormancy cycling behavior (Figure 4.2, 5°C experiments).

Yet, performing multiple replicates has advantages. Multiple replicates allow estimation of the error term by the normal approximation. Overdispersion of this error term can be attributed to container effects, or unidentified sources, during the experiment. When complex models are used, this can be highly advantageous while comparing dormancy cycling curves [123].

In the context of dormancy cycling, where indices (i.e. total germination or AUC) are used to summarize germination behaviors at timepoints, having multiple replicates

becomes disadvantageous. Concentrating multiple samples at a single timepoint decreases the number of samples available to other timepoints.

Setting up dormancy cycling experiments is logistically complex and time consuming. For example, the study visualized in the Figure 4.2 required 40 boxes each with six samples to be set in a single day to prevent variation in dormancy cycling behavior. Other large scale dormancy cycling screens also took all day and up to three people to set up. Therefore, increasing the number of timepoints while continuing to perform multiple replicates at each timepoint is unreasonable. In consideration of these, a compromise from the multiple replicates approach is required to be able to better assess dormancy cycling.

5.1.2. An Alternative Setup for Dormancy Cycling Experiments

Total germination of randomly sampled seeds from a single batch is distributed binomially. The error term for total germination can be derived using binomial estimation and does not require replicates [123]. Instead of replicates at a single timepoint, more timepoints can be sampled during dormancy cycling experiments especially under laboratory conditions without any replicates (a multiple timepoints approach). If possible, having replicates is obviously useful to pinpoint behavior at sampling timepoints.

The concern of outlier timepoints in the multiple timepoints approach is limited, since by "leaving k-out" techniques, possible outlier timepoints can still be detected and removed [124]. This can be accomplished even when there are no replicates, if sampling is frequent enough along dormancy cycling curve. If the desire is to compare dormancy cycling behavior, the possession of a complete dormancy cycling curve is evidently more important.

Curves can be fitted locally to describe dormancy cycling behavior to derive dormancy induction and release rates. Then, the effect of experimental factors on derived curve parameters can be estimated. Comparison of global trends during dormancy

cycling are more advanced and the interpretation wiil be harder. Modelling of global trends is guided by an estimation of the parameters that control dormancy cycling behavior such as temperature dependent dormancy induction and release rates [29]. When dormancy cycling is performed in the dark as in this study, temperature dependent dormancy induction and release rates change according to time (Figure 4.6). The estimation of a single release or induction parameter for all temperatures would not be the correct approach.

To settle whether performing multiple replicates or timepoints provides more realistic data, a simulation can be performed. This simulation should pick random timepoints for both a multiple replicates approach and a multiple timepoints approach to observe similarity of fitted dormancy cycling curves to the underlying data (i.e calculated by RMSE at random or equally distributed points along the time axis, not just on selected timepoints). Sudden responses and narrow peaks may be skipped in the multiple replicates approach (such as those that occur during the cold and warm stratification periods, Figure 4.2). In this case, the multiple timepoints approach would be predicted to have an advantage over the dormancy cycling period due to frequent sampling.

5.2. The Molecular Control of Dormancy in *Arabidopsis thaliana*

A simple interpretation would be as follows. *DOG1* and *MFT* are not absolutely required for PD as we observed lower dormancy in WT compared to single mutants (Figure 4.20). Their roles might be dependent on maturation conditions, for example *DOG1* might be more important for establishing PD at low maturation temperatures [68].

DOG1 and *MFT* are not absolutely required for SD induction but for an efficient one. During SD induction, the absence of one can be tolerated by the other. Yet, there is strong evidence for the presence of factors that can induce SD, independently (Figure 4.18). One of these is potentially responsive to osmotic conditions.

While this interpretation on the roles of *DOG1* and *MFT* in control of PD and SD is highly simplistic. Yet it fails to appreciate the current view on the molecular and ecological functions of *DOG1* and *MFT* in the literature.

5.2.1. The Role of *DOG1*

Firstly, the elephant in the room must be taken into notice. Lower dormancy of Col-0/WT seeds than *dog1-2* seeds is highly surprising and as far as experimental conditions are concerned unintentional (Figure 4.20). PD establishment is compromised in *dog1-2* seeds according to the literature [68]. In one of the experiment in the literature, when matured at 10°C, *dog1-2* seeds display lower dormancy compared to WT [68]. However, when maturated at 20°C, germination for both WT and *dog1-2* seeds are over 90%. This indicates that *DOG1* is more important for PD establishment in colder temperatures.

In this study, the seeds were matured at under a long day regime at 22/16°C (Table A.1). Both WT and *dog1-2* seeds display high temperature thermodormancy at PD (Figure 4.20). Therefore, *DOG1* is not integral to establishment of PD when matured at high temperatures. This indicates that *DOG1* is not important for PD establishment in warmer temperatures. WT seeds were lower in PD than *dog1-2* seeds due to biological variation.

Col-0 has D-SY haplotype of *DOG1*. Accordingly, SD induction is noticably slower during cold incubation in Col-0 background (Figure B.9) [100]. D-alleles of *DOG1* were referred to be not "functional". Yet, according to the results in this study, SD induction still occurs after transfer to 25°C and even in *dog1-2* seeds (Figures 4.19 and B.5). This is a strong evidence that *DOG1* is not absolutely required for SD induction.

E-alleles of *DOG1* were highlighted for cold induced SD [100]. Yet, seeds, used in the identification of *DOG1* haplotypes, were produced at 14°C with 6 weeks of ver-

nalization at 6°C [100]. We observed a higher SD induction in *dog1-2* seeds compared to WT. Lower PD can result in lower SD induction rates in *A. thaliana* [24, 25]. This would provide an explanation for the lower SD induction rate observed in WT compared to *dog1-2*. Therefore, it is possible that the effect of *DOG1* on cold-induced SD is related to PD levels [24, 25]. Factors important in SD induction might instead depend on the environmental context and the genetic background.

On the other hand, after SD induction is completed and germination levels are stabilized at 25°C/dark, *dog1-2* seeds cannot maintain SD as well as WT seeds when transferred to light. Germination occurs at small percentages late in the germination period resulting in higher AUC values than WT (Figures 4.19). *dog1-2/mft2* lines also displayed the same deficiency in the maintenance of SD when compared to *mft2*. Therefore, the role of *DOG1* during dormancy cycling is reserved to the maintenance of high levels of dormancy.

Overall, the results indicate that *DOG1* conditionally regulates the induction and maintenance of dormancy.

5.2.2. The Role of *MFT*

Differences in PD were observed between F3 and F4 generations of mutants at AUC levels at day 0 relative to WT (Figures B.5 and 4.19). This could be related to maturation conditions (Table A.1). However, the germination on day 0 were performed at different temperatures in these experiments, therefore judging the difference in germination potential relative to WT could be misleading.

mft2 seeds displayed increased higher temperature thermodormancy than WT and *dog1-2* seeds in WL. Yet, *mft2* seeds were more responsive to R and FR/R treatments than WT and *dog1-2* seeds. *mft2* seeds also had higher dark germination (Figure 4.20). This reveals that the mode of dormancy regulation is different between *DOG1* and *MFT*.

MFT has an inhibitory role in phytochrome signaling [108]. Accordingly, dark germinants were present after the transfer to 25°C in *mft2* and *dog1-2/mft2* mutants (Figure 4.18). Interestingly, *mft2* and *dog1-2/mft2* were induced into SD less rapidly than WT and *dog1-2* (Figures B.5 - Right and B.8).

Therefore, *MFT* is more important than *DOG1* during low dormancy, for the short period after 5°C phase. In the absence of *MFT*, seeds are less prominently blocked from germinating in the dark and are less efficiently induced into SD. In fact, the role of *MFT* in "spatial sensing" (another alias for low dormancy period) was speculated before and correlation of expression levels to temperature was opposite between Bur and Cvi during dormancy cycling in the field [122].

5.2.3. The role of *DOG1* and *MFT* in Phytochrome B Mediated Light Signaling

It was possible to decrease germination by FR treatment and to cycle back PhyB to trigger germination in all lines. In the absence of *MFT*, the inhibition of germination by FR treatment was less prominent compared to WT (Figure 4.21). This effect might be related to role of *MFT* in PhyB mediated light signaling [108].

The effect of absence of *DOG1* is less interpretable as seeds did not acquire the ability to germinate with R light following AR treatment. However, where the effect of FR treatment was observed, the germination was blocked almost completely. Since the dark germination of *dog1-2* seeds was limited as well, it can be suggested that *DOG1* is not involved in PhyB mediated light signaling. Higher sensitivity to R and FR-R treatments in *dog1-2/mft2* seeds can be explained by the inability to maintain dormancy states (Figure 5.1).

5.2.4. Problems in Assessing to Sensitivity to FR

There was either a strong interaction between all controlled parameters or the experimental procedure caused a significant variation in light responses due to an uncontrolled factor. A possible uncontrolled factor is FR intensity as the output of the FR light source might not be uniform. Seeds are highly sensitive to the differences in the intensity of light. This includes the range of FR light. Depending on the intensity of FR light, different pathways can be triggered accordingly [57-61].

The light intensity of FR light which needs to be around 2-3 μmol/m^{2}s was optimized for a small number of containers (12 petri dishes) by keeping the containers at a particular distance (Figure B.6). However, the number of containers (36 boxes) in later experiments prevented stacking samples at the required distance (Figure 4.21).

Samples were shuffled to even out the light intensity during light treatments. Yet, it is possible that the intensity at a single moment (the first or the last exposure moment) is more important than the average intensity experienced. This might be the reason behind the variation of response to FR treatment between temperatures. A consideration of the setup of the experiment and the obtained results supports this explanation. A single replicate of each ecotype was present within each box. Samples from the same temperature generally gave the same response to FR treatment across lines.

To reach a healthy conclusion for the roles of *DOG1* and *MFT* in PhyB mediated light signaling, it is required to repeat the experiment with a more uniform FR source. Increasing the number of replicates might also help as the experiment can be performed on multiple days per replicate. Lastly, decreasing the dependency between samples by sowing them in individual containers and packing them individually might help in the detection of outliers.

5.3. Dormancy Cycling Behavior of RIL Population and Parental Ecotypes

The central aim of this study was to identify genes controlling dormancy cycling in ecotypes adapted to different climates. These genes are potential sources of climate adaptation of dormancy cycling behavior and can be referred to as "climate adapted genes". Cvi and Bur ecotypes exhibit winter and summer annual life cycles putting them at opposite ends of a behavioral spectrum. [20]. For this reason, these ecotypes were chosen as the parental ecotypes to construct the Recombinant Inbred Line (RIL) mapping population. Using principal component analysis to analyze PD and dormancy cycling behaviors, Quantitative Trait Loci (QTLs) harboring genes involved in the regulation of these climate adapted behaviors can be identified.

Overall, we observed lower dormancy levels in Bur seeds than Cvi seeds across all experiments. Bur seeds was more sensitive to GA than Cvi seeds (Figure B.1). Cvi seeds reached higher germination percentages than Bur seeds only within dormancy cycling after a 25°C/dark period (Figure 4.5).

The direct comparisons of dormancy levels require germination of seeds in multiple conditions along the dormancy cycle (temperatures, osmotic potentials or GA dose response curves) [20], [89] (Figure 4.21). Not being able to achieve this inherently limits the interpretability of the results in terms of dormancy. However, germination potentials can be compared to the previous timepoints within the background. In that case, under one set of conditions dormancy cycling implies differences in dormancy cycling behavior (Figure 4.5). Following subsections attempt an explanation of the observed dormancy cycling behaviors.

5.3.1. Ecotypic Differences in PD

Difference in PD levels between the parental ecotypes were determined in terms of dose-sensitivity to dormancy breaking treatments (Figures B.1 and B.4). This difference can be attributed to adaptation to the prevailing climates at the geographic points

of origin. For example, Cvi originates from the arid climate of the Cape Verdi Isles, while Bur originates from wet climate of the Burren region of Ireland. Ecotypes from Mediterrenian region are generally more dormant compared to those from Northern and Central Europe [91], [100]. This difference in PD is also observed in RILs, where Cvi-like lines are more dormant compared to Bur.

Lower PD can cause increased sensitivity to dormancy breaking agents and vice versa. Interestingly, slopes of dose response curves are similar between ecotypes (Figure **??**). This similarity prevents a speculation about the mode of regulation. A genome-wide association study may detect loci related to changes in the sensitivity to dormancy breaking agents. Further dissection of measured sensitivities into thresholds and slopes might inform about the details of the regulatory mechanism.

QTL analysis identified three QTLs (Peaks 8-10) for PC7 which correlated positively with germination potential at 25°C/light for PD screening experiments (Table 4.1 and Figure 4.15). These QTLs all had the version from Bur ecotype as the effect allele. *MFT* is the most prominent candidate gene in the region. *MFT* mutation was highlighted for dark germination in this study. However, PC-7 did not correlate with dark germination. It is possible that the effect on PD in the mapping population is independent of dark germination in *mft2* which is in the Col-0 background. Alternatively, PD might be affected by another gene within this QTL.

Peak 9 included *ATHB20*, *RGL2* and *AHG3* within its confidence interval. A QTL was previously identified in the same region [67]. The peak centered at the tip of the chromosome, so the former is the most likely candidate gene. *ATHB20* mutants displayed higher seed dormancy [125]. *RGL2* encodes a DELLA protein that inhibits GA response during low dormancy periods [126]. *AHG3* encodes a PP2C promoting germination by decreasing ABA sensitivity [67].

Peak 10 did not include any genes yet associated with dormancy or germination. However, few QTLs were identified before in the same region and were named as

DOG4 [67] or D6-7 [127]. Yet, these QTLs were not associated with any genes within the confidence interval of Peak 10. Therefore, Peak 10 contains an unidentified gene or genes controlling PD levels.

Notice that Peaks 1 and 2 identified for PC-1, and Peak 7 identified for PC-4 also affected PD levels. However, their effects were not exclusive to PD (Table 4.1 and Figure 4.15). PC-1 was correlated with germination potential during dormancy cycling. PC-4 was correlated with germination potential at 10°C after 25°C/dark period. Peaks 1 and 7 were centered around *DOG1* but the effect alleles were different. Peak 2 was centered around *DOG6* which encodes for *ANAC060* [101]. The combined effect of these genetic factors on PD and SD screens emphasizes the effect of PD levels on the trajectory of dormancy cycling. The roles of *DOG1* and *DOG6* are detailed in the Literature Survey and below.

5.3.2. Ecotypic Differences in Cold-Induced SD

Dormancy release via cold stratification occurs at short periods of cold incubation. The cold stratification effect persists longer in Bur and Col-0 compared Cvi (Figures 4.2, 4.19 and 4.5). The onset of SD induction during cold incubation was accelerated in the winter annual Cvi.

Cvi has the ECCY haplotype while Bur and Col-0 ecotypes have D-SY haplotype for *DOG1* (Figure B.9). It is known that ecotypes with the ECCY haplotype are induced into SD by cold faster than D-SY haplotypes [100]. This explains why such a strong QTL (Peak 2) is identified in the region of *DOG1* region. The effect of *DOG1* on SD might be due to its effects on PD levels as argued during the discussion of dormancy cycling behaviors of mutants.

SD is induced in the Bur background upon prolonged cold incubation. Yet, the sensitivity to GA remains high (Figures 4.4 and 4.3). The continuation of high sensitivity is not due to saturation with high doses applied, as the ecotype was responsive

to lower GA doses applied in the other experiments (Figures 4.6 and 4.5). Due to this increased GA sensitivity during cold incubation, it was possible to detect the Peak 6 for PC-3 (Table 4.1, Figures 4.15 and 4.16). PC-3 correlates with GA sensitivity at 25°C during PD that persists after cold stratification (Figure 4.15).

The center of Peak 6 is the same as Peak 1. Peaks 1 and 6 include DOG6 that encodes for ANAC060 transcription factor [101]. *ANAC060* mutants have increased ABA sensitivity, but their roles in GA sensitivity of signaling is unstudied. Yet, ABA and GA signaling antagonistically regulate each other [18]. Therefore, decrease in ABA sensitivity might result in an increased GA sensitivity.

Peak 6 might be independent of ANAC060 as the expression level of *ANAC060* is similar between Cvi and Bur during dormancy cycling in the field (Figure B.10) [20]. Other genes within the confidence interval of Peaks 1 and 6 were investigated using the keyword "response to gibberellin" in TAIR annotations. This search returned only a single gene *SnRK2.2* which promotes ABI5 activity by phosphorylation with an antagonistic response between ABA and GA [126]. Increased and sustained GA sensitivity of Bur ecotype during cold stratification might be related to the differential regulation of this gene between ecotypes.

The expression levels of *SnRK2.2* are higher in the winter in Bur ecotype during dormancy cycling in the field (Figure B.11) [20]. The expression decreases at the onset of spring with dormancy induction. In Cvi, however, the expression levels do not follow dormancy levels (Figure B.11). This represents a possible difference between control of *SnRK2.2* expression by the environmental cues. Nonetheless, how increased expression of *SnRK2.2* would elevate GA sensitivity is unclear.

5.3.3. Ecotypic Differences in Warm Temperature Responses during Dormancy Cycling

The response to 25°C/dark differs between Bur and Cvi in both the mode and the rate. Bur is induced into SD at 25°C. Cvi does not lose sensitivity to GA and nitrate during incubation at 25°C/dark as opposed to 5°C/dark (Figures 4.3 and 4.4). When incubated directly at 25°C/dark dormancy levels are more stable in Cvi compared to Bur and no clear change in dormancy levels is observed (Figures 4.2, 4.3 and B.2). This indicates dormancy release at 25°C, if it occurs, is marginal in Cvi without a prior cold incubation period (Figures 4.2 and B.2).

SD is induced during cold incubation period is released during 25°C/dark in Cvi (Figure 4.5). In Bur, a rapid SD induction occurs 25°C/dark followed by a slow dormancy release (Figure 4.5). Increasing the duration of incubation at 5°C/dark accelerates the SD after the transfer to 25°C/dark in Bur (Figure 4.6). There is an ecotypic difference temperature regulation of dormancy cycling at 25°C/dark.

RILs that obtained the ability to germinate after the 25°C/dark phase were equally distributed across high and low dormancy lines after 5°C/dark phase (Figure 4.11). This indicates that the loci in regulation of dormancy cycling during 25°C/dark phase are independent of loci governing dormancy induction during 5°C/dark. Germination was limited in both ecotypes when germinated without GA after 25°C/dark phase (Figure 4.9, E. and F.). However, some RILs were able to germinate efficiently in the absence of GA, which indicates a possible transgression in the dormancy cycling trait (Figure 4.11).

Three QTLs, Peaks 3, 4 and 5, were identified for PC-2 that only correlated with the ability to germinate under three conditions after the incubation at 25°C/dark (Figure 4.15 and Table 4.1). Two of these, Peaks 3 and 5, were independent of all others that were identified. Peaks 4 and 5 were identified with a higher confidence for PC2 compared to Peak 3 (Table 4.1, LOD-scores >7.7). Notice that PC-1 and PC-4

also correlated with total germination after the 25°C/dark phase. The effect of QTLs identified for these PCs were associated with a wider range of screening conditions and were discussed above in relation to these conditions.

Peak 3 includes three dormancy or germination related genes within its confidence interval: *RDO2*, *PP2C5* and *DAG2*. *RDO2* encodes a transcription elongation factor, and its mutants show reduced dormancy [128]. *RDO2* is upregulated during seed maturation indicating that it might be required to induce PD during this period. The role of *RDO2* in SD has not been investigated. *PP2C5* is wrongly annotated in TAIR database as positive regulator of germination. *PP2C5* mutants have decreased stomatal closure and increased ABA insensitivity during germination (hence decreased dormancy) [129]. The role of *PP2C5* might be similar to *RDO5*, which is a pseudophosphatase that increases dormancy [102,103]. *DAG2* encodes a transcription factor containing a single zinc finger motif [130]. *DAG2* mutants show decreased germination rate and are less sensitive to GA with no difference in ABA sensitivity [131]. *DAG2* was mainly effective in promotion of PhyB dependent germination [130].

Peak 4 includes *DOG1* within the confidence interval but the effect allele was for Cvi and the center of the peak was more than 10 cM downstream of *DOG1* (which was exactly at the center of Peak 2). Interestingly, the center of Peak 4 is the same as previously identified *SET1* in the same mapping population [20]. If "peakdrop" parameter was removed Peak 4 and 5 merged and the resulting confidence interval included neither *DOG1* nor overlapped with confidence interval of Peak 2. Therefore, it is likely that Peak 4 is a QTL independent of *DOG1*. Peak 4 or *SET1* includes *AHG1* within the confidence interval [20]. *AHG1* encodes for a type 2C protein phosphatase that blocks ABA signaling [68].

5.3.4. Identification of a Candidate Gene within Peak 5 by WGCNA

Peak 5 is of higher interest over other identified peaks. Peak 5 governs dormancy cycling potentially through temperature signals during incubation at 25°C/dark.

DOG1 has an annotated role in cold temperature, but as of yet no natural variations were identified for the control of germination potential after SD induction at warm temperatures [101]. To identify a candidate gene within Peak 5, a weighted gene co-expression network analysis (WGCNA) was performed.

Genes with a high degree connectivity, transitivity and centrality (top 20% in the first two indices and top 30% in all ecotypes) in the latter were filtered. Candidate genes were identified within each metric itself. Only a single gene was commonly identified by filtering according to two indices (connectivity and transitivity): *ATHB-5*. No genes were identified commonly between all filtering methods.

ATHB-5 encodes a class I HDZip (homeodomain-leucine zipper) protein. *ATHB-5* is regulated by ABA signaling during seedling establishment [132]. During seed germination, *ATHB-5* expression is repressed by DELLA proteins, is responsive to GA and promotes *EXPA3* expression in hypocotyl [133]. Gene expression of *ATHB-5* increased with temperature in both ecotypes (Figure B.12). The temperature upregulation of *ATHB-5* was lower in Bur than Cvi ($p < 0.05$). There was significant negative correlation between *ATHB-5* expression and dormancy in Cvi, which was lost in Bur.

According to the literature *ATHB-5* is among genes that are upregulated during germination and gene expression is higher in low dormancy seeds (Figures B.13 and B.14) [134]. *ATHB-5* was in the brown module (Figures B.20 and B.21, Table B.3). *ATHB-5* can be treated as a candidate gene for the adaptivity of dormancy cycling in *A. thaliana*. It might be important for the germination potential as SD is released during the warm incubation period.

5.4. An Hypothesis on the Source of Local Adaptations in Dormancy Cyling in *Arabidopsis thaliana*

Gene expression values of 15416 genes that passed filtering were used in the network construction [20], [24]. Eight modules were detected in the network named with

different colors (Figures B.20 and B.21). 8920 genes were placed to a module while 6496 genes were not. None of the modules correlated significantly and in the same direction (sign of the correlation) with temperature or dormancy levels across all ecotypes. This indicates that the roles and the connections of modules might differ between ecotypes during dormancy cycling. GO analyses was performed for the identified modules (Tables B.1 - B.8)

All modules were correlated with dormancy in Cvi. Yet, only two modules significantly were weakly correlated with dormancy in Bur (pink and black modules, $p < 0.05$, Figures, B.20). The centrality indice was negatively correlated between the networks of Bur and Cvi ecotypes, while the connectivitiy indice was randomly distributed (Figures B.17 - B.19). The transitivity indice displayed a combination of the other figures, but also nodes (or genes) with maximum transitivity were present in ecotypes (Figure B.18).

In the network visualization, blue, yellow and pink modules were clustered together (Figures B.16 and B.15 - reft cluster) and turquoise, red, and brown modules were clustered together in both networks (Figures B.16 and B.15 - right cluster). In the Bur network, the modules in the left cluster had higher centrality, while in the Cvi network the modules in the right cluster had higher centrality. The black module switched clusters between networks but displayed low centrality in both. The black module was associated with gene ontology (GO) terms related to immune responses (Table B.1).

Interestingly, the green module also switched clusters and displayed high centrality in both networks (Figures B.16 and B.15, (Table B.4). The results of gene ontology (GO) analyses highlighted protein turnover related GO terms (proteasomal protein catabolic process; GO:0010498, ubiquitin-dependent protein catabolic process; GO:0006511, protein quality control for misfolded or incompletely synthesized proteins: GO:0006515 , protein deneddylation: GO:0000338 and translation: GO:0006412). This supports the proposal about the importance of translation in regulating dormancy cy-

cling [24].

The centrality of green module that is common to both networks indicates that constant protein turnover is required during dormancy cycling. Plants can not access the nutrient in the soil before seedling establishment. The degradation of storage proteins provide nutrients and necessary building blocks. The set of proteins required to establish the necessary environmental sensitivity differs between the periods of dormancy cycling [135]. In the high dormancy state, the sensitivity to terminating signals decreases. In concert the gene expression of seeds between high and low dormancy states differ [20], [24], [134]. Therefore, once the dormancy state is altered, the proteins that have become redundant in their function have to be degraded. This mechanism reasonably should constantly function during dormancy cycling.

The adaptivity in dormancy cycling behavior is dictated by ecotypic differences in genetic factors and molecular pathways (see the Literature Survey). The structural alterations in the network do not affect the connectivity as radically as they affect the centrality. The structural alterations result in a different set of modules with high centrality between networks. This could be explained by the following model.

A central module including protein turnover mechanisms is in regulation of the trajectory of dormancy cycling (Figure 5.2). Another set of modules are sensitive to environmental factors. Their environmental sensitivity and influence on the central module differ between ecotypes. This difference results in an increased centrality of the modules with the increased influence.

Due to their increased centrality in the respective networks, different modules would form shorter pathways between environmentally sensitive and the physically acting factors between ecotypes. This could be reflected as a differential sensitivity to environmental factors observed on the phenotypic level. Lastly, the central module might form a feedback on the environmental sensitive factors further determining the trajectory of dormancy cycling.

This hypothesis can be tested by blocking protein turnover, in which case dormancy cycling behavior would be expected to be compromised. The hub genes of the modules that become central in the respective networks can be followed in a dormancy cycling experiment to observe if their expressions differ between ecotypes.

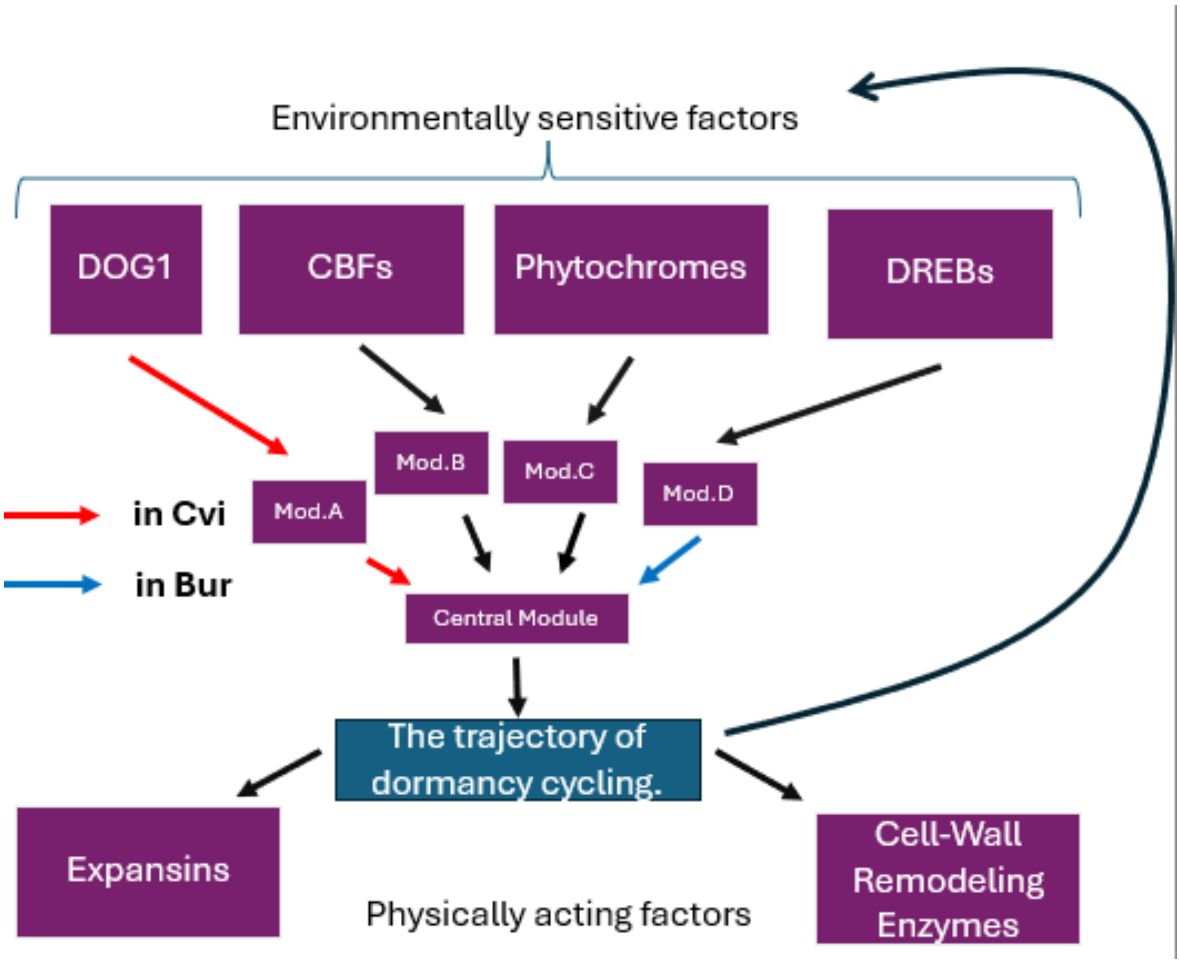

Figure 5.2. Graphical summary of the hypothesis on dormancy cycling adaptations. Interactions between factors, and their increased influence for Cvi (red) and Bur (blue) are hypothetical. Environmentally sensitive (DOG1, CBFs, phytochromes, DREBs) and physically acting factors (expansins and cell-wall remodeling enzymes) are examples.

5.5. Ecological Relevance of the Differences in Dormancy Cycling Behavior

5.5.1. Dormancy Cycling in the Winter Annual Life Cycle in Arid Climates

Cvi is a winter annual adapted to a climate with hot and dry summers [136]. Seeds of the winter annuals canonically germinate during decreasing temperatures in the autumn. The winter is spent in the vegetative state and surviving plants flower in the spring. The next generation of seeds are shed towards the end of the spring and spend the hot summer in the soil. Survival to reproduction during the summer is unlikely due to arid conditions. Therefore, a strong PD is selected for that persists during the summer to prevent germination.

Cvi seeds through their strong PD, do not germinate well prior to a dormancy breaking treatment (Figure 4.8). Cvi seeds can obtain the ability to germinate at higher temperatures in the dry state through dry AR (Figure B.4). Cvi seeds can also be released from PD via cold stratification (Figure 4.2). Yet, upon prolonged incubation at cold temperatures Cvi seeds are rapidly induced into a high SD and also lose their GA sensitivity (Figure 4.3).

Dormancy release by warm stratification was limited when Cvi seeds were incubated imbibed at 25°C/dark directly (Figure 4.2). In these conditions, a slight SD occurs according to the increase in the GA sensitivity of Cvi seeds (Figure 4.3). In Cvi, therefore, SD induction during the warm incubation was not observed. Higher rates of SD at lower temperatures for a winter annual that is adapted to a climate with hot and dry summers is sensible. As, if seeds were to germinate in the spring, the growing seasons would be shorter and the survival to reproduction would be less probable.

Seeds incubated at 25°C after a 5°C period regained their GA sensitivity through a SD release (Figure 4.5). However, Cvi germination percentages did not reach to high levels even when supplied with GA. Therefore, a bet-hedging behavior might be

more dominant, which prevents complete germination of the population. This would preserve the soil seed bank for multiple growing seasons. Dormancy release that occurs when the seeds are in the imbibed state at warm temperatures is one of the possible mechanisms to time the seedling emergence to the next autumn (at the start of the growing season).

Limitation of SD release in higher osmotic potential is another possible representation of a local adaptation to the local climate in Cvi. This mechanism can limit dormancy release during sporadic summer rains, where survival would be limited if germination occurs (Figure B.2). Accelerated SD induction at low temperatures with lower osmotic potential may be adaptive, if the growing season did not coincide with a rainy period (Figure 4.3).

5.5.2. Dormancy Cycling in the Summer Annual Life Cycle in Cool and Wet Climates

Bur displays a summer annual life cycle. The ecotype is adapted to a cool and wet climate [136]. Bur seeds predominantly emerge from the soil during increasing temperatures in the spring [20]. Most of the summer is spent in the vegetative state. Surviving plants flower late in the summer and at the beginning of the autumn. Seeds are shed and if germination is not triggered, they spend the cool winter conditions in the soil.

Bur seeds display low PD (Figure B.4). This suggests that Bur seeds can germinate under favorable conditions after seed shedding. Seeds are not rapidly induced into high levels of SD at cold temperatures (Figure 4.3). This response was related to a natural variation in the first exon of *DOG1* [100]. GA and nitrate sensitivities were rapidly lost during SD induction at high temperatures (Figure 4.5). While the sensitvities were maintained during SD induction at low temperatures for a longer period compared to Cvi (Figures 4.3) .

Decreased SD induction at cold temperatures can prepare Bur seeds to germinate at the beginning of the growing season in the spring, and an increased SD induction at high temperatures can prevent germination after the beginning of the growing seasons elapses. Conditions during summer may still be favorable for the germination, but it would be prevented by SD. Bur seeds release SD gradually over 25°C similar to Cvi (Figure 4.5). This slow SD release might time it to the autumn and may provide and explanation for the seedling emergence that was observed both during increasing and decreasing temperatures in the field [20].

The absence of an AR response in Bur in short terms (4 months) was observed before [137]. Bur seeds are low in PD, and can germinate over wide range of conditions readily upon imbibition even in the dark (Figure 4.20). Low dormancy is preserved at low temperatures (Figure 4.2). PD release by AR might not be essential in the summer annual life cycle [136]. The adaptation of Bur to a cool wet climate may have reduced the selection pressure for the retention of dry after-ripening [136]. Other effects of AR have not been completely lost, as AR resulted in increased sensitivity to FR-inhibition of PhyB mediated germination (Figure 4.21). It is possible that the effects of AR are modulated through independent molecular mechanisms.

5.5.3. The Osmotic Regulation of Dormancy Cycling in Cool and Wet Climates

In cool and wet climates, testing germination upon the first rainy period might be more favorable as the possibility of perishing due to drought is decreased. However, in arid climates the seasonal cycles of temperature might provide an information about the rainy periods that are sporadic or periodic. Testing germination in the first rainy periods would be in line with faster dormancy induction rates obtained at higher osmotic potentials in the dark. This would constitute an unfavorable conditions for germination since the light is absent even though the thermal and the osmotic conditions are permeable to germination.

If unfavorable osmotic conditions (low water potential) occur during favorable temperatures, (high temperatures) decreased SD induction rates might be more advantageous. In these conditions, the germination could take place after first rainy period if light is present (Figure 4.2). When both temperature (cold temperatures) and osmotic conditions are unfavorable, germination conditions can be tested for during the next rainy period as there are no risks of germination within the environmental status quo. Upon rainy period, SD induction might be accelerated if temperatures are still unfavorable (Figure 4.2).

5.5.4. Time Dependence of Temperature Responses in Dormancy Cycling

In Bur, SD can be induced after extended 5°C incubation. Extended periods of 5°C also decrease the sensitivity to a later 25°C phase. The adjusment of sensitivity to thermal switches depending on time might represent an adaptation. As far as the author is aware there are no similar experiments in the literature. A discussion of this interesting behavior will be accordingly limited.

Longer winters should decrease the extent of growing season. Bur seeds increase levels of their SD further at 25°C and do not enter dormancy cycling after extended an 5°C incubation. Considering the bolting time of Bur plants, a shortened growing season would prevent survival to reproduction (Table A.1).

In fact, climate phenomenon El Nino is known to drive milder winters and cooler, wetter summers in Northern Europe, while La Niña leads to colder winters, and warmer and drier summers. A systematical study of the presence of such a dormancy adaptation and its relationship with periodic climate patterns would be highly interesting [138, 139].

A time dependence in the dormancy cycling trait may also be studied for Cvi. For example, longer incubation durations at low temperature (winter conditions) might prime a more efficient SD release during the summer period (dry high temperature con-

ditions). The presence of such a behavior could be assayed efficiently under laboratory conditions. Comparison of dormancy release rates between AR and wet incubation at 25°C would be of interest as well.

5.5.5. Local Adaptations in the Regulation of Dormancy Cycling by Osmotic Signals

The modulation of SD induction by osmotic potential differs between ecotypes. SD induction rate was accelerated with lower water potentials at 5°C in Cvi (Figure 4.2). During incubation at 25 °C, there is a weak evidence that SD release rate was accelerated in Cvi by lower water potentials (Figures 4.2 and A.2). However, in Bur and Col-0, SD induction rate was limited at low water potentials (Figures 4.2 and B.8).

The decrease in SD induction rate at lower water potentials, that occurs in Col-0 background, was previously related to the decreased rate of metabolism at lower osmotic potentials [25]. In Cvi, dormancy SD induction rate was accelerated at lower potentials, instead (Figure 4.2). Therefore, high water potentials coupled with the absence of light and a suitable temperature for germination are indicators of unfavorable conditions depending on the ecotype. At low water potentials (-1.0 MPa), checking for germination with the arrival of the rain might be more advantageous after the experiencing suitable temperature regimes (25 °C following 5 °C) for germination for ecotypes adapted to cool and wet climates.

Conservation of this behavior between the summer annual Bur and the rapid cycling Col-0 backgrounds, and the absence in the winter annual Cvi background indicates to the presence of a natural variation for the osmotic responses in *Arabidopsis thaliana* during dormancy cycling. Osmotic and temperature signals are coupled differentially between ecotypes. (Figure 4.2). A potential natural variation in osmotic responses might be checked more throughly by manipulating osmotic potential while keeping temperatures stable during dormancy cycling.

Unfortunately, no PCs specifically correlated with germination potential after osmotic treatments (Figure 4.15). As a result, it was not possible to identify genetic sources for the regulation of osmotic signals. It is possible that osmotic potential responses are governed by many loci.

5.5.6. An Hypothesis on the Basis of Dormancy Cycling Behaviors

An overview of dormancy cycling behaviors reveals that SD release occurs while growing season is approaching [89]. It can be argued that SD induction is initiated as soon as the growing season begins but when favorable conditions are absent for the seedling development. Hence, the optimal utilization of the growing season might be selected for during the adaptation to local climate in the nature. In all climates, germination conditions are tested at the beginning of growing season by tracking precise seasonal patterns. In this paradigm, it can be then argued that differences between ecotypes are driven by two different selectional forces.

In an arid climate, it appears to be more favorable to skip the growing season in case favorable osmotic conditions did not coincide with the beginning of the growing season. In this case, dormancy cycling is regulated by the temperature which are modulated by the osmotic potential. Bet-hedging limits the dormancy release under imbibed conditions.

Conversely, in a cool and rainy climates. SD induction results in the high levels of dormancy in the absence of light with higher osmotic potentials. Temperature cycles provide information about the incoming growing season but dormancy is induced more rapidly when the light is absent for under favorable osmotic and thermal conditions. Therefore in a rainy climate, the range of unfavorable conditions are narrower (the absence of light, in permeable osmotic and thermal conditions) in the context of seed dormancy cycling compared to an arid climate (the absence of light and sub-optimal osmotic conditions, in permeable thermal conditions).

5.6. The Synthesis

PD can be established at a high or low dormancy state depending on the maturation conditions and genetic background (Figure 5.1). Seeds with high dormancy still germinate, but under a narrower range of conditions compared to low dormancy seeds. These range of conditions can be determined by the sensitivity to temperature, osmotic potential, and phytohormones.

DOG1 is induced by cold temperatures during seed maturation which correlates with high levels of dormancy. *DOG1* is required for maintenance of the high dormancy state. Yet, in the literature it has been suggested that *DOG1* is important for cold induced SD [100]. Alternatively, accelerated rate of cold induced SD is due to the effect of *DOG1* on PD. High levels of PD accelerate SD induction rate during prolonged unfavorable conditions (i.e. in the absence of light). In this perspective, the difference between *DOG1* haplotypes (D- and E- alleles) control PD levels established during seed maturation at cold temperatures.

Dormancy can be released via multiple mechanisms such as cold stratification, after-ripening, nitrate signaling within the dormancy cycling. In the low dormancy state, *MFT* inhibits the initiation of the germination program. Light signals remove the inhibition of *MFT* on the germination through phytochrome mediated signaling pathway. In the absence of *MFT*, dark germination occurs. There are other factors in control of dormancy cycling independent of *DOG1* and *MFT*. One of these factors, is *ATHB-5* which holds a potential to control the germination under the influence of SD after warm incubation (requires experimental validation).

The modulation of osmotic potential, temperatures and time and interactions between these factors on dormancy induction rates is ecotype-specific. The presence of this ecotypic specificity represent other genetic sources of local adaptations to be discovered.

5.6.1. Questions Against the Synthesis

One of the main arguments in this study is that PD levels determine the SD induction rates. This aspect of PD accounts for the regulation of *DOG1* on SD. In Bur ecotype, PD does not fit with this interpretation as PD is very low, yet SD can be rapidly released and induced subsequently at 25 °C (Figure 4.6). This can be attributed to a ecotype-specific effect (differences in the genetic background). This point is critical to the synthesis and requires systematical study.

6. CONCLUSION

Dormancy cycling is controlled by multiple loci that exert their effect during different temperature periods. Germination potential in states of PD and cold induced SD are governed by a similar set of factors including *DOG1*. However, germination potential under the influence of SD at warm temperatures are regulated by other loci. It was shown that *DOG1* is not required for SD induction at warm temperatures. SD induction is accelerated in higher osmotic potentials in the absence of *DOG1* and *MFT*, revealing that there are independent mechanisms involved in the environmental control of SD induction. Further studies can focus on the identification of factors involved in the osmotic regulation of SD induction. *ATHB-5* was identified by a network analysis as a candidate gene. *ATHB-5* is included in confidence interval of Peak 5 that controls germination potential during SD at warm temperatures. Further studies should verify if *ATHB-5* has an actual effect on SD state acquired at warm temperatures.

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APPENDIX A: SUPPLEMENTARY METHODS

A.1. Seed Sterilization

Seeds were sterilized before seed production or physiological experiments due to the presence of fungal spores whose growth may lead to viability loss if not treated. After treatment, viable spores may still be present if they penetrated the seed coat, however their population will be reduced, and their spread will be less likely. Two applied methods of sterilization are detailed below.

A.1.1. Surface Sterilization

Dry seeds were placed in a 1.5 ml Eppendorf tubes. Seeds were then suspended in a 2.5% bleach (Domestos, Unilever Thick Bleach Ultra White NaOCl <0.5%) solution for five minutes. The volume of seeds should not exceed one fifth of the volume of the tube. Seeds were thoroughly mixed with the solution by inverting and shaking the tube several times. After five minutes, the bleach solution was removed and seeds were rinsed off three times with de-ionized water. Then, seeds were suspended in de-ionized water for immediate use.

A.1.2. Dry Sterilization with Chlorine Vapor

This method was used to surface sterilize the RIL mapping population before seed production (Subsubsection A.3.3). Seeds were placed in a 1.5 ml Eppendorf tube sealed with a perforated cap. Tubes were placed in a desiccator jar with 50% bleach (Domestos, Unilever NaOCl <0.5%) solution in 100 ml beaker. Three ml of concentrated Hydrochloric acid HCl was added to bleach to produce chlorine gas. Contents were mixed rapidly, and the desiccator is sealed immediately.

Seeds were left for sterilization with chlorine fumes for three hours. The desiccator was opened under the fume hood and left under fume hood 5 minutes to release chlorine fumes which are highly toxic. Swiftly, perforated caps were recovered and lids were closed. Sterilized seeds were stored in -20°C until further use.

A.2. Preparation of Gibberellic Acid Stock Solution

The phytohormone, gibberellic acid (GA), was used throughout the study. GA is dormancy breaking agent and can promote germination of dormant seeds in a dose-dependent manner.

GA_{4+7} (Duchefa Biochemie - Haarlem / Netherlands) was used to make 500 μM stock solution. GA_{4+7} dissolved in 0.4 ml of 1M KOH and then mixed with 1 ml of Citrate Phosphate Buffer 100x stock solution (0.17 M Citric Acid and 0.33 M $K_2HPO_3 \cdot {}_3H_2O$, pH = 5.00). The solution was adjusted to pH 5.00 with HCl and KOH. Final solution was made to 100 ml with ddH_2O. The stock solution was filter sterilized, stored at 4°C and was kept sterile. Serial dilutions of GA stock were prepared in 1x Citrate Phosphate Buffer (1.7 mM Citric Acid and 0.33 mM $K_2HPO_3 \cdot {}_3H_2O$, pH = 5.00), stored for one week at at 4°C and were discarded afterwards.

A.3. Seed Production Methods

A.3.1. A General Outline of Seed Production

Use of fresh seeds is preferred in dormancy cycling experiments. Ideally, the schedule of seed production, especially the onset of reproductive phase (the period after bolting) should coincide to perform healthy comparisons between backgrounds. If it is not possible to synchronize the bolting times, the rest of the seed production conditions must be as uniform as possible. Conditions in maternal life cycle can affect the resulting seed characteristics, i.e. dormancy (see Literature Survey).

All seeds used in this study were produced in the same growth chamber. In all experiments, seed batches were chosen to provide comparability between backgrounds. The following growth chamber parameters were controlled; day-night cycle duration and temperature during each phase, humidity and light intensity. Growth conditions were similar between seed batches (except for seed batches #1, #6, and #7) and were standardized (Table A.1). Significant modifications were deemed necessary afterwards to decrease bolting time.

The growth chamber was programmed to a 16/8h day/night cycle with a diurnal range of 22/16°C at 50% relative humidity. Seed batches #1, #6 and #7 were produced with a diurnal range of 18/14°C, instead. The Light Emitting Diodes (LEDs) of growth room (in-built lighting system) were designed to support vegetative growth. To decrease bolting times, a supplementary lighting system was installed during the production of seed batches #1, #6 and #7.

As the installation date of supplementary ligthing system (02.02.2022) coincided with the production of seed batches #1, #6 and #7, the plants experienced two different light intensities during their growth. Before the installation, #1 and #6 experienced 200 μmol/m^2s light while #7 experienced 250 μmol/m^2s, only from the in-built lighting system. After the installation, the intensity of in-built lighting system was decreased to 168 μmol/m^2s for all three batches.

All other batches used in this study were grown after the installation of supplementary lighting system. The light dose was standardized for these batches. The in-built lighting system contributed 220 μmol/m^2s. The supplementary lighting system was composed of a couple of dimmed 25W incandescent bulbs which contributed 30 μmol/m^2s and of a 1 W Blue-LED (Cata CT-4071, Uğur Aydınlatma İstanbul, Türkiye). The dimmed incandescent lights mainly provided far-red wavelengths missing in the spectrum of in-built light system. The supplementary lighting system turned on earlier and off later than the in-built lighting system by 30 minutes to simulate sunrise and sunset.

Sterilized seeds were initially sown in separate containers rather than directly on soil. A dormancy breaking treatment was applied by scarification (rupturing of the seed coat with a syringe needle), cold stratification in the dark, nitrate treatment, GA treatment or a combination thereof. Applied sterlization method and dormancy breaking treatment are detailed in the relevant subsubsections.

Following the dormancy breaking treatments, seeds were germinated in the growth chamber (except for CB-Cvi, CB-Bur and RILs). All seeds were ensured to germinate before commencing with the production of next generation to prevent an artificial selection on dormancy levels.

A soil mixture was prepared prior to the date of seedling transplantation. Soil mixture was filled in cells of 5 and 6 cm in diameter and height, respectively (except for CB-Cvi, CB-Bur and RILs, where cell height was 4,5 cm). Cells were filled to the top with the soil mixture, placed in trays and watered from below. For all experiments a 3:1 part mixture of Klassmann Potground H90 and vermiculate (Serakulit, Turkey) were used (except for batches #1, 6, 7, 11 and 12, where Klassmann TS1 was used instead).

Each cell was sprayed with water before the transplantation of seedlings. This facilitates the transplantation procedure and aids the seedling survival. A single seedling was gently transplanted to each cell using forceps. The root should be submerged gently under the soil surface. If seedlings wilted or were weak, they were gently laid on top of the soil surface with minimal manipulation to prevent damage. The trays were covered with a layer of cling film for 3 days to preserve moisture at the soil surface. The plants were watered from below 2-3 times each week until the maturation-drying stage.

Once bolted, plants were reproductively isolated from each other. This was accomplished using the Ara-system (BETATECH BVBA - Ghent, Belgium) and an equivalent imitation system developed by the Seed-Lab. The imitation system was composed

of 0.33 ml plastic soda bottles as bases and 40 cm long tubes made from 100 µm thick transparent polyester sheets (Clear polyester film F102KRE, Ankara, Türkiye). The plants were pruned if they exceeded the height of the tubes.

When plants entered the maturation-drying stage (when two-thirds of the siliques turned yellow), watering was stopped. Plants were allowed to dry down for at most three weeks. Seeds were harvested by hand-threshing. For bulk collections of over 15 plants of a single genotype seeds were gathered in an envelope. Seeds were dried further for one week to limit after-ripening time. This drying was performed at RT (in the case of #1, 6, 7, 41 and 101).

If a lower number of plants were present, seeds were directly collected into an Eppendorf tube. In this case, perforated Eppendorf caps were used to dry seeds for a week over saturated $Ca(NO_3)_2$ solution ($\approx$ 55%RH) at 20°C (#11 and 22 [in envelopes]) or in the growth chamber (#55 and 57 [in envelopes], RILs, CB-Bur, CB-Cvi, Mutant F3 and Mutant F4). The seeds were then stored at -20°C until further use.

For ease of comparison between seed batches, details of seed production schedules are listed in Table A.1. The general procedure described above were followed in the production of all seed batches. Details about the sterlization method, the combination of dormancy breaking treatments and the container specifications are provided in the following subsubsections.

A.3.2. Seed Production of Ecotypes

Several seed batches of Burren (Bur) and Cape Verdi Isle (Cvi) and a single batch of Columbia (Col-0), ecotypes of *Arabidopsis thaliana*, were produced (Table A.1). Surface sterilized (Subsubsection A.1.1) seeds were sown in 9-cm Petri dishes or 8x12x3.5-cm polystyrene boxes (Hİ-PAŞ PLASTİK – Istanbul, Türkiye) containing two layers of Whatman 3MM chromatography paper (Interlab, Türkiye) and 5 or 8 ml of water respectively.

Table A.1. Seed production schedule.

Batch / Ecotype	**Sowing Date**	**Transplantation Date**	**Bolting Time (Days)**	**Harvest Time (Days)**	**Maturation Drying (Days)**
#1 / Cvi	19.10.2021	15.11.2021	44	127	14
#6 / Bur	18.10.2021	8.11.2021	87	149	14
#7 / Bur	18.10.2021	1.11.2021	95	164	14
#11 / Cvi	10.05.2022	2.6.2022	36	100	14
#22 / Bur	10.05.2022	20.05.2022	63	132	14
#41 / Cvi	28.09.2022	10.10.2022	37	109	14
#55 / Bur	28.10.2022	7.10.2022	63	165	14
#57 / Bur	18.10.2022	7.10.2022	63	167	16
#101 / Col-0	9.02.2023	17.02.2023	33	104	21
CB-Bur	29.07.2022	7.09.2022	60.33	119.67	7
CB-Cvi	29.07.2022	7.10.2022	17.33	87.67	7
RILs / Cvi x Bur	see Table A.3	see Table A.3	see Table A.3	see Table A.3	7
F3 Mutant - Col-0	27.06.2023	05.07.2023	Not recorded.	105	14
F4 Mutant - Col-0	17.10.2023	24.10.2023	27 for WT	97	14

When dormancy breaking was required 5 or 8 ml (for petri dishes or boxes, respectively) of 50 μM GA or 10 mM KNO_3 were used, alternatively. Following scarification (#1, #6 and #7), and cold stratification (#1, #6, #7, #11, #22, #41, #55, #57 and #101) seeds were germinated in the growth chamber. The remaining details on the growth schedule and the characteristics of each seed batch are in Table A.1.

A.3.3. Seed Production and Characterization of RILs

Seeds of F8 generation of the Cvi x Bur RILs (180 RILs with 2 parental lines) were obtained as the starting material [16]. An F9 population was grown using these seeds. Fresh seeds of the F9 generation were used in the genetic screening of dormancy cycling behavior in this unique population.

Seeds were dry sterilized with chlorine gas and then were stored at -20°C. Seeds were imbibed with sterile de-ionized water. Six seeds per line were plated on sterilized glass petri dishes (5.5 cm in diameter) containing a solid media (0.7% Agarose w/v, half-strength MS salts, pH $\approx$ 5.8) under laminar flow hood. Plates were sealed with parafilm and kept sterile until transplantation. No major contamination during incubation was observed.

To break dormancy, seeds were cold stratified at 5°C/dark for 3 days. Seeds were then germinated at 15°C/light. Seeds that did not germinate were treated with sterile 5-10 μl of 500 μM GA stock solution (Subsection A.2). After all seedlings produced first true leaves, three seedlings per line were randomly selected and transplanted to three distinct blocks in the growth chamber with identical setups. Positions of RILs were randomized within each block to ensure randomization of position related effects on seed production and neighbour interactions.

The seeds were sown on two separate occasions and the resulting seedlings were transplanted in seven different batches (Table A.3). Replacements were made in the week following the transplantation if any seedling did not survive. Details of seed pro-

duction schedule and additional information on different batches of RILs are detailed in Table A.3.

Table A.3. Seed production schedule of RIL batches.

Sowing & Transplantation Date - (No. of Lines)	Survival Rate (%)	Mean Bolting & Harvest Time (Days)
29.07.2022 & 17.08.2022 - (45)	95.56	44.27 & 105.1
29.07.2022 & 22.08.2022 - (45)	95.81	44.34 & 105.86
29.07.2022 & 24.08.2022 - (45)	93.33	45.91 & 107.23
29.07.2022 & 7.09.2022 - (32)	70.48	44.56 & 107.17
29.07.2022 & 13.09.2022 - (9)	97.67	46.59 & 105.67
28.09.2022 & 15.10.2022 - (11)	81.25	60.63 & 128.03
28.09.2022 & 22.10.2022 - (3)	36.36	36 & 87.25

No individuals survived the transplantation in a few lines that were sown in the first date (29.07.2022). These non-surviving lines composed the bulk of lines that were sown on the later date (28.09.2022, Table A.3). These lines differed significantly in mean bolting and harvest time (Table A.3). At the end, at least one plant from 179 RILs survived to harvest. There was only a single line that did not respond to any dormancy-breaking treatments.

Plants from each block represented independent biological replicates for the characterization of vegetative and reproductive traits measured during the life-cycle of the F9 generation. The following characteristics were recorded for each plant at the time of bolting. Bolting was determined as the initiation of the floral meristem and its extension to a height of 1 cm. Bolting time was recorded as the difference in time from transplantation to bolting. Rosette diameter was averaged by taking three different measurements across the rosette. Lastly, the number of green rosette leaves were counted at the time of bolting.

Seeds from each block were harvested individually and the following characteristics were performed for each plant at the time of harvest. The plant height, the number of primary inflorescences and the seed yield were measured. Seed yields were calculated by subtracting the weight of seed filled Eppendorf tube from the average weight of 10

empty Eppendorf tubes.

A.3.4. Generation and Verification of *dog1-2/mft2* Lines

Two mutant lines *dog1-2* and *mft2*, in Col-0 background, were crossed to generate double mutant lines over three generations. As mutant lines are compromised in PD, only cold stratification was applied at the first generation. However, none of the seeds survived the transplantation. Therefore, only in this case, seeds were directly sown in the soil after surface sterilization. Surviving seedlings were thinned down to a single seedling per pot. The same growth conditions were used in every generation.

For all crosses, pollens of *mft2* were used to pollinate emasculated flowers of *dog1-2*. Flowers of *dog1-2* were emasculated under light microscope by removing all anthers of a flower before the canonical stage 13 at which the anthesis occurs during flower development. Emasculated flowers were marked with an indelible pen and left for two additional days to mature. Maturation of the female organ corresponds to the erection and the whitening of the papillae on the stigma. A mature anther was plucked from an *mft2* plant and was lightly brushed against the naked stigmatic papillae to perform cross fertilization under the light microscope. After the verification of fertilization by the silique growth, *dog1-2* plants were continuously stripped of all non-emasculated flowers. Developing siliques were monitored and F1 seeds were harvested by cut-dropping the marked siliques into vials after a week of maturation drying.

In F1 generation, some plants were genotyped using the Polymerase Chain Reaction (PCR). Heterozygosity was observed at the *MFT* locus, verifying cases of successful cross-pollination. F2 seeds of genotyped plants were collected by hand-threshing, only. PCR primers and thermal cycle used in the verification are provided in Tables A.4 and A.5 in the Appendix section. *mft2* contains a T-DNA insertion, while *dog1-2* contains a point mutation leading to a premature stop-codon. Genotyping requires only agarose gel electrophoresis to discriminate mutant and wildtype (WT) fragments. However, genotyping for *DOG1* requires a digestion by MseI restriction enzyme to

discriminate mutant and WT fragments.

Table A.4. Primers used in the PCR verification of mutants.

Locus	**Primer Name**	**Primer Sequence**
DOG1	dog1-2_Mse-F	5-TTCTTTAGGCTCGTTTATGCTTTGTGTGGTT-3
DOG1	dog1-2_Mse-R	5-CTGACTACCGAACCAAAAAATTGAATTTAGTC-3
MFT	MFT-F	5-TCTAACCGAAAGCACCATGTC-3
MFT	MFT-R	5-CCGATGAGCTTTACACTCTCG-3
MFT	LBb1.3	5-ATTTTGCCGATTTCGGAAC-3

Table A.5. Thermal cycle used in the PCR verification of mutants.

Cycle Repeat	**Time (min)**	**Temperature(°C)**
1	4	95°C
40	0.5	94°C
40	0.5	57°C
40	1	72°C
	Return to row 2	
1	5	72°C

PCR screening detected no double homozygous mutants among thirty-six F2 plants. However, a plant was a homozygous mutant for *DOG1*, and heterozygous for *MFT*. The seeds of this plant were used in the production F3 generation.

Twenty-six F3 seedlings were screened using PCR and 9 were homozygous mutants for *MFT*. Three of these nine lines were checked for *DOG1* by PCR and expectedly all were found to be homozygous mutants.

The verified backgrounds were used in the seed production of *dog1-2/mft* double mutant lines. The seeds of F3 generation were used in the M-pilot experiment (Figure 3.5) to obtain an initial characterization of double mutants during the growth of F4 generation (Section A.3.5).

A.3.5. Seed Production of Mutant Lines

Finally, the F4 generation was grown from the seeds of double homozygous lines together with control lines (Col-0, *dog1-2* and *mft2*). The seeds were sown on two layers of Whatman 3 MM chromatography papers moistened with 8 mL of 10 mM KNO_3 in polystyrene boxes. After two days of cold stratification in the dark, the boxes were taken to the growth chamber for germination. Once all seeds have germinated, the seedlings were randomly selected to be transplanted. Seeds collected from F4 generation were used in the R-Experiment and RNA isolation (Figure 3.5).

A.4. Dormancy Cycling Setup for Physiological Experiments

Dormancy cycling under laboratory conditions consist of two main stages: an incubation and a germination test.

Before and during incubation period, all procedures were carried out in the dark under a green safe light (1 W green LED Cata CT-4071, Uğur Aydınlatma, İstanbul / Türkiye). Seeds were surface sterilized (Subsection A.1.1) and were sown in polystyrene boxes (8x12x3.5cm) (by samples of 6 x 40) containing an osmotic solution (PEG-8000). Setups of incubation containers and the adjustment of water potential of osmotic solutions are described in the Subsubsection A.4.1.

Boxes were wrapped in two layers of aluminium foil and placed in temperature-controlled incubators. Boxes were moved between incubators to inititate dormancy cycling depending on the scheme of the experiment. When required, samples were transferred to boxes containing fresh osmotic solutions. Temperature and osmotic

shifts that occur during these manipulations were designed to simulate the natural environment. Optimal conditions for the initiation of dormancy cycling differed between ecotypes.

During germination tests, samples were exposed to continuous light in a suitable temperature for germination (the definition dormancy requires absence of germination under favorable conditions). In addition, a dormancy breaking treatment was usually applied with GA or KNO_3 solutions. The response to these treatments during dormancy cycling was tracked. Tracking temporal change in a single condition provides information on the dynamic shifts in the range of germination conditions corresponding to dormancy cycling. Ideally, germination should be tracked in more than one condition, but logistically this is not practical.

The details of incubation and germination conditions, and the experimental design are detailed in the relevant sections.

A.4.1. Configuration of Incubation Containers

A lattice was formed at the bottom of polystyrene boxes (8x12x3.5cm) to hold the osmotic solution with plastic glass drying mats (Gondol Plastik - İstanbul / Türkiye) (Figure A.1). The lattice was filled with 25-29 ml osmotic solution. For denser liquids at lower osmotic potential, it is safe to use higher volumes. At higher osmotic potential, increased fluidity of the solution can lead to mixing of different genotypes or samples can be lost in the solution reservoir. Osmotic potentials of solutions are calculated using

$$\mathbf{\Psi} = (1.30[PEG]^2\mathbf{T} - 137[PEG]^2)/10 \tag{A.1}$$

where $\mathbf{\Psi}$ is the osmotic potential in MPa and $\mathbf{T}$ and [$\mathbf{PEG}$] are incubation temperature and Polyethylene Glycol-8000 concentration per gram water, respectively [140].

On top of a glass mat, a coarse nylon mesh with an opening of 1 mm was placed. The coarse mesh supports and equally distributes the weight of a single layer of Whatmann 3 MM chromatography paper on the lattice creating multiple contact points with the reservoir. The volume of the solution was more than 12 times the weight of chromatography paper to minimize the matrix effect of the chromatography paper [141]. Chromatography papers were autocleaved for long incubation periods. Using a lead pencil, the papers were labelled and divided into multiple sectors to place multiple samples prior to autoclaving. Seeds were sown on top of a 163 μm 43T grade polyester (BİTEKS TEKSTİL - Bursa, Türkiye) fine mesh (Figure A.1). A single fine mesh typically constitutes a single sample. Up to six samples were placed in a box.

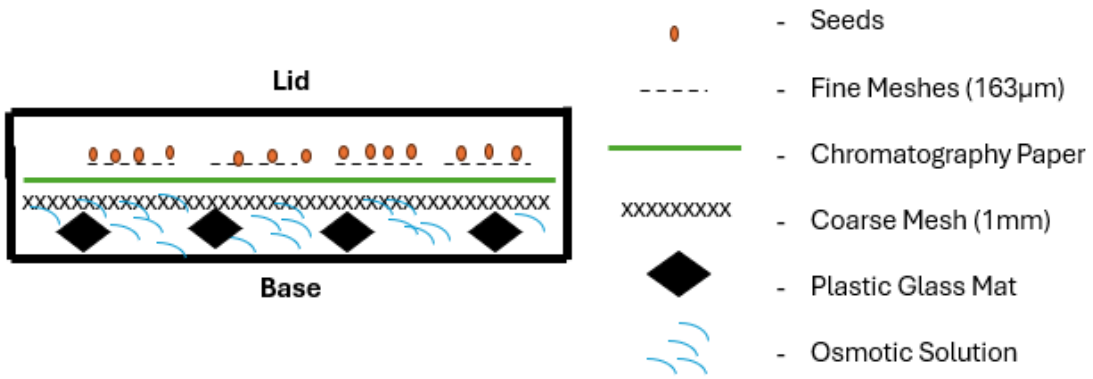

Figure A.1. Layout of an incubation box. (The experimental setup is adapted from [69]).

A.4.2. Configuration of Germination Containers

Samples from an incubation container were distributed to multiple germination containers at the time of transfer. Therefore, two samples from the same incubation box were not placed in the same germination box. If multiple lines are present, samples from each of them can be transferred to a common box. When hundreds of samples are present as in the case of dormancy cycling screening of mapping population, the transfer of samples to germination boxes was randomized. These practices ensure the

independency between replicates.

For germination incubators, two layers of Whatmann 3 MM chromatography paper, 7x10,5 cm for boxes or 7 cm in diameter for petri dishes, were saturated with ≈8 ml or ≈4 ml of germination solutions, respectively. Samples on mesh were transferred using a forceps to germination boxes. During a transfer, residual osmotic solution can be removed by gently running the mesh over the lip of donating box.

All germination tests were performed in temperature controlled incubators (NUVE TK120 - Ankara / Türkiye) under constant light. The light source consisted of 5 vertically situated fluorescent lamps (Philips MASTER TL-D Super 80 18W/865). Germination boxes were shuffled between different spots within the incubator each day.

A.4.3. Germination Scoring

Germination is defined by rupturing of endosperm by the embryo (germination *sensu stricto*). This typically occurs when the radicle penetrates through the micropylar endosperm (>99% of the time), but occasionally embryo was observed to "explode" out of the seed coat.

During germination scoring, seeds were removed from the containers upon germination. Observations started on the day of the transfer. Seeds that already germinated by the time of transfer (during the incubation period before exposure to light) are referred to as Dark Germinants. Frequency of observations to record germination and the extent of germination test were determined according to test temperature and sample behavior. Germination was recorded at least daily.

Liquids may evaporate from containers during the germination period. The saturation of chromatography papers was preserved by adding in de-ionized water as required (when saturation of paper was visibly lost).

At the end of observation period, ungerminated seeds were checked for viablilty. Scarification, cold stratification in the dark and GA treatment were applied in combination. Seeds that did not respond to these treatments were checked for seed hardness with forceps. If the embryo was intact these seeds were also counted as viable. Soft and infected seeds were counted as unviable and were not taken into account during the data analysis. Viability indicates potential to germinate in alternative but untested conditions due to the state of dormancy.

A.5. Configuration of Other Physiological Experiments on Ecotypes

A.5.1. Extended 25°C Experiment

To observe Cvi dormancy cycling behavior under long incubation conditions at 25°C, samples were incubated for up to 69 days in the dark at four different osmotic potentials; 0.0 MPa, -0.4 MPa, -0.8 MPa and -1.2 MPa (Figure A.2). Seeds from batch #1 were used (Table A.1). Germination was tested at 20°C under constant light with three different solutions; buffer control, 0.5 mM KNO_3 in buffer and 10 μM GA in buffer. Germination was scored for 21 days. Samples were equally distributed to germination containers at the time of transfer. Three independent replicates were measured for each condition.

A.5.2. Gibberellic Acid and Nitrate Dose Response Assays

Dose response assays were performed to determine the sensitivity of Bur and Cvi two dormancy breaking agents (GA and KNO_3). Seeds from batches #1 and #6 for Cvi and Bur were used, respectively (Table A.1). Serial dilutions of 500 μM GA and 10 mM KNO_3 stocks were prepared with the citrate phosphate buffer (Table A.6). Replicates of 4 x 40 seeds were exposed to constant light at 20°C at each concentration.

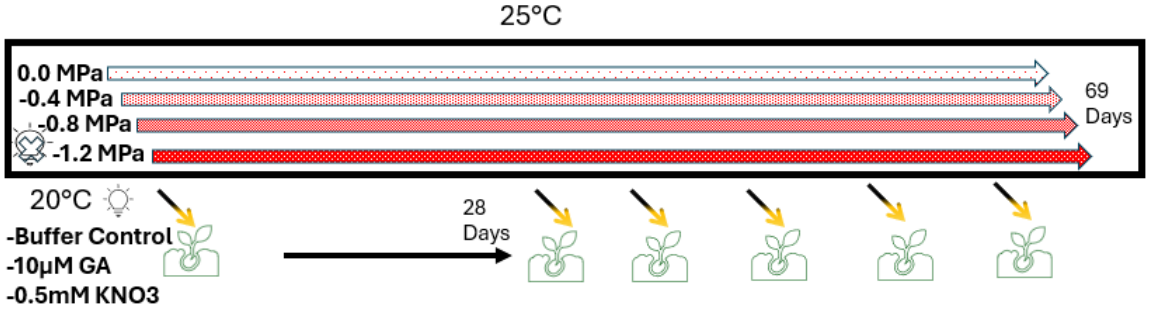

Figure A.2. Scheme of extended 25°C experiment. Samples were incubated at 25°C/dark (black box) for up to 69 days (red arrows) at different osmotic potentials (density of the arrows). Germination tests were performed on day 0 and starting from day 28 onwards at 20°C/light (yellow tipped arrows) in three germination solutions; buffer control solution, 10 μM GA and 0.5 mM KNO_3.

During the analysis, probit equivalents of germination proportions were calculated. Applied doses were transformed to logaritmic scale. Only experiments with total germination within 5% and 95% were used since probit equivalents are misleading outside the range.

Table A.6. Concentrations in dose response assay.

Chemical						
Gibberellic Acid (μM)	0	0.005	0.05	0.5	5	50
KNO_3 (mM)	0	0.01	0.1	0.5	5	10

A.5.3. Dry After-ripening Experiment

Seeds were dry after-ripened at high temperature to assess ecotypic differences in the rate of dormancy loss. Seeds from batches #11 and #22 for Cvi and Bur were used, respectively (Table A.1). Seeds were incubated in the dark at 25°C for up to 84 days (Figure A.3). At weekly intervals, germination was tested at 15°C and 20°C in buffer control using three replicates of 40 seeds. Germination was scored for 21 days.

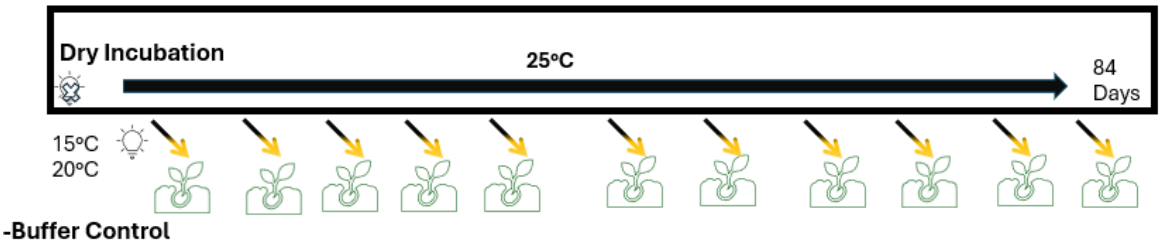

Figure A.3. Scheme of dry afterripening experiment. Seeds were incubated dry at 25°C up to 84 days (black arrow), in the dark (black box). Seeds were germinated weekly at 15°C and 20°C with constant light (yellow tipped arrows) in the buffer control solution.

A.6. Light Treatments for Assaying Wavelength Responsivity

Samples were subjected to different light treatments before germination in the dark. In the no light treatment (NL), samples were not exposed to light. In the white light treatment (WL), samples were incubated under constant light. In the red light treatment (R), samples were treated with 15 minutes of 27 μmol/m^{2}s 660 nm red light after 4 hours of dark incubation. In the far-red Light treatment (FR) , samples were treated with 15min of 2-3μmol/m^{2}s 730 nm Far-red light after 4 hours of dark incubation. In the FR-R treatment, FR treatment was followed by R treatment after 4 hours of dark incubation.

A.7. Data Analysis of Germination Experiments

Three main germination indices are used to describe the germination behavior (Figure A.5). Dark germination was calculated by dividing number of dark germinants at the time of transfer by the number of viable seeds at the end of scoring. Dark germinants are excluded in the calculation of remaining indices.

Figure A.4. Light treatments in the wavelength responsivity experiment. The time axis is not on scale. Samples were incubated in the dark (black) for 4 days before scoring germination. Before the dark incubation, samples were subjected to different light treatments 4 hours after seed imbibition.

Total germination percentage is calculated by dividing the number of germinants in the incubation period by the number of viable seeds. Area under curve (AUC) was calculated using trapz() function in the pracma package [142]. AUC corresponds to the integration of the germination curve over time the AUC contribution by the dark germinants (Figure A.5). AUCs were normalized by the maximum theoretical (Total Number of Seeds * Observation Days). The trapz() function performs trapezoidal integration and the value represents an overall summary of germination behavior.

Finally, germination rate to 50% of viable of seeds was estimated. The germination rate is the inverse of germination time (Figure A.5). If total germination does not reach 50%, the rate cannot be calculated.

The rate estimation by the probit method using glm() in the MASS package [143]. The inverse of time is used as the dose instead of time itself, which empirically provided a better estimate for a higher majority of experiments.

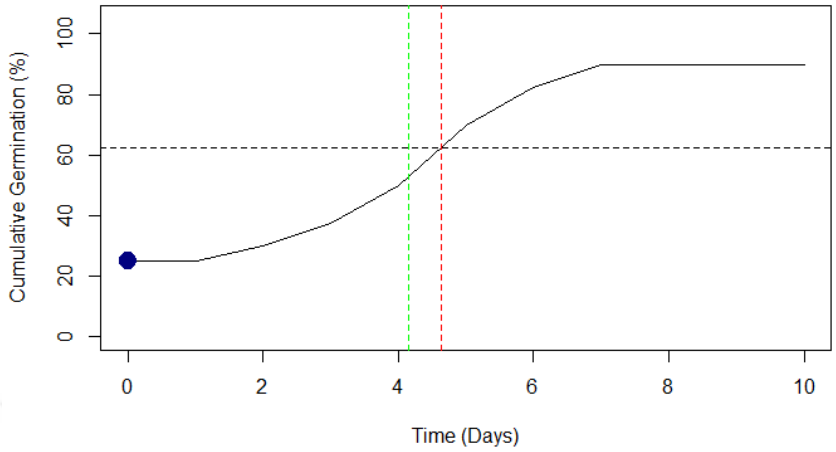

Figure A.5. An Example of Calculated Germination Indices. Dark germination percentage (blue point), time to 50% germination according to linear interpolation (dashed red line) and according to probit estimation (dashed green line), the total germination percentage (end point of the curve) are visualized.

However, if the probit method did not converge or returned an unreasonable estimate (i.e a negative estimate or inverse rate - time to 50% germination - exceeding the length of germination period), the rate estimation function, instead had a fallback to the linear interpolation method.

The linear interpolation method assumes that the distribution of germination is even between two timepoints between which 50% germination is exceeded. The two methods show high correlation (ρ >0.98 for over 1500 experiments performed during genetic screening).

Some examples of exemplary experiments are provided from RIL experiments for different germination behaviors (Figure A.6) and resulting germination indices (Table A.7). The fallback to linear interpolation was cancelled to show discrepancy in a couple of experiments.

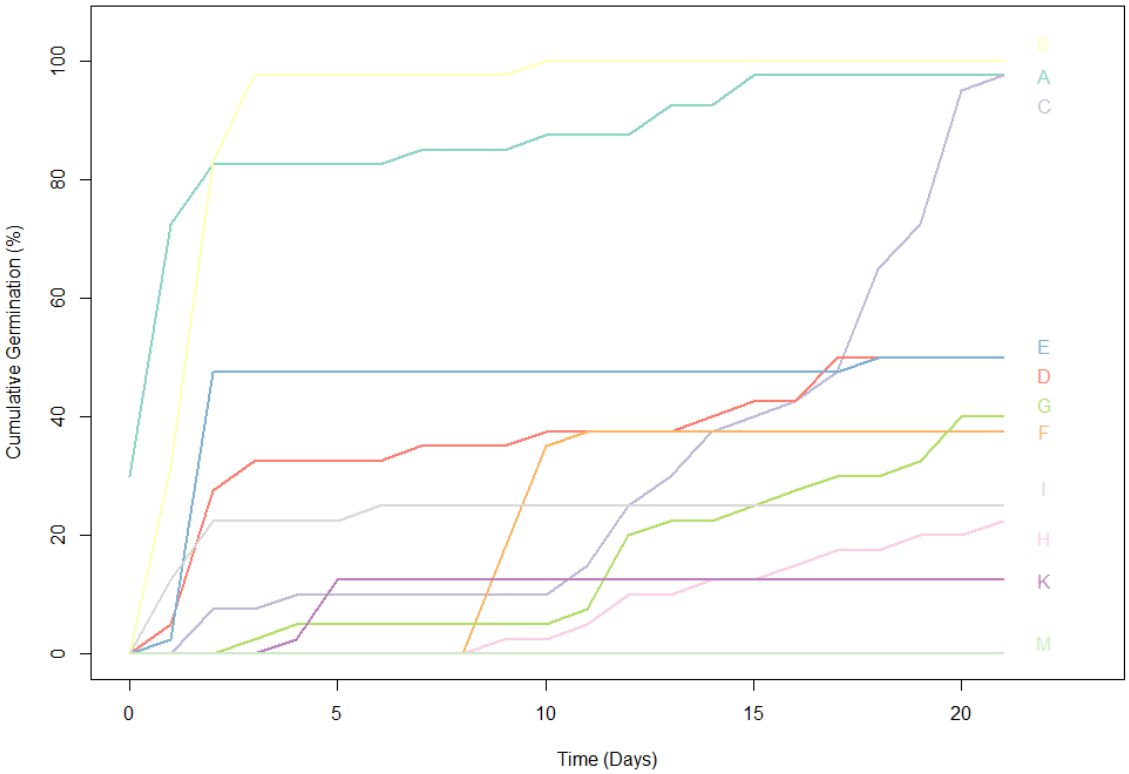

Figure A.6. Exemplary Germination Experiments.

Table A.7. Germination indices of the exemplary experiments. Exemplary experiments are in the Figure A.6

Experiment in Figure A.6	**Dark Germination (%)**	**Total Germination (%)**	**Rate - Linear (day^{-}1)**	**Rate - Probit (day^{-}1**
A	30	96,4	1,21	1,042
B	0	100	0,736	0,838
C	0	97,5	0,058	0,045
D	0	50	0,058	-0,059
E	0	50	0,055	0,055
F	0	37,5	NA	NA
G	0	40	NA	NA
H	0	22,5	NA	NA
I	0	25	NA	NA
K	0	12,5	NA	NA
M	0	0	NA	NA

A.8. Weighted Gene Co-expression Network Analysis

Data under the Bioproject numbers of PRJEB33535 [20] and PRJNA554414 [24] stored in National Center of Biotechnology Information sequence read archive were used to perform the analysis. The data stored under PRJEB33535 includes a set of transcriptomics data for dormancy cycling of Bur and Cvi ecotypes performed under field conditions over a year. The data stored under PRJNA554414 includes a set of transcriptomics data for dormancy cycling of Ler ecotype performed under field conditions over a year. The RNA-seq data for dormancy cycling of Ler is included to improve sensitivity in detection of modules. The data for Bur and Cvi is not complete due to loss of sequencing data of certain replicates before archival (personal communication by Prof. Dr. Steven Penfield). Particularly, all replicates for May and June where SD release occurs in Bur are completely missed. However mean FPKM values for all timepoints are available for illustration purposes.

Reads were mapped against TAIR10 genome release using HISAT2 [144]. Counts per genomic feature in TAIR10 annotation (version 2.2) were generated using featureCounts [145]. Filtered genes were transformed with varianceStabilizingTransformation() [146]. Resulting values were used to create a signed consensus network between three backgrounds using WGCNA pipeline [147]. Consensus networking is preferred to increase sensitivity in module detection. Ecotype specific modules might be present but they are eliminated in module detection as they are absent in other ecotypes. bicor() from WGCNA package was used to calculate correlations, minimum number of genes in a module was set to 50, mergeCutHeight was set to 0.25 to merge similar modules and other parameters were left at default values during network formation.

Eigengene (first PC of the module gene expression matrix) of resulting modules were calculated using complete FPKM values for Cvi and Bur. Eigengenes were in used in correlation calculations to dormancy and temperature. Three network indices were calculated to identify candidate genes using adjacency matrix of each ecotype. Degree connectivity (calculated by IntraModularConnectivity() in WGCNA package),

and transitivity (transivity() in igraph package with a hard threshold of 0.6) and betweenness centrality (evcent() in igraph package) were calculated [148]. Genes within Peak 5 that also have high figures for these indices were determined as candidates.

A.9. Network Visualization

Network visualization is a computationally demanding procedure, therefore genes were filtered according to degree connectivity (Figure B.17). Genes within top 3500 within degree connectivity were combined at the first filtering step. Topological overlap matrices of two networks for two ecotypes were filtered according to thresholds of 0.20 and 0.28 for Cvi and Bur, respectively. Networks were constructed using topological overlap values. Different thresholds had to be chosen as topological overlaps were overall lower in Cvi. The networks were visualized in Cytoscape (v3.10.2) [149].

A.10. Gene Ontology Analyses

Gene onotology (GO) analyses were performed using topGO package [150]. The GO results were calculated by fisher's test and "weight01" algorithm was used to specificity of results. The algorithm eliminates general parent terms which are less descriptive in favor of more descriptive child terms by comparison of significance values. GO analyses of modules were performed either by including all the genes in the module or by criterion of fuzzy module memberships (250 genes with the highest correlation to the module eigengenes). Top ten GO terms were ordered in significance and summarized for each module in a table format. In the table format, "Annotated" refers to the number of annotated genes associated with that GO-term included in the network construction. "Expected" refers to the number of genes associated with the GO-term expected to be included in the module considering its size. Lastly, "Significant" refers to the number of genes associated with the GO-term found in the module, actually. P-values reported were corrected according to benforenni's method for multiple testing [ref?].

A.11. Other Notes on Data Analysis

Broad-sense heritability H^2 was calculated by dividing genetic variance by phenotypic variance, block effects were observed to be minimal in the characterization data.

APPENDIX B: SUPPLEMENTARY RESULTS

B.1. Results of Other Physiological Experiments on Ecotypes

Rest of the physiological experiments characterized other interesting aspects of dormancy cycling behavior in Bur and Cvi. These results guided the experimental setup and contributed to the interpretation of the results given in the main body of the study.

B.1.1. Dose Response Assay

Bur and Cvi samples both germinate to high extent in the presence 50 μM GA and 10 mM KNO_3 when incubated at 25°C at all timepoints (Figures 4.3 and 4.4). The use of saturating doses of both dormancy breaking agents prevents tracking the changes in sensitivity. A dose response assay was performed to detect more enabling concentration to track changes in sensitivity at the same time in both ecotypes.

The sensitivity to dormancy breaking agents was exponential (Figure B.1). Slopes of the sensitivity curves were not significantly different between ecotypes (Bur-GA: CI[0.20±0.04], Cvi-GA: CI[0.15±0.03], Bur-KNO_3: CI[0.62±0.08], Cvi-KNO_3: CI[0.62±0.05]). Overall, seeds were more sensitive to GA compared to KNO_3. Compared to control treatments, the dose response indicated a threshold effect in all dose response assays. Sensitivity to both dormancy breaking agents was significantly higher in Bur than Cvi (Figure B.1 - lower values in log-dose indicate higher sensitivity and confidence intervals do not overlap). Overall, the results indicate that sensitivity differences in ecotypes depend on the threshold rather than the slope.

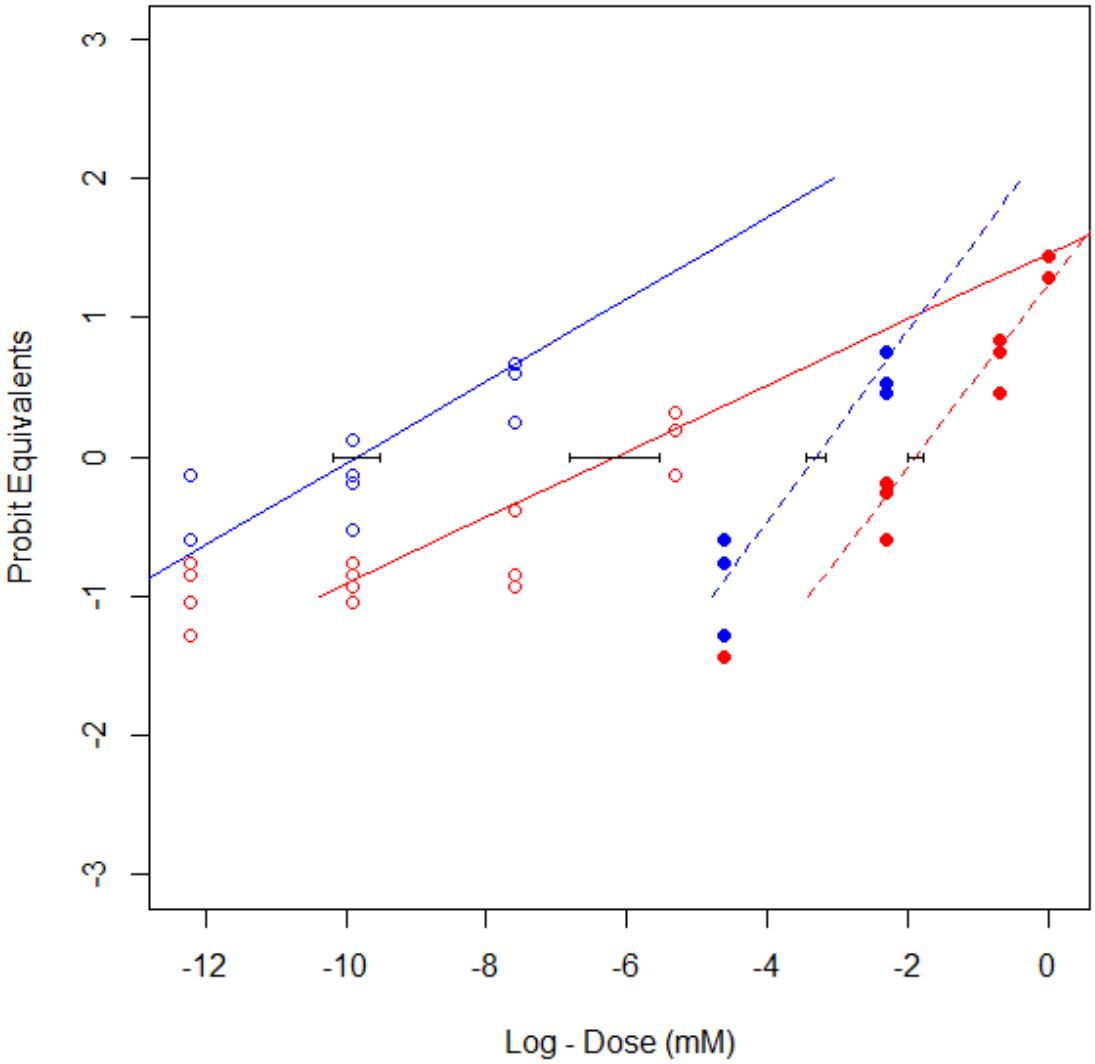

Figure B.1. Dose response assay. Dose responses of Bur (blue) and Cvi (red) ecotypes to GA (empty circles / solid lines) and KNO_3 (filled circles / dashed lines) are shown. Dose required for 50 % germination with 95% CI is estimated. (n = 4)

As a result of the dose response assay, ≈10 μM GA and ≈1 mM KNO_3 were determined as more appropriate to track dormancy cycling behavior of ecotypes. Loss in sensitivity to dormancy breaking agents and the tied changes in the depth or dormancy would be determined more efficiently at such concentrations rather than at saturating ones.

B.1.2. Extended 25°C Experiment

Samples were incubated for longer durations to observe if dormancy release is initiated with a direct incubation at 25°C, without experiencing prior incubation at a lower temperature (Figure B.2). Variation in germination percentages was only observed in 10 μM GA. While in other germination solutions, germination did not exceed 10% during incubation period. Without any incubation seeds germinated about 75% in 10 μM GA (Figure B.2 - Day 0 Controls). During the incubation period germination increased steadily until day 49 in all osmotic solutions (Figure B.2 - Right Pane / Incubation Period). Germination was higher in -1.2 MPa compared to other osmotic treatments. After day 49, germination decreased until the end of the experiment. This suggests that dormancy cycling occurs in Cvi without any transfer between different temperatures.

The results must be interpreted cautiously since error terms were high at all timepoints ($8{,}51 \pm 6{,}70$). The variation cannot be related to any controlled factors. It indicates a strong bet-hedging strategy in response to prolonged high temperature incubation that leads to high variation between samples.

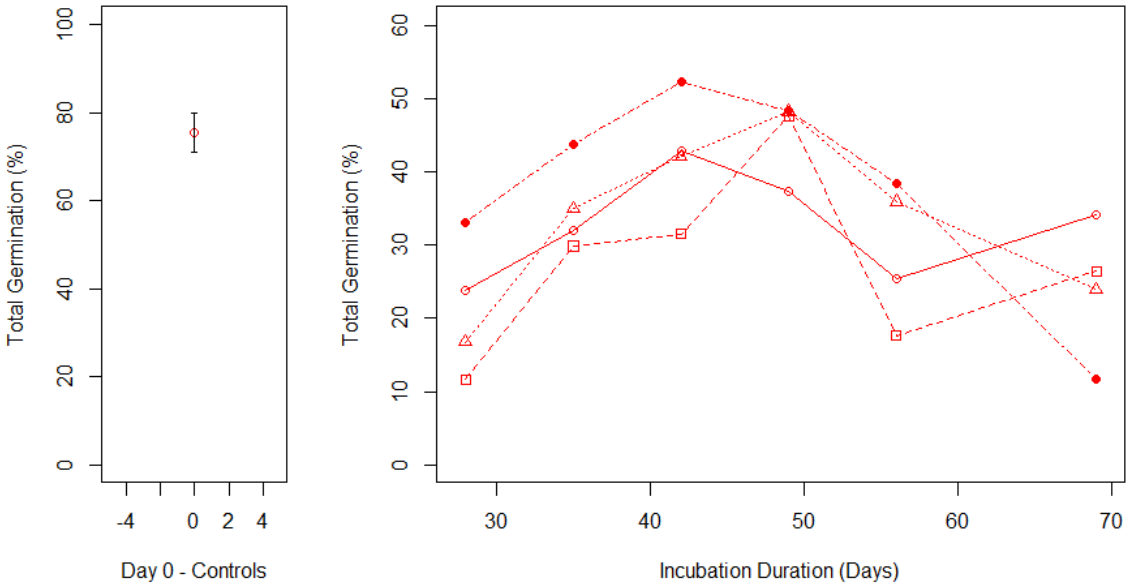

Figure B.2. Cvi samples in the extended 25°C experiment. Germination tests were at 20°C/light in 10 μM GA in buffer control. Incubations were at 25°C/dark at various water potentials: 0.0 MPa (empty circles / solid lines), -0.4 MPa (boxes, dashed lines), -0.8 MPa (triangles, dotted lines), -1.2 MPa (filled circles, dot-dashed lines) for up to 69 days. (Data represents the mean, n=3).

Bur seeds from #6 were also assayed after equivalent incubation conditions in three different germination solutions; buffer control, 0.1 mM KNO_3 and 0.1 μM GA in buffer control. Apart from dark germination in 0.0 MPa (43.61%±1.72), total germination did not exceed 10% during incubation period (Figure B.3). Bur germination did not fluctuate between replicates in any germination solution. Error terms between Bur germination percentages were low across all timepoints during incubation period (1.08±1.54). On the other hand the error term was high when assayed in 10 μM GA at the end of the dormancy cycling treatment (SE >20%, Figure 4.5). This might indicate an uncontrolled factor that leads to divergence in replicate samples during long incubations.

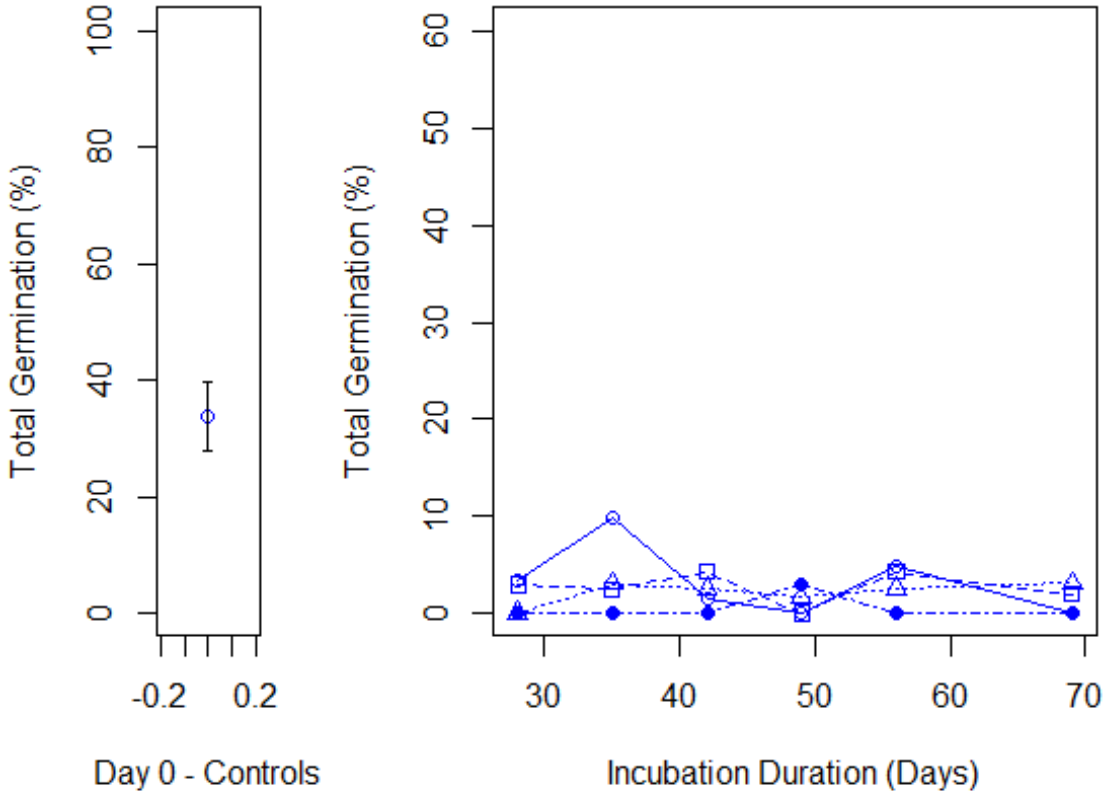

Figure B.3. Bur samples in the extended 25°C experiment. Germination tests were at 20°C/light in 0.1μM GA in buffer control. Incubations were at 25°C/dark at various water potentials: 0.0 MPa (empty circles / solid lines), -0.4 MPa (boxes, dashed lines), -0.8 MPa (triangles, dotted lines), -1.2 MPa (filled circles, dot-dashed lines) for up to 69 days. (Data represents the mean, n=3).

B.1.3. Dry After-ripening Experiment

Data for PD release in Cvi reflects the typical sigmoidal curve that is expected during dormancy release. Bur seeds, on the other hand were high in total germination without AR. During AR, the total germination in Bur did not increase and changes were stochastic. Seeds were still viable hence the decrease was not related to viability loss due to aging. Cvi seeds reached higher total germination figures earlier in 15°C as expected for an ecotype adapted to a winter annual life cycle ($AR_5$0 at 15°C = 32.16±1.44, $AR_5$0 at 20°C = 48.62±1.17). Bur total germination was higher at 20°C than 15°C as expected from an ecotype adapted to a summer annual life cycle, with no effect of AR ($F(1, 70) = [18.44]$, $p < 0.001$).

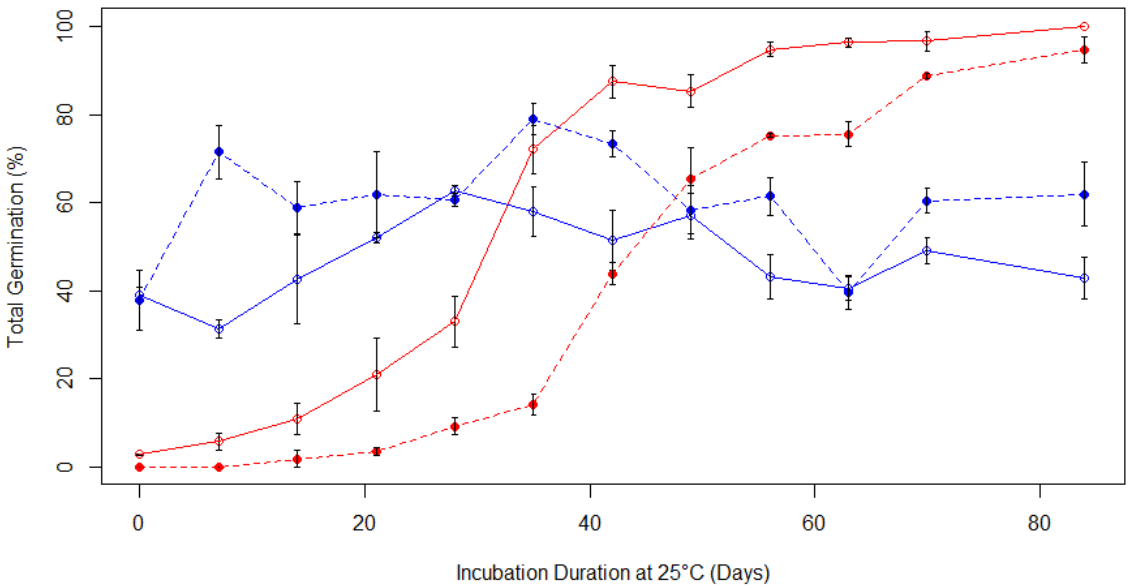

Figure B.4. Dry afterripening experiment. Germination was assayed for Cvi (red) and Bur (blue) at two temperatures, 15°C (empty circles /solid lines) and 20°C (filled circles /dashed lines) in buffer control. Dry afterripening was at 25°C. (Data are the mean ±SE, n=3).

B.1.4. Mutant Dormancy Cycling Experiment - M Pilot

At PD Col-0/WT seeds germinated less than other lines leading to lower AUC values (Figure B.5 - Right). *mft2* seeds germinated a day slower than other mutant lines, rather than less, resulting in slightly lower AUC values. Seeds germinated in full extent in germination tests during 5°C regardless of the background. Col-0/WT seeds were induced into SD quickly after transfer to 25°C and their germination percentages decreased quicker than in mutant lines. AUC values in other mutant lines started to decrease after 10 days of incubation in 25°C. AUC values for *dog1-2* seeds decreased more than *mft2* and double mutant at both temperatures.

Opposite to PD, *mft2* seeds germinated quicker than *dog1-2* seeds during the early period after transfer to 25°C, leading to slightly higher AUC values. For M-pilot experiment, regulation of dormancy cycling indicates that *DOG1* regulation is more dominant in PD, while *MFT* regulation is more dominant during low dormancy periods. In the absence of both genes, a dormancy induction is not apparent under these conditions.

Dark germination was observed only in *mft2* and *dog1-2/mft2* (Figure B.5 - Left). Dark germination was significantly higher in *mft2* lines compared to *dog1-2/mft2* when integrated over the period ($P < 0.05$).

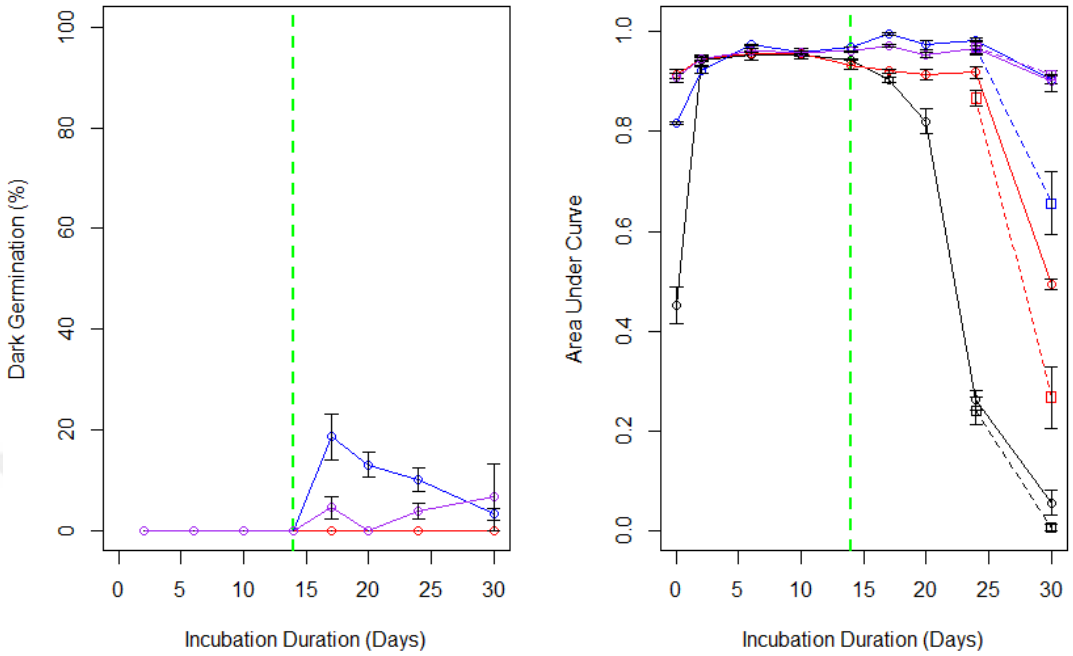

Figure B.5. The M-Pilot experiment. Incubations were at 5°C/dark, -1.0 MPa for 14 days (dashed green lines), then at 25°C/dark afterwards. Germination tests were at 20°C (circles / solid lines) or 25°C (squares /dashed lines) for Col-0/WT (black), *dog1-2* (red), *mft2* (blue) and *dog1-2/mft2* (purple). (Data are the mean, (Left) Dark germination (n = 6) and (Right) AUC values ±SE, (n = 3).

B.1.5. Effect of Dry After-ripening on Wavelength Responses

Seed batches #57 and #101 were used for Bur and Col-0, respectively. These experiments were performed to show that the setup of light treatments were functional and their effects were as expected. Additionally, the minimum length of AR treatment to obtain a response to light treatments was determined. Due to incubator availability light control (WL) was performed discontinuously at two temperatures.

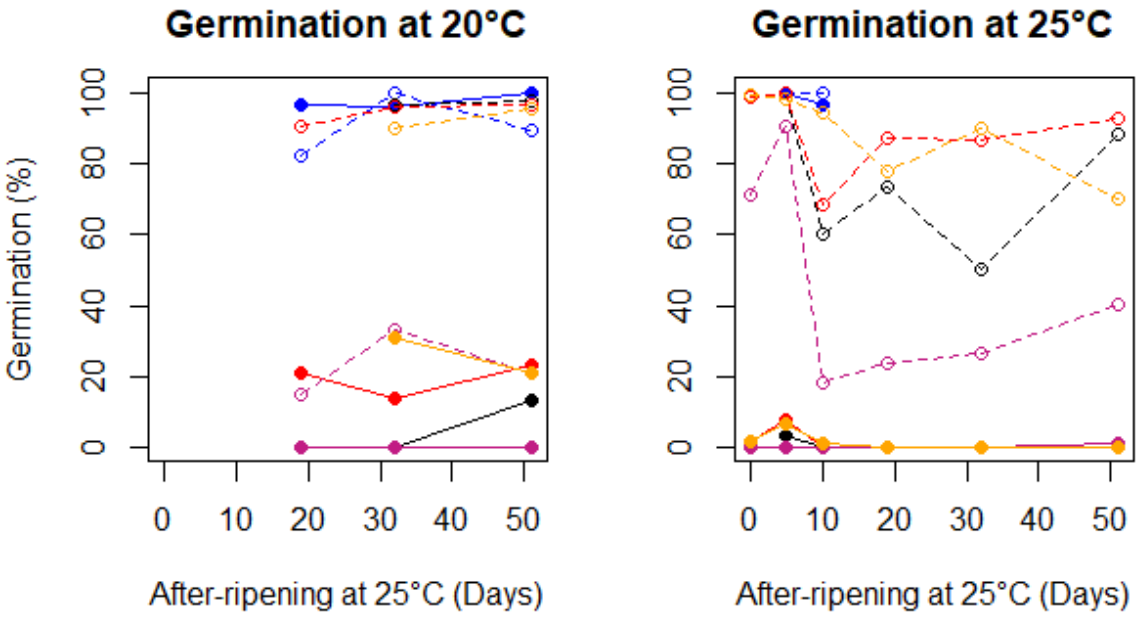

Figure B.6. Wavelength responses of ecotypes during dry after-ripening. Col-0 (solid lines / filled circles) and Bur (dashed lines / empty circles) were assayed for germination (Left) at 20°C/light (Right) or at 25°C/light for increasing durations of AR at 25°C/dark. Light treatments were described in Figure A.4; NL (black), WL(blue), R(red), FR(purple) and FR-R(orange). Data represent the mean (n =3).

Bur seeds required at least 10 days of AR to obtain a sensitivity to FR, which decreases germination (Figure B.6). Dark germination (NL) in Bur at 25°C also decreased with AR. However, dark germination was still at higher than germination after FR treatment, and were at similar levels to R treatment. FR-R treatment restored high germination in Bur. This indicates that the procedure succesfully cycles the state of phytochorome to manipulate germination.

Col seeds did not show any response to R treatment at 25°C, while 100% germination was seen in WL without AR. The germination with R treatment was marginal at 20°C and stable about 25% after 19 days of AR. Dark germination was lower in Col-0 compared to Bur.

B.2. Characterization of RILs at the Bolting

Characteristics recorded at the time of harvest were confounded by constant pruning of RIL plants due to spatial limitations. Height measurements were uniform within the population limited by the length of the material used to perform reproductive isolation (data not shown). Although they are normally distributed, yield measurements are biased due to external manipulations of plant height, hence they cannot be relied on (data not shown). It was assumed that such manipulation did not affect resulting dormancy behavior of the subsequent generation. Since almost all plants were pruned at some point to a certain extent, there were no records kept in detail about the extent of pruning.

All characteristics recorded at the designation of bolting (days from transplantation to bolting, number of green leaves, rosette diameter) estimate approximately the bolting time, either in real or in developmental time (Figure B.7). These traits have a higher relevance in a study concerning dormancy since dormancy and flowering time are regulated by some common genetic factors. Additionally, life cycle phenology concerns both major life cycle transitions which have been shown to be correlating at a certain extent in the nature. Average bolting time was 17.33±2.03 and 60.33±2.40 days for Cvi and Bur, respectively. Average rosette diameter was 25,89±3.23 and 93.33±7.98 mm for Cvi and Bur, respectively. Lastly the average number of green leaves at the time of bolting was 8.33±1.20 and 43.00±4.58 for Cvi and Bur, respectively.

Cvi bolting time was significantly shorter than Bur in both real time and in developmental parameters ($P < 0.012$). However, correlations between measurements were weak and ranged between 0.20-0.63. H^2 was equal to 0.76, 0.60 and 0.69 for bolting time, rosette diameter, and number of green leaves, respectively. High H^2 values for measurements at the bolting time indicated that genotypic factors were as expectedly more relevant than the block, the setups of which were identical, or other uncontrolled effects within the growth chamber. In contrast, H^2 for yield was equal to 0.41. This indicates that manipulations after bolting may have (pruning) led to the

decrease in the contribution of genetic variance.

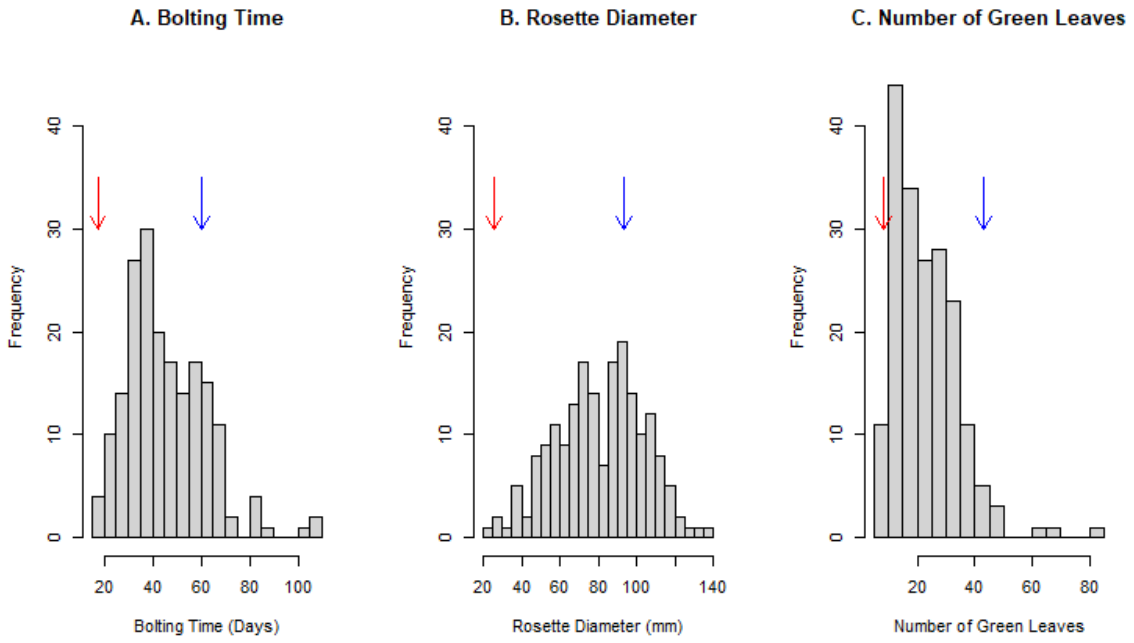

Figure B.7. Characteristics measured at the time of bolting for RILs. (A) Bolting Time, (B) Rosette Diameter and (C) Number of Green Leaves. All traits were measured at the time of bolting. Mean measurements for the parental ecotypes of Bur (blue arrows) and Cvi (red arrows) are at opposite ends of distributions.

B.3. Alternative Versions of Dormancy Cycling Figures

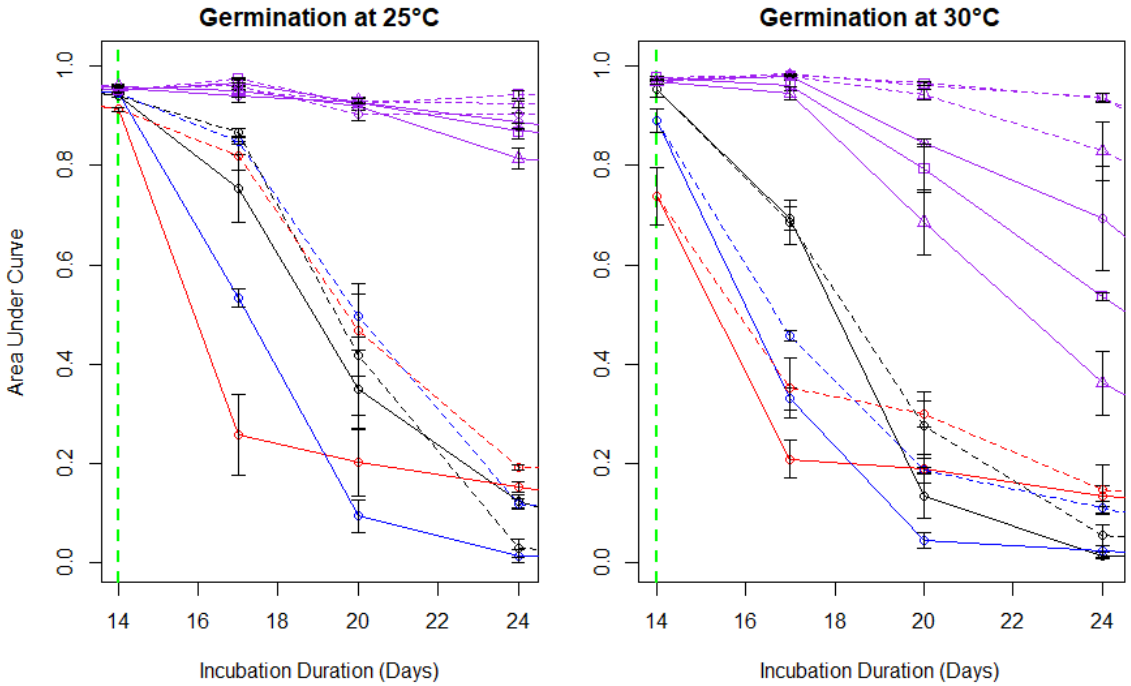

Figure B.8. Zoomed in between the days 14 and 24 in the R-Experiment. The figure caption is the same as in Figure 4.19.

B.4. Detection of Candidate Regulatory Genes within Identified Peaks in QTL Analyses

B.4.1. Determination of *DOG1* Haplotype for Bur Ecotype

The first exon of *DOG1* gene that defines the underlying haplotypes (Figure B.9). In the reference genome, the position of the 3-bp deletion is correct, while it is slightly shifted due to limited number of Bur and Col-0 ecotypes used in sequence alignment. Haplotypes were determined as in [100] using genotypic data in 1001 genomes database [151]. Col-0 and Cvi has D-SY and ECCY haplotypes, respectively (according to [100]). 3-bp deletion that is intergral to formation D-SY haplotype is present in Bur and if missing bases are completed by reference genome, the genomic sequence is the same as Col-0.

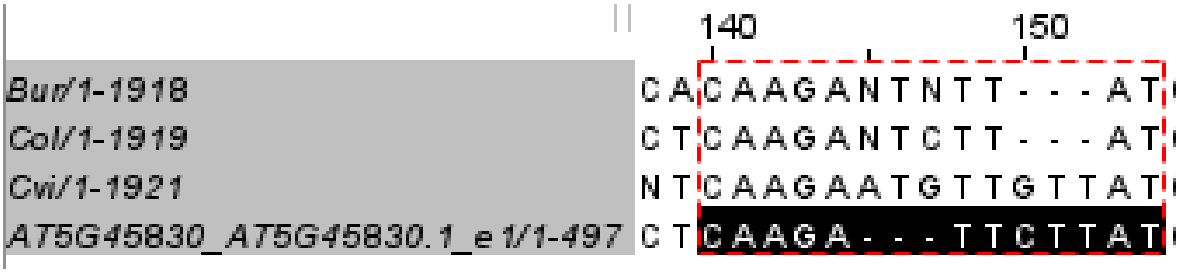

Figure B.9. *DOG1* haplotypes of the ecotypes used in the study. The sequence of *DOG1* in the reference genome is highlighted in black.

B.4.2. Gene Expression of Candidate Genes in Dormancy Cycling in the Field

In the figures B.10 - B.12, a transcriptome data during dormancy cycling in the field was used [20]. Pearson correlation of expression levels to dormancy and temperature is given within the figure. For Bur, the period with base water potentials higher and lower than -0.3 are indicated with purple and green lines, respectively. For Cvi, samples that did or did not require AR treatment are indicated with purple and green lines, respectively. Therefore, high dormancy periods are represented with purple, while low dormancy periods are represented with green.

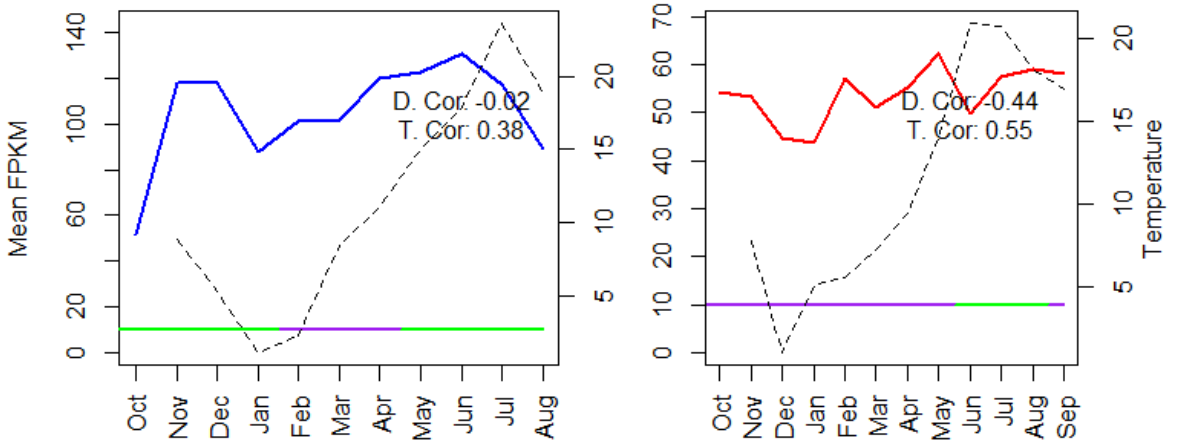

Figure B.10. Expression levels of *ANAC060* mRNA during dormancy cycling in the field. Mean expression levels (in FPKM) of *ANAC060* mRNA in Bur (blue solid line) and Cvi (red solid line) are shown during dormancy cycling in the field. Mean temperatures are provided recorded at the date of sampling (black dashed line).

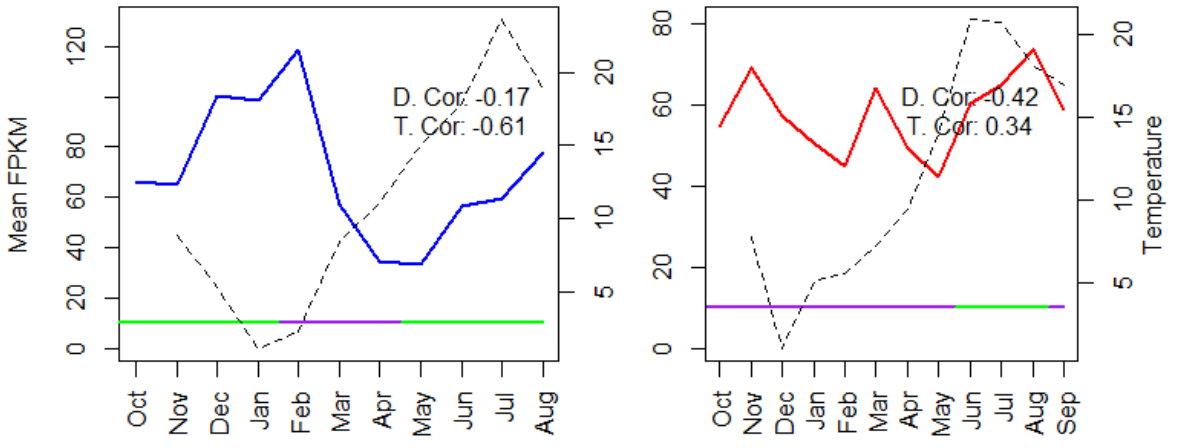

Figure B.11. Expression levels of *SNRK2.2* mRNA during dormancy cycling in the field. Mean expression levels (in FPKM) of *SNRK2.2* mRNA in Bur (blue solid line) and Cvi (red solid line) are shown. Mean temperatures are provided recorded at the date of sampling (black dashed line).

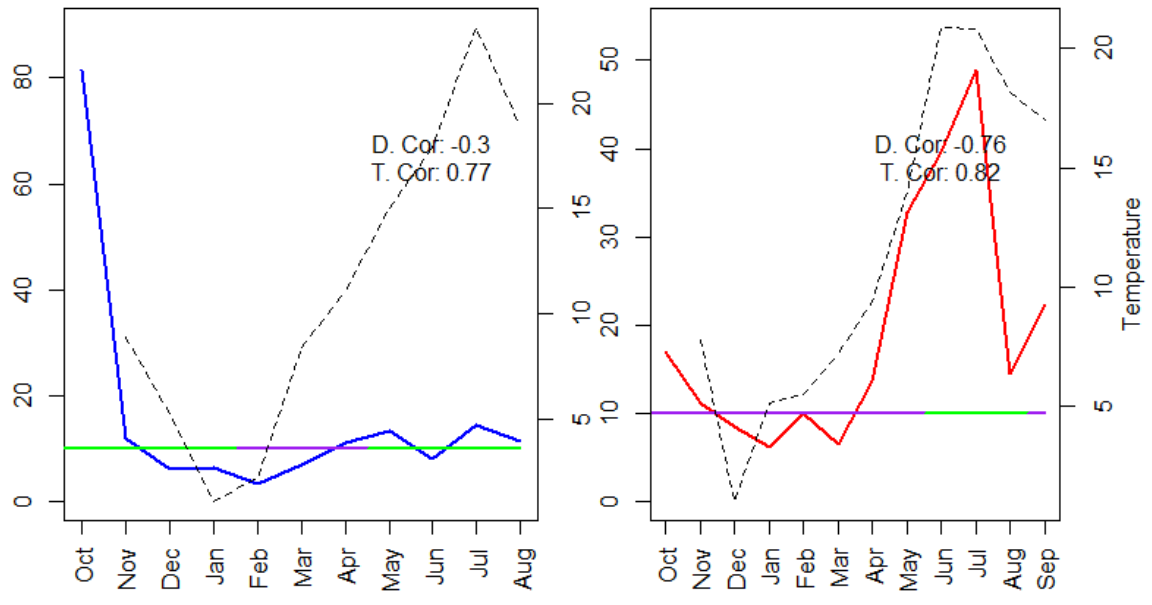

Figure B.12. Expression levels of *ATHB-5* mRNA during dormancy cycling in the field. Mean expression levels (in FPKM) of *ATHB-5* mRNA in Bur (blue solid line) and Cvi (red solid line) are shown during dormancy cycling in the field. Mean temperatures are provided recorded at the date of sampling (black dashed line).

B.4.3. Curated Information on *ATHB-5* in Public Databases

The microarray data in the Figure B.13 is from a series of dormancy cycling experiments in Cvi ecotypes was investigated for the gene expression of the candidate gene, *ATHB-5* [152, 153]. The experimental conditions for are detailed in the Table 1 in Finch Savage et.al (2007) [163]. In summary, the samples on the right in the Figure B.13 represent samples with low dormancy states (LIG, PDLN, PDN, DL, PDL, PDC) and have increased expression values (in red) for *ATHB-5* mRNA. The samples on the left in the Figure B.13 represent samples with high dormancy states (PDD, DDL, PD24h, PD30d, SD1 ,SD2).

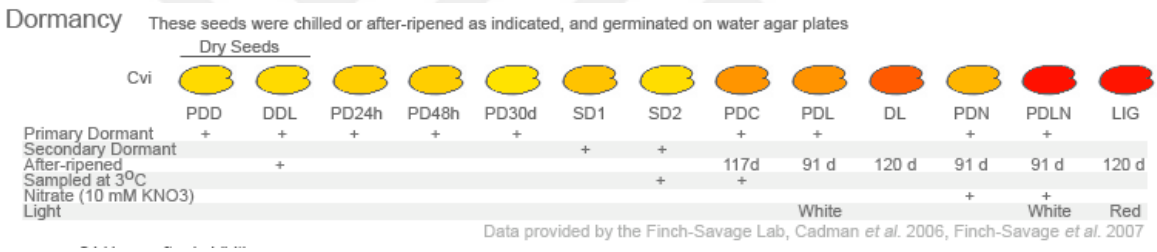

Figure B.13. Expression levels of *ATHB-5* between different dormancy states in Cvi. Data is from [152, 153].

The publicly available network in the Figure B.14 was constructed between a set of germinating and non-germinating samples in the presence of terminating signals (low vs high dormancy states, respectively). This network is different than the network constructed using WGCNA in this paper. The genes upregulated in the set of germinating samples are referred to as SAM-G, and the genes upregulated in the set of non-germinating samples are referred to as SAM-NG.

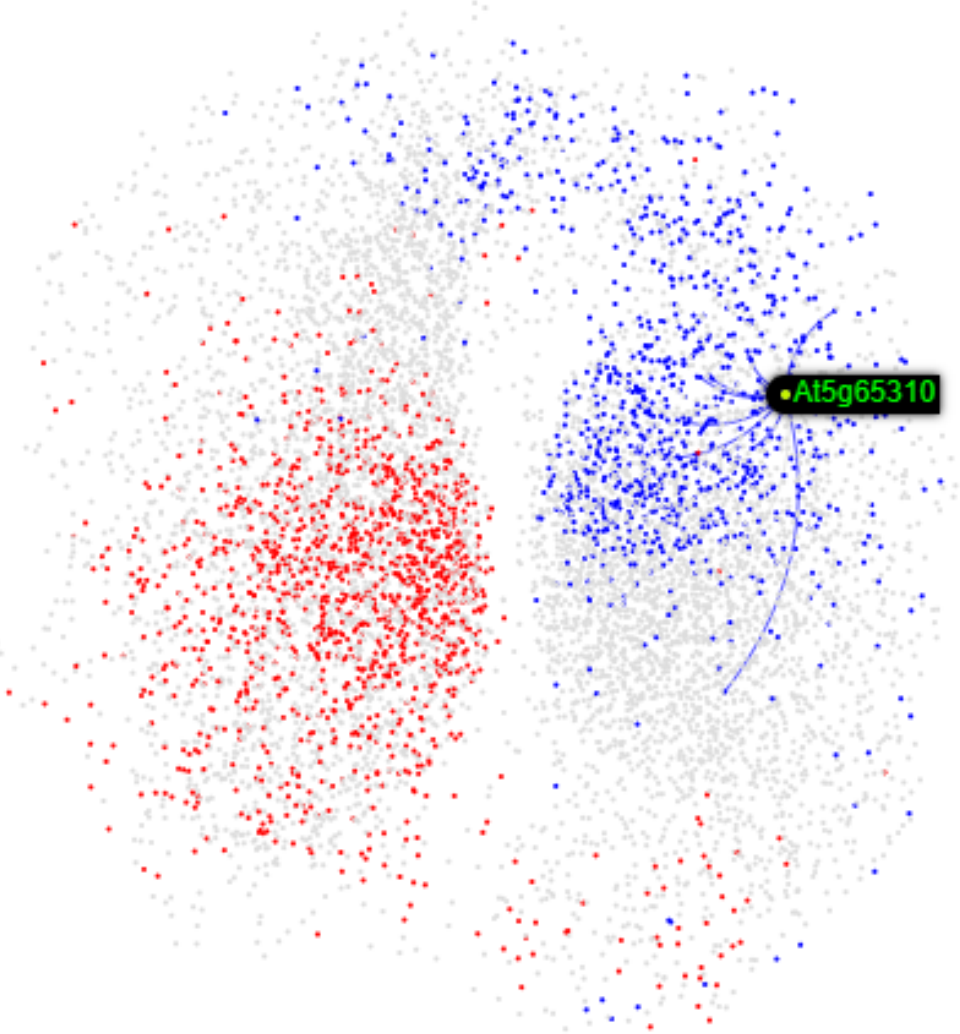

Figure B.14. Position of ATHB-5 in the SeedNet. Genes upregulated during germination and non-germination are highlighted in blue (SAM-G) and red (SAM-NG), respectively. Data is from [134].

B.4.4. Weighted Gene Co-expression Network Analysis Results

The resulting undirected co-expression network after filtering nodes for topological overlap values was composed of 533958 edges between 4235 nodes for Cvi and 681953 edges between 3968 nodes for Bur. The figures B.15 and B.16 are the visualized networks for respective ecotypes on the Cytospace software.

In the figures B.17 - B.19, only the genes included in summary of Bur network (Figure B.16). The results did not differ visually when Cvi network was used instead. Genes with lower degree connectivity in both ecotypes are missing as they were filtered for computational purposes during the network visualization.

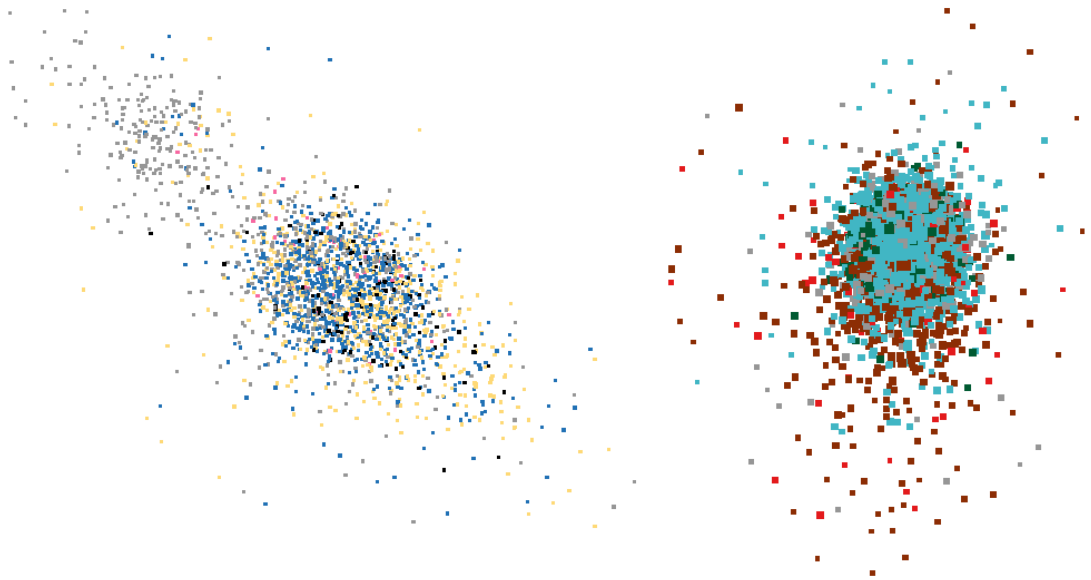

Figure B.15. WGCNA summary for Cvi ecotype. Nodes correspond to genes in the network. Edges were omitted due to visualization purposes, but there were no connections between two visually distinct clusters of nodes. Colors of the nodes represent the modules, while the size of the node correspond to centrality of the node in Cvi network.

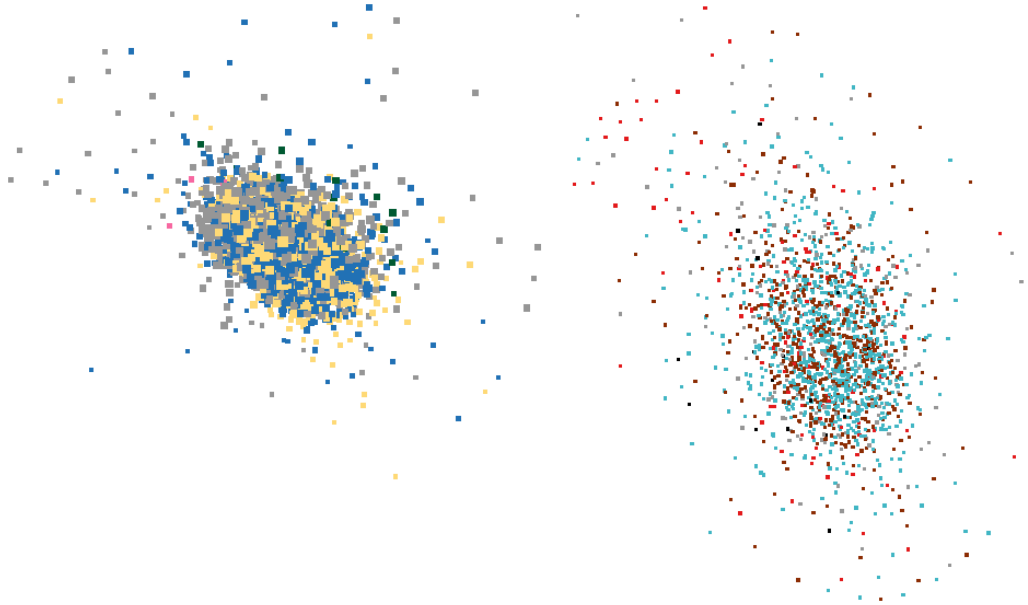

Figure B.16. WGCNA summary for Bur ecotype. Nodes correspond to genes in the network. Edges were omitted due to visualization purposes, but there were no connection between two visually distinct clusters of nodes. Colors of the nodes represent the modules, while the size of the node correspond to centrality of the node in Bur network.

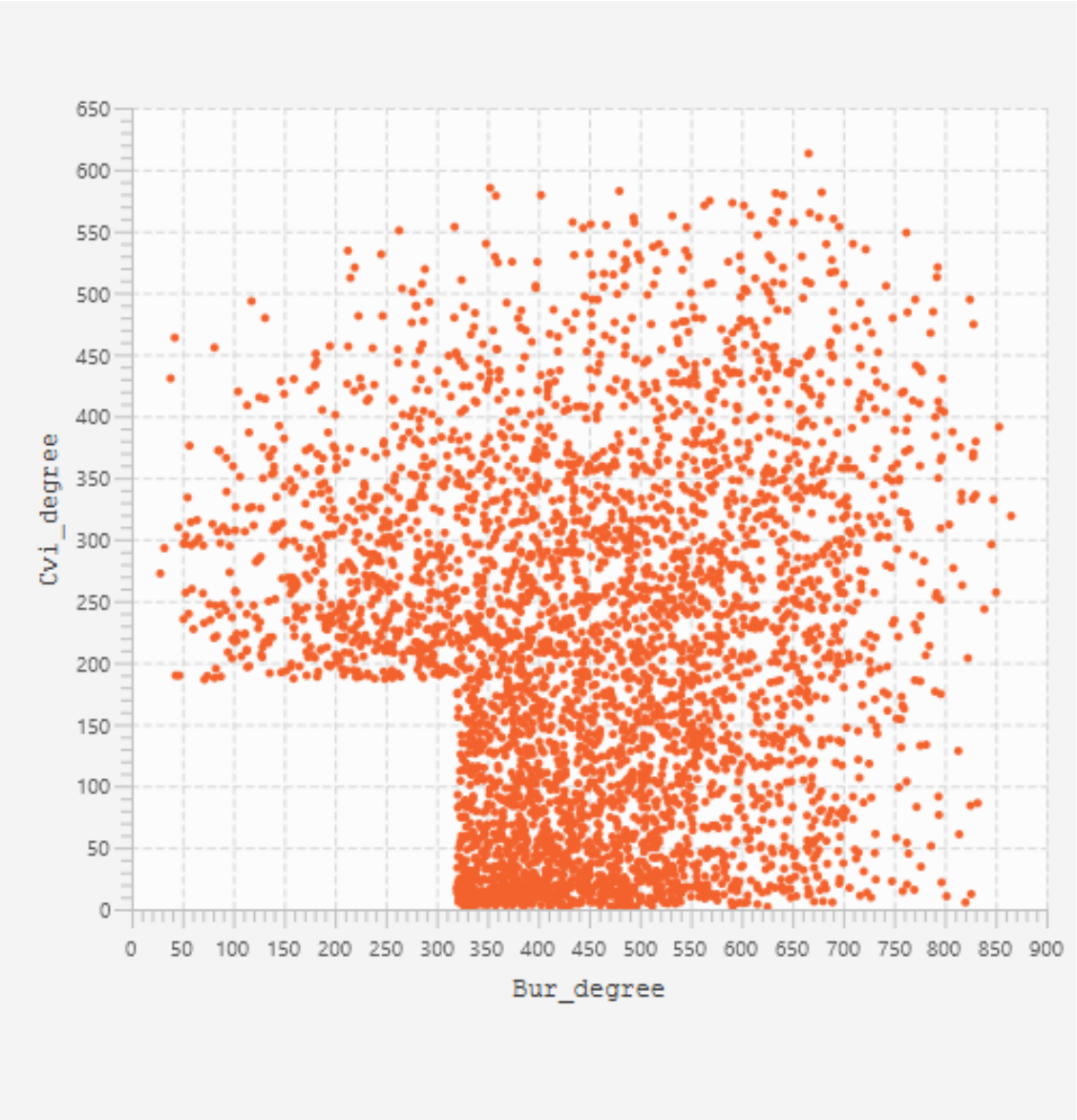

Figure B.17. Scatterplot of degree connectivity between Bur and Cvi networks. Degree connectivity was compared between Cvi (y-axis) and Bur (x-axis) networks.

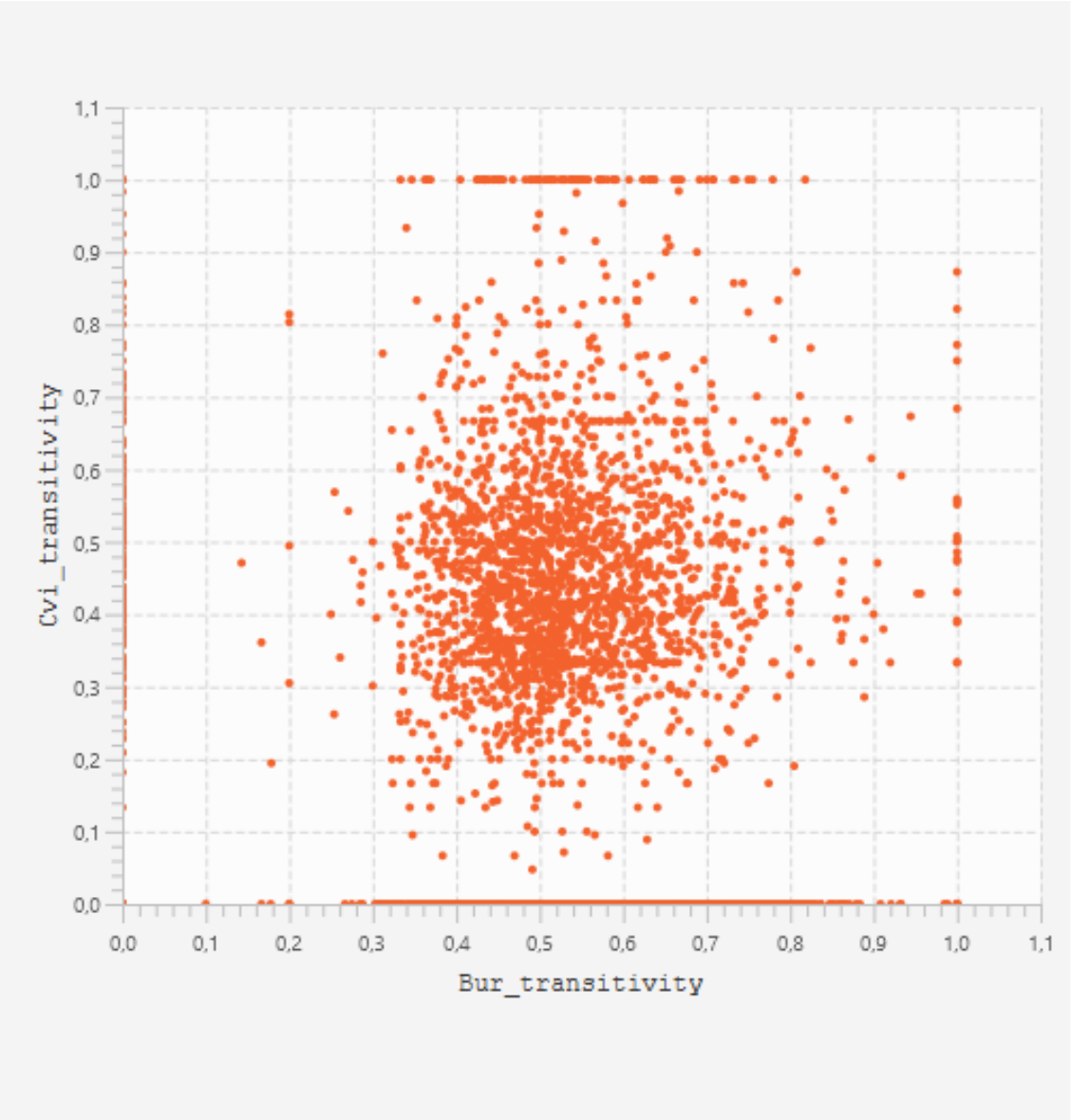

Figure B.18. Scatterplot of transivity between Bur and Cvi networks. Degree connectivity was compared between Cvi (y-axis) and Bur (x-axis) networks.

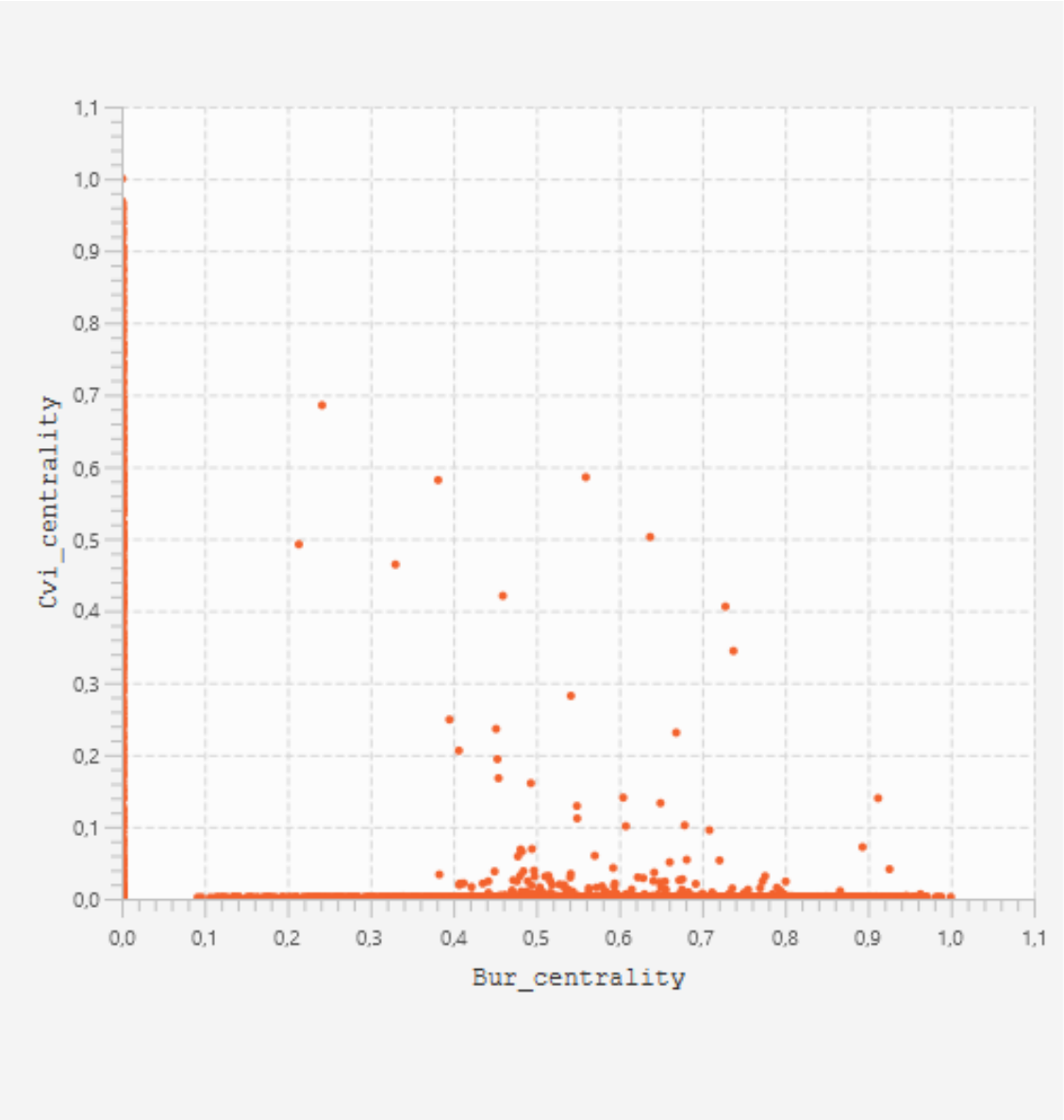

Figure B.19. Scatterplot of centrality between Bur and Cvi networks. Centrality was compared between Cvi (y-axis) and Bur (x-axis) networks.

B.4.5. GO Analyses of WGCNA Modules - Tables

Gene ontology analyses were performed for all the modules identified in the WGCNA (Tables B.1 - B.8).

Table B.1. GO analysis results for the black module (723 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0042742"	"defense response to bacterium"	673	57	30.52	0.00065
"GO:0007165"	"signal transduction"	1749	108	79.32	0.0019
"GO:0050832"	"defense response to fungus"	422	37	19.14	0.048
"GO:0051641"	"cellular localization"	977	48	44.31	0.0575
"GO:0010050"	"vegetative phase change"	12	4	0.54	0.0775
"GO:0002682"	"regulation of immune system process"	125	17	5.67	0.0915
"GO:0045088"	"regulation of innate immune response"	61	6	2.77	0.1415
"GO:0048497"	"maintenance of floral organ identity"	7	3	0.32	0.1415
"GO:0010200"	"response to chitin"	211	19	9.57	0.173
"GO:1901701"	"cellular response to oxygen-containing compound"	916	49	41.54	0.176

Table B.2. GO analysis results for the blue module (1566 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0046777"	"protein autophosphorylation"	142	35	14.34	2.15e-05
"GO:0018105"	"peptidyl-serine phosphorylation"	88	25	8.89	6e-05
"GO:0042742"	"defense response to bacterium"	673	106	67.95	0.000135
"GO:0006897"	"endocytosis"	74	22	7.47	0.00018
"GO:0007165"	"signal transduction"	1749	268	176.6	0.0012
"GO:0042147"	"retrograde transport, endosome to Golgi"	23	10	2.32	0.0018
"GO:0034727"	"piecemeal microautophagy of the nucleus"	6	5	0.61	0.00285
"GO:0051453"	"regulation of intracellular pH"	22	10	2.22	0.0032
"GO:0000422"	"autophagy of mitochondrion"	16	8	1.62	0.00325
"GO:0009751"	"response to salicylic acid"	271	49	27.36	0.0045

Table B.3. GO analysis results for the brown module (1322 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0006364"	"rRNA processing"	243	82	20.9	3.45e-10
"GO:0009451"	"RNA modification"	266	77	22.88	7e-08
"GO:0000470"	"maturation of LSU-rRNA"	43	16	3.7	7.5e-05
"GO:0006189"	"'de novo' IMP biosynthetic process"	12	7	1.03	9e-04
"GO:0009793"	"embryo development ending in seed dorman..."	580	82	49.89	0.0027
"GO:0044085"	"cellular component biogenesis"	1404	189	120.77	0.00275
"GO:0000462"	"maturation of SSU-rRNA from tricistronic..."	35	12	3.01	0.00465
"GO:0000460"	"maturation of 5.8S rRNA"	32	14	2.75	0.006
"GO:0006360"	"transcription by RNA polymerase I"	11	6	0.95	0.0065
"GO:0048316"	"seed development"	1087	147	93.5	0.011

Table B.4. GO analysis results for the green module (900 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0010498"	"proteasomal protein catabolic process"	254	36	15.21	1.55e-08
"GO:0006412"	"translation"	536	65	32.1	0.00011
"GO:0006511"	"ubiquitin-dependent protein catabolic pr..."	401	50	24.02	0.000175
"GO:0000338"	"protein deneddylation"	10	6	0.6	0.000385
"GO:1901259"	"chloroplast rRNA processing"	23	8	1.38	0.00175
"GO:0006364"	"rRNA processing"	243	37	14.55	0.0034
"GO:0032544"	"plastid translation"	20	7	1.2	0.0055
"GO:0006515"	"protein quality control for misfolded or..."	31	9	1.86	0.007
"GO:1902600"	"proton transmembrane transport"	49	11	2.93	0.011
"GO:0045039"	"protein insertion into mitochondrial inn..."	14	5	0.84	0.0485

Table B.5. GO analysis results for the pink module (294 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0051259"	"protein complex oligomerization"	42	11	0.77	3.1e-12
"GO:0009408"	"response to heat"	241	28	4.42	3e-09
"GO:0071456"	"cellular response to hypoxia"	183	17	3.36	2.25e-06
"GO:0006457"	"protein folding"	183	18	3.36	3.65e-06
"GO:0042542"	"response to hydrogen peroxide"	58	10	1.06	4.35e-06
"GO:0010286"	"heat acclimation"	56	7	1.03	0.009
"GO:0009651"	"response to salt stress"	490	20	8.98	0.036
"GO:0009415"	"response to water"	750	17	13.75	0.068
"GO:0009308"	"amine metabolic process"	92	4	1.69	0.161
"GO:0140021"	"mitochondrial ADP transmembrane transpor..."	5	2	0.09	0.1615

Table B.6. GO analysis results for the red module (792 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0010105"	"negative regulation of ethylene-activate..."	15	5	0.77	0.0335
"GO:0048831"	"regulation of shoot system development"	143	7	7.29	0.056
"GO:0009611"	"response to wounding"	498	39	25.4	0.261
"GO:0071230"	"cellular response to amino acid stimulus"	8	3	0.41	0.305
"GO:0009864"	"induced systemic resistance, jasmonic ac..."	8	3	0.41	0.305
"GO:0007166"	"cell surface receptor signaling pathway"	25	5	1.28	0.386
"GO:0071470"	"cellular response to osmotic stress"	37	6	1.89	0.438
"GO:0071577"	"zinc ion transmembrane transport"	9	3	0.46	0.4405
"GO:0051570"	"regulation of histone H3-K9 methylation"	9	3	0.46	0.4405
"GO:0009451"	"RNA modification"	266	21	13.57	0.4735

Table B.7. GO analysis results for the turquoise module (2156 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0006412"	"translation"	536	228	75.88	5e-29
"GO:0002181"	"cytoplasmic translation"	77	41	10.9	1.6e-16
"GO:0010035"	"response to inorganic substance"	1515	223	214.47	1.35e-12
"GO:0000028"	"ribosomal small subunit assembly"	25	15	3.54	7e-06
"GO:0006414"	"translational elongation"	44	20	6.23	6e-05
"GO:0000027"	"ribosomal large subunit assembly"	26	14	3.68	0.00011
"GO:0044085"	"cellular component biogenesis"	1404	295	198.76	2e-04
"GO:1903046"	"meiotic cell cycle process"	206	52	29.16	0.00046
"GO:0009089"	"lysine biosynthetic process via diaminop..."	14	9	1.98	0.00115
"GO:0042254"	"ribosome biogenesis"	329	102	46.58	0.0044

Table B.8. GO analysis results for the yellow module (1157 genes).

GO.ID"	Term	Annotated	Significant	Expected	p.value
"GO:0016567"	"protein ubiquitination"	487	73	36.82	9e-06
"GO:0006886"	"intracellular protein transport"	441	67	33.34	0.000125
"GO:0031146"	"SCF-dependent proteasomal ubiquitin-depe..."	52	15	3.93	0.000215
"GO:0006623"	"protein targeting to vacuole"	34	11	2.57	0.00125
"GO:0010228"	"vegetative to reproductive phase transit..."	304	45	22.98	0.0017
"GO:0044248"	"cellular catabolic process"	1376	172	104.04	0.0105
"GO:0006896"	"Golgi to vacuole transport"	18	7	1.36	0.0105
"GO:0007035"	"vacuolar acidification"	6	4	0.45	0.0215
"GO:0050793"	"regulation of developmental process"	864	89	65.33	0.0295
"GO:0009723"	"response to ethylene"	171	21	12.93	0.0475

B.4.6. Eigengene Expression of WGCNA Modules

Eigengene expressions were calculated using the complete FPKM means available for all timepoints, since some timepoints are missing the publicly available transcriptome data. The eigengene represent a summary of the expression profiles of the genes in the module. The correlation of eigengene to sample data (i.e. the correlation of green module to temperature) can differ between ecotypes. The difference in eigengene for modules, which were identified to be conserved in connection across all ecotypes, can be interpreted to be the driving force of ecotypic differences in dormancy cycling behavior.

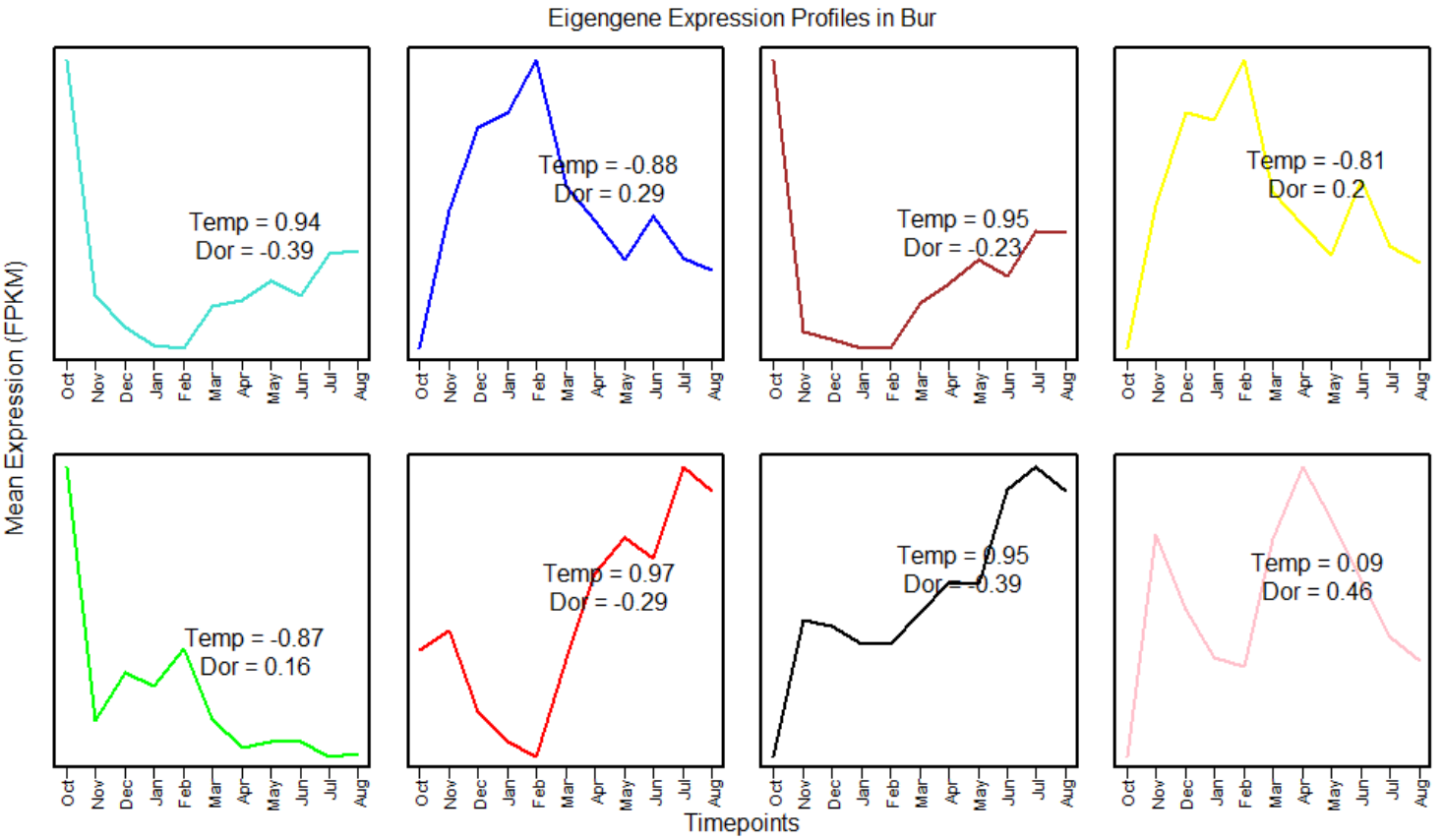

Figure B.20. Expression of module eigengenes in the Bur network. Expression of module eigengenes were plotted according to mean FPKM values per timepoint. Expression values are omitted as they are relative within modules and ecotypes, therefore not informative. Pearson correlation values to soil temperature and dormancy at the sampling date are shown within plot.

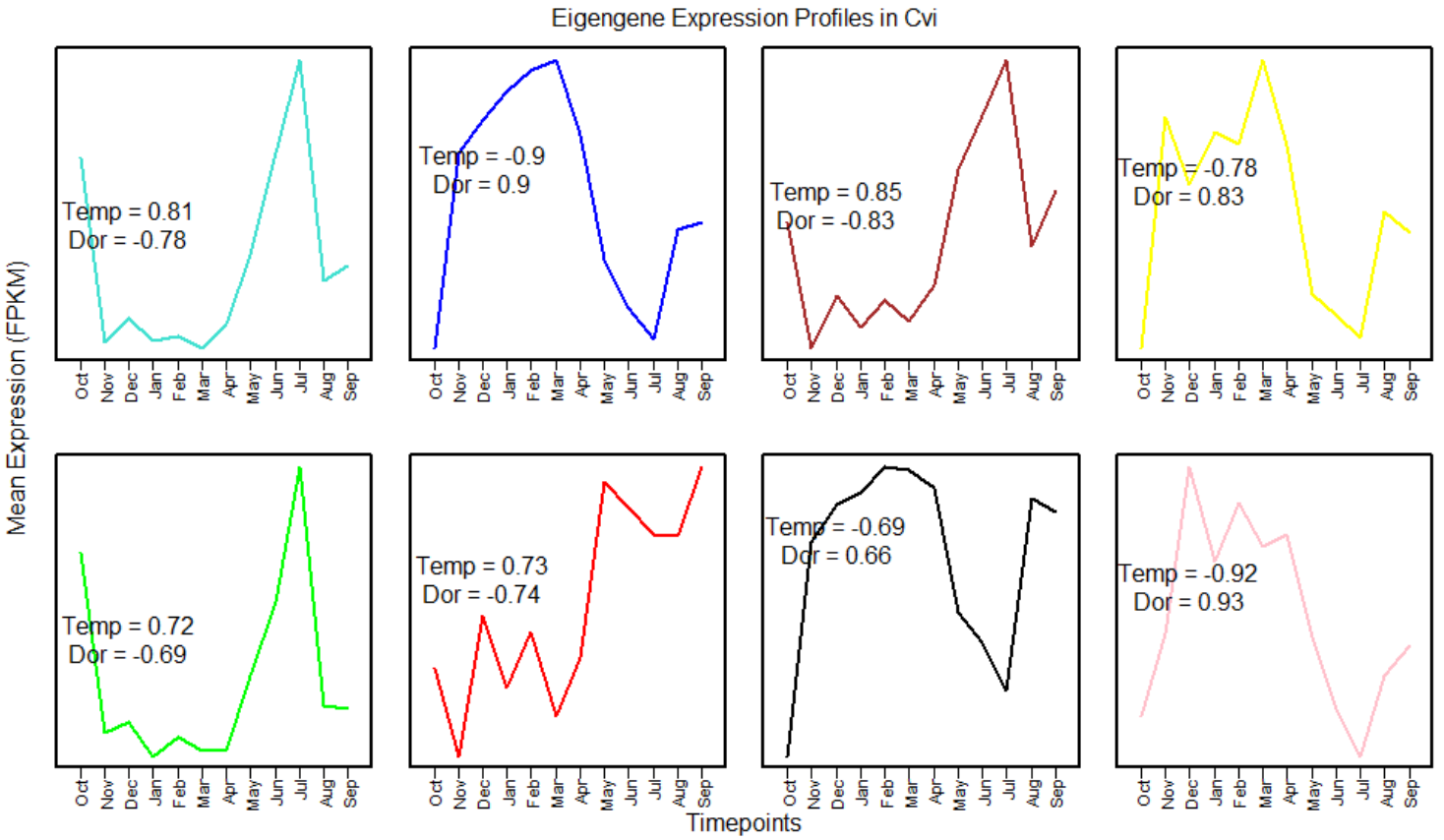

Figure B.21. Expression of module eigengenes in the Cvi network. Expression of module eigengenes were plotted according to mean FPKM values per timepoint. Expression values are omitted as they are relative within modules and ecotypes, therefore not informative. Pearson correlation values to soil temperature and dormancy at the sampling date are shown within plot.